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Pesticide Exposure and Stunting as Independent Predictors of Neurobehavioral Deficits in Ecuadorian School Children

Philippe Grandjean, MD, DMSc[a,b], Raul Harari, MD[c], Dana B. Barr, PhD[d], Frodi Debes, PsyD[a]

[a]Institute of Public Health, University of Southern Denmark, Odense, Denmark; [b]Department of Environmental Health, Harvard School of Public Health, Boston, Massachusetts; [c]Corporación para el Desarrollo de la Producción y el Medio Ambiente Laboral, Quito, Ecuador; [d]National Center for Environmental Health, Centers for Disease Control and Prevention, Atlanta, Georgia

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ABSTRACT

OBJECTIVES. To examine possible effects on blood pressure, neurological function, and neurobehavioral tests in school-aged children with and without prenatal pesticide exposure in an area where stunting is common.

METHODS. In a community of Northern Ecuador with intensive floriculture and a high female employment rate, we invited 79 children attending the 2 lowest grades of a public school for clinical examinations. In addition to a thorough physical examination, we administered simple reaction time, Santa Ana dexterity test, Stanford-Binet copying, and Wechsler Intelligence Scale for Children-Revised Digit Spans forward. Maternal interview included detailed assessment of occupational history to determine pesticide exposure during pregnancy. Recent and current pesticide exposure was assessed by erythrocyte acetylcholine esterase activity and urinary excretion of organophosphate metabolites.

RESULTS. All eligible children participated in the study, but 7 children were excluded from data analysis due to other disease or age >9 years. A total of 31 of the remaining 72 children were classified as stunted based on their height for age. Maternal occupational history revealed that 37 children had been exposed to pesticides during development. After confounder adjustment, prenatal pesticide exposure was associated with a higher systolic blood pressure than in the controls. On neurological examination, 14 exposed children and 9 controls showed ≥1 abnormalities. Of 5 neurobehavioral tests, the Stanford-Binet copying test showed a lower drawing score for copying designs in exposed children than in controls. Stunting was associated with a lower score on this test only, and both risk factors remained statistically significant in a multiple regression analysis with adjustment for demographic and social confounders. Increased excretion of dimethyl and diethyl metabolites of organophosphates was associated with increased reaction time and no other outcomes.

CONCLUSION. Prenatal pesticide exposure may cause lasting neurotoxic damage and add to the adverse effects of malnutrition in developing countries. The effects differ from those due to acute pesticide exposure.

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Key Words
blood pressure, maternal exposure, neuropsychological tests, neurotoxicity syndromes, organophosphorus compounds, prenatal exposure, delayed effects

Abbreviations
RfD—reference dose
WHO—World Health Organization

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Address correspondence to Philippe Grandjean, MD, DMSc, Department of Environmental Health, Harvard School of Public Health, Landmark Center 3E-110, 401 Park Dr, Boston, MA 02215. E-mail: pgrand@hsph.harvard.edu

THE DEVELOPING BRAIN is particularly susceptible to adverse effects of environmental toxicants and nutritional deficiencies.[1] During fetal development, the placenta offers some protection against unwanted compounds, but it is not an effective barrier against environmental neurotoxicants.[2] Furthermore, the blood-brain barrier, which protects the adult brain from many toxic agents, is not completely formed until ~6 months after birth[3] and, therefore, provides no protection during sensitive developmental stages. At the same time, the brain depends on appropriate supplies of essential nutrients, and malnutrition, as apparent from stunted growth, may result in delayed neurobehavioral development.[4–9]

The vulnerability of the brain originates from the combination of immaturity and ongoing development.[10] For optimal brain development to occur, a number of processes must take place within a tightly controlled time frame. If a developmental process is halted or inhibited, there is little chance for repair, and a small change may have substantial consequences, if the time schedule is disturbed. Acetylcholine is a major synaptic transmitter substance that also serves as neurotrophic signal during brain development.[11,12] Experimental studies in rodents suggest that cholinesterase inhibitors that are used as insecticides can interfere with brain development and lead to permanent damage.[11,13] Such interference might be temporary in the adult, but the disturbance could cause permanent abnormalities if it occurs during a sensitive stage of development.[14] Industrial chemicals are not routinely tested to identify such effects, and therefore the safety of currently used pesticides in this regard is unknown.

Despite the experimental evidence, epidemiological support on developmental neurotoxicity is very limited in regard to industrial chemicals, including pesticides.[1,2] In an anthropological study of children living in a Mexican community with pesticide exposure and one without, the draw-a-person test was administered; the random undifferentiated lines drawn by the exposed children averaged only 1.6 body parts per figure, whereas nonexposed children produced reasonably lifelike figures, averaging 4.4 body parts each.[15] However, the exposure classification was based only on residential proximity to farms that used high quantities of organophosphate, organochlorine, and pyrethroid insecticides. Although using an anthropological technique known as rapid assessment, this study suggests that pesticides with known neurotoxic potentials need to be evaluated with regard to their impact on nervous system development. A more recent study in California showed that reflex abnormalities in neonates were associated with increased urinary concentrations of organophosphate metabolites.[16]

Such effects need to be distinguished from those caused by nutritional deficiencies.[4–6,17] In rural populations, especially in developing countries, pesticide exposures may coincide with poverty and malnutrition.[18] Poor growth, or stunting, is frequently used as an objective indicator of malnutrition. The stunting manifests itself within the first 6 months after birth, and it has recently been reported in 1 out of every 4 children <5 years of age in Ecuador.[19] High-altitude hypoxia seems to have much less effect on growth, which is mainly affected by high rates of infection, poor nutrition, and other conditions of poverty common in both highland and lowland children.[7] Predictors of stunting include low maternal education, poor housing conditions, difficult access to health services, Amerindian ethnicity, high fertility, and a diet low in protein.[19] In Ecuadorian women, nutritional iron deficiency is particularly common because of low intakes of animal products.[8] Stunting, as a cumulative process that starts in utero, is also related to delayed mental development,[9] but clinical trials with nutritional supplements show that the effects of inadequate dietary intakes can be partially corrected.[20]

Likewise, in accordance with the fetal origins hypothesis, cardiovascular development is thought to be affected by prenatal factors, such as placental insufficiency, as indicated by low birth weight, thinness, and short body length at birth.[17] Still, genetic and environmental factors, including lower socioeconomic status, also seem to play a role and may, in some cases, explain the association.[21]

The need for better documentation of developmental neurotoxicity caused by widely used pesticides in developing countries requires resolution of a possible confounding problem, because pesticide exposures may occur concomitantly with malnutrition. Although joint effects of these 2 adverse health risks are possible, the effects of pesticides need to be distinguished from those caused by other factors.

We chose to carry out a study in the Tabacundo-Cayambe area in northern Ecuador, a rural region with 2 villages at an altitude of ~2800 m. The inhabitants are of indigenous and mixed origin (ie, Amerindian and Mestizos). In the Andean plateau, floriculture has become a major source of income over the last 15 years, and Ecuador is now one of the world's main producers of ornamental flowers. Approximately 150 flower plantations are in operation in this area, each with an average of >100 workers. At least half of these workers are women, primarily in childbearing age groups, who often continue to work during pregnancy.[22] Roses constitute the main product and are cultivated in greenhouses. These plantations apply ~30 different pesticides (insecticides, acaricides, and fungicides), but diethyldidithiocarbamate fungicides and organophosphate insecticides are most widely used. Although some plantations honor basic occupational safety principles, workers are present in the greenhouses during spraying operations or reenter after delays of only 30 minutes to a few hours.[22,23]

Occupational health studies have documented high exposure to pesticides; associated health problems include neurologic symptoms and signs.[22–24] However, previous studies have not focused on the risks to pregnant women and their children.

METHODS

The town of Tabacundo has 2 public elementary schools and a medical care center that belongs to the Ministry of Public Health. Eligible subjects included all of the children in the 2 lowest grades (called second and third) of 1 of these 2 schools. Through the school headmaster, an invitation to participate was extended to the parents, with a written description of the study. One of the teachers served as contact person. An informational meeting was held at the school on one evening, where the mothers signed the informed consent form, with the teacher signing on behalf of 2 illiterate mothers.

Examinations were scheduled at the nearby medical care center during normal school hours or in the late afternoon. The aim was to complete examinations of each child within 2 hours. The results of the examination were reported to the mother after completion of the clinical tests. The protocol was developed in accordance with the Helsinki convention and was approved by the Comité de Bioética of the Centro de Biomedicina, Universidad Central del Ecuador.

Skilled personnel interviewed the mothers and filled-in detailed questionnaires with ~150 items about the mother's own exposure history, demographic parameters, and the child's past medical history and current health. Each interview took ~1 hour. The questionnaires were then reviewed by a specialist in public health and occupational medicine, and a score was developed in regard to the standard of family housing (traditional/contemporary, running water, and sewage drainage), nutrition (2 or 3 meals per day and extent of animal protein in the diet), and potential maternal pesticide exposure during pregnancy. These categories were determined without knowledge about the child's clinical results.

Physical Examination

A physical examination was conducted by a local pediatrician with emphasis on neurologic functions: tendon reflexes of arms and legs; pronation/supination of forearms; finger-to-nose (open and closed eyes); finger opposition; sensitivity to touch of face, arms, and legs; Romberg's sign; walking on a straight line, on tiptoes, and on the sides of the feet; standing on one leg (10 seconds); and hopscotch. The blood pressure was measured under standardized conditions with the child relaxing in a chair. We used a small cuff appropriate to child size and a standard sphygmomanometer. The average of 2 measurements (in mm Hg) was used. Height (to the nearest half cm) and weight (in kg with 1 decimal) were measured using the routine procedures of the clinic. A capillary blood sample was obtained by a finger stick to determine the hematocrit. When indicated, referral was made and treatment initiated.

Neurobehavioral Tests

Given the time constraints of a schedule that could not last for >2 hours, neurobehavioral tests were chosen based on several considerations.[25,26] One concern was that the tests needed to be acceptable to the children and their parents (ie, painless, not too time-consuming, and appropriate for the age group). Rather than applying tests of general intelligence, we attempted to screen a range of specific brain functions. These tests also needed to be feasible and reasonably culture independent. Because a reliable power supply would be available, we included computerized tests of balance and reaction time. Using previous experience from a study in Brazil,[27] the following tests were considered feasible for children aged 7 years: Santa Ana Pegboard, Wechsler Intelligence Scale for Children-Revised Digit Spans forward, Stanford-Binet copying subtest, and finger tapping. Administration of the latter test had to be abandoned, because the equipment was not suitable for the children's small hand size.

To determine postural stability, we used the Catsys force plate. The child was asked to stand on the plate without moving, first with the eyes open and then closed. The total movement of the gravity center (sway area) was measured for 80 seconds in each condition, in accordance with the manufacturer's instructions.[28]

Simple Reaction Time

The Catsys equipment[28] includes a facility to test reaction time after a visual and auditory stimulus, where the child is required to push a button on a response rod, first with the right hand, then the left, with each condition lasting 120 seconds. This test and the continued performance test[29] were comparable in their sensitivity to prenatal methylmercury exposure (F.D., unpublished data, 2005). We used the average simple reaction time for the right hand and the left hand.

Santa Ana Form Board

In this test of motor coordination, square pegs with a cylindrical head fit into the 4 rows of square holes in the form board. Because this test is not commercially available, we have produced the board and pegs by computerized technology to ensure that all pegs and holes were the same sizes. Half of the circular area on the top of each peg is white, and the other half is black. The subject had to lift each peg and rotate it 180°. The score was the number of pegs turned in 30 seconds with the dominant hand, the nondominant hand, and both hands. We had previously used a test duration of 60 seconds,[27] but half the time was found to be more appropriate for the age

group examined in this study. This test measures motor coordination and dexterity and is known to be sensitive to developmental exposure to methylmercury.[27] Because of the low scores achieved on each condition, we used as the outcome variable the combined score for all 3 of the conditions.

Wechsler Intelligence Scale for Children-Revised Digit Span

In this IQ subtest,[30] the subject repeats strings of digits, and the score is the number of correct trials. We used the forward condition only, as was also the case in a previous study in Brazil.[27] This subtest reflects attention and short-term memory.

Stanford-Binet Copying Test

This test requires copying of visual designs[31] and assesses visuospatial and visuoconstructional function. We administered the second part of the test only, in its entirety, for all 16 of the geometrical figures. Each design was presented to each child, one at a time, at increasing degree of difficulty. The child was required to copy each design in freehand drawing. Using the standard scoring system, we obtained scores for correct designs both for the full series and for a more age-appropriate series of the first 8 designs (items 13–20). The drawings were scored after the completion of the examinations, but without knowledge of subject information, by a clinical neuropsychologist experienced in scoring of this test. In Brazilian children, this test has been found to be sensitive to methylmercury neurotoxicity.[27]

Exposure Assessment

Current exposure to pesticides was ascertained by 2 approaches. A blood sample (1 drop from a finger prick) was obtained to measure erythrocyte acetylcholine esterase activity using the Test-mater equipment and test solutions from EQM Research, Inc (Cincinnati, OH). This parameter reflects the combined impact of pesticide exposures on inhibiting acetyl cholinesterase activity. Because enzyme recovery is slow, it reflects a longer-term average exposure.[32]

In addition, spot urine samples were collected from the children to measure pesticide metabolites. These samples were collected into wide-mouthed Qorpak collection bottles, which were then immediately capped with the companion Teflon-lined screw cap and labeled. The samples were immediately refrigerated and then frozen within 4 hours of collection. The samples were subsequently shipped on dry ice to the Centers for Disease Control and Prevention in a Styrofoam container. Pesticide metabolite analyses were conducted by using an established gas chromatography-tandem mass spectrometry method under tight quality assurance.[33] All of the metabolite concentrations were quantified by using isotope dilution calibration and evaluated on both a whole-volume and creatinine-adjusted basis. Analytes included major organophosphate breakdown products that occur in urine, that is, 3 dimethylphosphate metabolites (dimethylphosphate, dimethyldithiophosphate, and dimethylthiophosphate) and 3 diethylphosphate metabolites (diethylphosphate, diethyldithiophosphate, and diethylthiophosphate). The level of detection was 0.3 μg/L for dimethylphosphate and dimethylthiophosphate and 0.1 μg/L for all of the other alkylphosphate metabolites measured.

Data Analysis

Prenatal pesticide exposure was treated as a dichotomous variable. Stunting was assessed by using the height-for-age z score, calculated from the anthropometric data of the children and the World Health Organization (WHO) Global Database on Child Growth and Malnutrition using Anthro 1.02 software (available at www.who.int/nutgrowthdb). As recommended by the WHO, children who had z scores lower than -2 were considered stunted.[34] Although stunting is usually determined from height-for-age data from children <5 years of age, tracking would also allow judgment of data for children who had just started school.[9]

From the creatinine-adjusted results on urinary pesticide metabolites, the children's individual exposures were estimated using calculated daily creatinine-excretion levels.[35] Because each pesticide may produce >1 metabolite, we summed molar concentrations of dimethyl (originating from compounds, eg, dichlorvos, malathion, and parathion) and diethyl metabolites (from, eg, chlorpyrifos and diazinon) as composite indicators of recent pesticide exposure. We assumed a worst-case scenario, where all of the metabolites originated from highly toxic pesticides with a reference dose (RfD) of 0.3 μg/kg per day. With a molecular weight of $\sim$300, the RfD would, therefore, correspond to $\sim$1 nmol/kg per day. Composite urinary dimethyl or diethyl metabolite-excretion rates above these limits were considered indicative of recent exposure. Because of skewed distributions of the urinary-excretion levels and results below the detection level, we first substituted nondetectable levels with 0.05 nmol/kg per day and then performed a logarithmic transformation of the composite metabolite concentrations.

Confounders were identified from a priori considerations of relevant factors that might influence nervous system development in this community. Because emphasis was on pesticide exposure, potential confounders were first evaluated in regard to their association with the exposure parameter, and those that showed a significant association were included in multiple regression analyses. For all of the neurobehavioral outcomes, age and sex were considered obligatory cofactors. Body weight was a mandatory covariate for blood pressure. Trauma, other injury, and meningitis in the past medical history were considered as a joint and mandatory risk

factor in regard to neuropsychological outcomes, as were maternal education (primary school only or above) and current nutrition (number of meals per day). Given the age adjustment, school grade was not regarded as an obligatory covariate. As suggested by recent studies on malnutrition,[8,19] socioeconomic variables also comprised maternal race, housing (traditional or other), running water at home, sewage drainage, number of siblings, and access to health care (childbirth taking place at home or at the hospital). Maternal smoking and alcohol use during pregnancy was also considered.

Standard parametric statistical tests were applied, but for comparisons of urinary organophosphate metabolites with many results below the detection limit, we used the Mann-Whitney test. Data analysis was conducted by using SPSS 12.0 (SPSS Inc, Chicago, IL).

RESULTS

All 79 eligible children participated in the examinations. However, 1 child had an acute upper respiratory infection requiring antibiotic treatment. Three additional children had clinical abnormalities on the physical examination, and 3 children were older than a limit of 9 years of age considered appropriate for the school grade. These 7 children were excluded from the data analysis. The total number of children included in the data analysis was, therefore, 34 boys and 38 girls. Relevant demographic information is given in Table 1. After these exclusions, a total of 37 children were found to have prenatal pesticide exposure from their mother's work during pregnancy, whereas 35 were free from such exposure. None of the mothers had worked as a pesticide applicator, and all of them reported that they had followed normal safety precautions. None of them had ever been poisoned by pesticides, and relevant symptoms recalled were weak, nonspecific, and without clear association to occupational exposures. In regard to paternal employment, 19 of the exposed children's fathers had worked in floriculture or agriculture during the pregnancy, and the same was true for 16 fathers of the children not prenatally exposed from their mother's work. Paternal work was otherwise mainly in construction trades. The 2 groups of children were remarkably similar. The only major difference was that almost all of the exposed children had access to running water at home. In addition, although not statistically significant, more often the exposed mothers had attended secondary school, and they also had fewer children (Table 1).

TABLE 1 **Demographic and Past Medical History Parameters for 35 Ecuadorian Primary School Children Without (Controls) and 37 Children With (Exposed) Prenatal Pesticide Exposure**

Parameter	Controls	Exposed	*P*
Gender, boy/girl, *n*	15/20	19/18	.47
School grade, 2nd/3rd, *n*	19/16	20/17	.98
Housing, traditional/contemporary, *n*	14/21	15/22	.96
Running water at home, yes/no, *n*	25/10	36/1	.002
Sewage drainage at home, yes/no, *n*	15/20	16/21	.97
No. of meals per day, 2/3, *n*	8/27	8/29	.90
Protein-rich diet, yes/no, *n*	18/17	21/16	.65
Chronic disease, yes/no, *n*	4/31	3/34	.64
History of trauma or accident, yes/no, *n*	12/23	10/26	.55
History of meningitis, yes/no, *n*	1/34	2/35	.59
No. of siblings, mean (SD)	3.4 (3.7)	2.5 (2.1)	.21
Delivery, clinic/at home, *n*	21/14	24/13	.67
Maternal smoking during pregnancy, yes/no, *n*	1/34	2/35	.59
Maternal alcohol drinking during pregnancy, yes/no, *n*	2/33	4/33	.43
Maternal age, mean (SD), y	31.5 (7.2)	30.7 (5.6)	.58
Maternal race, Amerindian/Mestizo-Hispanic, *n*	29/6	30/7	.97
Maternal education, primary school only/above, *n*	30/3	27/9	.23

The pesticide metabolite measurements indicated that the control children had slightly higher current exposure levels than the children with prenatal pesticide exposure (Table 2). A total of 21 children exceeded a summary RfD of 1 nmol/kg per day for the composite measures of dimethylphosphate or diethylphosphates metabolites; 12 of these children were controls, and 9 were prenatally exposed. The erythrocyte acetylcholinesterase activities were within expected ranges and similar in children exposed below and above the RfD level; urinary-excretion rates for individual metabolites or the 2 composite measures did not correlate with the enzyme activity.

In regard to anthropometric measures, exposed and unexposed children were quite similar (Table 3). The exposed children were slightly shorter, but their BMI was slightly higher. These tendencies were not statistically significant. Apart from 1 control child with a hematocrit of 32 in the absence of clinical anemia, all of the children had hematocrit values of ≥37 and were, therefore, considered normal. None of these parameters was related to current pesticide exposure.

The average systolic blood pressure was higher in the exposed children than in those not exposed (Table 4). An approximate 95th percentile of 113 mm Hg[36] was exceeded by 9 children, 7 of whom had prenatal pesticide exposure ($P = .09$). Diastolic blood pressure showed less clear association with prenatal pesticide exposure (Table 4), and 4 of 10 children with a diastolic blood pressure above a 95th percentile of 76 mm Hg[36] belonged to the exposed group ($P = .44$). Blood pressure was not significantly related to any of the anthropometric parameters or to current pesticide exposure.

Three children were left-handed, 2 of whom belonged to the exposed group. The neurologic examination showed normal sensitivity and normal Romberg test, but some abnormalities were observed, particularly in tendon reflexes, finger opposition, and standing on one leg for 10 seconds. At least 1 abnormality was present in 9 children from the control group and 14 from the exposed group ($P = .31$). The computerized posturography showed no difference between the 2 groups

TABLE 2 **Pesticide Exposure (nmol/kg per day) Calculated From Metabolite Concentrations in Urine for 35 Ecuadorian Primary School Children Without (Controls) and 37 Children With (Exposed) Prenatal Pesticide Exposure**

Analyte	Controls			Exposed			*P*[a]
	No. Detectable	Median	Maximum	No. Detectable	Median	Maximum	
Dimethyldithiophosphate	12	—	0.85	8	—	1.08	.21
Dimethylthiophosphate	7	—	4.12	5	—	4.77	.47
Dimethylphosphate	31	0.409	2.39	30	0.200	6.78	.06
Diethyldithiophosphate	0	—	—	0	—	—	1.00
Diethylthiophosphate	19	0.095	0.45	23	0.078	6.77	.48
Diethylphosphate	30	0.297	2.19	29	0.193	7.85	.05
All dimethyl metabolites	31	0.417	6.53	30	0.207	6.94	.07
All diethyl metabolites	31	0.426	2.49	33	0.273	14.62	.04

The level of detection was 0.3 μg/L for dimethylphosphate and dimethylthiophosphate and 0.1 μg/L for all other alkylphosphate metabolites measured. If detectable concentrations were present in less than half of the samples, no median is indicated.

[a] Mann-Whitney test.

TABLE 3 **Anthropometric and Other Clinical Results for 35 Ecuadorian Primary School Children Without (Controls) and 37 Children With (Exposed) Prenatal Pesticide Exposure**

Parameter	Controls ($N = 35$)		Exposed ($N = 37$)		*P*
	Mean ± SD	Range	Mean ± SD	Range	
Age, y	7.14 ± 0.85	5.71 to 8.63	7.09 ± 0.67	5.81 to 8.82	.78
Weight, kg	20.4 ± 2.9	15.1 to 29.0	20.1 ± 2.1	16.5 to 26.0	.56
Height, cm	113.2 ± 7.3	103.5 to 131.5	111.6 ± 5.1	100.5 to 123.0	.27
Height for age, *z*	−1.59 ± 0.99	−3.26 to 0.36	−1.84 ± 0.83	−3.65 to 0.35	.26
Weight for age, *z*	−0.84 ± 0.78	−2.26 to 0.63	−0.92 ± 0.64	−2.06 to 0.52	.64
Weight for height, *z*	0.34 ± 0.71	−1.02 to 1.55	0.50 ± 0.72	−1.07 to 1.04	.34
BMI, kg/m^2	15.9 ± 1.1	13.7 to 17.6	16.1 ± 1.1	13.9 to 18.6	.38
Head circumference, cm	50.7 ± 1.3	48 to 53	50.8 ± 1.4	47.5 to 53.5	.70
Hematocrit, %	42.3 ± 3.2	32 to 48	41.2 ± 2.2	37 to 46	.11
Acetylcholinesterase , U/g of hemoglobin	26.7 ± 3.0	20.1 to 32.7	27.0 ± 2.4	23.2 to 33.2	.59

TABLE 4 **Neurobehavioral Outcome Results (Mean ± SD) in 72 Ecuadorian Primary School Children in Relation to Pesticide Exposure**

Outcome	Prenatal Exposure		*P*	Current Exposure		*P*
	No ($N = 35$)	Yes ($N = 37$)		No ($N = 51$)	Yes ($N = 21$)	
Blood pressure						
Systolic	99.4 ± 7.8	104.0 ± 8.4	.02	102.2 ± 9.1	100.6 ± 6.3	.46
Diastolic	65.9 ± 9.4	68.5 ± 6.9	.19	67.8 ± 8.3	65.7 ± 8.0	.32
Sway area, cm^2						
Open eyes	385 ± 214	361 ± 200	.63	362 ± 170	401 ± 282	.49
Closed eyes	588 ± 282	570 ± 355	.82	559 ± 289	624 ± 388	.44
Santa Ana, total No. correct	30.3 ± 6.0	30.2 ± 6.3	.95	30.9 ± 6.5	28.6 ± 4.6	.15
Mean reaction time, ms	459 ± 134	437 ± 89	.43	426 ± 85	500 ± 153	.011
Digit Span forward	2.9 ± 1.3	3.1 ± 1.2	.58	3.1 ± 1.3	2.9 ± 1.1	.54
Stanford-Binet copying						
Designs 13–20	3.1 ± 1.6	2.3 ± 1.2	.02	2.8 ± 1.6	3.0 ± 1.7	.76
All designs	3.3 ± 1.8	2.5 ± 1.3	.03	2.7 ± 1.5	2.8 ± 1.4	.76

of children with open or closed eyes (Table 4), in agreement with the clinical Romberg test.

On the neuropsychological tests (Table 4), no difference was seen on simple reaction time, the Santa Ana form board score, and Digit Span. However, both copying test scores showed a statistically significant deficit in the exposed children compared with the controls. Similar bivariate analyses showed that stunting was also significantly associated with the copying scores but not with any of the other neuropsychological parameters.

Increased reaction time was associated with current pesticide exposure, as indicated by the urinary organo-

phosphate metabolite excretion, but no such association was seen with the other neuropsychological tests. Reaction time correlated with both composite dimethylphosphate ($r = 0.27$; $P = .02$) and composite diethylphosphate excretion ($r = 0.30$; $P = .01$). The correlation with the sum of all metabolites was even better (Fig 1). However, no association with acetylcholine esterase was seen. These associations were affected only marginally by age, gender, prenatal exposure, and stunting.

Blood pressure and the copying scores were then compared in groups stratified according to both prenatal pesticide exposure and stunting (Table 5). Stunting had no clear effect on blood pressure. The highest systolic blood pressure was seen in the exposed children, who were also stunted; prenatal pesticide exposure remained statistically significant in this subgroup.

The highest copying scores were obtained by children without signs of stunting who had also not been exposed to pesticides prenatally (Table 5). The lowest scores were seen in stunted children who had been exposed to pesticides prenatally. Both of these predictors showed statistically significant effects. Similar results were obtained with the full copying score.

Therefore, multiple regression analyses were conducted (Table 6). Age and gender were considered overall obligatory cofactors, and body weight was included as a recognized risk factor for blood pressure. Other anthropometric factors were also included in exploratory analyses but were not statistically significant and changed the regression coefficients for pesticide exposure only minimally. Height for age, also as a continuous parameter, did not approach statistical significance. After covariate adjustment, prenatal pesticide exposure was associated with a significant increase of 4.6 mm Hg in systolic blood pressure (Table 6); the parallel result for diastolic blood pressure was 2.7 mm Hg ($P = .16$).

The copying score was adjusted for age, gender, maternal education, and medical risk factors as a priori predictors of the neuropsychological outcome. In addition, running water at home was included as a significant covariate. The adjusted regression coefficients for both pesticide exposure and stunting were statistically significant (Table 6). When included in the regression

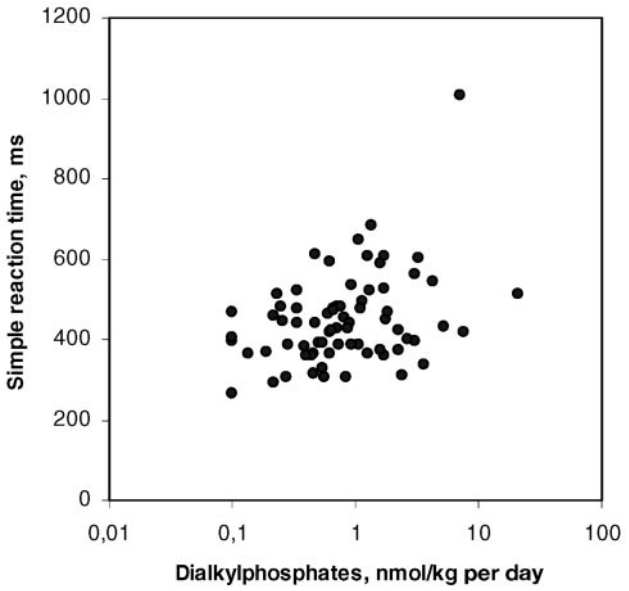

FIGURE 1
Average simple reaction time for right and left hands in relation to composite urinary excretion of dimethylphosphate and diethylphosphate metabolites in 72 Ecuadorian school children ($r = 0.36$; $P = .002$).

TABLE 5 **Averages and SD of Blood Pressures and Stanford-Binet Copying Scores for 72 Ecuadorian Primary School Children in Regard to Prenatal Pesticide Exposure and Stunting (Height-for-Age *z* Score of Less Than −2)**

Outcome	Unexposed		Exposed		*P*
	N	Mean ± SD	*N*	Mean ± SD	
Systolic blood pressure, mm Hg					
Not stunted	19	100.4 ± 6.9	22	103.3 ± 9.2	.26
Stunted	16	98.3 ± 8.9	15	105.0 ± 7.1	.03
P		.43		.55	
Diastolic blood pressure, mm Hg					
Not stunted	19	67.7 ± 8.9.	22	68.7 ± 7.4	.68
Stunted	16	63.8 ± 9.7	15	68.1 ± 6.3	.16
P		.23		.79	
Stanford-Binet copying score (13–20)					
Not stunted	19	3.68 ± 1.73	22	2.45 ± 1.14	.01
Stunted	16	2.38 ± 1.15	15	2.07 ± 1.16	.46
P		.02		.32	
Stanford-Binet copying, total score					
Not stunted	19	4.00 ± 1.97	22	2.68 ± 1.32	.02
Stunted	16	2.44 ± 1.26	15	2.13 ± 1.19	.50
P		.01		.21	

TABLE 6 **Multiple Linear-Regression Results for Independent Predictors of Blood Pressure and Stanford-Binet Copying Scores for 72 Ecuadorian Primary School Children**

Outcome and Predictors	β	SE	*P*
Systolic blood pressure, mm Hg			
Prenatal pesticide exposure	4.57	1.89	.018
Stunted	−2.81	2.41	.25
Weight, kg	−0.83	0.58	.16
Male gender	2.41	1.95	.22
Age, y	3.79	1.63	.024
Stanford-Binet copying score (13–20)			
Prenatal pesticide exposure	−1.01	0.37	.009
Stunted	−0.74	0.35	.038
Male gender	−0.13	0.35	.70
Age, y	0.27	0.23	.24
Mother primary school only	−0.01	0.24	.97
Risk factors in past medical history	−0.24	0.38	.53
Two meals per day only	−0.13	0.41	.76
No drinking water at home	−0.59	0.50	.25

model as a continuous variable, the height-for-age z score showed a $P > 0.05$ and caused a slight change in the regression coefficient for pesticide exposure to −0.92 ($P = .018$). As additional confounders, we examined other potential predictors of childhood neurobehavioral development, but none of them was statistically significant. Adding or removing these confounders from Table 1 only marginally affected the estimates for pesticide exposure and stunting. Virtually the same results were obtained with the copying scores for the complete series of designs. When an interaction factor between pesticide exposure and stunting was added, it showed a nonsignificant regression coefficient in the direction opposite to expectation, whereas both pesticide exposure and stunting remained significant.

DISCUSSION

In this study, prenatal pesticide exposure was associated with a higher blood pressure, an effect that seemed independent of stunting. Clinical neurologic examination showed a marginal increase in abnormalities among exposed children, and a lower score on the Stanford-Binet copying test was found in children with prenatal pesticide exposure and also in stunted children. Both risk factors remained statistically significant in adjusted analyses, and pesticide toxicity may, therefore, add to the adverse influence of malnutrition. Current pesticide exposure, as indicated by increased total excretion of dimethyl and diethyl metabolites of organophosphates, was associated with increased simple reaction time only, and this effect was independent of the other risk factors. These findings suggest that effects of prenatal pesticide exposure may be lasting and may differ from the effects of postnatal exposure.

The present study has aimed at exploring whether neurobehavioral deficits may be associated with prenatal pesticide exposure in a carefully chosen community, where social differences were thought to be minimal. Although nutritional deficiencies are widespread, pesticide exposure and stunting were not linked. The children examined attended the same school and were growing up under similar circumstances, thereby providing an opportunity to study children who were comparable in most respects, except for prenatal neurotoxicant exposure. Although employed mothers could be less capable of providing necessary parenting,[4] those who were capable of obtaining employment in floriculture seemed to be better educated and seemed to provide slightly better living conditions for their fewer children.

A cross-sectional study like the present one cannot provide any detailed information about dose-response relationships or the time of impact of toxic exposures. The dichotomous prenatal exposure score is crude and based only on maternal interview but allows statistical comparison of children with some degree of prenatal exposure to those with limited risk of such exposure. The magnitude and exact duration of the prenatal exposure is not known. Exposure misclassification is possible, but would be random, because the maternal interviews were conducted before the mothers received the results of the examinations of the children. Such misclassification is likely to result in a bias toward the null hypothesis, and any adjustment, if possible, would strengthen the findings of this study. Also, selection bias would be unlikely, because all of the eligible children participated in the examinations. Given the similarity between the exposed children and the controls in regard to past medical history and most other respects, confounding bias also seems unlikely to be a major problem in this study.

Women who do not work in floriculture may earn an income from handicraft, cooking, and similar occupations that do not entail exposures to pesticides or other neurotoxicants. Still, because of the widespread use of pesticides, such as for household purposes, the children could potentially be exposed from other sources (eg, from contaminated food or the environment). Yet, prenatal exposure was associated with neither a decreased current acetylcholine esterase activity nor an increased urinary metabolite-excretion rate, thus indicating no confounding in this regard.

Under the cross-sectional circumstances of the present study, assessment of stunting had to rely on current anthropometric measurements without the benefit of prospective growth data. Therefore, the height-for-age z score obtained at school age may be associated with some uncertainty in regard to malnutrition otherwise determined at ages <5 years, according to international recommendations.[37] However, most children are likely to have maintained their relative rank due to tracking and limited compensatory growth during the next couple of years, because the nutritional mechanism involved in stunting also leaves the child unable to catch up in growth.[38]

The standard requirement by the WHO of −2 as the stunting cutoff point for height for age[37] may be unreasonably strict for 2 reasons.[19] Populations at altitudes >3000 m may be affected by low oxygen tension, with a proportionately greater growth of the chest in comparison with other body proportions.[39] In addition, the Andean physique is typically short,[39] with mean adult heights of <150 cm,[8] thus suggesting that genetic factors may be of importance in regard to vertical growth. Furthermore, weight for height and BMI values in the present study deviate only minimally from expectation. Thus, the calculated height-for-age z scores likely exaggerate the extent of stunting in this population. Still, this dichotomous classification was a better risk indicator for decreased copying scores than was the continuous z score. In agreement with other research,[5] these findings, therefore, indicate that even a minor degree of stunting can impact brain development.

The neurobehavioral tests included in the present

study represent only a preliminary assessment of relevant brain functions. Although the tests were entirely feasible in this population, the results obtained must be interpreted in light of the likelihood of the tests being sensitive to neurotoxic damage. Thus, the computer-assisted tests provided excellent separation of performance results, but the traditional neuropsychological tests allowed fewer possible scores in the age range tested, thus substantially limiting the test sensitivity to small deficits.

Visuospatial performance, as assessed by the copying test, is important in regard to orientation in space and spatial performance. The adjusted regression coefficient for prenatal pesticide exposure was 4 times greater than the coefficient for age. This means that the deficit on the copying test associated with prenatal pesticide exposure corresponds to a developmental delay associated with being 4 years younger. Although the confidence interval is relatively wide, this statistically significant finding in a study of limited size suggests that the effect could be substantial. Preservation of spatial functions is likely of wider significance, because spatial perception and visuomotor coordination may be a prerequisite for other brain functions and their optimal development. Thus, increasing degrees of dysfunction on copying designs of the Rey-Osterrieth complex figure significantly predicted a greater probability of requiring remedial services in school.[40]

One previous study of children with developmental pesticide exposure in Mexico[15] found no exposure-related difference in growth patterns, but the exposed children showed deficits in eye-hand coordination, short-term memory, and the ability to draw a person. Although the present study found no effects on one test of motor coordination and did not use comparable memory tests, it replicated the findings in regard to spatial functions. The Mexican study used an ecological exposure classification that may have been imprecise and may have involved some degree of unmeasured confounding.[15] With exposure information available on an individual level, the present study, therefore, provides strong support for the notion of a pesticide effect on visuospatial development.

Increased simple reaction time was significantly associated with increased dimethylphosphate and diethylphosphate metabolite-excretion levels. Although not associated with erythrocyte acetylcholinesterase activity, the effect on reaction time could not be explained by any relevant cofactors assessed in this study. An increased reaction time is in accordance with observations in children and adults exposed to organophosphates,[41,42] and it could, therefore, likely reflect an effect of current pesticide exposures. On the other hand, concentrations of organophosphate metabolites in the urine were similar to levels reported for US children aged 6–11 years,[43] and the 90th percentile for composite metabolite excretions at 574 μg/g of creatinine was not exceeded by any of the Ecuadorian children. However, these results may not be directly comparable, because the metabolites may originate from different parent organophosphates. Likewise, the cutoff level of 1 nmol/kg per day for dimethyl and diethyl metabolite excretions may be considered arbitrary and difficult to interpret. At the same time, acetylcholinesterase activities were within anticipated ranges and did not show any decrease associated with urinary metabolite-excretion levels. The apparent increase in reaction time nonetheless suggests that adverse effects may occur in children even when biomarker results are not increased beyond ranges considered normal.

In regard to blood pressure, heart action and vascular resistance are regulated by the autonomic nervous system, and ventricular arrhythmias are well known from organophosphate pesticide poisonings.[44] Neurobehavioral research has only recently begun to include cardiovascular parameters, and adverse cardiovascular effects in children are now known to occur as a result of exposures to neurotoxicants, such as lead[45] and methylmercury.[46,47] In the present study, the exposure-related increase in systolic blood pressure and the smaller, not statistically significant, increase in diastolic blood pressure were independent of stunting and other covariates. Parallel to this increase, a higher number of exposed children exceeded the expected normal range of systolic blood pressure. Nutritional factors[48–50] and maternal smoking during pregnancy[51] may contribute to increased blood pressure in children. These parameters would be unlikely to explain the associations found, because stunting was of negligible importance, and only a few women smoked at all during pregnancy. Increased blood pressure, when present in childhood, is a risk factor for cardiovascular disease in later life,[36] and these results may, therefore, be important from a public health viewpoint.

In the present study, current pesticide exposure, which was independent of prenatal exposure, was not associated with the same outcomes that showed deficits after prenatal pesticide exposure. This observation is in accordance with the notion that prenatal and postnatal exposures may affect different nervous system functions in children.[1] Furthermore, the effects of prenatal pesticide exposure showed similarities with those associated with stunting, thus suggesting that malnutrition and toxicant exposures during early development may affect similar functions.

Within the limited literature on developmental neurotoxicity, the findings of the present study show parallels to another Mexican study.[52] This research showed that the height-for-age index was an independent risk indicator for low IQ scores, which were also affected by exposures to the neurotoxicants lead and arsenic.[52] In this study as well, the neurotoxicant effects seemed to be independent of the sequelae of malnutrition, although

the coexistence of these factors seemed to render a child potentially more vulnerable to delayed mental development. The findings of both studies underline the need to assess chemical exposures as a cofactor when studying the adverse effects of malnutrition.

The toxicological literature has convincingly documented the neurotoxicity of many pesticides, and the increased vulnerability of the developing nervous system is also well substantiated.[11–14,40,53] However, despite the extensive literature on pesticide poisonings in adults, very limited information is available on the effects of pesticides on the developing brain in humans.[1,2] Part of the reason for this lack of information is undoubtedly that the most widespread pesticide exposures occur in developing countries, where epidemiological studies may be difficult to carry out and where malnutrition and other potential confounders may compromise the results. On the other hand, extensive evidence has been gathered on the neurobehavioral consequences of malnutrition and stunting,[4–6,19,54] but these studies have not included pesticide exposure as a relevant cofactor that could impact on child development.

CONCLUSIONS

This study shows that maternal occupational exposure to pesticides during pregnancy is an important risk factor for the neurobehavioral development of a child. Statistically significant effects were increased blood pressure and decreased visuospatial performance. Although the latter outcome was also affected by stunting, the associations were robust to confounder adjustment. Furthermore, current pesticide exposure was associated with an increased simple reaction time, a parameter that was not affected by prenatal pesticide exposure or stunting. These results, therefore, suggest that prenatal pesticide exposure may adversely affect brain development, that the effects may resemble those caused by malnutrition, and that they differ from postnatal toxicity. Because developmental neurotoxicity is likely to cause permanent deficits, pesticide exposure during early life must be considered an important risk to human health that deserves attention by prevention authorities and the research community.

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