Occupational Exposure to Pesticides During Pregnancy and Neurobehavioral Development of Infants and Toddlers

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Background: Few studies have examined the effects of in utero exposure to organophosphate and carbamate pesticides on neurobehavioral development in infants and young children. This study considers the potential effects of maternal occupation in the cut-flower industry during pregnancy on neurobehavioral development in Ecuadorian children.

Methods: Data were collected during 2003–2004 for 121 children aged 3–23 months and living in the rural highland region of Cayambe, Ecuador. Children were administered the Ages and Stages Questionnaire and were given specific developmental tests including prehension (reach-and-grasp) and visual skills. Information was gathered on maternal health and work characteristics, the home environment, and child health status. Growth measurements and a hemoglobin finger-prick blood test were obtained. We conducted multiple linear and logistic regression analyses.

Results: Children whose mothers worked in the flower industry during pregnancy scored lower on communication (8% decrease in score, 95% confidence interval [CI]: −16% to 0.5%) and fine motor skills (13% decrease, 95% CI: −22% to −5), and had a higher odds of having poor visual acuity (odds ratio = 4.7 [CI =1.1–20]), compared with children whose mothers did not work in the flower industry during pregnancy, after adjusting for potential confounders.

Conclusions: Maternal occupation in the cut-flower industry during pregnancy may be associated with delayed neurobehavioral development of children aged 3–23 months. Possible hazards associated with working in the flower industry during pregnancy include pesticide exposure, exhaustion, and job stress.

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In utero exposure to neurotoxins may lead to deficits in neurobehavioral development. Previous research has focused mainly on the adverse neurobehavioral effects of fetal exposure to polychlorinated biphenyls,[1,2] organic solvents,[3] methylmercury,[4,5] lead,[6,7] and organochlorine pesticides.[8,9] Few studies have evaluated potential adverse neurobehavioral effects of in utero exposure to the organophosphate and carbamate classes of pesticides in infants. Animal studies suggest that exposure to these classes of pesticides during pregnancy, even at subtoxic levels to the mother, affects the neurologic development of the fetus, infant, and young child. Prenatal exposure causes disruption in brain development, leading to behavioral deficits, impaired cognitive and motor functions, and alterations in the cholinergic system that affects learning and memory processes.[10–14] Young et al[15] found associations between prenatal exposure to organophosphate pesticides and abnormal reflexes in neonates. Another study[16] showed that prenatal exposure to chlorpyrifos was associated with lower scores on the Bayley Scales of Infant Development and problems with attention deficit and hyperactivity in toddlers. Eskenazi et al[17] recently reported adverse associations of prenatal organophosphate with mental development and pervasive developmental problems in toddlers. Another recent study[18] reported associations between prenatal organophosphate pesticide exposure and deficits in neurodevelopment in school-aged children in a flower-growing region of Ecuador. Studies in both animals and humans have reported increased development of myopia after exposure to certain organophosphates.[19–21]

Globally, over $30 billion is spent every year on pesticides, one third of which is spent in the developing world.[22] In Ecuador, cut-flowers are now the country's third most important export. This industry depends heavily on the use of pesticides, with the major pesticides being in the organophosphate, carbamate, and dithiocarbamate classes. In the Ecuadorian cut-flower industry, approximately half of the workers are women of reproductive age (Centro de Estudios y Asesoría en Salud 2001, unpublished data). Typically, pregnant women work until 5 weeks before their due date, with many working until they give birth. Few studies have assessed the impact of pesticide use in the cut-flower industry on the health of the fetus, infant, and young child.[18,23,24]

The EcoSalud project, launched in 2001 by the Centro de Estudios y Asesoría en Salud in Quito, Ecuador, along with the Canadian International Development Research Center, addresses the regional impact of pesticide use in the cut-flower industry. As a component of EcoSalud, we studied the impact of potential pesticide exposure associated with the cut-flower industry on neurobehavioral development. Related analyses have described the main predictors of neurobehavioral development in this population, the ecologic effect of community of residence on child development, and the effect of household and community predictors of pesticide exposure on development in older children.[23–25] The present study considers the potential effects of maternal occupation during pregnancy on the neurobehavioral development of infants and toddlers ages 3–23 months in 3 communities in the Cayambe-Tabacundo region of Ecuador (2003–2004).

METHODS

Study Population

The study population included 3 communities selected for potential exposure status and having sufficient ties between researchers and community leaders to ensure accessibility to the community. Communities A and B were lower altitude and considered to have high potential for pesticide exposure given their proximity to the cut-flower industry; community C was higher altitude and further from the flower plantations, with fewer residents expected to work in the flower industry. To construct the sampling frame, a census was conducted by university students trained in door-to-door surveying techniques. Surveys obtained sociodemographic data on each family. Mothers with any children ages 3–61 months and who had lived in the communities for at least a year were eligible to participate. Up to 3 eligible children per mother were included. Approval for this project was obtained from the Institutional Review Board at the University of Michigan and from Centro de Estudios y Asesoría en Salud in Ecuador.

Procedures

The Ages and Stages Questionnaire,[26] a developmental screening test, was administered to the child. For a subgroup of children ages 9 to 18 months we, also administered specific tests examining visual acuity and prehension (reach-and-grasp) abilities. Information on sociodemographic characteristics, maternal occupational history, maternal and child health characteristics, and the child's socialization was obtained in a structured interview. A finger-prick blood test was obtained to assess the child's hemoglobin levels using the HemoCue testing kit (HemoCue, Inc., Lake Forest, CA,). Height (cm), weight (kg), and head circumference (cm) of the child were measured. In total, 219 mothers (91% of total eligible) and 283 children (91% of total eligible) participated in the study. The present analyses include infants ages 3–23 months (n = 121).

Exposure Measurement

Mothers were asked whether they worked outside the home during their pregnancy and whether they worked in the flower industry specifically, total months worked during pregnancy, total hours worked per week during pregnancy, primary job responsibility (harvest, postharvest), and whether pesticides were used in their workplace during pregnancy. Three dichotomous variables were used to assess mother's work history during pregnancy: (1) the mother worked outside the home ("paid work"); (2) the mother worked in the flower industry; and (3) pesticides were used in the workplace.

Neurobehavioral Development Assessment

Ages and Stages Questionnaire. The use of a parent-report screening test has been shown to be an effective and valid way in which to assess a child's developmental progress.[27,28] The Ages and Stages Questionnaire, a widely used screening instrument, is standardized for use in children aged 3–61 months. It is composed of 19 age-specific questionnaires that cover 5 broad developmental dimensions: communication, fine and gross motor skills, problem solving, and personal-social skills. Each domain is scored from 0–60 points, with 60 being a perfect score. A continuous score is calculated for each age-specific questionnaire, with scores summarized for each developmental domain. Unlike formal psychometric assessments such as the Bayley Scales of Infant Development, the Ages and Stages Questionnaire is a first-level comprehensive screening instrument.

Before administering the Ages and Stages Questionnaire (Spanish version), we first adapted it into the local vernacular, removing contextually inappropriate questions to prevent cultural and language bias. Testing was conducted using the home-visit procedure outlined in the manual, in which the tester attempts to elicit all behaviors directly from the child during the assessment. This procedure varies from one, which relies solely on parent-report and is more appropriate in a setting where the parent may not be able to complete the questionnaire on her own.[28] Testers brought all necessary materials to the interview, and mothers were encouraged to participate in the activities with their child throughout the session. The mother's report of her child's behavior was used only when a particular activity could not be elicited or carried out during the session.

Targeted Developmental Tests. Specific developmental tests were administered to infants ages 9–18 months, including tests of prehension (motor testing of reach and grasp)[29,30] and visual acuity.[31,32] All instruments were administered by 1 trained tester. Prehension tests were recorded using a video camera. The infant was first presented a 13-mm diameter multicolored wooden pellet attached by string to a plate within reaching distance of the infant. The child was given 3 opportunities to reach and grasp the pellet; each trial lasted 30 seconds. The infant was then presented with a larger 24-mm diameter pellet. For both pellet tests, the examiner observed whether the infant tried to grasp the pellet, whether they succeeded, whether they grasped at first contact, whether they used the table as a support, and the type of grasp (power-ulnar, power-radial, precision-multiple, or precision-pincer). Here, "power" refers to a grasp that involves the fingers and palm of 1 hand and "precision" refers to a grasp that involves the tips of fingers and the thumb of one hand.

To test for bi-manual coordination, the infant was presented a plastic translucent box (20 × 12 × 6 cm) with a symmetric rattle inside, and was encouraged to open the box and retrieve the rattle. Five 30-second trials were conducted for the box test. Infants were tested for their ability to try to open the box, to succeed in touching the rattle inside the box, to retrieve the rattle either with 1 hand or both, and whether they had good coordination when retrieving the rattle with 2 hands.

Visual acuity of infants 9–18 months of age was tested using the U.C. Berkeley Preferential Looking Test Cards, a procedure similar to that of the Teller Acuity Card Test.[31] Briefly, the procedure involved 6 large rectangular cards, each with a varying right-left location of black and white stripe grating which the infant observed. The presenter, unaware of the location of the stripe grating, observed the infant's looking preference. The distance that the infant exhibited preference was noted and visual acuity was calculated. The World Health Organization (WHO) classifications of visual impairment were used to assess visual acuity.[33]

Covariates

Standardized z-scores for anthropometric measures of malnutrition were calculated using the 1978 CDC/WHO growth reference curves, which are a normalized version of the 1977 National Center for Health Statistics growth reference curves.[34] Chronic malnutrition (stunting) was defined as a height-for-age z-score 2 standard deviations below the median. Presence of anemia was determined after taking into account the child's age and altitude of community of residence.[35] Information on birth weight was obtained from the child's vaccine record card or from the mother. Given the high frequency of missing information, birth weight was examined as a 3-level variable (<2500 g, ≥2500 g, missing).

The child's exposure to a developmentally stimulating environment was assessed by 2 variables: attendance at the daycare center (yes/no) and the type and frequency of stimulating activities the mother was involved with at home with each child. For the latter, we adapted a set of 6 questions from a UNICEF multicountry survey to assess home support for child development.[36] The 6 activities between mother and child included reading, counting and/or drawing, looking at pictures, singing songs, going out of the house together, and playing together. Mothers were also asked how they would rate their relationship with their husbands (or their families in the case of single mothers) to assess the home environment in which the child lives.

Data were collected on maternal age at pregnancy, alcohol use during pregnancy, pesticide use at home during pregnancy, receipt of prenatal care, and demographic information on the mother and father (eg, age, ethnicity, education level, predominant language preference of the mother, marital status). Data were also collected on monthly household income (US $0–150, $151–250, or >$250), and housing construction. Housing characteristics including roof, floor, and wall composition, type of water used in home, bathroom type, and access to electricity were summarized into a housing scale, with possible scores ranging from 0–7. This scale was then categorized as poorer (≤3), midlevel (4–5), and better (6–7) housing construction based on distribution quartiles.

Statistical Analysis

The child's health characteristics, maternal characteristics both current and during pregnancy, and the sociodemographic characteristics of the child's family were compared across categories of the maternal occupational exposure during pregnancy. Developmental delay was analyzed separately for each developmental domain. Two sets of sibling pairs were identified in our population; to eliminate any clustering in the sample, we randomly removed 1 sibling from each pair.

As none of the maternal exposure variables was associated with problem solving or personal-social skills, these developmental domains were not considered in the regression analysis. Regression models were constructed for 3 developmental domains (communication and gross, fine motor skills) to assess the effect of maternal occupational exposure to pesticides during pregnancy, after controlling for potential confounders. In this analysis we considered as potential confounders those sociodemographic and health variables reported in prior analyses of these data to be associated with neurobehavioral development.[23,25] Due to the limited sample size, only those variables found to be associated with each Ages and Stages Questionnaire domain and the exposure variable of interest were included in the regression model for a given domain.

Frequencies and percentages of prehension scores were calculated for infants ages 9–18 months. For the pellet tests, a dichotomous variable was constructed (succeeded in grasping the pellet, succeeded in grasping the pellet on first contact). Logistic regression models were constructed comparing those children who succeeded in grasping the pellet to those who succeeded in grasping the pellet on first contact. For the box test, there was a possible total score of 4 (tried to open the box, succeeded in touching the rattle, succeeded in retrieving the rattle, and retrieved the rattle with 1 or 2 hands). Most children tried to open the box, so comparisons were made only between those children achieving the highest 3 levels in the test sequence. Correlations between the trials were present for the box test and regression analysis accounted for this dependence using generalized estimation equations (GEE).

Visual acuity was analyzed for infants ages 9–18 months. Crude scores were recorded in the typical format of n/n (eg, 20/20). In the final analysis, however, only the denominator was considered, as only that value increases with declining visual acuity. We constructed a dichotomous variable for visual acuity: mild or moderate visual impairment (<200); severe or profound visual impairment (≥200).

We report associations and the percent difference in developmental scores between exposure groups. Effect size was calculated to compare the magnitude of effect of the main predictors of pesticide exposure on the developmental scores across exposure groups.[37] The measure of effect size, Cohen's *d*, is calculated by taking the difference in the mean score of each exposure group divided by the standard deviation and is independent of sample size. Effect size is cautiously interpreted as small ($d = 0.2$), medium ($d = 0.5$), and large ($d = 0.8$). Data were entered into SPSS 11.5 (SPSS Inc., Chicago, IL) and were analyzed in SPSS and SAS version 8 (SAS Institute Inc., Cary, NC). Nutritional data were analyzed in EpidInfo's NutStat program software (CDC, Division of Public Health Surveillance and Informatics, 2003).

RESULTS

Approximately half of the mothers in this analysis reported working outside the home during their pregnancy (n = 63; 52%). Of these, the majority worked in the flower industry (n = 53; 84%). All women who worked in the flower industry and half of the mothers involved in other work reported pesticide use in their workplace during pregnancy.

Several child and maternal characteristics differed by occupational status during pregnancy (Table 1). Flower workers had greater access to prenatal care in the first trimester (62% vs. 44%), had more maternal-child interaction (64% vs. 44%), higher monthly household income, and better housing construction. Most of the reported pesticide use in the workplace during pregnancy was reported by mothers who worked in the flower industry. The distributions of these characteristics for those children whose mothers reported pesticide use at work during the pregnancy and those children whose mothers reported employment in any paid work outside the home during pregnancy are similar to those presented for work in the flower industry during pregnancy (data not shown).

Table 2 presents the unadjusted mean Ages and Stages Questionnaire developmental scores by occupational status. Work in the flower industry during pregnancy and pesticide use at work during pregnancy were associated with lower communication scores. Greater total weekly hours worked during pregnancy (>45 h/wk) and pesticide use at home during pregnancy were associated with better gross motor scores. All measures of exposure except for pesticide use at home during pregnancy were negatively associated with fine motor scores.

Table 3 displays the frequencies for the prehension and visual acuity variables by occupational status. For the large-pellet test, higher frequencies of poorer grasp scores (<3) were found for work in the flower industry during pregnancy, use of pesticides in the work place during pregnancy, more hours worked per week during pregnancy, and working 6 months or more of the pregnancy. For a greater number of children with poor visual acuity, maternal work in the flower industry during pregnancy was reported.

Table 4 displays results of the unadjusted and adjusted regression models. On average, children whose mothers worked in the flower industry during pregnancy scored 8% lower on communication skills (−4.6 points, 95% CI = −9.6 to 0.3) and 13% lower on the fine motor skills (−8.0 points; −13 to −3) compared with children whose mothers did not work, after adjusting for key confounders. Inclusion of low birth weight in the regression models did not change the estimates of the effect of maternal work in the flower industry during pregnancy on neurobehavioral development (data not shown).

The odds of succeeding in grasping the large pellet upon first contact were approximately half for those children whose mother worked in the flower industry during pregnancy compared with those whose mothers did not work (95% CI = 0.22–0.93). The odds of poor visual acuity in infants whose mothers worked in the flower industry during their pregnancy were 4.7 times higher compared with those children whose mothers did not work in the flower industry during pregnancy (95% CI = 1.1–20). However, estimates are imprecise due to the limited size of the infant subsample.

We observed similar results for use of pesticides in the workplace, including lower fine motor score (13% decrease in score, 95% CI = −21% to −5%), lower communication (6% decrease in score, 95% CI = −15% to 2%), and poorer visual acuity (OR = 3.9; 0.94–16) scores among children whose mothers reported pesticide use in the workplace during pregnancy compared with those whose mothers did not. Paid work, which included many flower workers and some non-flower workers, also yielded similar results, with lower fine motor scores (12% decrease in score, 95% CI = −20% to −4%) and poorer visual acuity (OR = 3.3 [0.80–13]) among those who worked outside the home during pregnancy compared with those who did not work outside the home.

DISCUSSION

Maternal occupation in the cut-flower industry during pregnancy was associated with developmental deficits in children ages 3–23 months, particularly with fine motor skills and visual acuity. These results suggest that developmental hazards are associated with working specifically in the cut-flower industry during pregnancy compared with other work

TABLE 1. Characteristics of Participants and ORs With Maternal Occupation in the Flower Industry During Pregnancy for Infants 3–23 Months of Age (n = 121), Cayambe-Tabacundo Region, Ecuador, 2003

	No Work in Flowers (n = 68)[a] No. (%)	Work in Flowers (n = 53) No. (%)	OR (95% CI)
Child health characteristics			
Sex of child			
Male	39 (57)	29 (55)	1.11 (0.54–2.29)
Female[b]	29 (43)	24 (45)	1.00
Birth weight			
≥2500 g[b]	37 (54)	25 (47)	1.00
<2500 g	4 (6)	11 (21)	6.10 (1.25–14)
Missing data	27 (40)	17 (32)	0.75 (0.33–1.52)
Current health of child			
Excellent/good	42 (63)	24 (45)	2.03 (0.98–4.23)
Average/bad[b]	25 (37)	29 (55)	1.00
Anemia			
No	17 (25)	17 (32)	0.72 (0.32–1.57)
Yes[b]	51 (75)	36 (68)	1.00
Stunting			
No	37 (54)	30 (57)	0.92 (0.44–1.89)
Yes[b]	31 (46)	23 (43)	1.00
Stimulation at home			
3 or more activities	30 (44)	34 (64)	0.44 (0.21–0.92)
Less than 3 activities[b]	38 (56)	19 (36)	1.00
Maternal characteristics			
Mother's age			
≤25 y	42 (62)	30 (57)	1.24 (0.60–2.57)
>25 y[b]	26 (38)	23 (43)	1.00
Ethnicity of mother			
Indigenous	52 (78)	37 (71)	1.41 (0.61–3.23)
Mestizo/white[b]	15 (22)	15 (29)	1.00
Language most used			
Spanish/Quichua mix	11 (16)	3 (6)	3.22 (0.85–12)
Spanish[b]	57 (84)	50 (94)	1.00
Marital status			
Married[b]	34 (50)	26 (49)	1.00
Free union	21 (31)	19 (36)	1.25 (0.58–2.68)
Single/separated/widowed	13 (19)	8 (15)	0.75 (0.29–1.97)
Mother's education level			
None or partial elementary	15 (22)	10 (19)	0.82 (0.33–2.01)
Completed elementary school	43 (63)	31 (58)	0.82 (0.39–1.71)
Partial or completed high school[b]	10 (15)	12 (23)	1.00
Relations at home			
Calm/good	44 (65)	44 (83)	0.38 (0.16–0.90)
Indifferent/tense/violent[b]	24 (35)	9 (17)	1.00
Mother's age at pregnancy			
≥18 y	54 (79)	44 (83)	0.79 (0.31–1.99)
<18 y[b]	14 (21)	9 (17)	1.00
Month of pregnancy started prenatal visits (n = 107)[c]			
In the 1st trimester	25 (44)	31 (62)	0.48 (0.22–1.04)
After 3rd month[b]	32 (56)	19 (38)	1.00
Alcohol use during pregnancy			
No	26 (38)	19 (36)	1.12 (0.53–2.33)
Yes[b]	42 (62)	34 (64)	1.00

(*Continued*)

TABLE 1. (*Continued*)

	No Work in Flowers (n = 68)[a] No. (%)	Work in Flowers (n = 53) No. (%)	OR (95% CI)
Pesticide use at home during pregnancy			
No	48 (71)	45 (85)	0.43 (0.17–1.07)
Yes[b]	20 (29)	8 (15)	1.00
Socioeconomic characteristics			
Monthly household income (in US dollars)			
$0–150	39 (58)	21 (40)	0.43 (0.17–1.07)
$151–250	17 (26)	15 (28)	0.84 (0.41–1.73)
>$250[b]	11 (16)	17 (32)	1.00
Father's education level			
None or partial elementary	8 (14)	5 (11)	0.78 (0.24–2.54)
Completed elementary school	28 (50)	29 (66)	1.73 (0.84–3.56)
Partial or completed high school[b]	20 (36)	10 (23)	1.00
Housing construction			
Low	20 (29)	8 (15)	0.49 (0.23–1.01)
Medium	35 (52)	25 (47)	1.18 (0.53–2.67)
High[b]	13 (19)	20 (38)	1.00

[a]Includes mothers who worked during their pregnancy, but not in flowers AND those mothers who did not work outside the home during their pregnancy.
[b]Reference category.
[c]Fourteen women (12% of total N) reported not receiving any prenatal care.

TABLE 2. Unadjusted Means and Frequencies of Main Outcome Variables (Ages and Stages Questionnaire Sections) for Main Exposure Variables (Standard Deviations of the Means All in the Range of 11–17)[a]

	Total No.	Communication Mean (Difference) %[b]	Gross Motor Mean (Difference) %[b]	Fine Motor Mean (Difference) %[b]	Problem Solving Mean (Difference) %[b]	Personal-Social Mean (Difference) %[b]
Total months worked during pregnancy						
Did not work[c]	58	35.2	35.0	42.0	41.9	42.0
<6 mo	19	33.9 (−2)	39.5 (+8)	38.7 (−6)	40.0 (−3)	41.8 (0)
≥6 mo	44	35.8 (+1)	34.0 (−2)	34.9 (−12)	38.3 (−6)	39.8 (−4)
Total weekly hours worked						
Did not work[c]	58	35.2	35.0[c]	42.0	41.9	42.0
≤45 h/wk	33	36.7 (+3)	30.0 (−8)	37.7 (−7)	38.8 (−5)	39.7 (−4)
>45 h/wk	30	33.7 (−3)	41.8 (+11)	34.2 (−13)	38.8 (−5)	41.3 (+1)
Paid work during pregnancy						
No	58	35.2	35.0	42.0	41.9	42.0
Yes	63	35.2 (0)	35.6 (+1)	36.0 (−10)	38.8 (−5)	40.4 (−3)
Worked in flower industry						
No	68	37.0	35.8	41.5	41.3	42.1
Yes	53	32.9 (−7)	34.7 (−2)	35.6 (−10)	39.1 (−4)	40.1 (−3)
Pesticides used at work						
No	63	36.6	35.2	42.1	41.7	42.0
Yes	58	33.7 (−5)	35.4 (0)	35.4 (−11)	38.8 (−5)	40.3 (−3)
Pesticide used at home during pregnancy						
No	93	34.9	33.9	37.7	40.3	40.6
Yes	28	36.1 (+2)	40.2 (+11)	42.7 (+8)	40.4 (0)	43.3 (+5)

[a]Higher score corresponds to better developmental skills.
[b]Percent change was based on score difference divided by total possible score; for 3 level variables, "did not work" was the reference level.
[c]Reference category.

TABLE 3. Frequencies of Poorer Prehension and Visual Acuity for Main Exposure Variables for Infants Ages 9–18 Months, Cayambe-Tabacundo Region, Ecuador, 2003

	Total No.	Total No. Trials	Small Pellet Test (<3)[a] No. (%)	Large Pellet Test (<3) No. (%)	Box Test (<3)[b] Total No. Trials	Box Test (<3)[b] No. (%)	Visual Acuity (≥200)[c] Total No.	Visual Acuity (≥200)[c] No. (%)
Total months worked during pregnancy								
Did not work	58	99	35 (35)	25 (25)	160	57 (36)	33	4 (12)
<6 mo	19	36	16 (44)	14 (39)	60	21 (35)	12	2 (17)
≥6 mo	44	72	22 (31)	31 (43)	120	52 (43)	24	7 (29)
Total weekly hours worked								
Did not work	58	99	35 (35)	25 (25)	160	57 (36)	33	4 (12)
≤45 h/wk	33	66	22 (33)	31 (47)	110	45 (41)	22	6 (27)
>45 h/wk	30	42	16 (8)	14 (33)	70	28 (40)	14	3 (21)
Paid work during pregnancy								
No	58	99	35 (35)	25 (25)	160	57 (36)	33	4 (12)
Yes	63	108	38 (35)	45 (42)	180	73 (41)	36	9 (25)
Worked in flower industry								
No	68	114	42 (37)	32 (28)	185	68 (37)	38	4 (11)
Yes	53	93	31 (33)	38 (41)	155	62 (40)	31	9 (29)
Pesticides used at work								
No	63	108	39 (36)	29 (27)	175	63 (36)	36	4 (11)
Yes	58	99	34 (34)	41 (41)	165	67 (41)	33	9 (27)
Pesticide use at home during pregnancy								
No	93	171	57 (33)	59 (35)	280	105 (38)	57	11 (19)
Yes	28	36	16 (44)	11 (31)	60	25 (42)	12	2 (17)

[a]Pellet tests: frequency shown for those not successful in grasping the pellet on first contact.
[b]Box test: frequency shown for those not able to retrieve the rattle (1 or 2 hands) from the box.
[c]Visual acuity: frequency shown for those children with severe or profound visual impairment (≥200).

outside the home. Such hazards may include higher potential for pesticide exposure, exhaustion, and job stress.

The exposure profile of women who worked in the flower industry during pregnancy may differ from the profile of those who worked in other jobs. The attenuation of the effect of maternal occupation during pregnancy on neurobehavioral development when we considered any work outside the home suggests that work in the flower industry (as opposed to other employment) may explain the observed developmental delays. However, we had limited power to examine the independent effects of work in the flower industry compared with other types of paid work; the majority of the women who reported working outside of the home in a paid job during pregnancy reported working in the cut-flower industry and the majority of working women reported pesticide use at their workplace.

Developmental differences observed between children whose mother did or did not work in the flower industry during pregnancy may be due to factors other than pesticides. Approximately half of the mothers who worked in the flower industry during pregnancy reported working more than 45 hours per week, and almost three-quarters of those mothers reported working 6 months or more of their pregnancy. Long work days, job stress, and difficult work responsibilities where women are on their feet most of the day could contribute to adverse pregnancy outcomes. Furthermore, flower laborers work in a greenhouse setting where heat and exhaustion may also play a role in maternal and fetal health.

Protective equipment and clothing aid in reducing the harmful effects of exposure to pesticides on health of agricultural laborers.[38,39] In the present study, mothers were asked about current use of protective gear and clothing in the workplace. The majority of working mothers reported using protective gloves and rubber boots. However, only about half reported having access to masks and plastic aprons. In the flower industry, the majority of women work either in harvest or packaging, where masks may help reduce exposure to pesticides by inhalation. We were limited in our assessment of the impact of using protective gear, as we did not have data specific to the pregnancy period.

Our results suggest that employment in the cut-flower industry during pregnancy may adversely affect visual acuity of infants ages 9–18 months in this population possibly due to in utero exposure to organophosphate and carbamate pesticides. Acetylcholinesterase inhibitors such as the organophosphate and carbamate classes of pesticides interfere with neurite outgrowth and subsequent myelin production. Animal studies suggest myelination is associated with visual acuity.[40] However, we did not have direct measures of pesticide

TABLE 4. Unadjusted and Adjusted Regression Models for Developmental Outcomes for Maternal Occupation in the Flower Industry During Pregnancy

	Unadjusted Mean Score Difference (95% CI)	Adjusted Mean Score Difference (95% CI)	% Difference
ASQ (n = 121)			
Communication	−4.06 (−9.18 to 1.06)	−4.64[a] (−9.60 to 0.31)	−8
Gross motor	−1.09 (−6.76 to 4.58)	−1.82[b] (−7.38 to 3.75)	−3
Fine motor	−5.90 (−10.75 to −1.06)	−8.04[c] (−12.98 to −3.10)	−13
Bi-manual coordination (n = 336)[a]			
Box test	−0.04 (−0.75 to 0.67)	−0.47[d] (−1.19 to 0.26)	−1
	OR (95% CI)	**OR[e] (95% CI)**	
Prehension (n = 195)[f]			
Small-pellet test	1.42 (0.77 to 2.64)	1.4 (0.69 to 2.8)	
Large-pellet test	0.56 (0.31 to 1.02)	0.45 (0.22 to 0.93)	
Visual acuity (n = 69)			
	3.5 (0.95 to 12.7)	4.7[g] (1.1 to 20)	

[a]Adjusted for child's age, mother's education.
[b]Adjusted for anemia, stunting, housing construction, pesticide use at home during pregnancy.
[c]Adjusted for anemia, stimulation at home, housing construction.
[d]Adjusted for child's age, presence of anemia, stunting, and child stimulation at home.
[e]The odds of succeeding in grasping the large pellet upon first contact adjusted for child's age, presence of anemia, stunting, and correlations among trials.
[f]Total number of trials.
[g]Adjusted for anemia, alcohol.

exposure, and our sample size was limited. Domestic pesticide use is widespread in this region of Ecuador, and we were not able to obtain information on the type or quantity of pesticides used on domestic crops. It is possible that mothers who did not work in the flower industry were exposed through domestic pesticide use.

There are limitations to our general developmental screening tool. Ideally, we would have liked to use a battery of developmental tests. However, these tests are expensive, difficult to administer in a field setting, and time-consuming. In a developing country like Ecuador, where assessment of neurobehavioral development must be conducted in a field setting with minimal cost, a screening test was the most appropriate option for this preliminary investigation. However, available screening tools such as the Ages and Stages Questionnaire, although standardized and validated in a large and multicultural population in the United States, have not been validated in rural Andean populations. Also, we would have liked to assess development using narrower age ranges, but given the limited sample size, we were not able to create smaller age groupings.

As increasing numbers of women in the developing world find employment outside of the home in large-scale agricultural industries and more pregnant women become exposed to agricultural chemicals, more attention needs to be placed on the unique risks of pesticide exposure on the maternal reproductive system and on fetal and infant health. Although flower workers are paid a higher wage compared with other types of work in the region, and have access to health care and daycare, there appear to be some aspects of this occupation that adversely affects child development. In an associated study focusing on the community of residence (higher vs. lower exposure) of children ages 3 to 23 months, associations were found between children living in the higher exposure communities and delayed neurobehavioral development after accounting for higher household monthly income and maternal education level, more maternal-child interaction, and fewer cases of anemia—all factors that should promote optimal neurobehavioral development.[23]

This study, one of few conducted in a developing country setting, provides preliminary evidence of the potentially harmful effects of maternal occupation in the cut-flower industry during pregnancy (with its potential for in utero exposure to organophosphate and carbamate pesticides) on neurobehavioral development.

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