Published in final edited form as:

Chromatographia. 2005 ; 62(11-12): 623–629.

Detection of Several Classes of Pesticides and Metabolites in Meconium by Gas Chromatography-Mass Spectrometry

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Abstract

A solid phase extraction method was developed to isolate multiple classes of parent pesticides from meconium. A methanolic/hydrochloric acid methyl ester derivatization with liquid-liquid extraction technique was also developed for the analysis of metabolites. Identification and quantitation was by electron impact gas chromatography-mass spectrometry. For the parent compounds and metabolites, recoveries in spiked meconium ranged between 72–109%, with coefficients of variation ranging from 1.55–16.92% and limits of detection between 0.01–4.15 $\mu\text{g g}^{-1}$. Meconium samples obtained from infants in the Philippines were assayed using these methods, and propoxur, cypermethrin, pretilachlor, malathion, 4,4'-dichlorodiphenyltrichloroethylene, bioallethrin, and cyfluthrin were detected.

Keywords

Gas chromatography-mass spectrometry; Solid phase extraction; Pesticides in meconium; Prenatal exposure

Introduction

Pesticide use has increased exponentially in recent years and has been linked to the increased incidence of neurodevelopmental deficits in children [1]. Prenatal exposure to pesticides has been associated with adverse pregnancy outcomes, such as birth defects and severe neurological disorders [1,2]. However, the effects of prenatal exposure to pesticides at ambient pollution concentrations are as yet unknown. Sensitive and specific methods for detecting prenatal exposure are therefore needed to study the incidence of such exposure and the deleterious effects associated with it. Currently, prenatal exposure to environmental toxicants is measured primarily by extracting pesticides or their metabolites from amniotic fluid, blood, or urine [3-5]. There are several disadvantages to utilizing these methods. Collection of amniotic fluid is highly invasive and is only performed for high-risk pregnancies [3]. Blood collection is also invasive, except in the case of umbilical cord blood. Many of the pesticides are highly lipophilic, and the relatively low lipid content of cord blood [3], amniotic fluid, and urine could result in underestimating exposure [4]. Urine collection in newborns can also be difficult and invasive. Additionally, pesticides and metabolites in blood and urine are transient and only reflect recent exposures.

Meconium is an ideal matrix for measuring prenatal exposure to xenobiotics due to the ease of collection, noninvasiveness, and the ability to measure a wide window of fetal exposure [6]. Meconium is formed starting from the 12th week of gestation and illicit drugs have been detected in meconium of spontaneously aborted fetuses as early as the 17th week of gestation [7]. Meconium can be collected for 1–3 days after birth and can analytically provide positive results of prenatal exposure to xenobiotics [6]. It is a repository for many compounds that the

fetus has been exposed to during gestation, including a wide variety of licit and illicit drugs, food additives, and heavy metals [6]. However, only a few studies have examined fetal exposure to pesticides by the analysis of meconium for either parent pesticides [8] or metabolites [9,10]. Ostrea et al. [8] investigated the levels of several pesticides in meconium samples (N=200) from Manila, Philippines. The compounds detected included lindane (73%), malathion (53%), diazinon (34%), and 4,4'-dichlorodiphenyltrichloroethylene (DDT, 27%). Whyatt and Barr [9] analyzed meconium from 20 infants in New York, NY for organophosphate metabolites and found diethylthiophosphate (DETP) in 100% of the samples analyzed, and diethylphosphate (DEP) in 95%. Hong and colleagues [10] collected 60 meconium samples in Rostock, Germany and detected 4,4'-dichlorodiphenyldichloroethylene (DDE), a metabolite of DDT, in 5% of the samples tested.

These researchers have demonstrated that several pesticides and metabolites can be detected in meconium. As yet, no study has reported detection of multiple classes of both parent pesticides and their metabolites in this matrix. Since actual exposure tends to come from a wide array of pesticides representative of varying classes [11], it is essential that methods exist for monitoring exposure to a broad range of compounds. The purpose of the present study was to develop and validate methods for the analysis of a broad spectrum of pesticides and their common metabolites in meconium by gas chromatography-mass spectrometry (GC-MS).

Experimental

Materials and Chemicals

Pesticide Mix 11 (custom synthesized by Cerilliant, Round Rock, TX), dissolved in hexane: acetone (90:10 (v/v)) 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%), 4,4'-dichlorodiphenyltrichloroethylene (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%). Internal standards, 1,4-dichlorobenzene-D₄ (1,4-DCB, certified assay: 99.0%, in methylene chloride) and 2-phenoxybenzoic acid (2-PBA, certified assay: 99.0%, in methanol), were obtained from Cerilliant (Round Rock, TX, USA). Malathion monocarboxylic acid (MMA, certified assay: 99.0%, in methanol) was obtained from Chem Service (West Chester, PA, USA). Pesticide Mix 568 was custom synthesized and purchased from EQ Laboratories, Inc. (Augsburg, Germany). This metabolite mixture in methanol contained: 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-2-pyridinol (TCP, certified assay: 98.8%), 3-phenoxybenzoic acid (3-PBA, certified assay: 99.2%), and 4,4'-dichlorodiphenyldichloroethylene (DDE, certified assay: 99.0%). Nitrogen (purity: 99.99%) and helium (purity: 99.999%) gases were purchased from Wilson Welding (Warren, MI, USA). All solvents were of analytical grade and used without further purification.

Study Population and Selection of Analytes

The study population was part of an ongoing study in Bulacan, Philippines, to determine fetal exposure to environmental toxicants. A survey of this agricultural province was conducted to determine the pesticides used most frequently on farms (N=82) and in homes (N=84), so that analytical procedures would be developed to test specifically for those compounds. If pesticide use was reported, the surveyor examined the container to ascertain the active ingredients or the brand name of the compounds used. Mother/infant dyads (N=166) were enrolled from this population and meconium was collected for pesticide analysis. The study was approved by the

Human Investigations Committees at both Wayne State University and at the University of the Philippines.

Pesticides reportedly used by at least 10% of those surveyed (Table 1) were selected for inclusion in the analytical method development, plus lindane and DDT. The choice of the metabolites for the pesticides was based on information in the literature (Table 1), availability of high purity standards, and the ability to incorporate the metabolite compounds into the analytical method.

GC-MS Instrumentation

A Hewlett Packard (HP) gas chromatograph (GC 6890) was utilized with helium as the carrier gas at a flow-rate of 1 mL min⁻¹. The injection port temperature was 250 °C and interface temperature was 280 °C. A DB-5MS 5%-phenyl-methylpolysiloxane capillary column (30 m × 0.25 mm ID × 1 µm, Agilent, Wilmington, DE, USA) was used for chromatographic separation of analytes. Detection was performed by an HP5973 Mass Selective Detector (MSD) using electron impact (EI). Identification and quantitation of compounds was performed using HP ChemStation software (version B.01.00) in selected ion monitoring (SIM) mode.

Calibration Standards

Calibrators were prepared using Pesticide Mix 11 (400 µg mL⁻¹) in serial dilution with hexane, to achieve concentrations of 0.10, 0.20, 0.39, 0.78, 1.56, 3.13, 6.25, 12.5, and 25 µg mL⁻¹. The levels of MMA and Pesticide Mix 568, both initially 400 µg mL⁻¹, were combined and serially diluted with methanol to generate concentrations of 0.10, 0.20, 0.39, 0.78, 1.56, 3.13, 6.25, 12.5, and 25 µg mL⁻¹. The internal standard for the parent pesticide analysis was 1,4-DCB (4000 µg mL⁻¹). Stock internal standard was prepared fresh prior to analysis each day by diluting in methanol to a concentration of 16 µg mL⁻¹. The internal standard for metabolite analysis was 2-PBA (400 µg mL⁻¹), which was diluted with methanol to 25 µg mL⁻¹.

Quality Control Materials

Meconium samples containing no detectable pesticides or metabolites (see limits of detection below, under “Quantification and Data Analysis”) were pooled and used as negative controls which were analyzed with each batch of samples. Aliquots of meconium were also utilized for preparation of spiked positive controls and spiked matrix calibrators. Spiked positive controls (N=3) at 6.25 µg g⁻¹ of parent pesticides and 4.15 µg g⁻¹ of metabolites for the corresponding assays, were used to monitor t_R (min), peak shape, target: qualifier ion ratios, percent recovery, inter-assay variation, and accuracy of the calibration curves. Mean percent recovery of spiked compounds was calculated as the measured concentration/spiked concentration × 100 for three spiked controls. The percent coefficient of variation (%CV) was calculated as the standard deviation/mean recovery × 100. For sample analysis to be considered valid, the negative control had to show the absence of any of the compound peaks, the recovery of compounds in spiked controls ranged between 80%–120%, and the CV% was <15%. If any of the quality control criteria were not met, the sample batch was reprocessed.

Sampling and Preparation

Laboratory Analysis of Parent Pesticides in Meconium—Meconium samples (N=166) were collected from the infants' diapers for two days after birth and pooled into sterile polypropylene containers (Phenix Research Products, Hayward, CA, USA). The samples were stored at -20 °C and shipped in bulk on dry ice to the laboratory at Wayne State University for analysis. The procedures for extraction and quantification of parent pesticides from meconium were adapted from Ostrea et al. [8], and were optimized for the compounds studied presently. Meconium (0.5 g) was weighed into a Mectest processor (Mectest Inc., Rowland

Heights, CA, USA) containing 5 mL methanol:phosphate buffer (75:25 (v/v), 0.1 M, pH 7.0) and was vortexed until homogeneous. Positive controls and calibrators were spiked with 100 μL of the appropriate concentration of pesticide mixture at this time and vortexed. The mixture was centrifuged for 30 min at 4500 g and the supernatant was saved for further analysis.

Solid phase extraction (SPE) columns (High Flow C18, Alltech, Deerfield, IL, USA) were conditioned with 3 mL acetonitrile:deionized water (90:10 v/v), dried by vacuum (-20 kPa) for 5 min, followed by 3 mL of methanol and then 3 mL of deionized water. An aliquot of meconium supernatant (2 mL) was passed through the column, which was then washed with 3 mL ammonium hydroxide: deionized water (20:80 v/v), then dried under vacuum for 5 min. The pesticides were eluted with 6 mL acetonitrile: deionized water (90:10 v/v) into a test tube which was then placed in a hot block at 35–40 $^{\circ}\text{C}$ and the eluate was evaporated under a gentle stream of nitrogen to approximately 400 μL . The eluate was transferred to a high recovery amber vial (Agilent), and the test tube rinsed with 200 μL acetonitrile: distilled water (90:10 v/v) and the volume was further added to the vial. The eluate was dried to completion under a gentle stream of nitrogen and reconstituted with 100 μL of methanol. The internal standard (1,4-DCB, 16 $\mu\text{g mL}^{-1}$) in methanol was added to a calculated concentration of 615.4 ng mL^{-1} , which is a modification of EPA Method 8270 [12] and the reconstituted sample was vortexed prior to analysis.

Laboratory Analysis of Pesticide Metabolites in Meconium—Meconium (0.5 g) was weighed into a Mectest processor tube. Phosphate buffer (0.1 M, pH 7.0, 2 mL) was added and the mixture was vortexed to homogenize. For calibrators and positive controls, 1 mL methanol containing the appropriate dilution of metabolites, along with internal standard (2-PBA), [13] was added to the meconium homogenate and samples were vortexed. For negative controls and samples, 1 mL methanol containing only 2-PBA was added, for a final concentration of 4.15 $\mu\text{g g}^{-1}$. Methanol (2 mL) was added and the mixture was vortexed in a Vibrax orbital shaker for 30 min at 2000 rpm (Fisher Scientific, Pittsburgh, PA, USA), followed by centrifugation at 4500 g for 30 min. The supernatant (4 mL) was transferred into a screw-capped test tube and methanolic/hydrochloric acid methyl ester derivatization [14] was performed by adding 610 μL of 10 M hydrochloric acid into each sample, topping with 100 μL toluene as a keeper for the volatile esters [15], then capping and heating the tubes for 30 min at 80 $^{\circ}\text{C}$ in a hot block. After cooling the mixture to room temperature in a water bath (5 min), the derivatized metabolites were extracted by adding 2 mL of toluene, then capping and vortexing the tubes for 10 min. Tubes were centrifuged at 4500 g for 30 min and the toluene layer (1.5 mL) was transferred to an amber vial, capped, and vortexed before analysis.

GC-MS Analytical Conditions

For parent pesticide analysis, 1 μL of extract was injected in the GC front inlet in splitless mode using the autosampler (HP 7683). The oven program commenced at an initial temperature of 70 $^{\circ}\text{C}$ held for 1 min. The temperature was increased at a rate of 10 $^{\circ}\text{C min}^{-1}$ to a final temp of 280 $^{\circ}\text{C}$ and held for 10 min. Total run time was 34 min. For the metabolites, 2 μL was injected into the GC-MS using the autosampler. The initial oven temperature of 100 $^{\circ}\text{C}$ was held for 1 min, increased at 4 $^{\circ}\text{C}$ to a final temp of 250 $^{\circ}\text{C}$ which was held for 5 min with a post-run of 5 min. Total run time was 43.5 min.

Quantification and Data Analysis

Target and qualifier ions for the various pesticides were determined from the GC-MS assay of pure standards and spiked meconium at low concentrations. The ions with the highest abundances and greatest stability across decreasing concentrations were selected as target and qualifier ions(s). For some compounds (propoxur, cyfluthrin, cyper-methrin, 2-IPP, and 3-PBA) only one qualifier was utilized, based on the prevalent ions as reported in the literature

[15] and logistical limitations associated with using the SIM mode. That is, the maximum number of ions that could be selected in SIM was a reason for limiting the ions. Quantitation of pyrethroids with stereogenic centers was performed by manually integrating the first elution peak of the three visible peaks for cyfluthrin and cypermethrin, as the response of the first peak was most consistent at the lower concentrations.

Spiked-matrix calibration curves were constructed for the quantitation of pesticide and metabolite compounds. Triplicate determinations were performed at each concentration level. The calibration curves for the analytes were constructed by plotting the mean response ratio (response of analyte/response of internal standard) against the amount ratio (concentration of analyte/concentration of internal standard). From the linear curve, the unknown and control sample concentrations were determined.

The curve fit for the calibration curves was a linear regression, except for TCP, where a quadratic fit was more appropriate. The limits of detection (LODs) for the individual pesticides were determined by the empirical method [16], taken as the lowest concentration which satisfied GC-MS criteria for positivity as follows: 1) a distinct peak was present at the correct retention time (± 0.03 min) as compared to the spiked positive controls, 2) the target and qualifier ions were present in the correct ratio(s), with an acceptability range of 20%–30%, 3) the spectral data closely resembled that of the analyte in spiked meconium, and 4) there was agreement among the five investigators regarding identity of the compound.

Results and Discussion

Analytical Method

Data were quantitated by manual integration using ChemStation software. The target and qualifier ions selected, t_R , and coefficients of determination (r^2) for the parent pesticides and metabolites are listed in Tables 2 and 3, respectively. Slopes were between 0.136–0.967 and intercepts were all negative, between -0.096 and -0.989 . The concentrations of the pesticides and metabolites are calculated by the ChemStation software, based on the calibration curves. For both methods, there was a lack of interference peaks at the corresponding t_R for each compound in the chromatograms for negative controls.

Method Optimization

The parent pesticide method was modified from Ostrea et al [8], as follows: 1) use of 75% methanol: 25% phosphate buffer to homogenize meconium; 2) centrifugation time was reduced to 30 min; 3) the Millipore filter extraction was omitted; 4) the concentration of the elution solvent was increased to 90%; 5) matrix-spiked calibrators were used rather than pure pesticide calibrators; and 6) lower pesticide and internal standard concentrations were employed to increase sensitivity.

The metabolite extraction technique was based on modifications to methods for extraction of pyrethroid and chlorpyrifos metabolites from urine [15,17,18]. Derivatization of samples involved a methanolic/HCl methyl esterification procedure [14]. We tested the effects of varying the concentration of methanol:phosphate buffer for suspension of meconium, as well as several derivatization and extraction times. The recovery of most compounds was highest at a 3:2 v/v ratio of methanol:phosphate buffer. Recoveries of MMA and TCP were sensitive to the duration of the methyl ester derivatization, and were optimized at 30 min. We discovered that topping with 100 μ L of toluene layer during derivitization was necessary to avoid evaporation of compounds, particularly TCP. Experimental comparison of extraction times of 5, 10, and 30 min demonstrated that 10 min provided the most efficient extraction with high recoveries.

Method Validation

Representative chromatograms of the spiked pesticides and metabolites extracted from meconium are shown in Fig. 1 and Fig. 2, respectively. The percent recovery and the inter-assay variation of the analysis of triplicate meconium controls (N=24) spiked with 11 pesticides at $6.25 \mu\text{g g}^{-1}$ are shown in Table 4. Recovery for the parent pesticides ranged from 82–109% and inter-assay variation was below 12% for all compounds. The limit of detection (LOD) in matrix-spiked standards for the parent compounds ranged from $0.10 \mu\text{g g}^{-1}$ for propoxur to $1.56 \mu\text{g g}^{-1}$ for lindane by the empirical method [16]. This method of determining the LODs was selected because it gives a realistic measure of sensitivity as the lowest concentration at which a compound can reliably be detected by GC-MS. As depicted in Table 5, recoveries for metabolites (N=21) at $4.15 \mu\text{g g}^{-1}$ ranged from 72–108% and CV's were below 10% for all compounds except DDE, which was 17%. LODs for the pesticide metabolites ranged from $0.31 \mu\text{g g}^{-1}$ for most compounds, to $4.15 \mu\text{g g}^{-1}$ for TCP.

Analysis of Pesticides and Metabolites in Meconium

In meconium samples collected from infants born in an area where heavy pesticide use was reported, propoxur was the most frequently detected pesticide (32.53%, Table 6), which reflects the high rate of use (73%) of propoxur-containing spray pesticide (Baygon®) in the homes. Fig. 3 shows a representative chromatogram of a meconium sample which contained propoxur. Other compounds detected in meconium were cypermethrin (6.02%), pretilachlor and DDT (1.81% each), malathion (1.20%), and bioallethrin and cyfluthrin (0.60% each). Pesticide metabolites were not detected in any of the samples analyzed.

Method Comparison

Only one other published study had previously examined prenatal parent pesticide exposure through analysis of meconium [8]. Our modifications of that method included the addition of several compounds to the analysis, shorter centrifugation time, omission of the Millipore filtration, injecting a smaller volume of extract into the GC, and using the SIM mode. We also utilized lower calibration standards, and matrix-spiked calibrators. These modifications resulted in a more rapid procedure and lower LODs in the present study.

None of the metabolites examined were detected in the present cohort. However, we have found DDE in samples obtained from another area of the Philippines using the method described in this report (unpublished data). Others who have studied pesticide metabolite presence in meconium detected DDE [10] and organophosphate metabolites [9]. Hong et al [10] detected DDE at a concentration of 11.1 ng g^{-1} , which is below our LOD. Our method was optimized for many classes of metabolites, especially the pyrethroids, whereas Hong and colleagues were selectively searching for DDE, allowing for a higher sensitivity for that compound. We did detect DDT, the parent compound for DDE, in nearly 2% of meconium samples in the present cohort. Whyatt & Barr [9] found DETP, an organophosphate metabolite, in meconium. We attempted to analyze for this compound using our current metabolite extraction method. However, SIM for DETP was discontinued due to difficulty in the chromatographic separation from TCP.

In developing the method for detecting pesticide metabolites, our focus was on extracting major metabolites of pyrethroids [13,15,18] and chlorpyrifos [17]. Many of the metabolites we attempted to detect were from pyrethroids and we were unsuccessful in detecting these compounds, probably due to their relatively short half-lives. Leng, et al. [13] demonstrated in an elimination experiment of cyfluthrin that metabolites are eliminated quickly, with a half-life of 6.4 h. Perhaps development of separate methods for the various metabolites would have yielded lower LODs; however, the cost, time, and volume of sample required rendered this approach impractical.

Conclusion

Sensitive and precise methods were developed for detecting a wide array of pesticides and a number of metabolites in meconium. Several pesticides were detected in the meconium of subject infants from an area where pesticide use is widespread. Propoxur, an acetylcholinesterase inhibitor, was detected in a high percentage of samples (32.53%). The clinical implications of this prenatal exposure on infant development are currently under study. As the burden of pesticides and other toxicants increases in the global environment, the exposure of the fetus to these toxicants, which are predominantly neurotoxins to the developing brain, cannot be overemphasized. The development of techniques to determine fetal exposure to these compounds will facilitate the identification of infants at risk and the institution of appropriate measures to initiate interventions and reduce further exposure.

Acknowledgements

This study is supported by the National Institutes of Health/National Institute of Child Health and Development (R01HD039428) and the U.S. Environmental Protection Agency (R829395). We would like to thank the following members of the research team in the Philippines for the recruitment of subjects and the collection of specimens: Maria Esterlita Villanueva-Uy, M.D., Essie Ann M. Ramos, M.D., Abner M. Hornedo, M.D., Patrocinio C. Mateo, M.D., Lilibeth R. Avendaño, Rubilyn S. Obando, Maribel V. Santiago, Roberta S. Briones, Rozza D.C. Villavicencio, and Cecilia C. Santiago.

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Fig 1.
A representative electron-impact total ion chromatogram of a meconium sample spiked with $6.25 \mu\text{g g}^{-1}$ of parent pesticides

Fig 2.

A representative electron-impact total ion chromatogram of a meconium sample spiked with $10.36 \mu\text{g g}^{-1}$ pesticide metabolites

Fig 3.
A representative selected ion monitoring (SIM) chromatogram of a meconium sample positive for propoxur ($0.82 \mu\text{g g}^{-1}$)

Table 1

Pesticides commonly used in the study site, the percentages of use reported in homes and on farms, and corresponding metabolites as determined from the literature

Class	Pesticide	Selected Metabolite(s)	References
Carbamate	Propoxur 73% ¹	2-IPP ²	[19,20]
Chloroacetanilide	Pretilachlor 28% ³	Not applicable	
Organochlorine	DDT ⁴	DDE ⁵	[19,21]
	Lindane	Not applicable	
Organophosphate	Chlorpyrifos 6%, 37% ^{1,3}	TCP ⁶	[17,19]
	Diazinon 12% ³	Not applicable	
	Malathion 15% ³	MMA ⁷	[19]
Pyrethroid	Bioallethrin 26% ¹	Not applicable	
	Cyfluthrin 73% ¹	<i>cis</i> -DCCA ⁸ , <i>trans</i> -DCCA ⁹	[13,15,19,22]
	Cypermethrin 31% ³	<i>cis</i> -DCCA ⁸ , <i>trans</i> -DCCA ⁹ 3-PBA ¹⁰	[13,15,19,23]
	Transfluthrin 11% ¹	<i>trans</i> -DCCA ⁹	[24]

¹ Percentage of homes reporting pesticide use (N=84)

² 2-Isopropoxyphenol

³ Percentage of farms reporting pesticide use (N=82)

⁴ 4,4' dichlorodiphenyltrichloroethylene

⁵ 4,4' dichlorodiphenyldichloroethylene

⁶ 3,5,6 Trichloro-2-pyridinol

⁷ malathion monocarboxylic acid

⁸ *cis*-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylic acid

⁹ *trans*-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylic acid

¹⁰ 3-Phenoxybenzoic acid

Table 2Target and qualifier ion(s), retention time (t_R) and coefficients of determination (r^2) for parent pesticides

Compounds	Target Ion m/z	Qualifier Ions m/z	t_R (min)	r^2
1,4-dichlorobenzene (internal standard)	152	150, 115	8.33	N/A
Propoxur	110	152	16.81	0.987
Diazinon	304	179, 137	18.84	0.996
Lindane	181	183, 109	19.03	0.986
Transfluthrin	163	91, 335	20.03	0.997
Malathion	173	127	20.76	0.986
Chlorpyrifos	197	314, 97	21.03	0.998
Bioallethrin	123	79, 136	21.85	0.991
Pretilachlor	238	176, 202	22.96	0.991
DDT	235	237, 165	24.07	0.996
Cyfluthrin	206	226	29.92	0.981
Cypermethrin	181	209	30.84	0.986

Table 3Target and qualifier ion(s), retention time (t_R) and coefficients of determination (r^2) for the pesticide metabolites

Compounds	Target Ion m/z	Qualifier Ions m/z	t_R (min)	r^2
2-IPP ¹	110	152	11.60	0.993
<i>cis</i> -DCCA ²	222	187,163	16.63	0.997
<i>trans</i> -DCCA ³	222	187,163	16.92	0.996
TCP ⁴	199	169,107	20.89	0.987
2-PBA ⁵	197	228	29.73	N/A
3-PBA ⁶	197	228	31.79	1.00
MMA ⁷	125	93,159	33.41	0.986
DDE ⁸	246	248,176	40.62	0.991

¹ 2-Isopropoxyphenol² *cis*-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylic acid³ *trans*-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylic acid⁴ 3,5,6 Trichloro-2-pyridinol⁵ 2-Phenoxybenzoic acid⁶ 3-Phenoxybenzoic acid⁷ malathion monocarboxylic acid⁸ 4,4' dichlorodiphenyldichloroethylene

Table 4

Recovery, precision, and limits of detection for the analysis of parent pesticides in meconium (N=24) spiked with pesticides at 6.25 $\mu\text{g g}^{-1}$

Parent Pesticide	Recovery (%)	CV(%) Inter-assay ^I	Limit of Detection ($\mu\text{g g}^{-1}$)
Propoxur	95.43	10.42	0.10
Diazinon	82.44	10.20	0.20
Lindane	87.73	9.32	1.56
Transfluthrin	105.17	6.62	0.39
Malathion	97.64	11.37	0.39
Chlorpyrifos	92.63	7.65	0.39
Bioallethrin	95.35	10.45	0.39
Pretilachlor	93.19	9.14	0.20
DDT	88.69	11.40	0.78
Cyfluthrin	109.25	8.50	0.39
Cypermethrin	105.10	7.51	0.78

^I Percent coefficient of variation, calculated as the standard deviation/mean recovery $\times 100$

Table 5

Recovery, precision, and limits of detection for the analysis of pesticide metabolites in meconium (N=21) spiked at 4.15 $\mu\text{g g}^{-1}$

Pesticide Metabolite	Recovery (%)	CV(%) ^I	Limit of Detection ($\mu\text{g g}^{-1}$)
2-IPP	76.77	1.55	0.31
<i>cis</i> -DCCA	81.00	6.15	0.31
<i>trans</i> -DCCA	83.13	3.96	0.31
TCP	103.30	3.83	4.15
3-PBA	92.60	2.69	0.31
MMA	108.00	9.79	0.62
DDE	72.27	16.92	0.31

^I Percent coefficient of variation, calculated as the standard deviation/mean recovery $\times 100$

Table 6

Percent positive and concentration of parent pesticides in meconium samples obtained in a study population (N=166)

Parent Pesticide	% Positive	Concentration ($\mu\text{g g}^{-1}$, mean $\pm$ SD)
Propoxur	32.53%	0.83 $\pm$ 0.22
Diazinon	0%	nd ¹
Lindane	0%	nd ¹
Transfluthrin	0%	nd ¹
Malathion	1.20%	2.15 $\pm$ 0.87
Chlorpyrifos	0%	nd ¹
Bioallethrin	0.60%	0.97
Pretilachlor	1.81%	0.90 $\pm$ 0.11
DDT	1.81%	0.93 $\pm$ 0.57
Cyfluthrin	0.60%	0.93
Cypermethrin	6.02%	1.20 $\pm$ 0.17

¹
nd = not detected