Measurement of Organophosphate Metabolites in Postpartum Meconium as a Potential Biomarker of Prenatal Exposure: A Validation Study

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Experimental data have linked exposure to prenatal organophosphates to adverse neurocognitive sequalae. However, epidemiologic research has been hampered by lack of reliable dosimeters. Existing biomarkers reflect short-term exposure only. Measurements of pesticides in postpartum meconium may yield a longer-term dosimeter of prenatal exposure. As the initial step in biomarker validation, this research determined background levels, detection limits, and stabilities of six organophosphate metabolites in meconium: diethylphosphate (DEP), diethylthiophosphate (DETP), diethyldithiophosphate (DEDTP), dimethylphosphate (DMP), dimethylthiophosphate (DMTP), and dimethyldithiophosphate (DMDTP). Calibration curves were also constructed. The meconium was collected from 20 newborns at New York Presbyterian Hospital; analyses were undertaken at the Centers for Disease Control and Prevention (CDC). DEP was detected in 19/20 samples (range 0.8–3.2 µg/g) and DETP was detected in 20/20 (range 2.0–5.6 µg/g). DMP and DEDTP were each detected in 1/20 (at 16 and 1.8 µg/g, respectively). DMTP and DMDTP were not detected. Detection limits were comparable to or lower than those in urine; levels were similar to those seen in adult urine in population-based research. Metabolites were stable at room temperature over 12 hr. Calibration curves were linear over the range tested (0.5–400 µg/g); recoveries ranged from 18% to 66%. Using isotope dilution, recoveries of each analyte in individual samples can be corrected automatically based on the recovery of the respective stable isotope-labeled analogue, making this method fully quantitative. Results indicate that measurements of organophosphate metabolites in meconium have promise as biomarkers of prenatal exposure. Further research is needed to determine the time frame of exposure represented by pesticide levels in meconium and to evaluate the dose–response relationship. *Key words*: biomarkers, meconium, organophosphates, pesticides, prenatal exposures. *Environ Health Perspect* 109:417–420 (2001). [Online 29 March 2001]
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Residential use of organophosphate insecticides is widespread in the United States (*1*). Resultant exposures can be appreciable and have been shown to approach or even exceed health-based standards (*2–6*). Many organophosphate compounds are lipophilic and readily cross the placenta (*7*). Experimental evidence has linked organophosphate exposure during gestation or the early postnatal period to adverse neurodevelopmental sequelae in offspring (*1,8*). Exposures during the spurt in brain growth (which in humans begins during the third trimester) may be particularly deleterious (*9–14*). However, epidemiologic research on this relationship is limited and has been hampered partly because of uncertainties in exposure estimates. Although biologic markers can be useful in understanding the role of environmental contaminants during fetal development (*15–17*), research on the effects of prenatal organophosphate exposure has been limited by the lack of biomarkers reflecting cumulative exposures. Available biomarkers, including blood and urine measurements, provide short-term dosimeters only (half-lives range from 10 to 30 hr) (*18–20*). Residential pesticide exposures are episodic, with high peaks after application and decreasing levels over time (*3*). Thus use of available biomarkers as dosimeters can lead to exposure misclassification if sample collection is not timed to pesticide application. Although erythrocyte acetylcholinesterase is a good biomarker for acute organophosphate exposure, large intraindividual (13%–25%) and interindividual (10%–40%) variability makes it unreliable as a dosimeter in low-level exposure settings unless preexposure values have been determined on each subject (*21–23*).

Measurements of organophosphates in meconium may yield a longer-term dosimeter of prenatal exposure. In human fetuses, meconium begins to accumulate in the bowels at approximately 16 weeks gestation and is generally not excreted until after delivery (*24*). Meconium represents the intestinal contents of the fetus and is a complex matrix, consisting mainly of water but also containing mucopolysaccharides, lipids, proteins, bile acids and salts, epithelial cells, cholesterol and sterol precursors, blood-group substances, squamous cells, residual amniotic fluid, and enzymes (*25*). Prior research on a broad range of xenobiotics indicates that metabolites of compounds to which the fetus has been exposed can be detected in meconium. These include metabolites of illicit drugs (*25–32*) , nicotine (*33*), alcohol (*34*), analgesics, antihistamines, anesthetics, the food additive butylated hydroxytoluene (BHT), and heavy metals (*26*). One study has also measured pesticide levels in meconium (*26*). The xenobiotics appear to enter the meconium as a consequence of bile excretion into the intestines and/or of swallowing by the fetus of amniotic fluid (*35*). Other mechanisms may be operating as well; drugs injected directly into the amniotic fluid of pregnant ewes were detected in meconium in significant concentrations even after the fetuses had undergone esophageal ligation to prevent swallowing (*36*). The authors reasoned that the drugs reached the fetal circulation by absorption across the umbilical cord or diffusion across the placental surface. Evidence suggests that the half-life of xenobiotics in meconium can be protracted and that measured levels may reflect exposures from the second trimester of pregnancy through delivery (*26,28,34, 35,37*).

Materials and Methods

After obtaining Institutional Review Board approval, we collected meconium samples from the diapers of 20 newborns without knowledge of prenatal pesticide use. Sample collection was conducted over a 3-week period by the postpartum staff at New York

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Presbyterian Hospital. Samples were transported to the Molecular Epidemiologic Laboratory at Columbia University and frozen within 8 hr of collection in all cases. At the end of the collection period, the samples were shipped on dry ice to the Centers for Disease Control and Prevention (CDC) for analysis.

Before analysis, samples were thawed and homogenized to ensure that the pesticides were distributed evenly throughout the meconium, and then lyophilized to remove residual water. Approximately 0.5–1 g dried meconium was suspended in 5 mL methanol. After the addition of a stable isotope-labeled internal standard, the suspension was mixed by rotation and centrifuged to separate the solids from the supernatant. The supernatant was removed, evaporated to dryness, and reconstituted in acetonitrile. The analytes in the acetonitrile were chemically derivatized to form their chloropropyl esters to make the analytes more suitable for analysis by isotope dilution gas chromatography–tandem mass spectrometry (ID GC–MS/MS). Analyses were undertaken by ID GC–MS/MS to evaluate background levels of six organophosphate metabolites: diethylphosphate (DEP), diethylthiophosphate (DETP), diethyldithiophosphate (DEDTP), dimethylphosphate (DMP), dimethylthiophosphate (DMTP), and dimethyldithiophosphate (DMDTP). These metabolites are common to 1 or more of 28 different organophosphates, as shown in Table 1, and have been measured extensively in biological samples as specific indicators of both occupational and environmental exposure to organophosphate pesticides (*38–43*).

To determine stability of the metabolites in meconium, aliquots of meconium from the 20 newborns were thawed, pooled, and kept at room temperature for 0–12 hr, with analyses performed every hour. For analyses to construct calibration curves and to determine recoveries, we spiked 0.5 g meconium with an appropriate concentration of standard and analyzed as described above. To evaluate the meconium matrix effects, we compared the calibration curve slopes and intercepts and the reconstructed ion chromatograms from the analysis of spiked meconium samples to those of pure standards analyzed using the same technique.

Results

Table 2 shows the levels of the six organophosphate metabolites in postpartum meconium samples from the 20 newborns. We verified that the measured metabolites were not present in the diapers themselves. DEP was detected in 19/20 (95%) of the samples (range 0.8–3.2 µg/g), and DETP was detected in 20/20 (100%; range 2.0–5.6 µg/g). DMP and DEDTP were each detected in 1/20 (5%) of the samples at levels of 16 µg/g and 1.8 µg/g, respectively. DMTP and DMDTP were not detected.

Table 3 shows the stability of the organophosphate metabolites in meconium at room temperature from 0 and 12 hr. Concentrations of DEP and DETP were stable over the entire period, with < 1.5% variability. Concentrations of DMP were more variable, but there was no trend with time. Levels of DEDTP were too low to determine stability.

Figure 1 shows calibration curves for the six metabolites, and Table 4 shows the R^2 of

Table 1. Organophosphate pesticides, common metabolites, and insecticidal uses.

Pesticides	Metabolites						Insecticidal uses[a]
	DMP	DMTP	DMDTP	DEP	DETP	DEDTP	
Azinphos-methyl	X	X	X				Crops, trees, ornamentals
Chlorethoxyphos				X	X		Crops (corn)
Chlorpyrifos				X	X		Crop, lawn/turf, residential, termiticide, ornamentals, pet collars, pasture, livestock[b]
Chlorpyrifos-methyl	X	X					Stored grain
Coumafos				X	X		Livestock
Diazinon				X	X		Crop, lawn/turf, residential/commercial
Dichlorvos (DDVP)	X						Pest strips, residential, food, storage/processing, livestock
Dicrotophos	X						Crops (cotton)
Dimethoate	X	X	X				Crops, ornamentals
Disulfoton				X	X	X	Crops, ornamentals
Ethion				X	X	X	Crops (citrus), livestock
Fenitrothion	X	X					Residential/commercial ant/roach bait
Fenthion	X	X					Livestock, mosquito control (Florida)
Isazofos-methyl	X	X					Registrations canceled
Malathion	X	X	X				Crops, livestock, lawn/turf, mosquito
Methidathion	X	X	X				Crops
Methyl parathion	X	X					Crops
Naled	X						Crops, greenhouse, flea collars, mosquito, fly
Oxydemeton-methyl	X	X					Crops
Parathion				X	X		Crops[c]
Phorate				X	X	X	Crops
Phosmet	X	X	X				Crops, ornamental, forestry, livestock
Pirimiphos-methyl	X	X					Stored corn, seed, grain, livestock, bulbs
Sulfotepp				X	X		Greenhouses, ornamentals
Temephos	X	X					Mosquito larva
Terbufos				X	X	X	Crops
Tetrachlorvinphos	X						Livestock, domestic animals (dogs/cats)
Trichlorfon	X						Ornamentals, turf, agricultural premises, nurseries, ants

[a]Sources on insecticidal uses from U.S. EPA (*47*). [b]Indoor uses being phased out. [c]Crop uses being phased out.

Table 2. Levels of six organophosphate metabolites in postpartum meconium samples collected from 20 newborns (µg/g).

SAMPLE	DEP	DETP	DEDTP	DMP	DMTP	DMDTP
1	1.90	2.00	ND	ND	ND	ND
2	1.40	3.80	ND	ND	ND	ND
3	1.70	4.30	ND	ND	ND	ND
4	2.00	2.30	ND	ND	ND	ND
5	3.20	3.50	1.80	ND	ND	ND
6	1.20	2.40	ND	ND	ND	ND
7	1.00	2.80	ND	ND	ND	ND
8	1.10	2.00	ND	ND	ND	ND
9	1.00	2.20	ND	ND	ND	ND
10	1.30	2.70	ND	ND	ND	ND
11	1.40	3.00	ND	ND	ND	ND
12	1.30	2.50	ND	ND	ND	ND
13	0.80	2.00	ND	ND	ND	ND
14	2.50	5.60	ND	ND	ND	ND
15	2.80	5.20	ND	ND	ND	ND
16	0.90	2.50	ND	ND	ND	ND
17	1.00	2.40	ND	16.00	ND	ND
18	ND	2.00	ND	ND	ND	ND
19	1.80	5.00	ND	ND	ND	ND
20	0.90	2.40	ND	ND	ND	ND

ND, not detected.

the calibration lines and the detection limits and percent recovery of the pesticides in meconium. All calibrations were linear over the entire range tested (Table 4). All R^2 values were > 0.99, and the standard error about the slope was < 4%. Minimal matrix effects were observed. Due to fewer interfering coextractants, limits of detection were comparable to or better than those observed previously in urine samples from population-based studies that have been analyzed at the CDC. As Table 4 shows, the recoveries of the dialkylphosphate metabolites from meconium range from 18% to 66%. Use of the isotope dilution technique allows complete and automatic correction for analyte recoveries for each sample, enabling a fully quantitative analysis of the meconium.

Discussion

Results from this initial validation study show that organophosphate metabolites can be detected in postpartum meconium. It is interesting that diethylphosphate and diethylthiophosphate were detected in 95%–100% of the samples. Both are metabolites of the organophosphates diazinon and chlorpyrifos as well as several additional organophosphates used primarily in agriculture (see Table 1), and our findings are consistent with the widespread residential use that has been reported for these two insecticides (*1,2,44*). These insecticides are also of concern because prenatal exposure to both chlorpyrifos and diazinon has been linked experimentally to adverse neurodevelopmental sequelae in the offspring (*1,8*). The other organophosphate metabolites were detected only once (dimethylphosphate and diethyldithiophosphate) or not at all (dimethylthiophosphate and dimethyldithiophosphate). As seen from Table 1, this may reflect the fact that they are metabolites of organophosphates with less frequent residential use.

Table 3. Concentrations of analytes in meconium stored at room temperature.

Time (hr)	DEP	DETP	DMP
0	0.81	2.6	6.7
1	0.82	2.6	—[a]
2	0.82	2.6	6.4
3	0.82	2.6	4.0
4	0.83	2.6	6.9
5	0.82	2.6	7.4
6	0.83	2.6	5.8
7	0.83	2.7	8.7
8	0.83	2.6	5.5
9	0.83	2.6	7.2
10	0.81	2.7	4.4
11	0.82	2.6	6.4
12	0.83	2.6	8.2
Mean	0.82	2.6	6.5
RSD	0.9	1.4	22

RSD, relative standard deviation.
[a]Measurement not taken.

Results also indicate that the measurement of organophosphate metabolites in meconium may have promise as a biomarker of prenatal exposure. Detection limits for the organophosphate metabolites in meconium are low and comparable to or better than those seen with adult urine (*45*). Further, metabolite levels in meconium are several orders of magnitude higher than those generally seen in umbilical cord blood samples (usually nanograms per liter) (*46*) and are similar to levels seen in adult urine in population-based studies (*45*). In addition, the pesticide metabolites appear stable in meconium over 12 hr at room temperature, which should facilitate ease of incorporation of meconium measurements into research protocols. Although recoveries of the metabolites in meconium varied, low or variable recoveries will not compromise analyses. Using isotope dilution, recoveries of each analyte in each individual sample can be corrected based on the recovery of its respective stable isotope-labeled analogue. Chemically, the isotopically labeled analogues behave identically to the analytes measured, but they can be distinguished according to their mass differences. Given these initial promising findings, further research is needed to determine the time frame of exposure represented by pesticide levels in meconium and to evaluate the dose–response relationship.

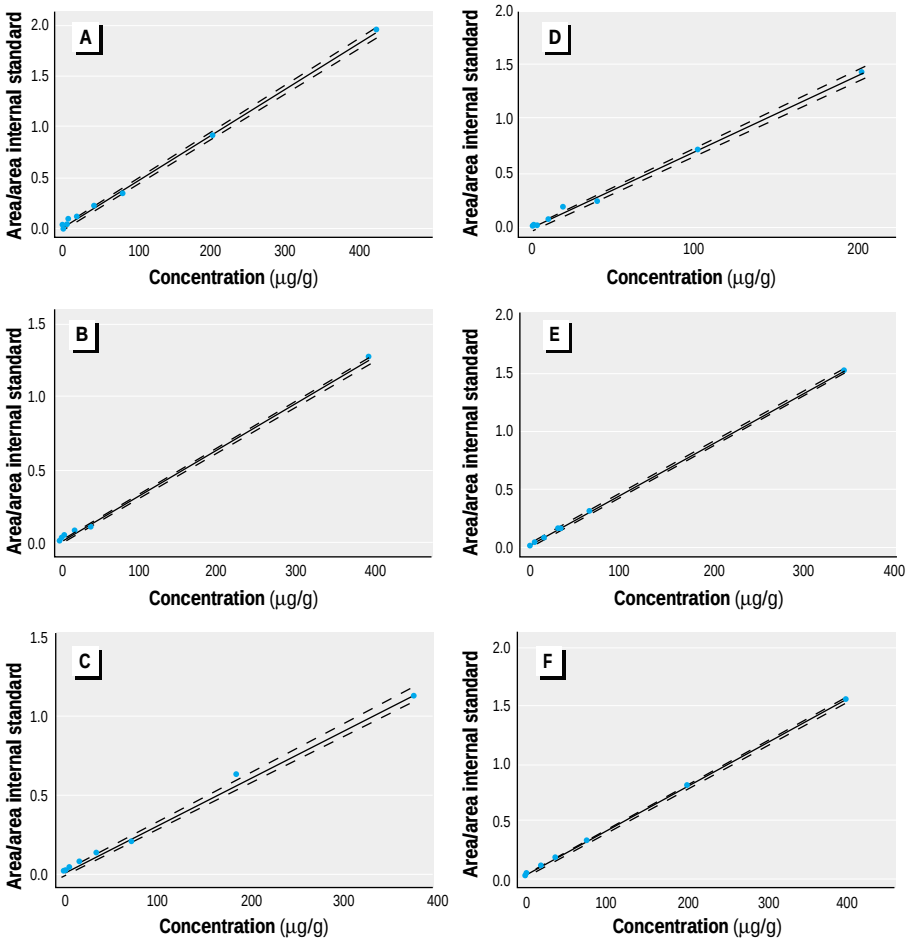

Figure 1. Standard curves for analytes in meconium: (*A*) DEP, (*B*) DETP, (*C*) DEDTP, (*D*) DMP, (*E*) DMTP, and (*F*) DMDTP. The solid lines are the linear regression lines, and the 95th confidence intervals are shown as dashed lines.

Table 4. Specifications of the analytic method.

Analyte	R^2 of calibration lines	Percent error about calibration slope	Percent recovery from meconium	Limit of detection (μg/g)
DEP	0.9929	3.0	26	0.2
DETP	0.9908	3.4	55	0.09
DEDTP	0.9969	2.0	62	0.05
DMP	0.9963	2.2	18	0.51
DMTP	0.9998	0.5	63	0.18
DMDTP	0.9995	0.8	66	0.08

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