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Assessment of the Levels of Persistent Organic Pollutants and 1-Hydroxypyrene in Blood and Urine Samples from Mexican Children Living in an Endemic Malaria Area in Mexico

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Abstract The aim of this study was to evaluate the exposure levels to persistent organic pollutants and 1-hydroxypyrene in children living in an endemic malaria zone in Mexico. The blood levels for 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane (DDT), 1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethylene (DDE) and lindane ranged from 15.4 to 17,886.5 ng/g lipid, 6,624.3 to 100,119.0 ng/g lipid, and 351.1 to 6,153.8 ng/g lipid, respectively. For total polychlorinated biphenyls the blood levels ranged from 2,584.9 to 14,547.9 ng/g lipid. Regarding urinary 1-hydroxypyrene levels, the mean level was 2.9 ± 3.1 μmol/mol creatinine. In conclusion, the children in our study are exposed to levels higher than normal to mixtures of environmental contaminants.

Keywords 1-Hydroxypyrene · DDT · Lindane · PCBs · DDE · POPs

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In developing countries as Mexico, the management of toxics is inadequate and thus, humans may be exposed at levels higher than normal in mining areas (metals); agriculture fields (pesticides); industrial zones (solvents, metals, etc.); landfills (hazardous waste); dwellings (biomass combustion); etc. (Trejo-Acevedo et al. 2009). In these polluted areas, children represent the most susceptible population, and indeed, chemical-induced health effects represent a public health issue for children living in these settings. In this regard, our group has been demonstrated that in hot spots sites children are exposed to levels higher than normal to several toxins, among these POPs and PAHs (Trejo-Acevedo et al. 2009; Martínez-Salinas et al. 2010).

POPs are substances that persist in the environment, bioaccumulate through the food web, and pose a risk of causing adverse effects to human health and the environment (UNEP 2011). These are widespread and, as a result of transport mechanisms from site of their use, they have been detected even in remote locations (e.g. the Arctic area). POPs includes chemicals such as dioxins/furans, polychlorinated biphenyls (PCBs), chlorinated pesticides (as DDT and its metabolites, hexachlorocyclohexane), brominated flame retardants, and perfluorinated compounds, among others. POPs are toxic to human health, being probable human carcinogens, having immunosuppressive activity, causing neurotoxicity, increasing risk of diabetes, cardiovascular disease, and hypertension, and being endocrine disruptive chemicals (UNEP 2011). In this regard, production prohibition or stronger restrictions in the application or emission of persistent organochlorines have been necessary; this was the aim of the Stockholm Convention on POPs (UNEP 2011). In May 2007, 147 countries were parties to the Convention. México signed the Convention in May 2001 and was ratified by our country in February 2003. This convention sought to determine baseline exposures to POPs in the general population; however, in

developing countries, the exposure to these chemicals in hot spots may be an issue of public health considering its magnitude (Trejo-acevedo et al. 2009). Furthermore, taking into account the scarcity of data in children, there is an urgent need to assess the exposure of this population group to POPs.

Interesting, another factor present in developing countries located in tropical areas is the use of solid or biomass fuels to cook and to heat homes, with an estimated 3 billion people exposed to smoke from burning these fuels in their own home. Biomass combustion has been associated with different pollutants as: carbon monoxide, formaldehyde, respirable particles, Toluene, Benzene and polycyclic aromatic hydrocarbons (PAHs), among others (Martínez-Salinas et al. 2010).

Therefore, the aim of this study was to evaluate the POPs and PAHs exposure levels in children living in a malaria zone in Quintana Roo state in Southeastern Region of Mexico. The community studied was Ramonal, Quintana Roo, Mexico, a community localized on Mexico-Belize border in the Hondo river (19°23′45″N and 88°36′30″W). The community is a tropical zone and is an important area for malaria located in Southeastern Region of Mexico (Fig. 1).

Materials and Methods

All children attending primary school located in the studied community were screened for study eligibility through in-person interviews. Children who had lived in the selected area since birth and who were 6–14 years old at the time of the study were eligible to participate. The parents of the children, who participated, were informed previously about the study and all gave their written informed consent prior to their inclusion in the study. After informed consent agreements were signed by all subjects, a questionnaire was circulated and blood samples were taken. During 2006, we studied a total of 45 healthy children who were residents of Ramonal. 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 ethics committee of the School of Medicine, Universidad Autonoma de San Luis Potosí.

Blood samples were drawn from a cubital vein into 10 mL vacuum tubes with heparin as anticoagulant for plasma collection. The tubes with blood were centrifuged at 1,200*g* for 10 min. The plasma was then transferred with hexane-rinsed Pasteur pipettes to hexane rinsed brown glass bottles. Plasma was stored at −20°C until analysis. Urine samples were taken in the morning (first morning urine), collected in sealable plastic bottles and stored in a deep freezer until analysis (−20°C).

Quantification of POPs was performed as reported by Trejo-Acevedo et al. (2009). Fourteen organochlorine pesticides (α,β,γ-hexachlorocyclohexane, hexachlorobenzene, aldrin, heptachloroepoxide, oxychlordane, α,γ-chlordane, trans-nonachlor, cis-nonachlor, DDE, DDT and mirex) and fourteen PCB congeners (International Union for Pure and Applied Chemistry No. 28, 52, 99, 101, 105, 118, 128, 138, 153, 156, 170, 180, 183, 187) were quantified. 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. Quantitative analyses were performed by gas chromatography coupled with a mass spectrometer (MS). 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. MS was operated in

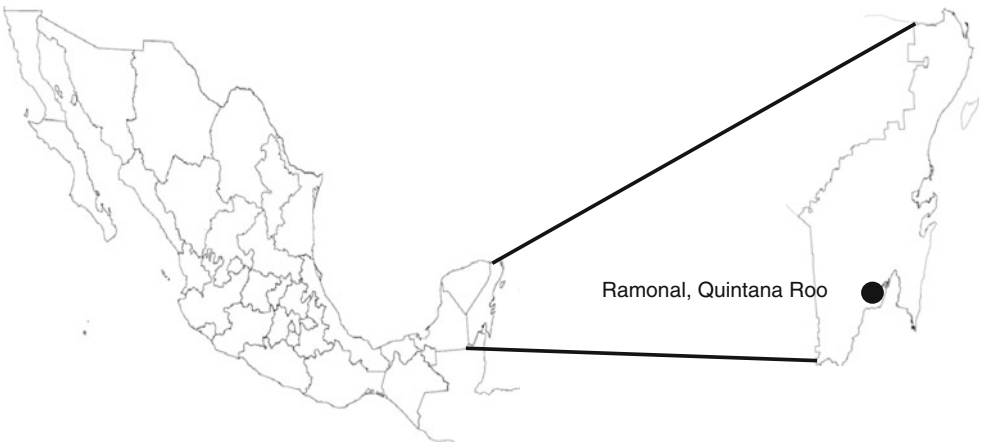

Fig. 1 Location of community studied

Selective Ion Mode (SIM). α-Hexachlorocyclohexane-C13, endrin-C13 and PCB-141-C13 were added as internal standards to all samples. Under these conditions and using data of nine replicates near the lowest concentration attainable on the calibration curve, the method detection limits for the pesticides and PCBs were approximately 0.30 μg/L. For quality control, organic contaminants in fortified human serum [National Institute of Standards and Technology (NIST) SRM 1958] were used; the recovery was 95 % ± 5 % for all compounds tested.

1-Hydroxypyrene (1-OHP) has been taken as a representative biomarker of exposure in populations exposed to PAHs' mixtures (Martínez-Salinas et al. 2010), taking into account that this compound is a pyrene metabolite, and in its turn, pyrene is often present in PAHs mixtures. 1-OHP was quantified following the method described by Martínez-Salinas et al. (2010). The analyses were performed by HPLC (HP1100, Agilent Technologies) using a fluorescence detector (G1321A). The pre-column was Zorbax SB-C18 and the column was a Zorbax Eclipse XDB-C18. The analysis temperature was set to 40°C, flow was adjusted to 1 mL/min and the injection volume was 20 μL. The eluent was 88:12 methanol: water and 1 % ascorbic acid. Data were collected and processed with HP ChemStation software. Urinary 1-OHP concentrations were adjusted by urinary creatinine. Under our conditions the method detection and quantification limits were 1.0 and 3.0 nmol/L respectively. Control quality was certified using standards: IRIS Clin Cal Recipe (Munich, Germany) 50013, 8867 and 50014 (9.1, 15.6 and 32.5 nmol/L 1-OHP), the recovery was 99 % ± 3 %.

To satisfy normality criteria the levels of POPs in plasma and 1-OHP in urine were logarithm-transformed. Therefore, all of the results are shown as geometric means. Mean levels of different POPs were compared, using one way analysis of variance (ANOVA), followed by Tukey test. For all statistical analyses we used JMP IN 7.0 software (SAS Institute, Inc., Cary, NC, USA).

Results and Discussion

Table 1 shows the results of children exposure to organochlorine pesticides. The levels for DDT ranged from 15.4 to 17,886.5 ng/g lipid, with mean levels of 2,547.8 ng/g lipid, for DDE the levels ranged between 6,624.3 and 100,119.0 ng/g lipid, with mean levels of 39,432.2 ng/g lipid. An important finding in our work is that we detected DDE in 100 % of blood samples studied and in only 70 % of blood samples were detected DDT (data no show). When compared the levels of DDT and DDE in children found in our study with levels found in other studies, we can note that were similar or higher than those previously reported in children from other communities of Chiapas (mean levels of 22,284 and 613 ng/g lipid for DDE and DDT, respectively) and Quintana Roo (mean levels of 10,767 and 2,851 ng/g lipid for DDE and DDT, respectively) in Mexico (two Mexican states located in the Southeastern Region of Mexico; Perez-Maldonado et al. 2010; Trejo-Acevedo et al. 2009). The Southeastern Region of Mexico was an important area for malaria, where DDT was applied indoors at a coverage of 2 g/m^2 every 6 months from 1957 (Perez-Maldonado et al. 2010). When comparing the levels found in this study with the levels found in children in NHANES III (12–19 years old),

Table 1 Blood concentrations of organochlorines pesticides in children

Compound	n	Mean ± SD[a]	Min–Max	PC25	PC50	PC75	PC90
HCB	45	nd	nd	nd	nd	nd	nd
Alfa HCH	45	nd	nd	nd	nd	nd	nd
Beta HCH	45	nd	nd	nd	nd	nd	nd
Lindane	45	1,273.1 ± 1,864.5	351.1–6,153.8	718.4	1,225.5	1,853.4	5,719.0
Aldrin	45	nd	nd	nd	nd	nd	nd
Heptaclor epoxy	45	nd	nd	nd	nd	nd	nd
Oxiclordane	45	nd	nd	nd	nd	nd	nd
Cis nonachlor	45	nd	nd	nd	nd	nd	nd
Clordane	45	nd	nd	nd	nd	nd	nd
DDE	45	39,432.3 ± 33,920.5*	6,624.3-143,717.1	24,350.0	39,934.0	740,636.0	100,119.0
DDT	45	2,547.8 ± 4,355.5	15.4–17,886.5	1,691.0	5,646.0	7,427.0	12,924.0
Trans nonachlor	45	nd	nd	nd	nd	nd	nd
Alpha chlordane	45	nd	nd	nd	nd	nd	nd
Mirex	45	nd	nd	nd	nd	nd	nd

Blood concentrations are shown in ng/g lipid. [a] Values are geometric means ± standard deviation (SD). *nd* non detected. Limit of detection (LOD) was approximately 0.3 μg/L for all compounds. * $p < 0.05$ when compared with all compounds

the difference is excessive for the children assessed in our study, as they had DDE levels approximately 340 times higher than children in the United States of America (NHANES III 2005).

The levels for lindane (γ-HCH) ranged from 351.1 to 6,153.8 ng/g lipid, with mean levels of 1,273.1 ng/g lipid. Moreover, in approximately 90 % of children participating in study was detected that pesticide (data no show). The levels found in our study are lower than detected by Trejo-Acevedo et al. (2009) in Puerto Madero, Chiapas, a community located in Southeastern Region of Mexico. However, when comparing the levels found in our work with levels found in children in NHANES III (12–19 years old), the difference is extreme for children assessed in our study, in NHANES III study no detectable levels of that pesticide were found in children living in United States of America (NHANES III 2005). Levels in children in this study also are higher than children living in an urban area in Germany with concentration of lindane of approximately <15 ng/g lipid (Heudorf et al. 2003). It is important to mentioning that Mexico has agreed to eliminate all agricultural, veterinary, and pharmaceutical uses of lindane through a prioritized, phase-out approach.

In Table 2, we depicted the exposure levels to PCBs in children. The mean level for total PCBs in blood was 5,892.1 ng/g lipid and the levels ranged from 2,584.9 to 14,547.9 ng/g lipid. The levels in this study are higher than levels found in different studies in Mexico (Trejo-Acevedo et al. 2009; Costilla-Salazar et al. 2011). For example, in a study performed in 2009, the total PCBs levels found in a community in Queretaro, Mexico was 1,253 ng/g lipid (Trejo-Acevedo et al. 2009). In other hand, in San Felipe Nuevo Mercurio, Zacatecas, Mexico, the mean levels found in children living in that community was 1,600 ng/g lipid (Costilla-Salazar et al. 2011). When compared PCBs levels in blood found in this study with levels reported in others studies around the world, we can note that children living in El Ramonal had higher levels (5,892.1 ng/g lipid) than children living in China (40.6 ng/g lipid; Shen et al. 2010) and children living in the Slovak Republic (352.8 ng/g lipid; Trnovec et al. 2010).

With regard to urine samples (Table 3), the 1-OHP levels found in this study (geometric mean ± SD: 3.4 ±4.7 μg/L and 2.9±3.1 μmol/mol creatinine) are higher than levels detected in children in other studies (NHANES III 2005; Schulz et al. 2009). For example, the geometric mean in

Table 2 Blood concentrations of polychlorinated byphenyls (PCBs) in children

Compound	n	Mean ± SD[a]	Min–Max	PC25	PC50	PC75	PC90
PCB 28	45	1,417.4 ± 1,626.2	153.4–5,239.9	915.5	1,757.6	3,128.8	5,239.9
PCB 52	45	nd	nd	nd	nd	nd	nd
PCB 99	45	1,347.8 ± 752.4	650.5–2,040.8	650.5	1,844.4	2,040.8	2,040.8
PCB 101	45	nd	nd	nd	nd	nd	nd
PCB 105	45	nd	nd	nd	nd	nd	nd
PCB 118	45	1,034.0 ± 661.4	587.9–2,811.3	815.4	939.3	1,237.7	2,811.3
PCB 128	45	nd	nd	nd	nd	nd	nd
PCB 138	45	nd	nd	nd	nd	nd	nd
PCB 153	45	nd	nd	nd	nd	nd	nd
PCB 156	45	nd	nd	nd	nd	nd	nd
PCB 170	45	1,215.2 ± 586.3	753.4–2,918.1	947.8	1,179.7	1,462.7	2,637.2
PCB 180	45	nd	nd	nd	nd	nd	nd
PCB 183	45	nd	nd	nd	nd	nd	nd
PCB 187	45	877.7 ± 269.4	439.7–1,537.8	845.8	845.8	1,068.4	1,388.2
Total PCBs	45	5,892.1 ± 3,895.7	2,584.9–14,547.9	4,175.0	6,566.8	8,938.4	14,117.4

Blood concentrations are shown in ng/g lipid. [a] Values are geometric means ± standard deviation (SD). *nd* non detected. Limit of detection (LOD) was approximately 0.3 μg/L for all compounds

Table 3 Urinary 1-OHP concentration in exposed children

Community	n	Mean	SD	PC25	PC50	PC75	PC90
1-OHP (μg/L)	45	3.4	4.7	0.9	3.6	5.5	12.3
1-OHP (μmol/mol creatinine)	45	2.9	3.1	1.2	3.1	4.1	13.5

Urinary 1-OHP concentrations are shown in μg/L and μmol/mol creatinine. Values are geometric means. *SD* standard deviation. Limit of detection (LOD) was 1.0 nmol/L

children aged 6–11 in NHANES III in the United States is approximately 0.05 μmol/mol creatinine (NHANES III). In Germany, a reference value of 0.5 μg/L was derived based on the representative data collection of the German Environmental Survey on children 2003–2006 (Schulz et al. 2009). German reference values are statistically derived values that indicate the upper margin (Pc95) of background exposure to a given pollutant in a given population at a given time. However, the levels found in this study are similar than found in children (mean levels ranged from 4.0 to 6.0 μg/L or from 2.6 to 4.5 μmol/mol creatinine) living in rural communities in Mexico, in those communities wood is also the principal energy source to cook and heat home (Martínez-Salinas et al. 2010).

Our study has some limitations such as the lack of information regarding other environmental media and dietary sources. However, our data indicates high exposure levels in children living in the community studied in this work. Children appeared to be particularly suitable for a monitoring program, as they are not directly exposed to occupational pollution. Thus, children normally reflect present trends of environmental exposure more accurately than adults (IPCS 2006). Moreover, the fact is well established that children are potentially at a higher risk than adults for adverse health effects from exposure to many environmental chemicals (IPCS 2006). Finally regarding health risks, it is difficult to associate a specific health effect with the levels found in the children studied. But, it is more complicated associate a health effect when the population are exposed to a chemical mixture. Therefore, more studies are needed in order to evaluate the health effects by exposure to multiple toxins. In conclusion, our results showed that in hot spots (as a malaria area) children are exposed to levels higher than normal to chemical mixtures of environmental contaminants.

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