The Secondary Exposure to Pesticides among Infants, Children and Adolescents study (ESPINA: Estudio de la Exposición Secundaria a Plaguicidas en Infantes, Niños y Adolescentes)

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Dedication

To my family and mentors for being an inspiration throughout my studies.

Abstract

INTRODUCTION:

Children of agricultural workers are at risk of pesticide contamination through secondary routes (e.g. take-home pathway). Animal and human studies suggest that organophosphate and carbamate pesticide intoxication in early childhood can affect childhood development but it is not clear whether secondary pesticide exposures, which tend to be chronic but in low amounts, are sufficient to affect physiologic processes and development in children. Objectives: The Secondary Exposure to Pesticides among Infants, Children and Adolescents (ESPINA) study (Spanish: Estudio de la Exposición Secundaria a Plaguicidas en Infantes, Niños y Adolescentes) evaluated the effects of secondary pesticide exposure on childhood growth and neurobehavioral development among children living in Pedro Moncayo County, Ecuador, a county with an active fresh-cut flower industry. The following hypotheses were addressed: compared to children without secondary pesticide exposure (operationalized as flower plantation worker (flower worker) cohabitation), exposed children have: 1) lower acetylcholinesterase (AChE) activity, 2) lower neurobehavioral development scores, 3) lower systolic blood pressure, 4) lower resting heart rate and 5) slower growth.

METHODS:

ESPINA included socio-economic, demographic and anthropometric (height and weight) information of children from 0 to 5 years of age who participated in 2004 in the Survey of Access and Demand of Health Services in the County of Pedro Moncayo (SAHS-PM 2004). Pedro Moncayo County, Pichincha, Ecuador, has one of the highest concentrations of flower plantations per capita worldwide and the flower industry employs 21% of adults of Pedro Moncayo. In 2008, ESPINA examined children, 4 to 10 years of age, who participated in the SAHS-PM 2004 and new volunteers living in the County to obtain anthropometric measurements,

a neurobehavioral developmental assessment and hemoglobin concentration and AChE activity. *Study Design*: This study is composed of: 1) a cross-section of 2004 to assess growth, 2) a cross-section of 2008 to assess neurobehavioral development, growth, heart rate, blood pressure and blood AChE levels; and 3) a longitudinal component (2004-2008) to assess growth.

RESULTS:

a) *Participant Characteristics:* From the SAHS-PM 2004, 922 (51% female) children were included. The mean age was 2.3 y (standard deviation (SD): 1.4 y), 63% cohabited with ≥1 flower worker with a mean of 2.0 flower workers at home. In ESPINA (2008), 313 (49% female, 78% mestizo) children participated; the mean age was 6.6y (SD=1.6 y), and 55% of participants cohabited with ≥1 flower worker. Flower worker cohabitants had a mean duration of cohabitation of 5.3 years and a mean of 1.5 flower workers at home. A total of 230 (49% female, 74% mestizo) children were examined in 2004 and 2008; the mean age was 6.4 years (SD=1.4 y) and 59% cohabited with at least one flower worker. Flower worker cohabitants had a mean duration of cohabitation of 5.1 years and a mean of 1.5 flower workers at home in 2008.

b) Flower worker cohabitation and AChE activity: 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.

c) Secondary Pesticide Exposure and Neurobehavioral Development: The range of scores among 13 NEPSY-II subtests was 5.9-10.9 units (SD: 2.8-4.9). Boys with AChE activity in the lowest vs. the highest tertile had adjusted odds ratios (OR) of 7.40 (95%CI 1.71-32.05), 9.39 (95%CI 2.36-37.38) and 2.35 (95%CI 1.03-5.34) of low scores (<9th percentile) of Attention and Executive Functioning Domain, inhibitory control and long term memory, respectively, after adjusting for age, gender, race, height-for-age z-score, household income, flower worker cohabitation status, maternal education, hemoglobin concentration. Children's cohabitation with a FW (vs. not) was associated with OR of low auditory attention score of 1.63 (95%CI 0.79,-3.37). AChE and FW cohabitation were not associated with other measures of neurobehavioral development.

d) Secondary Pesticide Exposure and Blood Pressure (and Heart Rate) among Children living in Agricultural Communities in Ecuador*:* AChE activity was directly associated with blood pressure: every U/ml decrease was associated with a mean decrease in SBP of 1.54 mmHg (95%CI -2.68, -0.40) and DBP of 1.52 mmHg (95% CI -2.55, -0.50), after adjustment for age, gender, race, height-for-age z-score, heart rate, hemoglobin concentration, income, residence distance to nearest flower plantation edge, pesticide use within household lot, pesticide use by contiguous neighbors and examination date. Flower worker cohabitation was associated with lower systolic blood pressure (SBP) by 1.79 mmHg (95%CI -3.57, -0.01). Every year of cohabitation was associated with an adjusted decrease of SBP of 0.32 mmHg (95%CI 0.02, 0.64). Further adjustment for AChE weakened these associations. Cohabitation with a flower worker was not related to diastolic blood pressure (DBP); resting heart rate was not associated with flower worker cohabitation or AChE activity.

e) Secondary Pesticide Exposure and Growth Cross-sectionally in 2004 (n=853), flower worker cohabitation was not associated with growth after adjusting for demographic and socio-economic factors. Longitudinally, child cohabitation with a flower worker was associated with decreased mean BMI-for-age (-0.36 standard deviations (SD), 95% CI: -0.66, -0.06) and weight-for-age (-0.33 SD,

95% CI: -0.61, -0.05). In 2008, flower worker cohabitation and lower AChE activity (per U/ml, mean=3.13 U/ml, SD=0.49) were both associated with larger head circumference (0.37 cm, 95% CI: 0.003, 0.74 and 0.75 cm, 95% CI: 0.30, 1.19, respectively).

CONCLUSIONS:

a) Cohabitation with a flower worker was related to lower AChE activity in children; this supports the hypothesis that indirect pesticide exposure from flower workers suffices to depress AChE activity, with greater suppression due to longer exposure.

b) Low AChE activity was associated with deficits on tasks reflecting memory, attention and inhibitory control in boys. These are critical cognitive skills that affect learning and academic performance

c) Our findings reflect physiologic reactivity in children with subclinical secondary exposures to pesticides and suggest vasodilation as an important mechanism of decreased blood pressure of cholinesterase inhibitors in this population. Although lower BP in isolation might be a beneficial effect of secondary pesticide exposure, the finding needs to be viewed with caution, as part of a pattern of generally adverse physiologic responses to pesticides.

d) Our findings suggest that indirect pesticide exposures (estimated by AChE activity and cohabitation with a flower worker) can affect growth and head circumference in children living in agricultural communities.

RECOMMENDATIONS:

Our findings support the hypothesis that take-home pesticide exposures have physiologic effects in children of flower plantations workers. This investigation reinforces the importance of reducing the amounts of pesticides introduced into the homes by agricultural workers. Interventions to reduce secondary exposures targeting flower plantation workers and their families are needed. It is important

to not only educate agricultural workers to adequately handle pesticides to reduce their contamination and that of their families, but also to provide adequate infrastructure to promote healthy practices including providing sufficient showers or and washing work clothes in the plantation.

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<u>CHAPTER 1: Introduction</u>

A. SPECIFIC AIMS

Agriculture is the greatest source of pesticide exposure, and children of agricultural workers are at risk of pesticide contamination. Organophosphates and carbamates are commonly used types of pesticides, and their lethal action is to inhibit the enzyme acetylcholinesterase (AChE). Animal studies have found brain development disruption associated with AChE inhibitor pesticides. The few existing human studies reported neurobehavioral developmental delays, (learning impairments, mental/motor developmental delays and attention deficit), smaller head circumference and higher blood pressure associated with prenatal pesticide exposure or early childhood intoxication. Additionally, studies suggest that chronic pesticide exposures during early childhood may have an impact on growth. Research has primarily been focused on the effects of direct pesticide exposure on various outcomes, including neurobehavioral development. Currently, it is not clear what effects secondary occupational pesticide exposure (e.g. parental agricultural work) has on child development.

The ESPINA study was conducted in the rural County of Pedro Moncayo, Ecuador, which has one of the highest concentrations of flower plantations per capita worldwide. The <u>*objective*</u> of this investigation is to evaluate the effects of secondary occupational pesticide exposure (operationalized as flower plantation worker (flower worker) cohabitation) on childhood development. The **hypotheses** addressed were: compared to non-exposed children, exposed children have 1) neurobehavioral delays, 2) lower acetylcholinesterase levels (a marker of pesticide contamination), 3 lower systolic blood pressure, 4) lower resting heart rate and 5) slower growth. To address the study hypotheses, the following <u>*specific aims*</u> were accomplished:

1. The relationship between secondary occupational pesticide exposure and children's height and weight was assessed among baseline (2004) study

participants (children younger than 5 years of age), This baseline cohort was established in 2004 through the Survey of Access and Demand of Health Services in the County of Pedro Moncayo (SAHS-PM 2004), Ecuador, which collected demographic, socio-economic and occupational information on 18,187 (71%) inhabitants of Pedro Moncayo and height and weight measurements on children <5 years.

2. The relationship of secondary occupational pesticide exposure and changes from 2004 in growth, prevalent neurobehavioral development, head circumference, heart rate, blood pressure and blood acetylcholinesterase (AChE) was assessed through an examination in 2008 of a sample of participants (children) from the 2004 cohort and new participants.
3. In 2008, subjects' parents were interviewed to collect more detailed occupational pesticide exposure, health and demographic information than in SAHS-PM 2004.

B. BACKGROUND, OBJECTIVES AND SIGNIFICANCE

B.1 Background

B.1.1 Indirect pesticide exposure from Agricultural workers. In spite of the technological revolution of the 20th and 21st centuries, agriculture still relies heavily on human labor force and pesticides. The agricultural industry has been the main user of pesticides (Kiely 2004) and the most important source of occupational pesticide exposure and intoxication. According to the National Institute of Censuses and Statistics (INEC) 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. Approximately 71% of the poisonings were due to organophosphate and carbamate pesticides (González-Andrade et al., 2010).

Agricultural workers in non-organic farms have direct and indirect exposure to pesticides in their daily activities and have a higher risk of pesticide intoxication.

Their family members are also at risk of pesticide contamination through indirect routes including proximity of worker's residence to plantation and worker take-home pesticides from work through clothes, boots, tools, skin, hair, and pesticide storage at home (Curl et al., 2002; Fenske et al., 2002; Gladen et al., 1998).

Once in the home, agricultural pesticides tend to accumulate because they are not readily cleared by environmental factors such as wind, sun or rain, thus, allowing for potential chronic exposure to its residents. Households of agricultural workers have been found to have higher pesticide concentrations in dust (Lu et al., 2000; McCauley et al., 2001; Simcox et al., 1995) while children of agricultural workers have been found to have higher pesticide metabolite concentrations than reference children (Fenske et al., 2000; Lambert et al., 2005; Simcox et al., 1995).

B.1.2 Pesticides: Overview. Organophosphates and carbamates are the most commonly used types of insecticides in agriculture, including the fresh-cut flower industry. Their lethal mechanism of action is to inhibit the enzyme acetylcholinesterase (AChE) which in turn regulates acetylcholine, an essential neurotransmitter required in various processes including cardiovascular, gastrointestinal, respiratory, neuromuscular, thermoregulatory, and behavioral (Guyton and Hall, 2006). The effects of these pesticides on health can be acute or chronic with particularly grave effects on children.

B.1.3. Pesticide and Acetylcholinesterase. AChE is a carboxyl ester hydrolase that regulates acetylcholine concentrations at nerve terminals by degrading it into acetic acid and choline. It is present in red blood cells, and in the central and peripheral nervous systems. Cholinesterase inhibiting pesticides, bind and inhibit AChE which causes an accumulation of acetylcholine at the nerve or myoneural terminal leading to an over stimulation followed by an inhibition of cholinergic neurotransmission at central and peripheral muscarinic and nicotinic receptors (Aaron CK, 2007; Abou-Donia, 2003).

AChE measurement is a good method to monitor a response to carbamates and organophosphate pesticides because it reflects longer term exposure than butyrylcholinesterase or metabolite quantification. Also, red blood cell AChE activity is similar to neuronal AChE, which makes this a better indicator of nervous AChE inhibition than plasma cholinesterase (butyrylcholinesterase) (Aaron CK, 2007). After an irreversible inhibition, the recovery time of AChE to normal levels occurs after 82 days for AChE (Mason, 2000), or about 0.5 to 1% per day.

Because of the long recovery time of AChE and Ecuador's year-round crop production and pesticide usage, we estimate that AChE measurements could reflect pesticide contamination at any given time of the year.

B.1.4. Pesticides and Child Health. Children are particularly vulnerable to pesticide exposures due to propitious behavior towards exposure (i.e. increased skin contact with floors/lawns due to crawling or playing, hand-to-mouth behaviors) and physiological immaturity (i.e. increased skin surface, eating and drinking for weight, increased breathing rates, decreased ability to detoxify pesticides and sensitive developing organs) (Cohen Hubal et al., 2000; Faustman et al., 2000).

Acute intoxication with cholinesterase inhibiting pesticides causes neurotoxicity particularly when irreversible inhibition of cholinesterase occurs. This causes a state of cholinergic excess which can manifest as excessive sweating, salivation and lacrimation, nausea/vomiting, diarrhea, bronchoconstriction, pupil constriction, visual disturbances, anxiety, confusion, insomnia, ataxia, muscle weakness with cramping, seizures, tachycardia, high blood pressure, and respiratory and cardiac failure (Aaron CK, 2007). Among cholinesterase inhibiting pesticides, organophosphates more likely inhibit cholinesterase irreversibly.

A different pathway of neurotoxicity of is the organophosphorous ester-induced delayed neurotoxicity (OPIDN), which occurs after single or cumulative exposures. It leads to ataxia many days later that is associated with primary axonopathy and secondary myelin degeneration in the distal segments of long tracts (sensory and motor) in the central and peripheral nervous systems. Although the mechanism is not understood, this neurotoxicity is the result of phosphorylation of neurotoxic esterase (also called neuropathy target esterase), and not due to AChE inhibition (Abou-Donia, 2003).

A third pathway of neurotoxicity induced by organophosphate pesticides has been defined as organophosphorous ester-induced chronic neurotoxicity (OPICN). OPICIN has a long-term onset that can be manifested as neurobehavioral deficits. The mechanism of neurotoxicity of OPICN is not clear but may evoke neuronal necrosis with intense organophosphate exposure and oxidative stress leading to neuronal apoptosis in subclinical doses (Abou-Donia, 2003).

In spite of the substantive information based on animal studies, there is still a degree of uncertainty about the long term effects of exposure to pesticides on children's development (Colborn, 2006). A prospective study of children intoxicated with organophosphates before the age of 3, found that such children had learning impairments and difficulty in restraining and controlling their active behavior (Kofman et al., 2006). Other studies have found associations between prenatal pesticide exposure with mental and motor developmental delays, attention and attention deficit hyperactivity disorder problems, pervasive developmental disorder problems, smaller head circumference and higher blood pressure (Berkowitz et al., 2004; Grandjean et al., 2006; Rauh et al., 2006).

Regarding growth and pesticide exposure, the association is unclear. Some studies have found inverse associations between prenatal organophosphate exposure with length and birth weight (Grandjean et al., 2006; Perera et al., 2005; Whyatt et al., 2004), while others did not find such association (Berkowitz et al., 2004; Eskenazi et al., 2004). These discrepancies may be due to differential exposure levels between the populations included in each study and differential methods to characterize the exposure throughout the pregnancy. Disruption of bone formation, bone growth and density has been described in organophosphate exposed individuals (Compston et al., 1999; Dahlgren et al., 2004; Stephen et al., 1998). These findings suggest that chronic pesticide exposures during infancy and childhood may have an impact on length or height.

Secondary occupational exposure to pesticides is an important source of subclinical (short-term) pesticide doses that can cause developmental delays which may be overlooked by parents and child screenings.

Findings in similar areas of Ecuador

Previous research on Ecuadorian children, in the vicinity of our study, have reported lower neurobehavioral scores on gross and fine motor skills and social skills among children living in communities with intense fresh-cut flower industry activity compared to communities distant to flower plantations (Handal et al., 2007). Additionally, prenatal maternal occupational pesticide exposures were associated with lower neurobehavioral development (Grandjean et al., 2006; Handal et al., 2008; Harari et al., 2010). Grandjean, et. al. in 2006 found that pesticide concentrations in Pedro Moncayo County children were similar to those of US children in a study that measured organophosphate metabolites on 72 children.

B.2 Thesis Objectives

The objective of this investigation was to evaluate the effects of secondary occupational pesticide exposure on childhood development among children of flower workers. The following hypotheses were addressed in this thesis: compared to children without secondary pesticide exposure, exposed children have 1) lower acetylcholinesterase activity (a marker of organophosphate and carbamate pesticide exposure), 2) lower neurobehavioral development scores, 3) lower systolic blood pressure, 4) lower resting heart rate and 5) slower growth.

B.3 Significance

Many investigations have focused on the effects of direct pesticide exposure on various outcomes, including neurobehavioral development. Animal studies have reported deleterious effects of direct pesticide exposure on neurological development in fetuses and young offspring. The few existing human studies add to this by identifying neurobehavioral developmental delays in children exposed in-utero or intoxicated in childhood. The effects of secondary exposure to pesticides, particularly from parental occupation, on children's development are unclear.

Secondary pesticide exposure among children of agricultural workers is of particular concern since agricultural workers can introduce pesticides into their homes throughout the length of their agricultural employment which may last decades. Areas with propitious weather year-round allow farms to use pesticides throughout the year to maximize their continuous crop yield, offering a venue for constant pesticide exposures. Even though the amount of pesticides introduced into the home by pesticide exposed agricultural workers may be small, we postulate that constant pesticide exposure for long periods of time, particularly during important growth and brain developmental periods, which occur in early childhood, may have negative consequences on the overall development of the child. This is an important public health problem given the high prevalence of secondary exposure worldwide. Advancements in this field could lead to the creation of new exposure standards and preventive programs to promote pesticide-free environments.

The foundation of this research is interdisciplinary and intercultural work that brings together the knowledge of US and Ecuadorian researchers with that of rural populations in the Andean Ecuador. This study combines efforts and expertise of professionals in the fields of Public Health, Medicine, Child Development and Environmental and Occupational Health.

C. PRELIMINARY STUDIES

In 2002-2003, the Principal Investigator conducted a pilot study (unpublished) with Fundacion Cimas del Ecuador to assess cholinesterase levels in flower plantation workers and their families in Pedro Moncayo County, using the EQM Testmate system (Suarez-Lopez, 2003). This study included 231 subjects of all ages and found that flower plantation workers and their families had an odds ratio (OR) of 6.69 (p= 0.004) of low cholinesterase values (2 standard deviations (SD) below the mean of the non-flower plantation group), adjusted for age, sex and hemoglobin concentration. Flower worker family members of all ages had an OR of 8.3 (p=0.03), and among children 10 years of age and younger, the OR was 7.12, but it was non-significant, probably due to a small sub-sample size. This preliminary study suggests that secondary occupational pesticide exposure may have tangible biological effects on children. This study has provided important insights on research management, logistics and cultural appropriateness for the proposed study in Pedro Moncayo County.

CHAPTER 2: METHODOLOGY

D. RESEARCH DESIGN

D.1. Study Design

The ESPINA study has the objective of evaluating the effects of secondary occupational pesticide exposure on childhood development among children of flower plantation workers. This study has the following designs:

A) 2004 Cross-Section - Growth: The relationship of cohabitation with a flower plantation worker (exposure) and children's height, weight and nutritional/growth status (BMI for age, height for age and weight for height) in 2004 (baseline) was assessed by including all children (<5 years) with valid height and weight measurements from the SAHS-PM 2004 (See E.3. Survey of Access Demand of Health Services in Pedro Moncayo, 2004). This survey was carried-out in Pedro Moncayo County by Fundacion Cimas del Ecuador.

B) 2008 Cross Section - Multiple outcomes: The relationship of cohabitation with a flower plantation worker and children's neurobehavioral development, head circumference, heart rate, blood pressure, height, weight, nutritional/growth status and blood AChE activity was assessed through child examinations and parental surveys in 2008. Participants were between 4 and 9 years old and were composed of children examined in the SAHS-PM 2004 and new volunteers.

C) 2004-2008 Cohort: Growth- Change in height, weight, and nutritional status from 2004 to 2008, among children exposed and unexposed to cohabitation with flower plantation workers, was assessed in the sample of children who were examined in 2004 and 2008.

D.2. Setting

Pedro Moncayo County has a population of approximately 26,000 people (51% female) (Instituto Nacional de Estadística y Censos, 2001) and is located in the province of Pichincha, Ecuador, 45 minutes northeast of Quito. It has the second largest area of flower plantations in Ecuador and one of the highest concentrations of flower plantations per capita worldwide. Workers in Ecuador's flower industry work throughout the year as the equatorial location and use of greenhouses provide for year-round growth of flowers. To provide a continuous supply of flowers, the work and application of pesticides are not a seasonal activity, which places the children of flower plantation workers at a more continuous risk of secondary pesticide exposure. More than 30 different pesticides are used in the Ecuadorian flower industry, of which, organophosphates and carbamates are the most commonly used (Breilh et al., 2005; Castelnuovo et al., 2000; Grandjean et al., 2006; Paz-y-Mino et al., 2002). The flowers are then sold within Ecuador and exported to Europe and North America.

D.2.1. Host Institution: Fundacion Cimas del Ecuador. This investigation was conducted with the assistance of Fundacion Cimas del Ecuador (CIMAS). CIMAS is a non-profit organization focused on studying public health, development and social-cultural problems in rural Ecuador. Through academic discipline and the participatory research-action approach, CIMAS has attempted to facilitate answers to some of the problems that affect Ecuador's most burdened populations. It supports processes of transference of knowledge, technology and resources, to empower organized social groups to create their own models of problem solving and development. The focus of CIMAS is in: 1) the development of academic programs partnered with Ecuadorian and North American universities, 2) implementation of epidemiologic research that utilizes a participatory model that supports the involvement of the affected population in the research approach, and 3) community support, particularly in Pedro Moncayo

County and other marginal, urban and rural communities in Ecuador. CIMAS is a founding member of the Pedro Moncayo Health Council and has had a working relationship with Pedro Moncayo County for over 20 years. Fundacion Cimas del Ecuador has had a partnership with the University of Minnesota since 1992 with the Minnesota Studies in International Development, and recently with the Medical School and School of Public Health.

CIMAS provided access to the SAHS-PM 2004, logistical assistance including digital maps, office space, research assistants and technical support.

D.3. Sample Size Calculation (2008)

In the SAHS-PM 2004, 1356 children (40% of all in Pedro Moncayo (Instituto Nacional de Estadística y Censos, 2001)) younger than 5 years of age had height and weight measurements in the SAHS-PM 2004 (See E.3. Survey of Access Demand of Health Services in Pedro Moncayo, 2004). A total of 922 of these had valid (biologically plausible) height and weight measurements.

Handal, et. al. in 2007 reported a study prevalence of neurobehavioral delays of 23% among children between 2 and 5 years of age in the vicinity of Pedro Moncayo County (Handal et al., 2007) Although their study population is not representative of the general population of the area because of the sampling scheme used, the study does provide a preliminary estimate of the prevalence of developmental delays in the area.

Because the effects of second-hand pesticide exposure on neurobehavioral development have not been described, we can only estimate the potential magnitude of the effect. A study of prenatal exposure to an organophosphate (chlorpyrifos) found that children with high prenatal exposures had OR of 2.37 (95%CI 1.08-5.19) for delays in mental development and 4.52 (95%CI 1.61-12.7) for delays in psychomotor development (Kofman et al., 2006).

Using conservative estimates of the prevalence of neurodevelopmental delays in Pedro Moncayo (20%) and strength of association of pesticide exposure and developmental delays (OR=2.3), the calculated sample size needed is 256 (128 exposed and 128 unexposed), based on 80% power and a two-sided significance of 0.05. Although studies involving pesticide exposure in this area have had study participation of greater than 90%, we assumed a proportion of 15% of non-participation and lost to follow-up.

We sought to include a total of 300 children (150 exposed and 150 unexposed). This sample size was easily attained because 21% of the adults in the SAHS-PM 2004 worked in a flower plantation. We estimated that 190 (14%) of the 922 potentially eligible children were exposed.

For attained sample size and detectable differences of various outcome variables of our study refer to sections H. PARTICIPANT CHARACTERISTICS and I. POWER, respectively.

D.4. Exposure definition

D.4.1. 2004 Cross-Section: The main exposure is defined as concurrent cohabitation with a flower plantation worker. Exposure information for the 2004 cross-section was obtained from the SAHS-PM 2004 (See E.3. Survey of Access Demand of Health Services in Pedro Moncayo, 2004 (SAHS-PM 2004)).

D.4.2. 2004-2008 Cohort and 2008 Cross Section: The main exposure is defined as having cohabited with a flower worker between birth and 5 years of age, for at least 1 year. By requiring at least 1 year of cohabitation we selected children with an important length of exposure which would reduce the probability of a type-2 error due to insufficient exposure. Exposure information was obtained from parental interviews (see E.5.1. Interviews).

We selected exposure between birth and 5 years of age because many crucial events of child development occur in this period and because the greatest detrimental effect caused by pesticides occurs in this age group. A study of children intoxicated with AChE inhibitors before the age of 3 years found them having learning and impulse control difficulties later in childhood (Kofman et al., 2006).

Non-exposure is defined as: 1) never cohabited with a flower plantation worker, 2) never inhabited a house where agricultural pesticides were stored, 3) having never been intoxicated with pesticides and 4) having no known direct contact with pesticides.

E. DATABASES AND DATA COLLECTION

E.1. Participant Ascertainment

The SAHS-PM 2004 intended to interview all people living in Pedro Moncayo County in 2004. Recruiters and interviewers conducted home visits to all houses in the county. Approximately 71% of the population was interviewed in this survey. In 2008, all children who had viable height and weight measurements from 2004 were contacted, through a home visit, to participate in the ESPINA study and recruited if they met the study's eligibility criteria. Participant volunteers were allowed to participate in the study if they met eligibility criteria.

Flower worker-cohabitant Children (Exposed): Children whose parents or other household members reported being employed in a flower plantation in the SAHS-PM 2004 were identified and screened for eligibility for study participation with a screening survey carried out at their homes.

Non-flower worker-cohabitant Children (Unexposed): Children whose parents or other household members did not report being employed in a flower plantation in the SAHS-PM 2004 were identified and screened for eligibility for study participation with a screening survey carried out at their homes.

E.2. Recruitment and Informed Consent

Subjects' parents were recruited at their homes by a trained interviewer. Parental and flower worker informed consent for the interview was sought in addition to parental permission of study participation of their children. Informed consent and parental permission of study participation were obtained before the pre-survey (see E.5.1. Interviews). Child assent of children, 7 years and older, was obtained prior to examination. Appendices 1a, 2a and 3a show the consent, parental permission and assent forms in Spanish. Appendices 1b, 2b and 3b show the English translations.

E.3. Survey of Access Demand of Health Services in Pedro Moncayo, 2004 (SAHS-PM 2004)

The ESPINA study analyzed data of the SAHS-PM 2004 and from data collected in 2008 (interviews and participant examination).

E.3.1. Overview. The SAHS-PM 2004 was developed by Fundacion Cimas del Ecuador with the approval of the Municipality of Pedro Moncayo and the County Health Council. It obtained information on socio-economic and demographic factors including education, housing, basic hygiene, employment, home geographical coordinates, height and weight measurements of children younger than 5 years of age. SAHS-PM 2004 obtained information through home visits of 18,187 (71% of the population) Pedro Moncayo County residents of all ages, and height and weight measurements on 1356 children (40% of all in Pedro Moncayo) (Instituto Nacional de Estadística y Censos, 2001). Of these children, 922 had valid height and weight measurements as determined by WHO standards and were included in the study. The ESPINA study used the variables listed in Table 1 from the SAHS-PM 2004.

E.3.2. Exposure information. The SAHS-PM 2004 contains information on employment of all people living in the surveyed houses. Current cohabitation with a flower worker was used as the exposure variable in the 2004 cross-sectional analysis (see D.4. Exposure definition).

E.4. Residence Distance to flower plantations

Although pesticide drift from flower plantations to the environment is thought to be minimal due to fully covered greenhouses, there may still be a sufficient drift to confound the results since flower plantation workers often live near their work. 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 (SILC: Sistema de Información Local y Comunitario), using portable global positioning system (GPS) receivers. Home coordinates for new and not previously coded homes were updated in 2006 and 2010 by Fundacion Cimas del Ecuador. In 2006, flower plantation edges (polygons) were created by measuring the geographic coordinates of each corner of the plantations' perimeter. Using ArcGIS 9.3 (Esri, Redlands, CA), the distance of each participant's home to the nearest 1 meter segment of flower plantation edge and to the flower plantation center were calculated. Additionally, the areas of flower plantations within a radius of 50m, 100m, 200m and 500m from the participant's homes were calculated.

E.5. Data Collection in 2008

Data collection had the following order: 1) parental and flower worker pre-survey, 2) anthropometry, 3) heart rate, 4) blood pressure, 5) AChE and hemoglobin measurements, 6) neurobehavioral assessment and 7) parental surveys. Subjects were provided with a small gift as a token of appreciation for their participation in the study.

The pre-survey and survey took place at participants' homes and participant examinations took place up to 3 weeks after the pre-survey at 1 or 2 schools in each parish.

E.5.1. Interviews. Participant parents and flower workers living in the same household as participant children were interviewed. The interviews consisted of a pre-survey that checked for eligibility of participation and was conducted prior to full enrollment in the study, and 2 surveys: 1 for participant parents and 1 specific for flower workers.

Interviewers and Training. Local community members with prior experience conducting surveys were trained to recruit participants and conduct interviews at their homes. Most interviewers had previous or current professional relations with Fundación Cimas del Ecuador.

Procedures. The pre-survey and surveys were revised by one staff member of Fundacion Cimas del Ecuador who was a resident of Pedro Moncayo County, to ensure that the language used was easily understood by the people of the County and was culturally appropriate. The interviews (in-person) of the pre-survey and surveys took place between 3 pm and 8 pm during weekdays, and between 8 am and 5 pm on weekends at participant's homes. Prior to the pre-survey, participants received an explanation of the study objectives with time for questions and answers, completed informed consent of study participation and permission of child participation. At the end of the pre-survey, participants were scheduled for examination visits.

<u>E.5.1.1. Pre-Survey.</u> The pre-survey obtained additional contact information and eligibility questions of child participation from children's parents or guardian. Appendix 4 shows the format and questions gathered from the pre-survey (in Spanish. See Table 1 for translations). The duration of the pre-survey was between 3 and 6 minutes.

<u>E.5.1.2. Parental Survey.</u> The parental survey obtained demographic, occupational, and exposure information (see Table 1) from 1 or 2 parents or guardian (see Appendix 5). The parental survey had a duration between 20 and 30 minutes.

E.5.1.3. Flower Worker Survey. Flower workers were interviewed to obtain occupational information, (e.g. job type and pesticide protection), and perceptions, attitudes and practices towards pesticides (see Table 1). The flower worker survey lasted between 4 and 6 min and was incorporated into the main survey (see Appendix 5).

E.5.2. Examination

Participant examinations included neuropsychological tests, anthropometry (height, weight, head circumference, body temperature, resting heart rate, blood pressure) and acetylcholinesterase and hemoglobin measurements. The total duration of the examination ranged between 1 and 2 hrs.

The examinations took place during the months of July and August 2008 in schools in each of the 5 Parishes of Pedro Moncayo County: Tupigachi (Escuela Maria de las Mercedes Suarez, Escuela Atahualpa, Escuela Mision Andina), La Esperanza (Escuela Leopoldo Chavez), Tocachi (Escuela Manuel Villavicencio), Malchingui (Escuela Pedro Moncayo) and Tabacundo (Escuela Pacifico Proaño). July and August were selected because schools were closed to students for summer break which would allow for quieter exam environments. Additionally, schools were familiar locations to the examined children which would benefit the child's performance in the neurobehavioral tests.

E.5.2.1. Neuropsychological Tests. *Equipment:* NEPSY: A Developmental Neuropsychological Assessment, Second Edition (NEPSY-II) was used to test neurobehavioral development (Korkman et al., 2007a). NEPSY-II is a test designed for children between 3 and 16 years of age, to investigate neuropsychological development through 32 (+4 delayed memory) subtests in 6 functional domains: 1) Executive Functioning/Attention (assesses deficits in attention and self-monitoring tasks), 2) Language (assesses various components of oral and written language that can relate to reading, writing, spelling and verbal problems), 3) Memory and Learning (comprehensively assesses immediate and delayed memory and can assess verbal and nonverbal processes involved in recall), 4) Sensorimotor Functioning (assesses motor skills, precision and mimicry), 5) Visuospatial Processing (assesses object discrimination, positional judgment and design copying) and 6) Social Perception (assesses interpretations of nonverbal social and behavioral cues of other individuals) (Ahmad and Warriner, 2001; Brooks et al., 2010b; Korkman, 1999; Korkman et al., 2007c).

The NEPSY test has direct and indirect evidence of validity for clinical use (Bandstra et al., 2002; Korkman et al., 2001; Till et al., 2001), assessing children with disorders including ADHD, learning disabilities, language disorders and autism (Brooks et al., 2010b; Korkman et al., 2007c). A validation study of NEPSY that controlled for cognitive ability, found that children with scholastic concerns, neurological conditions and controls had different test scores, even without adjustment for IQ (Schmitt and Wodrich, 2004). This finding supports neuropsychological validity of the test, and provides some justification for its use without IQ assessments. However, when analyzing domains and subtests, differences were not universally found.

NEPSY-II has improved validity and reliability of its psychometric properties, improved clinical sensitivity and improved construct validity over its predecessor. Because NEPSY-II is only available in English, it was translated into Spanish, adapting it to the culture and terminology of the population of Pedro Moncayo. We expect no significant difficulties or challenges to validity associated with the translation due to the simple nature of the assessments, many of which did not require verbal responses or more than 1 word translations. NEPSY has been successfully translated and adapted into other cultures and settings, and its results have been found to be relatively unaffected by language and cultural factors (Garratt and Kelly, 2008; Kofman et al., 2006; Mulenga et al., 2001).

Examiners and Training: Neurobehavioral tests were conducted by 4 trained examiners with psychological expertise. All examiners received over 40 hours of training on the NEPSY-II which included reading and audiovisual material, and practical training in class and with volunteer children. All examiners also received Human Subjects Protection training through the Collaborative Institutional *Training* Initiative (CITI) training program and the “Investigator 101” CD.

Procedures: Only research personnel and the participant were allowed in the room. In the rare instances where parents or participants requested parental presence in the exam, one parent was allowed to sit in the room outside the child’s line of sight maintaining strict silence. Presence of a parent during the test was noted.

The examination consisted of the General Battery of tests in the NEPSY-II examination, which included 12 subtests in 5 domains: Attention and Executive Functioning (Auditory Attention and Response set, Inhibition, Statue), Memory and Learning (Memory for Faces Immediate, Memory for Faces Delayed, Narrative Memory, Word List Interference), Sensorimotor (Visuomotor Precision), Language (Comprehension of Instructions, Speeded Naming) and Visuo-spacial processing (Design Copy, Geometric Puzzles). Subtests varied in complexity and number according to participant age. Appendix 6 lists the subtests conducted by age group and Appendix 7 describes each subtest. Examinations followed all procedures listed in the NEPSY-II Administration Manual (Korkman et al., 2007b).

Examiners were <u>unaware of exposure status</u> of the child. The NEPSY-II Clinical and Interpretive Manual (Korkman et al., 2007c) and the NEPSY II scoring assistant and administration planner software were used to calculate the scores of the test. The NEPSY-II examinations had durations ranging from 40 to 100 minutes.

<u>E.5.2.2. Weight.</u> *Equipment:* Weight was measured using the Tanita BF683W scale. This scale has an accuracy of 0.1 kg and allows easy tared weighing (adult holding child during weight measurement) because it stores and subtracts an adult’s weight, showing only the child’s weight.

Examiner Training: Anthropometric measurements were conducted by a physician or a nurse. All examiners were trained using the WHO Child Growth Standards: Training Course on Child Growth and Assessment and the "Anthropometry training video" in Spanish or English (World Health Organization, 2008). Additionally, examiners also received Human Subjects Protection training through the Collaborative Institutional *Training* Initiative (CITI) training program and the "Investigator 101" CD.

Procedures. The procedures for weight measurement described in the Training Course on Child Growth and Assessment (World Health Organization, 2008) were followed, namely:

- Children were weighed after their removal of outer clothing (jacket and shoes). If it was too cold to undress a child, or if the child resisted being undressed and became agitated, or if the parents objected, the child was be weighed clothed. Clothed weighing was noted in the recording.
- Tared Weighing: If the child was unable to stand still, tared weighing was done.
  - The adult (e.g. parent, examiner) removed his/her shoes and stepped on the scale to be weighed alone first. She/he adjusted any long garments that could cover the display of the scale.
  - After the adult's weight appears on the display, she/he remained standing on the scale.
  - The adult stood on the scale during the calibration period such that the scale was reset to include the adult's weight.
  - Adult then held the child.
  - The child's weight appeared on the scale's display.
  - If the adult was very heavy (e.g. more than 100 kg), a lighter person held the child on the scale.
- Individual Weighing: If child could stand sill, s/he stepped on the scale alone and stood very still.

E.5.2.3. Height. *Equipment:* Height was measured with a height board. Measurements were recorded to the nearest 0.1 cm.

Examiner Training: Anthropometric measurements were conducted by a physician or a nurse. All examiners were trained using the WHO Child Growth Standards: Training Course on Child Growth and Assessment and the "Anthropometry training video" in Spanish or English (World Health Organization, 2008).

Procedures: The procedures for height measurement described in the Training Course on Child Growth and Assessment (World Health Organization, 2008) were followed, namely:

- If the child had braids or hair ornaments that will interfere with length/height measurements, they were removed before measuring height to avoid delay between the measurements. Shoes were also removed.
- If the child was unable to stand still, upright recumbent length was measured.
- If the child is able to stand still, upright standing height was measured as follows:
    - The child stood on the baseboard with feet slightly apart, and his/her back of the head, shoulder blades, buttocks, calves, and heels touching the vertical board.
    - Child's head was positioned so that a horizontal line from the ear canal to the lower border of the eye socket runs parallel to the baseboard.
    - If necessary, the child's belly was gently pressed to help the child stand to full height.
    - Still keeping the head in position, the headboard was pulled down to rest firmly on top of the head, compressing the hair.

E.5.2.4. Head Circumference. *Equipment:* Metric plastic tape measure.

Examiner Training: Anthropometric measurements were taken by a physician or nurse. All examiners were trained using the WHO Child Growth Standards: Training Course on Child Growth and Assessment and the "Anthropometry training video" in Spanish or English (World Health Organization, 2008).

Procedures: Head occipito-frontal circumference was measured 2 times to the nearest 0.1 cm. The larger of the 2 measurements was used.

E.5.2.5 Blood Pressure and Heart Rate. *Examiners and Retraining:* Blood pressure and heart rate were measured by a physician or nurse. Examiners were retrained and evaluated, prior to data collection, emphasizing on patient positioning, no talking, and accurate observation of the blood pressure level by auditory and visual assessment and pulse palpation.

Procedures: Heart rate was measured through a 30 second auscultation, prior to blood pressure measurement. Blood pressure was measured with a pediatric Omron aneroid sphygmomanometer, appropriate for the size of the children. Blood pressure measurements followed the recommendations of the American Heart Association (Pickering et al., 2005). Measurements were taken after 3-5 minutes of rest. Children were in the seated position with the antecubital fossa supported at heart level, with uncrossed legs and both feet on the floor. Blood pressure measurements were measured twice.

E.5.2.6. Acetylcholinesterase and Hemoglobin. *Equipment:* AChE and hemoglobin concentrations were quantified with the EQM Test-mate ChE Cholinesterase Test System 400, using the EQM AChE Erythrocyte Cholinesterase Assay Kit 470. This portable unit has been found to be reliable and has been often preferred for field-based studies (Engel et al., 1998; Wilson et al., 1997). A preliminary study in Pedro Moncayo County, by the principal investigator of this study, found this method to be very acceptable by parents and children due to its rapid operation and minimal pain infliction (Suarez-Lopez, 2003).

Examiners and Training: Assays were conducted by a physician or a nurse or a medical student. Examiners were trained on the use of the Test-mate system and finger stick procedures, within 2 weeks prior to the beginning of data collection.

Procedures: Finger-sticks followed the National Committee for Clinical Laboratory Standards (Ernst and Clinical and Laboratory Standards Institute., 2008). AChE and hemoglobin were assayed in ambient temperatures between 15^{o} C and 30^{o} C to maintain validity and reliability of the test.

E.6. Reporting of Findings to Participants.

Participant parents were informed of the results of the 2008 examination via a results sheet delivered at the time of the parental survey. Appendix 7 shows a sample results sheet. This sheet was written in non-technical Spanish at an 8th grade level for easy comprehention. The exam results had interpretations of the values in addition to recommendations such as methods for secondary pesticide exposure reduction, nutritional changes and referal to health services, as appropriate.

E.7. Study Data.

Table 1 lists a summary of the variables available from the SAHS-PM 2004, parental interview, and examinations. For additional detail on the interview (pre-survey and survey) questions see E.5. Data Collection.

Gender appropriate z-scores for height-for-age, weight-for-age and BMI-for-age were calculated using normative data from the World Health Organization (WHO) Child Growth Standards (WHO Multicentre Growth Reference Study Group, 2006; World Health Organization, 2007). Delayed vertical growth (stunting) was determined as 2 SD below normal height for age.

Gender, height and age appropriate z-scores for systolic and diastolic blood pressure were calculated using normative data 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).

F. INSTITUTIONAL REVIEW BOARD APPROVALS

The ESPINA study received approval from the IRB of the University of Minnesota (code: 0802M26221) and Fundacion Cimas del Ecuador (code: 0352). Additionally, this investigation was approved by the Ministry of Public Health of Ecuador and the County Health Council of Pedro Moncayo County.

G. FUNDING

The ESPINA study received funding from the following sources:

- CDC/NIOSH Grant 1R36OH009402-01: Effects of Secondary Occupational Pesticide Exposure on Childhood Growth and Neurobehavioral Development
- Doctoral Dissertation Fellowship, Graduate School, University of Minnesota
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- Upper Midwest Human Rights Fellowship, University of Minnesota
- University of Minnesota Thesis Research Grant, University of Minnesota

H. PARTICIPANT CHARACTERISTICS

A total of 230 children had information from 2004 and 2008. In 2008, the mean age was 6.4 years (range: 3.8-9.4 y, SD=1.4 y), 49% were female, 72% mestizo and 26% native and 59% cohabited with one or more flower workers. Children who lived with a flower worker had a mean duration of cohabitation of 5.1 years and a mean of 1.5 flower workers at home in 2008.

The gender distribution was balanced among participants in 2004, 2008 and among participants who were examined at both time points (2004-2008 cohort). Overall, most participants were mestizo (mix of indigenous and white) and indigenous. 37%, 24% and 25% of participants were stunted in 2004, 2008 and among 2004-2008 cohort participants, respectively. Participant characteristics by exam year are listed in Table 2; 2008 neurobehavioral development mean scores and distribution are listed in Table 3.

H.1. 2004 Cross-Section

Among participants of the SAHS-PM 2004, *9*22 of 1356 (68%) had complete and viable height, weight and age data, and were included in the 2004 growth analysis. Fifty one percent were female, the mean age was 2.3 years (SD: 1.4), and 63% currently cohabited with 1 or more flower workers with a mean of 2.0 flower workers currently living at home. The median height-for-age z-score was 1.35 SD below the median WHO value, and the stunting prevalence was 37%. Approximately 32% lived in the Parish of Tabacundo, 28% in Tupigachi, 19% in Malchingui, 14% in La Esperanza, and 7% in Tocachi (Table 2).

Participants cohabiting with a flower worker, vs. not, had lower mean scores for SES variables including type of home and home roof material (Table 4). No other significant differences were found.

H.2. 2008 Cross Section

In 2008, 313 children, between 4 and 9 years of age participated in the ESPINA study examinations. Forty nine percent of participants were female, 76% mestizo, 22% indigenous, 1% white and 1% black (Table 2). The mean age was 6.6 y (SD=1.6 y). 49% (n=152) of participants currently cohabited with a flower worker and 55% (171) ever cohabited with a flower worker. Children who lived with flower workers had a mean duration of cohabitation of 5.3 years and a mean of

1.5 flower workers currently living at home. The median height-for-age z-score was 1.25 SD below the median WHO value, and the stunting prevalence was 24%. The overall neurobehavioral development means were lower in the study population compared to the NEPSY-2 standard population which had a mean of 10 for each of the subtests (Table 3). Twenty nine percent lived in the Parish of Tupigachi, 26% in Malchingui, 19% in Tabacundo, 14% in La Esperanza, and 12% in Tocachi. The median residence distance to a flower plantation was 350 m (10^{th}-90^{th} percentiles: 98-1000m).

No mean or proportion differences were observed in 2008 for age, gender, parental smoking, household income, BMI for age, head circumference, blood pressure, heart rate or hemoglobin concentration between participants cohabiting vs. not cohabiting with a flower worker (Table 5). Participants cohabiting with a flower worker had a greater proportion of indigenous, lower maternal education, lower height-for-age and weight-for-age z-scores and lower acetylcholinesterase activity. Children living with a Flower worker lived closer to the nearest flower plantation and had the following distribution: 29% lived in the Parish of Tupigachi, 26% in Malchingui, 19% in Tabacundo, 14% in La Esperanza, and 12% in Tocachi. 37% lived in the Parish of Tupigachi, 20% in Malchingui, 18% in Tabacundo, 10% in La Esperanza, and 15% in Tocachi.

H.3. Prospective Cohort 2004-2008

In 2004 and 2008, 230 children were examined; 49% were female, 72% mestizo, 26% indigenous and 2% white. The mean age was 6.4 years (SD=1.4 y). 54% currently cohabited with a flower worker while 59% had ever cohabited with a flower worker. Children who had ever lived with a flower worker had a mean duration of cohabitation of 5.1 years and a mean of 1.5 flower workers currently living at home in 2008. 37% lived in the parish of Tupigachi, 20% in Malchingui, 18% in Tabacundo, 10% in La Esperanza, and 15% in Tocachi (Table 2).

I. POWER

For the 2008 examination, a sample size of 256 participants (128 per exposure group) was calculated based on conservative estimates of prevalence of neurodevelopmental delays in Pedro Moncayo of 20% (Handal et al., 2007) and an OR of developmental delays for pesticide exposure of 2.3 (Kofman et al., 2006). The calculation was based on 80% power and a two-sided significance of 0.05. At the end of the examination period, we surpassed by 22% the target sample size.

In 2004, 922 participants were included in this study, 578 (63%) of whom currently cohabited with a flower worker. In the 2008 cross-section, 313 participants were examined, 171 (55%) of which had ever cohabited with a flower worker. In the 2004-2008 cohort, 230 participants were examined; 136 (59%) participants had lived with a flower worker at some point during their life.

Table 6 and Table 7 list the detectable difference of various outcomes according to the main exposure variable ("current cohabitation with a flower worker" in the 2004 cohort and "ever cohabitation with a flower worker" for the 2008 cross section and 2008 cohort). Calculations are based on the existing sample size for each exposure group among each of the three study groups: 2004 and 2008 cross sections and 2004-2008 cohort participants.

Table 1. Data obtained from SAHS-PM 2004, parental interview, and examinations.

Variable Type	Type	Source
A) Demographic		
Name of participant and parents	String	SAHS-PM 2004 Pre-survey Parental Interview
Contact information: address	String	SAHS-PM 2004 Pre-survey
Contact information: telephone number	String	Pre-survey
Participant's sex	Dichotomous	SAHS-PM 2004
Participant's age, date of birth	Continuous	SAHS-PM 2004 Pre-survey
Participant's race	Categorical	Parental Interview
Parental age	Numerical	SAHS-PM 2004, Parental Interview
Parental sex	Dichotomous	SAHS-PM 2004, Parental Interview
Parental race	Categorical	Parental Interview
Parental marital status	Categorical	SAHS-PM 2004, Parental Interview
Parental education	Numerical	SAHS-PM 2004 Parental Interview
Parental occupation	String	SAHS-PM 2004 Parental Interview
How many people smoke at home?	Numerical	Parental Interview
Household income	Categorical	Parental Interview
Household socio-economic status variables (e.g. type	Categorical	SAHS-PM 2004

of housing, access to potable water, waste disposal)		
Flower plantation geographic coordinates	Numerical	SAHS-PM 2006
Residential geographic coordinates	Numerical	SAHS-PM 2004, 2006 ESPINA 2008
B) Medical History		
Participant Disease: Heart, liver, other	String	Pre-survey
Participant Medication Usage	Dichotomous	Pre-survey
Does Child attend daycare in a flower plantation?	Dichotomous	Pre-Survey
Mother's parity	Numerical	Parental Interview
Mother's abortions or miscarriages	Numerical	Parental Interview
Mother's pregnancy complications	Dichotomous	Parental Interview
C) Pesticide Exposure (Interview only)		
Participant currently living with a flower worker?	Dichotomous	SAHS-PM 2004 Parental Interview
How many flower workers currently living at home?	Numerical	SAHS-PM 2004 Parental Interview
Participant ever lived with a flower worker?	Dichotomous	Pre-survey
Duration of flower worker cohabitation	Numerical	Pre-survey
Number of flower workers living at home	Numerical	Pre-survey
How old was child when s/he started living with a flower worker?	Numerical	Pre-survey
Participant's relationship with flower worker	String	Parental Interview
Agricultural pesticide storage at home	Dichotomous	Parental Interview
Agricultural pesticide use in residential perimeter?	Dichotomous	Parental Interview
Neighbor use of pesticides	Dichotomous	Parental Interview
Livestock in residential perimeter with ectoparasitic control with pesticides?	Dichotomous	Parental Interview
Has participant been in contact with pesticides? What type and source?	Dichotomous, String	Pre-survey Parental Interview

Has participant been intoxicated with pesticides?	Dichotomous	Pre-survey Parental Interview
Maternal occupational pesticide exposure during subject's pregnancy	Dichotomous	Parental Interview
Maternal cohabitation with a flower plantation worker during subject's pregnancy	Dichotomous	Parental Interview
Maternal cohabitation with other agricultural workers during subject's pregnancy	Dichotomous	Parental Interview
Maternal known exposures to pesticides during subject's pregnancy	Dichotomous	Parental Interview
Maternal pesticide storage or use at home during subject's pregnancy	Dichotomous	Parental Interview
Flower plantation areas + geographical coordinates	Numerical	SAHS-PM 2006
Flower Workers Only:		
Length of time working for flower industry	Numerical	Flower worker Interview
Amount of time working for flower industry since 2004	Numerical	Flower worker Interview
Did the flower worker work in a flower plantation in 2004?	Dichotomous	SAHS-PM 2004 Flower worker Interview
Job type and tasks performed in flower plantation	String	SAHS-PM 2004 Flower worker Interview
Hand washing frequency after work	Categorical	Flower worker Interview
Showering frequency after work	Categorical	Flower worker Interview
Location of shower (at work, at home, other)	Categorical	Flower worker Interview
Work clothes usage (use of gown, boots, hat etc.)	Categorical	Flower worker Interview

Closed storage provided for street clothes?	Dichotomous	Flower worker Interview
Work clothes washed at home?	Categorical	Flower worker Interview
Work boots/shoes brought home?	Categorical	Flower worker Interview
Work tools brought home?	Categorical	Flower worker Interview
Pesticide storage at home?	Categorical	Flower worker Interview
D) Examination Variables		
Neurobehavioral development	Numerical	ESPINA 2008
Resting heart rate	Numerical	ESPINA 2008
Blood pressure	Numerical	ESPINA 2008
Height	Numerical	SAHS-PM 2004 ESPINA 2008
Weight	Numerical	SAHS-PM 2004 ESPINA 2008
Head circumference	Numerical	ESPINA 2008
Temperature	Numerical	ESPINA 2008
Acetylcholinesterase activity	Numerical	ESPINA 2008
Hemoglobin concentration	Numerical	ESPINA 2008

Table 2. Participant Characteristics by Examination Year.

Characteristics	Examination Year(s) 2004[a]	2008	2004 and 2008[b]
	N=922	N=313	N=230
Demographic and Socio-economic			
Age, years	2.3 (1.4)	6.6 (1.6)	6.4 (1.4)
Gender, male (%)	51	51	51
Race, %			
Mestizo		72	68
Native		21	24
White		1	2
Black		1	0
Parental Smoking, current (%)		21	21
Maternal Education, years[c]		7.4 (3.8)	7.5 (4.0)
Maternal Education, categories[d]	3.4 (1.3)		
Monthly Income[e]		3.1 (0.8)	3.2 (0.9)
Ever cohabited with flower worker (%)		55	59
Current cohabitation with a flower worker (%)	63	49	54
Duration of Cohabitation (years)[f]		5.3 (1.9)	5.1 (1.8)
Distance of residence to nearest flower plantation edge, m		350 (185, 605)	334 (178, 620)
Parish of Residence (%)			
Tabacundo	32	19	18
Tupigachi	19	29	37
Malchingui	19	26	20
La Esperanza	14	14	10
Tocachi	7	12	15

Anthropometric			
Height, cm	82.5 (13.8)	112.1 (10.3)	110.9 (9.6)
Height-for-age, z-score	-1.35 (1.86)	-1.25 (0.98)	-1.32 (1.00)
Stunting prevalence	37%	24%	25%
Weight, kg	11.2 (3.6)	20.5 (4.5)	20.0 (4.1)
Weight-for-age, z-score	-0.86 (1.45)	-0.55 (0.90)	-0.57 (0.89)
BMI-for-age, z-score	0.01 (1.83)	0.34 (0.79)	0.38 (0.78)
Δ in Height, cm			27.9 (7.0)
Δ in Height-for-age, z-score[g]			0.73 (1.43)
Δ in Weight, kg			8.5 (2.7)
Δ in Weight-for-age, z-score[g]			0.55 (1.45)
Δ in BMI-for-age, z-score[g]			0.16 (1.79)
Head circumference, cm		50.6 (1.6)	50.6 (1.5)
Resting heart rate, bpm		84.8 (12.6)	84.8 (12.8)
Systolic Blood Pressure, mmHg		93.5 (8.5)	92.9 (8.2)
Systolic Blood Pressure, z-score[h]		0.00 (0.73)	0.0 (0.7)
Diastolic Blood Pressure, mmHg		49.2 (7.3)	49.5 (7.6)
Diastolic Blood Pressure, z-score[h]		-0.51 (0.61)	-0.5 (0.6)
Blood			
Acetylcholinesterase (U/ml)		3.14 (0.49)	3.12 (0.49)
Hemoglobin (mg/dl)		12.6 (1.2)	12.6 (1.2)

Table entries are percentage, mean (SD) or median (25^{th}-75^{th} percentile)

a. Missing data was not collected at this time period.

b. This is a subset of participants of 2004 and 2008.

c. Maternal education: Years of education after kindergarten.

d. Maternal education categories: 1=none 2=incomplete primary (K-6^{th})

3=complete primary 4=incomplete secondary 5=complete secondary 6=university/technical school.

e. Income categories (USD): 1=0-50 2=51-150 3=151-300 4=301-500 5=501-1000 6=>1000.

f. Includes only participants who had ever lived with a flower plantation worker.

g. Z-scores appropriate for gender. Normative data from the WHO growth standards.

h. Z-scores appropriate for age, gender and height.

Table 3. Overall mean and distribution of neurobehavioral subtests scaled scores in 2008. Sample sizes vary by subtest according to the age groups that each subtest was designed to examine.

Neurobehavioral Subtest Scaled Scores	Domain	N	Mean	SD	Min	Max
Auditory Attention	Attention and Executive Function	242	8.2	3.5	1	17
Response Set	Attention and Executive Function	128	8.8	3.2	1	14
Inhibition	Attention and Executive Function	233	7.1	3.1	1	14
Statue	Attention and Executive Function	174	10.2	2.9	1	15
Comprehension of Instructions	Language	313	7.3	3.0	1	16
Speeded Naming	Language	272	5.9	3.0	1	17
Memory for Faces	Memory and Learning	251	7.4	2.8	1	15
Narrative Memory, Free Recall	Memory and Learning	251	9.5	3.0	2	19
Memory for Faces Delayed	Memory and Learning	249	8.7	3.0	1	16
Word List Interference, Repetition	Memory and Learning	129	7.4	3.3	1	14
Visuomotor Precision	Sensorimotor	310	9.9	3.3	1	19
Geometric Puzzles	Visuospatial processing	313	8.5	3.2	1	15
Design Copy	Visuospatial processing	302	10.6	4.9	1	19

Table 4. Participant Characteristics in 2004, n=922.

	Children Cohabiting with a:		P-Value
	Flower worker	Non-Flower Worker	
	N=578 (63%)	N=344 (37%)	
Demographic and SES Characteristics			
Age, years	2.3 (1.4)	2.3 (1.4)	0.808
Gender, male	50%	47%	0.394
Household Head Education[a]	3.6 (1.2)	3.7 (1.3)	0.204
Home Type[b]	3.6 (0.9)	3.8 (0.6)	0.001
Home Roof Material[c]	2.9 (0.8)	3.1 (0.9)	0.010
Home Floor Material[d]	2.5 (0.9)	2.7 (0.9)	0.076
Type of Wastewater Disposal[e]	3.6 (1.3)	3.6 (1.3)	0.511
Anthropometric Characteristics			
Z-score height-for-age	-1.5 (-2.6, -0.5)	-1.5 (-2.5, -0.1)	0.080
Stunting	37%	36%	0.585
Z-score BMI-for-age	0.1 (-1.0, 1.1)	0.04 (-1.1, 1.4)	0.708
Z-score weight-for-age	-0.9 (-1.9, 0.1)	-0.6 (-1.7, 0.2)	0.101

Table entries are percentage, mean (SD), or median (25^{th}-75^{th} percentile).

a. Education: 1=none 2=incomplete primary (K-6^{th}) 3=complete primary. 4=incomplete secondary 5=complete secondary 6=university/technical school.

b. Home Type: 1=shack 2=room(rental) 3=apartment 4=house.

c. Home Roof Material: 1=zinc 2=fiber cement 3=tile 4=concrete.

d. Home Floor Material: 1=dirt 2=untreated wood 3=cement/brick 4=tile 5= hardwood/parquet.

Type of Wastewater Disposal: 1= None 2= Cesspool 3= Septic Tank 4=Latrine 5= Sewage.

Table 5. Participant Characteristics in 2008. N=313.

		Children Cohabiting with a:		P-Value
		Flower worker	**Non-Agricultural Worker**	
		N=171 (55%)	N=142 (45%)	
Demographic and SES Characteristics				
	Age, years	6.4 (1.6)	6.7 (1.6)	0.127
	Gender, male	52%	51%	0.894
	Race, mestizo	72%	86%	0.003
	Parental Smoking, current	19%	23%	0.484
	Maternal Education, years	6.8 (3.3)	8.1 (4.4)	0.003
	Monthly Income[a]	3.1(0.7)	3.1 (1.0)	0.946
	Residence distance to nearest flower plantation, m	321 (166, 610)	438 (212, 604)	0.021
Anthropometric Characteristics				
	Z-score height for age	-1.4 (-2.1, -0.7)	-1.1 (-1.9, -0.5)	0.038
	Stunting	27%	19%	0.075
	Z-score BMI for age	0.3 (-0.2, 0.8)	0.3 (-0.2, 1.0)	0.290
	Z-score weight for age	-0.7 (-1.2, -0.1)	-0.5 (-1.1, 0.3)	0.025
	Head circumference, cm	50.6 (1.6)	50.7 (1.5)	0.647
	Resting heart rate, bpm	85.3 (12.1)	84.4 (13.1)	0.535
	Systolic Blood Pressure, mmHg	92.7 (7.9)	94.3 (9.1)	0.097
	Systolic Blood Pressure, z-score[b]	-0.1 (-0.5, 0.4)	0.04 (-0.5, 0.4)	0.446
	Diastolic Blood Pressure, mmHg	49.5 (7.8)	48.9 (6.6)	0.541

	Diastolic Blood Pressure, z-score[b]	-0.6 (-0.8, -0.1)	-0.6 (-1.0, -0.2)	0.157
Blood Measurements				
	Acetylcholinesterase (U/ml)	3.08 (0.46)	3.21 (0.50)	0.022
	Hemoglobin (mg/dl)	12.5 (1.2)	12.7 (1.1)	0.180

Table entries are percentage, mean (SD), or median (25^{th}-75^{th} percentile).

[a] Income categories (USD): 1=0-50 2=51-150 3=151-300 4=301-500 5=501-1000 6=>1000.

[b] Z-scores appropriate for age, gender and height.

Table 6. 2004 Cross Section means, differences and detectable differences of various outcomes by current cohabitation with a flower worker.

	Current Cohabitation with a:		Absolute Observed Difference (SD[a])	Detectable Difference[b]
	Flower worker	Non-Agricultural Worker		
2004 Cross Section	**N=578 (63%)**	**N=344 (37%)**		
Height, cm	82.3 (14.0)	82.8 (13.4)	0.13 (13.8)	2.34
Height-for-age, z-score[c]	-1.44 (1.84)	-1.21 (1.88)	0.55 (1.86)	0.32
Weight, kg	11.2 (3.7)	11.3 (3.6)	0.05 (3.6)	0.61
Weight-for-age, z-score[c]	-0.92 (1.44)	-0.76 (1.46)	0.51 (1.45)	0.25
BMI-for-age, z-score[c]	0.00 (1.84)	0.04 (1.82)	0.09 (1.83)	0.31

Table entries are percentage or mean (SD).

a. Overall mean standard deviation.

b. Detectable difference for a 1-sided test, $\alpha=0.05$ and $\beta=0.80$.

c. Z-scores appropriate for gender. Normative data from the WHO growth standards.

Table 7. 2008 Cross Section and 2004-2008 Cohort means, differences and detectable differences of various outcomes by cohabitation (ever) with a flower worker.

	Ever Cohabited with a:		Absolute Observed Difference (SD[a])	Detectable Difference[b]
	Flower worker	Non-Agricultural Worker		
2004-2008 Cohort Change(Δ): 2008-2004	**N=136 (59%)**	**N=93 (41%)**		
Δ in Height, cm	27.7 (6.6)	28.4 (7.4)	0.7 (7.0)	1.98
Δ in Height-for-age, z-score[c]	0.69 (1.30)	0.83 (1.59)	0.14 (1.43)	0.41
Δ in Weight, kg	8.18 (2.27)	8.9 (3.22)	0.72 (2.7)	0.76
Δ in Weight-for-age, z-score[c]	0.52 (1.35)	0.6 (1.60)	0.08 (1.45)	0.41
Δ in BMI-for-age, z-score[c]	0.12 (1.69)	0.19 (1.93)	0.07 (1.79)	0.51
2008 Cross Section	**N=171 (55%)**	**N=142 (45%)**		
Anthropometric Characteristics				
Height, cm	110.8 (10.0)	113.5 (10.4)	2.7 (10.3)	2.91
Height-for-age, z-score[c]	-1.36 (1.00)	-1.13 (0.94)	0.23 (0.98)	0.28
Weight, kg	19.9 (4.0)	21.3 (4.9)	1.4 (4.5)	1.27
Weight-for-age, z-score[c]	-0.66 (0.82)	-0.43 (0.82)	0.23 (0.90)	0.26
BMI-for-age, z-score[c]	0.30 (0.71)	0.40 (0.87)	0.10 (0.79)	0.22
Head circumference, cm	50.6 (1.6)	50.7 (1.5)	0.1 (1.6)	0.45
Resting heart rate, bpm	85.3 (12.1)	84.4 (13.1)	0.9 (12.6)	3.56
Systolic Blood Pressure, mmHg	92.7 (7.9)	94.3 (9.1)	1.6 (8.5)	2.40
Systolic Blood Pressure, z-score[d]	-0.03 (0.69)	0.03 (0.78)	0.06 (0.73)	0.21
Diastolic Blood Pressure, mmHg	49.5 (7.8)	48.9 (6.6)	0.6 (7.3)	2.07
Diastolic Blood Pressure,	-0.47 (0.67)	-0.57 (0.53)	0.10 (0.61)	0.17

	z-score[d]				
Blood Measurements					
	Acetylcholinesterase (U/ml)	3.08 (0.46)	3.21 (0.50)	0.13 (0.49)	0.14
	Hemoglobin (mg/dl)	12.5 (1.2)	12.7 (1.1)	0.2 (1.2)	0.34

Table entries are percentage or mean (SD).

a. Overall mean standard deviation.

b. Detectable difference for a 1-sided test, α=0.05 and β=0.80.

c. Z-scores appropriate for gender. Normative data from the WHO growth standards.

d. Z-scores appropriate for age, gender and height.

CHAPTER 3: LOWER ACETYLCHOLINESTERASE ACTIVITY AMONG CHILDREN LIVING WITH FLOWER PLANTATION WORKERS

Summary

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.

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.

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.

Material and methods

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 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.

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 having 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.

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^{o}C and 28.4^{o}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.

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, to 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, 1–2, 3 and 4–6. 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).

Results

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.

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).

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).

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 <15th percentile and OR 5.53 (95% CI: 1.39 to 21.93) of AChE activity within 15th-33rd percentile, compared to the lowest tertile. There was no association between 1-2 or 3 bad practices (vs. none) and the 4 level AChE activity variable.

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.

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.

Tables

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 <15th 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)
15^{th} – 33^{rd} percentile[d]	1.23 (1.08, 1.41)	1.25 (1.08, 1.44)	1.24 (1.08, 1.43)
<15^{th} 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, 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 4. 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

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CHAPTER 4: ACETYLCHOLINESTERASE ACTIVITY FROM SECONDARY PESTICIDE EXPOSURES AND NEUROBEHAVIORAL DEVELOPMENT IN CHILDREN

Summary

BACKGROUND: Animal and human studies suggest that pesticide exposure in early childhood is harmful to neurodevelopment. We assessed the impact of secondary pesticide exposure on childhood neurobehavioral development in participants who lived in rural Ecuadorian communities with intense fresh-cut flower industry activity.

METHODS: In the Secondary Pesticide Exposure on Infants, Children and Adolescents (ESPINA) study, parental interviews, child neurobehavioral development (NEPSY-II test) and acetylcholinesterase (AChE) quantification (EQM Testmate) were conducted in 288 children aged 4-10y.

RESULTS: 56% of participants cohabited with ≥1 flower plantation workers (FW, mean duration= 5.3 years) and had a mean AChE activity of 3.1 U/ml (standard deviation (SD): 0.5). The range of scores among 13 NEPSY-II subtests was 5.9-10.9 units (SD: 2.8-4.9). Boys with AChE activity in the lowest vs. the highest tertile had adjusted odds ratios (OR) of 7.40 (95%CI 1.71-32.05), 9.39 (95%CI 2.36-37.38) and 2.35 (95%CI 1.03-5.34) of low scores (<9^{th} percentile) of Attention and Executive Functioning Domain, inhibitory control and long term memory, respectively, after adjusting for age, gender, race, height-for-age z-score, household income, flower worker cohabitation status, maternal education, hemoglobin concentration. Children's cohabitation with a FW (vs. not) was associated with OR of low auditory attention score of 1.63 (95%CI 0.79,-3.37).

CONCLUSIONS: Low AChE activity was associated with deficits on tasks reflecting memory, attention and inhibitory control in boys. These are critical cognitive skills that affect learning and academic performance. Added precaution regarding secondary pesticide exposure in the Ecuadorian flower industry would be prudent.

Introduction

Background: Pesticides are chemicals that are widely used in agriculture and regularly come into contact with human populations. Intoxication due to high exposure to pesticides in children and adults are associated with overt acute symptoms which can lead to fatal outcomes and typically occur in people at high risk of exposure (e.g. agricultural workers). However, the vast majority of pesticide exposure in a population likely occurs in small doses from indirect routes (e.g. ingestion of food exposed to pesticides or living near areas where pesticides are regularly used). Such exposures are not likely to be associated with overt symptoms, and may last for long periods of time.

Children are especially vulnerable to the noxious effects of pesticides due to physiologic and behavioral factors (Cohen Hubal et al., 2000; Faustman et al., 2000). During early childhood, the human brain develops at the greatest rate and is particularly vulnerable to developmental disturbances by neurotoxins (Huttenlocher and Dabholkar, 1997). Some of the most commonly used insecticides worldwide are cholinesterase inhibitors, (i.e. organophosphates (OP) and carbamates) pesticides that are known to suppress acetylcholinesterase (AChE) activity (Kwong, 2002) which an enzyme that has been used as an objective marker of exposure to these chemicals and whose inhibition could also directly affect brain development. Studies in rodents have found that crucial components of brain development that are mediated by acetylcholine (and thus its hydrolyzing enzyme AChE), occur in early childhood (Dori et al., 2005;

Hohmann, 2003). Acute intoxication with cholinesterase inhibitors can cause neurotoxicity due to cholinergic excess (Aaron CK, 2007) which occurs particularly with irreversible inhibition of cholinesterase as seen with OP pesticides. Non-cholinergic mechanisms of neurotoxicity of OP pesticides have also been proposed (Abou-Donia, 2003; Aldridge et al., 2005; Qiao et al., 2003; Slotkin, 2004a).

There is growing evidence in animals linking cholinesterase inhibitors with disruption of brain development (Byers et al., 2005; Roy et al., 2004; Santos et al., 2004; Slotkin et al., 2001), possibly at low levels of cholinesterase inhibition, and without symptoms of intoxication (Abou-Donia, 2003; Dam et al., 2000; Qiao et al., 2003; Slotkin, 2004b; Slotkin et al., 2001). Studies in children reported associations between prenatal pesticide exposure with mental and motor developmental delays, pervasive developmental disorder, decreased attention, working memory and intelligence quotient (Eskenazi et al., 2007; Marks et al., 2010; Rauh et al., 2011; Rauh et al., 2006). Associations between postnatal OP pesticide exposures and decreased learning, poor inhibitory control, attention and attention deficit hyperactivity disorder (ADHD) have also been reported (Bouchard et al., 2010; Kofman et al., 2006; Marks et al., 2010).

Children of agricultural workers have increased risk of pesticide contamination through close proximity of worker's residence to the plantation and take-home routes which include bringing home pesticide-impregnated work clothes, tools/objects, and home storage of agricultural pesticides (Coronado et al., 2011; Curl et al., 2002; Fenske et al., 2002; Gladen et al., 1998; Lambert et al., 2005; Lu et al., 2000; McCauley et al., 2001; Simcox et al., 1995). The amount of pesticide exposure reaching these children is usually small enough not to elicit clinical manifestations and, thus, likely to go unnoticed. However, children of agricultural workers may be exposed secondarily to pesticides for as many years as they cohabit with an agricultural worker, which for many may extend from birth through adolescence. These small but chronic pesticide exposures, particularly during early childhood, may have long lasting effects on children's development.

Of the studies that have examined low-level OP pesticide exposures and children's neurobehavioral development, most have assessed children younger than 3 years, only a few have included pesticide biomarkers and no adequately powered studies have analyzed the association of AChE with neurobehavioral development to our knowledge.

We tested the hypotheses that lower AChE activity and cohabitation with a flower plantation worker (flower worker), as indicators of pesticide exposure, are associated with lower neurobehavioral development (particularly with attention and executive function domain) in children of kindergarten and elementary school age living in agricultural communities, in 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).

Methods

Study Description

The ESPINA study examined children living in an area with a predominant agricultural economy with the purpose of characterizing the relationship between secondary pesticide exposures and child development (Suarez-Lopez et al., 2012). Participants resided in the Ecuadorian Andes, in Pedro Moncayo Canton, Pichincha, Ecuador. This Canton has substantial floricultural activity with an approximated production area of 1800 hectares (5.3% of the Canton's surface area) (Gobierno-Municipal-del-Canton-Pedro-Moncayo, 2011) and employs approximately 21% of adults (Suarez-Lopez et al., 2012). The floricultural industry has been reported to use a large number of insecticides, fungicides and herbicides. Among the most frequently used pesticides are OP (Harari, 2004), which account for a substantial proportion of intoxications in Ecuador (González-Andrade et al., 2010). Because of the substantial agricultural economy of Pedro

Moncayo Canton, residents likely have higher pesticide exposures than non-agricultural areas. A study that measured OP metabolites in 72 children in Pedro Moncayo Canton found that the pesticide metabolite concentrations there were similar to those of similarly aged US children (Grandjean et al., 2006), although this study did not separate out children who were cohabiting with agricultural workers.

Many study participants were identified from their participation in the 2004 Survey of Access and Demand of Health Services in Pedro Moncayo Canton conducted by Fundacion Cimas del Ecuador. This survey examined and interviewed families of 922 children in Pedro Moncayo Canton between the ages of 0 and 5 years in 2004. In 2008, ESPINA examined 313 children between 4 and 9 years of age, which included a subset of these children supplemented by new volunteers. Additional participant recruitment information has been described elsewhere (Suarez-Lopez et al., 2012). Additionally, we conducted in-person home interviews of 451 adults who lived with the examined children. The present report included 275 (88%) children who had all covariates of interest.

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 7 years of age and older. This study was approved by the Institutional Review Boards of Fundacion Cimas del Ecuador and the University of Minnesota.

Measures

Children’s parents and other adults were interviewed at home to obtain information on socio-economic status, demographics, health, and direct and indirect pesticide exposures of household members.

Children were examined in seven centrally located schools distributed in all parishes of Pedro Moncayo Canton during the summer months when school was out of session to ensure a quiet, familiar and child-friendly environment that was easily accessible to all families.

All examiners were unaware of the exposure status of participants. Children's height was measured using a height board following recommended procedures by the World Health Organization (World Health Organization, 2008). Erythrocytic AChE and hemoglobin concentrations were measured using the EQM Test-mate ChE Cholinesterase Test System 400 (EQM AChE Erythrocyte Cholinesterase Assay Kit 470) from a single finger stick blood sample. Blood samples were analyzed immediately at ambient temperatures within the recommended range (EQM Research, 2003). Erythrocytic AChE activity is similar to that of neuronal AChE (Aaron CK, 2007); therefore, it is a better indicator than butyrylcholinesterase of nervous system AChE. We previously found that AChE activity from a single measure can be a valid indicator of pesticide exposure in epidemiological studies of children (Suarez-Lopez et al., 2012). AChE activity change from an "unexposed" baseline was not calculated because 1) true unexposed values may not exist given that the vast majority of children in this study could have been exposed to pesticides in the environment since birth or even prenatally given that they have always lived in agricultural communities and 40% were born into families where a household member was a flower plantation worker.

Neurobehavioral development was assessed using the NEPSY-II test (Korkman et al., 2007a) by trained examiners. We conducted the General Assessment Battery of examinations which included age-appropriate sub-tests in 5 domains: Attention/Executive Functioning (subtests: Auditory Attention and Response set, Inhibition, Statue), Language (Comprehension of instructions, Speeded Naming), Memory and Learning (Memory for Faces (immediate and delayed), Narrative Memory, Word List Interference), Sensorimotor (Visuomotor Precision) and Visuospatial Processing (Design Copying, Geometric Puzzles). Descriptions of each subtest can be found in Supplementary Table #1 and additional information can be found elsewhere (Kemp and Korkman, 2010; Korkman et al., 2007c). Most children completed the general assessment battery within 50 to 80 minutes. This range is wide given that the NEPSY-II assesses fewer subtests or fewer items within a subtest among children in younger age groups. Table lists the age ranges that each subtest was designed to test.

The NEPSY-II test was translated into Spanish, adapting it to the culture and terminology of the population of Pedro Moncayo canton. The NEPSY test has been successfully translated and adapted into other cultures and settings, and its results have been found to be relatively unaffected by language (including examinee bilingualism) and culture (Garratt and Kelly, 2008; Kofman et al., 2006; Mulenga et al., 2001).

Each participant was examined in a quiet, well ventilated and well lit room, sitting in chairs/desks appropriate for their size. Participants were examined alone except in cases where the child experienced separation anxiety from their parents. In such cases (5 participants) one relative was allowed to be in the examination room and was instructed to remain silent and to sit between 2 and 4 meters away and outside of the line of sight of the child.

The distance of homes to the nearest flower plantation was calculated using ArcGIS 9.3 (Esri, Redlands, CA) from geographical coordinates obtained from portable global positioning system (GPS) receivers. Additional details regarding the geographical positioning data collection and calculations have been described elsewhere (Suarez-Lopez et al., 2012).

Statistical Analysis

Participant characteristics were calculated for all participants and for each AChE tertile. Because we measured erythrocytic AChE, participant characteristics by AChE tertiles were adjusted for hemoglobin concentration using linear regression.

The association between flower worker cohabitation and AChE with neurobehavioral development was analyzed using linear regression and logistic regression (dichotomous and polychotomous) using SAS Version 9.2 (SAS Institute Inc., Cary, NC). We analyzed interaction of gender with AChE based on previous reports in rats and humans of differential neurochemical and behavioral effects of pre/post- natal exposures of OP pesticides by gender (Dam et al., 2000; Levin et al., 2001; Marks et al., 2010; Slotkin et al., 2002) .

The NEPSY II scoring assistant software (NCS Pearson Inc., San Antonio, TX) was used to calculate primary scaled scores for each sub-test. The primary scaled scores are age-adjusted values based on a national normative sample of US children (Korkman et al., 2007c). To assess the associations of exposure on a conglomerate of related subtests, we calculated domain scores which were the average of primary scaled scores for each subtest within a domain. In subtests with more than one primary scaled score, an average subtest score was created from the available primary scaled scores. These average subtest scores were only used for the calculation of the domain score. For subtests that included time and error components (i.e. Inhibition, Speeded Naming, Visuomotor Precision) and correct and error components (i.e. Auditory Attention and Response Set), we used the combined scaled scores provided by the NEPSY II as primary scaled scores. In subtests that included more than one subtest component and provided more than one primary score (i.e. Auditory Attention and Response set, Inhibition and Word list interference), each component primary score was analyzed separately (i.e. auditory attention (AARS: auditory attention), response set (AARS: response set), inhibition naming trial (IN: naming trial), inhibition inhibition trial (IN: inhibition trial), inhibition switching trial (IN: switching trial), word list interference repetition (WI: repetition), word list interference recall (WI:

recall)). We excluded the IN: naming trial from the analyses because this part of the test only assesses children's ability to name a set of figures and does not by itself reflect abilities on inhibition. We did not calculate a score for Sensorimotor domain given that the domain comprised only one subtest (Visuomotor Precision).

Subtest or domain scores lower than 6 were considered "low" and corresponded to scores below the 9th percentile of the NEPSY II normative sample(Korkman et al., 2007c).

The associations between AChE and the various neurobehavioral development measures were adjusted for hemoglobin concentration, age, gender, race, height-for-age z-score, household income, flower worker cohabitation status and maternal education. Level of parental education is a proxy for socio-economic status and may be directly associated with neurobehavioral development (Brooks et al., 2010a). Flower worker cohabitation can be an indicator of pesticide exposure beyond cholinesterase inhibitors and could be associated with neurobehavior. The associations of flower worker cohabitation with neurobehavioral development were additionally adjusted for residential distance to the nearest flower plantation. In this population, we previously found that AChE activity was related to flower worker cohabitation and to proximity of homes to the nearest flower plantation (Suarez-Lopez et al., 2012).

Results

Participants had a mean age and standard deviation (SD) of 6.6 years and 1.6 years, respectively; 51% were male, 75% mestizo and 56% lived with a flower worker. Participant's characteristics are listed in Table 1. The overall means of AChE activity and hemoglobin concentration were 3.14 U/ml (SD=0.49) and 12.62 mg/dl (SD=1.16), respectively. AChE had crude direct associations with age, hemoglobin concentration and with NEPSY II's Design Copying subtest (Table 1), and borderline associations with flower worker cohabitation and WI: repetition.

Overall, the NEPSY-2 scores in our sample were lower but with similar variability than those of the test's normative sample which has a mean of 10 (SD=3) for each subtest (Table 2). Only 4 of 12 subtests had equivalent or higher mean scores than the normative sample: Statue, Narrative Memory, Design Copying and Visuomotor Precision.

Acetylcholinesterase and Neurobehavioral Development

AChE was associated primarily with subtests within the Attention/Executive Functioning (AEF) domain. In linear regression analyses, AChE had a borderline significant cubic association with AARS: auditory attention after adjustment (formula: 43.4*AChE-14.8*AChE2+1.6*AChE3, Table 2, Figure 1). There is an apparent threshold effect at approximately 3.5 U/ml of AChE activity. AChE had a positive adjusted association with IN: inhibition trial. Every unit decrease (≈2 SD) in AChE was associated with a scaled score decrease of 1.16 (95% CL: -2.27, -0.05, Table). There was a significant interaction of AChE with gender ($p<0.01$) in the association with IN: inhibition trial. This association was only present among boys, with a decrease in IN: inhibition trial score of 2.33 (95% CL: -3.95, -0.71) per unit decrease of AChE. There was also an interaction of AChE with gender on the association with AEF domain ($p<0.01$); boys had a stronger association than girls, although the association was non-significant.

AARS: Response set and WI: Repetition had similar association strengths as the IN: inhibition trial (score decrease of 1.20 and 1.34 per unit decrease of AChE, respectively, Table); however, these associations had wide, non-significant confidence intervals which may be a consequence of low statistical power reflected by the substantially lower number of children that we were able to examine using these tests (tests were designed to examine children of ages 7 years and older).

In logistic regression analyses, every SD decrease of AChE was associated with an odds ratio of 1.84 (95% CL 1.16, 2.92) of having a low AEF domain score (Table 2); males had a stronger association (OR: 2.94, 95% CL: 1.56, 5.56) than females (OR: 0.82, 95% CL: 0.36, 1.86). Similarly, when AChE was analyzed as tertiles the OR for low AEF domain score was strongest and significant only among males: (lowest AChE tertile (vs. highest) = OR: 7.40, 95% CL: 1.71, 32.05, middle AChE tertile (vs. highest) = OR: 1.72, 95% CL: 0.43, 6.90).

Compared to AEF Domain, SD decreases of AChE had minimally weaker but non-significant associations with both low AARS: response set (OR: 1.59, 95% CL: 0.86- 2.94, N: 116) and low IN: inhibition trial (OR: 1.41, 95% CL: 0.95-2.10, N: 217, Table 3). When analyzed as tertiles, AChE showed graded and significant associations with low values in both of these subtests. AChE in the lowest and middle tertiles (vs. the highest) were associated with: 1) OR of 4.73 (95% CL: 1.12, 20.07) and 1.40 (95% CL: 0.38, 5.18), respectively, for low AARS: response set, and 2) OR of 3.11 (95% CL: 1.28, 7.55) and 2.30 (95% CL: 1.03, 5.13), respectively, for low IN: inhibition trial scores. The association of AChE with low IN: inhibition trial was much stronger among males (AChE lowest tertile (vs. highest)= OR: 9.39, 95% CL: 2.36, 37.38; AChE middle tertile (vs. highest)= OR: 7.05, 95% CL: 2.11, 23.56) compared to females males (AChE lowest tertile (vs. highest)= OR: 1.34, 95% CL: 0.37, 5.0; AChE middle tertile (vs. highest)= OR: 0.74, 95% CL: 0.21, 2.61, Table 3).

Within the Memory and Learning Domain, the OR for low memory for faces delayed score was 1.35 (95% CL: 0.94, 1.95) per SD decrease of AChE. AChE tertile analyses yielded OR for low memory for faces delayed score of 2.35 (95% CL: 1.03, 5.34) and 2.10 (95% CL: 0.99, 4.46) for AChE in the lowest and middle tertiles (vs. the highest), respectively. AChE was not associated any other neurobehavioral development subtest. AChE was not associated with any other neurobehavioral development subtest.

Flower Worker Cohabitation and Neurobehavioral Development

Cohabitation with a flower worker was associated with an AARS: auditory attention score decrease of 1.01 (95% CI: -1.93, -0.08) after adjustment + residence distance to the nearest flower plantation. Every year of cohabitation was associated with an adjusted decrease of AARS: auditory attention score of 0.13 (95% CI: -0.28, 0.01). Flower worker cohabitation was associated with OR of low AARS: auditory attention of 1.63 (95% CI: 0.79, 3.37). Flower worker cohabitation was not associated with any other measure of neurobehavioral development.

Discussion

Lower AChE activity was associated with lower neurobehavioral development scores in tests assessing attention, inhibitory control and long term memory. The observed adverse effects of lower AChE on attention and inhibitory control were much stronger and only significant among males. Our findings support the concept that the cholinergic system plays an important role in brain development (Dori et al., 2005; Hohmann, 2003) and that disruptions via AChE inhibition is an important mechanism of neurotoxicity of OP pesticides.

We found greater associations of AChE inhibition with subtests within the Attention and Executive Functioning domain: a) the AARS: response set subtest assessed complex attention and inhibition of a previously learned stimuli in lieu of new stimuli; b) the Inhibition subtest also assessed inhibitory control, which is the ability to restrain an urge to engage in an inviting or automatic behavior, (e.g. the ability to resist playing outdoors with friends during homework time). Diminished executive functioning or inhibitory control may affect the child's capacity to plan and concentrate resulting in poor school performance (Korkman et al., 2007c). Impaired inhibitory control has been described as the primary deficit in ADHD (Barkley, 2003; Wodka et al., 2007), and such children in addition to children with mathematics disorder, language disorder and emotionally disturbed performed significantly worse than controls on the inhibition test of the NEPSY II (Korkman et al., 2007c). Additionally, children with ADHD performed lower on AARS, with greater difficulties in AARS: response set.

The stronger negative impact on neurobehavior that we observed in boys was also seen in a study of Mexican American children where boys with higher prenatal measures of OP metabolites had poorer attention scores than females (Marks et al., 2010). Studies in rats found that early postnatal exposure to the OP chlorpyrifos was associated with greater detrimental neurochemical and behavioral effects among males compared to females (Dam et al., 2000; Levin et al., 2001). The reasons for these gender differences are not yet understood but may be related to different brain development patterns affecting attention and inhibitory control by gender, as seen in a meta-analysis of children with ADHD (Hutchinson et al., 2008).

Previously, in this study population we found that the amounts of pesticides introduced into their homes by flower workers was sufficient to provoke decreases in AChE in children. In addition to cholinesterase inhibitors, flower workers likely introduce a variety of pesticides home for many years. Although there may be a degree of residual confounding in the inverse association that we found between flower worker cohabitation and AARS: auditory attention, this finding provides some insight on developmental disturbances from take-home pesticides by flower workers. A limitation of our study is the lack of pesticide metabolite measures. However, by analyzing OP pesticide metabolites without taking into consideration factors that affect OP pesticide metabolism (e.g. paraoxonase (PON) activity) we cannot obtain precise associations of pesticide exposure with neurotoxicity. Additionally, urinary metabolite levels may overestimate exposures because metabolite elevations may not only reflect exposure to OP pesticides, but also to less toxic ambient metabolites (Lu et al., 2005). AChE activity works relatively well in this context given that its inhibition is a physiologic response to the amount of pesticide exposure in relation to the individual's sensitivity and ability to metabolize OP pesticides. Additionally, when analyzing urinary metabolites of OP there are substantial analytical issues associated with larger daily variability within children compared to the variability between children (Griffith et al., 2011). Finally, AChE can reflect longer-term exposures than pesticide metabolite measures and may be superior when evaluating toxicity of carbamates and OP pesticides (Lotti, 1991).

AChE measurements in our study can reflect a year's average exposure to pesticides because AChE has a long recovery time (approximately 82 days after irreversible inhibition) (Mason, 2000), the sources of secondary pesticide exposures remain relatively constant (e.g. distance of homes to agricultural farms, consumption of pesticide exposed produce, cohabitation with an agricultural worker), and agricultural production and pesticide use uninterrupted throughout the year because of Ecuador's minimal seasonal temperature changes. The results of this cross-sectional study suggest that performance on tests assessing attention, inhibitory control and long term memory is affected by AChE activity. However, we cannot differentiate whether concurrent AChE activity or long term AChE activity (likely correlated with concurrent AChE activity) affected neurobehavior. The growing scientific literature leads us to believe that chronic pesticide exposure can have long lasting neurobehavioral delays.

Neurobehavioral development scores in this study population were lower than those of the normative sample of the NEPSY-II with the exception of the following subtests: Statue, Narrative Memory, Design Copying and Visuomotor Precision.

Potential explanations for this are less frequent standardized test taking exposures in Pedro Moncayo children which could decrease performance on these types of tests, and different cultural and social practices which influence perceptions and test-taking performance (Dalen et al., 2007). It is possible that a part of some of these subtest score differences can be the result of greater environmental pollutant exposures affecting the population of children living in agricultural communities as a whole.

Conclusions

In conclusion, lower AChE activity, reflecting OP and carbamate pesticide exposure, was associated with lower performance on assessments of attention, inhibitory control and long term memory in boys. These are critical cognitive skills that affect learning and academic performance. Our findings suggest that boys have greater sensitivity for neurobehavioral development delays from indirect, low-dose pesticide exposures. This is an important public health problem, particularly, in agricultural communities.

Tables

Table 1. Participant children's characteristics by acetylcholinesterase (AChE) activity, adjusted for hemoglobin concentration.

	All[a]	AChE Tertiles 1st	2nd	3rd	P-Trend
N	288	99	92	97	
AChE Range	1.44-4.69	1.44-2.93	2.94-3.32	3.33-4.69	
Demographic and SES Characteristics					
Age, years	6.6 (1.6)	6.4 (1.6)	6.4 (1.5)	7.0 (1.7)	0.001
Gender, male	51%	48%	49%	57%	0.324
Race, mestizo	75%	74%	76%	76%	0.873
Race, indigenous	23%	22%	23%	23%	0.941
Monthly income[b]	3.1 (0.8)	3.1 (0.9)	3.1 (0.8)	3.1 (0.9)	0.782
Maternal Education, years	7.4 (3.9)	6.9 (4.1)	7.5 (3.9)	7.7 (4.3)	0.310
Flower worker cohabitation	56%	68%	53%	47%	0.073
Duration of cohabitation, years	5.2 (1.9)	5.5 (2.0)	4.8 (1.9)	5.2 (2.1)	0.111
Anthropometric and Blood Measurements					
Height-for-age Z-score	-1.3 (1.0)	-1.3 (1.0)	-1.3 (1.0)	-1.2 (1.1)	0.747
Acetylcholinesterase, U/ml[a]	3.1 (0.5)	2.6 (0.3)	3.1 (0.1)	3.7 (0.3)	-
Hemoglobin, mg/dl[a]	12.6 (1.2)	12.0 (0.9)	12.5 (0.8)	13.4 (1.2)	<0.001
Neurobehavioral Development[c]					
Attention and Executive Functioning Domain (N=280)	8.6 (2.5)	8.6 (2.6)	8.7 (2.5)	8.3 (2.7)	0.505
AARS: Auditory Attention (N=223)	8.2 (3.5)	8.2 (3.7)	8.5 (3.5)	8.0 (3.8)	0.768
AARS: Response Set (N=116)	8.7 (3.1)	7.8 (3.5)	8.8 (3.2)	9.0 (3.5)	0.102
IN: Naming Trial (N=224)	7.0 (3.7)	7.0 (3.9)	6.9 (3.7)	7.1 (4.0)	0.786
IN: Inhibition Trial (N=217)	7.0 (3.1)	6.4 (3.3)	6.7 (3.1)	7.8 (3.3)	0.095
IN: Switching Trial (N=91)	7.2 (2.6)	6.8 (3.0)	7.1 (2.7)	7.4 (2.9)	0.267
Statue (N=158)	10.4 (2.8)	10.4 (2.9)	10.4 (2.8)	10.2 (3.0)	0.735
Memory and Learning Domain (N=283)	8.8 (2.1)	8.5 (2.2)	8.9 (2.1)	8.7 (2.3)	0.676

Memory for Faces (immediate) (N=231)	7.5 (2.8)	7.2 (3.0)	7.9 (2.9)	7.5 (3.1)	0.475
Memory for Faces (delayed) (N=229)	8.6 (2.9)	8.4 (3.1)	8.3 (3.0)	9.1 (3.2)	0.195
Narrative Memory (N=283)	9.7 (2.7)	9.5 (2.9)	9.9 (2.7)	9.7 (3.0)	0.621
WI: Repetition (N=117)	7.3 (3.4)	5.8 (3.6)	7.9 (3.4)	7.7 (3.6)	0.079
WI: Recall (N=117)	7.3 (3.4)	7.5 (3.8)	7.9 (3.5)	6.9 (3.8)	0.272
Visuospatial Processing Domain (N=286)	9.6 (3.1)	9.2 (3.3)	9.7 (3.0)	9.7 (3.4)	0.167
Design Copying (N=276)	10.6 (4.9)	9.9 (5.2)	10.5 (4.8)	11.5 (5.4)	0.040
Geometric Puzzles (N=285)	8.5 (3.2)	8.5 (3.5)	9.1 (3.2)	8.1 (3.6)	0.780
Language Domain (N=285)	6.6 (2.4)	6.5 (2.6)	6.8 (2.5)	6.8 (2.7)	0.122
Comprehension of Instructions (N=285)	7.3 (2.9)	7.1 (3.2)	7.6 (3.0)	7.3 (3.3)	0.211
Speeded Naming (N=225)	6.0 (3.0)	5.8 (3.2)	5.7 (3.1)	6.3 (3.3)	0.148
Sensorimotor Domain (N=282)	-	-	-	-	-
Visuomotor Precision (N=282)	9.9 (3.3)	10.3 (3.6)	9.4 (3.3)	10 (3.7)	0.790

Table entries are percentage, mean (SD) or median (25^{th}-75^{th} percentile)

[a] Not adjusted for hemoglobin concentration.

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

[c] Subtest sample size varies according to the age range it was designed to test. See Table for additional detail.

Subtest Abbreviations:

AARS: Auditory Attention and Response Set.

IN: Inhibition.

WI: Word List Interference.

Table 2. Adjusted[a] linear differences of neurobehavioral development scores per unit (≈2 SD) decrease of AChE activity.

N	Ages	Neurobehavioral Development Score	Score Difference (95% CL)
280	5-9	**Attention and Executive Functioning Domain[b]**	-0.34 (-1.05, 0.38)
		Males, N=144	-0.95 (-1.97, 0.06)
		Females, N=136	0.48 (-0.54, 1.49)
223	5-9	AARS: Auditory Attention	-0.22 (-1.41, 0.96)
		Cubic association formula[c]: $43.4*AChE-14.8*AChE^2+1.6*AChE^3$	
116	7-9	AARS: Response Set	-1.20 (-2.71, 0.30)
217	5-9	IN: Inhibition Trial[b]	**-1.16 (-2.27, -0.05)**
		Males, N=115	**-2.33 (-3.95, -0.71)**
		Females, N=102	-0.16 (-1.71, 1.39)
91	7-9	IN: Switching Trial	-0.78 (-2.26, 0.70)
158	4-6	Statue	0.34 (-0.84, 1.53)
283	4-9	**Memory and Learning Domain**	-0.28 (-0.92, 0.36)
231	5-9	Memory for Faces Immediate	-0.38 (-1.36, 0.59)
229	5-9	Memory for Faces Delayed[d]	-0.57 (-1.62, 0.48)
		Males, N= 121	-0.42 (-1.92, 1.08)
		Females, N-108	-0.70 (-2.26, 0.87)
283	4-9	Narrative Memory	-0.20 (-1.03, 0.63)
117	7-9	WI: Repetition	-1.34 (-2.99, 0.32)
117	7-9	WI: Recall	1.06 (-0.60, 2.72)
286	4-9	**Visuospatial Processing Domain**	-0.46 (-1.40, 0.48)

276	4-9	Design Copying	-0.94 (-2.45, 0.57)
285	4-9	Geometric Puzzles	-0.12 (-1.11, 0.87)
285	4-9	**Language Domain**	-0.44 (-1.18, 0.29)
285	4-9	Comprehension of Instructions	-0.20 (-1.08, 0.68)
225	5-9	Speeded Naming	-0.79 (-1.85, 0.28)
		Sensorimotor Domain	-
282	4-9	Visuomotor Precision	0.34 (-0.71, 1.39)

[a] Adjustments: age, gender, race, height-for-age z-score, income, maternal education, flower worker cohabitation status and hemoglobin concentration.

[b] AChE-gender interaction: p<0.01.

[c] P-cubic= 0.05.

[d] AChE-gender interaction: p<0.54.

Abbreviations:

AARS: Auditory Attention and Response Set subtest.

IN: Inhibition subtest.

WI: Word List Interference subtest.

Table 3. Adjusted[a] associations between acetylcholinesterase (AChE) and low neurobehavioral development scores.

N	Ages	Low[b] Score (Dependent Variable)	AChE (Independent Variable) Continuous OR (95% CL) per SD decrease	Tertiles (vs. Highest) OR (95% CL) Lowest	OR (95% CL) Middle
280	4-9	**Attention and Executive Functioning Domain**	**1.84 (1.16, 2.92)**	2.20 (0.80, 6.06)	0.95 (0.35, 2.60)
		Males, N=144	**2.94 (1.56, 5.56)**	**7.40 (1.71, 32.05)**	1.72 (0.43, 6.90)
		Females, N=136	0.82 (0.36, 1.86)	0.40 (0.07, 2.27)	0.49 (0.10, 2.35)
223	5-9	AARS: Auditory Attention	1.10 (0.72, 1.68)	0.84 (0.33, 2.16)	0.70 (0.29, 1.73)
116	7-9	AARS: Response Set	**1.59 (0.86, 2.94)**	**4.73 (1.12, 20.07)**	**1.40 (0.38, 5.18)**
217	5-9	IN: Inhibition Trial	**1.41 (0.95, 2.10)**	**3.11 (1.28, 7.55)**	**2.30 (1.03, 5.13)**
		Males, N=115	**1.81 (1.05, 3.09)**	**9.39 (2.36, 37.38)**	**7.05 (2.11, 23.56)**
		Females, N=102	1.19 (0.62, 2.30)	1.34 (0.36, 5.000	0.74 (0.21, 2.61)
91	7-9	IN: Switching Trial	0.86 (0.41, 1.79)	0.52 (0.09, 2.88)	0.45 (0.11, 1.91)
158	4-6	Statue	1.14 (0.53, 2.47)	0.22 (0.03, 1.89)	0.12 (0.01, 1.24)
283	4-9	**Memory and Learning Domain**	1.26 (0.71, 2.23)	1.96 (0.58, 6.69)	0.97 (0.27, 3.45)
231	5-9	Memory for Faces Immediate	1.28 (0.85, 1.93)	1.91 (0.77, 4.78)	1.40 (0.61, 3.23)
229	5-9	Memory for Faces Delayed	**1.35 (0.94, 1.95)**	**2.35 (1.03, 5.34)**	**2.10 (0.99, 4.46)**
		Males, N= 121	**1.69 (1.00, 2.85)**	**3.09 (0.93, 10.27)**	**3.15 (1.11, 8.98)**
		Females, N-108	1.12 (0.65, 1.94)	1.76 (0.54, 5.80)	1.21 (0.38, 3.90)
283	4-9	Narrative Memory	1.00 (0.65, 1.53)	1.18 (0.47, 2.96)	0.90 (0.38, 2.14)
117	7-9	WI: Repetition	1.35 (0.78, 2.34)	1.74 (0.50, 6.03)	0.53 (0.16, 1.76)
117	7-9	WI: Recall	0.74 (0.40, 1.37)	0.57 (0.14, 2.24)	0.42 (0.12, 1.46)

286	4-9	**Visuospatial Processing Domain**	0.77 (0.49, 1.20)	0.48 (0.19, 1.25)	0.56 (0.23, 1.35)
276	4-9	Design Copy	1.00 (0.68, 1.47)	0.90 (0.37, 2.16)	1.24 (0.57, 2.73)
285	4-9	Geometric Puzzles	0.82 (0.57, 1.18)	0.59 (0.27, 1.30)	0.53 (0.25, 1.12)
285	4-9	**Language Domain**	1.09 (0.73, 1.62)	0.96 (0.40, 2.28)	0.87 (0.38, 1.98)
285	4-9	Comprehension of Instructions	1.11 (0.81, 1.54)	0.99 (0.48, 2.04)	0.74 (0.38, 1.44)
226	5-9	Speeded Naming	1.27 (0.84, 1.91)	1.22 (0.50, 2.98)	1.04 (0.46, 2.34)
282	4-9	**Sensorimotor Domain**	-	-	-
282	4-9	Visuomotor Precision	0.96 (0.67, 1.39)	0.64 (0.28, 1.47)	1.24 (0.59, 2.61)

[a] Adjustments: age, gender, race, height-for-age z-score, income, maternal education, flower worker cohabitation status and hemoglobin concentration.

[b] Low: Scaled scores <6 (<9th percentile of the NEPSY II normative sample).

Abbreviations:

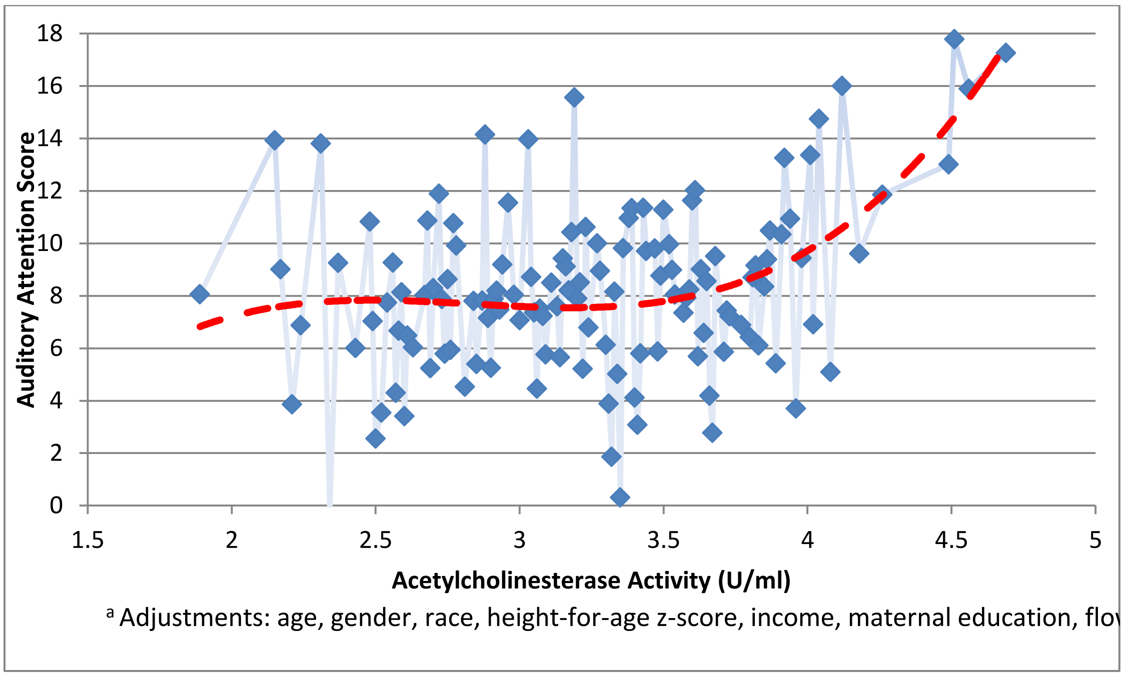
AARS: Auditory Attention and Response Set subtest.

IN: Inhibition subtest.

WI: Word List Interference subtest.

Figures

Figure 1. Adjusted[a] Association between Acetylcholinesterase Activity and Auditory Attention



Supplementary Material

Supplementary Table #1. Description of NEPSY-II subtests.

Subtest	Description	Concepts measured
Attention and Executive Functioning Domain		
Auditory Attention and Response Set		
AARS: Auditory Attention	Children are asked to touch a red circle when they hear the word "red" amidst consistent distracter words.	Selective and sustained auditory attention
AARS: Response Set	Children are asked to touch a red circle when they hear the word yellow and vice versa, but when they hear the word blue, they are asked to touch the color blue.	Complex attention and inhibition of a previously learned stimuli in lieu of new stimuli.
Inhibition		
IN: Naming	Naming of shapes (circles or squares) or direction of arrows (up or down).	Naming speed
IN: Inhibition	Naming the opposite shapes or direction of arrow (e.g. saying circle when seeing a square).	Inhibitory control
IN: Switching	Naming depends on color. White figures are named the correct shape name or arrow direction. Black figures are called the opposite shape name or opposite arrow's direction.	Inhibitory control and cognitive flexibility
Statue	Child must remain immobile with eyes closed for 75 seconds without responding to sound distracters.	Inhibitory control and motor persistence
Memory and Learning Domain		
Memory for Faces Immediate	Child looks at a set of pictures of faces. Immediately afterwards, the child is asked to select a previously seen face during various trials that include 3 faces (2 of which have not been shown previously).	Visual memory, discrimination and encoding of faces

Memory for Faces Delayed	Trials of face recall occur 15-20 minutes after the initial set of pictures of faces was shown.	Long term visual memory and recognition of faces
Narrative Memory	Child hears a short story and is asked to recall it. Child is then asked questions regarding missing components of the recalled story.	Memory, comprehension, expressive language
Word List Interference		
WI: Repetition	Child listens to two sets of words (2-5 words) and is asked to immediately repeat each set after it has been read to him.	Memory, receptive and expressive language
WI: Recall	Child listens to two sets of words (2-5 words) and is asked to recall both sets after repetition of the sets.	Memory, word recall after interference
Visuospatial Processing Domain		
Design Copying	Copying of a set of two-dimensional figures.	Visuospatial and visuomotor ability
Geometric Puzzles	Matching of figures outside a grid to those inside a grid.	Mental rotation, visuospatial analysis, attention to detail
Language Domain		
Comprehension of Instructions	Child must follow verbal instructions of increasing complexity.	Receptive language, semantic and sequential processing
Speeded Naming	Child names shapes, size and color or letters/numbers as quickly as possible from a list.	Processing speed, lexical access, expressive language
Sensorimotor Domain		
Visuomotor Precision	Child quickly draws a line inside of tracks of increasing complexity.	Graphomotor skills, precision, visuomotor coordination

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5. CHAPTER 5: ACETYLCHOLINESTERASE ACTIVITY IS RELATED TO BLOOD PRESSURE IN CHILDREN INDIRECTLY EXPOSED TO PESTICIDES

Summary

BACKGROUND: Acetylcholinesterase (AChE) inhibitors are commonly used pesticides that can effect hemodynamic changes through increased cholinergic stimulation. Children of agricultural workers are likely to have para-occupational exposures to pesticides, but the extent of physiologic impact of such exposures is unclear. We tested the hypothesis that secondary pesticide exposure decreases blood pressure and heart rate among children aged 4-9y who lived in agricultural (primarily floricultural) Ecuadorian communities.

METHODS: The Secondary Exposure to Pesticides among Infants, Children and Adolescents (ESPINA) study examined 275 children, 51% cohabited with ≥1 flower plantation worker (mean duration= 5.2y) and had a mean AChE activity of 3.14 U/ml (standard deviation: 0.49) measured by the EQM Testmate system.

RESULTS: Every U/ml decrease of AChE activity was associated with mean decreases in systolic blood pressure (SBP) of 1.54 mmHg (95%CI -2.68, -0.40) and diastolic blood pressure (DBP) of 1.52 mmHg (95%CI -2.55, -0.50), after adjustment for age, gender, race, height-for-age, heart rate, hemoglobin, income, home distance to nearest flower plantation, pesticide use within household, pesticide use by neighbors and examination date. Flower worker cohabitation was associated with lower SBP by 1.79 mmHg (95%CI -3.57, -0.01). Every year of cohabitation was associated with a decrease in SBP of 0.32 mmHg (95%CI 0.02, 0.64).

CONCLUSIONS: Our findings suggest vascular reactivity in children with subclinical secondary exposures to pesticides. Although lower blood pressure in isolation might be a beneficial effect, the finding needs to be viewed with caution as part of a pattern of generally adverse physiologic responses to pesticides.

Introduction

Cholinesterase inhibitors such as organophosphate and carbamate insecticides are commonly used pesticides in agriculture worldwide which can increase parasympathetic activity via inhibition of acetylcholinesterase (AChE) activity. Alterations in the cholinergic system can have varying effects on blood pressure regulation depending on whether nicotinic or muscarinic stimulation by acetylcholine predominates. Acetylcholine can lower blood pressure via vasodilation through nitric oxide, cyclooxygenase and endothelium derived hyperpolarizing factor pathways (de Wit et al., 1999; Hatoum et al., 2003a; Hatoum et al., 2003b; Leung et al., 2006) through the stimulation of muscarinic M3 receptors in vascular endothelium (Beny et al., 2008; Eltze et al., 1993; Gericke et al., 2011; Lamping et al., 2004), or it can increase blood pressure by stimulating nicotinic receptors in the sympathetic system (Claassen et al., 2009). Treatment with AChE inhibitors in older adults with neurogenic orthostatic hypotension has been found to improve orthostatic blood pressure presumably by stimulating adrenergic release through stimulation of nicotinic receptors in an environment of autonomic insufficiency (Gales and Gales, 2007; Singer et al., 2003). However, in the absence of autonomic insufficiency (the situation that is typical in children), it is unclear whether exposure to cholinesterase inhibitors can affect blood pressure.

Children of agricultural workers have a higher risk of pesticide contamination from secondary routes which include pesticide drift due to workers' residential proximity to plantations and take-home pesticide exposures by workers. (Coronado et al., 2011; Curl et al., 2002; Fenske et al., 2002; Gladen et al., 1998; Lambert et al., 2005; Lu et al., 2000; McCauley et al., 2001; Simcox et al., 1995; Suarez-Lopez et al., 2012). These secondary pesticide exposures are small enough to not elicit overt clinical manifestations and may remain unchanged throughout the duration of the child's cohabitation with the agricultural worker.

Assuming that in children the muscarinic response to AChE predominates, we tested the hypothesis that lower AChE activity from secondary pesticide exposure and cohabitation with a flower plantation worker are associated with lower blood pressure and heart rate among children living in agricultural communities in Ecuador where there is an active fresh-cut flower industry activity. The floricultural industry in Ecuador regularly uses organophosphate cholinesterase inhibitor insecticides (Grandjean et al., 2006; Harari, 2004), as well as carbamate and pyrethroid insecticides and fungicides.

Methods

Study Description

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) is a study of children living in Pedro Moncayo County, Pichincha, Ecuador. This county that has substantial floricultural activity with an approximated production area of 1800 hectares (5.3% of the Canton's surface area) (Gobierno-Municipal-del-Canton-Pedro-Moncayo, 2011) and employs approximately 21% of adults (Suarez-Lopez et al., 2012).

In 2008, the ESPINA study examined 313 children between 4 and 9 years of age which included a subset of children who participated in the 2004 Survey of Access and Demand of Health Services in Pedro Moncayo County (73%), conducted by Fundacion Cimas del Ecuador, and new volunteers (27%). We conducted in-person home interviews of 451 adults who lived with the examined children. Additional participant recruitment information has been described elsewhere (Suarez-Lopez et al., 2012). For these cross-sectional analyses, we included 275 (88%) children who had all covariates of interest.

Informed consent, parental authorization of child participation and child assent of participants 7 years of age and older was obtained. This study was approved by the Institutional Review Boards of Fundacion Cimas del Ecuador and the University of Minnesota.

Measures

In-person home interviews of children's parents and other adults obtained information on SES, demographics, health, and direct and indirect pesticide exposures of household members. Children's height was measured using a height board, following recommendations by the World Health Organization (World Health Organization, 2008). Resting heart rate was measured by a 30-second auscultation, prior to blood pressure measurement. Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured with a pediatric Omron aneroid sphygmomanometer, appropriate for the arm size of the children. Blood pressure measurements followed the recommendations of the American Heart Association (Pickering et al., 2005). Measurements were taken after 3-5 minutes of rest. Children were in the seated position with the antecubital fossa supported at heart level, with uncrossed legs and both feet on the floor. Blood pressure was measured twice.

Erythrocytic AChE activity and hemoglobin concentration 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).

AChE inhibition from an unexposed baseline was not calculated due to the cross-sectional design of the study and because no adequate unexposed AChE activity could be measured given that 56% of our study population had lived with a flower plantation worker at some point in their lives and 72% of those were born into a household of flower workers and potentially even exposed to pesticides in-utero. We previously found that AChE activity from a single measure can be a valid indicator of pesticide exposure in children in epidemiological studies (Suarez-Lopez et al., 2012).

The residential distance to the nearest flower plantation was calculated using ArcGIS 9.3 (Esri, Redlands, CA) from geographical coordinates obtained from portable global positioning system (GPS) receivers. Additional details regarding the geographical positioning data collection and calculations have been described elsewhere (Suarez-Lopez et al., 2012).

Statistical Analysis

We used multiple linear regression to calculate the associations between flower worker cohabitation, duration of flower worker cohabitation and AChE activity with SBP, DBP and heart rate. SBP and DBP were divided into octiles and adjusted (least square) means were calculated for each octile using the adjusted model (below). We plotted the association of blood pressure as a function of AChE and superposed the adjusted means for each octile to obtain a visual representation of model fit.

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

All associations were adjusted for age, gender, race, height-for-age z-score, heart rate, hemoglobin concentration, income, residence distance to nearest flower plantation edge, pesticide use within household lot, pesticide use by contiguous neighbors and examination date. We examined children during the months of July and August, roughly 2-3 months after the heightened fumigations of the Mother's Day (May) surge of flower production. Because 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 associated with Mother's Day production. The proportion of examined children who lived with a flower worker in early July does not necessarily represent the distribution of the overall group, and therefore examination date may confound the association. Data were analyzed with SAS Version 9.2 (SAS Institute Inc., Cary, NC).

Results

Participant Characteristics

Children who lived with a flower worker had similar distributions of age, gender, income and number of smokers at home compared to children who lived with non-flower workers, but were composed of a greater proportion of indigenous and lived closer to flower plantations. Participant characteristics are listed in Table 1.

Blood Pressure

AChE activity was directly related with blood pressure. Every U/ml decrease in AChE activity was associated with a decrease in a SBP of 1.54 mmHg (95% CI: -2.68, -0.40) and DBP of 1.52 mmHg, 95% CI -2.55, -0.50, Table 2). Our linear models closely follow the trends of blood pressure means for each octile, thus, providing visual evidence of good model fits (Figure 1).

Concurrent cohabitation with a flower worker was associated with lower SBP (1.79 mmHg 95%CI -3.57, -0.01) after adjustment (Table 2) and every year of cohabitation was associated with an adjusted decrease of 0.32 mmHg (95%CI -0.62, -0.02). Further adjustment for AChE activity weakened these associations between SBP and cohabitation with a flower worker (-1.66 mmHg, 95% CI: -3.43, 0.11) and duration of cohabitation (-0.28 mmHg, 95% CI: -0.58, 0.03, per year of cohabitation). Cohabitation with a flower worker was not related to DBP.

Resting heart rate was not significantly associated with cohabitation with AChE activity or with flower worker cohabitation (Table 2).

Discussion

AChE was directly associated with SBP and DBP even after adjustment for resting heart rate and hemoglobin concentration, thus suggesting vascular reactivity in children with subclinical secondary pesticide exposures

Cohabitation with a flower worker and duration of cohabitation were associated with lower blood pressure. We previously found that children living with flower plantation workers (vs. non-flower workers) were exposed, para-occupationally, to sufficient amounts of cholinesterase inhibitors to decrease AChE activity

(Suarez-Lopez et al., 2012). Exposure to cholinesterase inhibitor pesticides, as reflected by lower AChE activity, was an important mechanism of lower SBP among children who lived with a flower worker, given the weakened association observed after adjusting for AChE activity. Our findings indicate that the amount of para-occupational exposures to cholinesterase inhibitors (primarily organophosphate pesticides) is sufficient to decrease blood pressure in children.

The acetylcholine excess from AChE inhibition can increase the stimulation of both nicotinic and muscarinic receptors, thus producing systemic effects. Increased muscarinic receptor stimulation has been associated with increased salivation and lacrimation, nausea/vomiting, diarrhea, bradycardia and vasodilation, whereas nicotinic receptor stimulation is mainly associated with muscular weakness/paralysis, hypertension, and tachycardia (Kwong, 2002). Our findings of lower blood pressure without changes in heart rate associated with lower AChE activity suggest that cholinesterase inhibitor pesticide exposure induces vasodilation via a predominant stimulation of muscarinic M3 receptors in the vascular endothelium in children (Beny et al., 2008; Eltze et al., 1993; Gericke et al., 2011; Lamping et al., 2004). We can infer that these pesticide exposures do not decrease the production of catecholamines as a mechanism for lowering blood pressure, to the extent that cholinesterase inhibitors have been found to improve blood pressure changes in adults with neurogenic orthostatic hypotension by enhancing adrenergic release through stimulation of nicotinic receptors(Gales and Gales, 2007; Singer et al., 2003).

Although lower BP in isolation might be construed as a beneficial effect of secondary exposures to pesticides, the finding needs to be interpreted with caution, as part of a pattern of generally adverse physiologic responses to pesticides. These findings support the hypothesis that take-home pesticide exposures have physiologic effects in children of flower plantation workers.

Tables

Table 1. Participant Characteristics

		Concurrent Cohabitation with	
	All Participants	Flower plantation worker	Non-flower plantation worker
N	275	139 (50.6%)	136 (49.4%)
Demographic and Socio-Economic Status			
Age, years	6.6 (1.6)	6.4 (1.6)	6.8 (1.6)
Gender, male	51%	50%	52%
Race			
Mestizo	76%	68%	83%
Indigenous	22%	30%	14%
Other	2%	2%	3%
Monthly income[a]	3.1 (0.8)	3.1 (0.7)	3.1 (1.0)
Number of smokers at home	0.26 (0.46)	0.25 (0.45)	0.27 (0.46)
Residence distance to nearest flower plantation, m	346 (186, 605)	331 (170, 615)	415 (208, 602)
Duration of flower worker cohabitation, years		5.2 (2.0)	0.53 (1.52)
Number of bad practices		2.9 (0.93)	0
Anthropometric and Blood Measurements			
Height, cm	112.1 (10.3)	110.2 (10.2)	114.1 (10.1)
Height-for-age, z-score	-1.26 (0.96)	-1.41 (1.03)	-1.12 (0.86)
Systolic blood pressure, mmHg	93.1 (8.3)	92.1 (7.6)	94.2 (8.9)
Diastolic blood pressure,	49.4 (7.3)	49.3 (7.6)	49.5 (6.9)

mmHg			
Acetylcholinesterase, U/ml	3.14 (0.49)	3.08 (0.50)	3.20 (0.47)
Hemoglobin, mg/dl	12.6 (1.2)	12.5 (1.3)	12.7 (1.0)

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

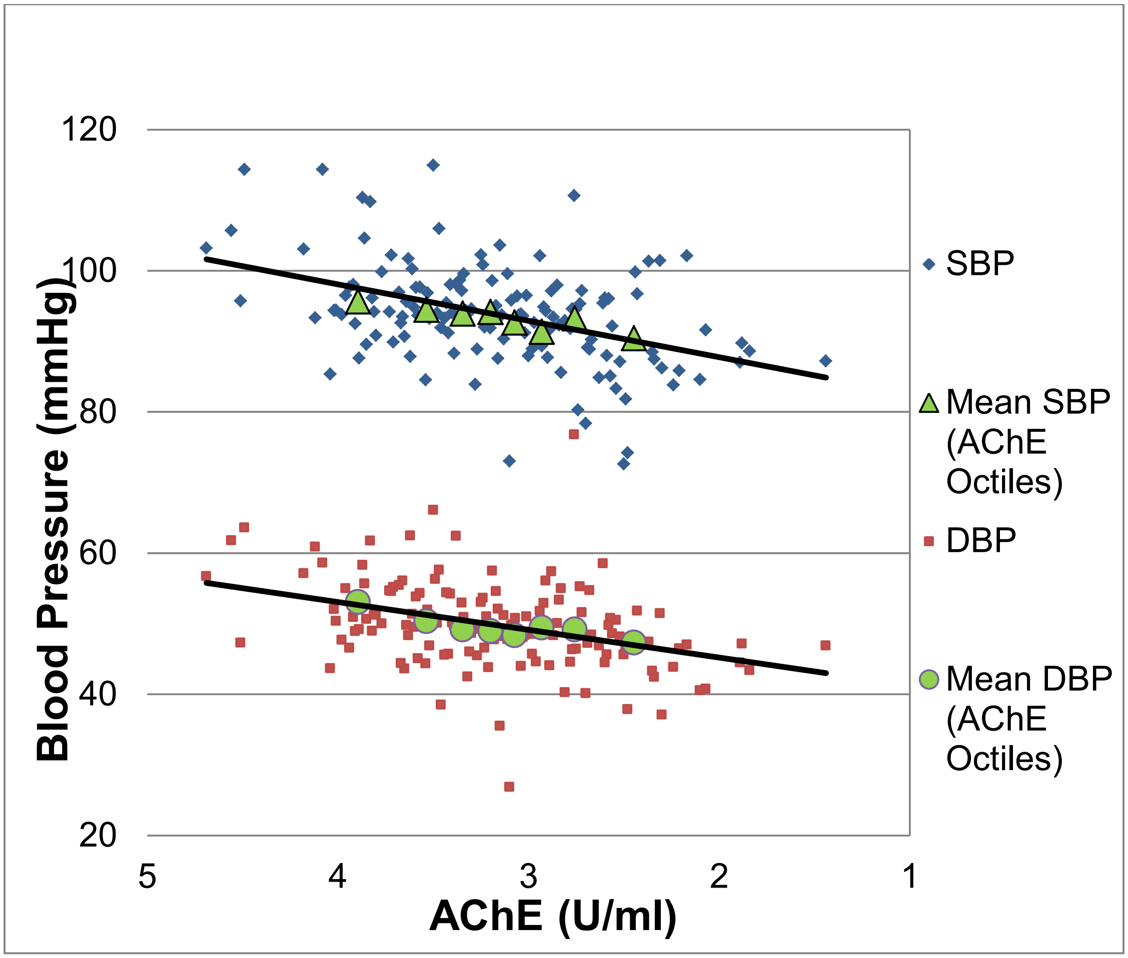
Table 2. Adjusted[a] associations between secondary pesticide exposure measures and blood pressure among children. N=275.

Predictor	**Outcomes** **Blood Pressure Difference mmHg (95% CI)**		**Heart Rate Beats per minute (95% CI)**
	Systolic	Diastolic	
AChE (per 1 U/ml decrease)	**-1.54 (-2.68, -0.40)**	**-1.52 (-2.55, -0.50)**	-2.56 (-6.32, 1.21)
Flower worker cohabitation (yes vs. no)	**-1.79 (-3.57, -0.01)**	-0.54 (-2.14, 1.07)	-1.78 (-4.66, 1.10)
Duration of cohabitation with a flower worker (per yr.)	**-0.32 (-0.62, -0.02)**	-0.12 (-0.39, 0.15)	-0.14 (-0.64, 0.35)

[a] Adjustment: age, gender, race, height-for-age z-score, heart rate, hemoglobin concentration, income, residence distance to nearest flower plantation edge, pesticide use within household lot, pesticide use by contiguous neighbors and examination date

Figures

Figure 1. Adjusted[a] associations between acetylcholinesterase (AChE) activity and blood pressure



[a] Adjustment: age, gender, race, height-for-age z-score, heart rate, income, residence distance to nearest flower plantation edge, pesticide use within household lot, pesticide use by contiguous neighbors, examination date and hemoglobin concentration.

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CHAPTER 6: SECONDARY PESTICIDE EXPOSURE IS ASSOCIATED WITH HEAD CIRCUMFERENCE AND GROWTH IN CHILDREN

Summary

BACKGROUND: Low-dose cholinesterase inhibitor pesticide exposures occur frequently, particularly in agricultural communities. The association of pesticide exposures and growth in children is not well understood; most of a few pertinent studies focused on birth outcomes. Some studies found greater head circumference with pesticide exposure. We hypothesized that acetylcholinesterase (AChE) inhibition and other surrogates of pesticide exposure are associated with decreased growth and greater head circumference in children.

METHODS: In 2004, we examined 853 children <5y who lived in agricultural (primarily floricultural) communities in Ecuador and re-examined 188 of these in 2008 in The Effects of Secondary Pesticide Exposure in Infants, Children and Adolescents (ESPINA) study. AChE activity was measured in 2008 with the EQM Testmate system.

RESULTS: Stunting prevalence was 39% and 26% in 2004 and 2008, respectively; 63% in 2004 and 55% in 2008 lived with a flower plantation worker. Cross-sectionally in 2004 (n=853), flower worker cohabitation was not associated with growth after adjusting for demographic and socio-economic factors. Longitudinally, child cohabitation with a flower worker was associated with decreased mean BMI-for-age (-0.36 standard deviations (SD), 95% CI: -0.66, -0.06) and weight-for-age (-0.33 SD, 95% CI: -0.61, -0.05). In 2008, flower worker cohabitation and lower AChE activity (per U/ml, mean=3.13 U/ml, SD=0.49) were both associated with larger head circumference (0.37 cm, 95% CI: 0.003, 0.74 and 0.75 cm, 95% CI: 0.30, 1.19, respectively).

CONCLUSIONS: Our findings suggest that indirect pesticide exposures (estimated by AChE activity and cohabitation with a flower worker) can affect growth and head circumference in children living in agricultural communities.

Introduction

Within the last decade, there has been increasing evidence of alterations in child development associated with organophosphate (OP) pesticide exposures in small doses and from indirect sources. OP pesticides are insecticides that can irreversibly inhibit acetylcholinesterase (AChE) activity and are widely used in agriculture. Studies assessing prenatal and postnatal OP exposures in urban and rural agricultural settings have reported associations with child physiologic and developmental alterations which include neurobehavioral delays and cardiovascular alterations (Bouchard et al., 2010; Eskenazi et al., 2007; Marks et al., 2010; Rauh et al., 2011; Suarez-Lopez, 2012a; Suarez-Lopez, 2012b).

The association between pesticide exposure and growth is less clear. Inverse associations between prenatal OP exposure with length and birth weight have been reported (Grandjean et al., 2006; Perera et al., 2003; Perera et al., 2005; Whyatt et al., 2004), and there is some evidence that AChE has a role in bone formation (Genever et al., 1999; Grisaru et al., 1999; Inkson et al., 2004). However, two larger studies did not find associations between maternal OP pesticide metabolite concentrations with fetal growth (Berkowitz et al., 2004; Eskenazi et al., 2004) but Berkowitz et al. 2004 found an increasing risk for low birth weight with decreasing maternal AChE. One study reported decreased cancellous bone formation and bone density among adult agricultural workers exposed to OP pesticides (Compston et al., 1999). Because bone health is an important component of growth it is plausible that children's vertical growth may be affected by pesticide exposures.

The association of exposure to cholinesterase inhibitors and head circumference is also unclear. Three studies reported no associations between maternal exposure to cholinesterase inhibitors and head circumference at birth (Berkowitz et al., 2004; Perera et al., 2003; Whyatt et al., 2004). One of these studies, however, found that low maternal paraoxonase-1 activity, an enzyme that hydrolyzes several OP insecticides (Costa et al., 2008), was associated with reductions in head circumference (Berkowitz et al., 2004). Alternatively, a study of Mexican-American children found that maternal urinary concentration of OP metabolites was associated with larger head circumference in children, but AChE activity at the umbilical cord was not associated with head circumference (Eskenazi et al., 2004).

Children living in agricultural communities are more prone to being exposed to pesticides and such exposures may last throughout childhood, particularly if they live in households with agricultural workers. Family members of agricultural workers are at risk of pesticide contamination through secondary routes (Coronado et al., 2006), which include proximity of worker's residence to plantations (pesticide drift) and take-home pesticides by the workers through clothes, hair, tools, and pesticide storage at home (Coronado et al., 2011; Curl et al., 2002; Fenske et al., 2002; Gladen et al., 1998). We previously found that children who lived with flower plantation workers (flower worker) in Ecuador had lower AChE activity than those who lived with non-agricultural workers and longer durations of cohabitation were associated with lower AChE activity (Suarez-Lopez et al., 2012). Studies in US agricultural populations found that family members 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).

The purpose of this investigation is to assess whether lower AChE activity, reflecting OP (and to some extent carbamate) pesticide exposure and cohabitation with a flower worker are associated with changes in growth and increased head circumference in a cohort of children living in Pedro Moncayo County, Ecuador. Pedro Moncayo County is located in the mountainous region of Ecuador and contains one of the largest areas of fresh-cut flower production in the country.

Methods

Study Description

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), is a cohort study analyzing the effects of secondary exposure to pesticides on child development. We included children younger than 5 years of age for whom we had anthropometric measurements as part of the 2004 Survey of Access and Demand of Health Services in Pedro Moncayo County.

The 2004 Survey of Access and Demand of Health Services in Pedro Moncayo County was conducted by Fundacion Cimas del Ecuador and had the objective of interviewing all residents of Pedro Moncayo County. Interviewers collected socio-economic status, demographic, occupational and health information of 18,187 residents (71% of Pedro Moncayo County's population) and collected both height (or length) and weight of 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). Of these children, we included 853 (93%) in this study who had all covariates of interest.

In 2008, we re-examined 230 of these children. We also conducted in-person home interviews of adults who lived with the examined children to obtain socio-economic status, demographic, health and pesticide exposure information of adults and children. Additional recruitment and data collection information has been described elsewhere (Suarez-Lopez et al., 2012). We included 188 (82% of re-examined children) who had all covariates of interest in both 2004 and 2008 for longitudinal analyses. This study was approved by the Institutional Review Boards of Fundacion Cimas del Ecuador and the University of Minnesota. Informed consent of surveyed parents was obtained in addition to parental permission for participation of each of their selected children and child assent of participants 7 years of age and older.

Measures

Children's parents and other adults in the household were interviewed at home to obtain socio-economic status, demographic, health and pesticide exposure information of adults and children. We obtained information of flower worker practices that could increase the amounts of pesticide inadvertently brought home as follows: frequency of hand washing, showering, removing work shoes and removing work clothes before leaving work (never, 1-2 days/week, 3-4 days/week, always), location of work clothes washing (e.g. home, flower plantation) and bringing work tools home. Most questions had 4 options: the worst practice (e.g. always bringing work clothes home) was coded 1 and the best practice (e.g. never bringing work clothes home) was 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. The household level value was assigned to each child within the household. Children of non-flower workers were assigned a value of 0. We then calculated the number of “bad practices” which were defined as codes 1 or 2 for each item. The maximum attainable number of bad practices was 6. We previously found that AChE activity decreased in this study population (reflecting greater cholinesterase inhibitor pesticide exposure) as the number of bad practices increased (Suarez-Lopez et al., 2012).

We calculated the duration of cohabitation for 2004 participants based on the 2008 responses and subtracting the elapsed time between examination years (mean: 2.8 years, range: 3.75-3.85 years).

Examinations in 2004 (height and weight) were collected at the time of the interview at the participants’ homes. In 2008, the examinations were conducted in seven centrally located schools distributed in all parishes of Pedro Moncayo Canton during the summer months when school was out of session.

Children’s standing height was measured using a height board after removing shoes and head ornaments. Participants were asked to stand straight with both heels, buttocks, shoulder blades and head touching the back of the height board. Children’s length was measured in children who were unable to stand using a length board. We converted length (recumbent) to height (standing) by subtracting 0.7cm from length. Z-scores for height-for-age, weight-for-age and BMI-for-age were calculated using the World Health Organization normative sample (World Health Organization). Stunting was defined as a z-score of height-for-age of less than or equal to -2.

In 2004, children’s weight was measured using an analog spring scale. Weight of children who could not stand was measured using a suspended baby spring scale. In 2008, weight was measured using a digital scale. Head (occipito-frontal) circumference was measured twice to the nearest 0.1 cm using a flexible plastic tape measure. The larger of the two measurements was used. Head circumference in 2004 was not measured.

Erythrocytic AChE activity and hemoglobin concentration were measured in 2008 using the EQM Test-mate ChE Cholinesterase Test System 400 (EQM AChE Erythrocyte Cholinesterase Assay Kit 470) from a single finger stick. We previously found that AChE activity from a single measure can be a valid indicator of pesticide exposure in epidemiological studies of children (Suarez-Lopez et al., 2012).

The residential distance to the nearest flower plantation was calculated using ArcGIS 9.3 (Esri, Redlands, CA) from geographical coordinates obtained from portable global positioning system (GPS) receivers. Additional details regarding the geographical positioning data collection and calculations have been described elsewhere (Suarez-Lopez et al., 2012).

Statistical Analysis

We used multiple linear regression to calculate the associations between flower worker cohabitation, duration of flower worker cohabitation, flower worker number of bad practices and AChE activity with z-scores for height-for-age, weight-for-age and body mass index (BMI)-for-age in cross-sectional analyses of 2004. We also used linear regression in the cross-sectional analyses of head circumference in 2008. We used multiple logistic regression to calculate odds ratios for presence of stunting. We analyzed longitudinally (2004-2008) the association between flower worker cohabitation and z-scores for height-for-age, weight-for-age and BMI-for-age using repeated measures regression to account for correlations between repeated tests within participants, using a compound symmetry covariance structure. Similarly, repeated measures logistic regression was used in analyses of stunting (dichotomous), using a compound symmetry covariance structure. Additionally, we tested quadratic and cubic associations between AChE and the various outcomes in all models.

Adjustment models varied according to year of analysis. We created 2 adjustment models for analysis of 2004 data: model-1 (age, gender, parish of residence) and model-2 (model-1 + socio-economic variables: education of the household head, type of home, home roof materials and type of wastewater disposal).

For 2008 cross-sectional analyses of height-for-age, BMI-for-age and weight-for-age, model-1 consisted of age, gender, race, hemoglobin concentration, residential distance to the nearest flower plantation and concurrent and either 2004: height-for-age, BMI-for-age or weight-for-age, depending on the (coinciding) outcome analyzed (e.g. for BMI-for-age analyses the adjustment included 2004 BMI-for-age). When the outcome was head circumference (2008), concurrent and 2004 height-for-age and weight-for-age z-scores were included. Model-2 in 2008 consisted of model-1 plus income, parental education (the mean of maternal and paternal years of education) and number of smokers living at home.

Model-1 in longitudinal analyses (2004-2008) consisted of age, gender, race, residential distance to the nearest flower plantation and year of examination. The corresponding model-2 included model-1 plus socio-economic variables of 2004 (education of the household head, type of home, home roof materials, type of wastewater disposal) and 2008 (income, parental education and number of smokers living at home). Data were analyzed with SAS Version 9.2 (SAS Institute Inc., Cary, NC).

Results

Participant Characteristics

In 2004, 49% of children were male with a mean age of 2.3 years, 63% lived with a flower plantation worker and 39% were stunted. Overall participant characteristics in 2004 and divided by flower worker cohabitation status are shown in Table 1.

In 2008, 52% of children were male, 70% mestizo and 28% indigenous; 63% of children lived with a flower plantation worker (mean duration: 5.1 years) and 26% were stunted. Children who lived with a flower worker were composed of a greater proportion of natives and had a substantially greater proportion of stunted. Participant characteristics in 2008 by flower worker cohabitation status and overall are shown in Table 2.

Growth

In 2004, flower worker cohabitation (vs. not) and duration of cohabitation had inverse but non-significant associations with height-for-age, BMI-for-age and weight-for-age in model-1 and 2 (Table 3). There was no effect modification by gender or age. In model-2, children who resided in the parish of Tupigachi had a lower z-score for weight-for-age compared to Tabacundo, the county seat (-0.33 SD, 95% CI: -0.59, -0.08).

In cross-sectional analyses of 2008 participants, children who lived concurrently with a flower worker had lower mean z-scores for height-for-age (-0.32 SD, 95% CI: -0.59, -0.04), BMI-for-age (-0.23 SD, 95% CI: -0.47, 0.01) and weight-for-age (-0.32 SD, 95% CI: -0.58, -0.06). Furthermore, children who lived with a flower worker had an odds ratio (OR) of being stunted of 2.55 (95% CI: 1.12, 5.80). Longer duration of cohabitation was associated with lower z-scores in BMI-for-age and weight-for-age and borderline lower height-for-age (Table 4). Increasing bad practices for take-home pesticide exposures by flower workers had borderline lower z-scores in BMI-for-age and weight-for age. AChE activity was not associated with either height-for age, BMI-for-age or weight-for-age.

In longitudinal analyses, cohabitation with a flower worker (vs. not) was associated with decreases in BMI-for-age and weight-for-age of 0.36 SD (95% CI: -0.66, -0.06) and -0.33 SD (95% CI: -0.61, -0.05), respectively (Table 5). Additional correlates for height-for-age in model-2 were male gender (-0.32, 95% CI: -0.62, -0.03) and indigenous race (-0.50, 95% CI: -0.86, -0.13). There was no effect modification by age, gender or race. Duration of flower worker cohabitation had borderline associations with BMI-for-age and weight-for-age. Cohabitation with a flower worker was not associated with increased odds of stunting in longitudinal analyses (OR: 1.18, 95% CI: 0.67, 2.08).

Head Circumference

Children who lived with a flower worker had a larger mean head circumference in model-2 (0.37 cm, 95% CI: 0.003, 0.74), and every year of cohabitation was associated with a non-significant increase of 0.05 cm (95% CI: -0.01, 0.12). Increasing number of bad practices was associated with an increase in head circumference (0.15 cm, 95% CI: 0.03, 0.027). Additionally, AChE activity was inversely (linear) associated with head circumference. Every 1 U/ml (≈2 SD) decrease in AChE activity was associated with an increase in head circumference of 0.75 cm (95% CI: 0.30, 1.19, Table 4).

Discussion

Our findings suggest that indirect pesticide exposures can affect children's growth as reflected by associations between AChE activity, cohabitation with flower workers and flower worker's practices that can increase the risk of para-occupational pesticide exposures with height-for-age, BMI-for-age, weight-for age and head circumference. The mechanisms for these associations may be multiple including nutritional (associated with education and socio-economic factors) and exposures to environmental pollutants from parental occupation via take-home routes.

We adjusted for various socio-economic status constructs including income, education and characteristics of participant's homes. However, it is possible that a degree of residual confounding by socio-economic status may exist in our analyses. It has been well characterized that socio-economic status influences child growth. A limitation of this study is a lack of environmental pollutant measurements or AChE activity measurements during multiple points in time to more comprehensively understand the driving factors of this association.

The inverse association between AChE activity (and surrogates of pesticide exposure including flower worker cohabitation and increasing number of "bad practices" by flower workers) and head circumference suggests that children with greater cholinesterase inhibitor pesticide exposures have greater brain growth, to the extent that head circumference is a good surrogate of brain size in children (Bartholomeusz et al., 2002; Lindley et al., 1999). A study of Mexican-American children living in an agricultural community in the US reported increased head circumference among children whose mothers had higher urinary concentrations of OP metabolites; however, umbilical cord AChE activity was not associated with head circumference (Eskenazi et al., 2004). This could indicate that pesticide exposure affects brain growth through pathways that do not necessarily involve AChE inhibition. A study of an agricultural community near areas with substantial OP pesticide usage in Argentina found that women whose first trimester of pregnancy coincided with the pesticide application period delivered neonates with a larger head circumference (Souza et al., 2005). Contrasting these findings, other studies found no association between maternal exposure to cholinesterase inhibitors and head circumference (Berkowitz et al., 2004; Perera et al., 2003; Whyatt et al., 2004)

In our study, AChE activity is an indicator of chronic pesticide exposures and may be representative of OP exposure at any given time of the year considering that AChE has a long recovery time after irreversible inhibition (Mason, 2000) and the sources of secondary pesticide exposures remain relatively constant throughout the year due, in part, to the equatorial location of the county which allows for year-round flower production (and pesticide use). Although our findings suggest a direct effect of AChE on brain development, we cannot exclude mechanisms outside of AChE inhibition associated with OP pesticides. Furthermore, because these are cross-sectional analyses, there is a possibility that children with larger brains have higher overall AChE activity. In this scenario we can expect increases in central nervous system AChE activity with greater brain mass; however, the degree to which brain size influences red blood cell AChE activity (our measurement of AChE) seems to be less plausible from a physiological stand point and is currently a topic that has not been studied.

Larger brain and head circumference have been associated with higher intelligence (Mcdaniel, 2005; Wicket et al., 2000); however, not all head circumference increases are beneficial. Periods of increased head circumference and macrocephaly have been associated in children with autism and other pervasive developmental disorders (Courchesne, 2004; Dementieva et al., 2005; Fidler et al., 2000; Lainhart et al., 2006; Stevenson et al., 1997). Although the effects of pesticides on the development of autism are not known, an association between autism spectrum disorders and residential proximity to agricultural plantations has been reported. (Roberts et al., 2007).

There is growing evidence from animal studies that cholinesterase inhibitor pesticides can disrupt brain development (Byers et al., 2005; Roy et al., 2004; Santos et al., 2004; Slotkin et al., 2001), at asymptomatic levels with low cholinesterase inhibition (Abou-Donia, 2003; Dam et al., 2000; Qiao et al., 2003; Slotkin, 2004b; Slotkin et al., 2001). AChE inhibition can increase acetylcholine concentrations which can disrupt the growth of the central nervous system given acetylcholine's important role in brain development (Dori et al., 2005; Hohmann, 2003). Studies in children reported associations between prenatal pesticide exposure with mental and motor developmental delays, pervasive developmental disorder, decreased attention, working memory and intelligence (Eskenazi et al., 2007; Marks et al., 2010; Rauh et al., 2011; Rauh et al., 2006).

In the ESPINA study, we previously found that lower AChE activity was associated with lower neurobehavioral development scores within the domains of attention/executive functioning and memory/learning (Suarez-Lopez, 2012a). Others have reported associations between postnatal OP pesticide exposures and decreased learning, poor inhibitory control, attention and attention deficit hyperactivity disorder (ADHD) (Bouchard et al., 2010; Kofman et al., 2006; Marks et al., 2010).

Conclusions

The present study supports the notion that para-occupational exposures to cholinesterase inhibitor pesticides are associated with decreased weight-for-age and BMI-for-age and increased head circumference among children living in agricultural communities. Further research is needed to more comprehensively understand the behavioral and physiological pathways involved in these associations.

Tables

Table 1. Participant Characteristics in 2004

		Concurrent Cohabitation with	
	All Participants	Flower plantation worker	Non-flower plantation worker
N	853	539 (63%)	314 (37%)
Demographic and Socio-Economic Status Characteristics†			
Age, years	2.3 (1.35)	2.3 (1.4)	2.3 (1.3)
Gender, male	49%	50%	46%
Parish of residence			
La Esperanza	14%	14%	14%
Malchingui	19%	16%	24%
Tabacundo	32%	34%	28%
Tocachi	8%	7%	8%
Tupigachi	27%	29%	26%
Household head education[a]	3.6 (1.2)	3.6 (1.2)	3.6 (1.3)
Type of home[b]	1.3 (0.8)	1.4 (0.9)	1.2 (0.6)
Home roof material[c]	2.0 (0.8)	2.1 (0.8)	1.9 (0.9)
Home wastewater disposal[d]	2.4 (1.3)	2.4 (1.3)	2.4 (1.4)
Duration of flower worker cohabitation		1.5 (1.5)	0.2 (0.7)
Anthropometric and Blood Measurements			
Height, cm	81.9 (14.0)	82.0 (14.2)	81.8 (13.7)
Weight, kg	11.2 (3.6)	11.2 (3.6)	11.2 (3.6)
Body mass index, m/kg^2	16.2 (2.6)	16.1 (2.5)	16.3 (2.6)
Height-for-age, z-score	-1.48 (1.86)	-1.55 (1.84)	-1.37 (1.90)

Stunted	39%	39%	39%
BMI-for-age, z-score	0.09 (1.82)	0.06 (1.82)	0.13 (1.82)
Weight-for-age, z-score	-0.88 (1.44)	-0.94 (1.42)-	0.77 (1.46)

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

[a] Education: 1=none 2=incomplete primary (K-6^{th}) 3=complete primary 4=incomplete secondary 5=complete secondary 6=university/technical school.

[b] Home type: 1=shack 2=room(rental) 3=apartment 4=house.

[c] Home roof material: 1=zinc 2=fiber cement 3=tile 4=concrete.

[d] Home wastewater disposal: 1= None 2= Cesspool 3= Septic Tank 4=Latrine 5= Sewage.

Table 2. Participant Characteristics in 2008

	All Participants	Concurrent cohabitation with Flower plantation worker	Concurrent cohabitation with Non-flower plantation worker
N	188	103 (55%)	85 (55%)
Demographic and Socio-Economic Status Characteristics			
Age, years	6.4 (1.4)	6.39 (1.4)	6.4 (1.4)
Gender, male	52%	51%	52%
Race			
Mestizo	70%	59%	82%
Indigenous	28%	39%	15%
Other	2%	2%	3%
Monthly income[a]	3.2 (0.9)	3.2 (0.7)	3.1 (1.0)
Number of smokers at home	0.24 (0.43)	0.23 (0.42)	0.25 (0.43)
Residence distance to nearest flower plantation, m	329 (180, 632)	321 (166, 634)	333 (190, 605)
Duration of flower worker cohabitation, years		5.3 (1.8)	0.7 (1.6)
Number of bad practices		2.9 (0.9)	0
Anthropometric and Blood Measurements			
Height, cm	110.91 (9.4)	109.7 (9.9)	112.4 (8.6)
Weight, kg	20.0 (4.0)	19.4 (3.9)	20.8 (4.1)
Body mass index, m/kg^2	16.1 (1.33)	15.9 (1.1)	16.3 (1.6)
Head circumference, cm	50.6 (1.5)	50.6 (1.7)	50.7 (1.3)
Height-for-age, z-score	-1.33 (0.99)	-1.55 (1.01)	-1.06 (0.90)
Stunted	26%	35%	15%
BMI-for-age, z-score	0.37 (0.79)	0.28 (0.67)	0.48 (0.90)
Weight-for-age, z-score	-0.59 (0.89)	-0.80 (0.80)	-0.32 (0.93)

Acetylcholinesterase, U/ml	3.13 (0.49)	3.09 (0.50)	3.17 (0.47)
Hemoglobin, mg/dl	12.5 (1.2)	12.5 (1.3)	12.6 (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 3. Associations of flower worker cohabitation with height-for-age, BMI-for-age and weight-for-age in 2004, N=853.

	Z-score Difference (95% CI)		
	Height-for-Age	**BMI-for-Age**	**Weight-for-Age**
Flower worker cohabitation vs. not			
Model-1	-0.12 (-0.38, 0.14)	-0.12 (-0.38, 0.13)	-0.16 (-0.36, 0.04)
Model-2	-0.07 (-0.33, 0.19)	-0.13 (-0.39, 0.12)	-0.14 (-0.34, 0.06)
Duration of flower worker cohabitation (per year)			
Model-1	-0.01 (-0.16, 0.18)	-0.11 (-0.30, 0.09)	-0.06 (-0.11, 0.10)
Model-2	-0.03 (-0.14, 0.20)	-0.11 (-0.31, 0.09)	-0.05 (-0.22, 0.11)

Model-1: age, gender, parish of residence.

Model-2: model-1 + socio-economic variables (education of the household head, type of home, home roof material, type of wastewater disposal).

Table 4. Associations of flower worker cohabitation with height-for-age, BMI-for-age and weight-for-age in 2008, N=188.

	Z-score Difference (95% CI)			**Head Circumference Difference (cm) (95% CI)**
	Height-for-age	**BMI-for-age**	**Weight-for-age**	
Flower worker cohabitation vs. not				
Model-1	**-0.33 (-0.59, -0.07)**	**-0.24 (-0.48, -0.13)**	**-0.36 (-0.61, -0.12)**	**0.43 (0.06, 0. 78)**
Model-2	**-0.32 (-0.59, -0.04)**	-0.23 (-0.47, 0.01)	**-0.32 (-0.58, -0.06)**	**0.37 (0.003, 0.74)**
Duration of cohabitation (per year)				
Model-1	-0.05 (-0.09, 0.001)	**-0.05 (-0.09, -0.01)**	**-0.06 (-0.10, -0.01)**	**0.06 (0.01, 0.13)**
Model-2	-0.04 (-0.09, 0.01)	**-0.05 (-0.09, -0.003)**	**-0.05 (-0.10, -0.01)**	0.05 (-0.01, 0.12)
Number of bad practices (per practice)				
Model-1	-0.07 (-0.16, 0.02)	-0.08 (-0.15, 0.002)	**-0.09 (-0.17, -0.01)**	**0.15 (0.03, 0.27)**
Model-2	-0.05 (-0.14, 0.04)	-0.07 (-0.16, 0.01)	-0.07 (-0.16, 0.01)	**0.15 (0.03, 0.27)**
Acetylcholinesterase (per decrease of 1 U/ml)				
Model-1	0.12 (-0.22, 0.45)	0.08 (-0.21, 0.37)	0.12 (-0.20, 0.44)	**0.78 (0.32, 1.24)**
Model-2	0.07 (-0.28, 0.42)	0.07 (-0.22, 0.37)	0.07 (-0.25, 0.40)	**0.75 (0.30, 1.19)**

Model-1: age, gender, race, hemoglobin concentration, residential distance to the nearest flower plantation and either: 2004 height-for-age, 2004 BMI-for-age or 2004 weight-for-age, depending on the (coinciding) growth outcome analyzed. When head circumference was the outcome, both 2004 and 2008 z-scores for-weight-for-age and height-for-age were included.

Model-2: model-1 + income, parental education and number of smokers at home.

Table 5. Longitudinal (2004-2008) associations of flower worker cohabitation with height-for-age, BMI-for-age and weight-for-age. N=188.

	Z-score Difference (95% CI)		
	Height-for-Age	**BMI-for-Age**	**Weight-for-Age**
Flower worker cohabitation vs. not			
Model-1	-0.17 (-0.45, 0.11)	-0.25 (-0.54, 0.03)	**-0.28 (-0.54, -0.02)**
Model-2	-0.16 (-0.46, 0.14)	**-0.36 (-0.66, -0.06)**	**-0.33 (-0.61, -0.05)**
Duration of flower worker cohabitation (per year)			
Model-1	-0.04 (-0.11, 0.02)	-0.05 (-0.12, 0.01)	-0.06 (-0.12, 0.001)
Model-2	-0.03 (-0.10, 0.03)	-0.06 (-0.13, 0.002)	-0.06 (-0.12, 0.001)

Model-1: age, gender, race, residential distance to the nearest flower plantation and year of examination.
Model-2: model-1 + socio-economic variables of 2004 (education of the household head, type of home, home roof materials, type of wastewater disposal) and 2008 (income, parental education, number of smokers at home).

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CHAPTER 7: DISCUSSION

This project provides adequate insight on the impacts of secondary pesticide exposure on childhood development. Currently, the ESPINA study is among the largest completed studies that have assessed neurobehavioral development in children indirectly exposed to pesticides.

We found that flower workers introduce into their homes a sufficient amount of cholinesterase inhibitor pesticides to decrease acetylcholinesterase activity and that longer duration of cohabitation was associated with lower acetylcholinesterase in children. Previous studies have found higher amounts of pesticide metabolites in children of agricultural workers, 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 greater AChE suppression. The inverse linear association between duration of cohabitation and AChE activity could be explained by: 1) an increased pesticide accumulation in the home which can be augmented by diminished 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, perhaps reflecting inadequate continuing education of flower workers; or 3) a combination of the two. We found that flower-worker families lived closer to flower plantations than non-flower-worker families and household proximity to a flower plantation was independently associated with AChE suppression in our study.

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. It is plausible that these pathways may play a significant role in exposing the families of flower workers to pesticides. For instance, dirty 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, and in cold water, which is less effective than hot water for removing pesticides. It would not be surprising to find children helping their parents wash clothes. 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.

Our results also bring insight on the effects of pesticides on the neurobehavioral domains of Memory and Learning, and Attention and Executive Function. Skills in these domains are essential for learning and academic performance. These results can guide future research to understand the pathways and toxic compounds that impact these and other areas. Our assessment of pesticide exposure was limited to an acetylcholinesterase measurement; thus, we were unable to capture the full scope of pesticide exposure. New investigations should include pesticide and persistent organic pollutant quantification to obtain a more compound specific effect on agricultural worker's family health and neurobehavioral development.

Flower worker cohabitation and increasing duration of cohabitation were associated with a decrease in SBP. AChE was directly associated with SBP and DBP, even after adjustment for resting heart rate and hemoglobin concentration. These findings reflect physiologic reactivity in children with subclinical secondary pesticide exposures and suggest that vasodilation from stimulation of muscarinic receptors in vasculature is an important mechanism of decreased blood pressure of cholinesterase inhibitors in children. Although lower BP in isolation might be a beneficial effect of secondary pesticide exposure, the finding needs to be viewed with caution, as part of a pattern of generally adverse physiologic responses to pesticides.

Finally, we found that indirect pesticide exposures can affect children's growth at different levels. Cohabitation with flower workers and flower worker's practices that can increase the risk of para-occupational pesticide exposures were associated with changes in height-for-age, BMI-for-age and weight-for age. The mechanisms for these associations may be multiple including nutritional (associated with education and socio-economic factors) and exposures to environmental pollutants from parental occupation via take-home routes. Although we adjusted for various socio-economic status constructs including income, education and characteristics of participant's homes, it is possible that some amount of residual confounding by socio-economic status may exist in our analyses. We found an inverse association of AChE activity and head circumference reflecting cholinesterase inhibitor pesticide exposure related to larger head circumference. This finding concurs with findings of a few studies where metabolites of organophosphate pesticide were associated with larger head circumference. However, this is the first study to report an association between AChE activity and head circumference. This association seems plausible given the important role of acetylcholine in brain development; excessive acetylcholine stimulation on growth could yield excessive brain growth; however, such growth may not necessarily be beneficial given our finding that lower AChE activity was related to poorer scores in attention, inhibitory control and long term memory.

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).

Conclusions and Recommendations

We conclude that secondary pesticide exposures can produce physiologic and neurobehavioral alterations in children. This investigation reinforces the importance of reducing the amounts of pesticides introduced into the homes by agricultural workers. Interventions to reduce secondary exposures targeting flower plantation workers and their families are needed. It is important to not only 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.

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9. APPENDICES

Appendix 1a. Consent Form for Parents (Spanish)

Nº de la Vivienda	☐☐	☐☐	☐☐☐	**URBANO**
		☐☐	☐☐☐	**RURAL**

Formulario de Consentimiento de Padres y Permiso de participación de los niños

Titulo del estudio: Efectos de la Exposición Secundaria Ocupacional de Pesticidas en el Crecimiento y Desarrollo Neurológico y Conductual de la Niñez.

Usted y su(s) hijos(as) están invitados a participar en la investigación de los efectos de la exposición de padres a pesticidas provenientes del trabajo en plantaciones florícolas en el desarrollo mental y crecimiento de los niños en el Cantón Pedro Moncayo.

Le pedimos que lea este documento y haga cualquier pregunta que tenga antes de aceptar a participar en el estudio.

Este estudio está dirigido por el Dr. José R Suárez López, miembro de la Fundación Cimas del Ecuador, la Universidad de Minnesota, Estados Unidos y el Area de Salud Tabacundo. Esta investigación se encuentra dentro del marco de prioridades del Consejo Cantonal de Salud de Pedro Moncayo.

Propósito:

El propósito de este estudio es el de evaluar los efectos de la contaminación por pesticidas en niños de trabajadores floricultores en el crecimiento en su crecimiento, desarrollo mental y de la conducta.

Procedimientos:

Si usted acepta a participar en este estudio, le solicitaremos que haga lo siguiente:

1) Se le solicitará que responda a las preguntas de una encuesta para obtener información demográfica (ej. edad, raza, sexo), socio-económica (educación, ocupación), y de salud suya y de su familia, así como información acerca de exposición a pesticidas dentro de la casa.

2) Su niño o niños que participen serán examinados por personal de investigación entrenado para obtener las siguientes medidas: altura, peso, tamaño de la cabeza, frecuencia cardiaca, presión arterial, nivel de acetilcolinesterasa en sangre (una enzima que mide exposición a pesticidas medido por 1 gota de sangre obtenida por un pinchacito en el dedo o

talón), concentración de pesticidas en orina (los participantes orinarán en un vaso recolector o un recolector de orina similar a un inodoro) y desarrollo neurológico y del comportamiento. Todos los exámenes son gratuitos.

Riesgos de la participación en este estudio

Los riesgos asociados con la participación en este estudio son mínimos. Los posibles riesgos son:

1) La información confidencial de los participantes puede perderse debido a robo o mala utilización. Protecciones apropiadas contra estos riesgos se tomarán como por ejemplo entrenamiento de investigadores en seguridad de la información y los nombres de los participantes no se escribirán en las hojas de recolección de información. La información será manejada con estricta confidencialidad.

2) La muestra de sangre y las mediciones de peso y talla pueden asustar a los niños. Los niños recibirán explicaciones de todos los procedimientos a realizarse.

3) Los potenciales riesgos de la toma de muestra sanguínea (1 gota de sangre capilar: en base a un pinchazo en un dedo o talón) son mínimos si se toman todas las medidas de control necesarias. Rara vez podría haber mareo o infección Cualquier otro método de obtención de sangre tiene similares o mayores probabilidades de desarrollar estos riesgos potenciales.

Beneficios de participar en el estudio:

Esta investigación puede no beneficiarle a usted o a sus niños. Este estudio podría beneficiar a aquellos niños con trastornos evaluados en este estudio. Padres de niños con problemas médicos detectados serán informados.

De encontrarse efectos deletéreos de de la exposición de padres a pesticidas provenientes del trabajo en plantaciones florícolas en el desarrollo de los niños, este estudio podría impulsar el desarrollo de intervenciones para prevenir o reducir los efectos negativos sobre el desarrollo de los niños viviendo con trabajadores floricultores.

Costos del Estudio:

Participar en este estudio <u>no le costará nada</u>. La participación en este estudio, la cual incluye costos de todos los exámenes, procedimientos y suministros serán gratuitos.

Compensación:

Si bien se consideran los riegos como mínimos, en el evento de que ocurra alguna lesión asociada con la investigación, habrá disponible tratamiento que incluye primeros auxilios, tratamientos de emergencia y seguimiento de ser necesarios. Cuidado de problemas que no sean controlados por el personal de investigación serán referidos a la atención médica más cercana y los costos del mismo serán cubiertos por los padres de los participantes o por su seguro de salud. Si piensa que su hijo(s) ha sufrido de una lesión relacionada con este estudio, por favor avise a los investigadores inmediatamente.

Como agradecimiento, su niño(s) participantes recibirán un pequeño regalo (ej. Juguete o útiles escolares) por participar en este estudio; el valor total que recibirán será de aproximadamente $4.

Confidencialidad:

La información recolectada será utilizada con fines de investigación y acción exclusivamente. La información con datos de identificación (por ejemplo nombres, apellidos, números de teléfono, dirección) nunca serán compartidos con ninguna institución externa. La información para uso investigativo también será removida de datos de identificación y a cambio los participantes recibirán un número de estudio. El archivo que contiene los nombres y números de estudio serán guardados en un archivo protegido con contraseña, guardado en una computadora protegida con contraseña, la cual es accesible únicamente por personal de investigación autorizado.

Todos los examinadores, entrevistadores e investigadores han cumplido entrenamiento de confidencialidad y seguridad de información.

Participación Voluntaria:

Su participación en este estudio es voluntaria. Su decisión de participar o dar consentimiento a la participación de su niño(s) no afectarán sus relaciones actuales o futuras con la Fundación Cimas del Ecuador, la Universidad de Minnesota o el Consejo Cantonal de Salud de Pedro Moncayo.

Contactos y Preguntas:

El investigador que está llevando acabo este estudio es: Jose R Suarez Lopez, MD, MPH, PhD (c)

Puede hacer las preguntas que tenga en este momento.
Si usted tiene preguntas luego, usted puede contactar a los investigadores en:
Dr. Jose Ricardo Suarez
Investigador
Fundación Cimas del Ecuador
Teléfono: (02)245 2300
(08)401 5028
Email: suar0026@umn.edu

Si usted quiere hablar con alguien que no es un investigador:
Dolores Lopez
Fundación Cimas del Ecuador
Teléfono: (02) 245 2509
Email: dlopez@cimas.edu.ec
Usted recibirá una copia de este formulario para su record.

Nº de la Vivienda	☐☐	☐☐	☐☐☐	URBANO
		☐☐	☐☐☐	RURAL

Declaración de Consentimiento

He leído la información de este documento. He hecho preguntas y he recibido respuestas. Yo doy consentimiento a participar en este estudio y doy consentimiento a la participación de mi niño(s) listado a continuación:

______________________________ ______________________
Nombre del Niño(a) Fecha Nacimiento: día/mes/año

______________________________ ______________________
Nombre del Niño(a) Fecha Nacimiento: día/mes/año

______________________________ ______________________
Nombre del Niño(a) Fecha Nacimiento: día/mes/año

______________________________ ______________________
Nombre del Niño(a) Fecha Nacimiento: día/mes/año

______________________________ ______________________
Nombre del Niño(a) Fecha Nacimiento: día/mes/año

____
Nombre de Madre, Padre o apoderado

X______________________________ Fecha de hoy ___________
Firma de Madre, Padre o apoderado

____
Nombre de Persona obteniendo consentimiento

______________________________ Fecha de hoy ___________
Firma Persona obteniendo consentimiento

Nº de la Vivienda	☐☐	☐☐	☐☐☐	**URBANO**
		☐☐	☐☐☐	**RURAL**

Permiso de re-contacto

Titulo del estudio: Efectos de la Exposición Secundaria Ocupacional de Pesticidas en el Crecimiento y Desarrollo Neurológico y conductual de la Niñez.

Este estudio está interesado en realizar un seguimiento al estado de salud de su niño en el futuro. Quisiéramos obtener su permiso de contactarles nuevamente en el futuro para solicitar su participación y la de su niño(s) para este u otros estudios. Aceptar esto no les obliga a participar en el futuro. Usted mantiene el derecho de no participar en estudios en el futuro y su decisión no afectara sus relaciones actuales o futuras con la Fundación Cimas del Ecuador, la Universidad de Minnesota o el Consejo Cantonal de Salud de Pedro Moncayo.

Declaración de re-contacto

He leído la información de este documento y autorizo a que se me contacte a mí y a mi niño(s) en el futuro para participación en este u otros estudios. Nosotros mantenemos el derecho de no participar en estudios en el futuro.

____
Nombre de Madre, Padre o apoderado

X______________________________ Fecha de hoy ___________
Firma de Madre, Padre o apoderado

____
Nombre de Persona obteniendo consentimiento

__________________________________ Fecha de hoy ___________
Firma Persona obteniendo consentimiento

Appendix 1b. Consent Form for Parents (English)

House Number	☐☐	☐☐	☐☐☐	URBAN
		☐☐	☐☐☐	RURAL

Original language: Spanish

Parent Consent and Child Participation Permission Form

Study title: Effects of Secondary Occupational Pesticide Exposure on Childhood Neurobehavioral Development and Growth

You and your child/children are invited to be in a research study of the effects of parental pesticide exposure from work in flower plantations on children's mental development and growth in Pedro Moncayo County.

We ask that you read this document and ask any questions you may have before agreeing to be in the study.

This study is conducted by Dr. Jose R Suarez Lopez, member of Fundacion Cimas del Ecuador, University of Minnesota, USA and the Health Area Tabacundo. This investigation is considered a priority by the Pedro Moncayo County Health Council.

Purpose:

The purpose of this study is to evaluate the effects of pesticide contamination of children of flower plantation workers on childhood growth and mental and behavioral (neurobehavioral) development.

Procedure:

If you agree to be in this study, we will ask you to do the following:

1) You will be asked to participate in a survey to obtain demographic (e.g. sex, race, age), socio-economic (e.g. education, occupation) and health information of you and your family, as well as information regarding pesticide exposure

2) Your participating child or children will be examined by trained research personnel to obtain the following measurements: height, weight, head circumference, heart rate, blood pressure, acetylcholinesterase levels in blood (an enzyme that measures pesticide exposure obtained by 1 drop of blood collected by finger stick or heel stick), pesticide concentrations in urine (participants will pee into a collection cup or urine collector similar to a toilet), and neurological and behavioral (neurobehavioral) development. **All exams are free of charge.**

Risks of Being in the Study:

The risks associated with your participation in this study are minimal. The possible risks associated with this study are:

1) Confidential data of the participants may be lost due to data theft or mishandling. Appropriate protections against these risks will be taken such as training of researchers in data security and the name of the participant will not be written on the data collection sheet. The information gathered will be managed with strict confidentiality.

2) Finger stick blood samples and height and weight measurements using a measuring board and scale may frighten children. Children will receive explanations of all procedures to be done.

3) The potential risks of the blood sample (1 drop of blood by finger stick or heel stick) are minimal if all necessary precautions are taken. Rarely there may be dizziness or infection. Any other blood sampling method has similar or greater chances of these possible risks.

Benefits of Being in the Study:

This investigation may not benefit you or your children directly. This study may benefit children with medical conditions detected in this study. Parents of children with detected medical problems will be informed.

If we find negative effects of parental pesticide exposure from work in flower plantations on the development of children, this study could lead to the development of interventions to prevent or reduce negative developmental effects on children living with flower plantation workers.

Costs of Study:

Participating in this study will not cost you anything. Participation in this study, which includes costs of all exams, procedures and supplies will be free of charge.

Compensation:

Although the associated risks are considered minimal, in the event that this research activity results in an injury, treatment will be available, including first aid, emergency treatment, and follow-up care as needed, for problems derived from this study. If you think that your child (children) have suffered a research-related injury, let the study investigators know right away.

For additional medical attention and follow-up, children will be referred to the services of the Health Area #13. The costs derived from these treatments will be billed to the participant's parents or to their health insurance.

As a token of appreciation, your child/children will receive a small gift (e.g. toy) for participating in this study.

Confidentiality:

The collected information will be used for research and action purposes only. Information with identifying information (e.g. names, last names, telephone numbers, address) will not be shared with any external institution. The information for research use will also be removed from identifying information and in exchange participants will receive a study number. The file that contains both names and identification numbers will be kept in a password protected and encrypted file, stored in a password protected computer accessible only to authorized research staff.

All examiners, interviewers and researchers have completed data confidentiality and data security training.

Voluntary Nature of the Study:

Your participation in this study is voluntary. Your decision whether or not to participate or consent to your child's participation will not affect your current or future relations with Fundacion Cimas del Ecuador, University of Minnesota or the Pedro Moncayo Health Council.

Contacts and Questions:

The researcher conducting this study is:

Jose R Suarez Lopez, MD, MPH, PhD (c)

You may ask any questions you have now.

If you have questions later, you may contact the researchers at

Dr. Jose Ricardo Suarez
Researcher
Fundacion Cimas del Ecuador
Phone: (02)245 2300
(02)245 2509
Email: suar0026@umn.edu

If you want to talk to someone other than the researchers

Dolores Lopez
Fundacion Cimas del Ecuador
Phone: (02) 245 2509
email: dlopez@cimas.edu.ec

You will be given a copy of this form to keep for your records.

Mother´s or Father´s code:

Statement of Consent:

I have read the information in this document. I have asked questions and have received answers. I consent to participate in the study and consent to the participation of my child/children listed below:

Child's Name ______________________________ Birth date ______

Child's Name ______________________________ Birth date ______

Child's Name ______________________________ Birth date ______

Child's Name ______________________________ Birth date ______

Name of Parent or Guardian ___________________________

Signature of Parent or Guardian_____________________ Date ___________

Name of Person Obtaining Consent ____________________________________

Signature Person
Obtaining Consent_________________________ Date ___________

Mother´s or Father´s code:

Re-contact Permission

Study title: Effects of Secondary Occupational Pesticide Exposure on Childhood Neurobehavioral Development and Growth

This study is also interested in conducting a follow up of your child's health in the future. We would like to obtain your permission to contact you once again to request for your participation and your child's (children's) participation. Agreeing to this does not bind you to participating in the study in the future. You still reserve the right to not participate in future studies and your decision will not affect your current or future relations with Fundacion Cimas del Ecuador, University of Minnesota or the Pedro Moncayo Health Council.

Statement of re-contact

I have read the information in this document and I authorize that my child (children) and I be contacted in the future to participate in this and other studies. We keep the right to not participate in future studies.

Name of Parent or Guardian ___________________________

Signature of Parent or Guardian_____________________ Date ___________

Name of Person Obtaining Consent ____________________________________

Signature of Person
Obtaining Consent _________________________ Date ___________

Appendix 2a. Consent Form for Parents (Spanish)

Nº de la Vivienda	☐☐	☐☐	☐☐☐	URBANO
		☐☐	☐☐☐	RURAL

Formulario de Consentimiento de los Floricultores, (<u>de no ser el padre, madre o apoderado del niño seleccionado</u>)

Titulo del estudio: Efectos de la Exposición Secundaria Ocupacional de Pesticidas en el Crecimiento y Desarrollo Neurológico y Conductual de la Niñez.

Usted está invitado a participar en la investigación de los efectos de la exposición de padres a pesticidas provenientes del trabajo en plantaciones florícolas en el desarrollo mental y crecimiento de los niños en el Cantón Pedro Moncayo.

Le pedimos que lea este documento y haga cualquier pregunta que tenga antes de aceptar a participar en el estudio.

Este estudio está dirigido por el Dr. José R Suárez López, miembro de la Fundación Cimas del Ecuador, la Universidad de Minnesota, Estados Unidos y el Área de Salud Tabacundo. Esta investigación se encuentra dentro del marco de prioridades del Consejo Cantonal de Salud de Pedro Moncayo.

Propósito:

El propósito de este estudio es el de evaluar los efectos de la contaminación por pesticidas en niños de trabajadores floricultores en el crecimiento en su crecimiento, desarrollo mental y de la conducta.

Procedimientos:

Si usted acepta a participar en este estudio, le solicitaremos que haga lo siguiente:

1) Se le solicitará que responda a las preguntas de una encuesta para obtener información demográfica (ej. edad, raza, sexo), socio-económica (educación, ocupación), y de salud suya y de su familia, así como información acerca de exposición a pesticidas dentro de la casa.

Riesgos de la participación en este estudio

Los riesgos para usted asociados con la participación en este estudio son mínimos. Los posibles riesgos son:

1) La información confidencial de los participantes puede perderse debido a robo o mala utilización. Protecciones apropiadas contra estos riesgos se tomarán como por ejemplo entrenamiento de investigadores en seguridad de la información y los nombres de los participantes no se escribirán en las hojas de recolección de información. La información será manejada con estricta confidencialidad.

Beneficios de participar en el estudio:

Esta investigación puede no beneficiarle a usted o a los niños que vivan con usted. Este estudio podría beneficiar a aquellos niños con trastornos evaluados en este estudio. Padres de niños con problemas médicos detectados serán informados.

De encontrarse efectos negativos de de la exposición de padres a pesticidas provenientes del trabajo en plantaciones florícolas en el desarrollo de los niños, este estudio podría impulsar el desarrollo de intervenciones para prevenir o reducir los efectos negativos sobre el desarrollo de los niños viviendo con trabajadores floricultores.

Costos del Estudio:

Participar en este estudio no le costará nada más que su tiempo.

Compensación:

Usted no recibirá compensación por participar en este estudio.

Confidencialidad:

La información recolectada será utilizada con fines de investigación y acción exclusivamente. La información con datos de identificación (por ejemplo nombres, apellidos, números de teléfono, dirección) nunca serán compartidos con ninguna institución externa. La información para uso investigativo también será removida de datos de identificación y a cambio los participantes recibirán un número de estudio. El archivo que contiene los nombres y números de estudio serán guardados en un archivo protegido con contraseña, guardado en una computadora protegida con contraseña, la cual es accesible únicamente por personal de investigación autorizado.

Todos los examinadores, entrevistadores e investigadores han cumplido entrenamiento de confidencialidad y seguridad de información.

Participación Voluntaria:

Su participación en este estudio es voluntaria. Su decisión de participar o dar consentimiento a la participación de su niño(s) no afectarán sus relaciones actuales o futuras con la Fundación Cimas del Ecuador, la Universidad de Minnesota o el Consejo Cantonal de Salud de Pedro Moncayo.

Contactos y Preguntas:

El investigador que está llevando acabo este estudio es:

Jose R Suarez Lopez, MD, MPH, PhD (c)

Puede hacer las preguntas que tenga en este momento.

Si usted tiene preguntas luego, usted puede contactar a los investigadores en:

Dr. Jose Ricardo Suarez
Investigador
Fundacion Cimas del Ecuador
Teléfono: (02)245 2300
(08)401 5028
Email: suar0026@umn.edu

Si usted quiere hablar con alguien que no es un investigador:

Dolores Lopez
Fundacion Cimas del Ecuador
Teléfono: (02) 245 2509
Email: dlopez@cimas.edu.ec
Usted recibirá una copia de este formulario para su record.

Nº de la Vivienda	☐☐	☐☐	☐☐☐	URBANO
		☐☐	☐☐☐	RURAL

DECLARACION DE CONSENTIMIENTO

Titulo del estudio: Efectos de la Exposición Secundaria Ocupacional de Pesticidas en el Crecimiento y Desarrollo Neurológico y conductual de la Niñez.

He leído la información de este documento. He hecho preguntas y he recibido respuestas. Yo doy consentimiento a participar en este estudio:

____
Nombres Completos

X_________________________ Fecha ___________
Firma

____
Nombre de Persona obteniendo consentimiento

X____________________________________ Fecha ___________
Firma Persona obteniendo consentimiento

PERMISO DE RECONTACTO

Este estudio está interesado en realizar un seguimiento en el futuro. Quisiéramos obtener su permiso de contactarle nuevamente para solicitar su participación para este u otros estudios relacionados. Aceptar esto no le obliga a participar en el futuro. Usted mantiene el derecho de no participar en estudios en el futuro y su decisión no afectara sus relaciones actuales o futuras con la Fundación Cimas del Ecuador, la Universidad de Minnesota o el Consejo Cantonal de Salud de Pedro Moncayo.

Declaración de re-contacto

He leído la información de este documento y autorizo a que se me contacte a mí y a mi niño(s) en el futuro para participación en este u otros estudios. Nosotros mantenemos el derecho de no participar en estudios en el futuro.

____
Nombres Completos

X__________________________ Fecha ___________
Firma

____
Nombre de Persona obteniendo consentimiento

____________________________________ Fecha ___________
Firma Persona obteniendo consentimiento

Appendix 2b. Consent Form for Flower Workers (English)

Flower worker´s code (if not the parents):
[ ][ ][ ][ ][ ]

House Number	[ ][ ]	[ ][ ]	[ ][ ][ ]	**URBAN**
		[ ][ ]	[ ][ ][ ]	**RURAL**

Original language: Spanish

Flower Worker Consent Form

Study title: Effects of Secondary Occupational Pesticide Exposure on Childhood Neurobehavioral Development and Growth

You are invited to be in a research study of the effects of parental pesticide exposure from work in flower plantations on children's mental development and growth in Pedro Moncayo County.

We ask that you read this document and ask any questions you may have before agreeing to be in the study.

This study is conducted by Dr. Jose R Suarez Lopez, member of Fundacion Cimas del Ecuador, University of Minnesota, USA and the Health Area Tabacundo. This investigation is considered a priority by the Pedro Moncayo County Health Council.

Purpose:

The purpose of this study is to evaluate the effects of pesticide contamination of children of flower plantation workers on childhood growth and mental and behavioral (neurobehavioral) development.

Procedure:

If you agree to be in this study, we will ask you to do the following:

1) You will be asked to participate in a survey to obtain demographic (e.g. sex, race, age), socio-economic (e.g. education, occupation) and health information of you and your family, as well as information regarding pesticide exposure

Risks of Being in the Study:

The risks associated with your participation in this study are minimal . The possible risks associated with this study are:

1) Confidential data of the participants may be lost due to data theft or mishandling. Appropriate protections against these risks will be taken such as training of researchers in data security and the name of the participant will not be written on the data collection sheet. The information gathered will be managed with strict confidentiality.

Benefits of Being in the Study:

This investigation may not benefit you or the children living with you directly. This study may benefit children with medical conditions detected in this study. Parents of children with detected medical problems will be informed.

If we find negative effects of parental pesticide exposure from work in flower plantations on the development of children, this study could lead to the development of interventions to prevent or reduce negative developmental effects on children living with flower plantation workers.

Costs of Study:

Participating in this study will not cost you anything more than your time.

Compensation:

You will receive no compensation for participating in this study.

Confidentiality:

The collected information will be used for research and action purposes only. Information with identifying information (e.g. names, last names, telephone numbers, address) will not be shared with any external institution. The information for research use will also be removed from identifying information and in exchange participants will receive a study number. The file that contains both names and identification numbers will be kept in a password protected and encrypted file, stored in a password protected computer accessible only to authorized research staff.

All examiners, interviewers and researchers have completed data confidentiality and data security training.

Voluntary Nature of the Study:

Your participation in this study is voluntary. Your decision whether or not to participate or consent to your child’s participation will not affect your current or future relations with Fundacion Cimas del Ecuador, University of Minnesota or the Pedro Moncayo Health Council.

Contacts and Questions:

The researcher conducting this study is:

Jose R Suarez Lopez, MD, MPH, PhD (c)

You may ask any questions you have now.

If you have questions later, you may contact the researchers at

Dr. Jose Ricardo Suarez
Researcher
Fundacion Cimas del Ecuador
Phone: (02)245 2300
(02)245 2509
Email: suar0026@umn.edu

If you want to talk to someone other than the researchers

Dolores Lopez
Fundacion Cimas del Ecuador
Phone: (02) 245 2509
email: dlopez@cimas.edu.ec

You will be given a copy of this form to keep for your records.

Mother´s or Father´s code:

Statement of Consent:

I have read the information in this document. I have asked questions and have received answers. I consent to participate in the study.

Name ___________________________

Signature_________________________ Date ___________

Name of Person Obtaining Consent ____________________________________

Signature Person
Obtaining Consent_________________________ Date ___________

Mother´s or Father´s code:
☐☐☐☐

Re-contact Permission

Study title: Effects of Secondary Occupational Pesticide Exposure on Childhood Neurobehavioral Development and Growth

This study is also interested in conducting a follow up in the future. We would like to obtain your permission to contact you once again to request for your participation for this and other related studies. Agreeing to this does not bind you to participating in the study in the future. You still reserve the right to not participate in future studies and your decision will not affect your current or future relations with Fundacion Cimas del Ecuador, University of Minnesota or the Pedro Moncayo Health Council.

Statement of re-contact

I have read the information in this document and I authorize that my child (children) and I be contacted in the future to participate in this and other studies. We keep the right to not participate in future studies.

Name ___________________________

Signature_________________________ Date ___________

Name of Person Obtaining Consent____________________________________

Signature of Person
Obtaining Consent _________________________ Date ___________

Appendix 3a. Assent Form for Children (Spanish)

Código de Niño: ☐☐☐☐☐

Nº de la Vivienda	☐☐ ☐☐ ☐☐☐	**URBANO**
	☐☐ ☐☐☐	**RURAL**

Formulario de Asentimiento del Niño.

Titulo del estudio: Efectos de la Exposición Secundaria Ocupacional de Pesticidas en el Crecimiento y Desarrollo Neurológico y conductual de la Niñez.

Investigador Principal: Dr. Jose Ricardo Suarez
Numero de teléfono: (02) 245-2300
(08) 401-5028

Objetivo (Porque estas aquí?)

Quisiéramos saber si la contaminación por pesticidas introducidos al hogar por trabajadores en las plantaciones florícolas tiene un impacto en el crecimiento, pensamiento y comportamiento de los niños.

Procedimientos: (Que pasará?)

Quisiéramos examinarte para medir que estatura tienes, cuanto pesas, cuan grande es tu cabeza, cuan rápido late tu corazón, cual es tu presión arterial, y examinar como piensas y te comportas. También te pediremos que orines en una taza para recolectar un poco de orina y quisiéramos obtener 1 gota de sangre con un pinchazo pequeño en tu dedo.

Riesgos: (Dolerá?)

El pinchazo en el dedo puede doler un poquito. El pinchazo durará menos de 1 segundo. Las otras pruebas son seguras y no te dolerán en lo absoluto.

Beneficios: (Como puede ayudarte?)

Este estudio puede no beneficiarte. Este estudio puede beneficiar a los niños que tienen ciertas enfermedades que estaremos evaluando. La información que obtengamos puede ayudar a otros niños en el futuro.

Procedimientos alternos. (Puedes decir "No"?)

Si. No tienes que realizar las pruebas si no quieres. Puedes parar durante cualquier tiempo si tú quieres. No tienes que responder ninguna pregunta o hacer algo que te haga sentir incómodo o triste. Nadie se molestará contigo si dices que “no” o si dices que “si” y luego cambias de parecer.

Compensación

Para agradecerte por tu participación, recibirás un pequeño regalo (por ejemplo crayolas o plastilina o un carrito de juguete, o cosas similares) por participar en el estudio.

Si tienes preguntas puedes hacerlas ahora

Tú has sido informado acerca del estudio
Tú has sido informado acerca de lo que tendrías que hacer
Tú has sido informado que no tienes que hacer ninguna de las pruebas si no quieres.
Tú has sido informado que puedes parar en cualquier momento que quieras, inclusive después de que hayas empezado tu participación.

____
Nombre del Niño(a)

X_______________________________ __________________
Firma del Niño(a) Fecha

____
Nombre de Persona obteniendo Asentimiento

X____ __________________
Firma de la Persona Obteniendo Asentimiento Fecha

Appendix 3b. Assent form for Children (English)

Child's code:	☐☐☐☐

House Number	☐☐	☐☐	☐☐☐	**URBAN**
		☐☐	☐☐☐	**RURAL**

Original language: Spanish

Child Assent Form

Study title: Effects of Secondary Occupational Pesticide Exposure on Childhood Neurobehavioral Development and Growth

Principal Investigator: Dr. Jose Ricardo Suarez
Phone number: (02) 245-2300
(02) 245-2509

Objective (Why are you here?)

We would like to find out if pesticide contamination brought home by flower plantation workers has an impact on growth, thought and behavior of children.

Procedures: (What will happen?)

We would like to examine you to measure how tall you are, how much you weigh, how big is your head, how fast your heart beats, what is your blood pressure, and to test how you think and behave. Also, we will ask you to pee in a cup to collect some urine and we will obtain 1 drop of blood from a small prick on your finger.

Risks: (Will it hurt?)

The finger prick could hurt a little. The finger prick will last for less than 1 second. The other tests are safe and will not hurt.

Benefits: (How may it help you?)

This study may not benefit you. This study may benefit children that have certain diseases that we will be testing. The information we learn may help other children in the future.

Alternative Procedures (Can you say "No"?)

Yes. You do not have to do the tests if you do not want to. You can stop at any time you want. You do not have to answer any question nor do anything that makes you feel uncomfortable or sad. No one will be upset with you if you say “no” or if you say “yes” and then change your mind.

Compensation

To thank you for your participation, you will receive a small gift (for example crayons or play dough or a miniature car, or similar items) for participating in the study.

You may ask any questions that you may have now

You have been told about the research study.
You have been told what you would have to do.
You have been told that you do not have to do any of the tests if you do not want to.
You have also been told that you can stop any time you want, even after you begin.

____
Name of Child

____ __________________
Signature of Child Date

____
Name of Person obtaining Assent

____ __________________
Signature of Person Obtaining Assent Date

Child’s code:					

Appendix 4. Pre-Survey

Appendix 4. PRE-Survey: Estudio del Efecto de la Exposición Secundaria a Plaguicidas en el Crecimiento y Desarrollo Neuro-conductual de los Niños

No. de Casa: ______________ No. Nuevo de Casa ______________ **Teléfonos:** ________________________ **Seleccione: Expuesto / NO Expuesto**

TAMIZAJE: PREGUNTAS EXCLUSIVAMENTE DE NIÑOS SELECCIONADOS														
	NOMBRES DE LOS NIÑOS SELECCIONADOS			Edad	Fecha N			Convivencia con Floricultor				Exposición a Plaguicidas		
	¿Cuál es el nombre completo del NIÑO SELECCIONADO?			Cuantos años cumplido tiene?	¿Cuál es la Fecha de Nacimiento?			Ha vivido alguna vez con un trabajador Floricultor en el pasado? No.... Si → Por cuánto tiempo?			Qué edad tenía el NIÑO cuando EMPEZÓ a vivir con el trabajador floricultor?	Han almacenado plaguicidas para fumigación en el hogar? No ....0 Si 1	Ha estado el niño en contacto con plaguicidas para agricultura? No ....0 Si 1	Se ha intoxicado o enfermado el niño con plaguicidas? No ....0 Si 1
				S1	S2			S3	S4		S5	S6	S7	S8
Código	Apellidos		Nombres	Año	Día	Mes	Año	No/Si	Años	Mes	---------	No/Si	No/Si	No/Si
	1													
	2													
	3													
	4													

	N.1 ¿Tiene el NIÑO(A) SELECCIONADO alguna enfermedad del: Hígado, corazón, otro: especifique	**N.2** ¿Está tomando el NIÑO SELECCIONADO medicamentos? NO ... SI .... ¿Cual?	**N.3** ¿Va el niño a una guardería dentro de una floricultora? NO....... SI..... ¿Cual?
1			
2			
3			
4			

Cuantos trabajadores floricultores viven en esta casa actualmente? ______

Como se llaman los floricultores? 1)____________________________________ 2)____________________________________

3)____________________________________ 4)____________________________________

Incluir **EXPUESTOS** si: (todas deben estar presentes)

a) S1 es mayor a 3 años y menor a 10 años
b) S3 es "Si"
c) S4 es mayor a 2 años
d) S5 es menor a 4 años

Incluir **NO EXPUESTOS** si: (todas deben estar presentes)

a) S1 es mayor a 3 años y menor a 10 años
b) S3 es "No"
c) S6 es "No"
d) S7 es "No"
e) S8 es "No"

Appendix 5. Survey (Spanish)

. Fundación Cimas del Ecuador

Appendix 5. Estudio de los Efectos de la Exposición Secundari
a Plaguicidas en Infantes y Niños (ESPINA)

University of Minnesota

Nº Encuesta

ENCUESTA PLAGUICIDAS
Floricultor

INFORMACIÓN GENERAL

1. IDENTIFICACIÓN DEL HOGAR

Nº de la Vivienda		URBANO
		RURAL

2. UBICACIÓN GEOGRÁFICA INEC

Parroquia	
Barrio / Comunidad:	

3. NOMBRE DE LOS NIÑOS(S) SELECCIONADOS EN EL HOGAR

Primer Nombre	Primer Apellido	CÓDIGO DEL NIÑO

4. DATOS DEL INFORMANTE

#1) Primer Nombre	Primer Apellido	¿Relación con niño?
#2) Primer Nombre	Primer Apellido	¿Relación con niño?

5. RESULTADO DE SEGUIMIENTO DE LA ENTREVISTA

Razón	Marcar con una "X"
1. Completa	
2. Rechazo	
3. Nadie en Casa	
4. Vivienda Desocupada	
5. Vivienda inhabitable / destruida	
6. Otra Razón	

6. PERSONAL RESPONSABLE

Nombre:	Código
Encuestador:	
Supervisor:	
Fecha: Año: Mes: Día:	

7. OBSERVACIONES

	SECCIÓN I. REGISTRO DE MIEMBROS DEL HOGAR					
TIPO	NOMBRES DE LOS MIEMBROS					
Escriba "F" si es Trabajador Floricultor ↓	¿Cuál es su nombre completo?					
			Apellidos		Nombres	
↓	F1		Paterno	Materno	Primero	Segundo
	Mamá	1				
	Papá	2				
	Niño	3				
	Niño	4				
	Niño	5				
	Niño	6				
	Otro	7				
	Otro	8				
	Otro	9				

SECCIÓN II. INFORMACION DEMOGRAFICA									
SEXO	EDAD	PARENTESCO	Raza	Trabajo de ADULTOS	Solo Floricultores		ESTADO CIVIL	NIVEL DE INSTRUCCIÓN	
Masc....M Femen...F	¿Cuántos años tiene?	¿Qué relación de parentesco tiene con el NIÑO SELECCIONADO? Niño Selección ......1 Padre..............2 Madre ....3 Hermano.............4 Primo ....5 Tío ....6 Otros parientes.....7 Otro no pariente....8	¿A qué grupo étnico pertenece? Indígena..............1 Mestizo/a.............2 Afro ecuatoriano......3 Mulato/a..............4 Blanco/a..............5 Otro, cuál?...........6	¿Que ocupación tiene? Ninguno ....0 Floricultor/Jornalero ......1 Agricult/Ganad ....2 Quehacer, Domestico.........3 Construcción................4 Comercio....................5 Manufactura/Artesanías......6 Transporte ....7 Estudiante ....8 Otros Cual?.................9 No responde 10	¿Cuánto tiempo ha trabajado en una floricultora en total? Solo Floricultor		¿Cuál es su estado civil o conyugal actual? Casado/a.......1 Separado/a.....2 Divorciado/a...3 Viudo/a........4 Unión libre....5 Soltero/a......6	¿Cuál es nivel y año de instrucción más alto que aprobó? Ninguno.......................1 Centro de alfabetización......2 Jardín de Infantes............3 Primaria......................4 Secundaria....................5 Superior no universitaria.....6 Superior universitaria........7 Post grado....................8	
	Años				Años	Mes		Nivel	Año Aprobado
D1	D2	D3	D4	D5	D6a	D6m	D7	D8	
	No			No	No	No	No	No	No
	No			No	No	No	No	No	No
	No			No	No	No	No	No	No
	No			No	No	No	No	No	No

Número de persona	SECCION IV. TABAQUISMO			SECCIÓN IV. SOLO MADRES Embarazo (Llenar para cada niño seleccionado)										
	Fuma? NO ... SI.....	¿Cuántos cigarrillos fuma por día?	Fumaba la madre durante el embarazo del niño (...nombre...)? NO ... SI	¿Fue la madre expuesta a plaguicidas DURANTE EL EMBARAZO del niño (.....)? NO ... SI......	¿Trabajó la madre en una floricultora DURANTE EL EMBARAZO del niño (.......)? NO ... SI......	¿Vivió la madre con un floricultor DURANTE EL EMBARAZO del niño (.....)? NO ... SI......			¿Almacenó plaguicidas en la casa para sembrar papas, etc. DURANTE EL EMBARAZO del niño (.....)? NO ... SI......	¿Cuántos embarazos ha tenido?	¿Cuántos hijos nacidos vivos ha tenido?	¿Cuántos arrojos o abortos ha tenido? Si no ha tenido abortos, registre 00	Tuvo complicaciones en su último embarazo? NO......0 SI.........1 Presión alta/pre. Eclampsia.......a Sangrado excesivo.....................b Parto pre-término.....................c Diabetes gestacional..................d Infección de niño o madre.........e Niño bajo de peso al nacer.........f Otro, (escriba) ....g Cod	¿Cuál?
	T1	T2	T3	M1	M2	M3			M6	X1	X2	X3	X4	X5
1			Llenar la información de estas preguntas para cada niño seleccionado											
2			Llenar la información de estas preguntas para cada niño seleccionado							No	No	No	No	
3	No	No								No	No	No	No	No
4	No	No								No	No	No	No	No
5	No	No								No	No	No	No	No
6	No	No								No	No	No	No	No
7			No	No	No	No	No	No	No					
8			No	No	No	No	No	No	No					
9			No	No	No	No	No	No	No					

SECCIÓN VI. EXPOSICION GENERAL Circule la respuesta
G1. Cuantas personas fuman en el hogar?
G2. Cuantos trabajadores floricultores viven en esta casa actualmente?
G3. Cuál es el ingreso económico familiar cada mes? A=$0-50 B=$51-150 C=$151-300 D=$301-500 E=$501-1000 F=1000-2000 G=$2000+ H= No sabe/no responde
G4. Se usan plaguicidas para fumigar en su propiedad de residencia (hogar, terreno)? 0 No / 1 Si
G5. Si la respuesta es SI, para que actividad usan los plaguicidas ustedes? ← **Ej. <u>Escribir</u> si siembran papas o frejol, etc.**
G6. Usa plaguicidas para matar a parásitos en sus animales? 0 No / 1 Si
G7. Ha estado el niño en contacto con plaguicidas? 0 No / 1 Si
G8. ¿Si la respuesta es SI, cual fue la fuente de la exposición, y en qué año? ← **Donde/Fuente de Exposición** ____ **En qué año?**_______________
G9. ¿Fumigan sus <u>vecinos inmediatos</u> con plaguicidas para agricultura? Ej. Para siembra de papas, frejol, etc. 0 No / 1 Si

3

Número de persona	SECCION VII. SOLO FLORICULTORES									
	LABORES EN LA FLORICOLA							PRACTICAS DE EXPOSICIÓN A PLAGUICIDAS		
	¿Trabaja Actualmente en una Floricultora? No ....0 Si ..... 1 →	¿Qué cargo ocupa ACTUALMENTE? Cosecha ....a Post cosecha ...b Fumigador ....c Clasificador ....d Empacador ......e Bodeguero ....f Riego ....g Otro ....h	¿Cuanto tiempo ha trabajado en una floricultora desde el 2004?		**¿En qué floricultora trabaja Actualmente?**	**¿Con que frecuencia cree usted que está en contacto con plaguicidas en el trabajo?** **Nunca ....1** **Rara Vez............2** **A veces ....3** **Frecuente........4** **Siempre 5**	**¿Con que frecuencia se lava las manos justo <u>ANTES</u> <u>DE SALIR</u> del trabajo?** **Días por semana:** **Nunca ....1** **1-2 Días............2** **3-4 Días ....3** **Siempre 4**	Solo para aquellos que se lavan las manos 2 o menos días por semana ¿Porque se lava las manos tan poco al salir del trabajo? Se olvida ....1 Pocos lavabos ....2 No hay agua ....3 No es necesario ....4 Otro, Cual? 5		**¿Con que frecuencia lleva los zapatos que usó en el trabajo a la casa?** **Días por semana:** **Nunca ....1** **1-2 Días............2** **3-4 Días ....3** **Siempre 4**
	Cod		Años	Meses				Cod	Cual	
	P1	**P2**	**P3**		**P4**	**P5**	**P6**	**P7**	**P8**	**P9**
1										
2										
3	No	No	No	No	No	No	No		No	No
4	No	No	No	No	No	No	No		No	No
5	No	No	No	No	No	No	No		No	No
6	No	No	No	No	No	No	No		No	No
7										
8										
9										

4

SECCION VII. SOLO FLORICULTORES

PRACTICAS DE EXPOSICIÓN A PLAGUICIDAS

Número de persona	¿Cuántos días a la semana se BAÑA EN EL TRABAJO antes de salir a la casa? Días por semana: Nunca ....1 1-2 Días ....2 3-4 Días ....3 Siempre 4		Solo para aquellos que se bañan 2 o menos días por semana Cod	Porque se baña tan poco al salir del trabajo? Se olvida ....1 No hay duchas..............2 Hay pocas duchas ....3 No hay agua ....4 No es necesario ....5 Otro, Cual? .... 6 ¿Otro, cual?	¿Le exigen que se ponga ropa de trabajo? NO ....0 SI 1	¿Con que frecuencia usa las siguientes prendas de TRABAJO? Siempre.......S A veces ....A Nunca ....N 1 BOTAS	2 MANDIL	3 PANTALON	4 OVEROL	5 GUANTES	6 GORRA	MASCARILLA	8 GAFAS	¿Cuántos días a la semana se cambia la ropa que usó en el trabajo ANTES DE SALIR del trabajo? Días por semana: Nunca ....1 1-2 Días ....2 3-4 Días ....3 Siempre 4	¿Con que frecuencia lleva la ropa de trabajo a la casa? Días por semana: Nunca ....1 1-2 Días ....2 3-4 Días ....3 Siempre 4	¿Donde lava la ropa de trabajo? Casa ....1 Contrata Lavandera......2 Floricultora ...3 Otro, Cual?......4	¿Trae usted herramientas de trabajo a la casa? (Tijeras, palas, baldes, equipos, plásticos, etc.) NO ....0 SI ....1 Cod	¿Cuales?
	P10		P11	P12	P13	P14								P15	P16	P17	P18	P19
1																		
2																		
3	No	No		No	No			No						No	No	No		No
4	No	No		No	No			No						No	No	No		No
5	No	No		No	No			No						No	No	No		No
6	No	No		No	No			No						No	No	No		No
7																		
8																		
9																		

Appendix 6. NEPSY-II: General Battery of Tests

Ages 3–4

Design Copying

Comprehension of Instructions

Geometric Puzzles

Narrative Memory

Speeded Naming

Statue

Visuomotor Precision

Ages 5-9

Speeded Naming

Auditory Attention and Response Set

Memory for Faces

Design Copying

Inhibition

Memory for Faces Delayed

Comprehension of Instructions

Geometric Puzzles

Narrative Memory

Statue (5–6 years)

Word List Interference (7–16 years)

Visuomotor Precision (5–12 years)

Appendix 7. Description of NEPSY-II subtests.

Subtest	Description	Concepts measured
Attention and Executive Functioning Domain		
Auditory Attention and Response Set		
AARS: Auditory Attention	Children are asked to touch a red circle when they hear the word "red" amidst consistent distracter words.	Selective and sustained auditory attention
AARS: Response Set	Children are asked to touch a red circle when they hear the word yellow and viceversa, but when they hear the word blue, they are asked to touch the color blue.	Complex attention and inhibition of a previously learned stimuli in lieu of new stimuli.
Inhibition		
IN: Naming	Naming of shapes (circles or squares) or direction of arrows (up or down).	Naming speed
IN: Inhibition	Naming the opposite shapes or direction of arrow (e.g. saying circle when seeing a square).	Inhibitory control
IN: Switching	Naming depends on color. White figures are named the correct shape name or arrow direction. Black figures are called the opposite shape name or opposite arrow's direction.	Inhibitory control and cognitive flexibility
Statue	Child must remain immobile with eyes closed for 75 seconds without responding to sound distracters.	Inhibitory control and motor persistence
Memory and Learning Domain		
Memory for Faces Immediate	Child looks at a set of pictures of faces. Immediately afterwards, the child is asked to select a previously seen face during various trials that include 3 faces (2 of which have not been shown previously).	Visual memory, discrimination and encoding of faces
Memory for Faces Delayed	Trials of face recall occur 15-20 minutes after the initial set of pictures of faces was	Long term visual memory and recognition of faces

	shown.	
Narrative Memory	Child hears a short story and is asked to recall it. Child is then asked questions regarding missing components of the recalled story.	Memory, comprehension, expressive language
Word List Interference		
WI: Repetition	Child listens to two sets of words (2-5 words) and is asked to immediately repeat each set after it has been read to him.	Memory, receptive and expressive language
WI: Recall	Child listens to two sets of words (2-5 words) and is asked to recall both sets after repetition of the sets.	Memory, word recall after interference
Visuospatial Processing Domain		
Design Copying	Copying of a set of two-dimensional figures.	Visuospatial and visuomotor ability
Geometric Puzzles	Matching of figures outside a grid to those inside a grid.	Mental rotation, visuospatial analysis, attention to detail
Language Domain		
Comprehension of Instructions	Child must follow verbal instructions of increasing complexity.	Receptive language, semantic and sequential processing
Speeded Naming	Child names shapes, size and color or letters/numbers as quickly as possible from a list.	Processing speed, lexical access, expressive language
Sensorimotor Domain		
Visuomotor Precision	Child quickly draws a line inside of tracks of increasing complexity.	Graphomotor skills, precision, visuomotor coordination

Appendix 8. Exam Results

Estudio de la Exposición Secundaria a Plaguicidas en Infantes y Niños (ESPINA)
Dr. José Ricardo Suárez. Tel: 245-2300 (08) 401-5028 Email: suarjo@hotmail.com

Quito, 12 de Noviembre de 2008

Nombre:	**Fecha Nacim (mes/día/año):**	**Fecha Examen (m/d/a):**
Codigo Estudio: **Tel:**	**No. de Casa:** **Parroquia:**	**Barrio:**

A su niño(a) se le realizaron exámenes para evaluar su crecimiento, nutrición, exposición a plaguicidas y desarrollo mental y de la conducta. Los resultados de estos se encuentran a continuación.

1. Crecimiento: Su niño(a) tiene un PESO NORMAL para su edad y talla, con un peso de 21kg, una talla de 116.4cm y un valor Z de IMC para la edad de -0.19. Además, tiene una TALLA BAJA para su edad con un valor Z de -2.11. Recomendamos que su niño(a) sea evaluado por un médico y/o nutricionista. El crecimiento del niño está principalmente ligado a la nutrición. Un niño con una nutrición balanceada en calorías totales, proteínas, carbohidratos, grasas y vitaminas no solo crece más y mejor, sino que está menos propenso a enfermarse. El sobrepeso (peso alto) en la niñez es un factor de riesgo para diabetes, presión alta, arteriosclerosis/problemas del corazón y circulatorios, entre otros.

2. Presión Arterial: Tiene un valor promedio de **83 / 47 mm/hg**. La presión arterial de su niño(a) **es normal para su edad.**

3. Hemoglobina: Tiene un valor de **11.7 mg/dl. Este valor es BAJO para su edad, es decir que tiene anemia. Recomendamos que acuda a su médico para que evalúe esta condición.** El nivel de hemoglobina es un indicador de la habilidad de células de la sangre de llevar oxigeno a los tejidos del cuerpo, lo cual es muy importante para su desarrollo. Generalmente refleja el nivel de nutrición particularmente en hierro, acido fólico, vitamina B12 y otros nutrientes. Para corregir la anemia (baja hemoglobina) se recomienda aumentar el consumo de productos que tengan alto contenido de estos nutrientes, como por ejemplo vegetales de hojas verdes, hígado, carne, pollo, pescado, germen de trigo, fruta, y combinarlos con frutas ricas en vitamina C, como limón o naranja, los cuales ayudan a la absorción del hierro. También se pueden tomar suplementos vitamínicos.

4. Acetilcolinesterasa ajustada a hemoglobina: Tiene un valor de **23.4. Este valor es NORMAL.** La acetilcolinesterasa es una enzima que está encargada de metabolizar la acetilcolina, la cual es una sustancia que está encargada de regular una gran cantidad de procesos en el organismo. Esta enzima se inhibe por acción de plaguicidas organofosforados y carbamatos. Las principales maneras por donde ingresan los plaguicidas al organismo son la piel, pulmones y tracto gastrointestinal. Las fuentes principales de introducción de plaguicidas al hogar son a través de trabajadores agricultores que están expuestos a plaguicidas en su trabajo, como por ejemplo trabajadores en plantaciones florícolas o agricultores que cultivan frejol, papas, etc. En estos casos la mejor manera de reducir la introducción de plaguicidas al hogar es la de dejar la ropa de trabajo en el trabajo, esto incluye los zapatos. Es decir no ingresar al hogar con ninguna prenda que estuvo en contacto con plaguicidas. Para trabajadores expuestos a plaguicidas: Si su trabajo no lava su ropa de trabajo, asegúrese de que se lave la ropa de trabajo separada del resto de ropa, usando guantes y preferiblemente con agua tibia. Antes de tener contacto con la familia, el trabajador deberá lavarse las manos y bañarse antes de saludar a la familia dado a que muchos plaguicidas permanecen en la piel y el pelo. Otro problema grave es el uso de plaguicidas en parcelas junto a su hogar. Si su vecino siembra productos como papas y frejol, los cuales tienden a ser fumigados, le recomendamos que hable con su vecino para que no use plaguicidas tan cerca a su hogar. Puede acercarse al Departamento de Control Sanitario del Centro de Salud de Tabacundo para exponer su problema si esto persiste.

5. Prueba Neuro-Conductual NEPSY-II. Esta prueba ayuda a evaluar dificultades sociales y del comportamiento en niños. Los resultados obtenidos en la prueba pueden ser utilizados para diagnosticar y ayudar en la planificación de la intervención de una variedad de trastornos.

Para evaluar el desempeño de su niño(a) en el NEPSY-II, revice la Tabla *de Descripción de Clasificación* y el *Gráfico de la Curva Normal* que se encuentran abajo. Estas herramientas le servirán como una guía para obtener un entendimiento general del desempeño de su niño(a) en la prueba. La *Tabla de Descripción de Clasificación* es una descripción de cada dominio en el que su hijo(a) fue evaluado. Al lado de esto, hay un resumen del desempeño de su hijo(a) en cada uno de los dominios. **Si su niño(a) tiene problemas "bastante por debajo del nivel esperado", le recomendamos que acuda a un psicólogo para su evaluación.**

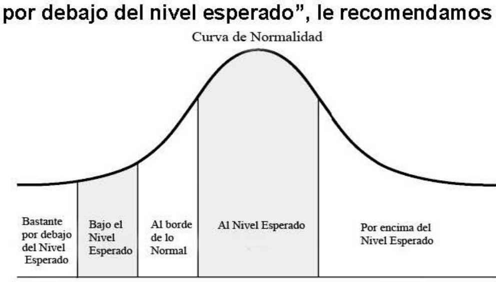

Tabla de Descripción de Clasificación	
Clasificación	**Descripción**
Encima del Nivel Esperado	Niños cuyos resultados caen dentro de este rango tienen destrezas más desarrolladas que 75% de otros niños
Al Nivel Esperado	Niños cuyos resultados caen dentro de este rango tienen destrezas que son iguales al 50% de otros niños.
Borde de lo Normal	Niños cuyos resultados caen dentro de este rango tienen destrezas menos desarrolladas que 75% de otros niños.
Bajo el Nivel Esperado	Niños cuyos resultados caen dentro de este rango tienen destrezas menos desarrolladas que 90% de otros niños.
Bastante por Debajo del Nivel Esperado	Niños cuyos resultados caen dentro de este rango tienen destrezas menos desarrolladas que 98% de otros niños.

Resumen de Resultados		
Dominio	**Descripción**	**Resultado**
Atención y Funcionamiento Ejecutivo	Estas pruebas miden que tan bien el niño(a) puede planificar, organizar, cambiar y controlar su comportamiento.	El desempeño de su niña(o) en este domino se encontro al Nivel Esperado
Lenguaje	Estas pruebas miden que tan bien el niño(a) puede entender y usar palabras y oraciones para comunicarse con otros.	El desempeño de su niña(o) en este domino se encontro al Nivel Esperado
Memoria y Aprendizaje	Estas pruebas miden como el niño(a) integra, almacena y recuerda información.	El desempeño de su niña(o) en este domino se encontro al Nivel Esperado
Sensorimotor	Estas pruebas miden que tan bien el niño puede controlar movimientos de la mano.	El desempeño de su niña(o) en este domino se encontro al Nivel Esperado
Procesamiento Visuo-espacial	Estas pruebas miden que tan bien puede el niño ver y organizar información visual	El desempeño de su niña(o) en este domino se encontro al Borde de lo Normal