FINAL PROGRESS REPORT

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TABLE OF CONTENTS

LIST OF TERMS AND ABBREVIATIONS

AChE – Acetylcholinesterase

AEF – Attention and Executive Functioning

BMI – Body mass index

DBP – Diastolic blood pressure

ESPINA – The Effects of Secondary Pesticide Exposure on Infants Children and Adolescents (Spanish: Efectos de la Exposición Secundaria a Plaguicidas en Infantes, Niños y Adolescentes)

FW – Flower worker

ML- Memory and Learning

OR – Odds ratio

SAHS-PM – Survey of Access and Demand of Health Services – Pedro Moncayo County

SBP – Systolic blood pressure

SD – Standard deviation

SES- Socio economic status

WHO – World Health Organization

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 pesticide intoxication in early childhood delays growth and neurodevelopment. Objectives: The Effects of Secondary Pesticide Exposure on Infants, Children and Adolescents (ESPINA) study evaluated the effects of secondary pesticide exposure on childhood growth and neurobehavioral development among children living in a county with an active Fresh-cut flower industry. The following hypotheses were addressed: compared to children without secondary pesticide exposure (operationalized as flower worker (FW) cohabitation), exposed children will have 1) lower acetylcholinesterase (AChE) concentration, 2) lower neurobehavioral development scores, 3) slower growth, 4) higher systolic blood pressure, and 5) lower resting heart rate. *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. In 2008, ESPINA examined children 4 to 9y living in the County to obtain anthropometric measurements, a neurobehavioral developmental assessment and hemoglobin and AChE measurements. Most of the children examined in 2008 were also examined in the SAHS-PM-2004. *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, head circumference, heart rate, blood pressure and blood AChE levels; and 3) a longitudinal component (2004-2008) to assess growth. *RESULTS*: a) *Participant Characteristics:* 922 (51% female) children were included from the SAHS-PM 2004; the mean age was 2.3 y (standard deviation (SD): 1.4), 63% cohabited with ≥1 FW with a mean of 2.0 FWs at home. In 2008, 313 (49% female, 78% mestizo) children participated; the mean age was 6.6y (SD=1.6), and 55% of participants cohabited with ≥1 FW. FW cohabitants had a mean duration of cohabitation of 5.3 years and a mean of 1.5 FWs at home. 230 (49% female, 74% mestizo) children were examined in 2004 and 2008; the mean age was 6.4 years (SD=1.4) and 59% cohabited with ≥1 FWs. FW cohabitants had a mean duration of cohabitation of 5.1 years and a mean of 1.5 FWs at home in 2008. *b) Flower worker cohabitation and AChE concentration:* After adjustment for gender, age, height-for-age, hemoglobin concentration, parish of residence, income, pesticide use within household lot and pesticide use by contiguous neighbors, children cohabiting (vs. not) with a FW had a lower mean of AChE by 0.10 U-ml (p=0.049). Their adjusted odds ratio (OR) for low AChE (≤2.4 U/ml) was 2.84 (95%CI: 1.09-7.39). Every year of cohabitation was associated with an adjusted AChE decrease of 0.02 U/ml (p=0.038). *c) Secondary Pesticide Exposure and Neurobehavioral Development:* Children's cohabitation with a FW was associated with a decrease of 1.17 units (p=0.020) in the Auditory Attention score (Attention and Executive Functioning (AEF) Domain) after adjustment for gender, age, race, hemoglobin concentration, height for age, maternal education, income and parish of residence. Every year of FW cohabitation was associated with an adjusted decrease of 0.15 units (p=0.055) of Auditory Attention. FW cohabitation was associated with an adjusted OR of 2.58 (95%CI 1.24-5.38) of having low Auditory Attention scores. Every unit decrease in AChE was associated with an adjusted decrease of 1.07 units

(p=0.057) of Inhibition (AEF Domain). Participants with AChE concentrations in the lowest vs. the highest tertile and middle vs. highest tertile had adjusted ORs of 3.27 (95%CI 1.32-8.12) and 2.35 (95%CI 1.05-5.29) for low Inhibition scores, respectively. For low Memory for Faces Delayed scores (Memory and Learning (ML) Domain), AChE concentrations in the lowest vs. the highest tertile and middle vs. highest tertile were associated with adjusted ORs of 3.59 (95%CI 1.33-9.64) and 2.58 (95%CI 1.05-6.34), respectively. AChE concentrations in the lowest vs. the highest tertile had an adjusted OR of 4.05 (1.02-16.16) for low Word List Interference Repetition scores (ML Domain). *d) Secondary Pesticide Exposure and Growth:* In 2004 and 2008, FW cohabitation was not associated with height-for-age after adjusting for age, gender, race, parish of residence and socio-economic variables (2004: household head education, type of home, home roof materials, home floor materials, type of wastewater disposal; 2008: income and maternal education). The number of FWs cohabiting with the participant (2004 and 2008), AChE concentration and duration of cohabitation (2008) had no adjusted effect on height-for-age, weight-for-age or BMI-for-age. Also, FW cohabitation was not associated with change between 2004 and 2008 in these three measures. *e) Secondary Pesticide Exposure and Blood Pressure and Resting Heart Rate.* Concurrent FW cohabitation was associated with a systolic blood pressure (SBP) decrease of 2.08 mmHg (p=0.029) after adjustment for age, gender, height-for-age, resting heart rate, hemoglobin concentration, parish of residence, and income. Duration of cohabitation was inversely related to SBP, with an adjusted decrease of 0.34 mmHg (p=0.032) per year of cohabitation. AChE was directly associated with diastolic blood pressure (DBP). Every SD (0.49 U/ml) of AChE was associated with a change of 1.56 mmHg (p=0.002) in DBP. AChE was not associated with systolic blood pressure or pulse pressure but was associated with a mean blood pressure change of 1.26 mmHg (p=0.005) per SD. Resting heart rate was not associated with FW cohabitation or AChE concentrations. *CONCLUSIONS*: 1) Indirect pesticide exposure from FW cohabitation suffices to depress acetylcholinesterase concentration, with greater suppression due to longer exposure. 2) FW cohabitation was associated with lower Auditory Attention scores, with a significant decrease with increasing length of cohabitation. AChE was inversely related to Inhibition (Attention and Executive Functioning Domain), Memory for Faces Delayed (Memory and Learning), and Word List Interference Repetition (Memory and Learning). These findings are in line with previous research where children exposed to pesticides had lower scores on attention, inhibition of active behavior, short-term memory, learning and motor development. 3) We found no association between FW cohabitation and AChE with height-for-age, weight-for-age or BMI-for-age in this population. These growth measures were mainly affected by SES. 4) FW cohabitation and duration of cohabitation were associated with a decrease in SBP which remained present even after adjusting for AChE. This may suggest a role of secondary exposure to non-cholinesterase inhibiting pesticides on SBP and/or the presence of additional behavioral/SES confounders that were not accounted for. AChE has primarily an effect on DBP as evidenced by its direct association with DBP and mean blood pressure but not with SBP or pulse pressure, even after adjustment for resting heart rate. *RECOMMENDATION*: Take home pesticide exposures need to be addressed in greater extent by flower plantations and their workers to reduce the burden of these contaminants on their families.

SECTION 1

1.1 Significant Findings

The amount of indirect pesticide exposure through flower worker (FW) cohabitation on children suffices to depress acetylcholinesterase (AChE) concentration. Increasing length of cohabitation was associated with lower AChE concentration.

Flower Worker Cohabitation was associated with lower Auditory Attention scores, with a significant decrease with increasing length of cohabitation. AChE concentration was inversely related to Inhibition (Attention and Executive Functioning Domain), Memory for Faces Delayed (Memory and Learning), and Word List Interference Repetition (Memory and Learning). Our findings suggest that secondary occupational pesticide exposure can have deleterious impacts on children's neurobehavioral development and is a public health problem. These findings are in line with previous research where children exposed to pesticides had lower scores on attention, inhibition of active behavior, short-term memory, learning and motor development.

FW cohabitation and duration of cohabitation were associated with a decrease in SBP which remained present even after adjusting for AChE. This may suggest a role of secondary exposure to non-cholinesterase inhibiting pesticides on SBP and/or the presence of additional behavioral/SES confounders that were not accounted for. AChE has primarily an effect on DBP as evidenced by its direct association with DBP and mean blood pressure but not with SBP or pulse pressure, after adjustment for various confounders including resting heart rate.

We found no association between FW cohabitation and AChE with height-for-age, weight-for-age or BMI-for-age in this population, in either the 2004 or 2008 cross-sections nor in the longitudinal study. These growth measures were mainly associated with socio-economic status.

1.2 Translation of Findings

Our study found that children cohabiting with a FW have greater amounts of pesticide exposure than children not cohabiting with a FW, and these exposures were associated with blood pressure and neurobehavioral developmental changes.

Our general recommendation is to increase efforts to reduce indirect pesticide exposures and increase health/disease awareness among families of FWs. Periodic health education targeted to FWs and their families, which includes potential short and long term effects of pesticide exposure with emphasis on children's health and preventive medicine, is advised. Additionally, recurrent pesticide exposure reduction education of FWs, their families and flower plantation staff, greater monitoring of take-home pesticide routes by flower plantations and regulating organisms, etc. Further analyses of the routes of indirect pesticide exposure to the families of FWs are needed to provide more detailed recommendations on the emphases of the intervention.

1.3 Relevance and Impact

This project provides insight on the impacts of secondary pesticide exposure on childhood development. Currently, this is one of the largest completed studies that have assessed neurobehavioral development in children indirectly exposed to pesticides. Our results bring insight on the impacts of pesticides on blood pressure and on the neurobehavioral domains of Memory and Learning, and Attention and Executive Function. 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 AChE 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.

This investigation reinforces the importance of reducing take-home pesticide exposures and including family members of flower plantation workers in pertinent pesticide safety and health prevention education.

SECTION 2

2.1 Scientific Report

I. INTRODUCTION

Indirect pesticide exposure from agricultural workers. In spite of the technological revolution of the 20th and 21st centuries, agriculture still relies heavily on a human labor force and pesticides. Agriculture has been the most important user of pesticides[1] and the most important source of occupational pesticide exposure and intoxication. It has been described that not only agricultural workers have a high risk of pesticide contamination,[2,3,4,5,6] but also their family members, usually through indirect routes.[7,8] The common sources of pesticide introduction into the homes are the workers themselves, who have been found to carry pesticides on their clothes, boots, tools, skin, hair, and have even been found to store pesticides at home.[8] 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 chronic exposure to its residents. Pesticide concentrations in household dust of pesticide exposed workers been found to be many times higher than those of non exposed workers.[9,10] Children of agricultural workers exposed to pesticides have been found to have higher pesticide metabolite concentrations than reference children.[9] These chronic pesticide exposures may have deleterious effects on children.

Pesticides: Overview. Organophosphates and carbamates are the most commonly used types of insecticides and their lethal mechanism of action is to inhibit the enzyme acetylcholinesterase (AChE). This enzyme regulates acetylcholine which is an essential neurotransmitter required in cardiovascular, gastrointestinal, respiratory, neuromuscular, thermoregulatory, and behavioral processes.[11] The effects of these pesticides on health can be acute or chronic with particularly grave effects on children.

Pesticides and Child Health. Studies in rodents have found that a crucial part of brain development mediated by acetylcholine occurs in early childhood.[12,13] Brain developmental disruption with cholinesterase inhibitors can lead to neurological abnormalities,[14,15,16,17] possibly at low levels of cholinesterase inhibition, and without symptoms of intoxication. [18,19] Additionally, certain OP pesticides have been found to have neurological effects beyond AChE inhibition. Impacts of AChE inhibitors on the brain include the hippocampus, cerebellum, forebrain and forebrain cholinergic projections to the cortex. Disruption of these areas may affect memory, cognition, behavior and motor coordination.

Although there is 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.[20] 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.[21] 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. [22,23,24]
Few studies have analyzed the effects of pesticides on growth, and the association is unclear. Some studies have found inverse associations between prenatal OP exposure with length and birth weight,[22,25,26] while others did not find such association.[24,27] These discrepancies may be due to differential exposure levels between the populations included in each study, as well as differential methods to characterize the exposure throughout the pregnancy. A disruption of bone formation, bone growth and density has been described in OP exposed individuals.[28,29,30] This finding suggests that chronic low-dose pesticide exposures during infancy and childhood may have an impact on length or height. Secondary occupational exposure to pesticides is an important source of pesticide contamination; few adequately powered studies have analyzed its effects on child development.

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

II. METHODS

Study Description. The Effects of Secondary Pesticide Exposure on Infants, Children and Adolescents (ESPINA) study is composed of 3 designs: 1) a cross-section of 2004 to assess growth (height and weight), 2) a cross-section of 2008 to assess neurobehavioral development, head circumference, heart rate, blood pressure and blood AChE; and 3) a longitudinal component to assess growth by contrasting 2004 with 2008 data.

This investigation included the information of children from 0 to 5 years of age, who had height and weight measurements in 2004 in the Survey of Access and Demand of Health Services in the County of Pedro Moncayo, Ecuador (SAHS-PM 2004). In 2008, ESPINA examined children 4 to 9 years of age living in the County obtaining various anthropometric measurements, a neurobehavioral developmental assessment and hemoglobin and AChE measurements. Many of these children were examined in the SAHS-PM 2004.

Setting. Pedro Moncayo County has a population of approximately 26,000 people (51% female) 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 and the industry employs 21% of the County's adults. Workers in Ecuador's flower industry work year-round as the equatorial location and use of green houses provides for year-round growth of flowers. To provide a continuous supply of fresh cut flowers, the work and application of pesticides are not a seasonal activity. This places the children of the workers at a more continuous risk of secondary occupational exposure to pesticides. More than 30 different pesticides are used in the Ecuadorian flower industry, of which, organophosphates and carbamates are the most commonly used.[22,31,32,33] The flowers are then sold within Ecuador and exported to Europe and North America.

Participant recruitment: The SAHS-PM 2004 intended to interview all people living in Pedro Moncayo County. Recruiters and interviewers conducted home visits to all houses in the County. In 2008, 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 eligibility criteria. Participant drop-ins were allowed to participate in the study granted they met eligibility criteria. Parental informed consent for the interview was sought in addition to parental permission of study participation of their children. Child assent of children 7 years and older was obtained.

Measures: The SAHS-PM 2004 consisted of: a) In-person Interviews: participant's parents received a home interview that asked for socio-economic status (SES), demographic, and health information of all members in the household. b) Examination: children's height (standing) or length (supine) and weight were measured using infant scales or standing scales.

The 2008 study was composed of: a) Interviews: in 2008, participant's parents received two home interviews that asked for SES, demographic, health, occupational, and pesticide exposure information of household members, focusing on the participating children. b) Examination: Participant examinations were conducted in 6 schools in Pedro Moncayo County. Examiners were blinded to exposure status. Children's height (standing), and head circumference were measured using standard procedures. Weight was measured using the Tanita BF-683W scale (digital), heart rate was measured through a 30 second auscultation, and blood pressure was measured twice using the pediatric Omron aneroid sphygmomanometer 0108M, appropriate for the size of the children. Blood pressure measurements followed the recommendations of the American Heart Association. AChE and hemoglobin concentrations were measured using the EQM Testmate ChE Cholinesterase Test System 400, using the EQM AChE Erythrocyte Cholinesterase Assay Kit 470. Neurobehavioral development was assessed with the NEPSY-II test.

Analyses: Height-for-age, BMI-for-age and weight-for-age z-scores were calculated using the World Health Organization (WHO) Child Growth standards macro for STATA. Stunting was defined as ≤-2 SD below the WHO median weight-for-height. Systolic and diastolic blood pressure z-scores were calculated using the formulas presented by the National High Blood Pressure Education Program Working Group on High Blood Pressure in Children and Adolescents.[34] Pulse pressure is the difference between systolic and diastolic blood pressure, and mean blood pressure is diastolic blood pressure plus one third of the pulse pressure.

Crude and adjusted mean differences between children cohabiting and not cohabiting with a FW were tested using ANOVA and multiple linear regression. Hypotheses were analyzed using multiple linear regression, logistic regression or polychotomous logistic regression. STATA 9 was used for dataset creation and SAS 9.2 for analysis.

III. RESULTS AND CONCLUSIONS

III.a) Participant Characteristics.

922 children were included from the SAHS-PM 2004; the mean age was 2.3 years (range: 0-5 years, standard deviation (SD): 1.4), 49% were female and 63% cohabited with ≥1 FW with a mean of 2.0 FWs at home (Table 1). The median height-for-age z-score was 1.5 SD below the median WHO value, and the stunting prevalence was 24%. Participants cohabiting with a FW, vs. not, had lower mean scores for SES variables including type of home, home roof material, home floor material and type of wastewater disposal. 32% lived in the Parish of Tabacundo, 28% in Tupigachi, 19% in Malchingui, 14% in La Esperanza, and 7% in Tocachi.

In 2008, 313 children participated in the study. The mean age was 6.6 years (range: 3.6-10 years, SD=1.6), 49% were female, 76% mestizo (mix of white and native) and 22% native. 20% of participant's parents smoked and 55% of participants cohabited with ≥1 FWs. FW cohabitants had a mean duration of cohabitation of 5.3 years and a mean of 1.5 FWs at home. The median height-for-age z-score was -1.3 SD and 37% were stunted (Table 2). There were no mean or proportion differences in 2008 in 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 FW (Table 2). Participants cohabiting with a FW had a greater proportion natives, lower maternal education, lower height for age and weight for age z-scores and lower acetylcholinesterase concentration. 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.

230 children had 2004 and 2008 information. In 2008, the mean age was 6.4 years (range: 3.8-9.4, SD=1.4), 49% were female, 72% mestizo and 26% native and 59% cohabited with ≥1 FWs. FW cohabitants had a mean duration of cohabitation of 5.1 years and a mean of 1.5 FWs at home in 2008.

III. b) Flower worker cohabitation and AChE concentration

The mean AChE concentration was 3.13 U/ml (SD 0.49, range: 1.44 - 4.69) and the mean hemoglobin concentration was 12.6 mg/dl (SD: 1.2, range: 9.3 – 17.4). After adjustment for gender, age, height-for-age, hemoglobin concentration, parish of residence, income, pesticide use within household lot and pesticide use by contiguous neighbors, children cohabiting (vs. not) with a FW had a lower mean of AChE by 0.10 U-ml (p=0.049). Their adjusted odds ratio (OR) of having low AChE (≤2.4 U/ml) was

2.84 (95%CI: 1.09-7.39) compared to non-FW cohabitants, and the OR of AChE concentrations within the lowest tertile compared to the highest was 2.73 (95%CI: 1.18-6.29). Length of FW cohabitation was inversely associated with AChE concentrations. Every year of cohabitation was associated with an adjusted AChE decrease of 0.02 U/ml (p=0.038) and an adjusted OR of having AChE concentrations in the lowest vs. highest tertile of 1.22 (95%CI: 1.06-1.14). FW cohabitation for more than 6 years vs. no cohabitation ever had an OR of 4.25 (95%CI: 1.41-12.82) for AChE concentrations in the first tertile vs. the third, an OR of 2.39 (95%CI: 0.77-7.45) for cohabitations lasting 4 to 6 years and an OR of 1.14 (95%CI: 0.34-3.88) for cohabitations lasting 1 month to 4 years.

CONCLUSIONS: Cohabitation with a FW was related to AChE suppression in children; this supports the hypothesis that indirect pesticide exposure from FWs suffices to depress acetylcholinesterase concentration, with greater suppression due to longer exposure.

III. c) Secondary Pesticide Exposure and Neurobehavioral Development

The mean and distribution of scaled scores of all neurobehavioral subtests are listed in

Table 3. Most subtests had lower scores than the normative data of the Nepsy II in which the mean was 10 for all subtests.

Children's cohabitation with a FW was associated with a decrease of 1.17 units (p=0.020) in the Auditory Attention scaled score (Attention and Executive Functioning Domain) after adjustment for gender, age, race, hemoglobin concentration, height for age, maternal education, income and parish of residence. Every year of FW cohabitation was associated with an adjusted decrease of 0.15 units (p=0.055) of Auditory Attention. FW cohabitation was associated with an adjusted OR of 2.58 (95%CI 1.24-5.38) of having low Auditory Attention scores, and every year of cohabitation was associated with an OR of 1.12 (95%CI 1.00-1.24). FW cohabitation was not associated with any other neurobehavioral test.

AChE concentration was directly related to the Inhibition scaled score (Attention and Executive Functioning Domain). Every unit decrease in AChE was associated with an adjusted decrease of 1.07 units (p=0.057). Participants with AChE concentrations in the lowest vs. the highest tertile and middle vs. highest tertile had adjusted ORs of 3.27 (95%CI 1.32-8.12) and 2.35 (95%CI 1.05-5.29) for low inhibition scores, respectively.

Similarly, AChE concentrations in the lowest vs. the highest tertile and middle vs. highest tertile were associated with adjusted ORs of 3.59 (95%CI 1.33-9.64) and 2.58 (95%CI 1.05-6.34) for low Memory For Faces Delayed scaled scores (Memory and Learning Domain), respectively. AChE concentrations in the lowest vs. the highest tertile had an adjusted OR of 4.05 (1.02-16.16) for low Word List Interference Repetition scaled scores (Memory and Learning Domain).

CONCLUSIONS: FW Cohabitation was Associated with lower Auditory Attention (Attention and Executive Function Domain) scaled scores, with a significant decrease with increasing cohabitation length. AChE was inversely related to Inhibition (Attention and Executive Functioning Domain), Memory for Faces Delayed (Memory and Learning), and Word List Interference Repetition (Memory and Learning). Our findings suggest that secondary occupational pesticide exposure can have deleterious impacts on children's neurobehavioral development and is a public health problem. These findings are in line with previous research where children exposed to pesticides had lower scores on attention, inhibition of active behavior, short-term memory, learning and motor development.

III. d) Secondary Pesticide Exposure and Growth

FW cohabitation was not associated with height-for-age in either minimally adjusted (age, gender, parish of residence) or fully adjusted (minimal adjustment + household head education, type of home, home roof materials, home floor materials, type of wastewater disposal) models in the 2004 cross-section. In the 2008 cross-section, children who cohabited with a FW vs. non-FW, had a height-for-age mean z-score lower by 0.24 SD (p=0.045) in the minimally adjusted model (age, gender, race, parish of residence), but the difference diminished to 0.2 SD and was non-significant in the fully adjusted model (minimal adjustment + income and maternal education) (p=0.111).

The number of FWs cohabiting with the participant had no effect on height-for-age in 2004 or 2008 in the fully adjusted models. Duration of cohabitation and AChE concentrations were not related to height-for-age in 2008 in fully adjusted models. Additionally, concurrent FW cohabitation was not associated (fully adjusted) with height-for-age, weight-for-age or BMI-for-age in 2004, nor in the amount of change between 2004 and 2008.

CONCLUSIONS: We found no association between FW cohabitation and AChE with height-for-age, weight-for-age or BMI-for-age in this population. These growth measures were mainly affected by SES.

III. e) Secondary Pesticide Exposure and Blood Pressure and Resting Heart Rate
Participants had comparable z-scores for systolic blood pressure (SBP) than US children, but had lower z-scores for diastolic blood pressure (DBP). Concurrent FW cohabitation was associated with a systolic blood pressure (SBP) decrease of 2.08 mmHg (p=0.029) after adjustment for age, gender, height-for-age, resting heart rate, hemoglobin concentration, parish of residence, and income. Further adjustment for AChE concentration only minimally diminished the association to 2.03 mmHg (p=0.033). Duration of cohabitation was inversely related to SBP, with an adjusted decrease of 0.34 mmHg (p=0.032) per year of cohabitation. This association weakened to 0.32 mmHg (p=0.040) when adjusting for AChE. Diastolic blood pressure (DBP) was not associated with concurrent FW cohabitation or with duration of cohabitation.

AChE was directly associated with diastolic blood pressure (DBP). Every SD (0.49 U/ml) of AChE was associated with a change of 1.56 mmHg (p=0.002) in DBP. Participants with AChE concentrations in the highest quartile compared to the lowest, had adjusted ORs of 3.23 (95%CI 0.97-10.80) and 4.18 (95%CI 1.13-15.51) of having a DBP in the third and fourth quartiles, respectively. AChE was not associated with systolic blood pressure. AChE was directly associated with mean blood pressure, with a change of 1.26 mmHg (p=0.005) per SD of AChE, but not with pulse pressure.

Resting heart rate was not associated with FW cohabitation or AChE concentrations.

CONCLUSIONS: FW cohabitation and duration of cohabitation were associated with a decrease in SBP which remained present even after adjusting for AChE. This may suggest a role of secondary exposure to non-cholinesterase inhibiting pesticides on SBP and/or the presence of additional behavioral/SES confounders that were not accounted for. AChE has primarily an effect on DBP as evidenced by its direct association with DBP and mean blood pressure but not with SBP or pulse pressure, after adjustment for various confounders including resting heart rate.

IV. DISCUSSION

This project provides insight on the impacts of secondary pesticide exposure on childhood development. Our study found that children cohabiting with a FW have greater amounts of pesticide exposure than children not cohabiting with a FW, and these exposures were associated with blood pressure and neurobehavioral developmental changes.

Currently, this is one of the largest completed studies that have assessed neurobehavioral development in children indirectly exposed to pesticides. Our results bring insight on the impacts of pesticides on blood pressure and on the neurobehavioral domains of Memory and Learning, and Attention and Executive Function. 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 AChE 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.

This investigation reinforces the importance of reducing take-home pesticide exposures and including family members of flower plantation workers in pertinent pesticide safety and health prevention education. Our general recommendation is to increase efforts to reduce indirect pesticide exposures and increase health/disease awareness among families of FWs. Periodic health education targeted to FWs and their families, which includes potential short and long term effects of pesticide exposure with emphasis on children's health and preventive medicine, is advised. Additionally, recurrent pesticide exposure reduction education of FWs, their families and flower plantation staff, greater monitoring of take-home pesticide routes by flower plantations and regulating organisms, etc. Further analyses of the routes of indirect pesticide exposure to the families of FWs are needed to provide more detailed recommendations on the emphases of the intervention.

Table 1. Participant Characteristics in 2004. N=922.

	Children Cohabitating with a: Flower worker	Non-Agricultural Worker	P-Value
	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, years[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[f]			
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 (25-75 percentile).

a. Education: 1=none 2=incomplete primary (K-6th) 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
e. Type of Wastewater Disposal: 1= None 2= Cesspool 3= Septic Tank 4=Latrine 5= Sewage
f. Normative data from The Fourth Report on the Diagnosis, Evaluation, and Treatment of High Blood Pressure in Children and Adolescents, Pediatrics, 2004;114:555-576

Table 2. Participant Characteristics in 2008. N=313.

	Children Cohabitating with a: Flower worker	Children Cohabitating with a: Non-Agricultural Worker	P-Value
	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[a]	6.8 (3.3)	8.1 (4.4)	0.003
Monthly Income[b]	3.1(0.7)	3.1 (1.0)	0.946
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[c]	-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[c]	-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 (25-75 percentile).

a. Education: 1=none 2=incomplete primary (K-6th) 3=complete primary 4=incomplete secondary 5=complete secondary 6=university/technical school
b. Income categories (USD): 1=0-50 2=51-150 3=151-300 4=301-500 5=501-1000 6=>1000
c. Normative data from The Fourth Report on the Diagnosis, Evaluation, and Treatment of High Blood Pressure in Children and Adolescents, Pediatrics, 2004;114:555-576

Table 3. Mean and distribution of scaled scores of neurobehavioral subtests.

NB Test (Scaled Scores)	Domain	N	Mean	SD	Min	Max
Auditory Attention Combined	Attention and Executive Function	242	8.2	3.5	1	17
Response Set Combined	Attention and Executive Function	128	8.8	3.2	1	14
Inhibition Combined	Attention and Executive Function	233	7.1	3.1	1	14
Statue Scaled Score	Attention and Executive Function	174	10.2	2.9	1	15
Comprehension of Instructions	Language	313	7.3	3.0	1	16
Speeded Naming Scaled Score	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
VP Combined Scaled Score	Sensorimotor	310	9.9	3.3	1	19
Geometric Puzzles	Visuospacial processing	313	8.5	3.2	1	15
Design Copy	Visuospacial processing	302	10.6	4.9	1	19

Table 4. Adjusted* association between Auditory Attention scaled score and flower worker cohabitation. N=217. Age: 5-9 years.

Auditory Attention Scaled Score	Predictor Variable	ND Score Change	OR	CL		P-value
				Lower	Upper	
Continuous	Flower worker cohabitation	-1.17				0.020
	Duration of Cohabitation (per yr)	-0.15				0.055
Low Score	Flower worker cohabitation		2.58	1.24	5.38	0.012
	Duration of Cohabitation (per yr)		1.12	1.00	1.24	0.042
	>0-5 vs. 0 yrs of Cohabitation		1.87	0.72	4.82	0.197
	>5 vs. 0 yrs of Cohabitation		2.30	1.07	4.96	0.034

* Adjusted for: gender, age, race, hemoglobin concentration, height for age, maternal education, income and parish of residence.

Auditory Attention (Attention and Executive Functioning Domain). Low: Poor selective and sustained attention; either slow responding or inattentive erroneous responding

Table 5. Adjusted* association between Inhibition Combined Scaled score and Acetylcholinesterase (AChE). N=218. Age: 5-9 years.

Inhibition Scaled Score	**Predictor Variable: AChE**	**ND Score Change**	**OR**	**Confidence Limits**		**P-value**
				Lower	**Upper**	
Continuous	Continuous: Per unit decrease	-1.07				0.057
	Quartiles: Per quartile decrease	-0.47				0.035
1st vs. 3rd Tertile	Continuous: Per SD (0.49) decrease		1.56	0.92	2.62	0.096
	1st vs. 3rd Tertile		3.63	1.23	10.73	0.020
	2nd vs. 3rd Tertile		3.76	1.35	10.42	0.011
Low Score	Continuous: Per SD (0.49) decrease		1.44	0.96	2.17	0.077
	1st vs. 3rd Tertile		3.27	1.32	8.12	0.011
	2nd vs. 3rd Tertile		2.35	1.05	5.29	0.039
* Adjusted for: gender, age, race, hemoglobin concentration, height for age, maternal education, income and parish of residence.						

Inhibition (Attention and Executive Functioning Domain): Low indicates poor inhibitory control

Table 6. Adjusted* association between Memory for Faces Delayed Scaled Score and Acetylcholinesterase (AChE). N=231. Age: 5-9 years.

Memory for Faces Delayed Scaled Score	**Predictor Variable: AChE**	**ND Score Change**	**OR**	**Confidence Limits**		**P-value**
				Lower	**Upper**	
Continuous	Continuous: Per unit decrease	-0.59				0.272
	Quartiles: Per quartile decrease	-0.32				0.119
1st vs. 3rd Tertile	Continuous: Per SD decrease		1.39	0.86	2.23	0.179
	1st vs. 3rd Tertile		2.61	0.92	7.39	0.071
	2nd vs. 3rd Tertile		2.82	1.06	7.53	0.038
Low Score	Continuous: Per SD (0.49) decrease		1.59	1.04	2.43	0.033
	1st vs. 3rd Tertile		3.59	1.33	9.64	0.011
	2nd vs. 3rd Tertile		2.58	1.05	6.34	0.039
* Adjusted for: gender, age, race, hemoglobin concentration, height for age, maternal education, income and parish of residence.						

Memory for Faces Delayed (Memory and Learning Domain): Low: Difficulties with recognition of newly learned faces for long-term memory

Table 7. Adjusted* association between Design Copy Scaled Score and Acetylcholinesterase (AChE) concentration. N=281. Age: 3-9 years.

Design Copy Scaled Score	Predictor Variable: AChE	ND Score Change	OR	Confidence Limits		P-value
				Lower	Upper	
Continuous	Continuous: Per unit decrease	-0.84				0.267
	Quartiles: Per quartile decrease	-0.60				0.048
1st vs. 3rd Tertile	Continuous: Per SD** decrease		1.72	1.07	2.76	0.026
	1st vs. 3rd Tertile		4.32	1.49	12.51	0.007
	2nd vs. 3rd Tertile		2.40	0.99	5.84	0.054
Low Score	Continuous: Per SD (0.49) decrease		1.01	0.67	1.51	0.980
	1st vs. 3rd Tertile		0.90	0.36	2.25	0.813
	2nd vs. 3rd Tertile		1.38	0.62	3.09	0.432

* Adjusted for: gender, age, race, hemoglobin concentration, height for age, maternal education, income and parish of residence.

Design Copy (Visuospacial processing Domain): Low: Visuoperceptual and construction problems in a two-dimensional copying task

Table 8. Adjusted* association between Word List Interference Repetition Scaled Score and Acetylcholinesterase (AChE) concentration. N=117. Ages: 7-9 years.

Word List Interference Repetition Scaled Score	Predictor Variable: AChE	ND Score Change	OR	Confidence Limits		P-value
				Lower	Upper	
Continuous	Continuous: Per unit decrease	-1.42				0.092
	Quartiles: Per quartile decrease	-0.75				0.029
1st vs. 3rd Tertile	Continuous: Per SD decrease		1.52	0.78	2.94	0.216
	1st vs. 3rd Tertile		4.30	1.00	18.52	0.050
	2nd vs. 3rd Tertile		0.72	0.22	2.41	0.596
Low Score	Continuous: Per SD (0.49) decrease		1.58	0.89	2.83	0.120
	1st vs. 3rd Tertile		4.05	1.02	16.16	0.047
	2nd vs. 3rd Tertile		0.60	0.19	1.93	0.390
* Adjusted for: gender, age, race, hemoglobin concentration, height for age, maternal education, income and parish of residence.						

Word List Interference Repetition (Memory and Learning Domain): Low: Limited verbal memory span. Limited capacity in working memory, possibly related to language difficulties

Table 9. 2004 Associations of Flower worker cohabitation and height-for-age z-score (ZHA). N=829.

	Mean	Difference ZHA	P-Value
Minimally Adjusted			
Flower worker Cohabitation	-1.48	-0.23	0.092
Non-flower worker Cohabitation	-1.25		
Fully Adjusted			
Flower worker Cohabitation	-1.46	-0.18	0.188
Non-flower worker Cohabitation	-1.28		
Minimal Adjustment: age, gender, parish of residence **Full Adjustment:** minimal adjustment + SES variables (household head education, type of home, home roof materials, home floor materials, type of wastewater disposal)			

Table 10. 2008 Associations of concurrent Flower worker cohabitation with height-for-age z-score (ZHA). N=289.

	Mean	**Difference ZHA**	**P-Value**
Minimally Adjusted			
Flower worker Cohabitation	-1.37	-0.24	0.045
Non-flower worker Cohabitation	-1.13		
Fully Adjusted			
Flower worker Cohabitation	-1.35	-0.20	0.111
Non-flower worker Cohabitation	-1.15		
Minimal Adjustment: age, gender, race, parish of residence **Full Adjustment:** Minimal adjustment + income and maternal education			

Table 11. 2004 Associations of Number of Flower worker cohabitating with participant and height-for-age z-score (ZHA). N=829.

	Change ZHA (per worker at home)	**P-Value**
Minimally Adjusted		
Number of Flower workers at home	-0.09	0.047
Fully Adjusted		
Number of Flower workers at home	-0.08	0.074
Minimal Adjustment: Age, gender, parish of residence **Full Adjustment:** Minimal adjustment + SES variables (household head education, type of home, home roof materials, home floor materials, type of wastewater disposal)		

Table 12. 2008 Associations of Number of Flower workers cohabitating with participant, duration of cohabitation and acetylcholinesterase (AChE) concentration with Height for age z-score (ZHA). N=289.

	Change ZHA (per unit)	**P-Value**
Minimally Adjusted		
Number of Flower workers at home	-0.05	0.361
Duration of Cohabitation (years)	-0.04	0.044
AChE (U/ml)*	-0.02	0.900
Fully Adjusted		
Number of Flower workers at home	-0.04	0.488
Duration of Cohabitation (years)	-0.03	0.118
AChE (U/ml)*	-0.06	0.663

Minimal Adjustment: Age, gender, race, parish of residence
Full Adjustment: Minimal adjustment + income, maternal education
* Additionally adjusted for hemoglobin concentration

Table 13. 2004- 2008 Associations of concurrent Flower worker cohabitation with Height for age, Weight for Age and BMI for Age z-scores. N=201.

	2004 Mean	**P-value**	**2004-2008 (Mean Change)**	**P-Value**
Height for Age z-score				
Flower worker Cohabitation	-2.11	0.849	0.68	0.428
Non-flower worker Cohabitation	-2.06		0.86	
Weight for Age z-score				
Flower worker Cohabitation	-1.26	0.463	0.50	0.432
Non-flower worker Cohabitation	-1.09		0.68	
BMI for Age z-score				
Flower worker Cohabitation	0.10	0.395	0.11	0.613
Non-flower worker Cohabitation	0.34		0.25	
Adjustment: age, gender, parish of residence, SES variables (household head education, type of home, home roof materials, home floor materials, type of wastewater disposal)				

2.2 Publications

None

2.3 Inclusion of gender and minority study subjects

Enrollment Table of children examined in ESPINA, 2008

This report format should NOT be used for data collection from study participants.

Study Title: **Effects of Secondary Occupational Pesticide exposure on Childhood Growth and Neurobehavioral development**

Total Enrolment: 313

Grant Number 1R36OH009402-01

TARGETED/PLANNED ENROLLMENT: Number of Subjects			
Ethnic Category	**Sex/Gender**		
	Females	**Males**	**Total**
Hispanic	153	160	313
Ethnic Category: Total of All Subjects *	154	159	313
Racial Categories			
Mestizo (Mix of Native and White)	116	122	238
Native Ecuadorian	35	34	69
White	1	3	4
Black or African Ecuadorian	0	2	2
Racial Categories: Total of All Subjects *	154	159	313

* The “Ethnic Category: Total of All Subjects” must be equal to the “Racial Categories: Total of All Subjects.”

2.4 Inclusion of Children

All subjects included in this study were children between the ages 0-5 years for the 2004 cross-section and 3-9 years at the 2008 examination. Inclusion of children was necessary to meet all aims of the proposed study, namely, to characterize the association between Secondary Occupational Pesticide exposure on Childhood Growth and Neurobehavioral Development. This age group was included due to the availability of an established cohort of children (ages: 0-5 years) who participated in the Survey of Access and Demand of Health Services in Pedro Moncayo County, in 2004. We carried out follow-up examinations in 2008 to the 2004 children in addition to new participants; therefore, the examined children were between 3 to 9 years of age.

2.5 Materials available for other investigators

Because the proposed investigation is expected to be the beginning of a prospective cohort study to determine the long term effects of secondary occupational pesticide exposure on childhood development, we expect to share the data upon completion of such prospective work. Data sharing will comply with the HHS/CDC data sharing agreement, with full compliance of the Health Insurance Portability and Accountability Act (HIPPA).

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