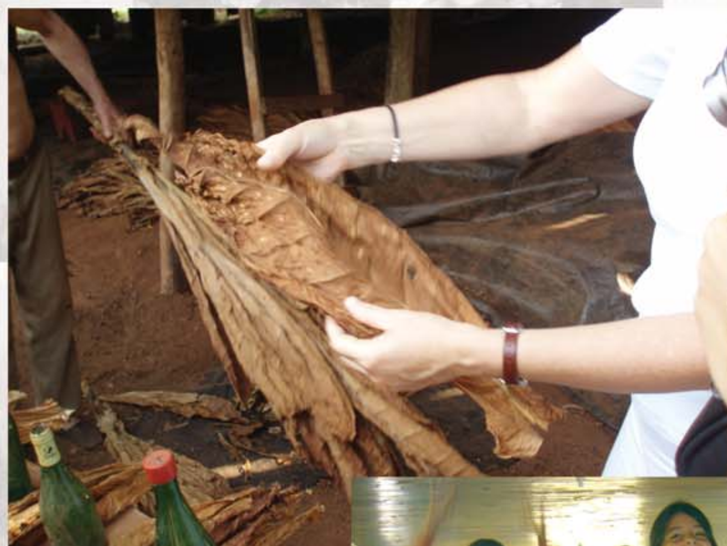
Children's Environmental Health in Argentina:

Evaluation of Organophosphate Pesticide Exposure in the Children of Tobacco Growers



Canadian Institute of Child Health
Institut canadien de la santé infantile

Sociedad Argentina de Pediatría
Por un niño sano en un mundo mejor

Asociación Argentina de Médicos por el Medio Ambiente, AAMMA

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Evaluation of Organophosphate Pesticide Exposure in the Children of Tobacco Growers

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Acknowledgements

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Children's Environmental Health in Argentina:
Evaluation of Organophosphate Pesticide Exposure in the Children of Tobacco Growers

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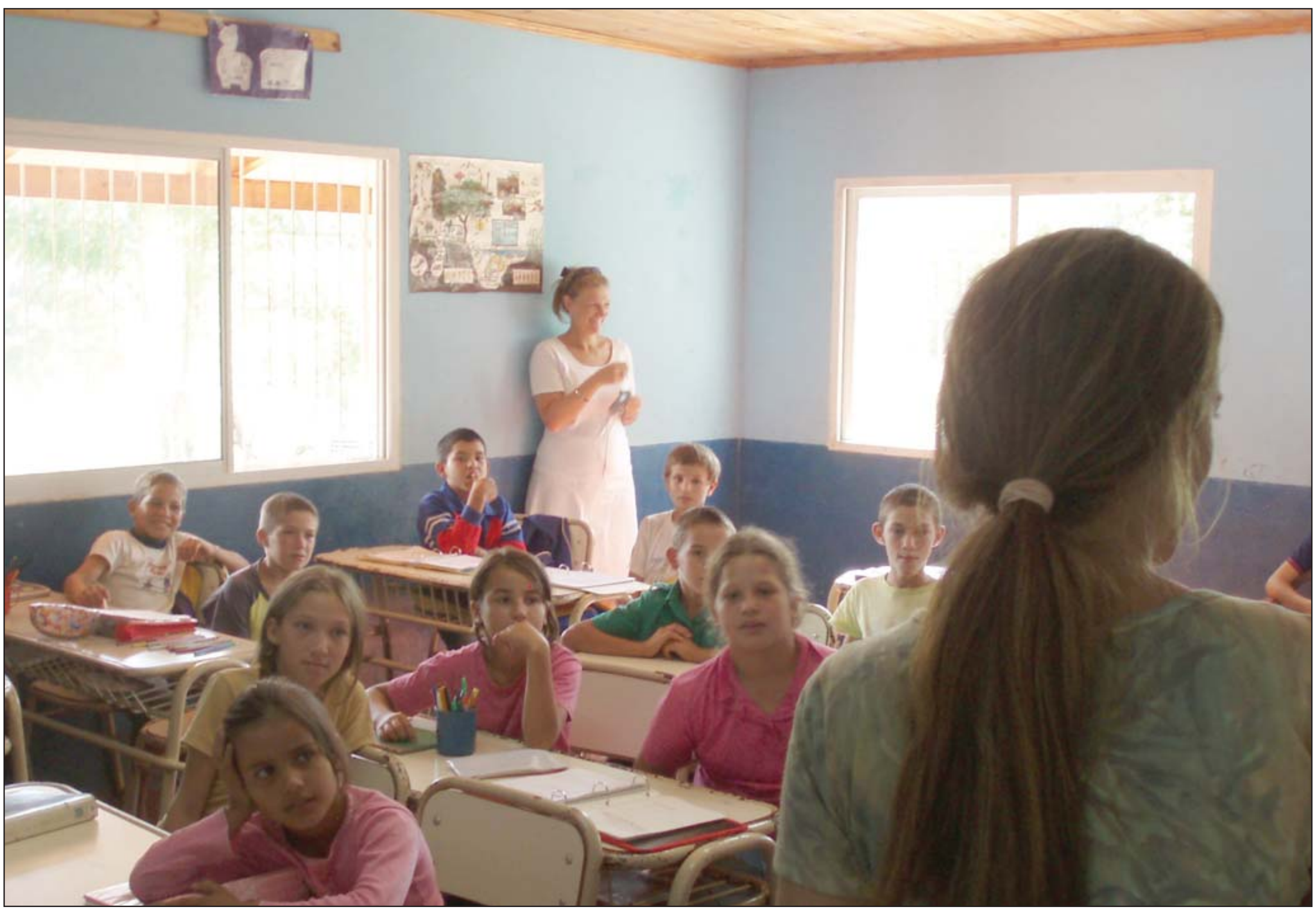
Children's Environmental Health in Argentina: Evaluation Of Organophosphate Pesticide Exposure In The Children Of Tobacco Growers is one of several activities that took place between 2004–2007 as part of a larger project entitled: *Measuring the Environmental Impact on Children's Health in the Southern Cone*. The main activities of the project included: a survey of 14,000 pediatricians, coordination of two case studies (one examining lead exposure in children and the other examining pesticide exposure in children); and, the development of *The Profile of Children's Environmental Health in Argentina*.

The Measuring the Environmental Impact on Children's Health in the Southern Cone Project is coordinated by a Steering Committee consisting of Canadian and Argentinean partners. In Canada the partners consist of: Health Canada; the University of Ottawa and the Canadian Institute of Child Health (CICH). In Argentina, the partners consist of: the Ministry of Health and Environment of Argentina, the Argentine Society of Paediatrics (SAP) and the Asociación Argentina de Médicos por el Medio Ambiente (AAMMA).



1.0 Introduction

1.1 Pesticides — Background

In the second half of the 20th century, synthetic chemical products have assisted progress in many areas, in particular; construction, transportation, communications and the production of goods and food[1]. Although the chemical products have contributed to society, their use can have risks. Chemical products are associated more and more with acute and chronic poisonings in all age groups resulting in negative effects for the environment and health[2].

Tens of thousands of synthetic chemical substances are currently available in the market, with many produced in high volumes (High Volume Production: HVP). It is conservatively estimated that over 5,000 chemical products are produced in high volumes[3] with the actual numbers considered much larger. Many of these products do not have information about safety or associated dangers, either because it has not been included in the packaging or because it is not available to the public[4].

Agrochemicals such as pesticides and fertilizers are products used throughout the world that have allowed for increased crop yield and consequently an increase in food production. Pesticides are used in agriculture, vector control programs, homes, gardens, schools, hospitals, companies, parks, clubs, as components in different industrial products, among others. According to the United Nations Environmental Program (UNEP) "*Pesticides are designed to kill, reduce or repel insects, weeds, rodents, fungi and other organisms that can threaten public health and the economies of nations. When these chemical products are mismanaged or are improperly handled they can affect human health.*"[5]

The incidence of pesticide poisonings is significant in developing countries and includes, among others, the accidental exposure of children, occupational exposure of young agricultural workers and exposure to outdated and inadequately stored pesticides. In the case of certain pesticides, chronic exposure to low doses can result in health effects including nervous system alterations, immune system deficiencies and cancer[1].

The consequences of pesticide exposure on the health of humans depends on numerous factors, including the type of pesticide and its toxicity, quantity or dose, duration, time and conditions of exposure. Several epidemiology studies have established statistical correlations between pesticide low dose exposure in the prenatal stage and increases in the number of spontaneous abortions, congenital malformations, child cancers and neurodevelopmental alternations. There is also concern over the potential links between pesticide exposure and immune system or endocrine function alterations. Comparisons of epidemiology studies that examine these linkages are limited due to different methods for determining exposure and lack of specificity in classification of resulting health effects.[6]

Once released into the environment, pesticides can be found in surface and underground water, air, soil and food products. Human exposure occurs while breathing, drinking, eating and through cutaneous absorption. UNEP states that "*The main risks of chronic low dose exposure to pesticides, linked to human health are related to cancer incidence, birth defects, nervous system and endocrine function alterations. However, the contribution of pesticides to the development of chronic diseases is unknown.*"[7] For this reason, it is necessary to conduct research studies that evaluate the long-term toxicity of chemical exposure, particularly chronic low dose exposure.

It is important to understand the benefits and risks related to the use of chemical products and achieve a balance. Specifically, it is imperative that safer options are promoted so that chemical products are safely used or even better, replaced by alternatives that protect health and promote sustainable development[1].

Pesticides are responsible for millions of acute poisoning cases every year of which at least one million require hospitalization. UNEP's Chemical

Products Division considers as "important" the number of children involved in these cases. Also, UNEP states that one to three out of every 100 rural workers across the world suffers acute[8] pesticide poisoning symptoms. Adolescents are often the victims of these acute cases[9]. Developing countries are the largest users of pesticides, consuming 75% of the world's production. In these countries, exposure to pesticides is of special concern since many of the people exposed to chemicals, especially children, already have their health compromised by other factors such as malnutrition[10].

This problem is accentuated by the irresponsible handling of agrochemicals and their containers (fertilizers as well as pesticides). In Argentina, agrochemical containers are produced, become obsolete and are disposed of inadequately. In many cases the containers are reused causing further exposures and when washed, the residues can cause contamination to water and soil.

Additionally, workers and their families are often directly or indirectly exposed to chemicals because protection standards recommended by manufacturers are not always followed when these products are used. It is normal for entire families to work together on agricultural tasks, including pregnant women and children. For this reason, exposure can begin very early in life or even prenatally.

Poverty, malnutrition and stress can increase the vulnerability of children to environmental exposures. Directly, these factors can exacerbate adverse health effects; indirectly, they perpetuate the poverty cycle leading to further degradation of the environment and further adverse health outcomes. Children represent the future of our society; protecting their health and maternal health by providing safe environments will allow them to reach their full potential.

1.2 Background information on tobacco cultivation in the Province of Misiones

Argentina, similar to other developing countries, faces severe problems caused by the intensive and extensive use of agrochemicals. The economy is based on agricultural activities and in 2006, more than 236 million kilolitres[11] of agrochemicals were sold in the country. This record amount for 2006 is a result of the continuous increase in agrochemical sales over the last decade.

Due to the wide variety of climates within Argentina, different crops are favoured over others in certain areas. Since the 80's, tobacco production has become very important in the Northern Provinces of Argentina, mainly in the Province of Misiones and there has been a growing external demand for this product.

According to data from the Tobacco Census[12], the exact number of registered and returning tobacco producers in 2004–2005 was 16,598. Tobacco producers, for the most part, are proprietors or occupants of small tracts of land and in general all members of the family work in the production. The average size of tobacco plantations, according to the 2001 National Census, was 17.3 hectares[13] with a total of 26,800 hectares used for this purpose.

In 2004–2005, when this study was conducted, three types of tobacco were planted in Misiones: Burley, Creole and to a lesser extent, Virginia. The first of these types is the most important as far as production value and quantity of producers that are growing it. There were 14,122 Burley growers in 2001–2002[12].Tobacco plantations tend to use a significant amount of agrochemicals. The most commonly applied are acaricides, fertilizers, insecticides, fungicides, bud inhibitors and nematicides.

Tobacco manufacturing companies work by means of contracts with small producers, financing all necessary supplies until harvest including: materials for the construction of storage facilities; seeds; and agrochemicals (fertilizers, insecticides and others).

Growers are committed to incurring these up-front costs (which they must pay back) and selling their product to the company. As part of this relationship, growers assume all risks for the success of the crop. Companies control the production process in its entirety through the sale of supplies, permanent supervision of the work process through instructors (each assist between 150 and 200 producers), price fixing and terms of delivery.

This type of agriculture demands that year after year chemical applications are repeated in connection with different growth stages from sowing until harvest. In the month of July, when seedlings are prepared, herbicides, insecticides and fungicides are used. In September, plants are transplanted to the field with insecticides, nematicides and acaricides used at this stage. Once in the field, these chemical products are applied regularly. In November, to avoid flowering and excessive budding, large quantities of insecticides are re-applied up until the harvest (December).

The cultivation of tobacco in this region requires the entire family be involved in much of the work. Children participate primarily during transplantation and harvest, both times when manpower demand is greatest. They also enter the fields to add fertilizers, eliminate overgrowths (weeds) and monitor growth.

Agrochemicals are generally applied by adults but on some occasions, older children participate in this activity. Often pesticide application is done without appropriate protection and under unfavorable weather conditions such as high temperatures and humidity.

In most of the plantations, the production area is not well differentiated from the place of residence. As the different stages of the cultivation process demand attention and permanent care, some production activities are conducted only a few meters from the family home. For example, during the preparation and care of seedlings.

Pesticide use related to tobacco growing presents a particularly high environmental and occupational risk in Misiones for the following reasons: the application of pesticides is done manually with workers in close contact with the chemicals (backpacks) and with little protection from exposure; extensive pesticide application is carried out at several times in one year and must be repeated every year; production activities are often located only a few meters from the family home; all family members, including pregnant women and children are directly or indirectly involved in activities using pesticides.

In 2004, when the study began, organophosphate wide spectrum insecticides, were the most frequently used pesticides for the production of tobacco leaves. Organophosphate compounds are among the most toxic insecticides available.

The physical-chemical characteristics of organophosphates (liposolubility and volatility) allow for quick entry into the organism through inhalation, ingestion and dermal absorption. The symptoms and signs of acute poisoning varies according to the dose and age of exposure; from migraines, dizziness, nausea, abdominal pain, diarrhea, anxiety and restlessness to more severe symptoms such as weakness, hypersecretion (sweating, salivation, rhinorrhea, bronchorrea), profuse diarrhea, blurred vision, confusion, vertigo, convulsions, coma, breathing difficulties and even death. The effect of organophosphates on human beings is related to the irreversible inhibition of the enzyme acetylcholinesterase and the associated accumulation of the neurotransmissor acetylcholine in neuronal unions. Chronic effects related to organophosphate exposure include congenital malformations, cancer, adverse effects on reproduction and neurological damage.

In 2005, tobacco companies began encouraging modifications in agricultural practice. To facilitate this, a new "technological package" called MIPE (Integral Management for Plagues and Diseases) was developed and sold to tobacco growers. This package replaced organophosphate pesticides with "neonicotinoids pesticides". Neonicotinoid pesticides are substances derived from nicotine that have less volatility than organophosphates (this allows less cutaneous absorbtion) and cause less acute toxicity in humans.

1.3 Background information on the sample site of Colonia Aurora, Province of Misiones

This study was conducted in the Municipality of Colonia Aurora, Department 25 of May (See Figure 1). The total population of the Municipality in 2001 was 8,776[14]. During 2001–2002 the number of tobacco growers in the Municipality was 1,273. For that same period, the number of family members working in tobacco plantations was 4,721.

44.2% of the Municipality's population possessed some or several characteristics classified as unsatisfied basic necessities[15]. A great proportion of the population also had incomplete primary education (62.9%) and 15.3% were illiterate. 86.6% of dwellings in the Municipality were type "B", presenting at least one of the following characteristics: no water access (pipes) inside the home, no flushing toilet, soil or other material used for flooring not including ceramic, tile, mosaic, wood, carpets, plastic, cement or fixed brick[16].

2.0 Objective

To determine the extent of exposure to organophosphate pesticides in the children of tobacco growers from the Municipality of Colonia Aurora, Province of Misiones, by comparing levels of biomarkers for pesticide exposure in the same individuals during periods of high and low pesticide use.

3.0 Sample population, materials and methods

This descriptive longitudinal study was conducted from July 2004 to December 2005 in El Progreso, Alicia Alta and Alicia Baja, all belonging to the Municipality of Colonia Aurora, Department 25 of May, Province of Misiones.

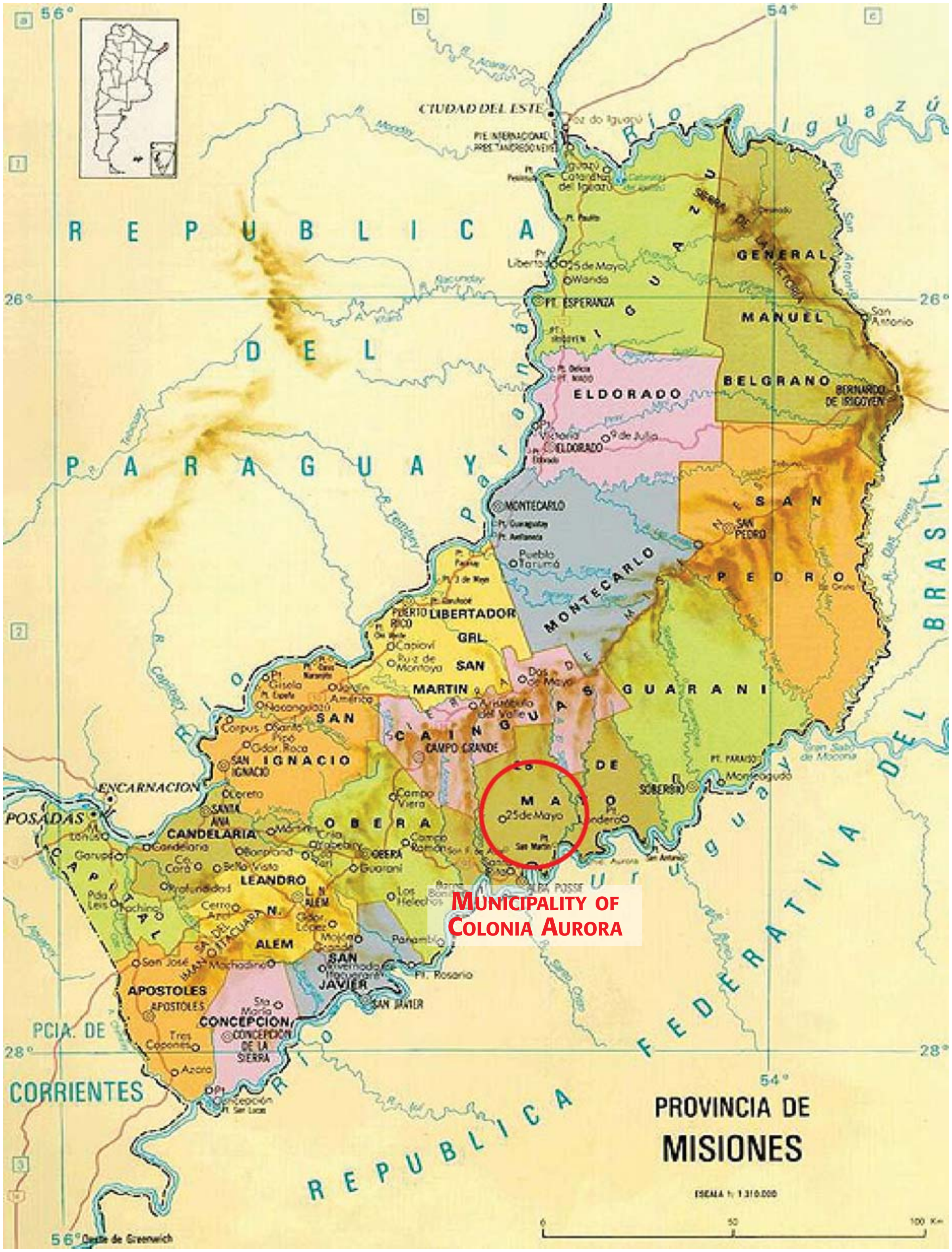
Participants in the study were children of tobacco growers, under 18 years of age that were living with their relatives in plantations located in the Municipality of Colonia Aurora. They were invited to participate through radio programs (local radio stations from Colonia Aurora, Alicia Alta and 25 of May), explanatory meetings and written information given to students that attended schools in the area. From those children who accepted the invitation, two convenient and non-random samples were developed. Participation in the study was voluntary, anonymous and free of charge.

Parents or guardians gave written informed consent and also provided answers to a questionnaire distributed at the time of blood collection. Children were excluded from the study if they were diagnosed with acute pesticide poisoning or had shown clinical symptoms of acute pesticide poisoning within 150 days. Children were excluded from the study if they were diagnosed with acute pesticide poisoning or had shown clinical symptoms of acute pesticide poisoning within 150 days prior to sampling, were dignosed with hepatic disease that could affect levels of cholinesterases, suffered from accute malnutrition, were pregnant or if the children refused at any stage of the study including during blood sampling.

Most children that participated came from homes with one or more unsatisfied basic necessities (UBN): unsafe housing; without a functioning bathroom; over-crowding; incomplete schooling of the parents; and families with less than subsistence level income. In general, homes lacked sewage systems, access to drinking water was restricted and waste collection services were not available.

The study was approved by the Ethics Committees of the National University of Misiones (Argentina) and University of Ottawa (Canada). Confidentiality of children's personal data was ensured through the Personal Data Protection Law 25.326[18]. The National University of Misiones took the responsibility of protecting and handling the collected information. Personal information was coded so that children were not identifiable by name.

Figure 1[17]: Map of Misiones, showing the Location of the Municipality of Colonia Aurora, Department 25 of May.



Source: Gobierno de la Provincia de Misiones, http://www.misiones.gov.ar/misiones/mapas/index.htm

3.1 Study Development

3.1.1 Information prior to the study stage

Prior to the study and before each sampling time, information meetings were conducted in schools from the Municipality of Colonia Aurora. Students, parents, administrative and educational staff, and doctors from the Colonia Aurora and Alicia Baja Health Centers participated in the meetings and supported the project.

At the meetings, information was provided on the importance of pesticide exposure generally and organophosphate exposure particularly, including the ways in which this can occur, potential health effects, special vulnerability of children, prevention and treatment options in case of acute poisoning. Also, information was provided on the characteristics of the study, the reasons for blood sampling twice and the way in which the sample was to be obtained. Students were invited to participate and parents were informed of the need to sign, after careful reading, the informed consent form and respond to the questionnaire.

As part of the meetings, students, parents and teachers were invited to ask questions to clarify any information they were given. Also, through the most popular radio networks, the population was informed about the study as well as the risk of pesticide exposure. All communications focused on informing without alarming.

3.1.2 Sampling time frames

Based on the usual cycle of pesticide application over the production year, two different months were chosen to represent high and low exposure times. The low exposure testing was done in May (when organophosphate pesticides were not used) and high exposure testing was done in October (when organophosphate pesticides are used).

Children joined the study in two stages. The first group joined in October 2004 and the second in May 2005. Children were categorized according to the high/low exposure time period.

Group A consisted of children that joined the study in October 2004. Two blood samples were taken, the first in October 2004 (high exposure) and the second in May 2005 (low exposure).

Group B consisted of children that joined the study in May 2005. Two blood samples were taken, the first in May 2005 (low exposure) and the second in October 2005 (high exposure).

Only children sampled at both high and low exposure periods were included in this analysis.

3.1.3 Data collection

The distribution and collection of questionnaires, blood sampling and presentation of results was carried out at the schools. Data on the children such as *personal data, place of residence* and *type of agricultural activity performed by relatives* was collected through the parent questionnaire. Prior to blood sampling, parents/guardians were asked about the presence of clinical signs or symptoms that could be related to pesticide exposure.

Blood sampling was conducted by professionals and students from the Exact, Chemical and Natural Sciences Faculty of the National University of Misiones. Blood samples were taken using sterile and disposable materials. At the same location, blood samples were centrifuged to separate plasma from erythrocytes. The samples were fractioned, labeled, refrigerated and appropriately treated for shipment to the laboratory of Toxicology and Legal Chemistry in Posadas. Determinations of the catalytic activity of cholinesterases were conducted the next day.

3.1.4 Release of results to parents

Results were presented to the parents 30 days after sampling, in sealed envelopes, during individual meetings with research personnel and in presence of school authorities or Health Center staff.

3.2 Biological Evaluation

Organophosphate pesticides have the capacity to irreversibly inhibit cholinesterase enzymes. For convenience and ease of analysis, the inhibition of cholinesterase enzymes was used as a marker of organophosphate exposure in this study.

The activity of two groups of isoenzymes was quantified:

Acetylcholine — hydrolase E.C. 3.1.1.7. (AChE) (Acetylcholinesterase, true Cholinesterase or Erythrocyte Acetylcholinesterase). Group of two isoenzymes located primarily in erythrocytes, nervous tissue, skeletal muscle and placenta. Their optimal substrate is acetylcholine, which is hydrolyzed specifically.

Acylcholine — acylhydrolase E.C. 3.1.1.8 (BChE) (Butyrylcholinesterase, Pseudocholinesterase or Plasma Cholinesterase). Group of several isoenzymes localized preferably in serum, plasma, brain, kidney, intestine and pancreas. It catalyzes hydrolysis with acetylcholine, but it possesses lower kinetic activity. It hydrolyzes the substrate butyrylcholine very rapidly.

Both groups of enzymes were analyzed because they possess different sensitivity to the action of organophosphate pesticides and different recovery times to the inhibition phenomenon (Table 1). The reference values for erythrocyte AChE (at 30°C) range from 7,120 to 11,760 International Units per litre of blood (IU/l)and the reference values for plasma BChE (at 30°C) range from 3650 to 9559 IU/l[19,20].

Limitations exist in the use of cholinesterases as biomarkers of exposure to organophosphates because accepted values, considered normal, vary a lot within a population and can present alterations as a result of diet or hepatic disease. For this reason it was decided to compare the values obtained, in the same individuals at both a time of high exposure and a time of low exposure.

A 15% to 29% decrease in cholinesterase activity at high exposure compared to low exposure in the same individual was considered as an indicator of organophosphate exposure. A decrease of 30% or more was considered as an indication of more serious exposure. With exposure to organophosphate pesticides producing cholinesterase inhibition levels of 60% to 70%, clear clinical signs and symptoms would be observed.[21] It was recommended that children with a 30% decrease in cholinesterase activity seek medical follow-up for 6 months.[22]

3.3 Analytical Techniques

Blood was used as the biological sample with heparin as an anticoagulant. The determination of cholinesterases was performed using a modified Ellman technique, a colorimetric assay where the rate of color production is measured spectrophotometrically (λ 405 nm). Acetycholine and butyrylcholine were used as substrates for the determination of erythrocyte AChE and plasma BChE respectively. Assays were processed in duplicate, not allowing differences greater than 10%.

3.4 Statistical Analysis

A database was created using Epi Info[23] software. The data was recorded as soon as it was received. Data was analyzed using the same software. Charts and figures were created using Microsoft Excel and Word.

TABLE 1. INTERPRETATION OF THE TYPE OF POISONING ACCORDING TO AChE AND BChE VALUES

Type of poisoning	AChE	BChE
Light or recent poisoning	Little or no decrease	Extremely decreased
Poisoning due to repeated doses (chronic exposure)	Decreased	Little or no decrease
Serious and recent poisoning	Extremely decreased	Extremely decreased

Source: World Health Organization. Field surveys of exposure to pesticidas. Standard Protocol. DOCUMENT VBC/82.1. Geneva: WHO, 1982.

The Student t-test was used to determine the significance between means by gender and age group. For comparison of mean values between low and high exposure times, where variances in samples differ, the Kruskal-Wallis test for two groups was used as equivalent to Chi Square. For both tests, a 95% or better probability ($p \leq 0.05$) was considered significant. The analysis was carried out by researchers from the National Institute of Epidemiology "Emilio Coni" and Memorial University of Newfoundland.

4.0 General results

4.1 Sample Characteristics

In October 2004, a time of high exposure to organophosphate pesticides, 60 children joined the study as part of Group A and a first blood sample was obtained. Twenty-nine were later released from the study because they were not present in May 2005 (low exposure) for the second sampling round. The total for Group A was 31.

In May 2005, a time of low exposure to organophosphate pesticides, 60 children joined the study as part of Group B and a first blood sample was obtained. Twenty-six were later released from the study because they were not present in October 2005 (high exposure) for the second sampling round. The total for Group B was 34.

The sample distribution according to gender and age is shown in Table 2.

There was no significant difference in AChE and BChE levels between females and males for Group A or Group B for either time period. There was no age related significant difference in AChE and BChE levels for either period when values are compared for children age 2 to 9 compared to age 10 or older.

4.2 Cholinesterase Levels in Blood

4.2.1 Acetylcholinesterase (AChE)

For erythrocyte acetylcholinesterase (AChE), thirty-one paired samples were analyzed from children in Group A, obtained in October 2004 and May 2005, and 34 paired samples from children in Group B, obtained in May and October 2005. Results for AChE are shown in Table 3.

Children in Group A, had a significantly lower mean AChE level during the high organophosphate exposure period compared with the mean level during the low exposure period. The mean level obtained during October 2004 was 15.3% lower than the mean from May 2005 for Group A. On the other hand, there were no differences observed in samples obtained during May 2005 and October 2005 for Group B.

4.2.2 Butyrylcholinesterase (BChE)

For plasma butyrylcholinesterase (BChE), twenty nine paired blood samples were analyzed from children in Group A, obtained in October 2004 and May 2005, and 32 paired samples from children in Group B, obtained in May and October 2005. The results for BChE are shown in Table 4

There were no statistically significant differences between BChE levels between high and low exposure times for October 2004 and May 2005 (Group A) or May 2005 to October 2005 (Group B).

Table 2. Gender and age distribution for Groups A and B

Age in years	Group A (n=31)				Group B (n=34)			
	Females	Males	Totals	%	Females	Males	Totals	%
<5	—	2	2	6.4	2	1	3	8.8
5–9	10	5	15	48.5	8	3	11	32.4
10–14	4	8	12	38.7	7	9	16	47.0
≥15	1	1	2	6.4	3	1	4	11.8
TOTALS	15	16	31	100.0	20	14	34	100.0

Table 3. Mean, median and range of AchE levels in Groups A and B, according to low or high exposure

AchE paired samples	Median IU/l	Median IU/l	Range IU/l	chi-square test*	*p*
Group A (n = 31)					
High exposure, October 2004	8845	8915	5425-11525		
Low exposure, May 2005	10439	10234	5572-15396	9.46	0.002
Group B (n = 34)					
Low exposure, May 2005	10843	10517	5279-16383		
High exposure, October 2005	10446	10117	6393-15288	0.686	0.400

** Kruskal-Wallis test for two groups equivalent to Chi Square*

Table 4. Mean, median and range of BchE levels in Groups A and B, according to low or high exposure

BchE paired samples	Median IU/l	Median IU/l	Range IU/l	chi-square test*	*p*
Group A (n = 29)					
High exposure, October 2004	6520	6530	4139-8318		
Low exposure, May 2005	6246	6185	4573-8640		ns
Group B (n = 32)					
Low exposure, May 2005	6774	6053	3852-16302		
High exposure, October 2005	6571	6342	4095-14248		ns

** Kruskal-Wallis test for two groups equivalent to Chi Square*

4.2.3 Cholinesterase inhibition

On an individual basis, organophosphate exposure can be assumed if exposure leads to a cholinesterase inhibition of 15% or more compared to the "non" exposed value. An inhibition of 30% or more indicates a more serious exposure. Table 5 shows the number and percent of children showing 15% or more and 30% or more inhibition for each cholinesterase for the two sample groups of children.

Forty-two percent of children in Group A showed 15% or more inhibition of AChE during high exposure compared to low exposure times and 32% in Group B. Three children out of 31 in Group A showed a 15% or more increase in AChE level during high exposure time. It should be noted however, that in Group B children, a total of 10 out of 34 children actually showed a 15% or more increase in AChE levels during high exposure time; almost the same number as presenting with 15% or more inhibition. Few children showed 15% or more change in BChE. None of the children reached 50% inhibition of either AChE or BChE.

Normal reference ranges for AChE and BChE have been well established[19,20] These reference ranges would normally include about 95% of the population. Any value falling below the normal reference range could be an indicator of cholinesterase inhibition and pesticide exposure. Table 6 shows the number and percent of children with cholinesterase values falling below the normal range during high and low exposure times for both groups of children.

Only a small percentage of children had cholinesterase values below the normal range for AChE and BChE at high and at low exposure times.

Table 5: Inhibition of Cholinesterases in the Two Sample Groups

	AChE		BChE	
Sample Group	15% or more inhibition at high exposure **n/total (%)**	30% or more inhibition at high exposure **n/total (%)**	15% or more inhibition at high exposure **n/total (%)**	30% or more inhibition at high exposure **n/total (%)**
Group A (Oct 04/May 05)	13/31 (42%)	6/31 (19%)	2/29 (7%)	0/29 (7%)
Group B (May 05/Oct 05)	11/34 (32%)	5/34 (15%)	3/32 (9%)	2/32 (6%)

Table 6: Cholinesterase Values below Normal Reference Range

	AChE		BChE	
Sample Group	<7120 IU/l at high exposure **n/total (%)**	<7120 IU/l at low exposure **n/total (%)**	<3650 IU/ at high exposure **n/total (%)**	<3650 IU/ at low exposure **n/total (%)**
Group A (Oct 04/May 05)	2/31 (6%)	2/31 (6%)	2/29 (7%)	0/31 (0%)
Group B (May 05/Oct 05)	1/34 (3%)	2/34 (6%)	1/33 (3%)	1/33 (3%)

5.0 Discussion

Children in Group A showed significantly higher level of AChE inhibition at high exposure time compared to Group B. There were no statistically significant differences with respect to AChE inhibition between the samples taken during periods of high and low exposure in Group B participants. As far as BChE, there were no statistically significant differences with respect to periods of high and low exposure in either Group.

5.1 AChE Compared to BChE as Indicator of Pesticide Exposure

In general, BChE provided little information in this study compared to AChE when results are compared between high and low exposure time. As indicated in Figure 2, BChE is most useful in indicating acute exposure whether light or extreme. AChE is most useful in indicating chronic exposure. These results suggest that we are dealing with repeated small dose exposure rather than acute in these children.

5.2 Difference in Results for Children in Group A and Group B

Children in Group A showed significantly higher level of AChE inhibition at high exposure time compared to Group B. Also, while Group A and Group B children showed similar percentages of children with 15% or more AChE inhibition at high exposure, Group B children also showed a relatively high number of children demonstrating 15% or more increase in AChE values at high exposure (reverse of what would be expected from pesticide exposure).

Children in Group A were sampled in October 2004 during a time of high pesticide application followed by sampling in May 2005 during a time of relatively low pesticide use. It should be noted, that information through radio programming, informative community meetings and education of parents/guardians regarding pesticide exposure and responsible use had just started at or near the initial sample time of October 2004. Also, for Group A, the first sample was taken during a time of high exposure. On-the-other-hand, children in Group B were first sampled at a time of low exposure in May 2005, 6 months after increased education and awareness in the community. For Group B children, the sample at high exposure time (October 2005) was a full year after community education and awareness activities had been underway. Therefore, results for Group B children showing less cholinesterase inhibition could be related to increased community education and awareness.

Another possible explanation for the differences between Group A and Group B could be related to changes in agricultural practices. In 2005, tobacco companies developed and sold to tobacco growers a new "technological package" that replaced organophosphate pesticides with "neonicotinoid pesticides". Neonicotinoid pesticides are substances derived from nicotine that are less volatile and cause less acute toxicity in humans than organophosphates.

The design of this study does not allow the direct analysis of the effect of the educational component of the project or the possible impact of changing agricultural practices. However, they could both be important factors in reducing pesticide exposure in children and could serve as a basis for expanding research in this population.

6.0 Strengths and limitations

6.1 Strengths

a. Community and parent education and awareness on pesticide hazards and means of reducing exposure were an integral part of the study protocol.

b. Sampling took place in the schools which provided a familiar and comfortable environment for participating children.

c. The study had strong support from the parent/guardians, school administrators, community, Municipality of Colonia Aurora, and local health professionals.

d. Participation in this study was anonymous and free of charge which re-enforced the open relationship between researchers and the parents/guardians and children.

e. At all the stages of the study parents (or guardians) were involved through repeated community or individual meetings.

6.2 Limitations

a. Many children received blood tests at only one time period and had to be excluded from the study.

b. Participation was non-random and voluntary which could create bias. The fact that parents/guardians wanted their children to be investigated could mean that they suspected probable pesticide exposure.

c. To obtain the strongest evidence for pesticide exposure, blood samples needed to be taken at times of high pesticide exposure and times with little or no pesticide exposure. However, children in this community may be chronically exposed to pesticides (home, school and community), even during times of low pesticide use. This reduced the statistical strength of comparisons made between the two sample time periods.

d. Although the children in this study acted as their own control, there was not a separate unexposed control group in which to compare results.

7.0 Conclusions and recommendations

7.1 Conclusions

- More than one third of children in this study showed a 15% or greater inhibition of AChE, a biomarker for chronic organophosphate pesticide exposure, during high pesticide exposure time compared to low exposure.
- Few children showed differences in either AChE or BChE of 30% or more.
- Few children had values during any exposure period which were below the normal reference range.

- Results suggest that children in Colonia Aurora were chronically exposed to organophosphate pesticides over the time of this study.
- Community discussions and parent/guardian education and awareness on pesticide exposure and responsible use could have contributed to a decrease in children's overall exposure to organophosphate pesticides over the time of this study.
- Changes in agriculture practices related to the increased use of neonicotinoid over organophosphate pesticides could have contributed to a decrease in children's overall exposure to organophosphate pesticides.

7.2 Recommendations

a. Community leaders in Colonia Aurora, in collaboration with education institutions and tobacco growers and processors, should continue to encourage community awareness regarding pesticide hazards, alternatives to using pesticides and means to reducing exposures.

b. Incorporate classes on children's environmental health in the educational curriculum of schools with special emphasis on issues related to pesticide exposure.

c. Increase awareness among health professionals, especially pediatricians, on prevention of acute and chronic pesticide exposures.

d. Consider the present study as a basis for developing future research in this field.

8.0 Endnotes

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