ORIGINAL ARTICLE

Assessment of long-term and recent pesticide exposure among rural school children in Nicaragua

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ABSTRACT

Objective This study assessed pesticide exposure of children in rural Nicaragua in relation to parental pesticide use, from around conception to current school age, as part of an epidemiological evaluation of neurodevelopment effects.

Methods We included 132 children whose parents were subsistence farmers or plantation workers, or had an agricultural history. As proxies for children's long-term exposures, we constructed cumulative parental pesticide-specific use indices for periods before and after the child's birth from data obtained using an icon-calendar-based questionnaire, of application hours (h) for plantation workers and subsistence farmers, and of kilograms of active ingredients (ai) only for subsistence farmers. Pesticide residues of TCPY, 3-PBA and 2,4-D were analysed in children's urine as indicators for current exposures.

Results Life-time indices were highest for the organophosphates chlorpyrifos (median 114 h (min 2; max 1584), 19.2 kg ai (min 0.37; max 548)) and methamidophos (84 h (6; 1964), 12.2 kg ai (0.30; 780)). The P50 values of children's urinary residues were 3.7 μg/g creatinine for TCPY, 2.8 for 3-PBA and 0.9 for 2,4-D; TCPY values are comparable with those in other countries, but 3-PBA and 2,4-D are considerably higher. The maximum levels for all three pesticides are the highest reported for children. Residues increased on days after application, but most high residue levels were unrelated to parental pesticide applications.

Conclusion Urinary pesticide residues reveal high environmental exposure among children in rural Nicaragua. The quantitative parental pesticide use indices as proxies for children's exposures during different periods may be useful for the evaluation of developmental health effects.

What this paper adds

- There is evidence that children of agricultural workers have more contact with pesticides than children in the general population, through parental take-home and farm proximity pathways, possibly inducing developmental effects at different times from pregnancy up to adolescence.
- Pesticide urinary residues showed high exposures among young children in rural Nicaragua.
- This study describes cumulative prenatal and postnatal parental pesticide-specific use indices for different periods of development, to be used as proxies of children's long-term pesticide exposures for outcome assessment.
- Children's pesticide exposures are a public health concern and require regulatory action by the Nicaraguan government as well as international agencies; policies for sustainable agricultural practices in developing countries, eliminating the risk of pesticide exposure at source, are recommended.

INTRODUCTION

In Central America, pesticides are used intensively in agriculture which employs about a quarter of the labour force.[1] In 2000, an estimated 45 million kg of pesticides were imported into the region (6.7 kg for each agricultural worker).[2] Some of the frequently pesticides in Central America are restricted in industrialised countries. For example, methamidophos (WHO category 1b) is a restricted use pesticide in the USA and may only be handled by certified operators.[3] Chlorpyrifos, although classified as moderately hazardous, is ranked fourth as a causal agent for occupational pesticide poisonings in Nicaragua, due to high volume of use and unsafe practices.[4]

The families of agricultural workers are in contact with pesticides. Children often play and work in or near pesticide treated fields, pesticides may drift, and parents may transport pesticides into the home.[5–7] Children are also exposed to pesticides through their diet. Organophosphate exposure has been related to diet,[8–10] as has exposure to pyrethroids[11] and 2,4-D.[12] In addition, children and adolescents are occupationally exposed to pesticides, especially on small holdings. The International Labour Organization (ILO) has estimated that some 3.2 million children are working in agriculture in Central America.[13] In Nicaragua, approximately 140 000 children aged 5–17 years work in subsistence farming and commercial food production (87% boys), which involves the handling of agrochemicals.[14]

Children's exposures to pesticides are a global concern because of their particular vulnerability due to differences in physiological characteristics as compared to adults.[15] Young children have a larger skin surface area relative to their body weight, a higher basal metabolic rate, and greater oxygen requirements. They also eat more food, drink more water for body weight, and breathe more air than adults. In addition, children's ability to metabolise, detoxify and excrete toxic compounds is immature.[16] Empirical evidence substantiates high susceptibility in humans to toxic insult in the developing nervous system from early gestation to adolescence, which can result in diverse outcomes ranging from structural abnormalities to functional deficits.[16 17]

In the US several studies have comprehensively assessed children's pesticide exposures by examining multiple sources and pathways, including dietary and water intake, domestic pesticide use, and parental take-home and farm proximity pathways. In Central America, some studies have shown important pesticide exposures among children in agricultural areas. Azaroff *et al* reported alkylphosphate residues in almost half of 358 urine samples taken from farmer's families in El Salvador.[18] In Nicaragua, children living close to a fumigation airport had lower cholinesterase levels than unexposed children.[19] Proximity to spraying and spray mixture preparation in homes were found to be important factors for children's exposure to diazinon and chlorpyrifos.[20] Only one study in Central America has linked children's pesticide exposure with a health outcome, specifically parental application of pesticides during critical windows of vulnerability for children with childhood leukaemia risk in Costa Rica.[21]

The objective of this study was to assess the pesticide exposure of children in rural Nicaragua in relation to parental pesticide use, from the time around conception up to current school age, as part of a broader epidemiological evaluation of pesticide-related neurobehavioral effects.

METHODS

Study population

This broad epidemiological study was conducted in the northwest area of Nicaragua from September to November 2007 and from May to October 2008. The villages studied were Los Zanjones/Calle Real in Posoltega, a highly agricultural area that produces peanuts for export and cereals and vegetables for subsistence, and Colonia 20 de Julio in Villanueva, a rural area with predominantly informal non-agricultural commercial activity. The study targeted all children aged 7–9 attending grade 1–3 classes in school. Contact with families was established with the help of the health authorities, school teachers, the Farm Worker's Association (Asociación de Trabajadores del Campo, ATC) and the Nicaraguan Agricultural Technology Institute (Instituto Nicaragüense de Tecnología Agropecuaria, INTA). A series of community meetings were first organised to explain the study to the families and children and to discuss procedures.

We assessed the pesticide exposure of 132 children. In the agricultural village, we included the data of 110 of the 126 children attending grade 1–3; 16 were excluded because of age or neurological disease or failure to interview the parents. In the non-agricultural village, of the 84 children attending grade 1–3 we included the data of 22 whose parents had worked previously in agriculture; the parents of 52 children had no agricultural history and the parents of 10 children could not be interviewed.

Exposure assessment

Data for exposure assessment were collected at two different times. During 2007 we collected long-term exposure data and during 2008 recent exposure data.

As regards long-term exposure, an icon-calendar-based form (ICBF)[22] was used to interview parents about their children's prenatal and postnatal pesticide exposures up to 2007. The ICBF was administered to 125 parents as only one ICBF was used for children who were siblings. However, the children's individual data differed for the exposure windows. The ICBF included one calendar sheet for each year, each marked with the 12 months. The ICBF covered pesticide use data from 1 year before birth up to the current year. Stickers were used to indicate life events and work, and colour pilots to mark the duration of each economic activity.

The interviewer asked the parents to provide the date of their child's birth and other important life events such as marriages, deaths, hurricane Mitch, presidential elections or inauguration of the school building. For each life event, an icon representing the event was placed at the appropriate date on the calendar. The subject was then asked detailed questions concerning his or her entire work history, first for the year preceding the birth, and next starting from the present moving backwards until the birth date. The interviewer prompted the subject's recall by referring to the life event icons previously placed on the calendar. In the ICBF we registered data on pesticide use for different crops and years, specific trade names or active ingredients, dose, number of applications during the spraying season, and usual hours of spraying, as well as use of personal protective equipment (PPE), personal hygiene, storage practice, and other practices. Most participants from the agricultural community were subsistence farmers (n=68) spraying with backpacks, 28 were plantation workers spraying with tractors, and seven were plantation workers who were also subsistence farmers.

Indices for retrospective pesticide use were constructed for the fathers and the sole mother applicator as proxies for the prenatal and postnatal exposures of the children. The indices referred to specific time windows and to total prenatal and postnatal exposures, which we refer to as lifetime exposures. For the subset of subsistence farmers, a retrospective cumulative pesticide-specific use index of total kilograms sprayed was constructed: dose (g/ha)×number of hectares×number of applications. For plantation workers we were able to collect data on the use of specific pesticides but not on dose and number of hectares sprayed, and therefore the pesticide use index was calculated as number of hours (number of days×number of hours per day). The latter was also used as a second index for subsistence farmers for comparing and combining groups. The indices of fathers who were both plantation workers and subsistence farmers were summed. The indices were not adjusted for use of PPE or other exposure determinants, because nobody had ever used PPE and there were no notable differences in other practices. Indices were constructed for five selected pesticides known to be neurotoxic and in use from 1997, the first year of our data collection: chlorpyrifos, methamidophos, cypermethrin, deltamethrin and 2,4-D. In addition, we constructed summary indices for the chemical groups organophosphates, carbamates and pyrethroids.

In 2007, when we administered the ICBF, we were unable to collect urine samples to simultaneously evaluate current exposure due to lack of farming activity because of hurricane Felix. Consequently, we planned to investigate the recent exposure of these same children in the 2008 agricultural seasons. However, unfortunately in this particular year, agricultural activities were at a low level and only 32 farmers (29%) were cultivating crops, this time because of delays in the delivery of seeds by state agencies. We analysed pesticide residues in the urine of children of farmers cultivating and not cultivating crops. Since farmers had explained to us that they follow the phases of the moon for spraying activities, we organised a sampling week around the full moon in July 2008. We asked the mothers of the 32 children whose parents were cultivating crops to write down the name of any pesticide used on a calendar we provided, and have the children carry it to school together with a urine sample, every day from Monday to Saturday. To obtain samples from the children of all families cultivating crops, we conducted similar sampling weeks in August and October 2008. In addition, we asked mothers of approximately half the families who were not cultivating crops to collect a urine sample from their children (n=42). We obtained 154 samples from the 32 children of fathers with crops (2–6 samples per child, 4.8 on average) and 57 samples from 42 children of fathers without current agricultural activity (15 children delivered two urine samples), for a total of 211 urine samples.

Immediately after collection, specimen samples were stored in a cool-box with frozen ice packs for transportation to the laboratory at CISTA/UNAN- León. Specimens were stored in the freezer at −20°C and subsequently shipped with blue ice to Sweden via express mail. The urine samples were analysed for the herbicide 2,4-D, and the insecticide metabolites 3-PBA (a general metabolite of pyrethroids) and TCPY (a specific metabolite of the organophosphate chlorpyrifos) at the laboratory of Occupational and Environmental Medicine of Lund University. Urine samples were hydrolysed and extracted using a mixed-mode solid-phase extraction cartridge. Extracts were analysed by high-performance liquid chromatography–tandem mass spectrometry using selected reaction monitoring in the negative ion mode.[23 24] The limits of detection (LOD) were 0.05 ng/ml for 2,4-D, 0.2 ng/ml for 3-PBA and 1 ng/ml for TCPY. Samples were analysed in duplicate, and the variation coefficients were 7% for 2,4-D, 9% for 3-PBA and 18% for TCPY. The quantified urine levels below the LOD were also used in the statistical analysis.

Data analysis

Statistical analyses of quantitative data were performed using SPSS software, release 16. For the two continuous indices of retrospective pesticide use, the median, minimum and maximum were computed for seven time windows: the trimester before conception, the first, second and third trimesters of pregnancy, the first year of life, age 1–5, and age 6 and older. In this paper, the first four windows (pre-conception and pregnancy) were merged into a single prenatal index.

Urine samples were categorised as unrelated or related to parental pesticide application, the latter provided between 24 and 72 h after application. Samples were categorised as related or unrelated separately for TCPY, 3-PBA and 2,4-D according to information from the mothers about specific pesticides sprayed. Geometric means, geometric standard deviations, and P25, P50, P75, P90 and P95 were calculated for the categories 'related' and 'unrelated' to parental pesticide applications, and for all urine samples combined. Adjustments of urine metabolites by urine density and by creatinine were highly correlated (Spearman r=0.92 for 2,4-D, 0.93 for 3-PBA, and 0.94 for TCPY). We used the creatinine adjusted values for comparison with other studies.

Ethical considerations

The study was approved by the Ethical Committee of the Faculty of Medical Sciences of UNAN-León. At the start of the study, researchers informed participants, including children, about the objectives and characteristics of the study in writing as well as verbally, and all parents signed an informed consent form. Analysis of pesticide biomarkers in Sweden was approved by the regional ethical board at the Medical Faculty, Lund University (Regionala Etikprövningsnämnden in Lund, dnr 208/2009).

RESULTS

Most of the fathers in the agricultural village were farmers (66%) who worked on small holdings of 0.3–8.8 hectares, cultivating corn, vegetables, rice, beans and other crops for food. The remaining 34% were workers on plantations. There was only one agricultural worker in 94% of the families. The agricultural community was surrounded by peanut plantations and 53% of the families lived in one-room houses, with dirt floors, roofs in very poor condition, and the kitchen and common family areas completely open to cultivated fields. All fathers in the non-agricultural village worked in occupations unrelated to agriculture, although some[22] had worked previously on plantations. The mothers in both communities were mainly housewives.

Pesticide exposure indices

A total of 47 pesticides were reported on the ICBF by the parents of the 132 children. These included 34 insecticides (organophosphates, carbamates and pyrethroids), seven herbicides (triazines, bipyridyliums and phosphonates), five fungicides (dithiocarbamates, triazoles and chloronitriles) and the fumigant aluminium phosphide. In recent years, the use of pyrethroids, mostly cypermethrin, had increased greatly and new herbicides (pendimethalyn, fomesafen, bentazon and fluazifop) and fungicides (tebuconazole, hexaconazole and mancozeb) had also been introduced.

Concerning exposure to the selected neurotoxic pesticides, 95 (53%) of the parents reported having used organophosphates, 100 (54%) pyrethroids, and 53 (29%) 2,4-D. Regarding indices for hours of use during the children's total lifetime (long-term exposure), the median (min; max) was 114 (2; 1584) for chlorpyrifos, 84 (6; 1964) for methamidophos, 81 (8; 1976) for cypermethrin, 40 (2; 265) for deltamethrin and 53 (2; 388) for 2,4-D. Regarding the indices for kilograms of active ingredient sprayed by the subsistence farmers during the children's lifetime, the median (min; max) was 19.2 (0.37; 548) kg for chlorpyrifos, 12.2 (0.30; 780) kg for methamidophos, 2.1 (0.01; 354) kg for cypermethrin, 0.97 (0.04; 22.7) kg for deltamethrin and 8.8 (1.1; 22.7) kg for 2,4-D.

As with the lifetime indices, all pesticide-specific indices for dose as well as hours of application showed ample gradients within all time windows. Table 1 gives the number of parents who sprayed the selected neurotoxic pesticides during the time windows with application hours and kilograms of active ingredient sprayed on average per year. The number of fathers who applied chlorpyrifos and deltamethrin decreased over time but the number using cypermethrin and 2,4-D increased. For the five pesticides, the median amounts sprayed per year by fathers were higher during the prenatal period and first year of life than during the later time windows. The number of hours of application of cypermethrin, deltamethrin and 2,4-D were also higher in the earlier periods, but the pattern for chlorpyrifos and methamidophos was not so clear. The median hours of application of the different pesticides during the children's lifetime were in general about twice as high for subsistence farmers compared to plantation workers. As expected, the median hours of application during the children's lifetime were between two and eight times higher for the children of parents currently engaged in agriculture than those not currently engaged.

Pesticide urinary metabolites

Pesticide urinary metabolites are shown in table 2. Most (n=203) samples contained quantities above the laboratory LOD for at least two of the three analysed residues: 74% contained

Table 1 Indices of parents' pesticide use in hours and kilogram of active ingredient (ai) by time windows

Long-term exposure (pre-conception, 2007), n=132	Chlorpyrifos n	Chlorpyrifos Median (min; max)	Methamidophos n	Methamidophos Median (min; max)	Cypermethrin n	Cypermethrin Median (min; max)	Deltamethrin n	Deltamethrin Median (min; max)	2,4-D n	2,4-D Median (min; max)
Periconception and pregnancy (1 year)										
Index in hours	39	30 (2; 384)	46	43 (3; 1003)	52	19 (2; 384)	19	18 (2; 312)	31	16 (2; 360)
Index in kg ai	37	7 (0.25; 135)	46	4 (0.16; 336)	52	0.7 (0.01; 197)	17	1.35 (1.35; 21)	31	5.5 (0.45; 97)
First year of life (1 year)										
Index in hours	36	19 (2; 144)	46	22 (2; 192)	55	22 (2; 120)	14	23 (8; 72)	33	24 (3; 114)
Index in kg ai	33	3.8 (0.13; 58)	43	2.4 (0.18; 48)	55	0.6 (0.02; 167)	14	0.8 (0.01; 3)	31	3 (0.3; 74)
Age 1–5 (average per year, 4 years)										
Index in hours	32	16 (2; 144)	52	16 (1; 96)	74	6 (0.5; 134)	13	4 (1; 18)	39	12 (0.5; 21)
Index in kg ai	37	1.3 (0.03; 66)	52	0.97 (0.04; 93)	72	0.16 (0.01; 58)	9	0.17 (0.01; 0.5)	39	0.44 (0.04; 47)
From age 6 (average per year, up to 2007, 2–4 years)										
Index in hours	27	40 (2; 320)	46	20 (2; 176)	85	8 (2; 288)	10	8 (2; 16)	44	6 (2; 48)
Index in kg ai	28	2 (0.04; 74)	46	1.4 (0.04; 75)	74	0.25 (0.01; 51)	8	0.41 (0.02; 1.34)	44	0.9 (0.10; 55)
Total prenatal and postnatal exposure (average per year, 8–10 years)										
Index in hours	46	11.7 (0.2; 176)	85	10 (0.6; 213.8)	96	8.6 (0.8; 197.6)	27	6.1 (0.1; 31)	52	0.99 (0.13; 44.3)
Index in kg ai	46	2.1 (0.05; 60.9)	85	1.2 (0.03; 86.7)	94	0.25 (0.01; 39.4)	23	0.2 (0.01; 2.52)	53	4.2 (0.25; 38.3)

TCPY, 97% 3-PBA and 99% 2,4-D. The P50 was 3.7 μg/g for urinary creatinine adjusted TCPY, 2.8 μg/g for 3-PBA and 0.9 μg/g for 2,4-D. The maximum levels of creatinine unadjusted (μg/l)/creatinine adjusted (μg/g of creatinine) were 303/595 for TCPY, 66/244 for 3-PBA and 7.4/14 for 2,4-D. The P50 for boys and girls were similar.

Children's urinary levels increased on the days following a parental pesticide application for the three evaluated pesticides. TCPY peaked 1 day after application from 3.9 to 16.5 μg/g (figure 1) based on eight datasets and 3-PBA peaked 4 days after application from 2.6 to 3.8 μg/g based on 22 datasets. However, the highest residue levels were observed mostly in children whose parents had not applied the pesticide in question; 18 of the 21 highest TCPY and 15 of the 21 highest 3-PBA urinary residue values (≥P90) were unrelated to parental pesticide application.

DISCUSSION

This study developed an exposure assessment strategy to be used for the evaluation of developmental neurotoxic effects among children in a poor area of rural Nicaragua. The ICBF allowed the construction of quantitative indices of parental exposure as proxies for prenatal and postnatal exposures of children, specifically cumulative pesticide-specific indices for hours of application for subsistence farmers and plantation workers, and, in addition, cumulative pesticide-specific dose indices for subsistence farmers. Urinary pesticide residues showed widespread current pesticide exposure among children, with increasing levels following parental sprayings, but also with high to extremely high levels in children whose parents had not sprayed.

Pesticide exposure assessment in children in epidemiological studies is difficult in any setting. Many studies have used very crude exposure indicators, such as history of residence in agricultural areas or paternal/maternal farming occupation during pregnancy or early childhood.[25] Pesticide biomarkers at the time of birth, specifically residues in the umbilical cord or meconium,[26] reflect recent exposures or exposures to highly persistent pesticides, but do not cover all prenatal pesticide exposures of concern. Recent birth cohorts prospectively follow-up children

Table 2 Pesticide urinary metabolites in 211 samples from 77 children from Los Zanjones/Calle Real

	All samples			Samples unrelated to parental pesticide application			Samples after parental pesticide application		
Number of samples (no. of children)	TCPY 211 (77)	3-PBA 211 (77)	2,4-D 211 (77)	TCPY 192 (69)	3-PBA 112 (55)	2,4-D 208 (76)	TCPY 19 (8)	3-PBA 99 (22)	2,4-D 3 (1)
% Above LOD	73.9	96.7	99	74	96	99	93.7	97	100
Creatinine unadjusted (μg/l)									
GM	2.0	1.5	0.5	2.0	1.7	0.5	2.0	2.4	0.4
P25	1.0	0.74	0.2	1.0	0.7	0.4	0.9	0.7	0.2
P50	1.7	1.3	0.5	1.7	1.3	0.5	2.3	2.1	0.4
P75	4.5	2.9	0.9	4.2	3.1	0.9	5.4	2.8	0.5
P90	9.5	5.9	1.7	9.7	7.4	1.7	7.1	5.2	0.5
P95	13.6	10.3	24	13.7	12.1	2.4	9.1	8.8	0.5
Max	302.8	66.2	7.4	302.8	66.2	7.4	9.1	22.9	0.5
GSD	3.2	3.2	2.5	3.3	3.1	2.2	2.8	2.4	2.7
Creatinine adjusted (μg/g creatinine)									
GM	4.3	3.2	1.0	4.3	3.7	1.0	3.9	5.0	0.8
P25	2.3	1.8	0.6	2.3	1.5	0.6	1.4	1.7	0.6
P50	3.7	2.8	0.9	3.6	2.8	0.9	3.8	4.7	0.8
P75	7.1	5.2	1.7	6.9	5.3	1.7	14.4	4.5	0.9
P90	16.5	9.4	2.9	16.2	10.2	2.9	17.4	8.2	0.9
P95	23.3	13.3	4.1	26.3	14.2	4.2	20	13.3	0.9
Max	594.9	243.8	14	594.9	243.8	14	20	21.4	0.9
GSD	2.9	2.3	2.2	2.9	2.9	1.1	3	2.4	1.1

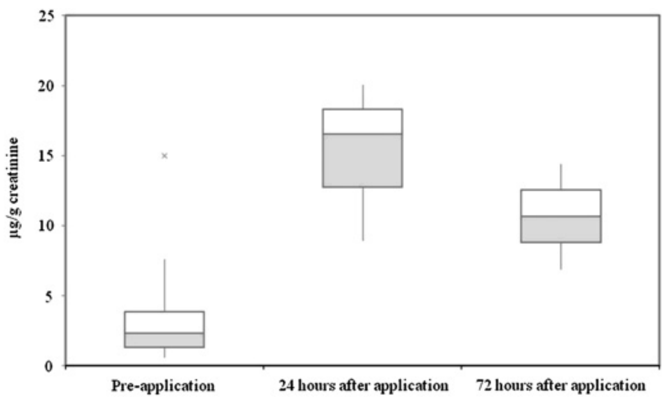

Figure 1 Pre and post application TCPY levels (µg/g creatinine) in eight children whose parents applied chlorpyrifos. The horizontal lines in each plot represent P25, P50 and P75.

from the prenatal period to the years after birth, through the mothers' urine levels during pregnancy and repeated pesticide residue analyses of children's urine and blood.[27] Although ideal, such an approach is currently beyond our capability in Nicaragua. The retrospective construction of cumulative pesticide-specific use indices for different time windows, as presented here, could be an option for improved quantitative exposure assessment in cross-sectional or case–control studies in developing countries, and perhaps beyond.

However, there are important limitations. Although the ICBF is well accepted by the farmers and seems to facilitate recall, farmers are using increasingly more pesticides and it is likely that errors occur in recalling pesticides used far back in time, especially when addressing narrow time windows. Recall of pesticide doses by subsistence farmers is likely subject to the same difficulty. In our particular study setting, the fact that 8 or 10 years before administration of the ICBF the number of pesticides in use was about half that used today, may have made recall somewhat easier. We checked the pesticides reported by the participants with technological recommendations for specific crops, issued periodically by the agricultural authorities, and found few large inconsistencies. In addition, we noticed a decreasing gradient over the years for the use indices of some of the traditional pesticides selected in this report, which is consistent with the emerging use of more modern compounds. Therefore, we anticipate that the indices will discriminate reasonably well between high and low levels of prenatal and postnatal exposures to specific pesticides.

We cannot compare the levels of prenatal and postnatal exposure in children with those from other settings based on the indices, because similar indices have not been previously reported in children. In one Costa Rican study on childhood leukaemia, parental exposure to pesticides was assessed with the ICBF and the data were used for modelling the children's exposures, resulting in sound outcome assessment.[21] However, quantitative results concerning hours of use and the quantity of

Table 3 Comparison of creatinine-adjusted urinary pesticides metabolites in this study with those in other studies

			TCPY			3-PBA			2,4-D		
Reference	**Study population**	**Sample description**	**P50**	**P75**	**Max**	**P50**	**P75**	**Max**	**P50**	**P75**	**Max**
Present study	N=184 Age 7–9 Nicaragua, rural children	Spot Related and unrelated to pesticide applications	3.7	7.1	595	2.8	5.2	244	0.9	1.7	14.0
Fourth CDC report, 2009[28] (1999–2000 survey)	N=483 Age 6–11 USA, general population	Spot Unrelated to pesticide application	3.2	6.4	12.0*	0.37	1.13	8.6*	<LOD	<LOD	1.30*
Fourth CDC report 2009[28] (2001–2002 survey)	N=580 Age 6–11 USA, general population		3.8	6.2	10.9*	0.38	0.86	3.4*	<LOD	0.49	1.55*
Arcury *et al*, 2007[29]	N=60 Age 1–6 North Carolina, farm workers' children	Spot No information about recent pesticide application	3.4			0.15			0.23		
Panuwet *et al*, 2008[30]	N=207 Age 12–13 years Chiang Mai, Thailand, children of agricultural and non-agricultural families	Spot Unrelated to pesticide application	2.7		38.9	0.17		39.7	0.19		1.87
	N=101 Children of agricultural families				1.08			39.7			1.08
	N=106 Children of non-agricultural families				19.9			4.8			1.87
Morgan *et al*, 2005[31]	N=129 Age 1.5–5 North Carolina, general population	48-h composites Related and unrelated to pesticide application	7.3	11.2	111						
Naeher *et al*, 2010[32]	N=203 Age 4–6 Jacksonville, Florida, general population	Spot Unrelated to pesticide application				2.5	5.0	141			
Lu *et al*, 2008[10]	Samples (children)=701 (33) Age 3–11 Seattle, Washington	Spot Conventional food Spot Organic food	3.7	7.5	14.7*						
Ortiz-Pérez *et al*, 2005[33]	N=32 Malarious communities San Luis Potosi, México	Related to deltamethrin application for vector control			148						

*95th percentile.
The data from Lu *et al* and Ortiz-Pérez *et al* are unadjusted for creatinine.

specific pesticides sprayed were not published. Another limitation of the ICBF method is that systematisation of data is a complex and laborious task, and apart from the technical recommendations of agricultural services, there were no objective use records for triangulation. More work needs to be done in developing countries to enhance the positive aspects of this interview method while simplifying the data analyses.

The children's urinary residue levels of 3-PBA and 2,4-D found in this study are clearly higher than reported in most or all previously published studies (table 3). With regard to TCPY urinary residues, median values were similar to those in other studies or even lower, as was the case for the urinary levels in children in North Carolina.[31] However, the maximum levels of TCPY residues found in the urine of children in this study are among the highest reported in the literature for children so far. To put the TCPY level in our study in perspective, the maximum value (303 μg/l creatinine-uncorrected and 595 μg/g creatinine-corrected) was higher than the maximum reported for post-application urinary values among sprayers (145 μg/l) in the same area in Nicaragua[20] and similar to the 430 μg/g reported in highly exposed Honduran banana workers.[34]

As expected, the children's urinary levels increased on the days following parental pesticide application (figure 1). However, high levels were often unrelated to any pesticide use by the parents. It is likely that pesticides drifting from neighbouring peanut plantations and other cultivated fields adds to children's exposure. Based on a report on land use, around 50% of agricultural land is used for plantation type crops. In the study area, the area of land used for peanut plantations far exceeded that of small farmers.

An important limitation of residue analysis of non-persistent pesticides is that the results reflect very recent exposures, that is, they are a picture of a given situation, and intra-individual levels may change markedly from day to day. Even if spraying occurs daily, the plantations are large and the location of spraying rotates, exposing different dwellings on different days. It is noteworthy that 80% of the dwellings are <100 m from a plantation and drift probably reaches most houses, contributing to children's exposures. This assumption is supported by the fact that only one of the participating farmers sprayed 2,4-D in 2008, but 99% of the children's urine samples contained 2,4-D residues at or above the LOD, with most children having levels higher than reported for other populations in the literature (table 3).

However, other factors can also influence urinary pesticide levels, such as the domestic use of agricultural pesticides. The highest TCPY level was found in a girl with no family agricultural activity in whose home chlorpyrifos was used to control ants a few days prior to sampling. In addition, dietary exposure could contribute to the internal dose. Several studies have detected organophosphate residues in food stuffs[35–37] and TCPY has been found in US children with no alternative explanation besides dietary intake. TCPY residues in children's urine practically disappeared after only 5 days after replacing conventional with organic food.[10] Although the median urinary TCPY values for these children exposed through their diet was similar to the median values observed in our study, extremely high levels as observed in our study have not been reported for dietary exposure. The same is true for 3-PBA and 2,4-D residues. It is also possible that the urinary residues may partly be the result of direct contact by the children with non-toxic TCPY and 3-PBA metabolites from environmental degradation of chlorpyrifos and pyrethroids, respectively. However, if this is the case, it still indicates a heavy burden of pesticides in the environment where these children live.[31 38]

Finally, it has to be kept in mind that an important restriction of pesticide residue analyses is that only few pesticides can be analysed for technical and cost reasons. The 32 farmers with spraying activities in 2008 documented the use of 16 different active ingredients during that cultivation period, and the technical guidelines for pesticide use on the peanut plantations in 2008 referred to 35 different active ingredients, for a total of 44 active ingredients. Therefore, our residue analyses covered only four (9%; chlorpyrifos, 2.4-D, cypermethrin and deltamethrin) of the 44 pesticides to which the children in this particular agricultural setting could have been exposed. However, although limited, the results suggest the likelihood of recent exposures to specific pesticides and long-term past exposures since similar conditions have prevailed for years.

Conclusions

In conclusion, we describe the assessment of prenatal and postnatal pesticide exposure in children with the aim of using quantitative exposure indices for developmental time windows in an epidemiological evaluation of neurobehavioral effects. The findings demonstrated recent pesticide exposure to highly toxic pesticides in children in an extremely poor population, at levels higher than reported in the general population in the USA and comparable to or higher than those reported in other developing countries. Parental application influenced children's pesticide exposure but the results also clearly indicated important environmental exposures beyond the family farm setting. High values are likely related to environmental contamination from neighbouring agricultural activities, especially surrounding plantations.

Finally, it should be kept in mind that this study assesses exposure to a limited number of selected neurotoxic pesticides, and that current exposures include an increasing number of new pesticides. Methods to assess such complex exposures must be developed further. The findings of this study should lead to regulatory action by the Nicaraguan government as well as international agencies to promote sustainable agricultural practices in developing countries, eliminating the risk of pesticide exposure at source.

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REFERENCES

1. **Partanen T,** Aragón A. *Perfiles de Salud Ocupacional en Centroamérica. Informe Regional. Serie Salud y Trabajo*. Instituto Regional de Estudios en Sustancias Tóxicas (IRET-UNA). Programa Salud y Trabajo en América Central. Heredia, Costa Rica. 2011
2. **Henao S,** Arbelaez P. Epidemiological situation of acute pesticide poisoning in the Central American Isthmus, 1992—2000. *PAHO Epidemiological Bulletin* 2002;**23**:3. http://paho.org/English/SHA/be_v23n3-plaguicidas.htm (accessed 30 Jan 2009).
3. **U.S Environmental Protection Agency (EPA).** *Office of Pesticide Program (OPP). Re-registration Elegilibiliy Decision for Methamidophos*. Washington, DC, 2006.
4. **Corriols M,** Marín J, Berroterán J, *et al*. Incidence of acute pesticide poisonings in Nicaragua: a public health concern. *Occup Environ Med* 2009;**66**:205—10.

5. **Curl CL,** Fenske RA, Kissel JC, *et al*. Evaluation of take-home organophosphorus pesticide exposure among agricultural workers and their children. *Environ Health Perspect* 2002;**110**:787—92.
6. **Thompson B,** Coronado GD, Grossman JE, *et al*. Pesticide take-home pathway among children of agricultural workers: study design. Methods and baseline findings. *J Occup Environ Med* 2003;**45**:42—53.
7. **Coronado GD,** Vigoren EM, Thompson B, *et al*. Organophosphate pesticide exposure and work in pome fruit: evidence for the take-home pesticide pathway. *Environ Health Perspect* 2006;**114**:999—1006.
8. **Aprea C,** Strambi M, Novelli MT, *et al*. Biologic monitoring of exposure to organophosphorus pesticides in 195 Italian children. *Environ Health Perspect* 2000;**108**:521—5.
9. **MacIntosh D,** Kabiru C, Echols SL, *et al*. Dietary exposure to chlorpyrifos and levels of 3,5,6-trichloro-2-pyridinol in urine. *J Expo Anal Environ Epidemiol* 2001;**11**:279—85.
10. **Lu C,** Barr DB, Pearson MA, *et al*. Dietary intake and its contribution to longitudinal organophosphorous pesticide exposure in urban/suburban children. *Environ Health Perspect* 2008;**116**:537—42.
11. **Schettgen T,** Heudorf U, Drexler H, *et al*. Pyrethroid exposure of the general population-is this due to diet? *Toxicol Lett* 2002;**134**:141—5.
12. **Nishioka MG,** Lewis RG, Brinkman MC, *et al*. Distribution of 2,4-D in air and on surfaces inside residences after lawn applications: comparing exposure estimates from various media for young children. *Environ Health Perspect* 2001;**109**:1185—91.
13. **Organización Internacional del Trabajo (OIT).** El trabajo infantil lo intolerable en el punto de mira. *Conferencia Internacional del Trabajo, 86ª reunión*, 1998. Ginebra, Switzerland.
14. **Organización Internacional del Trabajo (OIT)/Programa Internacional Para la Erradicación del Trabajo Infantil (IPEC).** *Informe de la Encuesta Nacional de Trabajo Infantil y Adolescente (ENTIA) 2000 en Nicaragua*, Julio 2004.
15. **Landrigan PJ,** Claudio L, Markowitz SB, *et al*. Pesticides and inner-city children: exposures, risks, and prevention. *Environ Health Perspect* 1999;**107**(Suppl 3):431—7.
16. **Selevan SG,** Kimmel CA, Mendola P. Identifying critical windows of exposure for children's health. *Environ Health Perspect* 2000;**108**:451—5.
17. **Adams J,** Barone S, LaMantia A, *et al*. Workshop to identify critical windows of exposure for children's health: Neurobehavioral work group summary. *Environ Health Perspect* 2000;**108**(Suppl 3):535—44.
18. **Azaroff LS.** Biomarkers of exposure to organophosphorous insecticides among farmers' families in rural El Salvador: factors associated with exposure. *Environ Res* 1999;**80**:138—47.
19. **McConnell R,** Pacheco F, Wahlberg K, *et al*. Subclinical health effects of environmental pesticide contamination in a developing country: cholinesterase depression in children. *Environ Res* 1999;**81**:87—91.
20. **Rodriguez T,** Younglove L, Lu C, *et al*. Biological monitoring of pesticide exposures among applicators and their children in Nicaragua. *Int J Occup Environ Health* 2006;**12**:312—20.
21. **Monge P,** Wesseling C, Guardado J, *et al*. Parental occupational exposure to pesticides and risk of childhood leukemia in Costa Rica. *Scand J Work Environ Health* 2007;**33**:203.
22. **Engel LS,** Keifer MC, Zahm SH. A comparison of a traditional questionnaire with an icon/calendar based questionnaire to assess occupational history. *Am J Ind Med* 2001;**40**:502—11.
23. **Lindh CH,** Littorin M, Amilon A, *et al*. Analysis of phenoxyacetic acid herbicides as biomarkers in human urine using liquid chromatography/triple quadrupole mass spectrometry. *Rapid Commun Mass Spectrom* 2008;**22**:143—50.
24. **Elfman L,** Hogstedt C, Engvall K, *et al*. Acute health effects on planters of conifer seedlings treated with insecticides. *Ann Occup Hyg* 2009;**53**:383—90.
25. **Petit C,** Chevrier C, Durand G, *et al*. Impact on fetal growth of prenatal exposure to pesticides due to agricultural activities: a prospective cohort study in Brittany, France. *Environ Health* 2010;**15**:71.
26. **Barr DB,** Ananth CV, Yan X, *et al*. Pesticide concentrations in maternal and umbilical cord sera and their relation to birth outcomes in a population of pregnant women and newborns in New Jersey. *Sci Total Environ* 2010;**408**:790—5.
27. **Eskenazi B,** Marks AR, Bradman A, *et al*. Organophosphate pesticide exposure and neurodevelopment in young Mexican-American children. *Environ Health Perspect* 2007;**115**:792—8.
28. **Department of Health and Human Services, Centers for Disease Control and Prevention, National Center for Environmental Health.** *Fourth National Report on Human Exposure to Environmental Chemicals*. Centers for Disease Control and Prevention. Atlanta, Georgia, 2009.
29. **Arcury TA,** Grzywacz JG, Barr DB, *et al*. Pesticide metabolite levels of children in eastern North Carolina farmworker households. *Environ Health Perspect* 2007;**115**:1524—60.
30. **Panuwet P,** Prapamontol T, Chantara S, *et al*. Urinary pesticide metabolites in school students from northern Thailand. *Int J Hyg Environ Health* 2009;**212**:288—97.
31. **Morgan MK,** Sheldon LS, Croghan CW, *et al*. Exposures of preschool children to chlorpyrifos and its degradation product 3,5, 6-trichloro-2-pyridinol in their everyday environments. *J Expo Anal Environ Epidemiol* 2005;**15**:297—309.
32. **Naeher LP,** Tulve NS, Egeghy PP, *et al*. Organophosphorous and pyrethroid insecticide urinary methabolite concentrations in young children living in a southeastern United State city. *Sci Total Environ* 2010;**408**:1145—53.
33. **Ortiz-Pérez MD,** Torres-Dosal M, Batres LE, *et al*. Environmental health assessemnt of deltamethrin in a malarious area de México: environmental persistence, toxicokinetics and genotoxicity in exposed children. *Environ Health Perspect* 2005;**113**:782—6.
34. **Wesseling C,** Aragón A, Rojas M, *et al*. *Efectos de Clorpirifos Sobre la Salud de Trabajadores Bananeros de La Lima, Honduras. Serie Salud y Trabajo, no. 1. Heredia*. Costa Rica: SALTRA, IRET-UNA, CISTA, UNAN-León, 2006. ISSN: 1659—2670.
35. **Wilson NK,** Chuang JC, Lyu C, *et al*. Aggregate exposures of nine preschool children to persistent organic pollutants at day care and at home. *J Expo Anal Environ Epidemiol* 2003;**13**:187—202.
36. **Food and Drug Administration (FDA).** Pesticide Program, Residue Monitoring 2004—2006, 2009. http://www.fda.gov/Food/FoodSafety/FoodContaminantsAdulteration/Pesticides/ResidueMonitoringReports/ucm125183.htm (accessed 29 Sep 2010).
37. **Lu C,** Schenck FJ, Pearson MA, *et al*. Assessing children's dietary pesticide exposure-direct measurement of pesticide residues in 24-hour duplicate food samples. *Environ Health Perspect* 2010. http://dx.doi.org (accessed 29 Sep 2010).
38. **Agency for Toxic Substances and Disease Registry (ATSDR).** *Toxicological Profile for Chlorpyrifos*. Centers for Disease Control and Prevention. Atlanta, GA, 1997.

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