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Maternal hair—An appropriate matrix for detecting maternal exposure to pesticides during pregnancy☆

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Abstract

The detection of exposure of pregnant women to toxicants in the environment is important because these compounds can be harmful to the health of the woman and her fetus. The aim of this study was to analyze for pesticides/herbicides in paired maternal hair and blood samples to determine the most appropriate matrix for detecting maternal exposure to these compounds. A total of 449 pregnant women were prospectively recruited at midgestation from an agricultural site in the Philippines where a preliminary survey indicated significant use at home and on the farm of the following compounds: propoxur, cyfluthrin, chlorpyrifos, cypermethrin, pretilachlor, bioallethrin, malathion, diazinon, and transfluthrin. Paired maternal hair and blood samples were obtained from each subject upon recruitment into the study (midgestation) and at birth and were analyzed for the above compounds, as well as lindane and DDT [1,1,1-trichloro-2-2-bis(*p*-chlorophenyl) ethane], and some of their known metabolites by gas chromatography/mass spectrometry. The highest exposure rate was seen for propoxur and bioallethrin and maternal hair analysis provided the highest detection rate for these two compounds, compared to blood, at both time periods: (1) At midgestation, 10.5% positive for propoxur in hair compared to 0.7% in blood ($P<0.001$) and for bioallethrin, 11.9% positive in hair compared to 0% in blood ($P\leqslant 0.001$), and (2) at birth, 11.8% positive for propoxur in hair compared to 4% in blood ($P\leqslant 0.001$) and for bioallethrin, 7.8% in hair compared to 0% in blood ($P\leqslant 0.001$). A small number of maternal hair samples were also positive for malathion, chlorpyrifos, pretilachlor, and DDT. Only a few of the pesticide metabolites were detected, principally 3-phenoxybenzoic acid, malathion monocarboxylic acid, and DDE [1,1,dichloro-2-2-bis(*p*-chlorophenyl)ethylene], and they were mostly found in maternal blood. There was a significant association between the use of the home spray pesticide, Baygon, and propoxur in maternal hair at birth ($P=0.001$) and between the use of a slow-burning mosquito coil and the presence of bioallethrin in maternal hair at midgestation and at birth ($P=0.001$, $P\leqslant 0.041$, respectively). There is significant exposure of the pregnant woman to pesticides, particularly to pesticides that are used at home. Our study demonstrates the advantages of analyzing maternal hair as a readily available biologic matrix for studying maternal exposure to toxicants in the environment, compared to blood. For propoxur, there was a 3- to 15-fold higher detection rate of the pesticide in maternal hair as compared to blood. As for the other pesticides, bioallethrin, malathion, chlorpyrifos, and DDT were exclusively found in maternal hair compared to blood. On the other hand, pesticide metabolites were infrequently found in maternal hair or maternal blood. Pesticides in blood most likely represent acute exposure, whereas pesticides in hair represent past and/or concurrent exposure. The high sensitivity, wide window of exposure, availability, and ease of hair collection are distinct advantages in using hair to detect exposure to pesticides among pregnant women. However, pesticides in maternal hair may also be secondary to passive exposure and therefore not truly representative of the internal pesticide dose. Finally, the analysis of

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maternal hair for pesticides as an index of maternal exposure to pesticides in the environment allows the institution of measures to prevent further exposure during pregnancy.
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1. Introduction

Due to widespread use of pesticides, vast quantities are released and dispersed into the environment and are found in the air, water, soil, food sources, and other biological materials (U.S. EPA, 1998). The exposure of the pregnant woman to toxicants in the environment, specifically pesticides, is harmful both to her and her fetus. The transfer of pesticides across the placenta, from the mother to the fetus, is likely governed by a number of factors that affect most xenobiotics, including the concentration gradient of the pesticide between the maternal and fetal blood, the surface area of the placenta, the thickness of the membrane barrier, and the diffusion constant, which is determined by the physicochemical characteristics of the pesticides, such as molecular weight, p*K*a (the pH at which the pesticide is 50% ionized), lipid solubility, and state of ionization (Ostrea et al., 2004). In animal and human studies, the organochlorines, being highly lipophilic, cross the placenta more readily than the other pesticides, such as parathion, chlorpyrifos, or carbamate, and with a positive correlation existing between maternal and cord blood levels (Abdel-Rahman et al., 2002; Sala et al., 2001; Abu-Qare et al., 2000; Covaci et al., 2002a, b; Waliszewski et al., 2001).

Some pesticides are neurotoxicants, and aberrations in neuronal proliferation, migration, differentiation, synaptogenesis, myelination, and apoptosis in the fetus have been described in animals and humans exposed to these compounds (Barone et al., 2000; Eriksson, 1997). Depending on the degree of maternal exposure to the toxicants, potential adverse effects in the infant may occur and these effects may be dose dependent. Gross neurologic damage has been reported in infants born to mothers who had accidentally ingested food heavily contaminated with polychlorbiphenyl (Chen et al., 1992; Rogan et al., 1988). Chromosomal abnormalities, DNA damage, and predisposition to leukemia have been observed in infants born to mothers who were antenatally exposed to pesticides (Au et al., 1999; Buckley et al., 1989; Daniels et al., 1997; Ford et al., 1998; Infante-Rivard et al., 1991, 1999; Shu et al., 1988; Ma et al., 2002). Most maternal exposures to toxicants in the environment are subtle and subclinical; however, serious concerns about their adverse effects on the fetus and the child have been raised, including developmental, learning, and behavioral difficulties, such as mental retardation, learning disability, attention deficit hyperactivity disorder, and autism (Boyle et al., 1994; California HHS, 1999; Schettler et al., 2000). Substantial evidence from animal and human data has demonstrated that a variety of chemicals commonly encountered in industry and the home can contribute to these disorders, even at low levels of exposure (Crump et al., 1998; Schantz and Bowman, 1989; Holene et al., 1998; Jacobson and Jacobson, 1990; Rosenstein and Chernoff, 1978). In one study, the carbamate, propoxur was observed to impair reflex development in the offspring of rats prenatally exposed to low levels of the pesticide (Rosenstein and Chernoff, 1978). In humans, abnormal reflexes in newborn infants, as assessed by the Brazelton Neonatal Behavioral Assessment Scale, were associated with maternal exposure to environmental organophosphates during pregnancy (Young et al., 2005).

It is therefore essential that reliable measures of exposure, particularly subclinical exposure, of pregnant women to toxicants in the environment be available to identify the women at risk and to initiate preventive measures to minimize further exposure. The aim of this study was to detect and compare pesticide exposure among pregnant women through the analysis of maternal hair and blood at midgestation and at birth.

2. Materials and methods

2.1. Study group

Pregnant women were prospectively recruited at midgestation from the Outpatient Clinic of the Provincial Hospital in Malolos, an agricultural town in the province of Bulacan, Philippines. Informed consent was obtained from the subjects and maternal blood and hair were obtained upon recruitment (Sample A) and at delivery (Sample B). Maternal hair samples about the size of a pencil eraser in diameter were obtained from the base of the scalp. The hair samples were wrapped in aluminum foil and placed in individual polyethylene bags. Blood samples were collected in 4-mL sterile Vacutainer test tubes containing EDTA. Blood samples were frozen at $-18\,^{\circ}C$ until the time of analysis and hair samples were stored at ambient temperature. The maternal blood and hair samples were analyzed for the following pesticides, which were commonly used in the study site, based on a preliminary survey: cyfluthrin, propoxur, chlorpyrifos, cypermethrin, pretilachlor, bioallethrin, malathion, diazinon, and transfluthrin. Lindane and DDT were also analyzed because our previous study in Manila, Philippines showed significant exposure to these pesticides (Ostrea et al., 2002). Some common metabolites of the pesticides were also analyzed, including 2-isopropoxyphenol [2-IPP] for propoxur, *cis*-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylic acid [*cis*-DCCA] and *trans*-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylic acid [*trans*-DCCA] for cypermethrin, 3-phenoxybenzoic acid for cyfluthrin, 3,5,6-trichloro-2-pyridinol for chlorpyrifos, malathion monocarboxylic acid [MMA] for malathion, and 1,1,dichloro-2-2-bis(*p*-chlorophenyl)ethylene [DDE] for DDT. All reagents used in the analyses were of analytical grade, including the pesticide and metabolite standards. The composition of the pesticide and metabolite standards and their purity are described by Corrion et al. (2005).

2.2. Hair analysis

2.2.1. Sample preparation and analysis for parent pesticides

The hair samples were pulverized into a fine powder in a ball mill (Retsch, Germany) using a 1.5-mL steel grinding cup with a 5-mm-diameter, steel grinding ball (Retsch, Germany). Fifty milligrams of powdered maternal hair was weighed into test tubes, and 2 mL hexane was added. Solid–liquid extraction of the pesticides was conducted for 6 h using an IKA Vibrax VXR orbital shaker (Fisher Scientific, PA). The hexane extracts were separated by centrifugation at 2900*g* for 15 min, and 1.6-mL aliquots were transferred to reactivials (Agilent Technologies, GA). The extracts were dried to completion by a gentle stream of nitrogen gas and then reconstituted to a final volume of 100 μL using hexane. Four microliters of the 16.0 $\mu g\,mL^{-1}$ 1,4-dichlorobenzene solution was added to the 100-μL concentrated extracts before the GC/MS assay. This step in the procedure is in accordance with EPA Method 8270 (U.S. EPA, 1996) but was modified to result in a 0.62 $\mu g\,mL^{-1}$ internal standard concentration.

2.2.2. Analysis of pesticide metabolites

For the analysis of metabolites in hair, 50-mg samples of powdered hair in screw-capped test tubes were spiked with 2-phenoxybenzoic acid (2-PBA, internal standard). The internal standard was also added to three spiked positive controls (46.86 $\mu g\,g^{-1}$) and to a negative control and allowed to air dry. A methanolic/hydrochloric acid methyl ester technique was used to derivatize the metabolites. This involved adding 1 mL of methanol and 1 mL of 10 N HCl to hair and heating the suspension at 80 °C for 20 min. After the mixture was cooled to room temperature in a water bath, 2 mL of toluene was added and liquid–liquid extraction of the metabolites was conducted for 20 min in an IKA Vibrax VXR orbital shaker (Fisher Scientific, PA). The toluene layer was separated by centrifugation at 2900*g* for 15 min and a 1.5-mL aliquot was transferred into a reactivial for the GC/MS assay.

2.2.3. Quality control

Mongoloid-type hair with pesticide and metabolite levels below the limits of detection was used as a blank sample. Quality control samples, composed of unspiked blank hair (negative control) and three-spiked blank hair (positive controls), were included in every batch of samples analyzed. Positive controls for the parent pesticides were spiked at a concentration of 31.25 $\mu g\,g^{-1}$ and allowed to air-dry prior to extraction. For the metabolites assay, positive controls were spiked with 2-PBA (internal standard) and pesticide metabolite mix (46.86 $\mu g\,g^{-1}$), allowed to air-dry, and then processed as described above. The mean percent recovery was calculated as the measured concentration divided by the calculated spiked concentration multiplied by 100. The interassay variation of the measured concentration was assessed by the percent coefficient of variation (%CV). This was calculated as the standard deviation of the measured concentrations ($N = 3$) divided by the mean and multiplied by 100. Batches for which the recovery did not fall within 80–120%, and/or with %CV greater than 10%, were re-analyzed.

2.2.4. Evaluation of analytical performance

Recoveries for the parent pesticides and pesticide metabolites were determined by the analysis of hair samples ($n = 24$, triplicates spiked on 8 separate days) spiked at concentrations of 31.25 and 46.86 $\mu g\,g^{-1}$, respectively. The limits of detection (LOD) for the individual parent pesticides and metabolites were determined using the empirical approach (Corrion et al., 2005). The LOD was defined as the lowest concentration, where, (a) the target (quantitation) and qualifier(s) peaks were present and (b) response ratios of quantitation versus qualifier(s) were within a $\pm 20\%$ range of uncertainty.

2.2.5. Hair-washing experiment

To assess the effect of hair washing on the levels of pesticides in hair, the following study was conducted. Subsets of maternal hair samples previously found positive for propoxur ($N = 12$) and bioallethrin ($N = 23$) were each divided into two groups. Samples comprising the unwashed group were reanalyzed. The hair-washing method was done according to the method of Nakao et al. (2002). For the washed group, 1 g of whole hair was washed with a 1:10 solution of commercial shampoo and deionized water for 5 min using an orbital shaker. Rinsing was done with deionized water until the wash water was clear of suds. Once dry, the hair was pulverized in a ball mill and then analyzed. Paired sample *t*-tests were used to determine if washing significantly reduced the concentration of propoxur and bioallethrin.

2.3. Blood analysis

Parent pesticides were extracted from whole blood by liquid–liquid extraction and, for the pesticide metabolites, the compounds were derivatized and extracted through an HCl/methanolic methyl ester derivatization following the method described by Corrion et al. (2005). Briefly, for the analysis of parent pesticides, 500 μL of sonicated whole blood was used. The pesticides were extracted using hexane and analyzed by GC/MS using 1,4-dichlorobenzene as an internal standard. For the analysis of pesticide metabolites, 500 μL of sonicated whole blood was suspended in 1 mL of phosphate buffer. The metabolites were derivatized using methanol and concentrated hydrochloric acid (10.0 N). The derivatized compounds were extracted using toluene and analyzed by GC/MS.

2.3.1. Parent pesticides

Compound separation was conducted using an Agilent gas chromatograph (Model 6890, Atlanta, GA) operating at 250 °C in splitless mode. One microliter of the concentrated hexane extract from the hair or blood sample was injected into the front inlet of the gas chromatograph. The flow of helium (carrier gas) was 1 $mL\,min^{-1}$ through a DB5-MS 5%-phenylmethylpolysiloxane capillary column (30 m × 250 μm × 1 μm, Agilent Technologies, Atlanta, GA). The oven program commenced at 70 °C and increased at a rate of 10 $°C\,min^{-1}$ to a final temperature of 300 °C, where it was held for 10 min. The total run time for the analysis was 34 min.

2.3.2. Pesticide metabolites gas chromatography

Two microliters of the toluene extract was injected into the GC front inlet set at 250 °C in the splitless mode, with helium (carrier gas) flowing at 1 $mL\,min^{-1}$. The metabolites were separated through a DB5-MS column (30 m × 250 μm × 1 μm) using an oven program starting at 100 °C and ramped at a rate of 4 $°C\,min^{-1}$ to 250 °C. This final temperature was maintained for 5 min followed by a postrun of 5 min. The total run time for the analysis was 43.5 min.

2.3.3. Mass spectrometry

Electron impact ionization and mass spectrometry were performed using a mass selective detector (MSD, Model 5973, Agilent Technologies, Atlanta, GA). To maximize sensitivity, the MSD was autotuned using perfluorotributylamine (PFTBA, tuning standard). Ionization of the analytes by electron impact (EI) was obtained using an emission current of 70 eV. Maximum abundance of pesticide and metabolite ion masses in the range of m/z 69–502, was achieved at repeller and electron multiplier voltages of 19.90 and 1318 V, respectively.

Fragment ions for the individual pesticides and metabolites were initially determined from the analysis of pure standards and matrix-spiked samples of known concentration in SCAN mode. Ions exhibiting significant abundance and stability across decreasing concentrations were chosen as either target or qualifier ions(s) for selected ion monitoring (SIM). In cases where only two predominant mass fragments exhibited stability, a single qualifier ion was selected. The target and qualifier ions for the individual parent pesticides and metabolites were cross-referenced with suggested ions from the literature.

2.3.4. Quantitation

A matrix-spiked calibration curve was used to determine the concentration of parent compounds and their selected metabolites in maternal hair and blood samples. Matrix-spiked calibrators were prepared by spiking blank hair and blood samples ($n = 3$ per concentration) with the calibration standards and then processed as described above. The range of concentration levels for the parent pesticides calibration curve was from 0.25 to 62.50 μg g^{-1} for hair and 0.10 to 25 μg mL^{-1} for blood. For the metabolites, the concentration levels ranged from 0.18 to 187.50 μg g^{-1} for hair and 0.13 to 33.33 μg mL^{-1} for blood. Calibration curves were constructed by plotting the mean response ratio (response of analyte/response of internal standard) against the mean amount ratio (amount of analyte/amount of internal standard). The target to qualifier ion(s) response ratios for the individual pesticides and metabolites were determined at each calibration level. The acceptable limits (% uncertainty) were set to $\pm 20\%$ of the mean target to qualifier ion response ratios. The limits of detection were established as determined by Corrion et al. (2005).

Quantitation of the pesticides and metabolites was done by manual integration of the quantifier and qualifier ion(s) using Chem Station software (Hewlett Packard, Version B.01.00). For the synthetic pyrethroids (cyfluthrin and cypermethrin), which exhibited three chromatographic peaks, the first eluting peak was used for quantitation due to its stability across decreasing concentrations.

2.4. Statistical analysis

Mean (standard deviation) and frequency values were calculated to describe the sociodemographic and environmental characteristics of the study population. The occurrence of pesticides in maternal hair and blood at midgestation (A) and at delivery (B) was compared using the Sign test. Pairwise agreement among the four measurements was evaluated by chi-squared tests and phi coefficients. When appropriate, i.e., when one or more cell expected values was less than 5, Fisher's exact test was used as the significance test of the phi coefficient (ϕ). The same comparisons for the concentrations of pesticides were done using the Wilcoxon matched-pairs signed rank test. Agreement among the pairs on the concentrations was assessed using Kendall's Tau (τ). The level of statistical significance was set at $p \leqslant 0.05$.

3. Results

3.1. Evaluation of analytical performance of maternal blood and hair analyses

Several classes of parent pesticides and their selected metabolites were effectively separated on the DB5-MS column using the oven programs we developed. Similar effective separation of parent compounds and metabolites were shown for blood (Corrion et al., 2005), and validation data for blood are presented in that paper. Limits of detection for blood in the current study ranged from 3.10–98.00 ng mL^{-1}. The parent pesticides and their respective classes and metabolites are listed in Tables 1 and 2, along with the target and qualifier ion(s), which were used for both matrices.

In maternal hair, matrix-spiked calibration curves were linear for all parent pesticides and pesticide metabolites with coefficients of linearity greater than 0.998. Optimum recovery rates using our 6-h hexane extraction method ranged from 87% to 112% at a spiked concentration of 31.25 μg g^{-1}. The interassay and intra-assay coefficients of variability for the analysis of parent pesticides were below 11%. Limits of detection by the empirical approach ranged from 30.50 to 488.00 ng g^{-1} hair. Recovery rate of the metabolites by liquid–liquid extraction of the acid digest ranged from 87% to 103% using a spiked concentration of 46.86 μg g^{-1}. Interassay and intra-assay coefficients of variability for the analysis of metabolites were less than 11%, with LODs in the range of 0.18–5.88 μg g^{-1}.

Table 1
Target and qualifier ions for the parent pesticides

Parent pesticides	Target ion (m/z)	Qualifier ion(s) (m/z)
Carbamate		
Propoxur	110	152
Organophosphate		
Diazinon	304	179, 137
Malathion	173	127
Chlorpyrifos	197	314,97
Organochlorine		
Lindane	181	183, 109
DDT[a]	235	237, 165
Pyrethroids		
Bioallethrin	123	79, 136
Transfluthrin	163	91, 335
Cyfluthrin	206	226
Cypermethrin	181	209
Chloroacetanilide		
Pretilachlor	238	176, 202

[a] 1,1,1-Trichloro-2-2-bis (*p*-chlorophenyl) ethane.

Table 2
Target and qualifier ions for the pesticide metabolites

Parent compound	Metabolites	Target ion (m/z)	Qualifier ion(s) (m/z)
Propoxur	2-isopropoxyphenol	110	152
Malathion	malathion monocarboxylic acid	93	159
Chlorpyrifos	3,5,6-trichloro-2-pyridinol	199	169, 107
DDT	DDE[a]	246	248, 176
Cyfluthrin	3-phenoxybenzoic acid	197	228
Cypermethrin	*cis-/trans*-DCCA[b,c]	222	187, 163

[a] 1,1-Dichloro-2-2-bis (*p*-chlorophenyl)ethylene.
[b] *cis*-3-(2,2-Dichlorovinyl)-2,2-dimethylcyclopropanecarboxylic acid.
[c] *trans*-3-(2,2-Dichlorovinyl)-2,2-dimethylcyclopropanecarboxylic acid.

3.2. Effect of preliminary hair washing on pesticide concentration in hair

Pesticides in hair have been reported to be removed from the hair surface by washing (Altshul et al., 2004; Nakao et al., 2002) and could therefore be an important factor in determining the presence and concentration of pesticides in maternal hair. There was no significant difference in the concentration of propoxur in the paired hair samples before and after washing ($P = 0.175$, Wilcoxon signed ranks test), but for bioallethrin, the concentration of the pesticide was significantly higher in the pre- compared to the postwashed hair samples ($P = 0.001$, Wilcoxon signed ranks test). However, the postwash concentration was indetectable in only one sample (4.7%. 1.27 versus $0\,\mu g\,g^{-1}$). All other samples that initially tested positive were still positive after washing. These results indicate that regular washing of the hair with shampoo can affect the concentration of bioallethrin, but not propoxur, in maternal hair.

4. Clinical study

A total of 449 pregnant women were studied with paired maternal hair (MH) and blood (MB) obtained at midgestation (A) and at delivery (B). The sociodemographic and environmental characteristics of the study population are shown in Table 3. The subjects had a mean age of 25.4 yr and a median gravidity of 2 and parity of 1. About 74% were married, 96.5% had attained at least a high school education, and 76.3% were homemakers. The average numbers of people and families per household were 5.4 and 1.6, respectively. The mean number of children under family support was 1.1 (range of 0–11) with a mean age of 3.1 yr. There were also other dependents under family support with mean number of 0.35 (range of 0–11) and mean age of 7.1 years (range of 0–84 yr). The average household monthly income was 5509 pesos (equivalent to US$100).

About 69% of the mothers lived in their own homes, although 2% were in makeshift homes. Sixty percent of the homes were classified in the low-class category (class D to E) according to a Philippine socioeconomic scale (Roberto, 1987, 2002), which ranged from Class A (highest) to class F (lowest). Approximately 74% of the homes were made of cement and wood and 5.4% of makeshift materials. About 13% of the homes were located in former transformer sites, 30% beside garbage dumps, 46% near rice fields, and 8.1% near a lead recycling plant.

The cleanliness of the home and surroundings was rated as fair. The toilet was predominantly of the water seal type (82.4%); water source was either piped in (52.6%) or well water (37.6%); waste disposal was by sewage (27.5%) or into canals (65%), and 62.5% had organized garbage collection. Most of the homes had problems with flies, roaches, and mosquitoes (89–98%). Home spray pesticide was used in 43% of the homes and the principal pesticide brand used was Baygon, which contained propoxur and cyfluthrin. A slow-burning mosquito repellant coil (Katol), which principally contained bioallethrin, was used in 55% of the households. Farm pesticides were used by 3% of subjects.

For the parent pesticides, most of the pesticides were found in maternal hair (Table 4) and consisted principally of propoxur (10.5% in MHA and 11.8% in MHB) and bioallethrin (11.9% in MHA and 7.8% in MHB). A small percentage of maternal hair was also positive for malathion (1.8% in MHA), chlorpyrifos (0.4% in MHB), pretilachlor (0.2% in MHA and MHB), and DDT (0.4% in MHA and

Table 3
Sociodemographic and environment characteristics ($N = 449$)

1. Mother	
Age—mean (sd)	25.4 (6.0) years
Gravida	2 (median)
Parity	1 (median)
Single	25.9%
Married	74.1%
Catholic	88.6%
Completed high school	69.5%
Occupation (homemaker)	76.3%
2. Father	
Mean age	28.4 (7.2)
Catholic	89.7%
Completed high school	67.8%
3. Household members	
Number of people in household	5.4 (2.9)
Number of families in household	1.6 (0.9)
Number of children under family support	1.1 (1.5) Range from 0 to 11
Mean age of children under family support	3.1 (3.7) yrs
Number of other dependents under family support	0.35 (1.0) Range from 0 to 11
Mean age of other dependents under family support	7.1 (17.5) yrs. Range 0–84 yrs
4. Mean monthly income (Philippine peso)	P 5509 (5755)
5. Socioeconomic score Roberto scale	
Class AB (highest)	6.0%
Class C (middle)	33.9%
Class D (Low)	50.1%
Class E (Very low)	10.0%
6. Home ownership	
Owns home	69.3%
Rents home	18.9%
Housing project	1.8%
Squatters	2.0%
7. Type of material of house	
Cement	14.8%
Wood	5.4%
Mixed	74.4%
Makeshift	5.4%
8. Cleanliness of house and surroundings	
Satisfactory	9.4%
Fair	73.5%
Poor	17.1%
9. Toilet facilities	
Flush	7.3%
Water seal	82.4%
Public toilet	4.9%
Pit privy	0.9%
Others	4.5%
10. Source of water	
Piped water	52.6%
Well	37.6%
Communal (artesian well)	7.7%
Others	2.1%
11. Waste disposal	
Sewage	27.5%
Canal	65.0%
Pit	7.4%

Table 3 (*continued*)

12. Organized garbage collection	62.5%
13. Location of home	
Near a former transformer site	13.2%
Near garbage dumpsite	29.6%
Near rice field	46.1%
Near lead recycling plant	8.1%
Near factory	31.3%
Battery	9.8%
Textile	37.1%
Food	12.6%
Chemical	9.1%
14. Pests at home	
Flies	92.9%
Cockroaches	89.3%
Mosquitoes	97.8%
Rats	69.2%
15. Use of home pesticide spray	43.1%
16. Brand of home pesticide spray used	
Baygon	90.0%
Raid	7.4%
Baygon water based	0.3%
Others	2.6%
17. Use of mosquito coil repellant	54.9%
18. Pesticide use on farm	3%
19. Use of gloves when handling pesticides	4.9%

0.7% in MHB). Maternal blood was only positive for propoxur (0.7% in MBA and 4.0% in MBB) and the levels were higher than those reported by Barr et al. (2002). The pesticides diazinon, lindane, transfluthrin, cyfluthrin, and cypermethrin were not detected and are not shown in Tables 4–6.

Based on the type of matrix (hair versus blood), propoxur was found at a significantly higher frequency in maternal hair compared to blood, both at midgestation (10.5% versus 0.7%, $P<0.001$) and at delivery (11.8% versus 4.0%, $P<0.001$)—see Table 5. However, there was no significant correlation between the frequency of propoxur in maternal blood versus hair either at midgestation ($\phi = -0.028$, $P = 1.000$) or at delivery ($\phi = -0.040$, $P = 0.709$). Bioallethrin was only detected in maternal hair, rather than compared to blood, both at midgestation (11.9% versus 0%. $P<0.001$) and at birth (7.8% versus 0%, $P<0.001$). Malathion was found at significantly higher frequency in maternal hair than in blood only at midgestation (1.8% versus 0%, $P = 0.008$).

Based on the timing of sample collection (midgestation versus delivery), propoxur was found at a similar frequency in maternal hair obtained at midgestation and at delivery (10.5% versus 11.8%, $P = 0.590$)—see Table 5. On the other hand, propoxur was found at a significantly lower frequency in maternal blood obtained at midgestation compared to at delivery (0.7% versus 4.0%, $P<0.001$). Bioallethrin and malathion were found at significantly higher frequency in maternal hair at midgestation compared to delivery (11.9% versus 7.8%, $P\leqslant 0.034$ and 1.8% versus 0.0%, $P\leqslant 0.008$, respectively). There was a significant correlation between the frequency of bioallethrin in maternal hair in samples obtained at midgestation and at delivery ($\phi = 0.202$, $P<0.001$).

The concentrations of the parent pesticides in positive maternal hair and blood samples are shown in Table 6. There was no significant difference in the concentration of propoxur in maternal hair at midgestation as compared to delivery ($P = 0.218$) and no significant correlation existed between the two concentrations ($\tau = 0.047$, $P = 0.294$). For bioallethrin, concentration in maternal hair was significantly higher in samples obtained at midgestation compared to delivery ($P<0.001$) and there was a significant correlation between the two concentrations ($\tau = 0.206$, $P<0.001$).

With regards to the pesticide metabolites, only a few metabolites were detected in the various matrices (Table 4) and consisted principally of 3-PBA (1.4% in MBB; concentration range = 0.300–0.310 $\mu g\,mL^{-1}$), MMA (0.2% in MHB; single sample concentration of 0.44 $\mu g\,g^{-1}$), and DDE (1.6% in MBA; concentration range = 0.270–0.720 $\mu g\,mL^{-1}$ and 0.2% in MBB; single sample concentration of 0.005 $\mu g\,mL^{-1}$).

There was a significant association between the use of the mosquito coil, Katol (which contains bioallethrin), and bioallethrin in maternal hair at midgestation ($\phi = 0.153$, $P\leqslant 0.001$) and at delivery ($\phi = 0.097$, $P = 0.041$) and a significant association between the use of Baygon insect spray (which contains propoxur and cyfluthrin) and propoxur in maternal hair at birth ($\phi = 0.153$, $P<0.001$), but not at midgestation ($\phi = 0.013$, $P = 0.787$).

5. Discussion

5.1. Hair analysis for pesticides

The detection of pesticide exposure among pregnant women is important because of the potential toxicity of these compounds and of the need to initiate measures that can prevent further exposure. A survey of the literature has shown that maternal blood, plasma, and serum (James et al., 2002; Jarrell et al., 1998; Klopov et al., 1998; Sandanger et al., 2004) and occasionally maternal urine (Berkowitz et al., 2004; Young et al., 2005) and amniotic fluid (Bradman et al., 2003; Foster et al., 2000) have been analyzed for this purpose. However, the pesticides found in these matrices are predominantly biomarkers of short-term exposure. On the other hand, hair has been analyzed to assess human exposure to organochlorines, including lindane and DDT (Dauberschmidt and Wennig, 1998; Nakao et al., 2002). It has been used to provide information on past occupational exposures of women to DDT (Covaci et al., 2002a, b) and in children to detect exposure to DDT and lindane.

There are several advantages of analyzing hair. Hair analysis is capable of detecting a wider window of exposure

Table 4
Incidence and median concentration of parent pesticides and metabolites in 449 paired maternal hair (MH) and maternal blood (MB) obtained at midgestation (A) and at birth (B)

Pesticides	MHA		MHB		MBA		MBB	
	Positive	Conc[a] (μg/g)	Positive	Conc[a] (μg/g)	Positive	Conc[a] (μg/mL)	Positive	Conc[a] (μg/mL)
Parent compound								
(1) Propoxur	10.5%	2.67	11.8%	0.58	0.7%	0.67	4.0%	0.77
(2) Malathion	1.8%	4.60	0.0%	0.0	0.0%	0.0	0.0%	0.0
(3) Chlorpyrifos	0.0%	0.0	0.4%	4.48	0.0%	0.0	0.0%	0.0
(4) Bioallethrin	11.9%	5.20	7.8%	2.40	0.0%	0.0	0.0%	0.0
(5) Pretilachlor	0.2%	2.68[b]	0.2%	2.55[b]	0.0%	0.0	0.0%	0.0
(6) DDT	0.4%	0.98	0.7%	1.22	0.0%	0.0	0.0%	0.0
Metabolites								
(1) 3-PBA	0.0%	0.0	0.0%	0.0	0.0%	0.0	1.4%	0.31
(2) DDE	0.0%	0.0	0.0%	0.0	1.6%	0.34	0.2%	0.005[b]
(3) MMA	0.0%	0.0	0.2%	0.44[b]	0.0%	0.0	0.0%	0.0

Note: 3-PBA (3-phenoxybenzoic acid); DDE (1,1,dichloro-2-2-bis(*p*-chlorophenyl)ethylene); MMA (malathion monocarboxylic acid).
[a]Median concentration of positive samples only.
[b]Only one positive sample.

Table 5
Comparison of the incidence (%) of parent pesticides detected in paired 449 paired maternal hair (MH) and blood (MB) samples obtained at midgestation (A) and at birth (B)

Parent pesticides	MHA	MHB	MBA	MBB	MHA vs MHB		MBA vs MBB		MHA vs MBA		MHB vs MBB	
					Sign test[a]	ϕ coeff[b]	Sign test[a]	ϕ coeff[b]	Sign test[a]	ϕ coeff[b]	Sign test[a]	ϕ coeff[b]
(1) Propoxur	10.5%	11.8%	0.7%	4.0%	0.590	0.032	0.001	0.123[c]	<0.001	−0.028[c]	<0.001	−0.040[c]
(2) Malathion	1.8%	0.0%	0.0%	0.0%	0.008		1.000		0.008		1.000	
(3) Chlorpyrifos	0.0%	0.4%	0.0%	0.0%	0.500		1.000		1.000		0.500	
(4) Bioallethrin	11.9%	7.8%	0.0%	0.0%	0.034	0.202[c,*]	1.000		<0.001		<0.001	
(5) Pretilachlor	0.2%	0.2%	0.0%	0.0%	1.000	−0.002[c]	1.000		1.000		1.000	
(6) DDT	0.4%	0.7%	0.0%	0.0%	1.000	−0.006[c]	1.000		0.500		0.250	

*$P<0.01$.
[a]P values from sign test.
[b]ϕ coefficient can only be calculated when pesticide is detected by both matrices.
[c]Fisher's exact test used to assess significance of ϕ coefficient.

to pesticides during pregnancy as compared to blood or urine due to the pesticide's accumulation into the growing hair shaft from the blood and with no active metabolism of pesticides in the hair (Tsatsakis et al., 1998; Tutudaki et al., 2003). Hair is also more convenient to analyze, compared to blood or other tissue, since its collection is easy and less invasive and requires simple handling and storage. However, most of the studies that have analyzed hair for pesticides have analyzed either for a single pesticide for a single class of pesticide.

5.2. Comparison of hair versus blood analysis for pesticides

This is the first study to compare the analysis of maternal hair and blood for several classes of pesticide. Whole blood, instead of serum or plasma, was used due to the high lipophilicity of pesticides, which favors the concentration of these compounds in the erythrocytes (Frenzel et al., 2000). The results of our study have shown several advantages of analyzing maternal hair for pesticides compared to whole blood. For propoxur, there was a 3- to 15-fold higher detection rate in maternal hair compared to blood. As for the other pesticides, bioallethrin, malathion, chlorpyrifos, and DDT were found exclusively in maternal hair compared to blood. On the other hand, pesticide metabolites were infrequently found in maternal hair or maternal blood and of the metabolites, only 3-PBA, MMA, and DDE were identified. Overall, therefore, hair is a more appropriate matrix to analyze for exposure of pregnant woman to pesticides compared to maternal blood and between parent pesticides and their metabolites, the former are the preferred compounds to measure quantitatively.

5.3. Comparison of maternal pesticide exposure during midgestation and at birth

The study was also designed to compare pesticide exposure during two periods of pregnancy—at midgesta-

Table 6
Comparison of the concentrations of parent pesticides in paired maternal hair (MH) and blood (MB) samples obtained at midgestation (A) and at birth (B)

Parent pesticides	MHA (μg/g)	MHB (μg/g)	MBA (μg/mL)	MBB (μg/mL)	MHA vs MHB		MBA vs MBB	
					Wilcox[a]	Tau[b]	Wilcox[a]	Tau[b]
(1) Propoxur	$N=47$	$N=53$	$N=3$	$N=18$	0.218	0.047	<0.001	0.122*
25%ile	0.58	0.52	0.66	0.77				
Median	2.67	0.58	0.67	0.77				
75%ile	2.78	0.72	0.68	0.77				
(2) Malathion	$N=8$	$N=0$	$N=0$	$N=0$	0.012		1.000	
25%ile	3.95							
Median	4.60							
75%ile	5.32							
(3) Chlorpyrifos	$N=0$	$N=2$	$N=0$	$N=0$	0.180		1.000	
25%ile		4.42						
Median		4.48						
75%ile		4.55						
(4) Bioallethrin	$N=53$	$N=35$	$N=0$	$N=0$	<0.001	0.206*	1.000	
25%ile	2.08	1.12						
Median	5.20	2.40						
75%ile	6.42	4.95						
(5) Pretilachlor	$N=1$	$N=1$	$N=0$	$N=0$	0.655	−0.002	1.000	
25%ile	2.67	2.55						
Median	2.67	2.55						
75%ile	2.67	2.55						
(6) DDT	$N=2$	$N=3$	$N=0$	$N=0$	0.345	−0.006	1.000	
25%ile	0.43	1.22						
Median	0.98	2.11						
75%ile	1.53	5.42						

Note: Sample size, 25th percentile, median and 75th percentile are given only for cases where pesticide was detected.
* $P<0.05$.
[a] P values from Wilcoxon signed rank test (Wilcoxon is based on entire sample $N=449$).
[b] Kendall's Tau can only be calculated when pesticide is detected (Kendall's Tau is based on entire sample, $N=449$).

tion and at birth—as an index of persistence of pesticide exposure throughout pregnancy. The frequency of propoxur in maternal hair was not significantly different at midgestation as compared to at delivery (10.5 versus 11.8%, $P<0.590$), which indicates the persistence of exposure to propoxur during these two time periods. On the other hand, the significantly higher frequency of bioallethrin and malathion in maternal hair at midgestation compared to birth (11.9 versus 7.8%, $P<0.034$ and 1.8 versus 0%, $P<0.012$, respectively) indicates a diminishing exposure to these pesticides toward delivery. The potential effects of variability in maternal hair washing may also factor into these differences (see below).

5.4. *Effect of preliminary hair washing on pesticide concentration in hair*

Pesticides in hair have been reported to be removed from the hair surface by washing (Altshul et al., 2004; Nakao et al., 2002) and could therefore be an important factor in determining the presence and concentration of pesticides in maternal hair. There was no significant difference in the concentration of propoxur in the paired hair samples before and after washing ($P=0.175$, Wilcoxon signed ranks test), but for bioallethrin, the concentration of the pesticide was significantly higher in the pre- compared to the postwashed hair samples ($P=0.001$, Wilcoxon signed ranks test). However, the postwash concentration was indetectable in only one sample (4.7%, 1.27 versus $0\,\mu g\,g^{-1}$). All other samples that initially tested positive were still positive after washing. These results indicate that regular washing of the hair with shampoo can affect the concentration of bioallethrin, but not propoxur, in maternal hair.

5.5. *Significance of pesticides in maternal hair*

The pesticides found in maternal hair are indicative of the exposure burden of the pregnant woman to these toxicants in the environment. How much of the pesticide is actually ingested by the pregnant woman and how much crosses the placenta and exposes the fetus are also important questions to answer and can be determined by the measurement of the pesticides in the fetal/infant compartments, e.g., infant blood, hair, and meconium. These studies are currently ongoing.

5.6. Significance of study

Our study is significant for many reasons. First, it demonstrates that maternal hair, compared to maternal blood, is a more sensitive matrix to analyze for pesticide exposure in pregnant women and pesticide identification can help target interventions that will minimize further exposure of women to pesticides during pregnancy. The study also indicates that, compared to agricultural pesticides, which were also used in the area, household pesticides constituted the major source of pesticide exposure among pregnant women in the study cohort. The significant association between the use of common pesticides at home and their presence in maternal hair indicates the improper use of these products at home, e.g., spraying of the pesticide by the pregnant woman herself and failure to observe appropriate reentry time in rooms that have previously been sprayed with pesticides. Due to a large number of pests (flies, mosquitoes and roaches) in the homes of the subjects in the study, spray pesticide use is commonly reported (43%), principally Baygon (90%), which contains propoxur and cyfluthrin. In a subset study of 136 subjects who used spray pesticides at home, 37.1% of the spraying was done by the pregnant woman and reentry time to the sprayed area was $\leqslant 60$ min in 74.1% of the cases. These practices may therefore account for the high pesticide exposure rate at home. Inadequate labeling of the pesticide spray is one of the reasons for the improper use of the products. Labels that warn that the product should not be used by pregnant women, as well as education of the user regarding appropriate reentry time in sprayed areas are important measures that can be instituted to reduce exposure to these toxicants in the environment.

Bioallethrin is a pyrethroid and is the principal component of a slow-burning mosquito coil repellant (Katol) that is reportedly used in 55% of the households. There was a highly significant association between the use of mosquito coils at home and the presence of bioallethrin in maternal hair (16.4 vs 6.5%, $P < 0.001$). Thus, increased education and awareness of the public that the mosquito coil contains a pesticide that can be inhaled or ingested are important measures that can reduce exposure to this compound.

5.7. Limitations of study

There are some limitations of this study. Although hair is a more appropriate matrix to test for maternal exposure to pesticides, than blood, the amount of pesticides found in hair does not necessarily reflect the internal dose in the mother. The pesticides could have been present in hair secondary to passive exposure, particularly since we did not wash the hair prior to the pesticide analysis. On the other hand, our decision to analyze unwashed hair provided important information on the general spectrum of pesticides that were present in the home or environment of the mother.

Some reports of pesticide analysis in blood and hair have used preliminary cleanup procedures. However, in our blood analysis, we have compared solid phase extraction (SPE) and liquid–liquid/solid–liquid extraction and found that the latter gave higher recoveries. For hair, solid–liquid extraction was preferred because the extract was clear and did not require a cleanup step. There are no interference peaks coinciding with the analytes of interest. The methods we have developed are capable of detecting a wide range of pesticides and are suitable for a large population study.

Due to the multiple and diverse compounds for analysis and the large number of samples that needed to be analyzed, our high throughput method had higher detection limits and was secondary to a number of factors, which included (1) rigid criteria for identification of compounds (correct retention time in the chromatogram, presence of target and qualifier ions in the mass spectrum, ratio of target to qualifier ions within the expected range, and concurrence among the research team on the final identity of the compound) and (2) multiple classes of pesticides being simultaneously analyzed. On the other hand, our limits of detection are within values found by other investigators using a similar method of detection (Ramesh and Ravi, 2004). The establishment of our LOD was purposely strict in order to increase the specificity of our method and lessen the chances of detecting false positives. Barr et al. (2002) reported a very sensitive method of pesticide analysis in blood with LODs in the pg/mL level. However, they also acknowledged that the specificity of the method was to some extent compromised by its high sensitivity.

6. Conclusion

In conclusion, there is significant exposure of pregnant woman to pesticides, particularly to those used at home. The study results demonstrate the usefulness of maternal hair as a readily available, minimally invasive, and sensitive biological matrix for studying potential maternal exposure to toxicants in the environment. Pesticides in blood most likely represent acute exposure, whereas pesticides in hair represent past and/or concurrent exposure. The wide window of exposure, availability, and ease of hair collection are distinct advantages in using hair to detect exposure to pesticides among pregnant women. On the other hand, pesticides in maternal hair may also be due to passive exposure and therefore not truly representative of the internal pesticide dose. Nonetheless, analysis of hair for pesticides can serve as a good index of maternal exposure to the pesticides in the environment, and therefore allows the institution of measures to prevent or minimize further exposure during pregnancy.

The exposure of pregnant women to household pesticides (principally propoxur and bioallethrin) was high, compared to farm pesticides, and may be due to the inappropriate use of pesticides at home and likely secondary to inadequate labeling of the pesticide products and ignorance about their use and potential danger. Appropriate measures, therefore, to address these problems will help reduce exposure of pregnant woman to these compounds, which can be toxic to her and to her fetus.

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