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Lower Acetylcholinesterase Activity among Children Living with Flower Plantation Workers

Jose R. Suarez-Lopez[a,b,*], **David R. Jacobs Jr.**[a,c], **John H. Himes**[a], **Bruce H. Alexander**[d], **DeAnn Lazovich**[a], and **Megan Gunnar**[e]

[a]Division of Epidemiology and Community Health, University of Minnesota, 1300 South 2nd Street, Suite 300, Minneapolis, MN 55454, USA

[b]Fundación Cimas del Ecuador, De los Olivos E14-226 y las Minas, Quito, Ecuador

[c]Department of Nutrition, School of Medicine, University of Oslo, Blindern 0316 Oslo, Norway

[d]Division of Environmental Health Sciences, University of Minnesota, 420 Delaware St. SE, MMC 807, Minneapolis, MN 55455, USA

[e]Institute of Child Development, University of Minnesota, 51 East River Parkway, Minneapolis, MN 55455, USA

Abstract

BACKGROUND—Children of workers exposed to pesticides are at risk of secondary pesticide exposure. We evaluated the potential for lower acetylcholinesterase activity in children cohabiting with fresh-cut flower plantation workers, which would be expected from organophosphate and carbamate insecticide exposure. Parental home surveys were performed and acetylcholinesterase activity was measured in 277 children aged 4–9 years in the study of Secondary Exposure to Pesticides among Infants, Children and Adolescents (ESPINA). Participants lived in a rural county in Ecuador with substantial flower plantation activity.

RESULTS—Mean acetylcholinesterase activity was 3.14 U/ml, standard deviation (SD): 0.49. It was lower by 0.09 U/ml (95% confidence interval (CI) −0.19, −0.001) in children of flower workers (57% of participants) than non-flower workers' children, after adjustment for gender, age, height-for-age, hemoglobin concentration, income, pesticide use within household lot, pesticide use by contiguous neighbors, examination date and residence distance to nearest flower plantation. Using a 4 level polychotomous acetylcholinesterase activity dependent variable, flower worker cohabitation (vs. not) had odds ratio 3.39 (95% CI 1.19, 9.64) for being <15th percentile compared to the highest tertile. Children cohabitating for ≥5 years (vs. never) had OR of 4.11 (95% CI: 1.17, 14.38) of AChE activity within <15th percentile compared to the highest tertile.

*__Corresponding Author:__ Jose R. Suarez-Lopez, Division of Epidemiology and Community Health, University of Minnesota. 1300 South 2nd Street, Suite 300, Minneapolis, MN 55454. Phone: (612) 626-8249. Fax: (612) 624-0315. suarez@umn.edu.

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CONCLUSIONS—Cohabitation with a flower worker was related to lower acetylcholinesterase activity in children. This supports the hypothesis that the amount of take-home pesticides from flower workers suffices to decrease acetylcholinesterase activity, with lower activity associated with longer exposure.

Keywords

Acetylcholinesterase; AChE; children; pesticide; organophosphate

1. Introduction

The agricultural industry, and specifically the fresh-cut flower industry, is a primary source of occupational pesticide exposure and intoxication. According to the National Institute of Censuses and Statistics of Ecuador, between 2001 and 2007 there were 14,145 pesticide poisonings, most commonly associated with work in the banana and fresh-cut flower industries. Seventy-one percent of the poisonings were due to cholinesterase-inhibiting pesticides (organophosphates and carbamates) (González-Andrade et al., 2010). The floricultural industry concomitantly uses various types of insecticides, fungicides and herbicides; among these, the most commonly used pesticides in the industry are organophosphate and carbamate insecticides (Castelnuovo et al., 2000; Harari, 2004; Paz-y-Mino et al., 2002). Because of inadequate regulation, safety practices and controls are, for practical purposes, determined by the flower plantations. Although some plantations follow adequate occupational safety precautions, it is not uncommon for unsafe practices to take place throughout the industry (e.g. short re-entry times (within a few hours) into greenhouses after pesticide fumigation) (Harari, 2004).

Family members of agricultural workers are at risk of pesticide contamination through secondary routes, which include proximity of the worker's residence to plantations and pesticide introduction into the home by the workers through clothes, hair, tools, and pesticide storage at home (Coronado et al., 2006; Curl et al., 2002; Fenske et al., 2002; Gladen et al., 1998). In US agricultural populations, households of agricultural workers had higher pesticide concentrations in dust (Curwin et al., 2005; Lu et al., 2000; McCauley et al., 2001; Simcox et al., 1995), while family members, including children, of agricultural workers had higher pesticide metabolite concentrations than non-agricultural worker families (Curwin et al., 2007; Fenske et al., 2000; Lambert et al., 2005).

While it is well understood that substantial acute exposures to cholinesterase-inhibiting pesticides cause toxicity in humans through excessive acetylcholine activity via suppression of acetylcholinesterase (AChE) activity (Kwong, 2002), it is uncertain whether secondary exposures to such pesticides are sufficient to depress AChE activity. Because of the indirect nature of secondary exposures, the amount of pesticides that come into contact with family members of agricultural workers is likely small; however, repeated low-dose exposures over long periods of time could inhibit cholinesterase activity without necessarily inducing overt symptoms (Kwong, 2002). AChE measurements have been widely used to monitor cholinesterase-inhibiting pesticide exposure and toxicity among agricultural workers. AChE is a carboxyl ester hydrolase present in the nervous system and in red blood cells that degrades acetylcholine into acetate and choline. Cholinesterase-inhibiting pesticides bind and inhibit AChE, causing an accumulation of acetylcholine at the nerve or myoneural terminal which causes an overstimulation followed by an inhibition of cholinergic neurotransmission at central and peripheral muscarinic and nicotinic receptors (Aaron CK, 2007; Abou-Donia, 2003). Acetylcholine is involved in a wide array of essential physiologic processes including cardiovascular, gastrointestinal, respiratory, neuromuscular, thermoregulatory, and behavioral (Guyton and Hall, 2006). The effects of cholinesterase-inhibiting pesticides on health can be acute or chronic and children are particularly sensitive to exposure to such chemicals (Faustman et al., 2000).

We hypothesized that the amount of secondary pesticide exposure from cohabitation with a flower plantation worker (flower worker) is sufficient to decrease AChE activity in children. We examined our hypothesis among children living in Pedro Moncayo County, Ecuador. Pedro Moncayo is primarily a rural county located in the Ecuadorian highlands and agriculture is its main source of income. The county has approximately 1800 hectares dedicated to flower production (5.3% of its surface area) (Gobierno-Municipal-del-Canton-Pedro-Moncayo, 2011), which are actively productive year-round due to the equatorial proximity of the county.

2. Material and methods

2.1. Study Description

We tested our hypothesis in participants of the study of Secondary Exposure to Pesticides among Infants, Children and Adolescents (ESPINA: Estudio de la Exposición Secundaria a Plaguicidas en Infantes, Niños y Adolescentes), a cross-sectional study with overarching aim to evaluate the effects of secondary exposure to pesticides on child development. The aim of the present report is to evaluate the association between flower worker cohabitation and children's AChE activity. Study participants resided in Pedro Moncayo County, Pichincha, Ecuador.

2.2. Recruitment

The recruitment objective was to enroll at least 260 children with an approximately balanced distribution of flower worker cohabitation status. The main source of ESPINA participants in 2008 was 922 children who participated in the 2004 Survey of Access and Demand of Health Services in Pedro Moncayo County, a survey that intended to interview all people living in Pedro Moncayo County, conducted by Fundacion Cimas del Ecuador. Through home visits, interviewers obtained socio-economic status, demographic, occupational and health information (of all members in the household) of 18,187 residents (71% of Pedro Moncayo County's population) and collected both height (or length) and weight of the 922 children younger than 5 years of age (27% of Pedro Moncayo County's children younger than 5) (Instituto Nacional de Estadística y Censos, 2001). Among adults who were interviewed, 21% worked for the flower industry in 2004.

To supplement recruitment, new volunteers (child and parent) living in Pedro Moncayo County were also recruited through community announcements performed by leaders and governing councils and word of mouth.

Because the 2004 Survey of Access and Demand of Health Services in Pedro Moncayo County included only residential addresses, participants were recruited at home. We were able to re-contact 419 children from the 2004 Survey of Access and Demand of Health Services in Pedro Moncayo County. Most losses were due to invalid or unlocatable addresses or because participants moved to a different house. A total of 124 new volunteers registered to participate.

Participation of children was sought after a pre-survey of the parents if they met the following criteria: exposed group: cohabitation with a flower worker for at least one year; unexposed group: never cohabited with an agricultural worker, never inhabited a house where agricultural pesticides were stored and havi ng no previous direct contact with pesticides. Multiple children per household were allowed to participate. Two hundred sixty six (63%) participants who were surveyed in 2004 and 86 (69%) new volunteers were eligible to participate, of whom 259 participants surveyed in 2004 and 84 new volunteers agreed to participate in the study. Twenty seven participants (47% of whom were cohabitants with a flower worker) did not show up for the examination appointments, 1 child did not assent to be examined and two children refused their examination.

We examined a total of 313 children 4 to 9 years of age in 2008. Of these children, 277 had all covariates of interest to our analyses and were included in this study (57% cohabited with a flower plantation worker). We obtained interview information from 451 adults who cohabited with the examined children (at least one parent). We were able to obtain information of practices related to secondary pesticide exposure from 141 (87%) flower workers.

Informed consent of surveyed parents was sought in addition to parental permission for participation of each of their selected children and child assent of participants who were 7 years of age and older. This study was approved by the Institutional Review Boards of the University of Minnesota and Fundacion Cimas del Ecuador.

2.3. Measures

ESPINA staff conducted home interviews of children's parents who lived with the examined children to obtain information on SES, demographics, health, and direct and indirect pesticide exposures of household members (e.g. pesticide usage at home, child pesticide exposure history, duration of cohabitation with a flower worker). Parents or other adults who were flower workers and lived with the children were interviewed to obtain information regarding practices associated with secondary occupational exposures to pesticides (pesticide introduction in to the home) to their family members.

Examinations were conducted in seven schools centrally located to participants' residences in all five parishes in Pedro Moncayo County. The vast majority of flower plantations were located in the eastern-most parishes of Tabacundo and Tupigachi, whereas the western parishes (Malchinguí, Tocachi, La Esperanza) had minimal floricultural activity.

Examiners were unaware of exposure status of the participants. Children's standing height was measured using a height board after removing shoes and head ornaments. Participants were asked to stand straight with heels, buttocks, shoulder blades and head all touching the back of the height board; assistance was provided to reach the desired posture (World Health Organization, 2008). AChE activity and hemoglobin concentration were measured from finger-stick blood samples using the EQM Test-mate ChE Cholinesterase Test System 400 (EQM AChE Erythrocyte Cholinesterase Assay Kit 470). The Test-mate system adjusts AChE concentration values for ambient temperature (EQM Research, 2003). Blood samples were analyzed immediately at ambient temperatures between 15.6°C and 28.4°C, which are within the recommended range.

Geographical coordinates of Pedro Moncayo County homes were collected in 2004 by Fundacion Cimas del Ecuador, as part of the System of Local and Community Information (Sistema de Información Local y Comunitario), using portable global positioning system receivers. Home coordinates for new homes and homes not previously coded were updated in 2006 and 2010 by Fundacion Cimas del Ecuador. In 2006, flower plantation edges (areal polygons) were created by measuring the geographic coordinates of each corner of each plantation's perimeter and plotting them on maps. Using ArcGIS 9.3 (Esri, Redlands, CA), the distance of each participant's home to the nearest 1 meter segment of flower plantation edge was calculated.

2.4. Statistical Analysis

The associations of flower worker cohabitation, duration of cohabitation and number of flower workers living at home with AChE activity among children were calculated using multiple linear regression (AChE continuous) and polychotomous logistic regression (AChE a 4 level polychotomous variable. The rationale for the 4 level AChE variable was as follows. The amount of pesticide taken home by flower workers likely varies; some workers may bring hardly any pesticides home while others may bring copious amounts. Thus, the extent of cholinesterase inhibition among children of flower workers likely varies as well. Because of the growing literature of developmental impacts of pesticides on child development (Eskenazi et al., 2007; Marks et al., 2010; Rauh et al., 2011; Rohlman et al., 2005), we are also interested in assessing the risk of flower worker's children of having more substantial pesticide exposures. Our model assumes that more exposed children have a more pronounced reduction in AChE than less exposed children, and that this would be reflected in our cross-sectional data in a larger effect size within lower levels AChE activity than within higher levels. To our knowledge, no AChE standards exist for children. Therefore, t o assess this, we divided the sample into tertiles, then divided the lowest tertile of AChE activity into two categories: an upper half (33^{rd} to 15^{th} percentiles, 2.68–2.93 U/ml) and a bottom half ($<15^{th}$ percentile, <2.68 U/ml ≈ $<10^{th}$ percentile of children who do not live with a flower worker).

No "unexposed" baseline was available to calculate AChE suppression. In our population of children, no adequate baseline AChE activity can be measured because most of the children who lived with a flower-worker were born into families with a flower worker (72%) and potentially even exposed to pesticides in-utero.

In addition, we obtained a dose-response association of secondary pesticide exposure with AChE activity by analyzing duration of cohabitation both as a continuous variable and as a 3-level categorical variable: 0= no cohabitation ever, 1= >0–4.9 years, 2= ≥5 years. The number of flower workers concurrently living at home was treated continuously and categorically: 0= none, 1= 1 flower worker, 2= ≥2 flower workers.

We report flower worker practices that would likely influence the amounts of pesticide inadvertently brought home. Most questions had 4 options: the worst practice (e.g. always bringing work clothes home) coded 1 and the best practice (e.g. never bringing work clothes home) coded 4. In dichotomous questions the worst practice was coded 1 and the best practice was coded 4. When there was more than one flower worker in a household, we included only the worst practice value among all such workers. We assigned the household level value to each child living in the household and assigned a value of 0 to children of non-flower workers. Because the composite of practices was assumed to be most relevant to low AChE, we calculated the number of "bad practices" which was defined as codes 1 or 2 for each item. Because the number of bad practices had an unbalanced distribution, we created a 4 category variable based on the number of bad practices: 0, any but ≤ 2, >2 but <4, and ≥4. We analyzed the association of the 4 level AChE variable as predicted from the number of bad practices using polychotomous logistic regression.

Height-for-age z-scores were calculated using the World Health Organization growth standards (World Health Organization).

Associations are presented according to levels of adjustment for pertinent covariates: 1) a minimum adjustment model included general confounders between secondary exposure and AChE activity (age, gender, race, hemoglobin concentration and examination date); 2) a multiple adjustment model to account for chronic nutritional status and additional potential sources of pesticide exposure (minimum adjustment + z-score height-for-age, income, pesticide use within household lot, pesticide use by contiguous neighbors); 3) a full adjustment model to control for potential pesticide drift from flower plantations (multiple adjustment + residence distance to the nearest flower plantation edge). Because we examined children during the months of July and August and AChE activity can reflect exposures to organophosphates within the previous 82 days (Mason, 2000), there is potential that children who were examined earlier in July may have lower AChE activity (from secondary sources) due to the heightened fumigations of the Mother's day (May) surge of flower production. This exposure gap may be differential by exposure status given that the proportion of examined children who lived with a flower worker in early July does not necessarily represent the distribution of the overall group. Examination date was not an effect modifier for the association between flower worker cohabitation and AChE (p=0.12). For these reasons we controlled for examination date in all models. Data were analyzed with SAS Version 9.2 (SAS Institute Inc., Cary, NC).

3. Results

3.1. Participant Characteristics

Child participants had a mean age of 6.6 years (standard deviation (SD): 1.6); 51% were male, and 76% mestizo (mix of white and indigenous) (Table 1). The mean (SD) height-for-age z-score was −1.25 (0.98) and 24% were stunted (height-for-age z-score <−2). Fifty-seven percent of children cohabited with at least one flower worker; their mean duration of cohabitation was 5.2 years, with 51% cohabiting less than 5 years, and 49% 5 years or more. Among cohabitants with flower-workers, 92% lived with 1 or 2 flower workers and 72% of children cohabiting with flower-workers had lived with a flower worker since birth. A greater proportion of children cohabiting with a flower worker was indigenous compared to non-flower workers. Children cohabiting with a flower worker had lower AChE and lived closer to the nearest flower plantation than cohabitants with non-flower workers. There were no significant differences in age, gender, household income or hemoglobin concentration between the groups.

The 451 adult participants (55% women) had a mean age of 33.5 years (range: 18–67 years); 50% were participant mothers, 41% fathers, and 48% were cohabitants with non-flower workers. Flower workers comprised 36% of adults. Fifty eight percent of flower workers were harvesters, 15% post-harvesters, 4% fumigators and 22% other.

3.2. Flower worker cohabitation and acetylcholinesterase activity

Children's cohabitation with a flower worker was associated with a mean AChE difference of −0.09 (95% CI: −0.19, 0.00) in the fully adjusted model (Table 2). Additional predictors of AChE activity in the fully adjusted model were age (0.05 U/ml increase per year of life, 95% CI: 0.02, 0.08), hemoglobin concentration (0.25 U/ml increase per mg/dl of hemoglobin, 95% CI: 0.21, 0.29), and residential distance to the nearest flower plantation edge (0.23 U/ml increase per 1000 meters greater distance, 95% CI: 0.11, 0.40). The associations between flower worker cohabitation and AChE did not differ by gender, race, age or height-for-age z-score (data not shown).

In the fully adjusted model, children who cohabited with a flower worker (vs. children of non-flower workers) had 3.39 (95% CI: 1.19, 9.64, Table 2) times the odds of having AChE activity within the lowest 15^{th} percentile (compared to the highest tertile). Children of flower workers had an OR of 2.34 (95% CI 1.01, 5.43) of AChE activity within 15^{th}–33^{rd} percentiles.

The number of flower workers cohabiting with children was not associated with differences in AChE activity (−0.02 U/ml per flower worker (95% CI: −0.06, 0.02), data not shown).

3.3. Duration of flower worker cohabitation and acetylcholinesterase activity

Greater duration of cohabitation was associated with lower AChE activity. Every year of cohabitation with a flower worker was associated with a fully adjusted mean difference of −0.02 U/ml (95% CI: −0.03, −0.002; Table 3). Every year of cohabitation was associated with an adjusted OR of 1.22 (95% CI: 1.02, 1.45) for AChE activity in the lowest 15^{th} percentile compared to the highest tertile. Children who cohabited with a flower worker for 5 years or more (vs. never) had an OR of AChE within the lowest 15^{th} percentile (compared to the highest tertile) of 4.11 (95% CI: 1.17, 14.38) and within the 15^{th} to 33th percentiles of 3.86 (95% CI: 1.44, 10.37).

3.4. Flower worker practices related to secondary pesticide exposure

The most common sources of pesticide introduction into the home were: washing work clothes at home (95%), never showering at work (45%), infrequent removal (38%), and bringing work shoes (18%) and tools (16%) home (Table 4). Among those who showered 2 or fewer days per week at work (n=62), the main reasons for not showering were: a) no working showers (52%), b) not enough time for showering (16%), c) thought showering was unnecessary (13%) and d) not enough showers (8%). The distribution of “bad practices” among flower workers (n=141) was: 1–2= 33%, 3= 40% and 4–6= 27%.

Among the 277 children, we had flower worker bad practices information of 233 children (parents of 44 children of flower workers had missing information). The parents of 52% (n=120) of children (the 120 non-flower worker cohabitants) had 0 bad practices, 17% (n=39 of flower worker cohabitants) had 1–2 bad practices, 18% (n=43 of flower worker cohabitants) had 3 bad practices and 13% (n=31 of flower worker cohabitants) had 4–6 bad practices.

The fully adjusted AChE activity means among children for the 4 level bad practices variable was: 0= 3.19 U/ml (95% CI: 3.13, 3.26), 1–2: 3.09 U/ml (95% CI: 2.97, 3.21), 3: 3.09 U/ml (95% CI: 2.97, 3.20) and 4–6: 3.02 U/ml (95% CI: 2.90, 3.15). Children whose parents had 4–6 bad practices had a lower mean AChE activity by 0.17 U/ml (95% CI: −0.31, −0.02) compared to children with no bad practices.

After full adjustment, 4–6 bad practices (vs. 0 bad practices) were associated with OR of 7.98 (95% CI: 1.45, 43.84) of AChE activity $<15^{th}$ percentile and OR 5.53 (95% CI: 1.39 to 21.93) of AChE activity within 15^{th}–33^{rd} percentile, compared to the lowest tertile. There was no association between 1–3 bad practices (vs. none) and the 4 level AChE activity variable.

4. Discussion

We found that cohabitation with a flower worker was related to lower AChE activity in children and longer duration of cohabitation was associated with lower AChE activity.

Because these flower workers are routinely exposed to organophosphate and carbamate pesticides, and those pesticides are known to reduce AChE, our findings support the hypothesis that flower workers in this population introduce pesticides into their homes in sufficient amounts to affect physiologic processes in children. Previous studies have found higher amounts of pesticide metabolites in children of agricultural workers (Fenske et al., 2000; Lambert et al., 2005; Simcox et al., 1995), but to our knowledge, no reports have associated cohabitation with an agricultural worker with AChE activity. Although we cannot assess temporality between exposure and outcome by repeated measures within participants given our cross-sectional study design, we did observe that longer duration of cohabitation was associated with lower AChE activity. The inverse linear association between duration of cohabitation and AChE activity could be explained by: 1) increased pesticide accumulation in the home which increases through the years due to regular occupational "take-home" pesticide exposures. Once in the home, pesticides can accumulate in the absence of environmental factors inside the home that degrade or dissipate pesticides, such as wind, sun or rain; 2) decreased safety practices to reduce home exposures with longer work experience. This may reflect inadequate continuing education of flower workers which may increase the amount of pesticides introduced into their homes as years go by.

The most frequent routes of occupational pesticide introduction into homes in our sample of flower workers were washing work clothes at home (work clothes can lodge pesticides), not consistently removing the work clothes before leaving work, not showering before leaving work (pesticides can adhere to skin and hair) and taking work tools home. Flower workers' performance of 4–6 "bad practices", reflecting potential for greater take-home pesticide amounts, was associated with substantially lower AChE activity, which provides a notion that the listed pathways of pesticide introduction into the home may indeed be the sources of secondary pesticide exposure to children. Washing work clothes at home can be a substantial source of secondary exposures because pesticide contaminated work clothes can remain inside the home without being washed for days and may come into contact with other dirty clothes or with people in the household. Clothes in Pedro Moncayo County are usually washed by hand, which increases the exposure potential for the washer (Laughlin, 1993). Clothes are usually washed in cold water, which is less effective than hot water for removing pesticides (Laughlin, 1993). It would not be surprising to find children helping their parents wash clothes. Additionally, children are prone to being in direct contact with their parents and can, therefore, have a higher likelihood of pesticide exposure if the parent is wearing work clothes at home or if they have pesticides on their skin or hair. Finally, children can be drawn to playing with work tools lying around in the house. The most frequent routes of pesticide introduction and those that are associated most closely with AChE inhibition is a topic of continuing research.

Flower-worker families lived closer to flower plantations than non-flower-worker families and household proximity to a flower plantation was independently associated with lower AChE activity in our study. Residential proximity to a flower plantation confounded (away from non-association) the relationship of flower worker cohabitation with AChE activity, but empirically, the amount of confounding was not large. Additionally, we found that AChE increased linearly with age. We recommend that analyses of AChE activity in children account for age.

Currently, it is not known how much clinical impact AChE suppression equivalent to the ones we observed can have on children in the short and long term. Due to the vulnerability of children to pesticides (Cohen Hubal et al., 2000; Faustman et al., 2000), chronic pesticide exposures during childhood could effect multi-systemic developmental impairments including neurobehavioral delays (Marks et al., 2010; Rohlman et al., 2005); this is a gap in the literature that needs to be addressed and is a further aim of the ESPINA study.

In agricultural populations such as that of Pedro Moncayo County where over 60% of children live with a flower worker, secondary pesticide exposures could have adverse effects on a large scale. While theoretically this county likely has higher pesticide exposures than non-agricultural areas, the levels may not be too different from those of countries with greater pesticide protection regulations. Grandjean, et. al., in a study that measured organophosphate metabolites on 72 children of similar ages in Pedro Moncayo County found that the pesticide metabolite concentrations there were similar to those of US children (Grandjean et al., 2006).

The AChE measurement is an appropriate method to assess past exposures to carbamate and organophosphate pesticides because it can reflect longer-term exposures than plasma cholinesterase (butyrylcholinesterase) or pesticide metabolite quantification (Lotti, 1991; Mason, 2000). Furthermore, AChE is likely superior to metabolite quantification when evaluating the toxic effects of organophosphates and carbamates (Lotti, 1991); however, AChE is less sensitive in detecting pesticide exposures (Barr and Needham, 2002). The AChE measurements in our study sample can roughly represent organophosphate pesticide contamination at any given time of the year, considering: 1) the long recovery time of AChE of approximately 82 days after irreversible inhibition, (Mason, 2000); 2) the sources of secondary pesticide exposures remain relatively constant throughout the year (e.g. flower worker pesticide exposure prevention practices, consumption of pesticide exposed produce, residential distance to agricultural farms); and 3) the year-round flower production (and pesticide use) in Ecuador, due to its minimal seasonal temperature changes and standard use of greenhouses in the industry.

We did not ask about frequency of consumption of “organic” (pesticide-free) products as a possible source of reduced pesticide exposure given that such products are rarely available or advertised as such by local growers in the county. We have no reason to believe that consumption of non-“organic” products would differ by flower worker cohabitation status.

A limitation in our study, as in any cross-sectional study, is the lack of multiple AChE measurements to allow for tracking activity over time and to provide more robust conclusions on the associations of AChE and flower worker cohabitation. Our findings, nonetheless, are substantive and internally valid because the duration of cohabitation was inversely related to AChE and is consistent with cumulative exposures.

A strength of this study is the inclusion of household distance to the nearest flower plantation in the analysis, which allows to disentangle the mechanisms of pesticide introduction into the home between pesticide drift from flower plantations and flower worker associated “take-home” pathways.

We highlight the importance of reducing the amounts of pesticides introduced into the homes by agricultural workers. It is important not only to educate agricultural workers to adequately handle pesticides to reduce their contamination and that of their families, but to provide adequate infrastructure to promote healthy practices including providing sufficient showers or washing work clothes in the plantation.

5. Conclusions

This study demonstrates a physiological effect of secondary occupational exposure to pesticides reflected by lower AChE activity in children of flower workers and particularly with longer cohabitation durations. We also demonstrate the utility of field AChE measurements as a marker of pesticide exposure in children. Our findings, in addition to the general background of exposure to pesticides, bring importance to assessing the effects of chronicity of low-level pesticide exposures on child development.

Highlights

- Families of agricultural workers have a higher risk of pesticide exposure
- We studied Ecuadorian children living with and without floricultural workers
- Floricultural workers’ children had lower acetylcholinesterase activity

- Longer cohabitation with flower workers associated with lower acetylcholinesterase

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Institutional Review Board Approval for Research:

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References

Aaron, CK. Organophosphates and carbamates. In: SM Haddad, LM.; Borron, SW.; Burns, MJ., editors. Haddad and Winchester's Clinical Management of Poisoning and Drug Overdose. Philadelphia: Saunders/Elsevier; 2007.

Abou-Donia MB. Organophosphorus ester-induced chronic neurotoxicity. Arch Environ Health. 2003; 58:484–497. [PubMed: 15259428]

Barr DB, Needham LL. Analytical methods for biological monitoring of exposure to pesticides: a review. J Chromatogr B Analyt Technol Biomed Life Sci. 2002; 778:5–29.

Castelnuovo, C., et al. Ecuador Trabajo Infantil en la Floricultura: Una Evaluación Rápida. Vol. Vol. 2011. Geneva: International Labor Organization/IPEC; 2000.

Cohen Hubal EA, et al. Children's exposure assessment: a review of factors influencing Children's exposure, and the data available to characterize and assess that exposure. Environ Health Perspect. 2000; 108:475–486. [PubMed: 10856019]

Coronado GD, et al. Organophosphate pesticide exposure and work in pome fruit: evidence for the take-home pesticide pathway. Environmental health perspectives. 2006; 114:999–1006. [PubMed: 16835050]

Curl CL, et al. Evaluation of take-home organophosphorus pesticide exposure among agricultural workers and their children. Environ Health Perspect. 2002; 110:787–792.

Curwin BD, et al. Pesticide contamination inside farm and nonfarm homes. Journal of occupational and environmental hygiene. 2005; 2:357–367. [PubMed: 16020099]

Curwin BD, et al. Urinary pesticide concentrations among children, mothers and fathers living in farm and non-farm households in iowa. The Annals of occupational hygiene. 2007; 51:53–65. [PubMed: 16984946]

EQM Research. Test-mate ChE Cholinesterase Test System (Model 400). Instruction Manual. Vol. Vol. 2011. Cincinnati: EQM Research; 2003.

Eskenazi B, et al. Organophosphate pesticide exposure and neurodevelopment in young Mexican-American children. Environmental health perspectives. 2007; 115:792–798. [PubMed: 17520070]

Faustman EM, et al. Mechanisms underlying Children's susceptibility to environmental toxicants. Environ Health Perspect. 2000; 108 Suppl 1:13–21. [PubMed: 10698720]

Fenske RA, et al. Biologically based pesticide dose estimates for children in an agricultural community. Environ Health Perspect. 2000; 108:515–520. [PubMed: 10856024]

Fenske RA, et al. Children's exposure to chlorpyrifos and parathion in an agricultural community in central Washington State. Environ Health Perspect. 2002; 110:549–553. [PubMed: 12003762]

Gladen BC, et al. Exposure opportunities of families of farmer pesticide applicators. Am J Ind Med. 1998; 34:581–587. [PubMed: 9816416]

Gobierno-Municipal-del-Canton-Pedro-Moncayo. El Cantón. Vol. Vol. 2011. 2011.

González-Andrade F, et al. Acute pesticide poisoning in Ecuador: a short epidemiological report. JOURNAL OF PUBLIC HEALTH. 2010; 18:6.

Grandjean P, et al. Pesticide exposure and stunting as independent predictors of neurobehavioral deficits in Ecuadorian school children. Pediatrics. 2006; 117:546–556.

Guyton, AC.; Hall, JE. Textbook of medical physiology. Philadelphia: Elsevier Saunders; 2006.

Harari, R. Seguridad, Salud y Ambiente en la Floricultura. Quito: IFA, PROMSA; 2004.

Instituto Nacional de Estadística y Censos. Censo de Población y Vivienda 2001. Vol. Vol. 2011. Quito: Instituto Nacional de Estadistica y Censos; 2001.

Kwong TC. Organophosphate pesticides: biochemistry and clinical toxicology. Therapeutic drug monitoring. 2002; 24:144–149. [PubMed: 11805735]

Lambert WE, et al. Variation in organophosphate pesticide metabolites in urine of children living in agricultural communities. Environ Health Perspect. 2005; 113:504–508. [PubMed: 15811843]

Laughlin J. Decontaminating pesticide protective clothing. Reviews of environmental contamination and toxicology. 1993; 130:79–94. [PubMed: 8419989]

Lotti M. The pathogenesis of organophosphate polyneuropathy. Crit Rev Toxicol. 1991; 21:465–487. [PubMed: 1666291]

Lu C, et al. Pesticide exposure of children in an agricultural community: evidence of household proximity to farmland and take home exposure pathways. Environ Res. 2000; 84:290–302. [PubMed: 11097803]

Marks AR, et al. Organophosphate pesticide exposure and attention in young Mexican-American children: the CHAMACOS study. Environmental health perspectives. 2010; 118:1768–1774. [PubMed: 21126939]

Mason HJ. The recovery of plasma cholinesterase and erythrocyte acetylcholinesterase activity in workers after over-exposure to dichlorvos. Occup Med (Lond). 2000; 50:343–347. [PubMed: 10975133]

McCauley LA, et al. Work characteristics and pesticide exposures among migrant agricultural families: a community-based research approach. Environ Health Perspect. 2001; 109:533–538. [PubMed: 11401767]

Paz-y-Mino C, et al. Cytogenetic monitoring in a population occupationally exposed to pesticides in Ecuador. Environ Health Perspect. 2002; 110:1077–1080. [PubMed: 12417477]

Rauh V, et al. Seven-year neurodevelopmental scores and prenatal exposure to chlorpyrifos, a common agricultural pesticide. Environmental health perspectives. 2011; 119:1196–1201. [PubMed: 21507777]

Rohlman DS, et al. Neurobehavioral performance in preschool children from agricultural and non-agricultural communities in Oregon and North Carolina. Neurotoxicology. 2005; 26:589–598. [PubMed: 16112324]

Simcox NJ, et al. Pesticides in household dust and soil: exposure pathways for children of agricultural families. Environ Health Perspect. 1995; 103:1126–1134. [PubMed: 8747019]

World Health Organization. WHO child growth standards : length/height-for-age, weight-for-age, weight-for-length, weight-for-height and body mass index-for-age : methods and development. Geneva: World Health Organization, Department of Nutrition for Health and Development;

World Health Organization. Training Course on Child Growth Assessment. Geneva: WHO; 2008.

Table 1

Children's characteristics by flower plantation worker cohabitation status.

		Cohabitation with	
	All Participants	**Flower plantation worker**	**Non-flower plantation worker**
	N=277	**N=157 (57%)**	**N=120 (43%)**
Demographic and Socio-Economic Status Characteristics[†]			
Age, years	6.6 (1.6)	6.4 (1.6)	6.7 (1.7)
Gender, male	51%	50%	53%
Race, mestizo	76%	71%	82%
Race, indigenous	22%	28%	15%
Monthly income[a]	3.1 (0.8)	3.1 (0.7)	3.1 (1.0)
Residence distance to nearest flower plantation, m	349 (189, 602)	321 (166, 615)	438 (222, 600)
Duration of flower worker cohabitation, years		5.2 (2.0)	
Number of flower workers living at home		1.5 (1.2)	
Anthropometric and Blood Measurements[†]			
Height-for-age Z-score	−1.25 (0.98)	−1.34 (1.01)	−1.15 (0.86)
Acetylcholinesterase, U/ml	3.14 (0.49)	3.08 (0.51)	3.22 (0.46)
Acetylcholinesterase activity			
$<10^{th}$ percentile (<2.67 U/ml)	14.4%	19.1%	8.3%
Hemoglobin, mg/dl	12.6 (1.2)	12.6 (1.2)	12.7 (1.1)

[†]Table entries are percentage, mean (standard deviation) or median (25^{th}–75^{th} percentile).

[a]Monthly income categories (USD): 1= 0–50, 2= 51–150, 3=151–300, 4=301–500, 5= 501–1000, 6=>1000

Table 2

Adjusted difference and odds ratios for acetylcholinesterase activity (AChE) according to flower worker cohabitation (yes vs no), among 277 children.

Outcome: AChE	**Models**		
	Minimum[a]	**Multiple**[b]	**Full**[c]
	Difference, U/ml (95% CI) for mean AChE		
Continuous	−0.10 (−0.20, −0.01)	−0.11 (−0.20, −0.01)	−0.09 (−0.19, 0.00)
	Odds Ratio (95% CI) for 4 level polychotomous AChE		
Highest Tertile[d]	1.00	1.00	1.00
Middle Tertile[d]	1.36 (0.68, 2.70)	1.48 (0.72, 3.03)	1.46 (0.71, 2.99)
15^{th} – 33^{rd} percentile[d]	2.30 (1.02, 5.18)	2.47 (1.07, 5.69)	2.34 (1.01, 5.43)
<15^{th} percentile[d]	3.36 (1.25, 8.99)	3.82 (1.38, 10.56)	3.39 (1.19, 9.64)

[a] **Minimum Adjustment**: age, gender, race, hemoglobin concentration and examination date

[b] **Multiple Adjustment:** minimum adjustment + z-score height-for-age, income, pesticide use within household lot, pesticide use by contiguous neighbors

[c] **Full Adjustment:** multiple adjustment + residence distance to nearest flower plantation edge

[d] **AChE cutoffs:** highest tertile: >3.32 U/ml, middle tertile: 2.94–3.32 U/ml, 15^{th}–33^{rd} percentile (upper half of lowest tertile): 2.68–2.93 U/ml, <15^{th} percentile (bottom half of lowest tertile): <2.68 U/ml

Table 3

Adjusted difference and odds ratios for acetylcholinesterase activity (AChE) per year of cohabitation with a flower worker, among 277 children.

Outcome: AChE	Models		
	Minimum[a]	Multiple[b]	Full[c]
	Difference, U/ml (95% CI) for mean AChE		
Continuous	−0.02 (−0.03, −0.003)	−0.02 (−0.04, −0.005)	−0.02 (−0.03, −0.002)
	Odds Ratio (95% CI) for 4 level polychotomous AChE		
Highest Tertile[d]	1.00	1.00	1.00
Middle Tertile[d]	1.04 (0.93, 1.17)	1.05 (0.93, 1.19)	1.05 (0.93, 1.19)
15th – 33rd percentile[d]	1.23 (1.08, 1.41)	1.25 (1.08, 1.44)	1.24 (1.08, 1.43)
<15th percentile[d]	1.19 (1.01, 1.41)	1.24 (1.04, 1.47)	1.22 (1.02, 1.45)

[a] **Minimum Adjustment**: age, gender, race, hemoglobin concentration and examination date

[b] **Multiple adjustment:** minimum adjustment + z-score height-for-age, income, pesticide use within household lot and pesticide use by contiguous neighbors

[c] **Full Adjustment:** multiple adjustment + residence distance to nearest flower plantation edge

[d] **AChE cutoffs:** highest tertile: >3.32 U/ml, middle tertile: 2.94–3.32 U/ml, 15th–33rd percentile (upper half of lowest tertile): 2.68–2.93 U/ml, <15th percentile (bottom half of lowest tertile): <2.68 U/ml

Table 2

Practices related to occupational pesticide introduction into homes among 141 adult flower plantation workers.

Practices	%
How frequently do you wash your hands right before leaving work?	
Always	97
How frequently do you shower at work right before going home (per week)?	
Never	45
1–2 days	7
3–4 days	19
Always	29
How frequently do you take work shoes to your home (per week)?	
Never	82
1–2 days	5
3–4 days	4
Always	9
How often do you remove your work clothes before leaving work (per week)?	
Never	1
1–2 days	37
3–4 days	22
Always	40
Where do you wash your work clothes?	
Home	95
Flower plantation	1
Other	4
Do you take tools from work to your home?	
Yes	16