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Environmental Pesticide Exposure and Neurobehavioral Effects among Children of Nicaraguan Agricultural Workers

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Abstract
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Background: Children exposed to pesticides are susceptible for neurodevelopmental disruption. Data from developing countries are scarce.

Aim: Assessing long-term and recent pesticide exposure in Nicaraguan children in relation to parental pesticide use and examining potential associated neurobehavioral effects.

Methods: In the first study, pre- and post-spraying urinary residues of the chlorpyrifos metabolite TCPY and diazinon metabolite IMPY were measured among 7 subsistence farmers and 10 plantation workers, and in one child per worker. In the second study, for 110 children in an agricultural village and 22 in a non-agricultural village, aged 7-9, parental pesticide use was assessed by hours of spraying and kilograms of active ingredients during pre-and-postnatal time windows, as proxies for children's long term pesticide exposures. Urinary TCPY, 3-PBA (pyrethroid metabolite), and 2,4-D were determined in 211 samples of 74 children of the agricultural village. IQ components and total IQ (WISC-IV) were evaluated in all agricultural village children. Behavior was evaluated with the Conners' Teacher Rating Scale-Revised: Short. Multivariate linear regression models assessed associations between long-term and recent exposure to organophosphates and pyrethroids and cognitive and behavioral scales.

Results: In study 1, post-spraying urinary levels of pesticide metabolites of subsistence farmers and their children were highly correlated ($r=0.85$), but not those of plantation workers and their children. In study 2, a wide range of exposures was reported by parents for all pesticides and time windows. The median urinary TCPY (3.7 µg/g creatinine), 3-PBA (2.8), and 2,4-D (0.9) were comparable to other studies for TCPY and 3-PBA but high for 2,4-D. Maximum levels were the highest reported for all compounds. Prenatal use of organophosphates affected working memory, and methamidophos also verbal comprehension and total IQ. Urinary TCPY was associated with poorer working memory. Organophosphate exposures were not associated with children's behavior. Pyrethroid exposure during the first year of life associated with poorer perceptual reasoning and behavior, and urinary 3-PBA with a number of cognitive functions and ADHD in girls but not in boys.

Conclusion: Nicaraguan children in poor agricultural areas are highly exposed to pesticides, which is influenced by parental pesticide use in subsistence farms. Organophosphate and pyrethroid exposures adversely affect their neurobehavioral development.

Keywords: pesticides, organophosphates, pyrethroids, children, cognitive function, behavioral outcomes, neurodevelopment

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To my daughters María Teresa y
Ana Lucía
My husband César Augusto
and my parents Antonio y Teresa

With special dedication to
My grandpa ”Papa Tomás”,
who is always alive in my heart

List of Papers

This thesis is based on the following papers, which are referred to in the text by their Roman numerals.

I Rodríguez, T., Younglove, L., Lu, C., Funez, A., Weppner, S., Barr, DB., Fenske, RA. (2006). Biological monitoring of pesticide exposures among applicators and their children in Nicaragua. International Journal in Occupational and Environmental Health 12, 312-320.

II Rodríguez, T., van Wendel de Joode, B., Lindh, CH., Rojas, M., Lundberg, I., Wesseling, C. (2012). Assessment of long-term and recent pesticide exposure among rural school children in Nicaragua. Occupational and Environmental Medicine 69, 119-125.

III Rodríguez, T., van Wendel de Joode, B., Hernández-Bonilla, D., García, L., Roque, E., Lundberg, I., Wesseling, C. Cognitive deficits in organophosphate exposed children of Nicaraguan subsistence farmers. Manuscript.

IV Rodríguez, T., van Wendel de Joode, B., Hernández-Bonilla, D., Soto, A., Balladares, S., Lundberg, I., Wesseling, C. Pyrethroids exposure and neurobehavioral performance in school age children in rural Nicaragua. Manuscript.

Contents

Abbreviations

2,4-D:	2,4 dichlorophenoxyacetic acid
AChE	Acetylcholinesterase
ADHD	Attention deficit hyperactivity disorder
ARYase	Arylesterase
ASQ	Ages and stages questionnaires
BARS	Behavioral Assessment and Research System
BCN	Central Bank of Nicaragua (Banco Central de Nicaragua)
BHC	Benzene hexachloride (gamma-hexachloro cyclohexane, Lindane)
BMI	Body Mass Index
CBCL	Child Behavior Checklist
CCCEH	Columbia Center for Children's Environmental Health. Children from a cohort of African American and Dominican mothers in New York City
CENOP	Center of neuropsychological testing evaluation and pedagogical orientation (Centre d'evaluation neuropsychologique et d'orientation pédagogique)
CHAMACOS	Center for the Health and Assessment of Mothers and Children of Salinas. Children from a cohort of Latina mothers in agricultural communities in California
CTRS-R:S	Revised Conners' Teachers Rating Scale, short version
DAPs	Dialkylphosphates
DBCP	1,2-dibromo-3-chloropropane
DDT	Dichlorodiphenyltrichloroethane
DMAP	Dimethyl alkyl phosphate
DQs	Children developmental quotients
dw	dry weight
U.S. EPA	United States Environmental Protection Agency
FAO	Food and Agriculture Organization
GABA	Gamma aminobutyric acid

GDS	Gesell Developmental Schedules
ICBF	Icon-calendar-based form
IMPY	2-isopropoxy-4-methyl-pyridinol
INIDE	National Information and Development Institute (Instituto Nacional de Información de Desarrollo)
IQ	Full-Scale intelligence quotient
K-CPT	Conners' Kiddie Continuous Performance Test
MDI	Mental Development Index
MINSA	Health Minister (Ministerio de Salud)
MSCEHC	Mount Sinai Children's Environmental Health Center
NBAS	Neonatal Behavioral Assessment Scale
NCTB	Neuropsychological core test battery
NEPSY-II	Neuropsychological Assessment
OPs	Organophosphastes
PDI	Psychomotor Development Index
PON1	Paraoxonase 1
PSI	Processing Speed Index
PENTB	Pediatric Environmental Neurobehavioral Test Battery
PON 1	Paraoxonase 1
PRI	Perceptual Reasoning Index
***p,p*'- DDE**	*p,p*'- dichlorodiphenyldichloroethylene
***p,p*'- DDT**	*p,p*'- dichlorodiphenyldichloroethane
TCPY	3,5,6-trichloro-2-pyridinol
UNAN	National Autonomous University of Nicaragua (Universidad Nacional Autónoma de Nicaragua)
VCI	Verbal Comprehension Index
WHO	World Health Organization
WISC-IV	Wechsler Intelligence Scale for Children, 4th edition
WISC-R	Wechsler Intelligence Scale for Children, revised
WMI	Working Memory Index

1. Introduction

In Nicaragua, agriculture is the most important economic activity. Use of toxic pesticides is widespread and high, both on plantations and among subsistence farmers (Corriols, 2009). With 42% of the population living in rural areas (INIDE, 2012), pesticide exposures and subsequent health effects have been a serious concern since decades (Corriols, 2009). Rural children are an especially important target group for research on pesticide hazards and risks.

There exists evidence, from studies in the United States, that children in agricultural communities have more contact with pesticides in comparison with children from urban areas, through a number of different pathways (Karr, 2012). In addition, recent studies, mostly from industrialized countries, are increasingly showing that pesticide exposure early in life can disrupt the normal development of the central nervous system causing impairments in children's cognitive and psychomotor performance (London et al., 2012).

In developing countries in general, and in Nicaragua specifically, very few studies have addressed pesticide exposures among children and their determinants. Nonetheless, important exposures occur in Nicaraguan children (Dowling et al., 2005). No previous studies have addressed long-term health outcomes from pesticide exposures among Nicaraguan children.

This thesis intends to fill some of the knowledge gaps by examining pesticide exposures in children of Nicaraguan farmers and plantation workers, both recent exposures by means of urinary residue analyses (papers I and II) and cumulative exposures by means of indices of parental pesticide use as proxies of children's long-term pesticide exposures during vulnerable prenatal and postnatal time-windows of development (paper II). This thesis then explores associations between these children's exposures to common neurotoxic pesticides and their performance on cognitive and behavioral tests, specifically for organophosphates (paper III) and pyrethroids (paper IV).

2. Background

2.1 Pesticides in an agricultural setting in Nicaragua

2.1.1 High pesticide use

In Nicaragua, with six million inhabitants, over 40% of the economically active population works in agriculture (INIDE, 2012). From the very beginning of introduction of pesticides on the market, in the 1950s, the agriculture in Nicaragua has been highly dependent on pesticides (FAO, 2012). Pesticide use started in cotton cultivation with the introduction of the dichlorodiphenyltrichloroethane (DDT) and, shortly after, the extremely toxic organophosphate insecticide methyl parathion. The use of insecticides in cotton plantations was massive. For example, 454 tons of methyl parathion were applied in 1952 (15 kg/Ha) (Duarte, 2004). DDT and carbamate insecticides were authorized in that same period by the Ministry of Health for malaria vector control (MINSA, 2001).

During the next two decades, besides the high use in cotton cultivation, new export crops (coffee, banana, sugarcane and tobacco) and the expansion of subsistence crops (beans, corn, rice, soy bean and vegetables) forced dramatically the increase of pesticide imports. The use of organochlorine insecticides (DDT, aldrin, chlordane, toxaphene, DBCP, BHC, endrin, mirex) and organophosphates (methyl parathion, malathion) was especially intensive (Duarte, 2000).

The analysis of import data evidences the continued use of organophosphates and carbamates classified by the World Health Organization (WHO) as class 1A (extremely hazardous) and 1B (highly hazardous). In the context of international regulations, at the end of the 1990s the use of pesticides gradually shifted from persistent (organochlorines) and highly toxic pesticides (carbamates and organophosphates) to less persistent and less acutely toxic pyrethroids (MAGFOR 2001; MAGFOR, 2004).

Today, Nicaragua imports just over ten thousand tons of active ingredient of pesticides, equivalent to 0.8 kg active ingredient per inhabitant per year in rural Nicaragua. Twenty-two percent of the import volume corresponds to highly or extremely highly hazardous pesticides from an acute toxicity perspective and almost 30% are neurotoxic (Bravo et al., 2011).

2.1.2 Pesticide use in conditions of poverty

Inadequate handling during preparation and application of pesticides by agricultural workers in Nicaragua has been repeatedly reported (Corriols, 2009; Aragón et al, 2001; Blanco et al, 2005; Rojas et al. 2009). The following reported practices could directly influence children's exposure to pesticides: storage of the chemicals under beds, in the kitchen or in places of easy access for children; inappropriate use of empty pesticide containers to store water, vegetable oils or food; deficient personal hygiene, washing of work clothes together with the clothes of the family, and dumping of empty pesticide containers near playing areas.

Besides inappropriate pesticide handling, rural families usually live in the middle of or close to the cultivated fields. The homes are very often one-room houses with dirt-floors, roofs in very poor condition and completely open to cultivated fields (Figure 1) (Rojas et al., 2009). In consequence, drifting of pesticides from the sprayed crops and contamination of indoor environments is highly probable.

Figure 1 One room-houses in Los Zanjones /Calle Real where this thesis was carried out. Areas where families expend most of the time are open to the cultivated fields.

2.1.3 Regulation of pesticide use in Nicaragua

In 1998, Law 274 was approved, the basic law for the regulation and control of toxic and hazardous pesticides and other high risk substances. This law also determines that the Ministry of Agriculture and Forestry (MAGFOR) is the authority in charge of its implementation. MAGFOR has restricted and banned a number of hazardous pesticides over the last two decades. Currently, uses of terbufos, carbofuran, methomyl, methyl parathion, methami-dophos, etoprophos, endosulfan, chlorpyrifos, paraquat and aluminum phosphide are restricted, whereas all uses of aldicarb, chlordane, heptachlor, chlordimeform, DBCP, DDT, aldrin, dieldrin, endrin, EDB, HCH/BHC, lindane, pentachlorophenol, toxaphene, 2,4,5-T and monocrotophos are cancelled (MAGFOR 2001; MAGFOR, 2004). Despite that Nicaragua has a fairly good pesticide legislation and the government assumedly follows international guidelines for pesticides regulation, such as the FAO Code of Conduct, structures and resources to ensure compliance are limited.

2.1.4 Evidence of environmental contamination by pesticides

The combination of high use of pesticides, precarious handling of pesticides, and poor regulatory implementation gives rise to frequent events of environmental pollution, which have been documented in Nicaragua since de-cades. The studies of environmental pesticide contamination have focused on water, sediments and, more recently, soil in agricultural areas. No studies in air have been carried out yet.

A screening of persistent chlorinated hydrocarbons was carried out in 1995 in the main lagoons on the Pacific coast of Nicaragua, where cotton cultivations predominated. In sediments, very high concentrations of total DDTs (270 ng g^{-1} dw) and toxaphene (420 ng g^{-1} dw) were found. Other compounds such as arochlors, chlordane, endosulfan, and dieldrin were also identified (Carvahlo et al., 1999). In water, the pesticides determined were dichlorvos (up to 410 ng g-1 dw), diazinon (up to 150 ng g^{-1} dw), chlorpyrifos (up to 83 ng g^{-1} dw), toxaphene (up to 17,450 ng g^{-1} dw), total DDTs up to 478 and arochlor up to 119 (Carvalho et al., 2002). In soils, many organochlorines were also identified with concentrations of most of these compounds being below 5 ng g^{-1}, but concentrations of toxaphene being extremely high (17000- 44000 ng g^{-1} dw) (Carvahlo et al., 2003).

A study carried out in 1999 in Posoltega, the same area where a part of this thesis was carried out, reported dieldrin, *p,p'*- DDE, fenthion, ethylparathion, methomyl and carbaryl in soils; and dieldrin, *p,p*'- DDE, DDT and cya-nazine in water in relation to cotton cultivation (Cuadra et al.,1999). In Río San Juan, the river that forms the border with Costa Rica, *p,p'*- DDE, lindane and *p,p*'- DDT were found in water, and dieldrin, *p,p'*- DDE and lindane in suspended particles, in relation to sesame, cane sugar and rice crops (PROCUENCA San Juan, 1997). Montenegro et al., (2007) showed hexachlorobenzene, dieldrin and DDT in well waters in villages close to banana plantations.

2.1.5 Evidence of human pesticide exposure and health effects in agricultural areas

Exposures to pesticides and related health effects have been documented in Nicaragua essentially in adult agricultural workers. Determinants of dermal exposure have been studied in subsistence farmers (Blanco et al., 2005) and measured with a visual semi-quantified fluorescent method (Aragón et al, 2006). Extensive dermal contamination post-application, especially on hands, arms, feet and back, was observed for most farmers. All the 32 agricultural workers participating in this study had at least one contaminated body part and no body part was free from contamination in all workers (Aragón et al., 2005).

Acute pesticide poisonings have been reported in relation to organophosphate and carbamate insecticides and the herbicide paraquat (Amador et al., 1993; McConnell et al., 1990; Rosenstock et al., 1991; McConnell and Hruska, 1993; Keifer et al., 1996 (a), Corriols et al., 2009).

In Nicaraguan agricultural workers with previous acute organophosphate poisoning, persistent neurological effects have been reported. Compared to a never poisoned control group, poisoned agricultural workers performed worse on five of six subtests of the World Health Organization Neuropsychological Core Test Battery (WHO-NCTB) and on additional tests that assessed together verbal and visual attention, visual memory, visuomotor speed, sequencing and problem solving, and motor steadiness and dexterity, two years after an episode of acute pesticide poisoning (Rosenstock et al., 1991). Of the workers previously poisoned with methamidophos, 25% had abnormally high vibrotactile thresholds (McConnell et al., 1994).

In a cohort study that followed men after poisoning with an organophosphate pesticide starting at hospital discharge, grip and pinch strength were impaired at two years of follow up among men severely poisoned with methamidophos and chlorpyrifos both occupationally and intentionally (Miranda et al., 2004). Decreased visuomotor performance and a marked increase in neuropsychiatric symptoms were also reported two years post-poisoning (Delgado et al., 2004).

In children, organophosphate exposure has been documented by means of decreased cholinesterase activity (Keifer et al., 1996 (b); McConnell et al., 1999) and urinary levels of the chlorpyrifos metabolite 3,5,6-trichloro-2-pyridinol (TCPY) (Dowling et al., 2005). Between 1995 and 2006, the pesticide poisoning surveillance system registered 2069 acute pesticide poisonings in children aged 5 to 14. Of these, 47% were caused by organophosphate pesticides, predominantly methamidophos and chlorpyrifos (Corriols and Aragon, 2010). Long-term health effects have not been investigated in children in Nicaragua.

2.2 Determinants and pathways of children's pesticide exposure in agricultural families

Determinants of exposure refer to factors that influence exposure in a broad sense, from proximal factors such as behavior, hygiene, weather or landscape up to more distal social, economic and climatic factors. The term 'exposure pathway' refers to the course of a contaminant from its origin to its endpoint, considering release at the source (for example a sprayed agricultural field), environmental fate and transport mechanisms through water, air, soil, and biota; the point of exposure or the place where someone can get in contact with the contaminant in the environment (for example home dust), the route of uptake (inhalation, ingestion or dermal contact), and the exposed receptor population (children in our case). Pathways of exposure characte-rize how children can come into contact with a pesticide. Knowledge on determinants and pathways, which interlink and overlap, is important in designing and implementing effective preventive and control measures.

In industrialized countries pathways for children's pesticide exposures in agricultural areas have been studied. Pathways have, for example, identified carry-home of pesticides by farmer and farmworker parents on their contaminated skin, clothes and shoes (Gomes et al., 1999; Simcox et al.,1995; Bradman et al., 1997; Loewenherz et al.,1999; Fenske et al., 2000; Lu et al., 2000; Mc Couley et al., 2001; Curl et al., 2002; Thompson et al., 2003; Coronado et al., 2006) and contamination of domestic dust (Simcox et al., 1995, Lu et al., 2000, Mc Couley et al., 2001). Of special importance is transfer of pesticides to the unborn child in pregnant women. Between 25 to 60% of pregnant farmworkers of the CHAMACOS cohort in Salinas Valley California incurred in risky behaviors with regard to basic norms of hygiene such as hand washing, bathing, and use of protective clothing, storage and washing of clothes, house cleaning, bringing home fruit and vegetables directly from the field, and wearing work clothes more than 30 minutes after work (Goldman et al., 2004).

In Posoltega, the same rural area where the fieldwork for papers II, III and IV was carried out, a parallel qualitative assessment of pesticide exposure of the children evidenced factors that indirectly influenced pesticide use by the parents and others that directly determined exposure of the children (Rojas et al., 2009). This study found that the economic dependence on farming for family subsistence and absence of feasible alternatives of employment, the presence of big peanut plantation around the villages with high volumes of pesticide use, the reluctance of poor farmers to use alternative pest control methods out of fear for crop loss, and child labor as a traditional part of the upbringing, were important determinants of children's exposures to pesticides. Poverty is the underlying determinant of these interconnected co-mmunity and family factors for contamination and exposures. In addition, the weak governmental enforcement of pesticide legislation, especially concerning working conditions and environmental contamination in relation to economically powerful export plantations (in this case peanut plantations surrounding the study community), is a more distal determinant of exposure among these rural children. This study also identified pathways, i.e. how the pesticides came into contact with the children, including take home, environmental exposures and direct manipulation by the child.

2.3 Neurotoxicity of pesticides

Organophosphate and pyrethroid insecticides directly target nervous tissue as their mechanism of toxicity.

2.3.1 Mechanism of neurotoxicity for organophosphates and pyrethroids

Organophosphates

The primary target of organophosphate insecticides (OP) is the enzyme acetylcholinesterase (AChE), which hydrolyzes the neurotransmitter acetylcholine (ACh) in both the peripheral and the central nervous system. The reaction between an OP and the active site in the AChE results in the formation of a temporary intermediate complex that is partially hydrolyzed leaving a phosphorylated (inactive) enzyme. Under normal conditions, AChE can be reactivated only at a very slow rate (Bjørling-Poulsen et al., 2008). Some OPs have the ability to bind persistently to the active site of AChE to produce an irreversibly inhibited enzyme by a mechanism known as aging (Pope et al., 1999). The excessive amount of undestroyed ACh cause nicotinic and muscarinic signs and symptoms. Nicotinic manifestations, occu-rring in severe cases and late in the course, comprise fasciculations, cramps, weakness, hypertension, tachycardia, mydriasis, pallor and neuromuscular paralysis. Muscarinic manifestations include bradycardia, hypotension, rhinorrhea, bronchorrhea, bronchospasm, cough, severe respiratory distress, hypersalivation, nausea and vomiting, abdominal pain, diarrhea, fecal incontinence, urinary incontinence, blurred vision, miosis, increased lacrimation and diaphoresis (Singh and Sharma, 2000).

In addition to excitatory effects in both central and peripheral nervous system, ACh also plays a key role in regulation of morphogenetic cell movements, cell proliferation, growth, and differentiation in the development brain. The interference in the cholinergic system by OPs may have morphological, neurochemical and functional effects (Lauder and Schambra 1999). Cell signaling cascades that control neural cell replication and differentiation appear to be among the most sensitive targets for OPs developmental neurotoxicity and, in particular, the regulation of cyclic AMP (cAMP). Animal studies confirm that inhibition of cell signaling cascades in developing brain, disrupts the processes of replication and differentiation of neurons, axonogenesis and synaptogenesis (Schuh et al., 2002; Yanai et al.,2002; Meyer et al., 2003, 2004, 2005; Curtin et al., 2006).

Pyrethroids

The effects of pyrethroids on the central and peripheral nervous system are complex and information regarding the potential developmental neurotoxicity of this class of compounds is limited. Effects in the nervous system in development have been associated with altered structure and function of voltage-gated sodium channels (Marban et al., 1998). The pyrethroids affect both the activation (opening) and inactivation (closing) of the channel, resulting in a hyperexcitable state producing abnormal repetitive nerve impul-ses (Ginsburg and Narahashi, 1993; Narahashi et al., 1998). Pyrethroids type I (without alfa-cyano-3-phenoxybenzyl moiety) hold sodium channels open for a relatively short time period (milliseconds) and type II (with alfa-cyano-3-phenoxybenzyl moiety) keep the channel open for a prolonged time period (up to seconds) (Soderlund, 2012). Additional mechanism for developmental neurotoxicity may involve antagonism of the inhibitory neurotransmitter gamma-aminobutyric acid (GABA) in chloride channels, (Abalis et al., 1986; Soderlund, 2012), modulation of nicotinic cholinergic transmission, enhancement of noradrenalin release, and direct actions on voltage dependent calcium or chloride ion channels (Soderlund et al., 2002). It is difficult to know the implications of these mechanisms in the process of maturation of nervous system because insufficient information is available in the scientific literature; the animal studies were conducted with inadequate study design, used formulated products, and/or had inadequate control group (Shafer et al., 2005).

2.3.2 Special vulnerability of children to toxic effects in the nervous system

The special vulnerability of children to effects of pesticides is due to both physiological and behavioral reasons. On the one hand, there are important differences in physiological characteristics between children and adults. Young children have a larger skin surface area relative to body weight, higher basal metabolic rate, and greater oxygen requirements; they also eat more food, drink more water per body weight and breathe more air than adults; children's ability to metabolize, detoxify and excrete toxic compounds is also immature (Selevan et al., 2000). On the other hand, some children's behavior, such as the hand to mouth behavior, playing on the ground or playing with empty pesticide containers or in the crop fields make them more susceptible to enter in contact with pesticides (Freeman et al., 2001; Freeman et al., 2005; Black et al., 2005). Finally, because children have more years of life ahead than most adults do, they have more time to develop chronic effects that may be initiated by early exposures (Landrighan et al., 1999).

The nervous system is in continuous process of maturation concluding well into adulthood, on average at the age of 27 (Needleman, 2006). Some maturation periods are most susceptible to the toxic damage of environmental exposures. An important window for development of cognitive and behavioral function is the maturation of the frontal lobe, which is not complete until the age of 6, which is actually the reason that the inclusion of the children to the formal education is set at this age (Needleman, 2006). Research to evaluate neurological effect of environmental exposures in early stages in the life should consider this time point (Lanphear et al., 2005; Needleman, 2006; Weiss, 2000; Weiss et al., 2004; Weiss and Bellinger, 2006)

2.3.3 Neurobehavioral effects of organophosphate and pyrethroid insecticides

A series of studies have shown a relationship between exposure to pesticides during pregnancy or early years of life and neurodevelopmental effects. Table 1 summarizes current evidence regarding effects in neurodevelopment in children exposed to organophosphates and pyrethroid insecticides. The evidence mainly came from three cohort studies in the United States: A cohort of children of Latina mothers' in agricultural communities in Salinas Valley California (CHAMACOS); a cohort of children of African American and Dominican mothers in New York City (CCCEH) and a multiracial cohort of children born in The Mount Sinai Hospital studied by the Mount Sinai Children's Environmental Health Center (MSCEHC).

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Table 1 Children's pesticide exposure and neurobehavioral effects: a review of studies from January 2001 to June 2012.

Country (re-ference)	**Study design / study population**	**Children's age at neurobehavioral testing**	**Pesticide / exposure assessment**	**Tools for outcome assessment**	**Neurobehavioral effects**
USA (Rauh *et al.*, 2012)	Longitudinal birth cohort / 40 (CCCEH)	5.9 - 11.2 years	Chlorpyrifos / chlorpyrifos in umbilical cord blood	Magnetic resonance imaging	High chlorpyrifos exposure was associated with enlargement of cortical regions related with attention, receptive language, social cognition, emotions and inhibitory control.
China (Guodong *et al.*, 2012)	Cross-sectional / 301	23 - 25 months	Organophosphates / DAP concentrations in children's urine samples	DQs based on GDS	No association was found between child urinary levels of DAPs and any of the DQs scores.
USA (Bouchard, *et al.*, 2011)	Longitudinal birth cohort / 329 (CHAMACOS)	7 years	Organophosphates / DAP concentrations in urine collected during pregnancy and from children at 6 months and 1, 2, 3.5 and 5 years of age	WISC-IV	Averaged maternal DAP concentrations were associated with poorer scores for WMI, PSI, VCI, PRI, and Full-Scale IQ.

Country (re-ference)	Study design / study population	Children's age at neurobehavioral testing	Pesticide / exposure assessment	Tools for outcome assessment	Neurobehavioral effects
USA (Rauh *et al.*, 2011)	Longitudinal birth cohort / 265 (CCCEH)	7 years	Chlorpyrifos / chlorpyrifos in umbilical cord blood	WISC-IV	For each standard deviation increase in exposure (4.61 pg/g), Full-Scale IQ declined by 1.4%, and WMI declined by 2.8%.
USA (Engel *et al.*, 2011)	Longitudinal birth cohort / 200: 12 months 276: 24 months 169: 6 to 9 years (MSCEHC)	12 months 24 months 6 - 9 years	Organophosphates / DAPs in maternal urine samples; PON1activity and PON1 polymorphism	BSID-II for children of 12 and 24 months and WPPSI-III for children 6 to 9 years	Decrement in mental development at 12 months among blacks and Hispanics. Associations appeared to be enhanced among children of mothers who carried the *PON1* Q192R QQ genotype. In school age children, prenatal total dialkyl- and dimethylphosphate metabolites were associated with decrements in perceptual reasoning in the maternal *PON1* Q192R QQ genotype.
USA	Longitudinal birth	36 months	Permethrin/	BSID-II	No associations with

Country (re-ference)	Study design / study population	Children's age at neurobehavioral testing	Pesticide / exposure assessment	Tools for outcome assessment	Neurobehavioral effects
(Horton *et al.*, 2011)	cohort / 342 for permethrin in personal air 272 for permethrin in plasma 230 subjects for pi-peronyl butoxide in personal air (CCCEH)		permethrin levels measured in maternal and umbilical cord plasma collected on delivery; permethrin and piperonyl butoxide levels measured in personal air collected during pregnancy		permethrin Piperonyl butoxide in personal air samples (>4.34 ng/m^3) scored 3.9 points lower on the MDI than those with lower exposures.
Philippines (Ostrea Jr. *et al.*, 2011)	Longitudinal birth cohort / 754	2 years	Pyrethroids, carbamates and organophosphates/ propoxur, cyflu-thrin, chlorpyrifos, cypermethrin, pretilachlor, bioallethrin, malathion, diazinon and transfluthrin in maternal hair and blood, infant's hair, cord blood and meco-nium	Griffiths mental developmental scale	Exposure to propoxur was negatively related to motor development, but was unrelated to social and performance deve-lopment. No significant relationships between pyrethroids and neurodeve-lopment at 24 months were found.

Country (re-ference)	**Study design / study population**	**Children's age at neurobehavioral testing**	**Pesticide / exposure assessment**	**Tools for outcome assessment**	**Neurobehavioral effects**
USA (Eskenazi *et al.*, 2010)	Longitudinal birth cohort / 353 (CHAMACOS)	2 years	Organophosphates / DAP concentrations in maternal urine during pregnancy; $PON1_{192}$ and $PON1_{-108}$ genotypes in mothers and children, and aryl-esterase (ARYase) and paraoxonase (POase) in pregnant mothers, cord and 2 years old blood.	MDI and PDI of the Bayley Scales and CBCL	Children with the $PON1_{-108T}$ allele had poorer MDI scores and somewhat poorer PDI scores. Association between DAPs and MDI scores was strongest in children with $PON1_{-108T}$ allele.
USA (Marks *et al.*, 2010)	Longitudinal birth cohort/ 654 331: 3.5 years 323: 5 years (CHAMACOS)	3.5 years 5 years	Organophosphates / DAP concentrations in maternal urine during pregnancy	CBCL Visual attention subtest of NEPSY-II K-CPT Hillside Behavior Rating Scale	Prenatal DAPs were not associated with maternal report of attention problems and ADHD at age 3.5. Prenatal DAPs were associated with maternal report of attention problems and ADHD at age 5. Prenatal DAPs were

Country (re-ference)	Study design / study population	Children's age at neurobehavioral testing	Pesticide / exposure assessment	Tools for outcome assessment	Neurobehavioral effects
					associated with scores on the K-CPT (8% of children scored ≥ 70% on the standardized ADHD Confidence Index scale). Some effects are stronger in boys than in girls.
USA (Bouchard *et al.*, 2010)	Cross sectional / 1139	8 - 15 years	Organophosphates / DAP concentrations in urine of children Data of the National Health and Nutrition Examination Survey (2000 – 2004)	A structured interview with a parent to ascertain ADHD diagnostic status	A 10-fold increase in DMAP concentration was associated with an odds ratio of 1.55 for ADHD.
Ecuador (Harari *et al.*, 2010)	Cross-sectional / 87	Children atten-ding Ecuadorian second and third grade	Organophosphates / maternal interview, DAPs in urine and erythrocyte acetylcholinesterase activity in children	Finger Tapping Task, Santa Ana Form Board, K-CPT (version 5), Copying Test of the Stanford-Binet (4th edition), Raven's Colored	Children with prenatal exposure from maternal greenhouse work showed consistent deficits for motor speed (Finger Tapping Task), motor coordination (Santa Ana Form Board),

Country (re-ference)	**Study design / study population**	**Children's age at neurobehavioral testing**	**Pesticide / exposure assessment**	**Tools for outcome assessment**	**Neurobehavioral effects**
				Progressive Matrices, and Digit Span of WISC-R	visuospatial performance (Stanford-Binet Copying Test), and visual memory (Stanford-Binet Copying Recall Test). Current exposure (DAPS and acetylcholinesterase) did not affect the outcomes.
Ecuador (Handal *et al*, 2008)	Cross-sectional / 121	3 - 23 months	Any pesticide / occupational history of mothers	Prehension (reach-and-grasp) and visual skills	Children whose mothers worked in the flower industry (exposed to pesticides) during pregnancy, scored lower on communication and fine motor skills and had a higher odds of having poor visual acuity.
USA (Sanchez-Lizardi *et al*., 2008)	Cross-sectional / 48	7 years	Organophosphates / DAPs in children's urine	Short form of the WISC-Third Edition, Children's Memory Scale; Wisconsin Card Sorting; Trail Making Test A &	Higher OP pesticide metabolite concentration levels were significantly correlated with poorer performance on some subtests of the Wisconsin Card Sorting Test

Country (re-ference)	Study design / study population	Children's age at neurobehavioral testing	Pesticide / exposure assessment	Tools for outcome assessment	Neurobehavioral effects
				B; CBCL/4-18 and the Tea-cher Report Form	
Brazil (Eckerman *et al.*, 2007)	Cross-sectional / 56	10 - 18 years	Any pesticide /chronic pesticide exposure index	BARS	Impairment of tapping, digit span, and selective attention
USA (Engel *et al.*, 2007)	Longitudinal birth cohort / 311 (MSCEHC)	Neonates	Organophosphates / DAPs in maternal urine; PON1 activity	Brazelton NBAS	DAPs related to increase in the number of abnormal reflexes, and increase in the proportion of children with more than three abnormal reflexes. There was a strong interaction between PON1 expression levels and total dimethyl alkyl phosphates on risk of abnormal reflexes.
USA (Eskenazi *et al.*, 2007)	Longitudinal birth cohort / 6 months (n = 396) 12 months (n = 395) 24 months (n = 372)	6, 12 and 24 months	Organophosphates / six nonspecific DAP metabolites, malathion / specific metabolite MDA,	CBCL	At 24 months: Bayley MDI Risk of Pervasive Developmental Disorder

Country (re-ference)	Study design / study population	Children’s age at neurobehavioral testing	Pesticide / exposure assessment	Tools for outcome assessment	Neurobehavioral effects
	(CHAMACOS)		and chlorpyrifos / specific metabolite TCPY in pregnant mothers and child urine		
Ecuador (Handal *et a*l., 2007)	Cross-sectional / 283	3 - 61 months	Any pesticide / Community of residence	ASQ	Deficits on gross and fine motor and socio-individual skills
USA (Rohlman *et al.*, 2007)	Cross-sectional / 175 119 adolescents and adults working in agriculture 56 not working in agriculture	Adults 28 ± 7.6 years Adolescents 15.7 ± 1.6 years	Any pesticide / years working in agriculture.	Finger tapping, digit symbol, simple reaction time, digit span, progressive ratio, selective attention, serial digit learning, continuous performance, math-to-sample, reversal learning.	In adults and adolescents low levels of pesticides over many years of agricultural work are asso-ciated with neurological impairment as measured by the selective attention, digit symbol, and reaction time tests. Years of experience handling pesticides were inversely associated with deficits in neurobehavioral performance.
USA (Rauh *et al.*,	Longitudinal birth cohort / 254	12, 24 and 36 months	Chlorpyrifos / chlorpyrifos levels in	PDI and MDI of Bayley Scales	Highly exposed children scored

Country (re-ference)	**Study design / study population**	**Children's age at neurobehavioral testing**	**Pesticide / exposure assessment**	**Tools for outcome assessment**	**Neurobehavioral effects**
2006)	(CCCEH)		umbilical cord plasma	CBCL	on average 6.5 points lower on the PDI and 3.3 points lower on the MDI and had more attention problems, ADHD, and pervasive developmental disorder problems at 3 years of age compared with those with lower levels of exposure
Ecuador (Grandjean *et al.,* 2006)	Cross-sectional / 79	7 years	Organophosphates / erythrocyte AChE activity and urinary excretion of DAPs in urine of children.	Santa Ana Form Board, WISC-R, Stanford-Binet copying subtest, Finger tapping task, Reaction time test	Mother's occupation in floriculture associated with deficits on visuo-spatial and response speed. DAP metabolites associated with reaction time deficits.
Israel (Kofman *et al.,* 2006)	Cross-sectional / 52 26 exposed 26 unexposed	6 - 12 years	Organophosphates / organophosphate poisoned during infancy vs kerosene poisoned vs matched unpoisoned controls	Neuropsychological battery	Deficits on long-term memory, learning, inhibitory motor control

Country (re-ference)	Study design / study population	Children's age at neurobehavioral testing	Pesticide / exposure assessment	Tools for outcome assessment	Neurobehavioral effects
USA (Young *et a*l., 2005)	Longitudinal birth cohort / 381 (CHAMACOS)	≤ 2 months	Organophosphates / urinary levels of DAPs in maternal urine at 14 and 26 weeks of pregnancy and 7 days post-partum.	Brazelton NBAS	Prenatal exposure was related with an increase in number of abnormal reflexes and more than three abnormal reflexes.
USA (Rohlman *et a*l, 2005)	Cross-sectional / 78	48 - 71 months	Any pesticide / Agricultural communities vs non-agricultural communities	Finger tapping Match-to-sample	Children from agricultural communities performed poorer on measures of response speed (Finger tapping) and latency (Match-to-sample) compared to children of non-agricultural communities.
USA (Ruckart *et al.*, 2004)	Cross-sectional / 226 107 exposed 119 unexposed	6 years	Methyl parathion / urinary *para*-nitrophenol levels in children and environmental wipe samples for methyl parathion.	PENTB	High exposure to Methyl parathion was associated with poor performance in tasks involving short-term memory and attention.

Country (re-ference)	Study design / study population	Children's age at neurobehavioral testing	Pesticide / exposure assessment	Tools for outcome assessment	Neurobehavioral effects
			High exposure was defined as household Methyl parathion ≥ 1,000 µg/100 cm^2 or a urinary PNP level ≥ 300 ppb		
USA (Rohlman *et al.*, 2001)	Cross-sectional / 147 96 adolescents currently working in agriculture 51 adolescents currently non-migratory and not working in agriculture	13 - 18 years	Any pesticide / occupational exposure	BARS	Deficits on response speed, Attention and complex functioning

ADHD: Attention deficit hyperactivity disorder; AChE: acetyl cholinesterase; ARYase: Arylesterase; ASQ: Ages and stages questionnaires; BARS: Behavioral Assessment and Research System; BSID-II: Bayley Scales of Infant Development, version II; CBCL: Child Behavior Checklist; CCCEH: Columbia Center for Children's Environmental Health. Children from a cohort of African American and Dominican mothers in New York City; CHAMACOS: Center for the Health and Assessment of Mothers and Children of Salinas. Children from a cohort of Latina mothers in agricultural communities in California; DAPs: Dialkylphosphates; DMAP: Dimethyl alkyl phosphate; DQs: Children developmental quotients; GDS: Gesell Developmental Schedules; IQ: Full-Scale intelligence quotient; K-CPT: Conners' Kiddie Continuous Performance Test; MDI: Mental Development Index; MSCEHC: Mount Sinai Children's Environmental Health Center; NBAS: Neonatal Behavioral Assessment Scale; NEPSY-II: Neuropsychological Assessment; PDI: Psychomotor Development Index; PENTB: Pediatric Environmental Neurobehavioral Test Battery; PON1: Paraoxonase 1; WISC-IV: Wechsler Intelligence Scale for Children, 4th edition; WISC-R: Wechsler Intelligence Scale for Children, revised; WPPSI-III: Wechsler Preschool and Primary Scale of Intelligence - Third Edition.

3. Objectives

The overall objective of this thesis was to examine the exposure to pesticides in Nicaraguan children in relation to parental pesticide use, and the occurrence of associated neurobehavioral effects, in a developing country context.

The specific objectives were

- To measure current pesticide exposure of children in rural Nicaragua in relation to parental pesticide use.
- To construct retrospective pre and postnatal quantitative indices of parental pesticide use in rural Nicaragua as proxies for children's life-long pesticide exposures.
- To explore the relationships between pre and postnatal exposure to organophosphate and pyrethroid insecticides and cognitive performance and behavior in school-aged rural Nicaraguan children.
- To explore the relationships between recent exposure to organophosphate and pyrethroid insecticides and cognitive performance and behavior in these children.

4. Methods

This thesis is based on two separate data collections. The first study was conducted in 2003 and assessed exposures to the organophosphate insecticides chlorpyrifos and diazinon among pesticide applicators and their children. The second study, carried out during 2007-2008, assessed children's long-term pesticide exposures through indices of parental pesticide use and children's recent exposure through urinary residue levels, and examined associations between long-term and recent pesticide exposures with neurobehavioral effects.

4.1 First data collection and paper I.

4.1.1 Study population

The study population included farm workers and their children from two areas in Nicaragua: 7 smallholders using the organophosphate chlorpyrifos on corn crops in León, and 10 workers on two banana plantations in Chinandega who sprayed diazinon. One child of each agricultural worker, between 2 and 12 years of age and residing with the applicator, was included in the study.

4.1.2 Exposure assessment

Urine samples were collected from each pesticide applicator and from his child. A complete set of urine samples included four voids on the day of the application (before and immediately after spraying, at midday, and at the end of the workday), and three voids the day after the application (morning, midday and afternoon) for the sprayers and their children.

4.1.3 Data analysis

Concentrations of 3,5,6-trichloro-2-pyridinol (TCPY), the specific metabolite of chlorpyrifos, and 2-isopropoxy-4-methyl-pyridinol (IMPY), the specific metabolite of diazinon, were log-normally distributed and the geometric mean, range and percentiles were calculated. Linear regression was used to examine the relationship between adult and child metabolite levels.

4.2 Second data collection and papers II, III and IV.

4.2.1 Study population

This cross-sectional study was conducted in the northwest of Nicaragua. The study initially targeted the populations of children aged 7 to 9, attending grade 1 to 3, of two communities, an agricultural community versus a non-agricultural community. The communities were Los Zanjones/Calle Real in Posoltega, a highly agricultural region that produces peanut for exportation and cereals and vegetables for subsistence, and Colonia 20 de Julio in Villanueva, also a rural area with predominantly informal nonagricultural commercial activity. In the agricultural community, 110 children were included in the study population (96% response) and in the non-agricultural community 74 (88% response). All children from Los Zanjones/Calle Real had a parent working in agriculture at the time of the study. In Colonia 20 de Julio no agricultural activity was registered since one year before the study, but 22 parents had been employed earlier in large scale agriculture.

The three papers used different subsets of these 284 children. The construction of life-long exposure indices, reported in Paper II, was based on data for all children of fathers with a pesticide use history from both communities (n=132). During the fieldwork, however, important differences between the two communities became apparent, which could influence the neurobehavioral performance of children. To avoid confounding from socioeconomic disparities and differences in quality of schooling, the neurobehavioral assessment was restricted to the children of the agricultural community (n=110) who had similar socioeconomic backgrounds and all went to the same school (Papers III and IV). Recent exposure and associated neurobehavioral effects were examined in a subset of the children of the agricultural community (n=74) (Papers II, III and IV).

4.2.2 Exposure assessment

Long-term pesticide exposure

Due to Hurricane Felix, no pesticides were applied in the agricultural community at the time of neurobehavioral testing in 2007, and, therefore, no recent exposure occurred. An icon-calendar-based form (ICBF) (Engel et al., 2001; Monge et al., 2004; Monge et al., 2007) was used to interview the parents about children's prenatal and life-time pesticide exposures. The ICBF was applied to parents of all 110 children of the agricultural community and to 22 parents of the children in the non-agricultural community with a history of agricultural work in the past. Some children were siblings and only one ICBF was applied to their parent, but their individual data differed for exposure windows. The ICBF covered pesticide use data from one year before birth throughout pregnancy up to the current year. Stickers were used to indicate life events and work history, to facilitate recall of pesticide use. The data collected were from one year before birth to the year of interview (2007). 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. All farmers used manual backpack sprayers and some of the fathers had worked on plantations with tractor spraying. None had ever used protective equipment.

At the time of the interviews, most (n=74) were subsistence farmers spraying with backpacks, 28 were plantation workers spraying with tractor, and 8 were plantation workers who also owned a piece of land. Indices for pesticide use were constructed for the fathers and one widow mother. In some subsistence farming families (n=9) mothers also had participated in pesticide related tasks. For subsistence farming, a pesticide specific use index of total kg used in a lifetime was constructed: dose (g/ha) * # ha * frequency (# application days). For plantation work we could not collect data on pesticide dose, and therefore the pesticide index was calculated as total number of hours in a lifetime: # application days * # hours per day. The latter was also used as a second index for subsistence farmers to be able to combine the groups. When fathers were both plantation worker and subsistence farmer simultaneously, their indices of hours of spraying were summed. Indices were constructed for five specific known neurotoxic pesticides (chlorpyrifos, methamidophos, cypermethrin, deltamethrin and 2,4-D), chemical groups of organophosphates, carbamates and pyrethroids, and for total pesticide use, by adding specific pesticide use scores.

Recent exposure

In 2008, pesticide residue analyses in urine samples was added to the exposure assessment strategy to assess recent pesticide exposure of the children in relation to parental applications. However, also in 2008, only about half of the farmers were cultivating crops, this time due to delays in delivery of the seeds by the state agencies.

Effective urine sampling was difficult, because the fathers often changed their pesticide spraying plans at the last moment. Therefore, mothers filled in information in a calendar about the use of pesticide during the week of full moon, when farmers usually spray. At the same time, the children were asked to bring in a urine sample daily from Monday to Saturday. Samples were then related to parental pesticide applications with the help of the moon calendar. A complete set of urine samples for children, whose father had sprayed pesticides, consisted in four voids, one per day during 4 days (the day before application, the day of application, and 24 and 48 hours after application). Of the children, whose fathers did not spray pesticides, one or two voids were randomly selected during this sampling week. A total of 211 urine samples were collected for 74 children, 154 samples from 32 children of fathers cultivating agriculture produce and 57 samples of 42 children of fathers without agriculture activity.

The urine samples were analyzed for the chlorpyrifos metabolite TCPY, the general pyrethroid metabolite 3-phenoxybenzoic acid (3-PBA), and 2,4-D according to Lindh et al. (2008) adjusted for urinary creatinine levels. Creatinine levels were analyzed using an enzymatic method described by Mazzachi et al., (2000).

4.2.3 Outcome assessment

Neurobehavioral tests for children were selected based on the recommendations of a workshop in Costa Rica, in February 2006, with participation of experts from North and Latin America (UNA, 2006). The testing was done at the schools of the communities, by psychologists trained and supervised by a neuropsychologist.

Cognitive performance of the children was evaluated with the Wechsler Intelligence Scale for Children (WISC IV) (Weschler, 2003). Twelve sub-tests grouped in four cognitive indices and total IQ were applied (Table 2).

Table 2 Cognitive indices and sub-test of WISC IV (Anderson et al., 2001; Braga and Campoz Da Paz; Wechsler, 2007)

Cognitive indices	Functions assessed	Sub-tests
Verbal Comprehension Index (VCI)	o ability to listen to a question, o draw upon learned information from both formal and informal education, o reason through an answer, and express their thoughts aloud.	Similarities Vocabulary Comprehension
Perceptual Reasoning Index (PRI)	o ability to examine a problem o draw upon visual-motor and visual-spatial skills o organize their thoughts, create solutions, and then test them.	Block design Picture concepts Matrix reasoning Picture completion
Working Memory Index (WMI)	o ability to memorize new information, hold it in short-term memory o manipulate the information to produce some result or reasoning processes.	Digit Span Letter-number sequencing
Processing Speed Index (PSI)	o ability to focus attention and quickly scan o discriminate between, and sequentially order visual information o persistence and planning ability	Coding Symbol search Cancellation

Behavior of the children was measured using the revised Conner's Teachers Rating Scale short version (CTRS-R:S). The CTRS-R:S is a commonly used measure of behavioral problems in children. Several abbreviated forms exist; in this thesis the revised short-form of 28 items was used (Conners, 1997). Questions were responded on a 4-point scale: ''never'' (0), "occasionally" (1), "often" (2) and ''very often'' (4) and the answers were grouped in three scales (Oppositional, Cognitive Problems/Inattention, and Hyperactivity). In addition, the test yields an 'ADHD Index', which partially overlaps with the hyperactivity scale. Raw scores were calculated by summation of points for each question comprised in every sub-scale.

In addition we applied a test for general intelligence of the mother, independent of language and reading and writing skills (Raven, 2003) and a child development questionnaire (CENOP FL, 2012). A complete physical and neurological examination was conducted, and children's height and weight and body mass index (BMI) were registered.

An overview of the periods of data collection, study populations, and exposure and outcome assessment in studies 1 (Paper I) and 2 (Paper II, III and IV) is presented in Table 3.

Table 3. Overview of the study methods

	2003 Paper I	**2007 Paper II, III and IV**	**2008 Paper II, III and IV**
Study population	-10 banana plantation worker and child pairs -7 farmer and child pairs	Initial study population: 110 children of an agricultural community and 74 children of a non-agricultural community (including 22 with history of parental pesticide use in agriculture) -Paper II: 132 children with a parental history of pesticide use in agriculture (110 + 22) Paper III and IV: 110 children of the agricultural community	74 children of the agricultural community
Exposure assessment	Urinary metabolites of fathers and children 221 urine samples -Urinary TCPY (chlorpyrifos metabolite) -Urinary IMPY (diazinon metabolite)	Long term exposure indices based on data collected by ICBF -Exposure indices in hours and kg of a.i for total pyrethroids and cypermethrin, and for total organophosphates, chlorpyrifos and methamidophos. -Time windows: prenatal, first year of life, age over 1 to age in 2007.	Urinary metabolites -154 samples of 32 children whose parents were cultivating crops -57 samples of 42 children whose parents were not cultivating crops -Urinary 3-PBA (general pyrethroid metabolite) -Urinary TCPY (chlorpyrifos metabolite)

	2003 Paper I	2007 Paper II, III and IV	2008 Paper II, III and IV
Cognitive assessment		**WISC-IV** -Verbal Comprehension Index (VCI) o Similarities o Vocabulary o Comprehension o Block design -Perceptual Reasoning Index (PRI) o Picture concepts o Matrix reasoning o Picture completion -Working Memory Index (WMI) o Coding o Symbol search o Cancellation -Processing Speed Index (PSI) o Digit Span o Letter-number sequencing -Total IQ	**WISC-IV (shortened test battery)** Verbal comprehension domain o -Similarities o -Vocabulary o -Comprehension Perceptual reasoning domain o Matrix reasoning o Picture completion Working memory domain o Coding o Symbol search Processing speed domain o Digit span o Letter-number sequencing
Behavioral assessment		**CTRS-R:S** -Oppositional -Cognitive problems/inattention -Hyperactivity -ADHD index	**CTRS-R:S** -Oppositional -Cognitive problems/inattention -Hyperactivity -ADHD index

4.2.4 Data analysis

Indices of pesticides use were normalized by log10 conversion. Because no normative data for cognitive and behavioral tests exist for Central American populations, the raw scores were used for sub-tests of WISC-IV. Linear regressions were performed to examine, for each time window, associations between indices of kg of a.i. and the outcome variables, adjusting for potential confounders. In the models with urinary metabolites as the explanatory variable, the geometric mean and the maximum urinary TCPY for each child (1 to 6 samples) were used.

To construct the models, we evaluated whether potential confounders changed the crude regression coefficient by more than 10% in bivariate analyses. For the cognitive outcomes these covariates were children's school year, maternal years of education, the Raven test score and age of children.

Maternal years of education and Raven test score were correlated (r= 0.59, p< 0.05) and also children's school year and children's age (r= 0.64, p< 0.01). We retained children's school year and maternal years of education in the models for the cognitive outcomes, and excluded Raven test score and children's age since they did not further influence the effect estimates. In the models for behavioral outcomes, covariates were children's sex and age. Models for cognitive and behavioral outcomes in relation to organophosphate exposures were also adjusted for life time indices of pyrethroid use, and viceversa. Models assessing effects from recent exposures were in addition adjusted for lifetime exposure indices. Weight, height or body mass index as proxies for nutritional status, years of education of the father, fathers' age, and mothers' age had no effect on any of the coefficients and were therefore not included.

4.3 Ethical considerations

Study 1 (Paper I) was approved by the Ethical Committee of the Faculty of Medical Sciences of UNAN-León (08-2003) and by the Human Subject Committee at the University of Washington (03-5936-E-01).

Study 2 (Paper II, III and IV) was approved by the Ethical Committee of the Faculty of Medical Sciences of UNAN-León (150-2007). 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).

5. Results

5.1 Children's pesticide exposure

5.1.1. Recent pesticide exposure (Papers I and II)

This thesis found evidence that children of agricultural families in Nicaragua are highly exposed to pesticides.

Pesticide residues in fathers and their children were found before and after parental pesticide application (Paper I). Detectable levels of TCPY were found in 48 of 53 samples (91%) of small-scale farmers, and in 37 of 54 samples (69%) taken from their children. Concentrations of TCPY peaked in the fathers 27 hours post application (geometric mean 25.8 µg/L, a 30-fold increase, $p<0.01$) and at 8.5 hours in the children (geometric mean of 2.99 µg/L, a 3-fold increase $p>0.05$). A high correlation was observed between urinary levels of TCPY of fathers and their children in the evening samples of the day that chlorpyrifos was sprayed (beta = 1.29; CI = 0.19–2.90; R2 = 0.73; Pearson r= 0.85, p= 0.03). Although the geometric mean of urinary TCPY was clearly higher in adults than in children (25.8 µg/L vs 3.0 µg/L), the highest TCPY concentration observed among the children (125 µg/L) approached the highest concentration among the fathers (147 µg/L). The relationships between pesticide application by fathers and children's exposure was less evident in plantation workers spraying diazinon, for whom the metabolite IMPY was found only in 3 of the 57 samples of their children. Instead, unexpectedly, TCPY was detected in 57 of 58 of the plantation workers' samples (98%), and in 45 of 56 of their children's samples (79%), ranging from non-detectable to 109 µg/L for adults and from non-detectable to 20.2 µg/L for their children.

Also in the agricultural community of subsistence farming surrounded by peanut plantations, measurable quantities of pesticide residues were found in the urine of all children with 96% of the children's urine samples containing at least two and 71% containing all three residues that were analyzed (Paper II). The P50 for TCPY was 3.7 µg/g creatinine, for 3-PBA 2.8, and for 2,4-D 0.9; maximum levels were the highest reported in the literature for all three pesticides. TCPY peaked 1 day after application from 3.9 to 16.5 µg/g 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, those over the 90th percentile, were observed predominantly 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.

5.1.2 Children's long term pesticide exposure (Paper II)

Parents of the 132 schoolchildren of the agricultural community and the nonagricultural community with a pesticide use history reported together 47 different active ingredients on the ICBF, including 34 organophosphate, carbamate and pyrethroid insecticides. For all pesticides, there was an ample gradient both in hours and dose of active ingredient used by the parent (Table 4).

Table 4. Parental use indices of five neurotoxic pesticides during their children's life-time

Specific pesticide	Index in hours	Index in kg of a.i
Chlorpyrifos, median (min; max)	114 (2; 1584)	19.2 (0.37; 548)
Methamidophos, median (min; max)	84 (6; 1964)	12.2 (0.30; 780)
Cypermethrin, median (min; max)	81 (8; 1976)	2.1 (0.01; 354)
Deltamethrin, median (min; max)	40 (2; 265)	0.97 (0.04; 22.7)
2,4-D, median (min; max)	4.2 (2; 38,8)	8.8 (1.1; 22.7)

For these five pesticides, the median of kg of a.i sprayed per year by fathers were higher during the prenatal period and first year of life than other time windows. A pattern of change in the number of hours of application between periods was not so clear (Table 5).

Table 5. Median of hours and kg of a.i of relevant neurotoxic pesticides used by time-windows

	Chlorpyrifos	**Methamidophos**	**Cypermethrin**	**Deltamethrin**	**2,4-D**
Indices in hours					
Periconception and pregnancy (1 year)	39.0	43.0	19.0	18.0	16.0
First year of life (1 year)	19.0	22.0	22.0	23.0	24.0
Age 1 to 5 (average per year, 4 years)	16.0	16.0	6.0	4.0	12.0
From age 6 (average per year, up to 2007, 2 to 4 years)	40.0	20.0	8.0	8.0	6.0
Indices in kg of a.i					
Periconception and pregnancy	7.0	4.0	0.7	1.4	5.5
First year of life (1 year)	3.8	2.4	0.6	0.8	3.0
Age 1 to 5 (average per year, 4 years)	1.3	1.0	0.2	0.2	0.4
From age 6 (average per year, up to 2007,	2.0	1.4	0.3	0.4	0.9

5.2 Neurobehavioral effects in early school-age children in relation to organophosphate and pyrethroid exposure

5.2.1 Children's long term pesticide exposure and neurobehavioral effects (Paper III and Paper IV)

Cognitive effects

In general, a poorer performance of children highly exposed to organophosphate and pyrethroids on most of sub-test of WISC-IV was observed. The prenatal index in kg of total use of organophosphates was associated with lower scores in Verbal Comprehension Index (VCI), Perceptual Reasoning Index (PRI), Working Memory Index (WMI), and total IQ. The adjusted beta indicated that the Working Memory Index score decreased 3.2 points for each 10-fold increase in kg of total organophosphate used by fathers, and similar decreases were observed in working memory index with the specific OPs chlorpyrifos and methamidophos. The prenatal index for methamidophos was also associated with decreased scores on the comprehension and perceptual reasoning indices and on total IQ.

The index in hours for pyrethroid use during the first year of life was significantly associated with poorer performance on the Perceptual Reasoning Index (PRI), with the PRI score decreasing 1.1 point for each 10-fold increase in hours of pyrethroid use. No associations were observed between hours of pyrethroid spraying by the fathers and the indices for verbal comprehension, working memory and processing speed, or for total IQ for any of the time windows.

No significant associations were found between exposure indices of organophosphates and pyrethroids for the age older than 1 and cognitive performance. No relevant differences between sexes were observed in cognitive assessment in relation to exposure to pesticides.

Effects in behavior

The organophosphate exposure indices in kg of a.i in none of the time windows associated with the scales of the CTRS-R:S. Regarding pyrethroids, we observed increasing scores on the hyperactivity subscale and ADHD index scores with increasing hours of use, during the first year of life as well as in the time windows after age one.

The association between parental pesticides use indices as a proxy of children long-term exposure and neurobehavioral outcomes is summarized in Figure 2.

5.2.2 Children's recent pesticide exposure and neurobehavioral effects (Paper III and Paper IV)

Cognitive effects

After adjusting for confounders a 10-fold increase in urinary residue values of the chlorpyrifos metabolite TCPY was related with a non-significant 3.2 points decrease on the Working Memory Index, significantly for a subtest of this domain, Letter-number sequencing.

A poorer performance on most cognitive sub-test was noted with increases in 3-PBA urinary residue levels. Scores on Vocabulary (verbal comprehension), Picture Completion (perceptual reasoning) and Letter-number Sequencing (working memory) decreased 2.9, 2.4 and 0.4 points, respectively, in association with 10-fold increases in urinary levels of 3-PBA. Different effect patterns occurred for boys and girls. For boys, scores on the Vocabulary and Coding tests decreased significantly with increasing 3-PBA, whereas for girls scores on the Symbol search (processing speed).

Effects in behavior

TCPY urinary values were not related to scales of the Conners' Teacher Rating Scale. With regard to 3-PBA no behavioral effects were seen in boys but ADHD scores in girls increased 8.5 points for each 10 folds increase in 3-PBA urinary levels.

The association between urinary pesticides metabolites and neurobehavioral outcomes is summarized in Figure 3.

Long-term pesticides exposures indices	Prenatal	First years of life	From age 1 (Average per year, up to 2007)
Total organophosphates Index in kg of a.i, median (min-max)	7.2 (0.2; 470.9)	3.1 (0.1; 105.7)	1.6 (0.0; 148.7)
Chlorpyiphos Index in kg of a.i, median (min-max)	7.0 (0.3; 135.0)	3.8 (0.1; 58.0	1.4 (0.1; 63.0)
Methamidophos Index in kg of a.i, median (min-max)	4.8 (0.2; 336.0)	1.5 (0.1; 93.0)	1.5 (0.1; 93.0)
Total Pyrethroids Index in hours, median (min-max)	38.0 (2.0; 420.0)	25.0 (2.0; 120.0)	10.0 (2.0; 186.0)
Cypermethrin Index in hours, median (min-max)	32.0 (2.0; 384.0)	24.0 (2.0; 120.0)	7.0 (0.6; 186.0)

and

Cognitive indices of WISC-IV	
Verbal Comprehension Index(VCI), median (min-max)	68 (45-96)
Perceptual Reasoning Index (PRI), median (min-max)	67 (45-105)
Working Memory Index (WMI), median (min-max)	80 (52-113)
Processing Speed Index (PSI), median (min-max)	78 (50-115)
Total IQ, median (min-max)	67 (41-94)

Significant associations	
WMI and log10 of total organophosphate exposure index in kg of a.i during prenatal window, adjusted beta	-3.2
WMI and log 10 of chlorpyrifos exposure index in kg of a.i during prenatal window, adjusted beta	-3.5
WMI and log 10 of methamidophos exposure index in kg of a.i during prenatal window, adjusted beta	-3.5
VCI and log 10 of methamidophos exposure index in kg of a.i during prenatal window, adjusted beta	-3.8
Total IQ log 10 of methamidophos exposure index in kg of a.i during prenatal window, adjusted beta	-3.3
PRI and log 10 of pyrethroids exposure index in hours during the first year of life, adjusted beta	-1.1

and

Behavioral scales of CTRS-R:S	
Oppositional, median (min-max)	3.0 (0-15)
Cognitive problems/ Inattention, median (min-max)	4.0 (0-15)
Hyperactivity, median (min-max)	6.0 (0-21)
ADHD Index, median (min-max)	12.0 (0-34)

Significant associations	
Hyperactivity and log 10 of pyrethroids exposure index in hours during the first year of life, adjusted beta	1.6
Hyperactivity and log 10 of pyrethroids exposure index in hours from age older than 1, adjusted beta	2.1
ADHD index log 10 of pyrethroids exposure index in hours during the first year of life, adjusted beta	2.2

Models for cognitive indices (WISC IV indices scores) are adjusted by mothers' years of education, children's school grade, and log 10 of indices of use of pyrethroids or organophosphate for the respective time windows (negative coefficientes indicate worse performance).

Models for CTRS-R:S are adjusted by children's age, gender and log 10 indices of use of pyrethroids or organophosphate for the respective time windows (positive coefficients indicate worse performace).

Figure 2. Associations between long-term pesticide exposure indices and cognitive and behavioral performance among Nicaraguan school children.

Urinary pesticides metabolites	
Urinary 3,5,6-Trichloro-2-pyridinol (TCPY) median (min; max), μg/ g creatinine of children repeated samples	3.3 (0.5; 594.9)
Urinary 3-phenoxybenzoic acid (3-PBA) median (min; max), μg/ g creatinine of children repeated samples	3.2 (3.2; 243.9)

and

Cognitive sub-test	
Similarities, median (min-max)	3.5 (0-10)
Vocabulary, median (min-max)	20 (13-39)
Matrix reasoning, median (min-max)	5 (0-13)
Picture completion, median (min-max)	7.0 (2-16)
Coding, median (min-max)	26 (1-59)
Symbol search, median (min-max)	9.0 (0-28)
Digit span, median (min-max)	11.0 (6-15)
Letter-number sequencing, median (min-max)	9.0 (6-13)

Significant associations	
Letter-number sequencing and log 10 of TCPY, adjusted beta	-1.0
Letter-number sequencing and log 10 of 3-PBA, adjusted beta	-4.1
Vocabulary and log 10 of 3-PBA, adjusted beta	-2.9
Picture completion and log of 10 3-PBA, adjusted beta	-2.9

and

Behavioral scales	
Oppositional, median (min-max)	0 (0-15)
Cognitive problems/ Inattention, median (min-max)	0 (0-15)
Hyperactivity, median (min-max)	4 (0-21)
ADHD Index, median (min-max)	11 (0-36)

Significant associations	
Hyperactivity and log 10 of 3-PBA, adjusted beta	6.5
ADHD index and log 10 of 3-PBA, adjusted beta	1.6
ADHD index and log 10 of 3-PBA, adjusted beta (girls)	8.5

Models for sub-test of WISC IV are adejusted by mothers'years of education, children's school grade and log 10 of urinary 3,5,6 Trichloro-2-pyridinol (TCPY) or 3-phenoxy benzoic acid (3-PBA) (negative coefficients indicate worse performance).

Models for CTRS-R:S are adjusted by choldren's age, children gender and log 10 of urinary 3,5,6-Trichloro-2-2 pyridinol (TCPY) or log 10 of urinary 3-Phenoxybenzoic acid (3-PBA) (negative coefficients indicate worse performance).

All models were also adjusted by life-time long-term exposure indices

Figure 3. Associations between urinary pesticide metabolites and cognitive and behavioral performance among Nicaraguan school children.

6. General discussion

6.1 Main findings

This thesis examined the exposure to pesticide in Nicaraguan children in relation to parental pesticide use, and the occurrence of associated neurobehavioral effects, in a developing country context. In summary we reported evidence that children of agricultural families are highly exposed to pesticides and this exposure is influenced by occupational activities of fathers as well as by environmental pesticide contamination. The main cognitive effects from long-term pesticide exposure were a poorer performance in working memory in association with prenatal kg of organophosphate sprayed by fathers, specifically chlorpyrifos and methamidophos, poor performance in verbal comprehension and total IQ scores associated with prenatal kg of methamidophos, and poor performance in perceptual reasoning with hours of use of pyrethroids during the first year of life. Urinary TCPY was also, not significantly, related to working memory but it was not associated with behavioral outcomes. Urinary 3-PBA was associated negatively with a number of cognitive functions and with ADHD in girls but not in boys.

6.1.1 Children's pesticide exposure

The observed urinary levels of pesticide metabolites in children evidence that children of agricultural families in Nicaragua are highly exposed to pesticides. Although the geometric mean of TCPY were similar to the findings in other studies, the geometric mean of 3-PBA and the maximum for both TCPY and 3-PBA are the highest reported in the international literature for children (Figure 4). Comparison with pesticide levels in other studies that have evaluated cognitive and/or behavioral outcomes was difficult, because these studies used other pesticide biomarkers, such as urinary dialkylphosphates (Guodong et al., 2012; Bouchard et al., 2011; Engel et al., 2007), chlorpyrifos in umbilical cord blood (Rauh et al., 2012, 2011, 2006) or specific pesticides measured in maternal blood or meconium (Ostrea Jr et al., 2011; Horton et al., 2011).

The long-term exposure indices cannot be contrasted with other studies because the only previous study that has utilized data of parents' pesticide use collected with ICBF as a proxy of children's exposures, did not publish quantitative summaries of pesticide use and only reported the association between these data and childhood leukemia (Monge, 2006).

It seemed that pesticides exposure in children from agricultural areas is influenced by fathers' occupation. A take-home pathway was evidenced by the correlation observed between TCPY levels in adult and child samples taken in the evening of the chlorpyrifos' application day reported (Paper I) and the increasing urinary levels of pesticide metabolites on the days following parental application (Paper II). These results are consistent with other reports of take-home (Gomes et al., 1999, Simcox et al., 1995, Bradman et al., 1997, Loewenherz et al.,1999, Fenske et al., 2000, Lu et al., 2000, Mc Couley et al., 2001). However, it is evident that other pathways besides the take-home occurred in our study population. For example, the highest levels of urinary pesticides metabolites were mostly unrelated to any pesticide application by the parents suggesting the possibility of pesticides drifting from neighboring plantations or small cultivated fields of neighbors, as well as domestic pesticide use.

The parallel qualitative study in the same agricultural area where the data collection for this thesis was conducted, mentioned previously in section 2, identified through interviews and focus group discussions important pathways of children's exposure (Rojas et al., 2009). The pathway of environmental exposures appeared in association with pesticide applications in the proximity of houses and schools, in particular due to the drift of pesticide used in peanut plantations. Take-home pathway was also important in relation to the parents' behavior after pesticide applications such as getting home with their contaminated clothes, tools and application equipment, the use of leftovers of agricultural pesticides for domestic pest control, the storage of pesticides inside their homes, and the inadequate disposal and/or re-use of empty pesticide containers. Direct manipulation of pesticides by the child also occurred, which was gender-differentiated. Boys helped their fathers in the fields with pesticide application. Girls helped their mothers in cleaning activities at home, doing laundry of contaminated work clothes, and with domestic pest control. The qualitative information about pathways and routes of pesticide uptake is summarized in in Table 6.

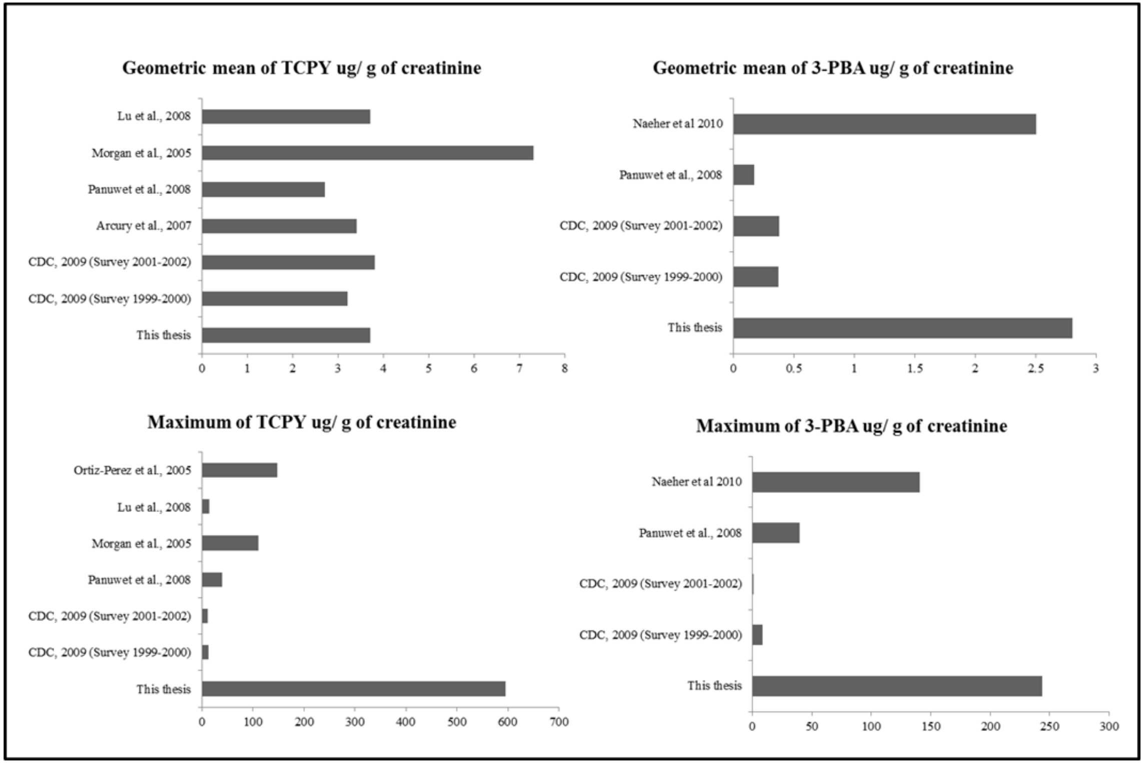

Figure 4. Comparison with other studies of the geometric means of the urinary chlorpyrifos' metabolite 3,5,6-trichloro-2-pyridinol (TCPY) and the non-specific pyrethroid metabolite 3-phenoxybenzoic acid (3-PBA.)

Table 6. Pathways of children's pesticide exposure among rural Nicaraguan children

Pathway	Source of contamination	Environmental substrate for contaminant transportation	Point of exposure	Route of exposure	Receptor population
Environmental	Pesticide application in surrounding peanut plantation Pesticide use in small farms	Air Water Dust Surfaces	In the households At school In the fields	Inhalation Dermal contact Ingestion	Boys and girls
Take-home	Domestic application of leftovers of agricultural pesticides Empty pesticide containers Pesticides stored inside home Parents' contaminated clothes	Air Domestic dust	In the households	Inhalation Dermal contact	Boys and girls Girls
Child labor	Pesticide application equipment Recently sprayed field	Air Surfaces	In the fields	Inhalation Dermal contact	Boys
	Domestic pesticide use Contaminated dust and surfaces at home Parents' contaminated clothes and shoes	Air Water Surfaces	In the households In the fields	Inhalation Dermal contact	Girls

6.1.2 Children's pesticide exposure and neurobehavioral effects

Effects from long-term exposures during vulnerable time windows

The association between organophosphate exposure and cognitive effects found in this thesis is congruent with previous studies. Decrease in working memory performance and also full scale IQ, have been reported in association with maternal urinary dialkylphosphate levels (Bouchard et al., 2011; Engel et al., 2011) and chlorpyrifos levels measured in umbilical cord blood (Rauh et al., 2011). The poor performance in perceptual reasoning observed in relation to methamidophos has also been reported in relation to maternal urinary dialkylphosphate levels (Bouchard et al 2011; Engel et al., 2011). Some additional studies have reported cognitive impairment in association with organophosphate exposure but direct comparisons are difficult because other cognitive tools were used and/or other age groups were studied (Sanchez-Lizardi et al, 2008, Eskenazi et al., 2007; Handal et al., 2008; Harari et al., 2010). However, regardless the age groups or the method for cognitive evaluation used, all findings are related with poor capacity of holding in mind and integrating information in memory.

The data presented here demonstrate an association between hours of use of pyrethroids during the first year of life and a poorer performance on perceptual reasoning. No other epidemiological studies have reported neurobehavioral toxicity in relation to pyrethroids. One study examined whether permethrin levels, measured during pregnancy in mothers' plasma and as individual airborne levels, as well as in cord blood affected children's cognition. No associations were reported with permethrin but decrease in cognitive performance was associated with piperonyl butoxide a synergist of pyrethroids insecticides (Horton et al., 2011), which could be also a proxy for pyrethroid exposures. Despite the almost total lack of epidemiologic evidence, animal studies have demonstrated neurotoxic effects of pyrethroids on the developing brain. In rats and mice exposed to pyrethroids during pregnancy and early postnatal days, hyperactivity and dose dependent decreases in learning capacity have been reported (Shafer et al., 2005; Lazarini et al., 2001; Sinha et al., 2006).

Behavioral effects

Unlike other studies, no behavioral effects in relation to pre or postnatal organophosphate exposures were detected in this study. Attention problems, attention-deficit/hyperactivity disorder problems, and pervasive developmental disorder problems have been reported in association with chlorpyrifos concentrations in umbilical cord blood (Rauh et al., 2006), children's urinary dialkyl phosphates (Bouchard et al., 2010) and maternal urinary dialkyl phosphates (Eskenazi et al., 2007; Marks et al., 2010). A possible explanation is that Conners' Behavior Rating Scale is self-reported by teachers and it is possible that some abnormal behaviors were not reported because they are tolerable within the Nicaraguan cultural context. Nonetheless, behavioral effects were observed for postnatal pyrethroid exposures. ADHD in girls but not in boys was associated with 3-PBA, in our knowledge no previous studies have examined this potential association.

6.2 Validity

In this thesis, misclassification of long-term pesticide exposure is a main concern for bias. Parents were asked to recall pesticide applications as far as 10 years back and, in consequence, it is likely that both omissions and overestimations of dose and hours of spraying occurred. Farmers reported 47 different active ingredients on the IBCF during the lifetime of their children, but there were no governmental statistics on pesticide use available to check the validity of the data obtained from the questionnaires. The use of the ICBF for quantifying children's life time exposure was extremely labor-intensive both with regard to applying the questionnaire and with systematizing the data for analyses. However, the ICBF has advantages that justify its use, since the farmers liked the method and did not get tired during the often rather prolonged interview. Overall, the recall of farmers seemed acceptable, particularly in the case of the "most used" pesticides.

The data on child exposure were derived from parental pesticide use data, which is only a proxy for their true exposures. However, the results of the assessment of children's urinary residue pre and post parental pesticide application (paper I) evidenced a reasonable relationship between fathers' pesticide use and children's exposure among farmers, also in accordance with another Nicaraguan study (Dowling et al., 2005).

In addition, child exposure was affected by air and water contamination caused by pesticide drifts, runoff and leakage from the plantations, none of which were considered in the individual exposure indices. It is likely though that environmental exposures were similar for most children, since the plantations surrounded the entire village and most dwellings were close to the border of the plantations (Rojas et al., 2009). Likewise, we did not consider applications of agricultural and domestic use pesticides in homes in the exposure indices. It remains unclear whether all these factors combined directed the bias towards above or below true exposures. On the positive site, there was no interference from recent exposures since the outcome data were collected at a moment during which no agricultural activities were taking place. In our opinion kg of a.i is a better indicator of children's exposure than hours of application, since farmers can relate directly the quantity of pesticide sprayed with the extension of cultivated area, whereas possibly some farmers report the total hours of the working day instead of hours of pesticide application. The association of cognitive and behavioral outcomes with pyrethroids exposure was reported with indices in hours of use not to lose the sub-set of peanut plantation workers who reported high pyrethroid use and for whom the data about quantities applied was not available.

Recent exposures, as assessed through urinary metabolite levels, correspond to a particular moment in time. This means that determining the contribution of recent exposure to observed neurobehavioral effects is difficult since neurological impairment from pesticides tends to be chronic in nature. One indication that urinary pesticides residues may, up to a point, be a proxy for high usual exposures was the fact that the variability of TCPY and 3-PBA levels in this study were much higher between children than within children (in the 32 with repeated measurements).

Bias may also derive from the selection of the tests for outcome assessment, which followed the recommendations of an expert workshop (Anonymous, 2006), where the expertise did not represent Nicaraguan rural conditions. It is known that the WICS-IV is highly influenced by social-cultural factors (Cronbach et al., 1990; Shuttleworth-Edwards et al., 2004), but since our outcome assessment included only participants from the same community with similar living conditions, the comparisons made within this study population are valid. Likewise, the Working Memory Index, where we found the strongest effects, has been documented as adequate for different cultures due to a low culturally acquired content need for performance on the digit span and letter-sequencing sub-tests (Wechsler, 2003; Shuttleworth-Edwards et al., 2004).

Strengths of this study worth noting are the high response rates (96% in 2007 and 100% in 2008) and the fact that all the participating families had similar socio-economic background and all children attended the same school, thereby reducing the possibility of confounding.

7. Conclusions

- The findings of this thesis demonstrated important long-term and recent pesticide exposures to highly toxic pesticides among children in an extremely poor population.
- Parental pesticide use influenced children's exposure but environmental contamination was also an important contributor to total children's exposure.
- Prenatal as well as recent exposure to organophosphates influenced negatively children's cognitive performance, in particular in relation to working memory, and total IQ. Exposure to pyrethroids in the first year of life influenced negatively children's performance on perceptual reasoning, and increased hyperactivity and ADHD scales of behavior.
- Recent exposures to pyrethroids also seemed to influence children's neurobehavioral functioning.

8. Implications

Given that almost half of Nicaraguan population lives in agricultural areas and the extensive use of pesticides for agricultural pest control, a large number of children are potentially exposed to developmental neurotoxic substances. Subsequently, the potential health effects in children are a major public health concern. This thesis adds to the growing body of evidence that exposure to pesticides early in the life, may result in cognitive and behavioral deficits, these will negatively affect future achievements of the already socially disadvantaged children of this study.

Although agricultural workers received educational training through governmental institutions and some non-governmental organism, the health topics are usually around occupational acute poisoning prevention. It is necessary educational plans for reduce risky behaviors that increase the contact the whole family to the pesticides. On the other hand, increased efforts aimed at reducing pesticide are mandatory. The findings of this study should lead to regulatory action by the Nicaraguan government as well as international agencies to promote alternatives for pest control eliminating the risk of children pesticide exposure at source.

This thesis is the only one that associated neurobehavioral outcomes with long-term exposure in children. Although, a clear gap in the contribution of environmental contamination and other potential sources for children's contact with pesticides exist, relevant associations were noted. Further research is necessary in order to refine the use of these indices for outcome assessment and more studies are necessary in order to explore potential effects in other organs and system highly vulnerable during the childhood such us endocrine and immune system.

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I come from the so-called third world. Which is the second?

Vengo del llamado tercer mundo. ¿Cuál es el segundo?

Isabel Allende

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"La única manera de detener el uso de venenos,
es si otras fuentes de empleos existan
porque sin estas,
si no nos morimos por el veneno, nos vamos a morir de hambre"

Una madre de Los Zanjones, Posoltega, Nicaragua

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