Environmental Research 117 (2012) 17–26

Contents lists available at SciVerse ScienceDirect

Environmental Research

journal homepage: www.elsevier.com/locate/envres

Indigenous children living nearby plantations with chlorpyrifos-treated bags have elevated 3,5,6-trichloro-2-pyridinol (TCPy) urinary concentrations ☆

Berna van Wendel de Joode [a,*], Douglas Barraza [a,b], Clemens Ruepert [a], Ana María Mora [a,c], Leonel Córdoba [a], Mattias Öberg [d], Catharina Wesseling [a], Donna Mergler [e], Christian H. Lindh [f]

[a] Central American Institute for Studies on Toxic Substances (IRET), Universidad Nacional, Heredia, Costa Rica
[b] Technology and Agrarian Development Group, Wageningen University, the Netherlands
[c] University of California at Berkeley, USA
[d] Institute of Environmental Medicine, Karolinska Institute, Stockholm, Sweden
[e] Center for Interdisciplinary Research in Biology, Health, Environment and Society (CINBIOSE), University of Quebec at Montréal, Canada
[f] Department of Occupational and Environmental Medicine, Lund University Hospital, Lund, Sweden

ARTICLE INFO

Article history:
Received 2 September 2011
Received in revised form
13 April 2012
Accepted 18 April 2012
Available online 28 June 2012

Keywords:
Children
Pesticides
Chlorpyrifos
Biomarkers
Banana
Developing countries

ABSTRACT

Background: The US Environmental Protection Agency voluntary phased-out residential use of chlorpyrifos in 2001. In contrast, in Costa Rica, chlorpyrifos-treated bags are increasingly used to protect banana and plantain fruits from insects and to fulfill product standards, even in populated areas.
Objectives: To evaluate children's exposure to chlorpyrifos in villages situated nearby banana plantations and plantain farms in Costa Rica.
Methods: The study targeted two villages with use of chlorpyrifos-treated bags in nearby banana plantations and plantain farms and one village with mainly organic production. For 140 children from these villages, mostly indigenous Ngäbe and Bribri, parent-interviews and urine samples ($n=207$) were obtained. Urinary 3,5,6-trichloro-2-pyridinol (TCPy) levels were measured as a biomarker for chlorpyrifos exposure. In the banana and plantain village also environmental contamination to chlorpyrifos was explored.
Results: Children from the banana and plantain villages had statistically significant higher urinary TCPy concentrations than children from the referent village; 2.6 and 2.2 versus 1.3 μg/g creatinine, respectively. Chlorpyrifos was detected in 30% of the environmental samples as well as in 92% of the hand/foot wash samples. For more than half of the children their estimated intake exceeded the US EPA chronic population adjusted dose. For some, the acute population adjusted dose and the chronic reference dose were also exceeded.
Conclusions: Our results suggest that children living nearby plantations with chlorpyrifos-treated bags are exposed to chlorpyrifos levels that may affect their health. Interventions to reduce chlorpyrifos exposure are likely to improve children's health and environment in banana and plantain growing regions.

1. Introduction

Pesticides constitute one of the most pressing environmental health problems in many tropical countries. In Costa Rica, pesticide use is very high (Bravo et al., 2011), especially on bananas grown for export purposes with more than 40 kg active ingredients of pesticides applied per hectare per year (Wesseling et al., 2001; Ramírez et al., 2009). Pesticides are increasingly used by indigenous plantain farmers in the Talamanca region (Polidoro et al., 2008, Barraza et al., 2011). To protect the fruits from insects and fulfill product standards, banana or plantain bunches are generally covered with a plastic bag treated with chlorpyrifos (O,O-diethyl O-3,5,6-trichloro-2-pyridyl phosphorothioate) at a 1% concentration.

Abbreviations: ADD, absorbed daily doseaPAD-acute population adjusted dose; cPAD, chronic population adjusted dose; GM, geometric mean; GSD, geometric standard deviation; LOD, limit of detection; RfD, Reference dose; RBC, red blood cell; TCPy, 3,5,6-trichloro-2-pyridinol; OP, organophosphate; US, United States; US EPA, United States Environmental Protection Agency

☆ **Grant/Support:** International Development Research Center (IDRC), Canada; Swedish Research Council for Environment, Agricultural Sciences, and Spatial Planning; The Swedish Agency for International Development Cooperation; The Department for Research Cooperation; The Swedish Environmental Protection Agency; Erasmus-Columbus 2013 (ERACOL).
The authors declare they have no competing financial interests.

* Corresponding author.
E-mail addresses: bvanwen@una.ac.cr, bernavanwendel@gmail.com (B. van Wendel de Joode).

0013-9351/$ - see front matter Crown Copyright © 2012 Published by Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.envres.2012.04.006

Chlorpyrifos is a chlorinated organophosphate (OP) insecticide with widespread agricultural and residential use since 1965, with moderate acute toxicity (WHO-IPCS, 2005). Due to concern about neurodevelopmental effects, the U.S. Environmental Protection Agency (EPA) voluntary phased-out residential use of chlorpyrifos in 2001 (US EPA, 2002). Chlorpyrifos is a neurotoxinant which inhibits plasma and red blood cell (RBC) acetylcholinesterase (McCollister et al., 1974; Coulston et al., 1972; Kisciki et al., 1999; Nolan et al., 1984). Recent studies suggest there are other neurotoxicological mechanisms in addition to cholinesterase inhibition at lower doses (Eaton et al., 2008; Nomura et al., 2006; Schuh et al., 2002; Rush et al., 2010; Slotkin et al., 2010; Seidler and Slotkin, 2011). In addition, three US children's cohort studies have reported neurodevelopmental deficits, including decreased full-scale IQ and working memory up to the age of 7 years in relation to prenatal exposures to organophosphates in general (Eskenazi et al., 2007; Engel et al., 2007; Rosas and Eskenazi, 2008; Bouchard et al., 2011) and chlorpyrifos in particular (Berkowitz et al., 2004; Rauh et al., 2006; Lovasi et al., 2011; Rauh et al., 2011).

Chlorpyrifos is metabolized to the specific metabolite 3,5,6-trichloro-2-pyridinol (TCPy), which after oral exposure is mainly (70%) excreted in the urine, with a half-life in adults of 27 h (Nolan et al., 1984). After dermal exposure, only 3–4% is excreted in urine (Meuling et al., 2005). TCPy has frequently been used as a specific biomarker in urine for chlorpyrifos exposure (Barr et al., 2005). Results from two studies conducted in the US, showed increased urinary TCPy levels for children whose parents' reported chlorpyrifos applications in their garden (Fenske et al., 2000) or whose father had applied chlorpyrifos at work prior to urine sampling (Curwin et al., 2007). Curwin et al. (2007) also found an association between chlorpyrifos indoor dust levels and farmer children's TCPy urinary levels, whereas Fenske et al. (2000) did not. Two studies performed in Nicaragua, showed an approximately eightfold increase in children's urinary TCPy levels after paternal chlorpyrifos application (Rodríguez et al., 2006, 2011). Researchers also detected TCPy in 79% of the repeat urine samples from children ($n=10$) of diazinon applicators on large-scale banana plantations, likely, due to contact with chlorpyrifos-treated bags on banana plantations.

In almost all studies on children's chlorpyrifos exposure, the pathway of exposure to chlorpyrifos was either as a sprayed liquid formulation in farm or residential areas or as residues in food (Eaton et al., 2008). To our knowledge, none of the studies specifically addressed children's exposures from chlorpyrifos-treated plastic bags used in agriculture. The main aims of this study were to (1) evaluate and compare the levels of urinary TCPy from children living near banana plantations and plantain farms, two of which used chlorpyrifos-treated bags; (2) determine whether children's estimated doses exceed US government recommended guidelines, (3) examine factors explaining children's urinary TCPy concentrations; and (4) explore environmental contamination of chlorpyrifos.

2. Materials and methods

2.1. General context

The data of this cross-sectional study were collected between February and August 2007, as part of a larger project on neurobehavioral effects of pesticide exposure in children in three villages situated in the Caribbean lowlands of Talamanca, Limón, Costa Rica. This larger project used an ecosystem approach to human health (Forget and Lebel, 2006; Webb et al., 2010), examining social (Barraza et al., 2011) and environmental determinants in relation to human health. The ecosystem health approach draws on local knowledge and community participation to bring about collective and individual changes to improve health and well-being (Mertens et al., 2005). Accordingly, during initial fieldtrips to the Talamanca area of Costa Rica, representatives from local community, governmental and non-governmental organizations were identified and their active collaboration was sought (Barraza et al., 2011). With their help, three villages were selected based on children's potential exposure to pesticides used in banana plantations and plantain farms. The project was approved by the Bio-ethics Committee of the Universidad Nacional in Costa Rica (CECUNA-03-2007).

2.2. Study population

2.2.1. Banana village

The village, Daytonia, situated outside the Bribri Indigenous Territory, was inhabited almost exclusively by indigenous Ngäbe migrants from Panama and surrounded by two large-scale banana plantations with intensive chemical pesticide use. The distance between the houses and the banana plantations ranged from 15 to 80 m. The school and soccer-field, where local children usually played, were in close proximity to the plantations, without any physical barrier. The bananas were exported to the US and Europe.

2.2.2. Plantain village

The village, Shiroles, situated in the Bribri Indigenous Territory, consisted of indigenous Bribri plantain smallholders who produced part of their production for export to US and Europe. They used a similar application scheme of chemical pesticides as in large-scale banana farming, including chlorpyrifos-treated bags. The smallholders' plantations were located at a distance of 2 km from the village. Only a few families lived close to the plantations.

2.2.3. Organic village

The population of the third village, Amubrë, situated in the Bribri Indigenous Territory, consisted of mainly Bribri organic banana and plantain farmers. Only 12% of the farmers reported pesticide use (Table 1), including chlorpyrifos-treated bags.

The target population included all children aged 6–9 years from all three villages. In each village a meeting was organized at the primary school to explain the purpose of the study and to ask for their collaboration. The parents who did not attend the school meeting, about 40% in each village, were visited at their homes. Of these, 26 (12%) could not be located and 4 (2%) refused to participate. In total, parents' informed-consent was obtained for 188 out of 218 children, an overall 86% response rate that was similar for the three villages. Of the 188 participating children, 165 (88%) provided a urine sample, and for 140 (74%) a parent-interview was administered during home visits as 25 parents could not be located during the interviewing period. Thus, complete data were available for 140 children, which is equivalent to 64% of the target population of 218 children.

2.3. Use of chlorpyrifos at banana and plantain plantations

A structured questionnaire on pesticide use was administered to plantain smallholders, who reported placing chlorpyrifos-treated bags over the plantain bunches each week. Information on pesticide use at large-scale banana plantations was obtained from a previous study in which the continuous and permanent use of chlorpyrifos-treated bags was reported (Bravo et al., 2007), assuming a similar use at the banana plantations in this study.

2.4. Parent interviews

Structured interviews were administered to the parents to collect information on exposure-related factors, medical history and socio-economic aspects. Also, information on pesticide use at home was obtained.

2.5. Urine sampling analysis

First morning urine voids were obtained from 140 children. In addition, we obtained repeat samples from 40 children (between 2 and 10 samples), resulting in 207 samples in total (see Table 2).

Samples were stored in a fridge at 4 °C and frozen at −20 °C at the end of the day. The samples were stored at −20 °C until transported to the Department of Laboratory Medicine at Lund University Hospital, Sweden. TCPy was analyzed using liquid chromatography tandem mass spectrometry (LC/MS/MS). The sample preparation of the urine was performed using a modification of the method by Lindh et al. (2008). Briefly, 1 mL of urine was acidified and hydrolyzed overnight, then extracted using SPE columns prior to analysis. TCPy was analyzed using mass spectrometric parameters according to Olsson et al. (2004), however [$^{2}H_3$]-2,4-dichlorophenoxyacetic acid was used as an internal standard. All samples were analyzed in duplicates with a coefficient of variance of 10%. For each duplicate, its mean value was used in further calculations. The limit of detection (LOD) for TCPy in urine was 1.0 μg/L. The creatinine levels were analyzed using an enzymatic method described by Mazzachi et al. (2000) in order to adjust for urine dilution.

Table 1
Distribution of socio-demographic characteristics in a banana, plantain and organic village in Talamanca, Costa Rica..

	Banana	**Plantain**	**Organic**
Number of children	38	48	54
% Girls	55	42	58
Average age in years (SD)	8.1 ± 0.8	7.7 ± 0.8[a]	8.0 ± 0.9
% Children assisting			
First grade	45	54	35
Second grade	42	25	39
Third grade	13	21	26
% Children lived in this village since birth	61[b]	90	92
Language spoken at home (%)			
Spanish only	47	90[c]	39
Spanish and Bribri	0%	10%	61%
Spanish and Ngäbe	53%	0%	0%
Parents living together (%)	90%[d]	60%	71%
One or both parents own a piece of land (%)			
Plantain	0	95	8
Plantain and banana	0	5	43
Several crops	0	0	49
Mother's occupation (%)			
Farmer	0	44	62
Farm worker	16	4	2
Housewife	78[e]	23	10
Other	6	29	26
Fathers occupation (%)			
Farmer	0	97	88
Plantation or farm worker	100	3	0
Other	0	0	12
Mothers' age, average in years (SD)	32.0 (5.2)	32.7 (7.6)	32.0 (7.5)
Fathers' age, average in years (SD)	36.0 (5.8)	38.4 (10.2)	35.7 (7.0)
Mother's education, average in years (SD)	3.8 (3.8)[f]	6.3 (2.6)	6.7 (3.7)
Father's education, average in years (SD)	3.9 (3.6)[g]	5.6 (3.4)	6.5 (4.6)
Pesticide use at farm, including chlorpyrifos treated bags (%)	100[h]	97[i]	12[i]

[a] Lower than banana village (Tukey–Kramer HSD, $p=0.09$).
[b] Less than plantain and organic village (Chi-Square $p<0.0001$).
[c] More frequent than banana and organic village (Chi-Square $p<0.0001$).
[d] More frequent than plantain and organic village (Chi-Square $p=0.002$).
[e] More frequent than plantain and organic village (Chi-Square $p=0.004$).
[f] Lower than plantain (Tukey–Kramer HSD $p=0.0012$) and organic village (Tukey–Kramer HSD $p<0.0001$).
[g] Lower than organic village (Tukey–Kramer HSD $p=0.007$).
[h] Observed by the authors.
[i] Reported by the parents

2.6. Comparison of children's adsorbed daily dose with reference dose

For each child, the absorbed daily dose (ADD) (μg/kg/day) was estimated using a first order kinetic model at assumed steady state (adapted from Curwin et al., 2007): $\text{ADD}=((C\times R_{\text{mw}}\times P_{\text{cre}}\times \text{CF})/\text{BW})\times(\ln 2/t½)\times t$, where C is the measured concentration of TCPy (μg/g creatinine); R_{mw} is the molar adjustment factor (=1.77) between chlorpyrifos and TCPy: the ratio of parent pesticide and pesticide metabolite molecular weights; P_{cre} is the daily mean creatinine excretion of 0.624 g/day, as calculated from nocturnal urine collections in children by Fortin et al. (2008); CF is a correction factor used to account for incomplete excretion of TCPy in urine after oral chlorpyrifos exposure: an excretion rate of 70% was used (Nolan et al., 1984); BW is body weight (kg); t1/2 is the half-life of 27 h (Nolan et al., 1984); and t is the assumed dosing interval of 24 h.

To evaluate whether children's ADD formed a health risk, children's ADD values were compared with the US EPA (2002) reference dose (RfD). The RfD is an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime, generally expressed in units of milligrams per kilogram of bodyweight per day (mg/kg/day) (www.epa.gov/iris/rfd.htm). For chlorpyrifos, RfDs for acute and chronic exposure are 5 and 0.3 μg/kg/day, respectively. ADD values were also compared with US EPA's population adjusted dose for infants, children and females aged 13–50 years. To account for increased susceptibility and sensitivity to chlorpyrifos among neonates when compared with adults and for possible effects of chlorpyrifos on brain development, acute (aPAD) and chronic population adjusted dose (cPAD) are a ten-fold lower than the RfD, 0.5 and 0.03 μg/kg/day, respectively (US EPA, 2002).

2.7. Environmental sampling and analyses

To explore the presence of chlorpyrifos in environmental media in the banana and plantain village, where chlorpyrifos-treated bags were extensively used, the following samples were collected: hand and foot wash, mattress dust, house dust, soil, and water. In the banana village, passive and active air samples were also obtained. All of the personal samples were obtained from six, randomly selected, boys ($n=3$) and girls ($n=3$) in both the banana and plantain village. Due to logistical problems, environmental and personal samples were not taken in the organic village.

3. Sample collection procedures

3.1. Hand and foot wash samples

Hand and foot wash samples were collected, using a procedure adapted from Brouwer et al. (1992, 2000). Children were asked to wash their hands for 30 s in a zip-lock bag with 200 mL of a

Table 2
Distribution of children's urinary TCPy concentrations with median Absorbed Daily Dose (ADD) estimates for a banana, plantain and organic village in Talamanca, Costa Rica.

Urine concentrations	N[a]	K[b]	> LOD[c]	S_B^2 [d]	S_W^2 [e]	Mean (SD)	GM	Percentiles					Median (90th percentile) ADD in μg/kg/day[f] 50th and 90th percentiles
								25th	50th	75th	90th	Max	
Banana	78	38	77%										
Unadjusted (μg/L)				1.0	1.8	2.1 (1.4)	1.6[g]	0.5	1.7	2.9	4.3	6.6	P50th = 0.10
Creatinine adjusted (μg/g)				1.2	1.1	3.0 (1.9)	2.6[h]	1.4	2.5	3.3	4.9	11.2	P90th = (0.20)
Plantain	73	48	66%										
Unadjusted (μg/L)				1.6	2.4	3.8 (4.8)	2.0[g]	0.5	2.9	5.3	7.8	26.8	P50th = 0.10
Creatinine adjusted (μg/g)				1.7	1.9	4.0 (5.2)	2.h[f]	1.0	2.1	5.1	9.2	30.7	(P90th = 0.46)
Organic	56	54	30%										
Unadjusted (μg/L)				–[i]	–[i]	1.1 (1.1)	0.8[g]	0.5	0.5	1.4	2.6	6.4	P50th = 0.05
Creatinine adjusted (μg/g)				1.0	1.7	1.8 (1.7)	1.3[h]	0.7	1.2	2.1	3.6	8.4	(P90[th] = 0.14)

[a] Total number of samples.
[b] Number of children.
[c] Percentage of samples above Limit of Detection (LOD).
[d] Estimated variance component between children.
[e] Estimated variance component within children.
[f] Mean creatinine excretion rates calculated from nocturnal urine collections in children (Fortin et al., 2008) were used to estimate the amount of creatinine excreted per day (g/day). For chlorpyrifos, US EPA RfDs for acute and chronic exposure are 5 and 0.3 μg/kg/day, respectively, whereas acute (aPAD) and chronic population adjusted dose (cPAD) are 0.5 and 0.03 μg/kg/day, respectively.
[g] Geometric mean of children's urinary TCPy concentrations (μg/L) was highest for the plantain village, followed by the banana and the organic village (Tukey–Kramer $p < 0.04$ for the three comparisons).
[h] Geometric mean of children's urinary TCPy concentrations (μg/g creatinine) were similar for the plantain and banana villages, and lowest for the organic village (Tukey–Kramer $p < 0.001$ for both comparisons comparisons).
[i] Could not be estimated.

isopropanol/water mixture 1:1 (V/V). Subsequently, children washed each foot for 15 s in a zip-lock bag with isopropanol/water, using one of their just cleaned hands.

3.2. Mattress dust

Two dust samples were collected from each child's mattress, according to a slightly adapted procedure as described by Jarvis et al. (2007). Of each mattress, two areas of approximately 1 m^2 were vacuumed during 4 min each.

3.3. House dust

Inside each child's house two surface wipe samples were taken of window and doorposts, according to the NIOSH procedure 9100 'Lead in Surface Wipe Samples' using sterile cotton gauzes (Cutisoft Curity™, 10 × 10 cm^2, 12 layers, BSN Medical, Germany. Gauzes were used dry. The wiped areas ranged from 42 to 1254 cm^2 (median 338 cm^2).

3.4. Soil

In total, four top soil samples were obtained from two playing areas in both the banana village and the plantain village with a gouge arable land auger (13 cm diameter, Eijkelkamp, Netherlands) to a depth of approximately 20 cm, by taking 30 samples distributed over the playing area and mixed in the field to a sample of approximately 1 kg.

3.5. Water

Seven drinking water samples at children's houses/school were collected for pesticide residue analysis in pre-cleaned 1 L glass bottles. Finally, six surface water samples were collected in streams or creeks nearby the villages.

3.6. Active air

In the banana village, a high volume air sampler (HiVol) with a flow of approximately 600 L/min and containing a pre-combusted glass-fiber filter, polyurethane foam (PUF) disks and XAD-resin filled mesh cylinders, was placed for 24 h at the public primary school using the same procedure as described by Gouin et al. (2008). The particle phase was collected with the pre-combusted glass-fiber filter (GFF, Whatman, 11 cm diameter) and the gas phase with the XAD-2 resin (Supelco, Supelpak-2, precleaned Amberlite XAD-2 resin, 20/60 mesh) sandwiched between two PUF plugs (Supelco, precleaned large PUF plug, 6 × 3.8 cm^2).

3.7. Passive air

In the banana village, two passive air samplers (PUF disk PAS (PUF-PAS) as described by Shoeib and Harner (2002) were attached outside the school and a nearby house during 86 day at 2.5 m' height.

All samples were transported at 4 °C and stored at −20 °C until analysis.

4. Chemical analysis of environmental samples

The hand and foot wash samples were extracted twice with petroleum-ether, after adding of sodium chloride, the solvent extracts were dried with sodium sulphate and concentrated by a rotavapor and finally under nitrogen to 0.1 mL. LOD was 1.0 ng/both hands or both feet.

Dust (LOD = 0.05 μg/g dust), wipe, soil (LOD = 1 ng/g dry weight) and low flow air samples (LOD = 0.1 ng/m^3) were microwave-extracted in two steps in acetone/hexane 1:1 (V/V) mixture (Mars 5 CEM Corporation Matthews, NC). The passive and

HiVol air samples were extracted twice with acetone-hexane (1:1) using an ultrasonic bath for 20 min.

The drinking and surface water samples (LOD=0.01 μg/L) were extracted with SPE cartridges (Isolute ENV+200 mg/6 mL) conditioned with ethyl acetate, water, and methanol. The cartridges were eluted with ethyl acetate and concentrated to approximately 0.1 mL with nitrogen gas. Details of the applied procedure are described in Polidoro et al. (2009).

After extraction, all samples were concentrated to 0.1 mL under nitrogen, and finally were dissolved in acetone-cyclohexane (1:9) to achieve a final extract volume of 1 mL.

We performed analytical determination of the chlorpyrifos using gas chromatography (GC) with electron capture detector (ECD), flame photometer detector (FPD) and mass spectrometry (MS).

Ethion was used as an internal standard. For all samples, media recovery tests were performed. Chlorpyrifos recovery was above 70% for all media. Results were not corrected for recovery.

Air concentrations of chlorpyrifos captured with the PAS-PUF samplers were estimated with the mean uptake rate of 5.9 m^3/d determined in the Gouin et al. (2008) study elaborated in Costa Rica.

4.1. Statistical data analyses

Data were analyzed in JMP 8 (SAS Institute, Cary, NC).

To compare socio-demographic characteristics between villages, Chi-Square Test was used for categorical measures and Tukey–Kramer (HSD) Test was used for continuous measures.

Urine samples with TCPy levels below limit of detection (LOD) were set at LOD/2 (Hornung and Reed, 1990) at 0.5 μg/L. Descriptive statistics were run for urinary levels. Log-transformed TCPy levels followed approximately a normal distribution. As log-transformed creatinine-adjusted values (μg/g creatinine) fitted better (Shapiro-Wilk=0.99; p=0.04) than crude values (μg/L) (Shapiro-Wilk 0.87; $p<0.0001$), creatinine-adjusted values were used in subsequent data analysis Tukey–Kramer (HSD) Test was used to test whether urinary TCPy levels differed among the villages. To estimate variance components between (S_B^2) and within children (S_W^2) mixed linear regression models were applied, using the method of restricted maximum likelihood (REML) and a compound symmetric variance matrix (van Wendel de Joode et al., 2005).

To identify exposure affecting factors, univariate regression analysis (Standard Least Squares) were run with log10-transformed TCPy metabolite levels in urine as dependent variable and individual covariates, such as village, sex, and grade, as independent variable (Table 3). For children with repeated measurements, the geometric mean (GM) of their repeated measures was calculated and used in the regression analysis. Independent variables were included in the multivariate model if its P-value was ≤ 0.10 in the univariate analysis, or if adding the variable to the multivariate model changed the beta by more than 20%. The variables were retained in the model if their P-value was ≤ 0.20 (Table 3). To ensure that covariates were not highly correlated, Spearman's correlation coefficients were estimated before adding factors to the model. We also verified whether the fixed effects included in the models showed interaction. Residuals of the regression models were tested for normality and outliers. For all statistical tests, the significance level was set at 5%.

5. Results

5.1. Socio-demographic characteristics

Table 1 contains the socio-demographic characteristics for the three villages. Although there were relatively fewer girls in the plantain village, the proportions were not significantly different between villages (Chi-Square, P=0.17). Children's mean age was slightly lower in the plantain village as compared to the banana and organic villages (Tukey–Kramer HSD p=0.09 and p=0.15, respectively). Most parents in the plantain and organic villages reported their child had lived in this vilage since birth (90% and 92% respectively), compared to 61% in the banana village (Chi-Square, $p<0.0001$). In the banana and organic village, many families reported speaking their indigenous language at home, 53% and 61%, respectively, whereas in the plantain village, it was only 10% (Chi-Square $p<0.0001$). In the plantain and organic village, more children had divorced or separated parents than in the banana village (Chi-Square, P=0.002). Dietary habits were similar for the three villages. Diets consisted of rice, black beans, eggs, corn tortillas and sometimes chicken, fish and organic plantain.[1] In the banana village mothers were more often housewives than in the plantain and organic village, where most mothers were farmers. Fathers in the banana village were mostly plantation workers whereas in the plantain and organic village mostly farmers. Mothers' and fathers' age was similar for the three villages. Mothers' educational level was lower in the banana village as compared to the plantain and organic village (Tukey–Kramer $p \leq 0.001$ and $p \leq 0.0001$, respectively). Fathers' educational level was also lower in the banana village as compared to the organic village (Tukey–Kramer HSD p=0.007). Mother's and father's educational level were moderately correlated (Spearman's ρ=0.56, $p<0.0001$). None of the parents reported applying chlorpyrifos at home.

5.2. Children's urinary TCPy concentrations

Descriptive data on urine samples and distribution of metabolite concentrations in the three villages are presented in Table 2.

Geometric Mean (GM) creatinine-adjusted TCPy concentrations were similar for children from the plantain and banana village, 2.2 and 2.6 μg/g creatinine, respectively; about twice the GM of children from the organic village (1.3 μg/g creatinine) (Tukey–Kramer HSD $p<0.001$ for both comparisons).

For children with repeat samples, TCPy creatinine adjusted concentrations were similar when comparing different months, overall and when comparing children for each village separately (Tukey Kramer HSD $p>0.15$ for all comparisons) (results not tabulated).

TCPy metabolite concentrations in urine adjusted for creatinine varied more for children from the plantain village as compared to the banana village: plantain village $S_B^2=1.7$, $S_W^2=1.9$ and banana village: $S_B^2=1.2$, $S_W^2=1.1$. Within the plantain and banana villages, variability in TCPy concentrations between children was similar as variability within children (between days). For children from the organic village, estimated variability between children ($S_B^2=1.0$) was smaller than within children ($S_W^2=1.7$). However, in this village repeated samples were only obtained for two children.

Overall, mean urinary TCPy concentrations (μgTCPy/g creatinine) were higher in boys than girls (GM=2.4 versus 1.8 μg/g creatinine, p=0.02). However, this was driven by differences in TCPy concentrations between boys and girls from the plantain village (GM=3.1 versus 1.5 μg/g creatinine, p=0.002); in the other two villages TCPy urinary levels were similar for both sexes (see also Annex A: Fig. A1a–c). On the other hand, 90th-percentiles TCPy urinary concentrations (μgTCPy/g creatinine) were similar for boys and girls from the plantain village: boys=9.7 and girls=8.6 μg TCPy/g creatinine, respectively.

[1] During interviewing, parents indicated they consumed non-bagged plantain.

Table 3
Univariate and multiple regression analyses of factors that may explain children's TCPy concentrations in urine ($n=140$).[a] Regression models were run with log10(μgTCPy/creatinine) as dependent variable and the different factors as independent variable. Values of 'inversed' log-10 estimates are presented.

Univariate

Factor	Estimated least square mean (se)[b]
Village	
Banana	3.0 (1.1)***
Plantain	2.2 (1.1)
Organic	1.3 (1.1)
Sex	
Boys	2.2 (1.1)#
Girls	1.7 (1.1)
Grade	
First	2.3 (1.1)#
Second	1.8 (1.1)
Third	1.6 (1.2)
Child has lived in village	
Since birth	1.8 (1.1)*
1–5 years	2.8 (1.2)
Language at home	
Only Spanish	2.0 (1.1)
Spanish and indigenous	1.9 (1.1)
Parents	
Live together	1.9 (1.1)
Separated/divorced	2.0 (1.1)

	Intercept (se)	Beta of factor (se)
Child's age (years)	3.6 (1.9)	0.9 (1.1)
Mother's age (years)	1.5 (1.4)	1.0 (1.0)
Mother's education (years)	2.9 (1.1)	0.93 (1.02)***
Father's age (years)	2.3 (1.6)	1.0 (1.0)
Fathers education (years)	2.6 (1.1)	0.95 (1.02)

Multivariate

Factor	Estimated least square mean (se)[b]
Village	
Banana	3.1 (1.2)***
Plantain	2.5 (1.1)
Organic	1.7 (1.1)
Sex	
Boys	2.6 (1.1)
Girls	2.2 (1.1)
Village × sex	
Boys-Banana	3.0 (1.2)*
Boys-Plantain	3.5 (1.2)
Boys-Organic	1.6 (1.2)
Girls-Banana	3.3 (1.2)
Girls-Plantain	1.7 (1.2)
Girls-Organic	1.8 (1.2)
Grade*	
First	2.4 (1.1)
Second	1.8 (1.1)
Third	1.6 (1.1)

	Intercept (se)	Beta of factor (se)
Mother's education (years)	3.2 (1.2)	0.95 (1.02)**[c]

# $p < 0.10$.
* $p < 0.05$.
** $p < 0.01$.
*** $p < 0.001$ Tukey–Kramer (HSD) Test.
[a] Regression models were run with log 10 (μgTCPy/creatinine) as dependent variable and the different factors as independent variable. Values of 'inversed' log-10 estimates are presented.
[b] se = standard error.
[c] A factor of 0.95, means a 5% decrease in TCPy concentrations in urine per mother's education year. For example, for the overall median TCPy concentration of 2.1 μg/g creatinine this means a decrease of 0.1 μg/g creatinine TCPy per education year ($2.1 \times 0.95 = 2.0$, a 0.1 decrease).

5.3. Comparison with reference dose

For children from the banana, plantain and organic village, respectively, the median estimated absorbed daily doses (ADD) were 0.10 (90th percentile at 0.20), 0.10 (90th percentile at 0.46) and 0.05 (90th percentile at 0.14) μg/kg/day (Table 2). Assuming urinary TCPy *concentrations reflected chlorpyrifos exposure only, the estimated ADD exceeded the chronic RfD of 0.3 μg/kg/day, established by the US EPA, in 6% (banana village), 19% (plantain village) and 2% (organic village) of the samples, whereas the acute RfD was not exceeded. Using an alternative and more conservative approach by subtracting the estimated ADD (geometric mean) in the organic village from the TCPy levels measured in the banana and plantain village, the estimated ADD still exceeded the chronic RfD in 2% and 13% of the samples from the banana and plantain village, respectively.

If we compare the ADD with the cPAD, which includes an extra safety factor of 10 to ensure safety for children, 97%, 82% and 68% of the samples exceeded cPAD, in the banana, plantain and organic villages, respectively. After subtracting the geometric mean level of TCPy measured in children from the organic village, still 67% and 55% of the children exceeded the cPAD in the banana and plantain villages, respectively.

In addition, in the plantain village, the estimated ADD exceeded aPAD in 4% of the samples, whereas in the banana and organic village the estimated ADD did not exceed aPAD.

5.4. Factors explaining children's urinary TCPy concentrations

In Table 3 results of univariate and multivariate analysis are presented. Values of 'inversed' log-10 estimates are shown. The variables village, sex, sex × village, grade, and mother's education were associated with TCPy concentrations and explained 29% of total variance.

5.5. Environmental exposure

In Table 4, results of environmental samples are presented for the banana and plantain village. Chlorpyrifos was detected in all hand and foot wash samples except from one child of plantain village. Hand- and foot wash chlorpyrifos concentrations were moderately correlated (Spearman's $\rho=0.76$ ($p=0.005$)). Girls ($n=6$) seemed to have lower levels than boys ($n=6$) in particular for foot wash samples, but differences were not statistically significant, possibly due to small sample size: for hands, GM = 10 versus 24 ng/both hands respectively (Tukey–Kramer

Table 4
Concentrations of chlorpyrifos in samples from multiple media at children's homes in a banana and plantain village in Talamanca, Costa Rica.

Medium	LOD	Village	Detected/N	Mean (SD)[a]	GM[a]	Max
Hand wash (ng/both hands)	1.0	Banana	6/6	33 (25)	26	67
		Plantain	5/6	20 (20)	9	52
Foot wash (ng/both feet)	1.0	Banana	6/6	56 (36)	47	108
		Plantain	5/6	31 (32)	13	89
Children's mattress dust (μg/g)	0.05	Banana	2/10[b]	0.22 (0.01)	0.22	0.22
		Plantain	6/10[c]	6.3 (14.0)	0.91	34.9
Indoor dust (μg/g)	0.05	Banana	3/12	0.53 (0.48)	0.33	1.2
		Plantain	1/12	–	–	0.10
Outside air (high volume) (ng/m^3)	0.1	Banana	3/3	3.1 (1.2)	2.9	4.9
Outside air (passive sampler) (ng/m^3)	0.1	Banana	2/2	8.3 (1.1)	8.2	9.3
			–			
Outdoor soil (ng/g dry weightps)	1.0	Banana	1/2	–	–	5
		Plantain	0/2	–	–	–
Drinking water (μg/L)	0.,01	Banana	0/5	–	–	–
		Plantain	0/2	–	–	–
Surface water (μg/L)	0.,01	Banana	2/4[d]	–	–	0.02
		Plantain	0/2		–	–

[a] Mean, SD = standard deviation and GM = geometric mean were calculated for samples with detectable chlorpyrifos levels.
[b] For one child this sample could not be obtained as he did not sleep on a mattress.
[c] For one child this sample could not be obtained as the house did not have electricity.
[d] 2 out of 4 samples above the LOD, however, below the Limit of Quantification (LOQ) of 0.02 μg/L and therefore Mean, SD, and GM could not be estimated.

HSD $p=0.27$) and for feet 13 versus 49 ng/both feet, respectively (Tukey–Kramer HSD $p=0.13$). Children's chlorpyrifos hand and foot wash levels seemed somewhat higher in the banana village than in the plantain village, but again differences were not statistically significant (Tukey–Kramer HSD $p=0.20$ and $p=0.15$ for hand and foot wash samples, respectively). Chlorpyrifos was detected more frequently in children's mattress dust in the plantain village (60% of samples) as compared to the banana village (20% of samples) (Chi-square, $p=0.06$). Chlorpyrifos was also detected in indoor dust in children's houses in the banana village (3/12), and the plantain village (1/12). In addition, chlorpyrifos was detected in soil from the banana village, whereas in the plantain village it was not. None of the drinking water samples of the banana and plantain village contained detectable chlorpyrifos levels. However, 2 out of 4 surface water samples in the banana village did. Both active and passive air samples collected in the banana village showed measurable chlorpyrifos concentrations.

6. Discussion

The results of our study suggest the use of chlorpyrifos-treated bags in banana plantations and plantain farms results in environmental exposure in children. Children from the banana and plantain villages had statistically significant higher urinary TCPy concentrations than children from the organic village. The exposure to chlorpyrifos was confirmed by detected levels in environmental samples as well as hand/foot wash samples. For some of the children, their estimated daily intake exceeded the chronic RfD established by US EPA. For more than half of the children their estimated intake was above the cPAD, and for some children their intake was above the aPAD.

Due to economic and logistic constraints, environmental samples were few and only performed in the banana and plantain village, allowing only loose comparisons between sex, villages and with other studies. Another limitation of our study is that we did not measure TCPy in environmental samples, as chlorpyrifos can degrade in the environment into several metabolites including TCPy (Morgan et al., 2005, 2011).

In addition, for indoor dust sampling, the wiped surface areas were small, resulting in few samples above LOD (16%). Nonetheless, the results indicate environmental contamination to chlorpyrifos; i.e. almost all of the hand and foot wash samples contained detectable chlorpyrifos levels. Assuming an average surface of 400 cm^2 for both hands (US EPA, 2009), GMs of hand-wash samples of our children from banana villages seemed to be higher than levels reported by Morgan et al. (2005, 2011) for children from homes and US day care centers where chlorpyrifos was sprayed. The chlorpyrifos concentrations measured in outdoor air were about a ten-fold higher as the concentrations measured outdoors by Morgan et al. (2005, 2008) and comparable with the indoor air samples measured by the same authors.

Overall, our results suggest that the general living environment of children from the banana village is contaminated with chlorpyrifos, detected in air, soil, surface water, mattress and house dust samples, and in all the hand and foot wash samples. When performing the fieldwork of this study, we observed children swimming in the banana canals. The general living environment of children from the plantain villages seems to be less contaminated, possibly because plantain plantations are located at about 2 km distance from the village. Children from the plantain villages seem to be mainly exposed to chlorpyrifos when helping their parents at the farms. However, the results of our hand and foot wash samples do not reflect these exposures, since they were collected at the end of the day and children had already showered or had not gone to the plantations on the sampling day. Also, some mothers indicated that they stored bags at home (Barraza et al., 2011), which might explain the relatively high levels found in some mattress dust samples.

The GMs of TCPy urinary concentrations in children from the banana and plantain village were comparable with levels measured in children from Nicaraguan small-scale farming families after their fathers applied chlorpyrifos (Rodríguez et al., 2006, 2011). and several studies performed in the USA in agricultural school-aged children (Alexander et al., 2006; Arcury et al., 2007; Fenske et al., 2000) or the general population (Barr et al., 2005; Lu et al., 2008; Morgan et al., 2005, 2011). Curwin et al. (2007) reported somewhat higher TCPy levels, but this may be because they used an immune-assay method to determine TCPy. Part of the TCPy concentrations in urine from US-children can be explained by residential use before its ban in 2001, and chorpyrifos and TCPy exposure through food (Lu et al., 2008; Eaton et al., 2008; Morgan et al., 2011).

On the contrary, in our study TCPy urinary concentrations seem to reflect mainly chlorpyrifos exposure from the treated bags. First, the children's TCPy urinary concentrations measured in the organic village suggest only a low background exposure from the ingestion of chlorpyrifos or TCPy-contaminated food. The diet of the children of the three villages is similar and the plantain consumed locally is not pesticide treated. During interviews, parents commented that they prefered organic plantains because they were cheaper and tasted better than non-organic plantains.

Second, children's urinary TCPy concentrations varied most in the plantain village, more fluctuating chlorpyrifos exposures to sometimes low and sometimes high levels in children from this village. The finding that the boys from the plantain villages had higher mean TCPy urinary concentrations than girls suggests that boys had more contact with the chlorpyrifos-treated bags, possibly because of helping more often at the plantations, or performing other tasks, than the girls. In contrast, children's TCPy levels in the banana village varied very little, indicating a continuous exposure to relatively low levels of chlorpyrifos. The smaller differences between children of the banana plantation village are consistent with their type of exposure; they all live permanently very nearby (15–80 m) the banana plantations, both girls and boys, and may constantly receive exposure to low levels of chlorpyrifos. This contrasts with the more fluctuating exposure of the children in the plantain village who do not live nearby the field but play and assist their parents on the farms after school and in the weekends.

Although many uncertainties exists in the calculation of ADD, such as variability of creatinine excretion (Fortin et al., 2008), and the scarce database for determination of t½ and absorption rate (Nolan et al., 1984), it still gives a rough estimate of the internal chlorpyrifos dose in the children and is valuable when comparing with established RfD. An additional uncertainty is whether urinary TCPy concentrations reflected chlorpyrifos exposure only, as chlorpyrifos can degrade to in the environment into several metabolites including TCPy which is less toxic than chlorpyrifos (Eaton et al., 2008; Morgan et al., 2005, 2011). Nevertheless, even assuming only half of the TCPy concentrations reflected chlorpyrifos exposure and thus lowering the estimated ADD by a factor two, the chronic RfD and aPAD were still exceeded for some children from the plantain village and the cPAD was still exceeded for most children from the banana (82%) and plantain (64%) village.

As far as we know, this is the first study that assesses children's exposure to chlorpyrifos-treated bags used at banana plantations and plantain farms in the tropics. One of the strengths of this study is its considerable size ($n=140$). Also, we studied three well defined and contrasting populations and included all children aged 6-9, in those populations. The participation rate was good with very few actual refusals; non-participation was related to parents not being available for interviewing since they were not in the village at the time the study was performed. Only few children forgot to bring their urine sample to school.

In this study, the children living in villages nearby banana plantations and plantain farms had statistically significant higher urinary concentrations of TCPy, as compared to children living in a village with almost exclusively organic production. Our results suggest that children living nearby plantations with chlorpyrifos-treated bags are exposed to chlorpyrifos levels that may affect their health, as estimated daily intake exceeded US EPA chronic RfD, aPAD and cPAD. Interventions to reduce chlorpyrifos exposure, such as the substitution of the chlorpyrifos-treated bags by agro-ecological pest control methods, are likely to improve children's health and environment in banana and plantain growing regions. Global regulatory and policy interventions related to chlorpyrifos have a potential to improve children's health and environment in tropical regions from where fruits are exported to countries that are in the process of phasing out this chemical due to national health concerns.

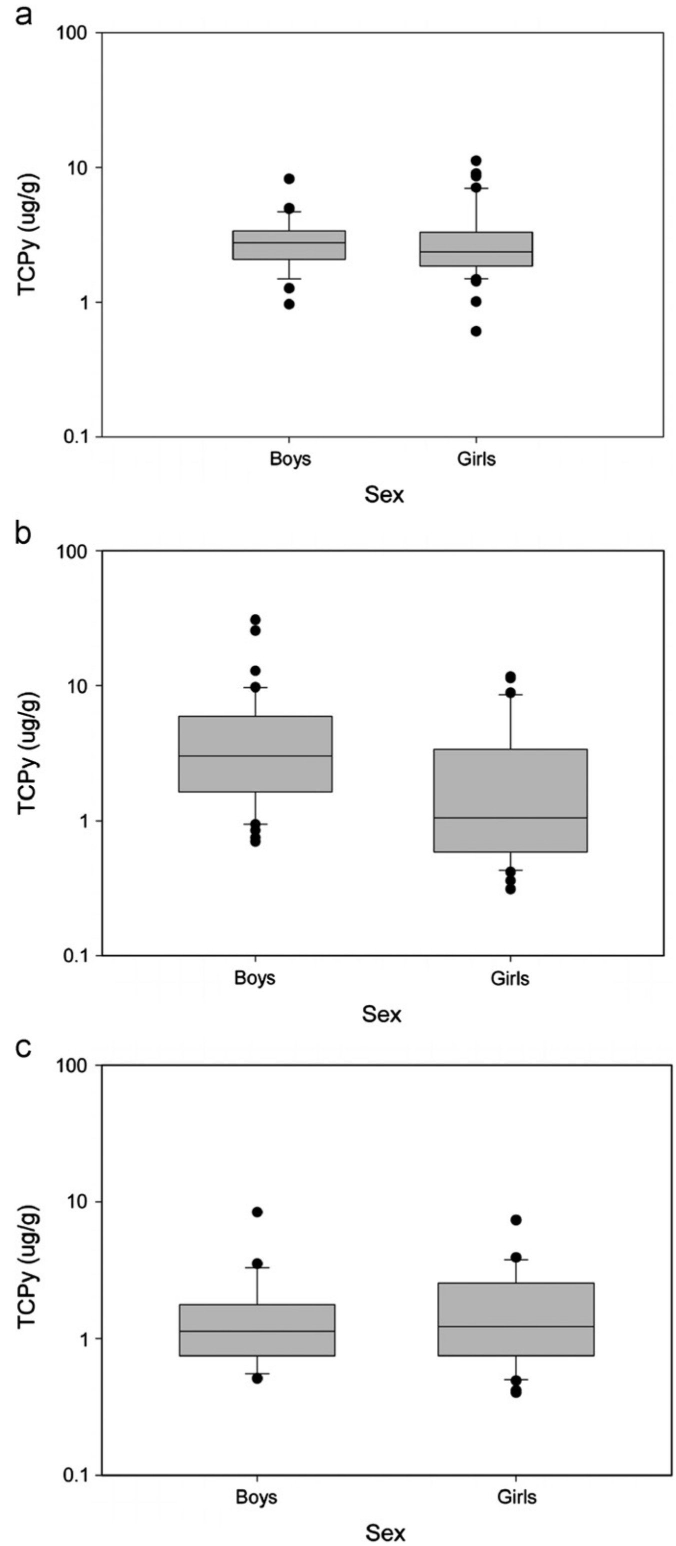

Fig. A1. (a)–(c) Distribution of children's urinary TCPy concentrations by sex for a banana (1a), plantain (1b) and organic (1c) village in Talamanca, Costa Rica; the horizontal lines present the 25th, 50th, and 75th percentiles.

Acknowledgment

Mariam Gutiérrez for recollecting samples, Dario Villagra for applying questionnaires to parents, Marilú Morera and Jairo García for their help with environmental sampling and chemical analysis, Åsa Amilon for excellent help with the analysis of urine samples. School directors and teachers for coordinating parent meetings. Kathia Almengor for coordinating home visits.

Annex A

See Fig. A1 here.

Appendix B. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.envres.2012.04.006.

References

Alexander, B.H., Burns, C.J., Bartels, M.J., Acquavella, J.F., Mandel, J.S., Gustin, C., Baker, B.A., 2006. Chlorpyrifos exposure in farm families: Results from the farm family exposure study. J. Exp. Science. Environ. Epidemiol. 16, 447–456.

Arcury, T.A., Grzywacz, J.G., Barr, D.B., Tapia, J., Chen, H., Quandt, S.A., 2007. Pesticide urinary metabolite levels of children in eastern North Carolina farmworker households. Environ. Health. Perspect. 115, 1254–1260.

Barr, D.B., Allen, R., Olsson, A.O., Bravo, R., Caltabiano, L.M., Montesano, A., Nguyen, J., Udunka, S., Walden, D., Walker, R.D., Weerasekera, G., Whitehead Jr, R.D., Schober, S.E., Needham, L.L., 2005. Concentrations of selective metabolites of organophosphorus pesticides in the United States population. Environ. Res. 99 (3), 314–326.

Barraza, D., Jansen, K., van Wendel de Joode, B., Wesseling, C., 2011. Pesticide use in banana and plantain production and risk perception among local actors in Talamanca, Costa Rica. Environ. Res., 708–717.

Berkowitz, G.S., Wetmur, J.G., Birman-Deych, E., Obel, J., Lapinski, R.H., Godbold, J.H., Holzman, I.R., Wolff, M.S., 2004. In utero pesticide exposure, maternal paraoxonase activity, and head circumference. Environ. Health Perspect. 12 (3), 388–391.

Bouchard, M.F., Chevrier, J., Harley, K.G., Kogut, K., Vedar, M., Calderon, N., Trujillo, C., Johnson, C., Bradman, A., Barr, D.B., Eskenazi, B., 2011. Prenatal exposure to organophosphate pesticides and IQ in 7-year old children. Environ. Health Perspect.. [Apr 21 Epub ahead of print].

Bravo, V., Rodríguez, T., van Wendel de Joode, B., Canto, N., Calderón, G.R., Turcios, M., Menéndez, L.A., Mejía, W., Tatis, A., Abrego, F.Z., de la Cruz, E., Wesseling, C., 2011. Monitoring pesticide use and associated health hazards in Central America. Int. J. Occup. Environ. Health 17 (3), 258–269.

Bravo V., Partanen T., Wesseling C., 2007. ''Health risk indicators for pesticide use: banana cultivation in the Atlantic Region of Costa Rica, 2006''. Salud Pública de México. Edición Especial 2, 2007. Volumen 49. ISSN 0036-3634. Abstracts. Proceedings of the 19th Conference of the International Society of Environmental Epidemiology (ISEE). Ciudad de México, del 5 al 9 de septiembre de 2007. p. 575.

Brouwer, D.H., Brouwer, E.J., van Hemmen, J.J., 1992. Assessment of dermal and inhalation exposure to zineb/maneb in the culture of flower bulbs. Ann. Occup. Hyg. 36, 373–384.

Brouwer, D.H., Boeniger, M., van Hemmen, J.J., 2000. Hand wash and manual skin wipes. Ann. Occup. Hyg. 44 (7), 501–510.

Coulston, F., Golberg, L., Griffin, T., 1972. Safety Evaluation of Dowco 179 in Human Volunteers. Unpublished report from the Institute of Experimental Pathology and Toxicology, Albany Medical College. Albany, NY. MRID No. 00030754, 00095175.

Curwin, B.D., Hein, M.J., Sanderson, W.T., Striley, C., Heederik, D., Kromhout, H., Reynolds, S.J., Alavanja, M.C., 2007. Pesticide dose estimates for children of Iowa farmers and non-farmers. Environ. Res. 105, 307–315.

Eaton, D.L., Daroff, R.B., Autrup, H., Bridges, J., Buffler, P., Costa, L.G., Coyle, J., McKhann, G., Mobley, W.C., Nadel, L., Neubert, D., Schulte-Hermann, R., Spencer, P.S., 2008. Review of the toxicology of chlorpyrifos with an emphasis on human exposure and neurodevelopment. Crit. Rev. Toxicol. 38 (Suppl 2), 1–125.

Engel, S.M., Berkowitz, G.S., Barr, D.B., Teitelbaum, S.L., Siskind, J., Meisel, S.J., Wetmur, J.G., Wolff, M.S., 2007. Prenatal organophosphate metabolite and organochlorine levels and performance on the Brazelton Neonatal Behavioral Assessment Scale in a multiethnic pregnancy cohort. Am. J. Epidemiol. 165, 1397–1404.

Eskenazi, B., Marks, A.R., Bradman, A., Harley, K., Barr, D.B., Johnson, C., Morga, N., Jewell, N.P., 2007. Organophosphate pesticide exposure and neurodevelopment in young Mexican–American children. Environ. Health Perspect. 115, 792–798.

Fenske, R.A., Lu, C., Simcox, N.J., Loewenherz, C., Touchstone, J., Moate, T.F., Allen, E.H., Kissel, J.C., 2000. Strategies for assessing children's organophosphorus pesticide exposures in agricultural communities. J. Expo. Anal. Environ. Epidemiol. 10, 662–671.

Forget, G., Lebel, J., 2001. An ecosystem approach to human health. Int. J. Occup. Environ. Health 7 (2 Suppl.), S3–38.

Fortin, M.C., Carrier, G., Bouchard, M., 2008. Concentrations versus amounts of biomarkers in urine: a comparison of approaches to assess pyrethroid exposure. Environ. Health 7, 55.

Gouin, T., Wania, F., Ruepert, C., Castillo, L.E., 2008. Field testing passive air samplers for current use pesticides in a tropical environment. Environ Sci Technol. 42 (17), 6625–6630.

Hornung, R.W., Reed, L.D., 1990. Estimation of average concentration in the presence of nondetectable values. Appl. Occup. Environ. Hyg. 5 (1), 46–51.

Jarvis, D., Zock, J.P., Heinrich, J., Svanes, C., Verlato, G., Olivieri, M., Villani, S., Ponzio, M., Leynaert, B., Sunyer, J., Dahlman-Hoglund, A., Chinn, S., Luczynska, C., Norbäck, D., Burney, P., 2007. Cat and dust mite allergen levels, specific IgG and IgG4, and respiratory symptoms in adults. J. Allergy Clin. Immunol. 119 (3), 697–704.

Kisciki J.C., Seip C.W., Combs M.L., 1999. A Rising Dose Toxicology Study to Determine the No-Observable-Effect-Levels (NOEL) for Erythrocyte Acetylcholinesterase (AchE) Inhibition and Cholinergic Signs and Symptoms of Chlorpyrifos at Three Dose Levels. Unpublished Report from MDS Harris. MRID No. 44811002.

Lindh, C.H., Littorin, M., Amilon, A., Jönsson, B.A., 2008. Analysis of phenoxyacetic acid herbicides as biomarkers in human urine using liquid chromatography/triple quadrupole mass spectrometry. Rapid Commun. Mass Spectrom. 22 (2), 143–150.

Lovasi, G.S., Quinn, J.W., Rauh, V.A., Perera, F.P., Andrews, H.F., Garfinkel, R., Hoepner, L., Whyatt, R., Rundle, A., 2011. Chlorpyrifos exposure and urban residential environment characteristics as determinants of early childhood neurodevelopment. Am. J. Public Health 101 (1), 63–70.

Lu, C., Barr, D.B., Pearson, M.A., Waller, L.A., 2008. Dietary intake and its contribution to longitudinal organophosphorus pesticide exposure in urban/suburban children. Environ Health Perspect. 116 (4), 537–542.

Mazzachi, B.C., Peake, M.J., Ehrhardt, V., 2000. Reference range and method comparison studies for enzymatic and Jaffé creatinine assays in plasma and serum and early morning urine. Clin. Lab. 46 (1-2), 53–55.

McCollister, S.B., Kociba, R.J., Humiston, C.G., McCollister, D.D., 1974. Studies on the acute and long-term oral toxicity chlorpyrifos (0,0-diethyl-0(3,5,6-trichloro-2-pyridyl) phosphorothioate). Food Cosmet. Toxicol. 12 (1), 45–61.

Mertens, F., Saint-Charles, J., Mergler, D., Passos, C.J., Lucotte, M., 2005. Network approach for analyzing and promoting equity in participatory ecohealth research. Ecohealth 2, 113–126.

Meuling, W.J.A., Ravensberg, L.C., Roza, L., van Hemmen, J.J., 2005. Dermal absorption of chlorpyrifos in human volunteers. Int. Arch. Occup. Environ. Health 78, 44–50.

Morgan, M.K., Sheldon, L.S., Croghan, C.W., Jones, P.A., Robertson, G.L., Chuang, J.C., Wilson, N.K., Lyu, C.W., 2005. 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. 15 (4), 297–309.

Morgan, M.K., Sheldon, L.S., Jones, P.A., Croghan, C.W., Chuang, J.C., Wilson, N.K., 2011. The reliability of using urinary biomarkers to estimate children's exposures to chlorpyrifos and diazinon. J. Expo. Sci. Environ. Epidemiol. 21 (3), 280–290.

Nolan, R.J., Rick, D.L., Freshour, N.L., Saunders, J.H., 1984. Chlorpyrifos: pharmacokinetics in human volunteers. Toxicol. Appl. Pharmacol. 73 (1), 8–15.

Nomura, D.K., Durkin, K.A., Chiang, K.P., Quistad, G.B., Cravatt, B.F., Casida, J.E., 2006. Serine hydrolase KIAA1363: toxicological and structural features with emphasis on organophosphate interactions. Chem. Res. Toxicol. 19 (9), 1142–1150.

Olsson, A.O., Baker, S.E., Nguyen, J.V., Romanoff, L.C., Udunka, S.O., Walker, R.D., Flemmen, K.L., Barr, D.B., 2004. A liquid chromatography-tandem mass spectrometry multiresidue method for quantification of specific metabolites of organophosphorus pesticides, synthetic pyrethroids, selected herbicides, and deet in human urine. Anal. Chem. 76 (9), 2453–2461.

Polidoro, B.A., Dahlquist, R.M., Castillo, L.E., Morra, M.J., Somarriba, E., Bosque-Pérez, N.A., 2008. Pesticide application practices, pest knowledge, and cost-benefits of plantain production in the Bribri-Cabécar Indigenous Territories, Costa Rica. Environ. Res. 108 (1), 98–106.

Polidoro, B.A., Morra, M.J., Ruepert, C., Castillo, L.E., 2009. Pesticide sequestration in passive samplers (SPMDs): considerations for deployment time, biofouling, and stream flow in a tropical watershed. J. Environ. Monit. 11 (10), 1866–1874.

Ramírez, F., Chaverri, F., de la Cruz, E., Wesseling, C., Castillo, L., Bravo, V., 2009. Importación de plaguicidas en Costa Rica: período 1977–2006. IRET—Universidad Nacional, Heredia, Costa Rica.

Rush, T., Liu, X.Q., Hjelmhaug, J., Lobner, D., 2010. Mechanisms of chlorpyrifos and diazinon induced neurotoxicity in cortical culture. Neuroscience 166 (3), 899–906.

Rauh, V.A., Garfinkel, R., Perera, F.P., Andrews, H.F., Hoepner, L., Barr, D.B., Whitehead, R., Tang, D., Whyatt, R.W., 2006. Impact of prenatal chlorpyrifos exposure on neurodevelopment in the first 3 years of life among inner-city children. Pediatrics 118, e1845–e1859.

Rauh, V., Arunajadai, S., Horton, M., Perera, F., Hoepner, L., Barr, D.B., Whyatt, R., 2011. 7-Year Neurodevelopmental Scores and Prenatal Exposure to Chlorpyrifos, a Common Agricultural Pesticide. Environ. Health Perspect.. (April 21. Epub ahead of print).

Rodríguez, T., Younglove, L., Lu, C., Funez, A., Weppner, S., Barr, D., Fenske, R., 2006. Biological monitoring of pesticide exposures among applicators and their children in Nicaragua. Int. J. Occup. Environ. Health 12 (4), 312–320.

Rodríguez, T., van Wendel de Joode, B., Lindh, C.H., Rojas, M., Lundberg, I., Wesseling, C., 2011. Assessment of long-term and recent pesticide exposure among rural school children in Nicaragua. Occup. Environ. Med.. (July 1. Epub ahead of print).

Rosas, L.G., Eskenazi, B., 2008. Pesticides and child neurodevelopment. Curr. Opin. Pediatr. 20 (2), 191–197. Review.

Schuh, R.A., Lein, P.J., Beckles, R.A., Jett, D.A., 2002. Noncholinesterase mechanisms of chlorpyrifos neurotoxicity: altered phosphorylation of Ca2+/cAMP response element binding protein in cultured neurons. Toxicol. Appl. Pharmacol. 182 (2), 176–185.

Seidler, F.J., Slotkin, T.A., 2011. Developmental neurotoxicity targeting hepatic and cardiac sympathetic innervation: Effects of organophosphates are distinct from those of glucocorticoids. Brain Res. Bull. 30, 85 (3–4), 225–230.

Shoeib, M., Harner, T., 2002. Characterization and comparison of three passive air samplers for persistent organic pollutants. Environ. Sci. Technol. 36 (19), 4142–4151.

Slotkin, T.A., Lobner, D., Seidler, F.J., 2010. Transcriptional profiles for glutamate transporters reveal differences between organophosphates but similarities with unrelated neurotoxicants. Brain Res. Bull. 83 (1-2), 76–83.

US EPA, 2002. Interim Reregistration Eligibility Decision for Chlorpyrifos (Case No. 0100) EPA 738-R-01-007. Health Effects Division, Office of Pesticide Programs, US Environmental Protection Agency, Washington, DC.

US EPA, 2009. Highlights of the Child-Specific Exposure Factors Handbook. Final Report. US Environmental Protection Agency, Washington, DC, EPA/600/R-08/135, 2009.

van Wendel de Joode, B., van Hemmen, J.J., Meijster, T., Major, V., London, L., Kromhout, H., 2005. Reliability of a semi-quantitative method for dermal exposure assessment (DREAM). J. Expo. Anal. Environ. Epidemiol. 15 (1), 111–120. (January).

Webb, J.C., Mergler, D., Parkes, M.W., Saint-Charles, J., Spiegel, J., Waltner-Toews, D., Yassi, A., Woollard, R.F., 2010. Tools for thoughtful action: the role of ecosystem approaches to health in enhancing public health. Can. J. Public Health 101 (6), 439–441.

Wesseling, C., Aragón, A., Castillo, L., Corriols, M., Chaverri, F., de la Cruz, E., Keifer, M., Monge, P., Partanen, T.J., Ruepert, C., van Wendel de Joode, B., 2001. Hazardous pesticides in Central America. Int. J. Occup. Environ. Health 7 (4), 287–294.

World Health Organization, 2005. The WHO Recommended Classification of Pesticides by Hazard and Guidelines to Classification: 2004. Geneva.