

Home proximity to flower plantations and higher systolic blood pressure among children

Jose R. Suarez-Lopez^{a,*}, Vennis Hong^b, Kelsey N. McDonald^c, Jose Suarez-Torres^d, Dolores López^d, Franklin De La Cruz^d

^a University of California, San Diego, La Jolla, CA, 92093, USA

^b University of California, Los Angeles, Los Angeles, CA, 90095, USA

^c Macalester College, St. Paul, MN, 55105, USA

^d Fundacion Cimas del Ecuador, Quito, Ecuador

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ABSTRACT

Background: Pesticide drift from agricultural plantations increases the chemical exposure potential of people living nearby. Some studies have described positive associations between pesticide exposures and blood pressure (BP) in adults, whereas limited evidence in children suggests negative associations. This study characterized the association between home proximity to plantations and BP among children living in a flower-growing county in Ecuador.

Methods: We included 310 4–9-year-old children living in Pedro Moncayo County, Ecuador as part of The ESPINA study. We calculated age, gender and height-specific BP z-scores. Geographic coordinates of homes and flower plantations were collected using GPS receivers and satellite imagery. Exposure-outcome associations were analyzed using linear regression.

Results: The mean home distance to the nearest flower plantation was 449 m (SD: 347) and the median plantation area within 150 m of participants' homes was 989 m² (25th–75th percentile: 492–3164) among those with non-zero values. Children living closer to plantations had lower AChE activity. Systolic BP z-score increased with greater residential proximity to plantations (0.24 SD per 1000 m [95% CI: 0.01, 0.47]) and with greater areas of flower plantations within 150 m of homes (0.03 SD per 1000 m² [0.00, 0.06]), after adjusting for socio-economic, anthropometric and other factors. Further adjustment for acetylcholinesterase and hemoglobin strengthened these associations.

Conclusions: Proximity of homes to flower plantations and greater plantation areas within 150 m from homes were associated with higher systolic BP, independent of cholinesterase activity. This suggests that non-cholinesterase inhibitor pesticide drift from agricultural plantations may be sufficient to induce physiologic changes on children living nearby.

1. Introduction

People living near agricultural fields are known to have an increased risk of chronic exposure to pesticides, which in turn may alter physiological processes. Pesticide exposures appear to affect the cardiovascular system, and could increase the risk for cardiovascular conditions including arrhythmia, coronary artery disease, and congestive heart failure (Hung et al., 2015). High blood pressure is an important risk factor for cardiovascular disease (Kannel, 2000; Rapsomaniki et al., 2014) and a limited number of studies have

described associations between history of pesticide exposures and blood pressure in children and adults (Gordon and Padnos, 2000; Grandjean et al., 2006; Samsuddin et al., 2016; Smith and Gordon, 2005; Suarez-Lopez et al., 2013).

Among mosquito control workers in Malaysia, duration and magnitude of pesticide exposure were associated with elevated diastolic blood pressure (DBP) and systolic blood pressure (SBP) (Samsuddin et al., 2016). Among Sicilian women, twice the incidence rate of gestational hypertension was noted among those occupationally exposed to pesticides compared to those who had indirect exposures or were not

Abbreviations: AChE, acetylcholinesterase activity; SBP, Systolic blood pressure; DBP, Diastolic blood pressure

* Corresponding author. Department of Family Medicine and Public Health, 9500 Gilman Dr. MC: 0725, La Jolla, CA, 92093-0725, USA.

E-mail addresses: jrsuarez@ucsd.edu (J.R. Suarez-Lopez), vennishong@ucla.edu (V. Hong), kmcdonal@macalester.edu (K.N. McDonald), jsuarez@cimas.edu.ec (J. Suarez-Torres), dlopez@cimas.edu.ec (D. López), fcruz@cimas.edu.ec (F. De La Cruz).

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exposed to pesticides (Ledda et al., 2015). In a study of Ecuadorian school children, maternal work in agriculture during pregnancy, but not concurrent pesticide exposure of children, was associated with elevated systolic blood pressure in children (Grandjean et al., 2006). These positive associations from epidemiological studies were also observed in experimental studies in which rats treated with the organophosphate chlorpyrifos, a cholinesterase inhibitor, developed increases in SBP and DBP (Gordon and Padnos, 2000; Smith and Gordon, 2005). In contrast to these findings, we previously observed lower systolic blood pressure among children who lived with a flower plantation worker, and observed a positive association between acetylcholinesterase (AChE) activity and SBP and DBP, among children growing up in agricultural communities in Ecuador (Suarez-Lopez et al., 2013). AChE is a physiological marker of exposure to cholinesterase inhibitor pesticides; lower values reflect greater exposure (Taylor, 2011). The differing associations observed across the studies mentioned above may be due to differences in the age-groups of participants and in the construct of exposure used (e.g. urinary metabolites vs AChE activity vs history of work in agriculture). One construct of exposure which has not been studied in this context is home proximity to agricultural pesticide spray sites.

Several studies suggest that pesticide drift may play an important role in pesticide exposures and health of families living within the vicinity of the application sites. For instance, a 20% increase in an urinary organophosphate metabolite per mile of greater residential proximity to farmland was observed among families living in agricultural areas in Washington State, USA (Coronado et al., 2011). Similarly, dose-response relationships have been described between acreage of farmland within 750 m of homes and herbicide levels in house dust among families in rural Iowa, USA (Ward et al., 2006). These effects have been observed at smaller distances in two pediatric studies, in which children living within 60 m from fruit orchards and agricultural fields had higher pesticides levels in house dust and urinary metabolite levels than those living farther away (Bradman et al., 2011; Lu et al., 2000). Residential distance to plantations is an indicator of pesticide drift for many classes of pesticides, as fungicides, insecticides and herbicides are usually used concomitantly in agriculture.

There is some evidence that proximity to pesticide spray sites may affect the health of children. Pregnant mothers in California who lived near fields where organophosphate or pyrethroid pesticides were used were found to have offspring with lower cognitive function in childhood, or with greater risk of having autism spectrum disorders (Gunier et al., 2016; Roberts et al., 2007; Rowe et al., 2016; Shelton et al., 2014). Additionally, there is some evidence that proximity of pregnant women's homes to pesticide spray sites is associated with certain heart defects in their offspring (Carmichael et al., 2014). More studies assessing various health components of children living near agricultural sites are needed, as it is important to understand how far residential zones and schools should be from pesticide spray sites to minimize the potential for exposure and related adverse health problems.

The aim of this study was to determine whether proximity of homes to flower plantations was associated with blood pressure alterations among a cohort of Ecuadorian children who did not work in agriculture but lived in agricultural communities in Pedro Moncayo County, Ecuador. The floricultural industry in Pedro Moncayo has a prevalent use of insecticides (organophosphates, neonicotinoids and pyrethroids) and fungicides (quinone outside inhibitors, azoles, dithiocarbamates) (Grandjean et al., 2006; Handal et al., 2016; Harari, 2004; Suarez-Lopez et al., 2017).

2. Materials and methods

2.1. Study description

The study of Secondary Exposures to Pesticides among Children and Adolescents (ESPIÑA, Exposición Secundaria a Plaguicidas en Niños y

Adolescentes) (Suarez-Lopez et al., 2012) is aimed at understanding the effects of asymptomatic pesticide exposures on child development in a population of children living in Pedro Moncayo County, Pichincha, Ecuador. With approximately 1800 hectares of flower plantations (5.3% of the County's surface area) (Gobierno Municipal del Cantón Pedro Moncayo, 2011), a sizeable portion of the residents of this Andean county live in the vicinity of a flower plantation.

We examined 313 boys and girls of ages 4–9 years in 2008, 73% of whom were recruited using contact information from their participation in the 2004 Survey of Access and Demand of Health Services in Pedro Moncayo County, a large representative survey of Pedro Moncayo County conducted by Fundación Cimas del Ecuador in collaboration with the communities of Pedro Moncayo county. The remaining 27% of participants were recruited by word of mouth or community announcements. We also conducted home interviews of 451 parents and agricultural workers who lived with the examined children. The ESPINA study was carried out in response to the needs defined locally by the people and leaders of Pedro Moncayo County. Additional recruitment information has been described elsewhere (Suarez-Lopez et al., 2012). For the present analyses, we included 310 (98%) children who had all covariates of interest.

The ESPINA study was approved by the Institutional Review Boards of Fundación Cimas del Ecuador, the University of Minnesota, the University of California San Diego, Universidad San Francisco de Quito and the Ministry of Public Health of Ecuador. Informed consent, parental authorization of child participation and child assent of participants 7 years of age and older were obtained.

2.2. Survey

Information on socio-economic status, demographics, health, and pesticide exposure histories of participant children, children's parents and other adults in the household were obtained through home interviews by trained interviewers.

2.3. Examination

Children's heights were measured using a height board, following recommendations by the World Health Organization (World Health Organization, 2008). Resting heart rates were measured during a 30-s auscultation. Blood pressure was measured with a pediatric Omron aneroid sphygmomanometer, appropriate for the arm sizes of the children. Blood pressure measurements followed the recommendations of the American Heart Association (Pickering et al., 2005). Children were seated with uncrossed legs and both feet on the floor with the antecubital fossa supported at heart level. Two measurements were taken after at least 5 min of rest. The average of both blood pressure measurements were used in these analyses. Interpretation of blood pressure levels in pediatric populations is conducted using age, gender and height specific blood pressure z-scores (National High Blood Pressure Education Program Working Group on High Blood Pressure in Children and Adolescents, 2004). Such blood pressure z-scores were calculated using formulas from the Fourth Report on the Diagnosis, Evaluation, and Treatment of High Blood Pressure in Children and Adolescents (National High Blood Pressure Education Program Working Group on High Blood Pressure in Children and Adolescents, 2004).

2.4. Biological measures

Erythrocytic AChE activity and hemoglobin concentrations were measured from a single finger stick sample using the EQM Test-mate ChE Cholinesterase Test System 400 (EQM AChE Erythrocyte Cholinesterase Assay Kit 470).

Table 1

Demographic, social-economic characteristics and physiological variables distributed by tertiles of residential distance to the nearest flower plantation edge.

	Total	Tertiles of residential distance to the nearest flower plantation			p-trend ^b
		1 st	2 nd	3 rd	
Range, m	0–1553	0–232	233–532	533–1553	
Mean (SD), m	449 (347)	128.5 (67)	369 (93)	844 (291)	
N	310	103	103	104	
Age (years)	6.6 (1.6)	6.46 (1.6)	6.74 (1.63)	6.53 (1.60)	0.01
Gender (female) %	49	46	50	51	0.84
Race					
Mestizo (%)	76	72	71	86	0.84
Indigenous (%)	22	28	23	14	0.76
Other (%)	2	0	6	0	–
Lived with floricultural worker %	49	52	50	45	0.01
Income level ^a	3.1 (0.8)	3.2 (0.8)	3.2 (0.9)	2.9 (0.7)	0.01
Maternal education (years)	7.3 (3.8)	7.1 (3.6)	7.8 (4.1)	6.9 (3.8)	0.78
Height (m)	112.1 (10.3)	110.7 (9.7)	113.2 (10.2)	112.6 (10.8)	0.02
SBP (mmHg)	93.4 (8.3)	94.6 (7.2)	93.3 (7.8)	92.3 (9.7)	0.10
DBP (mmHg)	49.3 (7.2)	48.6 (6.1)	49.3 (6.8)	50.0 (8.3)	< 0.01
SBP z-score	–0.01 (0.71)	0.16 (0.62)	–0.05 (0.67)	–0.14 (0.81)	0.01
DBP z-score	–0.51 (0.60)	–0.54 (0.49)	–0.53 (0.60)	–0.46 (0.70)	< 0.01
Heart Rate (beats/min)	84.9 (12.6)	84.7 (12.0)	84.3 (12.0)	85.6 (12.9)	0.59
Hemoglobin (mg/dl)	12.6 (1.2)	12.6 (1.1)	12.67 (1.1)	12.6 (1.3)	< 0.01
Acetylcholinesterase (U/ml)	3.14 (0.49)	3.08 (0.48)	3.13 (0.51)	3.20 (0.48)	< 0.01

SBP = systolic blood pressure and DBP = diastolic blood pressure.

^a Data presented are percentage or mean (SD). Monthly income categories (USD): 1 = 0–50, 2 = 51–150, 3 = 151–300, 4 = 301–500, 5 = 501–999, 6 = > 1000.^b P-trend corresponds to the continuous variable of residential distance to the nearest flower plantation.

2.5. Geospatial information

Geographical coordinates of Pedro Moncayo County homes were obtained through portable global positioning system receivers in 2004, 2006 and 2010 by Fundacion Cimas del Ecuador, as part of the System of Local and Community Information (Sistema de Información Local y Comunitario [SILC]). Flower plantation edges (areal polygons) were created in 2006 from satellite imagery. Residential distances from the nearest 1-m segment of flower plantation edge were calculated using ArcGIS 9.3 (Esri, Redlands, CA). To further quantify the amount of potential exposure from pesticide drift, areas of flower plantations within a 150 m radius of each participant's home were calculated.

2.6. Statistical analysis

We calculated the mean and standard deviation of characteristics of all participants, and by strata of tertiles of residential distance to the nearest flower plantation. To test whether values differed across tertiles of distance, we calculated p-trend values using simple linear regression and with residential distance to the nearest plantation as a continuous variable.

We analyzed the association of children's blood pressure with residential distance to the nearest flower plantation and areas of flower plantations within 150 m of their residence using multiple linear regression. We analyzed the variable of residential distance to the nearest flower plantation as a continuous variable and as tertiles. We also calculated the least square mean SBP and DBP for each tertile of residential distance to the nearest flower plantation and we tested the pair-wise differences between tertiles (reference category: tertile 1).

Areas of flower plantations within 150 m of the children's residence was analyzed as a continuous variable and as a 3-category variable: the first category included residences without flower plantations within the buffer; the second and third categories included the lower and upper 50th percentile of non-zero flower plantation area values, respectively. Least-square means were calculated for each category of area of flower plantations within 150 m of children's residence, and blood pressure differences were calculated between the lower and upper 50th percentiles and the reference category (no flower plantations within 150 m of

residences).

We ran multivariable linear regression models adjusting for several covariates, selected *a priori*, associated with exposure to pesticides and blood pressure. Model 1 adjusted for age, gender, race, height, income, maternal education, examination date, and cohabitation with a floricultural worker. We further adjusted for heart rate to control for transient blood pressure effects related to heart rate; the outcome of our hypothesis focuses on chronic (rather than transient) blood pressure changes associated with the exposure. In adolescents, heart rate is positively associated with blood pressure (Christofaro et al., 2017). Model 1 adjusted for examination date considering that participants in our study were examined between 63 and 100 days after the end of a peak-pesticide spray season (the Mother's day flower harvest); we previously observed that examination date was positively associated with AChE activity (Suarez-Lopez et al., 2017), affecting particularly children living in close proximity to plantations. We adjusted for cohabitation with a floricultural worker since we found that children living with flower plantation workers had lower AChE activity compared to children not living with agricultural workers (Suarez-Lopez et al., 2012). Both examination date and cohabitation with floricultural workers would hence be potential confounders considering that we previously described a strong positive association between AChE activity and both SBP and DBP in ESPINA study participants, and children living with flower plantations workers had lower blood pressure compared to children not living with agricultural workers (Suarez-Lopez et al., 2013). Model 2 further adjusted for AChE activity and hemoglobin concentration. As a standard practice, models including (red blood cell) AChE activity are further adjusted for hemoglobin concentration to account for AChE differences related to hematocrit. We tested effect modification by sex and age using interaction models that included the exposure, effect modifier and the interaction term (exposure*effect modifier). For a visual depiction of the association between residential distance to flower plantations and blood pressure, we plotted the model-2 adjusted least squared means of blood pressure (systolic and diastolic) across deciles of distance. Data were analyzed with SAS Version 9.4 (SAS Institute Inc., Cary, NC).

3. Results

3.1. Participant characteristics

The mean age of children was 6.6 years (SD: 1.6), 49% were female, 76% mestizo (mix of white and indigenous), 22% indigenous, and 49% lived with a flower worker (Table 1). Compared to children in the United States, blood pressure z-scores (age, gender and height appropriate) were similar for SBP (mean: -0.01 SD, SD: 0.71) but lower for DBP (mean: -0.51 SD, SD = 0.60). The residences of 19.4% of the children were within 150 m of a flower plantation. Among these residences, the median (25th–75th percentile) area of flower plantations was 989 m² (492, 3164), with a range of 43 to 27,457 m². Height, DBP, DBP z-score, and AChE levels were positively associated with residential distance to the nearest flower plantation, whereas flower worker cohabitation, income, SBP, and SBP z-score were negatively related. The distribution by gender and race did not vary by residential distance to the nearest flower plantation (Table 1).

3.2. Blood pressure and heart rate as a function of residential distance to the nearest flower plantation edge

SBP and DBP z-scores were positively related to residential proximity (hence, negatively related to distance) to the nearest flower plantation edge. In model 1, for every 1000 m greater proximity to a plantation, SBP increased by 2.44 mmHg (95% CI: 0.00, 4.88) and SBP z-score increased by 0.24 SD (0.01, 0.47), Table 2 and Fig. 1. This association was strengthened when further adjusted for hemoglobin and acetylcholinesterase activity in model 2 (SBP per 1000 m greater proximity = 2.98 [95% CI: 0.49, 5.47]; SBP z-score per 1000 m greater proximity = 0.29 SD (95% CI: 0.06, 0.53). In model 1, children living within 233 m (tertile 1) of a plantation had the highest SBP mean (95.0 mmHg) which was significantly higher by 2.2 mmHg and 2.6 mmHg than those of children living within 233–532 m (tertile 2), and 533–1553 m (tertile 3), respectively (Table 2). Residential distance to the nearest flower plantation edge was not associated with DBP or z-

score of DBP. There was a significant association with pulse pressure (Model 1: β per 1000 m = -3.9 mmHg [95% CI: -6.2 , -1.6]) and no association with heart rate (Model 1: β per 1000 m = 0.7 bpm [95% CI: -3.3 , 4.7]). There was no evidence of effect modification by age or gender on any of the associations.

3.3. Blood pressure and heart rate as a function of surface areas of flower plantations within 150 m of homes

SBP was positively associated with areas of flower plantation within 150 m of children's residences. For every 1000 m² of flower plantation areas, SBP increased by 0.32 mmHg (95% CI: 0.01, 0.64) and SBP z-score increased by 0.03 (0.00, 0.06) in model 1 (Table 3). These associations were strengthened, although minimally, after further adjusting for AChE and hemoglobin (model 2). Participants with the highest areas of flower plantations near their homes had the highest SBP and SBP z-scores (Table 3). In both models, we observed statistically significant differences in SBP and SBP z-score between the upper 50th percentile of areas of flower plantations (category 3) and having no areas of flower plantations within 150 m of residences (category 1). No consistent associations were observed with DBP, and no associations were observed with heart rate (Model 1: β per 1000 m² = 0.10 bpm [95% CI: -0.41 , 0.62]). Pulse pressure was significantly associated with areas of flower plantation within 150 m of homes (Model 1: β per 1000 m² = 0.42 mmHg [95% CI: 0.12, 0.72]). There was no evidence of effect modification by age or gender on any of the associations.

4. Discussion

The present study analyzed the associations between blood pressure and 2 constructs of off-target pesticide drift from pesticide spray sites: residential distance to flower plantations and areas of flower plantations near homes. SBP and pulse pressure, but not DBP or heart rate, was positively associated with residential proximity (-1^{st} distance) to flower plantations and with areas of flower plantations within 150 m of the homes. These findings suggest that off-target drift of pesticides may

Table 2

Adjusted associations between residential distance to the nearest flower plantation and blood pressure among children.

	Range, m N	Tertiles of residential distance to the nearest flower plantation			Difference (β) per 1000 m decrease (95% CI) ^a
		1st 0–233	2nd 233–532	3rd 533–1553	
		103	103	104	
Model 1					
SBP, mmHg	Mean	95.1 (93.7, 96.5)	92.8 (91.4, 94.2)*	92.4 (91.0, 93.8)*	2.44 (0.00, 4.88)*
	Difference vs tertile 1	–	-2.2 (-4.1, -0.2)*	-2.6 (-4.6, -0.6)*	
SBP z-score, SD	Mean	0.14 (0.01, 0.27)	-0.07 (-0.20, 0.06)*	-0.11 (-0.24, 0.02)*	0.24 (0.01, 0.47)*
	Difference vs tertile 1	–	-0.21 (-0.39, -0.02)	-0.26 (-0.44, -0.06)	
DBP, mmHg	Mean	48.8 (47.6, 50.0)	48.9 (47.7, 50.1)	50.2 (48.9, 51.4)	-1.47 (-0.68, 3.62)
	Difference vs tertile 1	–	-0.13 (-1.86, 1.61)	1.38 (-3.16, 0.39)	
DBP z-score, SD	Mean	-0.55 (-0.66, -0.45)	-0.54 (-0.64, -0.43)	-0.44 (-0.55, -0.33)	-0.11 (-0.30, 0.08)
	Difference vs tertile 1	–	0.02 (-0.14, 0.17)	0.12 (-0.04, 0.27)	
Model 2					
SBP, mmHg	Mean	95.1 (93.7, 96.5)	92.9 (91.5, 94.2)*	92.2 (90.9, 93.6)*	2.98 (0.49, 5.47)*
	Difference vs tertile 1	–	-2.2 (-4.2, -0.3)*	-2.8 (-4.9, -0.8)*	
SBP z-score, SD	Mean	0.15 (0.02, 0.28)	-0.06 (-0.19, 0.07)*	-0.12 (-0.26, 0.01)*	0.29 (0.06, 0.53)*
	Difference vs tertile 1	–	-0.21 (-0.40, -0.03)*	-0.28 (-0.47, -0.09)*	
DBP, mmHg	Mean	48.9 (47.7, 50.2)	49.0 (47.8, 50.2)	49.9 (48.7, 51.2)	-0.75 (-2.93, 1.44)
	Difference vs tertile 1	–	0.08 (-1.63, 1.80)	1.01 (-0.76, 2.79)	
DBP z-score, SD	Mean	-0.54 (-0.65, -0.44)	-0.53 (-0.64, -0.42)	-0.46 (-0.56, -0.35)	-0.05 (-0.25, 0.14)
	Difference vs tertile 1	–	0.01 (-0.14, 0.17)	0.09 (-0.07, 0.24)	

Values presented are Mean (95% CI) or blood pressure difference per 1000 m.

SBP = systolic blood pressure. DBP = diastolic blood pressure (mmHg).

Model 1 covariates: age, gender, race, height, income, maternal education, heart rate, examination date, cohabitation with a floricultural worker.

Model 2 covariates: model 1 + hemoglobin concentration and acetylcholinesterase activity.

^a Calculation based on a natural log-transformed variable of distance.

* P_{trend} or $P_{\text{difference}} < 0.05$.

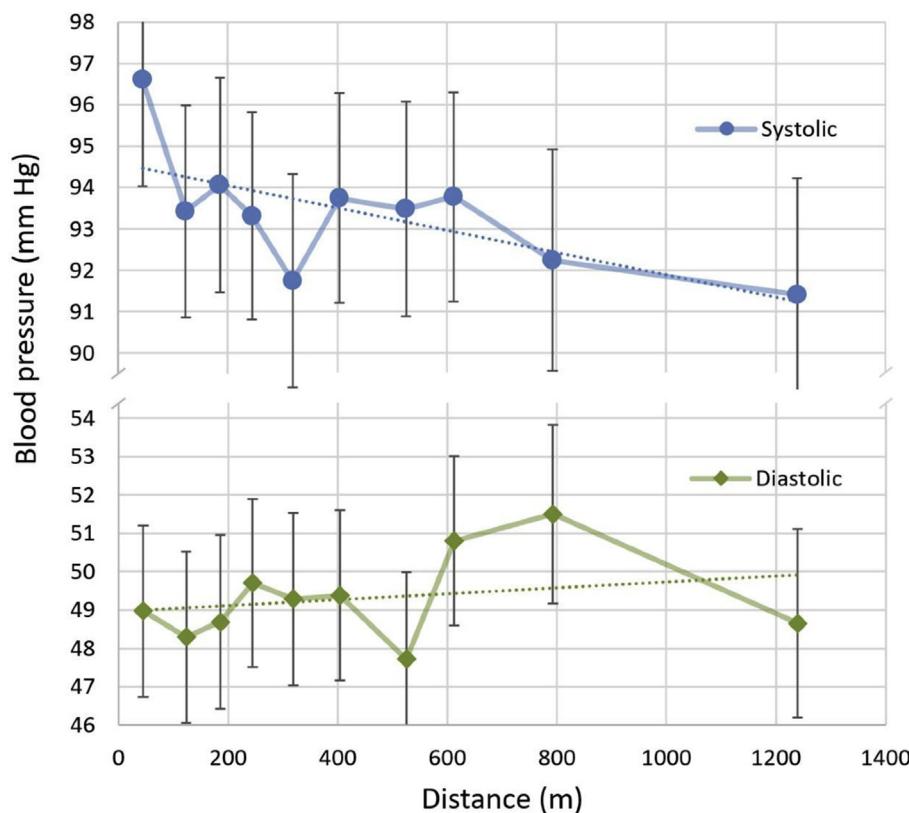


Fig. 1. Deciles of residential distance to the nearest flower plantation and blood pressure among children, n = 310.

Dotted lines depict the slope of the associations using residential distance to the nearest flower plantation as a continuous variable.

Adjusted for age, gender, race, height, income, maternal education, heart rate, examination date, cohabitation with a floricultural worker, AChE, and hemoglobin concentration.

Table 3

Associations between surface areas of flower plantations with 150 m of children's homes and blood pressure.

Range, m ² N	Categories of areas of flower plantations within 150 m of participants' homes			Blood pressure difference (β) per 1000 m ² (95% CI)
	1	2 ^a	3 ^a	
1	93.0 (92.2, 93.9)	93.8 (91.3, 96.3)	96.0 (93.2, 98.7)	0.32 (0.01, 0.64)*
0	–	0.72 (–1.93, 3.38)	2.91 (0.04, 5.79)*	0.03 (0.00, 0.06)*
250	0	43–989	990–27,457	
		32	28	
Model 1				
SBP, mmHg	Mean	93.0 (92.2, 93.9)	93.8 (91.3, 96.3)	96.0 (93.2, 98.7)
	Difference vs Tertile 1	–	0.72 (–1.93, 3.38)	2.91 (0.04, 5.79)*
SBP z-score	Mean	–0.04 (–0.13, 0.04)	0.03 (–0.21, 0.26)	0.23 (–0.03, 0.48)
	Difference vs Tertile 1	–	0.07 (–0.18, 0.32)	0.27 (0.00, 0.54)*
DBP, mmHg	Mean	49.5 (48.7, 50.3)	47.0 (44.8, 49.2)	50.0 (47.6, 52.4)
	Difference vs Tertile 1	–	–2.53 (–4.87, –0.20)*	0.50 (–2.02, 3.03)
DBP z-score	Mean	–0.49 (–0.56, –0.42)	–0.71 (–0.90, –0.52)	–0.44 (–0.65, –0.23)
	Difference vs Tertile 1	–	–0.22 (–0.43, –0.01)*	0.05 (–0.17, 0.27)
Model 2				
SBP, mmHg	Mean	93.0 (92.1, 93.9)	94.0 (91.5, 96.5)	96.1 (93.3, 98.8)
	Difference vs Tertile 1	–	1.00 (–1.67, 3.67)	3.07 (0.19, 5.94)*
SBP z-score	Mean	–0.05 (–0.13, 0.04)	0.05 (–0.19, 0.29)	0.24 (–0.02, 0.49)
	Difference vs Tertile 1	–	0.09 (–0.16, 0.35)	0.28 (0.01, 0.55)*
DBP, mmHg	Mean	49.4 (48.7, 50.2)	47.4 (45.2, 49.5)	50.2 (47.8, 52.6)
	Difference vs Tertile 1	–	–2.09 (–4.42, 0.24)	0.75 (–1.75, 3.25)
DBP z-score	Mean	–0.50 (–0.56, –0.43)	–0.68 (–0.87, –0.49)	–0.43 (–0.64, –0.22)
	Difference vs Tertile 1	–	–0.19 (–0.39, 0.02)	0.07 (–0.15, 0.29)

Values presented are Mean (95% CI) or blood pressure difference per 1000 m.

SBP = systolic blood pressure, DBP = diastolic blood pressure.

Model 1 covariates: age, gender, race, height, income, maternal education, heart rate, examination date, cohabitation with a floricultural worker.

Model 2 covariates: model 1 + hemoglobin concentration and acetylcholinesterase activity.

^a Median split of non-zero values.

* P_{trend} or $P_{\text{difference}} < 0.05$.

reach children living near plantations in sufficient amounts to alter their blood pressure.

To our knowledge, this is the first study to describe associations between home proximity to agricultural crops and alterations in blood pressure. Our findings concur with the few existing studies on the

relationship between pesticide exposures and blood pressure. Among experimental studies, rats were found to have increases in systolic, diastolic, and mean blood pressure for up to 56hrs after administration of chlorpyrifos (Gordon and Padnos, 2000; Smith and Gordon, 2005). Among human studies, adult male mosquito control workers in

Malaysia with self-reported exposure to pesticides were found to have increased heart rate, and elevated aortic and brachial SBP and DBP (Samsuddin et al., 2016). Among Sicilian women, the incidence of gestational hypertension was twice as high among women occupationally exposed to pesticides (12%) compared to those who had indirect or domestic exposures (6% and 7% respectively), and four times greater than the incidence among unexposed women (4%). (Ledda et al., 2015). Finally, a pilot study of Ecuadorian children aged 5–9 years living in the same area as our participants, reported a positive correlation between maternal work in floriculture during pregnancy and children's SBP (Harari et al., 2010). However, concurrent exposures to pesticides were not associated with blood pressure.

The findings in the present study contradicted our hypothesis that greater residential proximity would be associated with lower blood pressure, which was based on the previously observed positive associations between AChE activity and blood pressure in this cohort of Ecuadorian children (Suarez-Lopez et al., 2013). One explanation for this discrepancy is that AChE activity is a marker of exposure to cholinesterase inhibitors, whereas residential distance to flower plantation is an indicator of pesticide drift for many classes of pesticides. Floriculture in Pedro Moncayo county actively uses many classes of fungicides, insecticides and herbicides (Harari, 2004; Suarez-Lopez et al., 2017). In fact, 23 different carbamates and organophosphate insecticides were reported to be used by flower plantations in Pedro Moncayo, over 50 different fungicides, including azoles, quinone outside inhibitors and dithiocarbamates (Suarez-Lopez et al., 2017), and many other insecticides and herbicides. Currently it is unknown how pesticides such as fungicides, neonicotinoids, pyrethroids and others may affect the cardiovascular system; research is needed in this area.

In this study, home proximity to flower plantations and greater areas of flower plantations within 150 m of homes were positively associated with SBP and pulse pressure but not with DBP or heart rate. The reasons for this are unclear. Pulse pressure, calculated as the difference between SBP and DBP, increases with age. In older adults, this increase is largely attributed to stiffness of the large arteries and early pulse wave reflection (Nichols et al., 1992; O'Rourke and Nichols, 2005), whereas peripheral vascular resistance and increases in stroke volume appear to be more important contributors to hypertension in younger adults (Franklin et al., 1997; Messerli et al., 1983). In children, diabetes is one of the most important predictors of increased pulse pressure (Dost et al., 2014). We can only speculate that the chronic drift of non-cholinesterase inhibitors onto homes nearby could increase the peripheral vascular resistance of children; cholinesterase inhibition appears to have opposing effects. In this cohort, we previously observed lower SBP and DBP, but no changes in heart rate, associated with lower AChE activity likely from greater cholinesterase inhibitor insecticide exposure (Suarez-Lopez et al., 2013). This suggested that cholinesterase inhibitor pesticides may primarily induce vasodilation in children, perhaps through stimulation of muscarinic M3 receptors in the vascular endothelium (Bény et al., 2008; Eltze et al., 1993; Gericke et al., 2011; Lamping et al., 2004). In the present study, this likely explains why the associations in Model 1 were further strengthened after adjusting for AChE activity and hemoglobin. The present analyses suggest that non-cholinesterase inhibitor agro-chemicals may be responsible for the greater blood pressure seen in children living near plantations.

We observed a crude positive association between AChE activity and residential distances of homes to plantations (Table 1), which suggests that cholinesterase inhibitors, and plausibly other pesticides, reached children in greater amounts if they lived closer to flower plantations. This provides some validation of residential distance to plantations as a construct of pesticide exposure. An extensive assessment of the associations of AChE activity and residential distance to plantations, and areas of flower plantations near homes is described in a separate manuscript (in preparation). Proximity of homes to agricultural crops has been found to be an informative predictor of pesticide exposures due to off-target drift of pesticides from plantations onto

neighboring residential areas. For instance, a study on children living in Washington State found that families living within 60 m of orchards had higher pesticide concentrations in house dust and in urine compared to children living at greater distances (Lu et al., 2000). Families living more than a quarter mile from orchards had lower concentrations of dust and urine metabolites and no detectable levels of metabolites on their hands. Similarly, young children living less than 60 m from an agricultural field in California had increased concentrations of organophosphate urinary metabolites (dimethyl alkylphosphates and diethyl alkylphosphates) (Bradman et al., 2011). These effects have also been observed in studies assessing pesticide impact at greater distances from agricultural sites. For instance, Coronado et al. (2011) observed a 20% decrease in urinary concentrations of the organophosphate metabolite dimethylthiophosphate per mile decrease in proximity from farmland. Similarly, Ward et al. (2006) observed a dose-response relationship between acreage of farmland within 750 m and risk for detecting herbicides in house dust, suggesting that families surrounded by greater areas of farmland are exposed to greater amounts of pesticides than those surrounded by smaller areas of farmland.

Families living near agricultural sites are also at increased risk for illness. One population-based case-control study in California observed positive associations between pesticide use within 500 m of a mother's home during pregnancy and some specific heart defect phenotypes (Carmichael et al., 2014). In a birth cohort in California, it was found that greater use of pesticides within 1 km of homes of pregnant women were associated with decreases in cognitive function of their children at 7-years (Gunier et al., 2016) and 10-years (Rowe et al., 2016) of age. For each standard deviation increase in agricultural use of total organophosphates (237 kg) there was a 2 point decrease (15% of a standard deviation) in Full-Scale IQ (Gunier et al., 2016). Two case-control studies of autism spectrum disorders (ASD) in California found that pregnant mothers had greater odds of having children later diagnosed with ASD if they lived near organophosphate or pyrethroid pesticide spray sites (Shelton et al., 2014), or where organochlorine pesticides were detectable (Roberts et al., 2007).

The pesticide exposure levels that children in Pedro Moncayo County are subjected to may not be very different from those of similar communities in the United States and other developed countries. A study of Ecuadorian children living in the vicinity of the present study area reported that urinary metabolite concentrations of organophosphate pesticides were similar to those of a representative sample of children in the United States (Barr et al., 2003; Grandjean et al., 2006). The cholinesterase inhibitor pesticide exposure, assessed through urinary metabolites levels, among children in Pedro Moncayo county have been found to be similar to those observed among children living in agricultural communities in the United States (Barr et al., 2003; Grandjean et al., 2006). Additionally, AChE levels in our cohort were on par to those of Hispanic children living in agricultural settings in Oregon (Higgins et al., 2001; Suarez-Lopez et al., 2013, 2012), after accounting for slight age differences between the groups.

Limitations of this study include the inability to account for factors related to blood pressure such as prenatal toxicant exposures, perinatal health history, or concurrent and previous dietary patterns (e.g., salt and fat consumption). Additionally, we did not account for direction of prevailing winds in this area during the examination periods. This potential non-differential misclassification of the amount of pesticide drift from plantations to homes may have biased our findings towards the null (Gordis, 2014), as distance to plantations includes participants with higher and lower exposure potential from living either downwind or upwind from the plantations. Also contributing to potential exposure misclassification is the lack of information regarding which flower fields used pesticides and which did not. Organic (non-pesticide using) flower plantations are few in Pedro Moncayo. In 2007, only 0.65% of agricultural land in Ecuador was managed without pesticides (organic) (Willaer et al., 2008) and the United States, the biggest importer of Ecuadorian Flowers, reported that only 0.6% of flower sales were

organic in 2016 (United States Department of Agriculture, 2016).

Additionally, we do not have precise information regarding the exact locations in which participants spend most of the time, as pesticide drift from plantations may occur at various sites in the county. However, children were examined during the summer vacation and a majority of the daytime was probably spent at or near their homes.

Another limitation is that we were unable to discern which pesticide or class of pesticides were driving the associations described in these analyses. Although a growing number of pesticides can be quantified in urine, methods to quantify many pesticides in biospecimens, particularly fungicides and most herbicides, have not been developed. Finally, the crude validation of residential proximity to plantations as a construct of increased pesticide exposures through assessment of AChE activity is limited as its quantification occurred at 1 point in time so we cannot measure actual depression of activity.

5. Conclusions

Greater proximity of homes to plantations and greater areas of flower plantations in the vicinity of homes were associated with greater systolic and pulse pressures but had no association with DBP or heart rate in this cohort of 4-to-9 year-old children living in agricultural communities in Ecuador. These associations were independent of cholinergic activity. These findings indicate that pesticide drift from agricultural plantations may be sufficient to induce physiologic changes on children living nearby.

Declarations of interest

None.

Conflicts of interest

The authors declare no conflict of interest.

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