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Assessment of the Levels of Hexachlorocyclohexane in Blood Samples from Mexico

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Abstract The aim of this study was to evaluate hexachlorocyclohexane (HCH) exposure in children living in nine hot spots in four Mexican states. We analyzed HCH (α, β, and γ-isomers) in blood using gas chromatography/mass spectrometry. HCH exposure level in 261 children was assessed and approximately 75 % of the children studied had detectable levels of HCH. These levels ranged from 188 to 40,096.7 ng/g lipid. The highest mean levels were found in Lacanja (5,446.9 ng/g lipid), an indigenous community in Chiapas, Mexico. Our data indicate high exposure to HCH in children living in these communities.

Keywords Children · Hexachlorocyclohexane · Lindane · Mexico · POPs

Organochlorine pesticides (OCPs) are capable of persisting in the environment, being transported between phase media and accumulating to high levels, thus may pose a risk to human health and the environment (Xu et al. 2010). Consequently, most OCPs are designated as persistent organic pollutants (POPs). Hexachlorocyclohexane (HCH) is used collectively for the eight isomers of 1,2,3,4,5,6-hexachlorocyclohexane. The eight isomers differ in their axial-equatorial substitution pattern around the ring. These eight isomers are denoted by Greek letters (α, β, γ, δ, ε, η, and θ). The γ-isomer, also known as lindane, has the highest pesticidal activity; however, technical mixtures of all isomers have been widely used as commercial pesticides (Breivik et al. 1999). γ-HCH is used as an insecticide for fruits, vegetables, rice paddies, Christmas trees, and animals, and as a seed treatment. Medicinally, γ-HCH has been applied topically to treat individuals with lice and scabies (NARAP 2006).

There is no primary production of lindane in Mexico and no reports of historical production exist. Approximately 20 tonnes of lindane per year were imported and subsequently formulated in Mexico (NARAP 2006). Lindane was used in Mexico to protect agricultural crops and for ectoparasite control in livestock including ticks and common fly larvae (NARAP 2006). Lindane was also used in Mexico as a flea treatment in domestic animals (NARAP 2006). It was registered in Mexico for use in public health campaigns for the treatment of lice and scabies and was previously used to control scorpions but this is no longer recommended by the Ministry of Health (NARAP 2006). The extraordinary stability and persistence of lindane, together with its lipophilic properties, has led to its accumulation in the food chain and considerable health and ecotoxicological effects (Xu et al. 2010).

Since January 2005, as a precautionary initiative, pollutant release and transfer register reporting is mandatory for industry in Mexico, and it is mandatory to report the industrial release of lindane. Moreover, in June 2005, Mexico submitted a proposal to the Stockholm Convention to add lindane to Annex A for elimination (NARAP 2006). In 2009, lindane was added to Annex A of the Stockholm Convention (UNEP 2011).

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Several regional and global POP treaties and/or initiatives have been negotiated to identify POPs and develop risk management measures to reduce exposure in humans and the ecosystem to these toxic substances (Buccini 2003; Zhang et al. 2005). Among these treaties, the Stockholm Convention on POPs, which came into effect on 17 May 2004, is the most important milestone. This convention sought to determine baseline exposure to POPs in the general population (POPs 2009); however, in developing countries, exposure to these chemicals in hot spots may be an issue of public health considering its magnitude. Furthermore, taking into account the scarcity of data in children, there is an urgent need to assess exposure to POPs in this population.

Therefore, the aim of this study was to evaluate HCH exposure in children living in hot spots in Mexico.

Materials and Methods

In order to obtain a gradient of lindane exposure, nine communities were selected (Table 1, Fig. 1). The population studied lived in the following Mexican states: Chihuahua (Agua Caliente, San Juan de Dios and Morelos); Quintana, Roo (Ramonal, Allende and Sabidos); Chiapas (Lacanja and Victoria) and Oaxaca (Ventanilla). We studied a total of 261 healthy children (aged 4–12 years; Table 1) during 2006–2008. The children attending public schools at the sites were screened for study eligibility through personal interview with their parents and the children had similar ethnic and socioeconomic backgrounds. After informed consent agreements were signed by parents, a questionnaire was circulated and blood samples were obtained. The questionnaire registered characteristics such as source of drinking water, occupational history of parents, age, weight, height, exposure to medicaments, environmental tobacco smoke exposure and infectious diseases in the last month. The study was approved by the ethical committee of the School of Medicine, Universidad Autonoma de San Luis Potosi. Blood samples were drawn from the cubital vein into 10 mL vacuum tubes with heparin as anticoagulant for plasma collection. The tubes containing the blood samples were centrifuged at 3,000 rpm for 10 min and the plasma was then transferred with hexane-rinsed Pasteur pipettes to hexane-rinsed brown glass bottles. Plasma was stored at −20°C until analysis.

Table 1 Characteristics of sampled sites

Community	n	Characteristics
Agua Caliente, Chihuahua (Agua)	15	Rural community localized in an endemic malaria zone and with agriculture and livestock activities
San Juan de Dios, Chihuahua (San Juan)	39	Rural community localized in an endemic malaria zone and with agriculture and livestock activities
Morelos, Chihuahua (Morelos)	47	Rural community localized in an endemic malaria zone and with agriculture and livestock activities
Ramonal, Quintana Roo (Ramonal)	33	Rural community localized in an endemic malaria zone and with agriculture, fishing and livestock activities
Allende, Quintana Roo (Allende)	21	Rural community localized in an endemic malaria zone and with agriculture, fishing and livestock activities
Sabidos, Quintana Roo (Sabidos)	42	Rural community localized in an endemic malaria zone and with agriculture, fishing and livestock activities
Lacanja, Chiapas (Lacanja)	28	Rural community localized in an endemic malaria zone and with agriculture and livestock activities
Victoria, Chiapas (Victoria)	21	Rural community localized in an endemic malaria zone and with agriculture, fishing and livestock activities
Ventanilla, Oaxaca (Ventanilla)	15	Rural community localized in an endemic malaria zone and with agriculture, fishing and livestock activities

Quantification of HCH (α, β, and γ isomers) was performed as reported by Trejo-Acevedo et al. (2009). Briefly, a 2 mL aliquot of plasma was first extracted with a mixture of ammonium sulfate/ethanol/hexane (1:1:3), and the extract was then concentrated and cleaned up on Florisil columns. The quantification was performed using a HP 6890 gas chromatograph coupled with a HP 5973 mass spectrometer as described below. α-Hexachlorocyclohexane-C13 was used as the internal standard. A HP5-MS column, 60 m × 0.25 mm ID, 0.25-μm film thickness was used (J&W Scientific, Bellefonte, PA, USA). Column temperatures were: initial, 100°C (2 min), final, 310°C (rates: 20°C/min up to 200°C, 10.0°C/min up to 245°C, 4.0°C/min up to 280°C and 30°C/min up to 310°C). Injector temperature was 270°C operated in pulsed splitless mode. Helium was used as the carrier gas at a linear velocity of 1.0 mL/min. Under these conditions and using data of nine replicates near the lowest concentration attainable on the calibration curve, the method detection limits for α, β, and γ hexachlorocyclohexane were approximately 0.30 μg/L. For quality control, Organic Contaminants in Fortified Human Serum (National Institute of Standards and Technology (NIST) SRM 1958) was used; recovery was 95 % ± 5 % for the three isomers.

To satisfy normality criteria, the levels for lindane were logarithm-transformed. Therefore, all the results are shown

Fig. 1 Location of communities studied

Table 2 Total HCH (α, β, and γ-isomers) in blood samples of children (ng/g lipid)

Community	n	GM	SD	Min	25	50	75	Max	%Pos+
Agua	15	2,552.6	5,212.6	971.2	1,108.0	2,105.0	6,412.0	15,542.0	80
San Juan	39	1,840.5	4,114.6	580.1	1,008.0	1,478.0	3,275.0	5,911.0	69
Morelos	47	1,740.2	3,335.0	188.0	758.0	1,511.0	4,962.0	8,935.0	79
Ramonal	33	1,658.8	1,646.1	658.1	927.0	1,701.8	2,255.7	5,352.6	55
Allende	21	1,412.0	947.9	675.7	774.5	1,326.5	2,421.4	3,224.9	62
Sabidos	42	1,849.2	1,325.0	575.4	1,239.4	1,905.0	3,113.9	4,667.7	38
Lacanja	28	5,446.9*	18,450	393.9	591.0	9,467.0	34,479.0	40,096.0	100
Victoria	21	1,219.1	8,358.2	393.9	761.0	1,033.0	1,363.0	4,716.0	95
Ventanilla	15	1,907.4	1,656.9	997.6	1,513.8	1,701.3	2,066.6	5,723.0	100

GM geometric mean, *SD* standard deviation, *Max.* maximum concentration, *Min.* minimum concentration; detection limit (LOD = 0.0003 mg/kg), *n* number of blood samples analyzed

* $p < 0.05$ when compared to other communities. +% Children with detectable levels of HCH (α,β, and γ-isomers)

as geometric means. Mean levels for lindane were compared between communities, using one way analysis of variance (ANOVA), followed by Tukey's test. Jmpin Start Statistics Software 7.0 (SAS Institute) was used for all statistical analyses.

Results and Discussion

The results are shown as the sum of α, β, and γ-isomers and are represented as total HCH. Table 2 shows the HCH exposure levels in children living in the nine communities studied (α, β, and γ isomers). The levels ranged from 188 to 40,096.7 ng/g lipid and the highest mean levels were found in Lacanja (5,446.9 ng/g lipid), an indigenous community in Chiapas state (Table 2). The children living in the other eight communities had levels lower than those found in Lacanja and the levels between these communities were similar (mean levels between 1,200 and 2,500 ng/g lipid). An important finding in our study is that all the studied communities had children with detectable levels of lindane (Table 2). Moreover, approximately 75 % of the children studied had detectable levels of HCH (Table 2). The levels found in this study are similar or higher than those previously reported in children from other communities in Mexico (Trejo-Acevedo, et al. 2009). A mean of

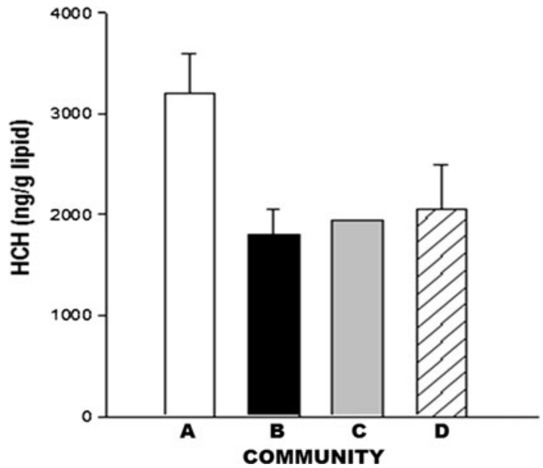

Fig. 2 Total HCH (α,β, and γ-isomers) in blood samples of children (ng/g lipid) grouped by Mexican state. *$p < 0.05$ when compared to other three communities. *A* Chiapas; *B* Quintana Roo; *C* Oaxaca; *D* Chihuahua

4,000.0 ng/g lipid of γ-HCH was found by Trejo-Acevedo et al. (2009) in nine communities in Mexico. When comparing the levels found in our work with levels found in children in NHANES III (12–19 years old), the difference was significant for children assessed in our study, as they had HCH levels approximately 300 times higher than children in the United States of America (Nhanes 2005). Levels in children in this study were also higher than children living in an urban area of Germany where a concentration of lindane of approximately <15 ng/g lipid was detected (Heudorf et al. 2003). It is important to mention that the major isomer (highest blood concentrations of the three isomers) found in our study was γ-HCH (data not shown).

When grouped by Mexican state: (a) Chiapas; (b) Quintana Roo; (c) Oaxaca and (d) Chihuahua; the highest levels of HCH (α, β, and γ isomers) were found in children living in communities in Chiapas state (Fig. 2). While, the levels in children living in the other three communities studied were similar (Fig. 2). In this regard, Trejo-acevedo et al. (2009) found γ-HCH in children living in sites with poor economic conditions. We studied four Mexican states, Chiapas is the poorest state of the four sampled. Moreover, in several other studies performed by our group, high mean levels of other contaminants such as DDE, polycyclic aromatic hydrocarbons (PAHs), among others, were found in this region (Trejo-Acevedo et al. 2009; Martínez-Salinas et al. 2010). Generally the levels of several contaminants found in Chiapas were higher than those found in other regions of Mexico. Exposure to lindane in Mexico could be due to lindane-containing shampoos which are still used for the control of scabies and lice.

A wide variety of toxicological effects are recorded for lindane and the other isomers of HCH, such as reproductive and neurological impairments (ATSDR 2011). Lindane has also demonstrated the potential to adversely affect the endocrine system in animals (ATSDR 2011). The effects of acute exposure to high concentrations of lindane range from mild skin irritation to dizziness, headaches, diarrhea, nausea, vomiting, and even convulsions and death (ATSDR 2011). Toxicological data indicate that chronic exposure to lindane at high concentrations can adversely affect the liver and nervous system of animals, and may cause cancer and possibly immune system suppression (ATSDR 2011). In this respect, it is difficult to associate a specific health effect with the levels found in the children studied. Therefore, more studies are needed in order to evaluate the health effects due to lindane exposure.

However, Mexico has agreed to eliminate all agricultural, veterinary, and pharmaceutical use of lindane through a prioritized, phase-out approach. Reasonable timeframes for a voluntary phase-out are currently being negotiated between the Federal Commission for Sanitary Risks Protection, Ministry of Health (COFEPRIS) and industry. In Mexico, lindane is currently authorized for use in livestock, as a seed treatment in six crops, as a flea treatment in domestic animals, and for public health campaigns. Lindane is also authorized for pharmaceutical use to control scabies and lice (NARAP 2006). Therefore, more biomonitoring studies are necessary in order to evaluate the phase-out of lindane in Mexico. In this regard, our group has shown the usefulness of three aspects of biomonitoring programs: (1) identification of high risk populations exposed to chemical compounds (those living in hot spots); (2) surveillance of the general population in order to prevent an increase in baseline exposure; and (3) assessment of the intervention programs developed for the reduction of exposure. Biomonitoring of toxins on a global scale can be the first step towards the prevention of toxin-induced illnesses in vulnerable populations.

Our study has some limitations such as the lack of information regarding environmental media and dietary sources. However, our data indicate high exposure levels in children living in the communities studied in this work. In order to analyze the possible sources of HCH, a comprehensive study of the environmental fate and distribution of the insecticide in the studied area must be conducted.

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