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# Combined analysis of prenatal (maternal hair and blood) and neonatal (infant hair, cord blood and meconium) matrices to detect fetal exposure to environmental pesticides☆

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## Abstract

**Objective—**The aim of this study was to determine optimum biomarkers to detect fetal exposure to environmental pesticides by the simultaneous analysis of maternal (hair and blood) and infant (cord blood, infant hair or meconium) matrices and to determine if a combination of these biomarkers will further increase the detection rate.

**Patients and methods—**Pregnant women were prospectively recruited from an agricultural site in the Philippines with substantial use at home and in the farm of the following pesticides: propoxur, cyfluthrin, chlorpyrifos, cypermethrin, pretilachlor, bioallethrin, malathion, diazinon and transfluthrin. Maternal hair and blood were obtained at midgestation and at delivery and infant hair, cord blood and meconium were obtained after birth. All samples were analyzed by gas chromatography/mass spectrometry (GC/MS) for the above pesticides and some of their metabolites.

**Results—**A total of 598 mother/infant dyads were included in this report. The highest rates of pesticide exposure were detected in meconium (23.2% to propoxur, 2.0% to pretilachlor, 1.7% to cypermethrin, 0.8% to cyfluthrin, 0.7% to 1,1,1-trichloro-2,2-bis,*p*-chlorophenylethane (DDT) and 0.3% to malathion and bioallethrin) and in maternal hair (21.6% to propoxur, 14.5% to bioallethrin, 1.3% to malathion, 0.8% to DDT, 0.3% to chlorpyrifos and 0.2% to pretilachlor). Combined analysis of maternal hair and meconium increased detection rate further to 38.5% for propoxur and to 16.7% for pyrethroids. Pesticide metabolites were rarely found in any of the analyzed matrices.

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**Conclusions—**There is significant exposure of the pregnant woman and her fetus to pesticides, particularly to the home pesticides, propoxur and pyrethroids. Analysis of meconium for pesticides was the single most sensitive measure of exposure. However, combined analysis of maternal hair and meconium significantly increased the detection rate. A major advantage of analyzing maternal hair is that prenatal pesticide exposure in the mother can be detected and intervention measures can be initiated to minimize further exposure of the fetus to pesticides.

### Keywords

Pesticides; Pregnancy; Meconium; Prenatal exposure to pesticides; Hair analysis; Blood analysis; Propoxur; Bioallethrin; Pyrethroid

## 1. Introduction

There is widespread use of pesticides and human exposure to these compounds is inevitable (US Environmental Protection Agency (USEPA), 2004; Waliszewski et al., 1998, Lucena et al., 2007; Sekiyama et al., 2007; Ye et al., 2008; Chevrier et al., 2008; Tsatsakis et al., 2008). The exposure of the pregnant woman to pesticides is of major concern since a majority of the pesticides are neurotoxicants and the fetus is at greater risk, compared to the adult, to the toxic effects of these chemicals due to the rapid state of growth of its brain at this stage of development (Bruckner, 2000; Eriksson, 1997; Barone et al., 2000). Most of the maternal exposures to environmental pesticides are probably subtle and result in little or no recognizable effects in the pregnant woman. Yet, serious concerns have been raised about their adverse effects on the fetus and of their potential role in subsequent developmental, learning and behavioral difficulties in children (Boyle et al., 1994; California Health and Human Services (HHS), 1999; Schettler et al., 2000; Grandjean et al., 2006). 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 pesticides (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). Thus, reliable biomarkers of fetal exposure to environmental pesticides are needed to identify infants who are at risk to adverse outcomes from these neurotoxicants. There are a few reports of analysis of infant cord blood (Whyatt et al., 2003, 2004; Ostrea et al., 2008), meconium (Ostrea et al., 2002, 2008; Whyatt and Barr, 2001; Ortega Garcia et al., 2006; Bielawski et al., 2005) or infant hair (Ostrea et al., 2008) to detect prenatal exposure to pesticides. Similarly, in pregnant women, maternal hair and blood have been analyzed for pesticides (Ostrea et al., 2006). However, no study has yet been conducted that compares and correlates simultaneous analysis of both maternal and fetal matrices. The aim of this study was therefore to determine optimum biomarkers to detect fetal exposure to environmental pesticides by the analysis of maternal (hair and blood) and infant (cord blood, infant hair or meconium) matrices and to determine if a combination of biomarkers will further increase the detection rate.

## 2. Materials and methods

Pregnant women were prospectively recruited from the Outpatient Clinic of the Provincial Hospital in Malolos, an agricultural town in the province of Bulacan, Philippines. Our preliminary survey of pesticide use in the region showed the predominant use of the following compounds at home or in the farm: cyfluthrin/ propoxur (73%), chlorpyrifos (37%), cypermethrin (31%), pretilachlor (28%), bioallethrin (26%), malathion (15%), diazinon (12%)

and transfluthrin (11%). This study was approved by the Human Investigation Committees at the Wayne State University, the University of the Philippines and the Bulacan Provincial Hospital. An informed consent was obtained from the mothers for their participation in the study, as well as that of their infants. Maternal blood (MB) and hair (MH) were obtained from the subjects upon initial recruitment (sample A) and at delivery (sample B). Hair was obtained from an inconspicuous area of the mother's scalp and was cut from the base of the scalp. The blood and meconium samples were frozen at −18 °C until the time of analysis. The hair samples were stored at 4°C. After birth, cord blood was collected into tubes containing EDTA as anticoagulant. While in the nursery, meconium and infant hair samples were obtained. For the hair samples, a small, pencil (width) size sample of hair was obtained from the infant's nape and placed in aluminum foil within sealed plastic bags. Meconium was collected by the nurse directly from every diaper of the infant during the first 2 days of life using previously published procedures of meconium collection (18, 22). If there was inadequate collection of meconium in the nursery, meconium collection was continued at home. The mother was instructed on how to save the infant's diapers and a member of the research team would visit the home daily to collect meconium from the diapers. The samples were analyzed for the pesticides which, from the survey, were commonly used in the study site: cyfluthrin, propoxur, chlorpyrifos, cypermethrin, pretilachlor, bioallethrin, malathion, diazinon, transfluthrin. Lindane and 1,1,1-trichloro-2,2-bis, *p*-chlorophenylethane (DDT) were also analyzed based on our previous study in Manila, Philippines that showed substantial exposure to these pesticides (Ostrea et al., 2002). Some known metabolites of these 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, 3,5,6-trichloro-2-pyridinol for chlorpyrifos, 3-phenoxybenzoic acid (3-PBA) for cyfluthrin, malathion monocarboxylic acid [MMA] for malathion and 4,4′-dichlorodiphenyldichloroethylene [DDE] for DDT.

The pesticides and their metabolites were analyzed by gas chromatography/ mass spectrometry (GC/MS) using published procedures (Bielawski et al., 2005; Posecion et al., 2006; Corrion et al., 2005). The analytical performance data for these various matrices are summarized as follows.

### 2.1 Hair

In hair, matrix-spiked calibration curves were linear for all parent pesticides and pesticide metabolites with coefficients of linearity greater than 0.998 (Ostrea et al., 2006; Posecion et al., 2006). We evaluated the extraction efficiency of parent pesticides at a spiking concentration of 1.56 μg $g^{-1}$. This single level (representing a mid-level concentration) is within the range of concentration of parent pesticides detected in maternal hair samples. Optimum recovery rates using our 6-h hexane extraction method ranged from 87% to 112%. The inter-assay and intra-assay coefficients of variability for the analysis of parent pesticides were below 11%. Limits of detection (LOD) by empirical approach ranged from 30.50 ng $g^{-1}$ for propoxur, diazinon and DDT to 488.00 ng $g^{-1}$ hair for bioallethrin. Recovery rates of the metabolites by liquid-liquid extraction of the acid digest ranged from 80% to 120% using a spiked concentration of 46.86 μg $g^{-1}$. Inter-assay and intra-assay coefficients of variability for the analysis of metabolites were <11%, and the LOD ranged from 0.18 (2-IPP, 3-PBA and DDE) to 5.88 μg $g^{-1}$ (for MMA).

### 2.2. Blood

Whole blood, instead of serum or plasma was used due to the high lipophilicity of peticides, which favors the concentration of these compounds in erythrocytes (Frenzel et al., 2000). In blood, the matrix-spiked calibration curves were linear for all parent pesticides and pesticide

metabolites with coefficients of linearity greater than 0.990 (Corrion et al., 2005). Recovery studies were done using blood spiked with parent pesticides (1.56 $\mu gmL^{-1}$) and pesticide metabolites (0.78 $\mu gmL^{-1}$). Optimum recovery rates ranged from 84% to 142% for parent compounds and 54–122% for the metabolites. The inter-assay and intra-assay coefficients of variability for the analysis of spiked controls were below 14.4%. The LOD by the empirical approach for parent compounds ranged from 0.003 (for propoxur, diazinon, pretilachlor and DDT) to 0.098 $\mu gmL^{-1}$ (for bioallethrin, cyfluthrin and cypermethrin). The LOD for the metabolites ranged from 0.20 $\mu gmL^{-1}$ (for 2-isopropoxyphenol, *cis*-/*trans*-DCCA, 3,5,6-TCP and MMA) to 0.78 $\mu gmL^{-1}$ (for 3-PBA and DDE).

### 2.3. Meconium

The matrix-spiked calibration curves were linear for all parent compounds and metabolites with coefficients of linearity greater than 0.981 (Bielawski et al., 2005). Recovery studies were done on spiked meconium at concentrations of 6.25 $\mu g\ g^{-1}$ for parent pesticides and 4.15 $\mu g\ g^{-1}$ for pesticide metabolites. Recovery ranged from 82.4% to 109.3% for parent pesticides and 72.3% to 108.0% for pesticide metabolites. The LOD for the parent compounds ranged from 0.098 $\mu g\ g^{-1}$ for propoxur to 1.56 $\mu g\ g^{-1}$ for lindane. Inter-assay variability was <11.4% for all compounds. LODs for pesticide metabolites ranged from 0.312 $\mu g\ g^{-1}$ for most compounds, to 4.15 $\mu g\ g^{-1}$ for 3,5,6 trichloro-2-pyridinol. Parent pesticides recovery spiked at 6.25 $\mu g\ g^{-1}$ ranged from 82.4% to 109.3% and metabolites, spiked at 4.15 $\mu g\ g^{-1}$ from 72.3% to 108.0%.

For the GC/MS identification of pesticides in the different matrices, the following criteria were strictly followed to ensure the highest sensitivity and specificity of the test: (1) a distinct peak of the pesticide in the chromatogram at the correct retention time (± 0.03 min) compared to the positive controls, (2) the target and qualifier mass ions were present in the correct ratio range and (3) there was independent agreement among the analytical investigators regarding the identity of the compound.

## 3. Statistical analysis

Mean (standard deviation) and frequency distribution were calculated to describe the demographic and socioenvironmental characteristics of the study population. When appropriate, median and interquartile ranges are presented. For statistical comparison, the units of pesticide concentrations in hair and meconium were expressed in $\mu gmL^{-1}$ to be uniform with the concentration unit in blood. The prevalence of exposure for each pesticide was compared among the five matrices by the Cochran *Q* test. A significant Cochran Q test was followed by the McNemar tests to compare the prevalences for pairs of matrices. Agreement between matrices for prevalence was assessed with Cohen's Kappa. The concentrations of pesticides among all matrices were compared using the Friedman test. A significant Friedman test was followed by Wilcoxon Signed Rank tests to compare the concentrations for pairs of matrices. When appropriate, exact *p*-values were calculated for the Cochran *Q*, McNemar, Friedman and Wilcoxon Signed Rank tests using StatXact (StatXact Version 7.0.0 (2005) [Computer software]. Cambridge, MA: Cytel Inc.). A *p*-value of $\leq 0.05$ was set as the level of statistical significance.

## 4. Results

Mother/infant dyads who had all five matrices available for analysis, were included in this report (N = 598). The demographic and environmental characteristics of the study population were as follows: Mean maternal age was 25.7 years with median gravidity of 2 and parity of 1. About 74% were married, 68.1% attained at least a high school education and 76.8% were homemakers. For the infants, mean gestational age was 38.7 weeks and 53.8% were males.

About 59.2% of the subjects lived in their own homes, although 7.4% lived in poor, makeshift homes. The socioeconomic status (SES) was assessed using the Roberto Scale (Roberto, 2002) which is a widely used socioeconomic scale in the Philippines based on home structure and appearance as compared to the Hollingshead Four Factor Index (Hollingshead, 1965). The latter, a standard test of SES in the United States, was not used since it was not applicable to the Philippine cohort due to culture differences. The Roberto scale ranges from A (highest) to E (lowest). About 59% of the homes were in the class D and E category. The cleanliness of home and surroundings was rated as fair (72.6%). The toilet was predominantly water seal (82.1%); water source was either piped in (53.1%) or from a well (38.9%); waste disposal was via sewer (27.3%) or canal (60.9%); 59.0% had organized garbage collection. Most homes had problems with flies (91.6%), roaches (90.0%) and mosquitoes (98.0%). Pesticide spray was used in 38.9% of the homes and the principal pesticide used was Baygon™ (92.1%) which contains propoxur and cyfluthrin. Spraying of home pesticides was done by 39.9% of mothers. Re-entry time after spraying of a room was < 60 min in 69.4% of the users. The slow burning mosquito coil Katol™ which contained bioallethrin was used in 53.1% of the households. Farm pesticides were used by 16.8% and only 4.2% used gloves to handle pesticides.

The prevalence of fetal exposure to pesticides based on the analysis of maternal blood and hair, infant hair, cord blood and meconium is shown in Table 1.

Among the fetal matrices (infant hair, cord blood and meconium), the highest number of pesticides detected and exposure rate was found in meconium (Table 1). A total of 7 out of 11 (64%) pesticides were found in meconium: propoxur (23.2%), pretilachlor (2.0%), cypermethrin (1.7%), cyfluthrin (0.8%), DDT (0.7%) and bioallethrin and malathion (0.3%). In contrast, cord blood was only positive for propoxur (1.8%) and only one infant hair sample was positive for chlorpyrifos (0.2%). The prevalences of propoxur ($p < 0.001$), pretilachlor ($p < 0.001$, exact test) and cypermethrin ($p < 0.001$, exact test) were significantly higher in meconium compared to cord blood or infant hair. Lindane, diazinon and transfluthrin were not found in any of the infant matrices. The concentration of pesticides in meconium, infant hair and cord blood are shown in Table 2 and median and interquartile ranges are given for cases with positive concentrations. The concentrations of propoxur ($p < 0.001$), pretilachlor ($p < 0.001$, exact test) and cypermethrin ($p < 0.002$, exact test) were significantly higher in meconium compared to cord blood and infant hair.

For the maternal matrices, the prevalence of pesticides was highest in maternal hair compared to maternal blood (Table 1). Propoxur (21.6%) and bioallethrin (14.5%) were the principal pesticides found in maternal hair and a small percent of hair samples were positive for malathion (1.3%), DDT (0.8%), chlorpyrifos (0.3%) and pretilachlor (0.2%). There was a significantly higher prevalence of propoxur in maternal hair taken at birth (hair B) compared to midgestation (hair A) (14% vs. 9.9%, $p<0.025$). Maternal blood was only positive for propoxur (3.5%) and DDT (0.2%). The prevalence of propoxur ($p<0.001$), malathion ($p<0.008$, exact test) and bioallethrin ($p<0.001$) were significantly higher in maternal hair compared to blood. Diazinon, lindane, transfluthrin, cypermethrin and cyfluthrin were not found in maternal hair or blood. The concentration of the parent pesticides in maternal hair and blood samples are shown in Table 2. There was no significant difference in the concentration of propoxur in maternal hair at midgestation as compared to at birth (0.42 versus 0.24 μg $mL^{-1}$, $p = 0.501$). In contrast, bioallethrin concentration was significantly higher in maternal hair at midgestation compared to birth (median of 2.19 versus 0.87 μg $mL^{-1}$, $p<0.005$).

An overall comparison of the five matrices (maternal hair and blood and infant hair, cord blood and meconium) showed that the highest number and exposure rate to pesticides was detected in meconium followed by maternal hair (Table 1). A total of 7 out of 11 pesticides (63.6%) were found in meconium and 6 of 11 pesticides (54.5%) in maternal hair. Concordance in the

detection of propoxur and grouped pyrethroids (bioallethrin, cypermethrin and cyfluthrin) between meconium and maternal hair were as follows: positive meconium for propoxur versus positive maternal hair A for propoxur (Cohen's Kappa = 0.003, $p = 0.926$); positive meconium for propoxur versus positive maternal hair B for propoxur (Cohen's Kappa = 0.125, $p = 0.001$); positive grouped pyrethroids in meconium versus positive grouped pyrethroids in maternal hair A (Cohen's Kappa = 0.039, $p = 0.248$) and positive grouped pyrethroids in meconium versus positive grouped pyrethroids in maternal hair B (Cohen's Kappa = −0.014, $p = 0.676$). Combining meconium and maternal hair analysis increased the detection rate for exposure to both propoxur and the combined pyrethroids (cyfluthin, cypermethrin and bioallethrin)—see Table 3. For propoxur, the increase was from 23.2% for meconium to 30.8% for meconium plus maternal hair A, 32.1% for meconium plus maternal hair B and 38.5% for meconium with maternal hair A and B ($p<0.001$). For pyrethroids, the detection rate also increased from 2.8% for meconium alone to 11.9% for meconium and maternal hair A, 11.4% for meconium and maternal hair B and 16.7% for meconium and maternal hair A and B ($p<0.001$).

Pesticide metabolites were not found in any of the matrices analyzed, except in one meconium sample which was positive for DDE, a DDT metabolite.

## 5. Discussion

The objective of this study was to determine reliable measures of fetal exposure to environmental pesticides. A few studies have reported on the analysis of cord blood, maternal blood or meconium for pesticides: cord blood and maternal blood for chlorpyrifos, diazinon and propoxur (Whyatt et al., 2003, 2004) and meconium for organophosphates (Whyatt and Barr, 2001), DDE (Hong et al., 2002), organochlorines (Ortega Garcia et al., 2006) and other pesticides (Ostrea et al., 2002, 2008; Bielawski et al., 2005). However, this is the first study to simultaneously analyze and compare five matrices (maternal hair and blood, cord blood, infant hair and meconium) to determine the optimum matrix or combination of matrices to detect antenatal pesticide exposure.

For the infant matrices (cord blood, infant hair or meconium), meconium was the best matrix for this purpose. Of eleven pesticides analyzed, eight were detected in meconium with a high prevalence rate for propoxur (23.2%). In contrast, cord blood and infant hair were each only positive for a single pesticide, propoxur and chlorpyrifos, respectively. Furthermore, the concentrations of the pesticides detected were also significantly higher in meconium than cord blood and infant hair. Pesticides that partition and accumulate in adipose tissues such as organochlorines were poorly found in cord blood, infant hair or meconium. Although DDT was found in meconium, the frequency of detection was low (0.7%). The use therefore of cord blood, infant hair and meconium as matrices for the detection of fetal exposure to these compounds is a recognized limitation of the study. However, access to fetal or infant adipose tissue is normally not feasible; thus its diagnostic use in clinical settings may not be practical. On the other hand, failure to detect lipophilic pesticides from the analysis of non-adipose tissue matrices should not imply non-exposure to these types of pesticides. We did not include the analysis of infant's urine due to inherent problems and difficulty associated with urine collection in infants. Besides, there are added limitations associated with the interpretation of urine results for pesticides particularly if only spot samples are collected (Barr and Needham, 2002). Cord blood also had an advantage over urine since parent pesticides are more readily detectable in blood compared to urine (Barr and Needham, 2002).

The high rate of detection of pesticides in meconium is consistent with the reported high rates of detection of most xenobiotics in meconium which include illicit drugs, licit drugs, nicotine metabolites and alcohol metabolites (Ostrea, 1999). This is attributed to the repository nature of meconium thus providing a wide window of exposure to xenobiotics. Meconium is formed

at around the third or fourth month of gestation and most xenobiotics that the fetus is exposed to during gestation are deposited in meconium, through fetal swallowing and/or bile secretion from that period up to the time of birth (Ostrea et al., 1989). Since meconium, unlike fetal urine, is not normally excreted in utero, compounds that deposit in meconium accu mulate and increase in concentration thus enhancing their detection. In contrast, pesticides in cord blood represent acute exposure and may not be readily detected due to their low concentrations in the blood as a result of the metabolism, excretion and deposition in tissues of the pesticides. A highly sensitive technique to detect pesticides in blood has recently been published (Barr et al., 2002) with LOD's of three orders of magnitude lower than the LOD in this report. However, the specificity of the method was compromised since in many instances, only a single mass ion, often not the molecular ion, was used for compound identity. Thus, the methodology was associated with an imprecision that was abot double that of methods using higher detection limits (Barr et al., 2002). In our study, we used stringent criteria for the identity of any compound, including (i) appropriate retention time in the chromatogram based on positive controls, (ii) the presence in the mass spectra of specific mass and qualifier ions and (iii) appropriate mass/qualifier ion ratios. Our adherence to these standard GC/MS criteria may have decreased the sensitivity of methodology, but retained the high specificity inherent to GC/MS analysis.

We did not detect any pesticides in infant hair except in one sample that was positive for chlorpyrifos. It appears that the deposition of pesticides in infant hair does not occur as readily compared to other compounds such as illicit drugs, nicotine, and most recently, fatty acid ethyl esters (Ostrea, 1999; Koren et al., 2002; Berkowitz et al., 2003). The pharmacokinetics and tissue distribution of pesticides in the fetus is largely unknown. Fetal metabolism of pesticides is low due to the poor detoxification mechanisms (Waliszewski et al., 1998). Furthermore, fetal hair starts to grow at approximately 6–7 months of gestation (Koren et al., 2002) so that the timing of maternal exposure during pregnancy could also influence incorporation of pesticides into the growing hair shaft. It is also likely that due to the small amount of hair that could be collected from the newborn infant, the limited sample size for analysis prevented the detection of minute quantities of pesticides in infant's hair. In contrast, our results with infant hair analysis differed markedly from the results with maternal hair due, in part, to more hair sample that could be obtained from the mother compared to newborn hair. Overall, newborn hair is not ideal for the analysis of pesticides because of low concentration of pesticides in infant hair and the limited amount of hair that could be collected for analysis. Furthermore, it has been reported that pesticide metabolites tend to partition predominantly towards blood rather than hair (Altshul et al., 2004).

With regards to maternal matrices, the analysis of maternal hair showed significantly higher detection rates for pesticides compared to maternal blood. When combined with meconium analysis for pesticides, the analysis of maternal hair at midgestation and at birth increased the detection rate of prenatal exposure to pesticides by almost two fold. For propoxur, the increase was from 23.25% with meconium alone to 38.5% with meconium plus maternal hair A and B and for pyrethroids, from 2.8% for meconium alone to 16.7% with meconium plus maternal hair A and B. However, unlike fetal matrices (meconium, cord blood or infant hair) which when positive for pesticides are indicative of active fetal exposure to these compounds, this relationship is not necessarily valid for maternal hair. Inherent in maternal hair analysis is the difficulty in distinguishing between active and passive exposure to pesticides. In this study, we purposely did not wash the maternal hair prior to analysis for pesticides because we were interested in maternal exposure to the pesticide, regardless of whether it was active or passive. We have conducted some preliminary study on hair washing before analysis of the hair for propoxur and bioallethrin. There was no 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-washed

compared to the post-washed hair samples ($p = 0.001$, Wilcoxon signed ranks test). However, the post wash concentration was undetectable in only one sample whereas all other samples were still positive after washing. The low concordance between the prevalence of propoxur and pyrethroids in maternal hair and meconium strongly suggests passive rather than active exposure in maternal hair. Nonetheless, there is ample justification and clinical use for the prenatal analysis of maternal hair for pesticides since important information on exposure can be provided (whether active or passive), which therefore provides an opportunity to initiate intervention measures during pregnancy that can reduce further exposure of the fetus to the pesticides.

Pesticide metabolites were rarely detected in the present study in any of the matrices analyzed despite a few publications that have reported on detecting pesticide metabolites in meconium, e.g., DDE (Hong et al., 2002) and organophosphate metabolites (Whyatt and Barr, 2001). Hong et al. (2002) randomly sampled 60 meconium samples in Germany and detected DDE in 3 of them. However, the pesticide metabolite concentration they detected was 11.1 ng $g^{-1}$, which is lower than our LOD for DDE. Our method had a higher LOD since it was optimized to detect many classes of metabolites, especially the pyrethroids, whereas Hong and colleagues were selectively searching for DDE. However, we did find one meconium sample positive for DDE. Whyatt and Barr (2001) found diethylthiophosphate (DETP), an organophosphate metabolite, in 100% of meconium samples studied in New York. We attempted to analyze for this compound using our current meconium liquid-liquid extraction method. However, it was discontinued due to difficulty in the chromatographic separation of DETP from TCP. The survey had reported higher use of malathion and chlorpyrifos, for which we had specific metabolites that we could accurately measure, than for diazinon, for which DETP would be a potential metabolite.

Overall, this study has shown that exposure to home, rather than farm, pesticides was the major source of pesticide exposure in the pregnant woman and her fetus even in an agricultural environment. This observation parallels reports of high exposure rate to home pesticides among pregnant women and their infants residing in urban areas (Whyatt et al., 2003, 2004; Ostrea et al., 2002). Thus, whether in the urban or rural areas, home pesticides constitute a high health risk in pregnant women and are likely related to the widespread and inappropriate use of pesticides at home. In our study, due to the widespread problems of pests at home, including flies, mosquitoes and roaches, spray pesticides were commonly used (38.2%), principally Baygon™ (91.5%) which contains propoxur and cyfluthrin. Inappropriate use of these home pesticides was evident since 39.9% of the spraying was done by the pregnant woman and reentry time to the sprayed area was ≤60min in 73.2% of the cases. Poor education and inadequate labeling on the safe use of the pesticide are major reasons for its improper use. The pesticide labels do not warn that the product should not be used by the pregnant woman, nor explicitly instruct on the appropriate reentry time of the sprayed area. Corrective measures to minimize further pesticide exposure in our study site have been instituted as a result of our findings. Assessment of clinical outcomes in the child in relation to prenatal and ongoing exposure to pesticides, are also under way.

In conclusion, our study has demonstrated that compared to cord blood or infant hair, meconium is the most sensitive matrix to analyze for fetal exposure to pesticides. The accumulation of pesticides in meconium, the ease of meconium collection and the large amount of sample that could be obtained for analysis are all factors that contribute to the increased sensitivity of this matrix. The prenatal analysis of maternal hair significantly adds to the detection rate of fetal exposure to pesticides and also provides the important advantage of initiating intervention measures during pregnancy that will reduce further fetal exposure to these potent neurotoxicants.

## Abbreviations

DDT, 1,1,1-trichloro-2,2-bis, *p*-chlorophenylethane
DDE, 4,4′-dichlorodiphenyldichloroethylene
2-IPP, 2-isopropoxyphenol
3-PBA, 3-phenoxy-benzoic acid
*cis*-DCCA, *cis*-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylic acid
*trans*-DCCA, *trans*-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylic acid
MMA, malathion monocarboxylic acid
LOD, limit of detection
GC/MS, gas chromatography–mass spectroscopy
SES, socioeconomic status
DETP, diethylthio-phosphate

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**Table 1**

Prevalence (*N*, percent positive) of parent pesticides in meconium, cord blood, infant hair, maternal hair and blood at midgestation (A) and at delivery (B)

| Toxin | Source | | | | | | | | | Overall test *p*-value[†] |
|---|---|---|---|---|---|---|---|---|---|---|
| | Infant | | | Mother | | | | | | |
| | Meconium | Cord blood | Hair | Hair A | Hair B | Blood A | Blood B | Hair[a] | Blood[a] | |
| Propoxur | 139 (23.2) | 11 (1.8) | 0 | 59 (9.9) | 84 (14.0) | 3 (0.5) | 19 (3.2) | 129 (21.6%) | 21 (3.5%) | <0.001 |
| Diazinin | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | N/A |
| Lindane | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | N/A |
| Transfluthrin | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | N/A |
| Malathion | 2 (0.3) | 0 | 0 | 8 (1.3) | 0 | 0 | 0 | 8 (1.3%) | 0 | <0.001[b] |
| Chlorpyrifos | 0 | 0 | 1 (0.2) | 1 (0.2) | 1 (0.2) | 0 | 0 | 2 (0.3%) | 0 | 0.676[b] |
| Bioallethrin | 2 (0.3) | 0 | 0 | 57 (9.5) | 52 (8.7) | 0 | 0 | 87 (14.5%) | 0 | <0.001 |
| Pretilachlor | 12 (2.0) | 0 | 0 | 1 (0.2)[c] | 0 | 0 | 0 | 1 (0.2%) | 0 | <0.001[b] |
| DDT | 4 (0.7) | 0 | 0 | 2 (0.3) | 3 (0.5) | 1 (0.2) | 1 (0.2) | 5 (0.8%) | 1 (0.2%) | 0.064[b] |
| Cyfluthrin | 5 (0.8) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | <0.001[b] |
| Cypermethrin | 10 (1.7) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | <0.001[b] |

*N* = 598.

[†] *p*-Value based on Cochran *Q* test.

[a] Samples A and B are combined for maternal hair and for maternal blood.

[b] Exact test using StatXact.

[c] *N* = 597.

**Table 2**

Concentration (μg $mL^{-1}$) of parent pesticides in meconium, cord blood, infant hair and maternal hair and blood at midgestation (A) and at delivery (B)

| Toxin | Source | | | | | | | | | Overall test *p*-value[†] |
|---|---|---|---|---|---|---|---|---|---|---|
| | Infant | | | Mother | | | | | | |
| | Meconium | Cord blood | Hair | Hair A | Hair B | Blood A | Blood B | Hair[a] | Blood[a] | |
| Propoxur | 0.33 (0.24–1.50) | 0.77 (0.77–0.77) | 0 | 0.42 (0.24–1.11) | 0.24 (0.21–0.25) | 0.67 (0.66–0.68) | 0.74 (0.68–0.77) | 0.25 (0.22–0.42) | 0.73 (0.67–0.77) | <0.001 |
| Diazinon | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | N/A |
| Lindane | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | N/A |
| Transfluthrin | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | N/A |
| Malathion | 4.15 (2.92–5.38) | 0 | 0 | 1.72 (1.62–2.12) | 0 | 0 | 0 | 1.72 (1.62–2.13) | 0 | <0.001[b] |
| Chlorpyrifos | 0 | 0 | 2.16 | 1.83 | 1.77 | 0 | 0 | 1.80 (1.77–1.83) | 0 | 0.676[b] |
| Bioallethrin | 1.2 (0.61–1.79) | 0 | 0 | 2.19 (1.08–2.74) | 0.87 (0.40–2.05) | 0 | 0 | 2.05 (0.60–2.49) | 0 | <0.001 |
| Pretilachlor | 0.52 (0.40–1.16) | 0 | 0 | 1.07[b] | 0 | 0 | 0 | 1.07 | 0 | <0.001[b] |
| DDT | 1.75 (1.10–2.88) | 0 | 0 | 0.41 (0.17–0.65) | 0.81 (0.56–1.86) | 0.56 | 0.53 | 0.65 (0.40–1.16) | 0.56 | 0.054[b] |
| Cyfluthrin | 2.22 (1.51–3.98) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | <0.001[b] |
| Cypermethrin | 2.21 (1.85–2.43) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | <0.001[b] |

$N = 598$.

Median and interquartile range given for cases with positive concentration.

[†] *p*-Value based on the Friedman test.

[a] Samples A and B are combined for maternal hair and for maternal blood.

[b] Exact test using StatXact.

**Table 3**

Prevalence (*N*, percent positive) of parent pesticides based on combined analysis of meconium and maternal hair at midgestation (A) or at delivery (B)

| Toxin | Source | | | | Overall test p-value[†] |
|---|---|---|---|---|---|
| | Meconium | Meconium and maternal hair A | Meconium and maternal hair B | Meconium and maternal hair A and B | |
| Propoxur | 139 (23.2) | 184 (30.8) | 192 (32.1) | 230 (38.5) | <0.001 |
| Pyrethroids | 17 (2.8) | 71 (11.9) | 68 (11.4) | 100 (16.7) | <0.001 |

*N* = 598.

[†] *p*-Value based on Cochran *Q*-test.