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Detection of prenatal exposure to several classes of environmental toxicants and their metabolites by gas chromatography–mass spectrometry in maternal and umbilical cord blood

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Abstract

We developed a sensitive method to detect several classes of pesticides and their metabolites in maternal and cord whole blood using electron-impact gas chromatography–mass spectrometry (GC–MS). The method can detect parent and metabolite compounds at levels of <0.10 and 0.20 μg/mL, respectively, with high accuracy and recovery. Analysis of blood from mother–infant dyads from an area of high pesticide use in the Philippines showed detectable levels of propoxur, 3-phenoxybenzoic acid (3-PBA), and 1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethylene (*p*,*p*′-DDE) in maternal and umbilical cord blood. GC–MS analysis of several classes of parent pesticides and their metabolites in maternal and cord blood provides a sensitive and specific method to detect pesticide exposure during pregnancy.

Keywords: Pyrethroids; Pesticides; Whole blood; Parental pesticide exposure; Cord blood; GC–MS; Environmental pollutants; Carbamates; Organophosphates; Herbicide

1. Introduction

The use of pesticides is widespread and global. In the United States, annual expenditure on pesticides since 1980 has nearly doubled from 5.8 to 11.2 billion dollars (US$) [1]. While the role of pesticides is important, particularly from the standpoint of agricultural and health issues, the ubiquitous presence of these compounds in the environment poses significant public health concerns as well.

Pesticide residues found in the population and at all levels of the food chain indicate persistent use and bioaccumulation that ultimately lead to elevated levels of these toxicants in humans. Robust methods are therefore needed to evaluate human exposure and health risks. However, while the action of many pesticides is known in the targeted species, few studies of human exposure in the pregnant woman and her fetus have been conducted. Recent studies have shifted focus from acute toxicity to effects of chronic low-level environmental exposures and the threats of such exposure, particularly in young children and infants [2–8]. An increasing incidence in children of mental retardation, learning disabilities, autism, and attention deficit-hyperactivity disorders is now suspected to be related to pesticide exposure [7].

Maternal exposure to pesticides may occur via ingestion of contaminated food or water, inhalation of contaminated air, or dermal absorption. However, it is the chemical equilibrium between the mother and her fetus through the placenta, which actively distributes these toxicants in the fetus. The transfer of lipids and lipoproteins from maternal tissues to the developing fetus favors the passive diffusion of xenobiotics across the placental membrane [9]. Additionally, significant

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partitioning between the maternal and fetal compartments leads to deposition in the lipid-rich tissues of the fetus, where poor enzymatic activity increases the accumulation of lipophilic toxicants [10]. Considering that the majority of maternal exposure is at sub-acute levels, few observable effects in the mother and fetus are likely to be present at birth. Therefore, for a clear estimation of health risks to the newborn, a valid assessment of the total amount of maternal and fetal exposure to pesticides is necessary.

Physiologically, the fetus is more susceptible to pesticide exposure than adults. Neonates have a higher percentage of total body water and less body fat to serve as storage sites for these lipophilic compounds [11]. Less deposition of toxicants can lead to higher circulating blood levels in the newborn. Additionally, renal clearance rates and hepatic enzyme activity can vary markedly throughout gestation and following birth. Low clearance rates or hepatic metabolism may result in greater toxicity in the fetus and infant. Cantalamessa et al. showed that 8-day-old rats lack permethrin-specific and cypermethrin-specific esterases necessary for pyrethroid metabolism [12]. Toxicity of permethrin to the neonatal rat was nearly five-fold while toxicity to cypermethrin was nearly six-fold compared to adult rats; furthermore, the findings by Cantalamessa suggest that neonatal rats lack the enzymes necessary to catalyze the metabolism of pyrethroids, ultimately increasing their susceptibility to these pyrethroid compounds.

Rapid development of the central and peripheral nervous systems renders the fetus more vulnerable to the neurotoxic effects of pesticides. Disturbances in neuronal growth may alter cell development at critical periods, ultimately interfering with hormones (i.e., endocrine disruptors), neurotransmitters, and other neurotrophic factors, which are crucial for normal brain function [13–15]. Unfortunately, few studies have provided reliable data to measure fetal exposure to environmental pesticides. To advance research in this area, we developed a sensitive and specific method of detecting eleven pesticide compounds and their major metabolites in maternal and umbilical cord whole blood. The advantage of using whole blood over serum or plasma is due to the high lipophilicity of pesticides which favor the concentration of these compounds in the erythrocytes [16]. In addition, while other matrices are available for analysis, we chose to analyze whole blood because blood volume is highly regulated such that water intake and other factors do not significantly alter it.

Our analytical method utilized liquid–liquid extraction of the toxicants in whole blood and subsequent analysis by electron-impact GC–MS. The method was then applied in a large, clinical study to detect maternal and fetal exposure to pesticides in a region of the Philippines known for significant use of agricultural and household pesticides. A preliminary survey of the region indicated high use of several classes of pesticides, including pyrethroids (bioallethrin, cyfluthrin, cypermethrin, and transfluthrin), carbamate (propoxur), organophosphates (chlorpyrifos, diazinon, and malathion), organochlorines (*p,p′*-DDT and lindane), and an herbicide (pretilachlor). We undertook this analysis to identify biomarkers of exposure in blood to assist in the development of suitable exposure intervention programs if necessary.

2. Experimental

2.1. Materials

All parent pesticides (Pesticide Mix 11) and internal standards (1,4-dichlorobenzene-D_4 (1,4-DCB, certified assay: 99.0%, dissolved in hexane) and 2-phenoxybenzoic acid (2-PBA, certified assay: 99.0%, dissolved in methanol)) were obtained from Cerilliant (Round Rock, TX, USA). Pesticide Mix 11 was dissolved in hexane and 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′*-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%). Malathion monocarboxylic acid (MMA, certified assay: 99.0%, dissolved 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). Pesticide Mix 568 was dissolved in methanol and contained: 2-isopropoxyphenol (certified assay: 98.5%), *cis*-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylic acid (certified assay: 99.5%), *trans*-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylic acid (certified assay: 99.5%), 3,5,6-trichloro-2-pyridinol (certified assay: 98.8%), 3-phenoxybenzoic acid (certified assay: 99.2%), and *p,p′*-DDE (certified assay: 99.0%). Nitrogen (99.99% pure) and helium (99.999% pure) were purchased from Wilson Welding (Warren, MI, USA). All solvents were analytical grade and used without further purification.

2.2. Instrumentation

The analyses were performed using a Hewlett-Packard 6890 gas chromatograph interfaced to a Hewlett-Packard 5973 mass spectrometer equipped with a 7683 Series Autosampler/Injector. Analytical separation was achieved on a 30 m J&W DB-5MS capillary column ([5%-phenyl]-methylpolysiloxane, 0.25 mm I.D., 1 μm film thickness) obtained from Agilent (Wilmington, DE, USA). Data was processed using the Enhanced Data Analysis software package (HP Chem Station version B.01.00 software) supplied with the mass spectrometer.

2.3. Calibration standards

For the parent pesticides, nine pure standards were prepared to encompass the entire calibration curve range. By

serial dilution in hexane, solutions with concentrations of 0.10, 0.20, 0.39, 0.78, 1.56, 3.13, 6.25, 12.5, and 25 μg/mL were prepared from the 400 μg/mL pure standard Pesticide Mix 11. For the pesticide metabolites, nine pure standards were prepared from the separate solutions containing 400 μg/mL Pesticide Mix 568 and 400 μg/mL MMA by serial dilution in methanol. The pure standards had concentrations of 0.10, 0.20, 0.39, 0.78, 1.56, 3.13, 6.25, 12.5, and 25 μg/mL.

2.4. Internal standards

To control for instrumental variation during analysis, the extracts of all samples were spiked with an internal standard. Stock internal standard solutions for parent pesticide analysis were prepared daily by diluting 1,4-DCB (4000 μg/mL) in 1 mL hexane. Prepared standards and samples were later spiked with 4 μL of the diluted internal standard (16.0 μg/mL) to yield a final concentration of 0.62 μg/mL. For pesticide metabolite analysis, 2-PBA (400 μg/mL) was diluted to 50 μg/mL with methanol. The resulting internal standard solution was used for spiking blood samples to yield a final concentration of 2.34 μg/mL.

2.5. Quality control materials

Whole blood quality control samples were prepared using samples collected from subjects with no known exposure to the pesticides being analyzed (i.e., analysis of these samples did not detect levels of contamination above the LOD for each compound). The samples were divided into 0.5 mL aliquots and stored at −20 °C until used. For quality assurance, each sample batch included three spiked positive controls and one negative control. Because no quality control material was commercially available, we used spiked, pooled blood at a concentration of 1.56 or 0.78 μg/mL for parent pesticides or metabolite analysis, respectively. Each run was considered acceptable if: (1) the mean recovery was ≥80%, and (2) the coefficient of variation was <15%. Mean recovery was defined as the calculated concentration of the spiked control divided by the expected concentration multiplied by 100. In instances where these criteria were not met, analysis of the entire sample set was repeated.

2.6. Sample preparation

For each analysis, spiked calibrators, quality control samples, and whole blood samples were prepared identically unless noted. Frozen blood samples were thawed at room temperature and then vortexed to ensure homogeneity prior to the analysis.

For parent pesticide analysis, a 500 μL aliquot of whole blood was transferred to a test tube. Calibrators and positive controls were spiked with 100 μL of pesticide standard. Samples were sonicated in an ultrasonic water bath for 30 min to disrupt the erythrocytes and leukocytes. Following sonication, 1 mL of buffered methanol (25:75 (v/v), methanol/phosphate buffer, pH 7.0) was added and samples were vortexed for 10 s. The pesticides were extracted by adding 3.1 mL of hexane to all unknown samples and the negative control, while 3 mL of hexane was added to the spiked positive controls. The samples were vortexed using an IKA Vibrax VXR orbital shaker from Fisher Scientific (Pittsburgh, PA) for 1 h. The samples were then centrifuged for 30 min at 3000 × *g*. A 2.8 mL aliquot of the supernatant was transferred to a clean test tube and dried to completion under a gentle stream of nitrogen. The concentrate was reconstituted in 100 μL of hexane, vortexed for 30 s, and transferred to a reactivial containing a glass insert. Our prepared standards and samples were spiked with 4 μL of the diluted stock internal standard (16 μg/mL) to yield a final concentration of 0.62 μg/mL, representing a modification made to the final concentration of internal standard used in EPA Method 8270 [17]. The reactivial was capped and vortexed before being analyzed.

For metabolite analysis, a 500 μL aliquot of whole blood was transferred to a test tube. A 500 μL aliquot of the diluted internal standard 2-PBA (50 μg/mL) was added and each sample was vortexed [18]. Calibrators and positive controls were spiked at this time. The whole blood was suspended in 1 mL of phosphate buffer (pH 7.0). For derivatization, separate 1 mL aliquots of methanol and concentrated hydrochloric acid (10.0 M) were added, and the samples were then vortexed gently and capped. The samples were derivatized using a methanolic/hydrochloric acid methyl ester derivatization procedure and heated at 80 °C for 20 min as described by Kitson et. al. [19]. A 2 mL aliquot of toluene was added to each tube. The samples were capped tightly, and the derivatized pesticide metabolites were extracted by vortexing for 20 min in a Vibrax orbital shaker. The samples were centrifuged for 15 min at 3000 × *g*. A 1.5 mL aliquot of the extract was transferred to a reactivial for analysis.

2.7. GC–MS analysis conditions

For parent pesticide analysis, one microliter of the concentrated extract was injected using splitless injection GC–MS. The initial column temperature of 70 °C was held for 1 min and increased at a rate of 10 °C/min to a final temperature of 300 °C, held for 10 min. The carrier gas was helium at a flow rate of 37 cm/min. Total analysis run time was 34 min. The injector and transfer line temperature were maintained at 250 and 280 °C, respectively, and the mass spectrometer was operated in selected ion monitoring (SIM) mode at +70 eV with a dwell time of 40 ms.

All instrumental conditions for pesticide metabolite analysis were identical unless noted. For metabolite analysis, 2 μL of the concentrated extract was injected using splitless injection GC–MS. The initial column temperature of 100 °C was held for 1 min and increased at a rate of 4 °C/min to a final temperature of 250 °C, held for 5 min. Total analysis run

Table 1
GC–MS Retention time (RT) with target and qualifier ions for the parent pesticides

	Target Ion (*m*/*z*)	Qualifier Ion(s) (*m*/*z*)	RT (min)
Internal standard			
1,4-Dichlorobenzene	152	150, 115	8.43
Carbamate			
Propoxur	110	152	16.95
Chloroacetanilide			
Pretilachlor	238	176, 202	23.07
Organochlorines			
p,*p*′-DDT[a]	235	237, 165	25.05
Lindane	181	183, 109	19.16
Organophosphates			
Chlorpyrifos	197	314, 97	21.18
Diazinon	304	179, 137	18.98
Malathion	173	127	20.91
Pyrethroids			
Bioallethrin	123	79, 136	21.99
Cyfluthrin	206	226	30.79
Cypermethrin	181	209	31.82
Transfluthrin	163	91, 235	20.18

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

time was 43 min. The metabolite method had a dwell time of 100 ms.

2.8. Quantification and data analysis

Chromatographic peaks from each spiked standard, quality control sample, or whole blood sample were integrated using the Hewlett-Packard Chem Station software. Tables 1 and 2 list the target and qualifier ion(s) monitored, as well as the retention time (RT) for each parent pesticide or metabolite compound, respectively. Target and qualifier ions were selected after injecting the pure standard (for metabolites, the pure standard was first derivatized) in SCAN mode, thereby determining the fragment ions with the highest abundances; chosen target and qualifier ions were then cross-referenced with existing methodologies. Though most compounds had two qualifier ions, propoxur, cyfluthrin, cypermethrin, 2-isopropoxyphenol, and 3-phenoxybenzoic acid each had one qualifier ion, in accordance with previous literature [20,21]. We selected target and qualifier ions with ratios that were consistent and stable over time. Though we could have selected a second qualifier ion for some compounds, other fragment ions showed inconsistent or weak abundances at low concentrations; for example, for propoxur only two ions are in high abundance (*m*/*z* 110 and 152). To ensure specificity as a condition of quality control and for peak verification in exposed subjects, target to qualifier ratios were considered acceptable within a range of ±20%.

Table 2
GC–MS retention time (RT) with target and qualifier ions for the pesticide metabolites

	Target ion (*m*/*z*)	Qualifier ion(s) (*m*/*z*)	RT (min)
Internal standard			
2-Phenoxybenzoic acid	197	228	29.73
Metabolite			
2-Isopropoxyphenol[a]	110	152	11.60
cis-DCCA[b]	222	187, 163	16.63
trans-DCCA[c]	222	187, 163	16.92
3,5,6-Trichloro-2-pyridinol[d]	199	169, 107	20.89
3-Phenoxybenzoic acid[e]	197	228	31.79
MMA[f]	125	93, 159	33.41
p,*p*′-DDE[g]	246	248, 176	40.62

[a] Metabolite of propoxur.
[b] *cis*-3-(2,2′-dichloro-vinyl)-2,2′-dimethylcyclopropane carboxylic acid, metabolite of the pyrethroids.
[c] *trans*-3-(2,2′-dichloro-vinyl)-2,2′-dimethylcyclopropane carboxylic acid, metabolite of the pyrethroids.
[d] Metabolite of chlorpyrifos.
[e] Metabolite of the pyrethroids.
[f] Malathion monocarboxylic acid, metabolite of malathion.
[g] 1,1-Dichloro-2,2-bis(*p*-chlorophenyl)ethylene, metabolite of *p*,*p*′-DDT.

Our parent pesticide method contains synthetic pyrethroid pesticides, namely cyfluthrin and cypermethrin, which are often prepared as racemic mixtures containing cyclopropane rings with stereogenic centers. The different physicochemical properties of the resulting diastereomers allow for their adequate separation on the achiral DB-5MS column, as shown in Fig. 1. For the purpose of quantitation, we integrated the first elution peak of the three visible peaks for each analyte, as it yielded the most consistent response at lower concentrations [22].

Calibration curves were constructed with several different pesticide or metabolite concentrations as previously indicated. Three repeat determinations were performed for each concentration with the mean response ratio plotted versus the amount ratio on the calibration curve. The response ratio was determined as the response of the analyte of interest divided by the response of the internal standard. The amount ratio was defined as the concentration of the analyte divided by the concentration of the internal standard.

Linear regression analysis data is shown in Table 3. From the linear curve, the unknown and control sample concentrations could be extrapolated. The lowest concentration level at which the analyte response could be determined and the relative response ratios of the qualifier ions fell within the accepted range was considered to be the empirical limit of detection (LOD), listed in Table 3 [23,24].

2.9. Study subjects

We also analyzed whole blood obtained from pregnant women and their newborn infants who were participants in a clinical study to detect prenatal exposure to pesticides. The cohort resided in a rural area in the province of Bulacan, Philippines, where a preliminary survey reported high use of pesticides in the home (43.1%) and farm (22.1%) and minimal use of gloves while handling pesticides. Whole blood was collected from the pregnant mother at the time of recruitment

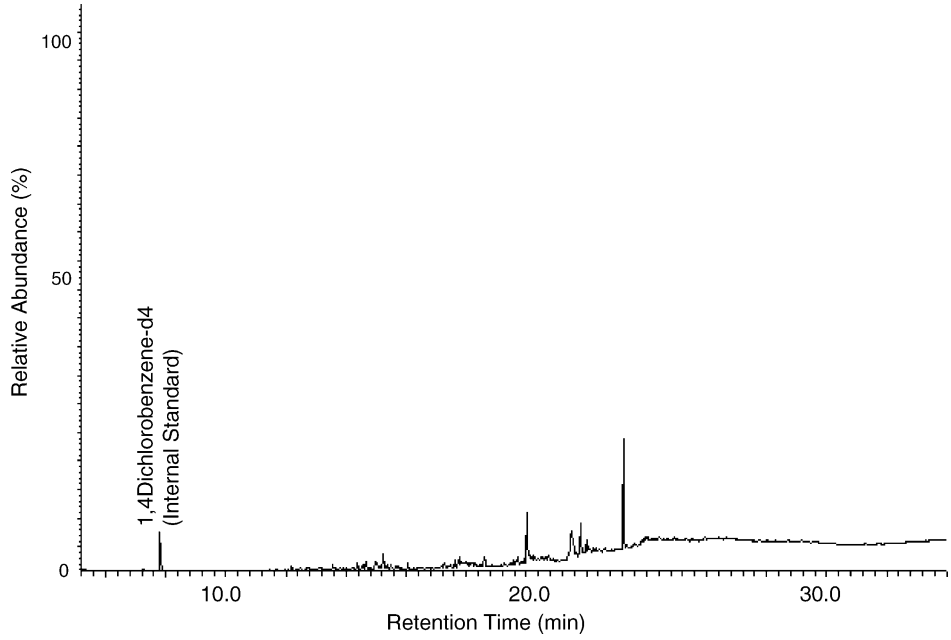

Fig. 1. A representative electron-impact total ion chromatogram of a whole blood negative control sample showing no interference peaks.

Table 3
Analytical performance data for the parent pesticide and metabolite methods

	R^2[a]	LOD (μg/mL)[b]	Recovery (%)[c,d]	CV (%)[d,e]
Parent pesticide				
Propoxur	0.998	<0.10	99.4	1.13
Pretilachlor	0.998	<0.10	116.9	14.4
p,p′-DDT	0.997	0.20	84.0	4.2
Lindane	0.998	<0.10	142.1	0.8
Chlorpyrifos	0.997	<0.10	137.5	5.1
Diazinon	0.997	<0.10	117.8	1.9
Malathion	0.997	0.20	139.6	1.5
Bioallethrin	0.997	<0.10	116.9	14.4
Cyfluthrin	0.987	<0.10	122.1	5.7
Cypermethrin	0.990	1.56	107.7	5.8
Transfluthrin	0.997	<0.10	124.1	4.7
Metabolite				
2-Isopropoxyphenol	0.998	<0.20	101.7	8.3
cis-DCCA	0.996	<0.20	109.2	5.6
trans-DCCA	0.998	<0.20	105.2	9.7
3,5,6-Trichloro-2-pyridinol	0.994	<0.20	54.4	13.9
3-Phenoxybenzoic Acid	0.999	0.78	67.6	3.9
MMA	0.994	<0.20	121.9	9.7
p,p′-DDE	0.998	0.78	98.2	10.6

[a] Obtained from linear regression analysis of the [analyte]/[internal standard] vs. the response (analyte)/response (internal standard).
[b] Empirical limit of detection (μg/mL).
[c] Presented as measured concentration divided by expected concentration multiplied by 100.
[d] For parent pesticides, $n = 45$ samples spiked at 1.56 μg/mL; for pesticide metabolites, $n = 21$ samples spiked at 0.78 μg/mL.
[e] Inter-assay coefficient of variability, calculated from analysis of quality control samples.

at mid-gestation (MBA) and at delivery (MBB). Cord blood was collected from the infant at birth. The blood was collected in Vacutainer test tubes containing EDTA and stored at −18 °C until the samples were sent on dry ice to our research laboratory at Wayne State University. The study was approved by the institutional review boards at Wayne State University and the University of the Philippines, Manila.

3. Results and discussion

3.1. Analytical method

The parent pesticides and their respective classes are listed in Table 1 along with the target and qualifier ion(s) and their expected retention times used to quantify each analyte of interest. Similar data are presented in Table 2 for the pesticide metabolites. The analysis of whole blood samples produced no interference peaks that might compromise the quantification of the individual compounds of interest, as indicated by the lack of peaks eluting in the negative control at the same retention time as the analytes of interest in the spiked control taken from the same blood sample (Fig. 1).

3.2. Method optimization

To facilitate the recovery of compounds with unique structural characteristics, we chose to develop separate methodologies for the parent pesticides and their respective metabolites. Parent pesticide compounds were extracted using a liquid–liquid extraction technique whereas most previous studies have employed solid phase extraction measures

[16,25–36]. The extraction period was considered a critical parameter in the development of the sample preparation method. Several solvents (hexane, methanol, and toluene—alone and in combination) and various solvent volumes were tested for extraction efficiency. The maximum recovery was optimized for all analytes in both the parent and metabolite methodologies, and was obtained with hexane and toluene, respectively. After trying several extraction times (10, 20, and 60 min), employing the 1 h extraction time for parent pesticides quantitatively enhanced recovery, though greatest recovery for metabolites was attained when samples were extracted for 20 min. The length of methyl ester derivatization for the metabolite protocol was optimized (i.e., the derivatized analytes displayed the highest abundance) at 20 min after trying 0, 20, 60, 90, and 120 min. Additionally, for each method of analysis, a multitude of centrifugation times and speeds were analyzed and compared for robustness. Liquid–liquid extraction of these pesticides from whole blood represents a sensitive and time-efficient method of analysis.

3.3. Method validation

Fig. 2 shows a representative total ion chromatogram for whole blood spiked with a known quantity of the parent pesticide compounds. Following calibration curve generation, R^2 values were determined by linear regression analysis. These values as well as the empirical LODs, recoveries, and coefficients of variability (CVs) of our analytes are shown in Table 3. Recoveries and CVs were determined from $n = 45$ samples spiked with 1.56 μg/mL of Pesticide Mix 11. The response for each analyte was linear over the validated range. Sensitivity was evaluated by determining the LOD for each analyte based on several criteria including a group evaluation of retention time (RT ± 0.03 min) and spectrum data, as well as verifying peak target:qualifier ion ratios within ±20%. The empirical method of LOD determination, consisting of measuring progressively more dilute concentrations of analyte, was chosen whereby the LOD represents the lowest concentration at which the results still satisfy our pre-determined criteria [23,24]. Therefore, the empirical LOD method provides a value that represents the actual limit of the feasibility of our assay, a value that meets all analytical acceptance criteria.

Most parent compounds yielded efficient recovery with relatively low CVs, not exceeding 15% for any of the analytes. Unfortunately, the recoveries of some compounds including lindane, chlorpyrifos, and malathion were considerably higher than those of other compounds using this method. High recovery for these compounds may indicate day-to-day variation in GC–MS instrumentation, resulting in varying responses for certain compounds. Other factors contributing to high recoveries may include minor errors in spiking volumes that may lead to overestimation of compound recovery, or the pure standards may undergo evaporation (particularly those dissolved in hexane), thereby concentrating the spiking solution with time. Additionally, the sensitivity of the calibration curves for these compounds may have lessened with repeated sample analysis. Nonetheless, these compounds were not detected in samples nor in the negative control.

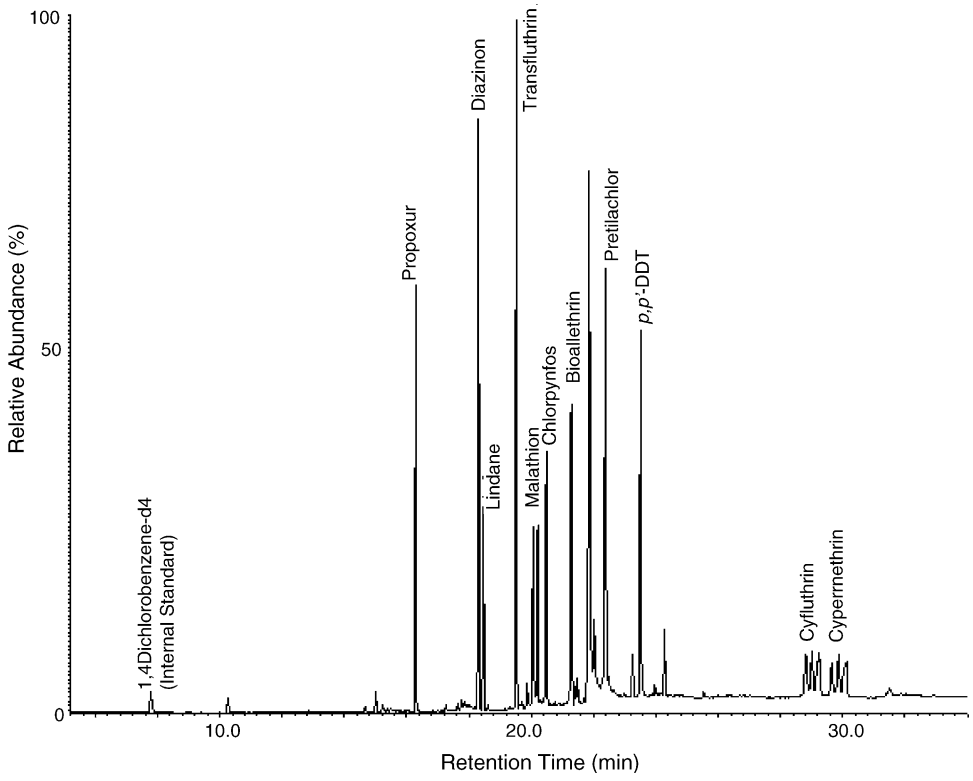

Fig. 2. A representative electron-impact total ion chromatogram of a whole blood sample spiked with 1.56 μg/ml of the parent pesticide compounds.

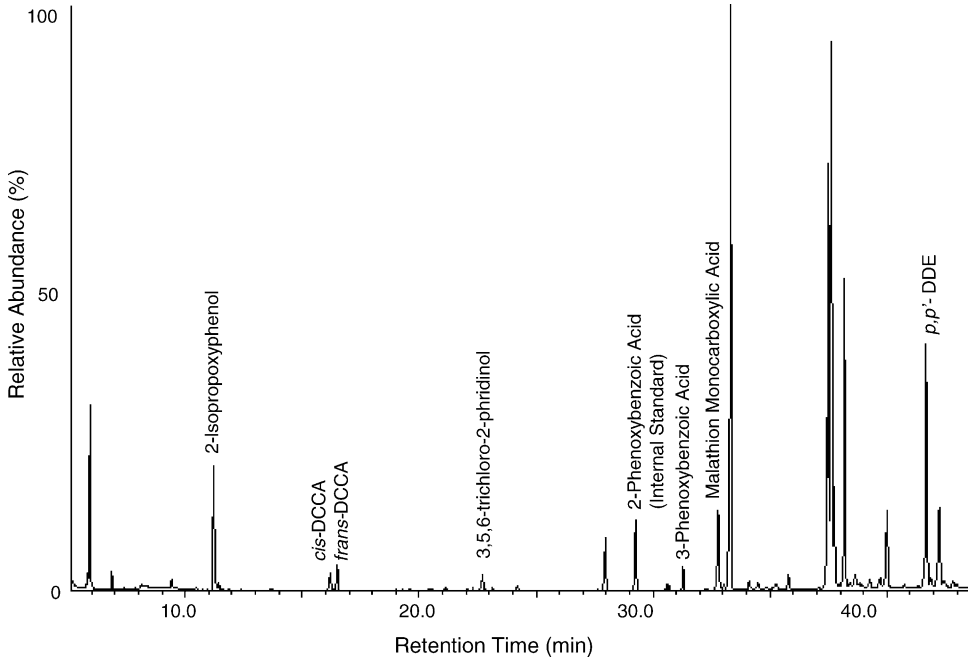

Fig. 3. A representative electron-impact total ion chromatogram of a whole blood sample spiked with 0.78 μg/ml of the pesticide metabolites compounds.

Pesticide metabolite analysis also employed liquid–liquid extraction following methyl ester derivatization to increase analyte volatility. Derivatization of several of the metabolite compounds significantly improved chromatographic and spectrometric resolution. A representative total ion chromatogram of a spiked whole blood sample is shown in Fig. 3. LODs, recoveries, and CV data are listed in Table 3. Again, the analytes were linear over the concentration range. The recovery and CV values were gathered from $n = 21$ samples spiked with 0.78 μg/mL of pesticide metabolites. Though most metabolite compounds showed efficient recovery, the relatively low recoveries of 3,5,6-trichloro-2-pyridinol and 3-PBA are a consequence of adjustments necessitated by the development of a multi-pesticide metabolite analysis method, though the relatively low CVs illustrate the good reproducibility of the method. Despite the low recovery of 3-PBA, positive samples (i.e., samples with calculated concentrations greater than the LOD) were detected for this compound.

3.4. Analysis of parent pesticides and their metabolites in maternal blood and umbilical cord blood

The application of each method was investigated using whole blood from pregnant women recruited from the Bulacan province of the Philippines. Maternal blood was collected at initial interview following enrollment in the study (MBA) and following delivery (MBB); cord blood was collected from the infant at birth. Of the mother–infant dyads analyzed, 1.1% were positive for propoxur in MBA ($n = 277$), 11.3% in MBB ($n = 177$), and 6.9% in cord blood ($n = 173$). Of the twelve infant cord blood samples found positive for propoxur, corresponding maternal propoxur exposure was detected in 33%.

Metabolite analysis of blood showed that 3.5% of MBB samples ($n = 174$) were positive for 3-PBA; *p,p′*-DDE was isolated in 2.5% of MBA samples ($n = 283$) and 0.6% of MBB samples. A representative total ion chromatogram from a maternal whole blood sample positive for *p,p′*-DDE is shown in Fig. 4. No metabolites were found in cord blood. We presume that *p,p′*-DDE was detected in the absence of *p,p′*-DDT because the storage of *p,p′*-DDE in tissues is a consequence of ingestion of *p,p′*-DDE previously degraded in the environment, rather than from direct consumption and metabolization of *p,p′*-DDT [35].

3.5. Method comparison

Our liquid–liquid extraction methods for the detection of several classes of pesticides and their primary metabolites are simple, rapid, and sensitive as means of exposure analysis in a human population. Most recent exposure analysis studies have focused on blood serum or plasma as the preferred matrices of interest [25,28,31–34,36–44]. However, these studies have not acknowledged that representative concentrations of highly lipophilic pesticides are more likely to be found in whole blood, and no published study to date has concomitantly analyzed maternal whole blood and cord blood. Additionally, since blood is a complex matrix, quantification methods of pesticide exposure have primarily focused on a single group of pesticides (i.e. organophosphates or pyrethroids).

Waliszewski and Szymczynski developed a technique for analyzing organochlorines in 5 mL whole blood using GC with an electron capture detector [44]. Their reported recoveries and CVs for *p,p′*-DDE and *p,p′*-DDT were comparable

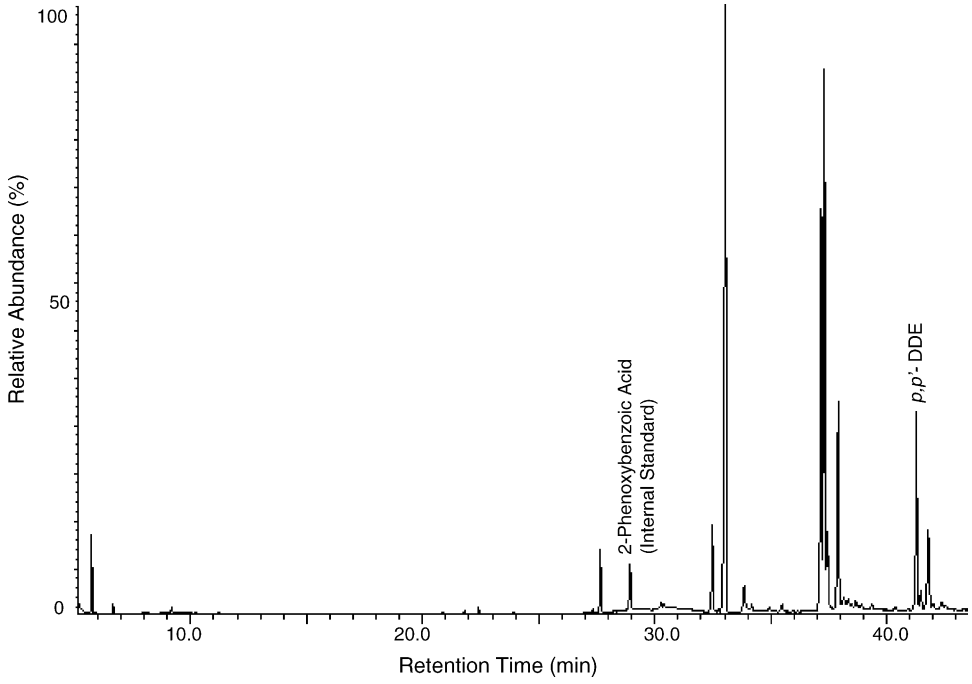

Fig. 4. A representative electron-impact total ion chromatogram of a maternal whole blood sample positive for *p,p′*-DDE (0.79μg/ml).

with our study, however, they did not extrapolate their method for whole blood to a human population for exposure analysis. More recently, Ramesh and Ravi developed a method of analysis for whole blood using GC–MS, exclusively investigating pyrethroid parent compounds with similar chemical structures [42]. Again, our multi-class method yielded comparable sensitivity for the corresponding compounds, and also included several common pyrethroid metabolites that were not incorporated in the Ramesh and Ravi method. In comparing the retention times for the compounds of interest, our GC oven method was considerably shorter in duration (34 min versus 75 min), providing a more robust and time-efficient technique. Furthermore, when Ramesh and Ravi applied their method of analysis to a human population continuously exposed to different pyrethroid formulations, none of the 45 whole blood samples analyzed contained detectable levels of pyrethroids. The authors attributed their findings to rapid excretion of the compounds or limitations of the experimental design. Though we did not find the parent pyrethroid compounds in any of our samples, we did detect 3-PBA, a common metabolite of the pyrethroids, in 3.5% of the MBB samples, suggesting that whole blood extraction methods for metabolite analysis are desirable for transient biological compounds, including the pyrethroids.

4. Conclusion

Clearly, biological monitoring using blood as a biomarker of pesticide exposure has merited attention from the scientific community, and its significance is reflected in the wealth of studies concerned with the detection and quantification of low-level exposures. However, the ubiquitous nature of pesticides requires analytical methods that sensitively and efficiently detect compounds from several diverse classes simultaneously to more accurately reflect a person's exposure. Though incidental exposures generally result in very low concentrations as a consequence of rapid excretion, the presence of these compounds signifies health risks for adults and children, as well as the developing fetus. We conclude that our novel method for whole blood analysis is a suitable tool to investigate the exposure of pregnant women and their newborns to environmental pollutants.

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