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Detection of Exposure to Environmental Pesticides During Pregnancy by the Analysis of Maternal Hair Using GC–MS

N. Posecion Jr^{1,□}, E. Ostrea Jr^{1,2}, D. Bielawski¹, M. Corrion¹, J. Seagraves¹, and Y. Jin¹

¹ Department of Pediatrics, Wayne State University School of Medicine, 421 E Canfield Boulevard, Detroit, MI 48201, USA

² University of the Philippines Institute of Child Health and Human Development, Manila, Philippines

Abstract

Several classes of pesticides were extracted from maternal hair by solid-liquid extraction. Analysis of their selected metabolites involved a methanolic/hydrochloric acid methyl ester derivatization and liquid–liquid extraction. Gas chromatography electron impact mass spectrometry was used to detect and quantify the pesticides and metabolites. Recovery of parent compounds and metabolites from the analysis of spiked hair ranged from 87 to 112% with coefficients of variation less than 11%. Limits of detection ranged from 0.031 to 5.88 $\mu\text{g g}^{-1}$. Analysis of hair samples from pregnant women in the Philippines showed maternal exposure during pregnancy to bioallethrin, propoxur, chlorpyrifos, pretilachlor and malathion.

Keywords

Gas chromatography-mass spectrometry; Pesticides; Hair analysis

Introduction

Pesticides are a group of environmental pollutants that pose a significant risk to human health. Due to their constant use and chemical stability, these toxic pollutants are widespread and may persist as contaminants in the ambient environment. The exposure of pregnant women to pesticides in the environment is a major concern because of their potential adverse effects on brain development in the fetus and infant [1]. Certain pesticides are neurotoxic and can be transferred to the fetus through the placenta during pregnancy [2], or to the nursing infant through the breast milk [3]. Exposure to pesticides during these periods is critical, due to the vulnerability of the developing brain to environmental agents and their potential harmful effects on neurodevelopment in children [1,4]. Animal studies on organophosphate exposure during gestation show that pesticide exposure causes cellular deficits in the developing brain and contributes to sub-optimal neurobehavioral functioning in the offspring [5]. In a similar study, Young et al. [6] suggest that this interference in brain development in the human fetus and infant can occur even at subclinical levels of exposure in the mother, and could result in long lasting damage to brain function in later life. Thus, due to the high vulnerability of the fetus to neurotoxic pesticides, reliable and sensitive methods to detect the exposure of pregnant women to environmental pesticides are needed to prevent further exposure and resulting toxicity.

Pesticide exposure during pregnancy has been detected by the analysis of maternal blood [3] and urine [7]. However, pesticides in these matrices are biomarkers of short-term or recent exposure and do not show past or cumulative exposure. Maternal hair analysis is capable of

detecting a wider period of exposure during pregnancy as compared to maternal blood or urine. This is due to hair's ability to accumulate pesticides incorporated by blood into the growing hair shaft [8–11], which occurs in a dose-related manner [12]. Since no active metabolism and excretion of pesticides occurs in hair, the pesticides present provide a cumulative estimate of exposure during pregnancy. As a biologic tissue, hair is also more convenient to analyze because its collection is easy, less invasive, and less expensive compared to blood or other tissue sampling. Hair also requires simple handling and storage.

Hair analysis as a tool for assessing risks of human exposure to environmental pesticides has been used successfully in the biomonitoring of exposure to organochlorine pesticides, including lindane and dichloro-dimethyl-trichloroethane or DDT [13,14]. It has also been used to provide information on past occupational exposures of women in Greece to DDT [15] and to detect exposure of children to DDT and lindane from indoor pollution [16]. However, pregnant women can be concurrently exposed to more than one pesticide and to date, analytical methods that measure exposure to several classes of pesticides by the analysis of human hair have not yet been reported. Furthermore, analytical techniques that assay for pesticide metabolites in hair, which are unique biomarkers of endogenous exposure, are lacking [17].

The aim of this study was to determine the utility of maternal hair analysis as a diagnostic tool for determining exposure to several classes of pesticides during pregnancy. Specifically, this work involved the development of an extraction method and GC–MS assay of a number of classes of pesticides from maternal hair, including, carbamates, organophosphates, organochlorines, pyrethroids, and a chloroacetanilide herbicide. A separate method to analyze for several metabolites was also developed.

Experimental

Pesticide Standards

Analytical standards for the parent pesticides were obtained from Cerilliant (Austin, TX, USA). The parent pesticide stock solution, Pesticide Mix 11 ($400\text{ }\mu\text{g mL}^{-1}$), was composed of: propoxur (certified assay: 99.0%), diazinon (certified assay: 99.0%), lindane (certified assay: 99.0%), transfluthrin (certified assay: 99.0%), malathion (certified assay: 99.0%), chlorpyrifos (certified assay: 99.0%), *p,p'*-dichloro-diphenyl-trichloroethane (*p,p'*-DDT, certified assay: 99.0%), bioallethrin (certified assay: 99.0%), pretilachlor (certified assay: 97.0%), cyfluthrin (certified assay: 97.0%), and cypermethrin (certified assay: 99.0%) in hexane:acetone (90:10 (v/v)). The internal standard, 1,4-dichlorobenzene ($4,000\text{ }\mu\text{g mL}^{-1}$, certified assay: 99.0%) was prepared in methylene chloride. For the pesticide metabolites, Malathion monocarboxylic acid (MMA) in methanol ($400\text{ }\mu\text{g mL}^{-1}$, certified assay: 99.0%) was obtained from Chem Service (West Chester, PA, USA). The internal standard, 2-phenoxybenzoic acid (2-PBA, $400\text{ }\mu\text{g mL}^{-1}$, certified assay: 99.0%) was prepared by Cerilliant (Austin, TX, USA) in methanol. Pesticide Mix 568, a pesticide metabolite mix ($400\text{ }\mu\text{g mL}^{-1}$) composed of 2-isopropoxyphenol (2-IPP, certified assay: 98.5%), *cis*-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylic acid (*cis*-DCCA, certified assay: 99.5%), *trans*-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylic acid (*trans*-DCCA, certified assay: 99.5%), 3,5,6-trichloro-pyridinol (3,5,6-TCP, certified assay: 98.8%), 3-phenoxybenzoic acid (3-PBA, certified assay: 99.2%), and *p,p'*-4,4-dichloro-diphenyl-dichloroethylene (*p,p'*-DDE, certified assay: 99.0%) in methanol was ordered from Dr. Ehrenstorfer, GmbH (Augsburg, Germany). Helium (purity: 99.99%) and nitrogen (purity: 99.99%) were purchased from Wilson Welding (Warren, MI, USA).

Instrumentation

An Agilent gas chromatograph (GC 6890) coupled with a Mass Selective Detector (MSD 5973N) (Agilent Technologies, Atlanta, GA, USA) was used for the assay. Separation of analytes by gas chromatography was done using a J & W DB-5MS capillary column (5%-phenyl-methylpolysiloxane, 30 m $\times$ 0.25 mm $\times$ 1.00 μ m, Agilent). An Agilent 7683 injector and autosampler were used to inject the samples into the GC-MS column. Ionization was accomplished by electron impact (EI) and the MSD operated under selected ion monitoring (SIM) mode. The HPChem Station software (Version B.01.00) was used for data analysis and quantitation.

Calibration Standards

A standard stock solution of parent pesticides with a concentration of 50 μ g mL⁻¹ in hexane was prepared from the pure standard (Pesticide Mix 11, 400 μ g mL⁻¹). Nine spiking solutions with concentrations ranging from 0.10 to 25 μ g mL⁻¹ were prepared from this stock solution by serial dilution with hexane. Spiking solutions for the pesticide metabolites were prepared from aliquots of Pesticide Mix 568 (400 μ g mL⁻¹) and MMA (400 μ g mL⁻¹). Eleven concentration levels ranging from 0.006 to 6.25 μ g mL⁻¹ were prepared by dilution of the stock solutions with methanol.

For the analysis of parent pesticides, a 16.0 μ g mL⁻¹ solution of the internal standard, 1,4-dichlorobenzene was prepared by diluting 4 μ L of the concentrated solution (4,000 μ g mL⁻¹) with 1 mL hexane. Four microliters of the internal standard were added to 100 μ L of unknown samples and to the quality control samples to yield a final concentration of 0.62 μ g mL⁻¹. For the metabolites analysis, a 25 μ g mL⁻¹ solution of the internal standard, 2-PBA in methanol was prepared from the pure standard (400 μ g mL⁻¹). Unknown hair samples and quality control samples were spiked with 125 μ L of this solution to yield a final concentration of 2.34 μ g mL⁻¹.

Sampling and Preparation

Maternal hair samples were obtained from participants in an ongoing study of maternal/fetal exposure to pesticides conducted in an agricultural town in Bulacan, Philippines [18]. The maternal hair samples were Asian black hair type and were obtained upon recruitment of pregnant women at mid-gestation into the study. Hair care regimen was not considered as a criterion for recruitment into the study. Hair specimens, about the size of a pencil eraser in diameter, were cut from the occipital area of the scalp [19]. The specimens were collected and sealed using a Psychomedics hair specimen collection kit (Psychomedics, CA, USA). All hair specimens were packed in a secondary, self-sealing polyethylene bag and shipped to the Pediatrics Laboratory at Wayne State University, in Detroit, Michigan, USA for pesticide analysis. Maternal hair samples were pulverized into a fine powder using a ball mill [9,12,14,20]. A 1.5 mL steel grinding cup with a 5 mm (diameter) steel grinding ball (Retsch, Germany) was used for this procedure.

Analysis of Parent Pesticides in Maternal Hair

For the analysis of parent pesticides, 50 mg samples of powdered hair were weighed out in test tubes, and 2 mL hexane was added. Three positive controls were spiked at a concentration of 31.25 μ g g⁻¹ and allowed to air dry. Once dry, 2 mL of hexane was added to the positive controls and to the negative control. Solid-liquid extraction of the pesticides was conducted for 6 h using an IKA Vibrax VXR orbital shaker (Fisher Scientific, PA, USA). The hexane extracts were separated by centrifugation at 2,900g for 15 min, and 1.6 mL aliquots were transferred to reactivials (Agilent). The extracts were dried to completion using nitrogen and then reconstituted to a final volume of 100 μ L using hexane. Four microliters of the 16.0 μ 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 [21] but was modified to result in a 0.62 $\mu\text{g mL}^{-1}$ internal standard concentration.

Analysis of Pesticide Metabolites in Maternal Hair

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 $\mu\text{g g}^{-1}$) and to a negative control and allowed to air dry. A methanolic/hydrochloric acid methyl ester technique [22] was used to derivatize the metabolites. This involved adding 1 mL of methanol and 1 mL of 10 N HCl to the hair and heating the suspension at 80°C for 20 min. After cooling the mixture 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). The toluene layer was separated by centrifugation at 2,900g for 15 min and a 1.5 mL aliquot was transferred into a reactivial for the GC–MS assay.

Quality Control

Black Asian hair with no detectable levels of pesticides and metabolites was used as 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. The mean relative percent recovery was calculated as the measured concentration divided by the calculated spiked concentration multiplied by 100 [23,24]. The inter-assay variation of the measured concentration was assessed by the percent coefficient of variation (% CV) which was calculated as the standard deviation of the measured concentrations divided by the mean and multiplied by 100. Batches for which the recovery did not fall within 80–120%, and/or having a % CV greater than 10%, were re-analyzed.

Gas Chromatography

Separation of the parent compounds involved injecting 1.0 μL of the sample into the front inlet of the gas chromatograph operating at 250 °C in the splitless mode. The flow of helium (carrier gas) was 1 mL min^{-1} . 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. For the metabolites, 2.0 μL 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 oven was programmed to start 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 post run of another 5 min.

Mass Spectrometry

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 a repeller and electron multiplier voltage of 19.90 and 1,318 V, respectively.

Fragment ions for the individual pesticides and metabolites were 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). 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.

Quantitation and Identification

A matrix-spiked calibration curve was used to determine the concentration of parent compounds and their selected metabolites in maternal hair samples. Matrix-spiked calibrators were prepared by spiking blank hair samples with the calibration standards. Once dry, the spiked calibrators were processed as described above. The range of concentration levels for the parent pesticides calibration curve was from 0.25 to 62.50 $\mu\text{g g}^{-1}$. For the metabolites, the concentration levels ranged from 0.18 to 187.50 $\mu\text{g g}^{-1}$. 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 pesticides and metabolites were identified by retention times and target and qualifier ions. The target to qualifier ion 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 limit of detection (LOD) for the individual analytes was established by the empirical approach [18,25,26], where the lowest concentration level at which all target and qualifier ions were detected, and where the target to qualifier ion response ratios were within the acceptable limits, was considered as the empirical limit of detection.

Quantitation and identification of the pesticides and metabolites was done by manual integration of the target and qualifier ion(s) using the HP Chem Station software. 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 [18,26].

Extraction Efficiency

The extraction efficiency for the parent pesticides was determined using spiked powdered hair [9,12]. Samples ($n = 3$) were spiked at a concentration of 1.56 $\mu\text{g g}^{-1}$ and processed as described above. The percent absolute recovery, calculated as the response of the analyte in the spiked sample divided by the response of the analyte in a pure standard at the same concentration multiplied by 100 [23,24], was used to evaluate the efficiency of the extraction. Absolute recoveries for the metabolites were not determined, as the derivatized form of the metabolites were not commercially available.

Results and Discussion

GC-MS Method

Several classes of parent pesticides and their selected metabolites were effectively separated on the DB5-MS column. Representative total ion chromatograms for the parent compounds (Fig. 1) and metabolites (Fig. 2) in maternal hair show adequate baseline separation of the individual analytes using the GC parameters established for each method. Run times were of reasonable length, 34 min for the parent compounds and 43.5 min for metabolites. Analysis of blank maternal hair showed minimal matrix effects on chromatographic separation of the analytes. In negative control hair, no interfering peaks were seen co-eluting at the expected retention times of the analytes.

Matrix spiked calibration curves for the individual analytes were mostly linear except for malathion which displayed a quadratic fit. The coefficients of determination for the parent compounds were greater than 0.998 with slopes ranging from 0.020 to 0.648, and intercepts between -0.169 and 0.280 . For the metabolites, the coefficients of determination were greater than 0.973. Slopes of the calibration lines ranged from 0.0082 to 2.46 with intercepts in the range of -0.025 – 0.00007 . The list of target ion and qualifier ion(s), retention times, and coefficients of determination for the parent pesticides and metabolites are given in Tables 1 and 2.

Our GC–MS parameters have been previously reported in our analysis of pesticides in blood [26] and meconium [19] for determining pesticide exposure during pregnancy. The extraction and clean-up procedures for these matrices were developed and validated individually. The use of a common GC–MS protocol was designed to provide a high output assay capable of detecting and quantitating pesticides from several maternal and infant compartments, including maternal hair, maternal blood, infant hair, cord blood, and meconium.

Method Optimization

Previous reports involving acid or alkaline hydrolysis of hair indicate that these methods of disintegrating the hair matrix are unsuitable for a multi-class pesticide assay, due to the lability of organophosphates, carbamates, and pyrethroids under these conditions [9,27]. The solvent extraction procedure has been used successfully to extract diazinon from the powdered hair matrix with good overall recoveries [9,12]. Using this technique we developed a 6 h hexane extraction procedure to extract several classes of pesticides from powdered hair. Prior experiments involving extraction times of 1, 3 and 6 h showed that optimum recovery was achieved at 6 h. The percent absolute recovery of the parent pesticides from the spiked powdered hair samples ranged from 72 to 127% (Table 3). Based on these data, the efficiency of our hexane extraction method for the different classes of pesticides ranged from 84 to 91% for the organophosphates; 80–111% for the organochlorines, 80–127% for the pyrethroids; 72% for the carbamate; and 105% for the chloroacetanilide.

Extraction of several classes of pesticides by solvent extraction from the solid matrix has been reported by Liu and Pliel [28] who analyzed dog hair for 34 pesticides including organochlorines, organophosphates and pyrethroids using hexane or hexane/ethyl acetate as solvent. They reported that although the hexane/ethyl acetate (4:1 v/v) combination favored the extraction of the slightly polar pyrethroids, this resulted in more background noise and diminished the sensitivity as compared to hexane alone. For the present study, non-target peaks were not detected co-eluting with the target compounds as a result of using hexane. However, this has resulted in lower recoveries of propoxur and cypermethrin which are slightly polar analytes.

Analytical Performance

Data from the “parent pesticide” analysis of triplicate spiked samples ($31.25 \mu\text{g g}^{-1}$) analyzed on eight separate days (Table 3) showed good accuracy and adequate extraction of the parent compounds from hair. The percent relative recovery of the parent compounds ranged from 87 to 112%. Inter-assay variation of the method was less than 11.00% with limits of detection in the range of $0.031\text{--}0.488 \mu\text{g g}^{-1}$. The analytical method is simple and rapid and can be done within a day as compared to other methods involving overnight acid digestion of the matrix [14,15] or solvent extraction of the analytes [9].

The analytical performance data for the pesticide metabolite method is given in Table 4. Our methanolic/hydrochloric acid methyl ester derivatization and liquid–liquid extraction technique resulted in adequate derivatization and extraction of several classes of metabolites in hair. Recovery of the metabolites was determined to be optimum using a 20 min derivatization time at 80°C , as compared to a lengthier procedure (i.e., 120 min) previously reported by Ormand et al. [29]. The metabolite method had good accuracy and precision. Percent relative recoveries calculated from samples ($n = 3$) spiked at a concentration of $46.86 \mu\text{g g}^{-1}$ and analyzed on eight separate days, ranged from 87.00 to 103.00% with between assay variation of less than 11%. The limits of detection for the analysis of pesticide metabolites ranged from 0.18 to $5.88 \mu\text{g g}^{-1}$. Absolute recoveries for the pesticide metabolites were not determined, as the derivatized form of the metabolites were not commercially available.

Human Study

Our analysis of maternal hair samples ($n = 282$) detected propoxur, bioallethrin, chlorpyrifos, malathion, and pretilachlor in the hair of pregnant women (Table 5). The exposure rate was highest to the indoor pesticides, bioallethrin (16.67%) and propoxur (13.12%), which are commonly used in 26 and 73% of homes, respectively [18]. Bioallethrin, the active ingredient in a slow burning mosquito coil (Katol™) was detected with the highest mean concentration in maternal hair followed by propoxur, the primary component of the kerosene based spray pesticide, Baygon™. A representative total ion chromatogram for a maternal hair sample positive for bioallethrin is shown in Fig. 3. Although less prevalent, maternal exposure to the commonly used agrochemicals in the area, including chlorpyrifos, pretilachlor, and malathion was also detected.

Data on the levels of pesticides found in human hair have been reported for many organochlorines [10,13–16] which we did not detect in this cohort. However, the mean concentrations of bioallethrin and propoxur in positive maternal hair samples are higher than previously reported organochlorine levels. The levels of malathion and chlorpyrifos detected in this study are also higher than those reported by Liu and Pleil [28].

Despite the detection of parent pesticides in the hair of pregnant women, our method did not detect the presence of their metabolites. Preliminary results from the study of Altshul et al. [10] suggest that pesticide metabolites tend to partition more towards blood rather than into hair, and especially with the non-persistent types [30] as these substances are biotransformed by the liver into more water-soluble compounds that are eventually excreted in the urine. Fritz and Balikova [31], in their review of the mechanisms of drug incorporation in hair, indicated that acidic pesticide metabolites may not readily deposit in hair. They reported that the passive diffusion of drugs from blood into the growing hair cells was favored by the chemicals' lipophilicity and basicity. The diffusion of acidic drugs or their metabolites, like 11-nor- Δ^9 -tetrahydro-cannabinol-11-carboxylic acid, however, are limited and are found in very low concentrations in hair. Thus, DDE which is more lipophilic and a persistent type, can be detected in hair [10,11] but acid metabolites of pesticides, such as MMA, 3-PBA, *cis*- and *trans*-DCCA, may require more sensitive methods for their detection and quantitation.

The high prevalence of exposure to bioallethrin and propoxur in this study population demonstrates the tendency of many indoor pesticides to bioaccumulate in the confinement of homes and thus be readily detected in hair due to indoor air pollution. Our method was able to show a high prevalence of maternal exposure to propoxur and bioallethrin, confirming their high rate of use in homes. Both pesticides are a cause of great concern as propoxur and bioallethrin have been reported to impair brain development in animals exposed to these toxicants in-utero [32,33].

Limitations of the Study

The empirical limits of detection of our methods are higher in comparison with other published values [10,13,15,28]. This lack of sensitivity may be attributed to the stricter guidelines involved in establishing the empirical limit of detection. However, this approach provides very strong specificity, resulting in a low probability of false positive results.

Our results suggest that the exposure of pregnant women to these pesticides may partly be passive in origin and may not be reflective of endogenous exposure since the samples were not decontaminated prior to the assay. Thus, the levels detected in maternal hair are a gross measure (i.e., exogenous and endogenous) of exposure to pesticides during pregnancy, and are not strictly indicative of internal body dose. Conversely, the method is simple and is capable of handling a large volume of samples, providing a rapid screening tool for detecting exposure

to a broad spectrum of environmental pesticides. Since the collection and handling of hair specimens is easier as compared to blood and urine, this method is of great value especially in the identification of exposed populations for biomonitoring studies or for implementation of preventive measures.

Conclusion

We have developed analytical methods for the detection of a broad spectrum of pesticides and their metabolites in maternal hair with good precision and accuracy. The methods are simple and provide a rapid screening tool for the detection of exposure to environmental pesticides. The application of these methods, particularly towards the analysis of parent pesticides in the hair of pregnant women in a study population in the Philippines, showed significant exposure of the women to household and agricultural pesticides.

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Fig 1. Total ion chromatogram (TIC) of maternal hair sample spiked with $31.25 \mu\text{g g}^{-1}$ of parent pesticide compounds

Fig 2. Total ion chromatogram (TIC) of maternal hair sample spiked with $46.86 \mu\text{g g}^{-1}$ of the pesticide metabolite compounds

Fig 3.
Total ion chromatogram (TIC) of maternal hair sample positive for bioallethrin ($299.70 \mu\text{g g}^{-1}$)

Table 1Retention time (t_R), target and qualifier ion(s), and coefficients of determination (r^2) for the parent pesticides

Target compounds	t_R (min)	Target and qualifier ions (m/z)	r^2
Internal standard			
1,4-Dichlorobenzene	8.22	152, 150, 115	N/A
Carbamate			
Propoxur	16.69	110, 152	0.998
Organophosphate			
Diazinon	18.69	304, 179, 137	0.999
Malathion	20.60	173, 127	0.998
Chlorpyrifos	20.88	197, 314, 97	1.000
Organochlorine			
Lindane	18.88	181, 183, 109	1.000
<i>p,p'</i> -DDT ^a	23.91	235, 237, 165	0.998
Pyrethroid			
Bioallethrin	21.66	123, 136, 79	0.999
Transfluthrin	19.88	163, 335, 91	1.000
Cyfluthrin	29.51	206, 226	1.000
Cypermethrin	30.43	181, 209	1.000
Chloroacetanilide			
Pretilachlor	22.77	238, 176, 202	1.000

^a 1,1,1-Trichloro-2,2-bis(*p*-chlorophenyl)ethane

Table 2Retention times (t_R), target and qualifier ion(s), and coefficients of determination (r^2) for the pesticide metabolites

Target compounds	t_R (min)	Target and qualifier ions (m/z)	r^2
Internal standard			
2-Phenoxbenzoic acid	29.06	197, 228	N/A
2-Isopropoxyphenol ^a	11.15	110, 152	0.998
<i>cis</i> -DCCA ^b	16.09	222, 187, 163	0.996
<i>trans</i> -DCCA ^c	16.38	222, 187, 163	1.000
3,5,6-Trichloro-2-pyridinol ^d	20.47	199, 169, 107	0.990
3-Phenoxybenzoic acid ^e	31.10	197, 228	0.999
Malathion monocarboxylic acid ^f	32.78	125, 159, 93	0.973
<i>p,p'</i> -DDE ^g	39.76	246, 248, 176	0.999

^a Metabolite of propoxur^b *cis*-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane carboxylic acid, metabolite of cyfluthrin and cypermethrin^c *trans*-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane carboxylic acid, cyfluthrin, cypermethrin and transfluthrin^d Metabolite of chlorpyrifos^e Metabolite of cypermethrin^f Metabolite of malathion^g 1,1-Dichloro-2,2-bis(*p*-chlorophenyl) ethylene, metabolite of *p,p'*-DDT

Table 3

Absolute recovery and analytical performance data for the analysis of parent pesticides in hair

Parent pesticide	Absolute recovery (%)	Relative recovery (%)	Limit of detection ($\mu\text{g g}^{-1}$)
Propoxur	72.25	96.53	0.031
Diazinon	84.92	87.20	0.031
Lindane	80.34	106.86	0.245
Transfluthrin	85.28	111.68	0.245
Malathion	90.57	102.05	0.123
Chlorpyrifos	88.39	110.65	0.245
Bioallethrin	99.53	101.82	0.488
Pretilachlor	105.15	102.54	0.061
<i>p,p'</i> -DDT ^a	111.11	110.07	0.031
Cyfluthrin	127.46	111.49	0.245
Cypermethrin	80.19	110.49	0.245

^a 1,1,1-Trichloro-2,2-bis(*p*-chlorophenyl)ethane

Table 4

Analytical performance data for the analysis of pesticide metabolites in hair

Pesticide metabolite	Recovery (%)	Coefficient of variation (%)	Limit of detection ($\mu\text{g g}^{-1}$)
2-IPP ^a	103.29	8.65	0.18
<i>cis</i> -DCCA ^b	94.02	7.95	0.37
<i>trans</i> -DCCA ^c	90.61	6.99	0.37
3,5,6-TCP ^d	95.94	9.67	2.94
3-PBA ^e	100.11	6.93	0.18
MMA ^f	86.89	6.03	5.88
<i>p,p'</i> -DDE ^g	90.61	10.57	0.18

^a2-isopropoxyphenol^b*cis*-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylic acid^c*trans*-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylic acid^d3,5,6-trichloropyridinol^e3-phenoxybenzoic acid^fMalathion monocarboxylic acid^g1,1-Dichloro-2,2-bis(*p*-chlorophenyl) ethylene

Table 5

Results of the analysis of Philippine maternal hair samples for parent pesticides

Parent pesticide	% Positive (<i>n</i> = 282)	Mean concentration($\mu\text{g g}^{-1}$) $\pm$ SD
Propoxur	13.12	2.80 $\pm$ 0.05
Diazinon	0.00	ND
Lindane	0.00	ND
Transfluthrin	0.00	ND
Malathion	2.84	4.85 $\pm$ 1.23
Chlorpyrifos	0.35	4.58
Bioallethrin	16.67	6.78 $\pm$ 2.10
Pretilachlor	0.35	2.68
<i>p,p'</i> -DDT ^a	0.00	ND
Cyfluthrin	0.00	ND
Cypermethrin	0.00	ND

ND not detected

^a 1,1,1-Trichloro-2,2-bis(*p*-chlorophenyl)ethane