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Prevalence of Fetal Exposure to Environmental Toxins as Determined by Meconium Analysis

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Abstract

Objective: The primary objective was to determine whether environmental pollutants, specifically lead (Pb), cadmium (Cd), mercury (Hg), arsenic (As) and organochlorine and organophosphate pesticides can be detected in meconium. Study design: Prospective, cohort study. Infants were randomly recruited from the nurseries of five hospitals in Manila, Philippines. Their stools (meconium) were collected and analyzed for heavy metals by atomic absorption spectrophotometry and for pesticides by gas chromatography/mass spectrometry (GCMS). Results: A total of 426 infants were studied. The exposure rate (based on meconium analysis) and the median concentration of the pollutants in the positive samples were as follows: lead (26.5%; 35.77 μg/ml), cadmium (8.5%; 13.37 μg/ml), mercury (83.9%; 3.17 ng/ml), chlordane (12.7%; 22.48 μg/ml), chlorpyrifos (11.0%; 8.26 μg/ml), diazinon (34.3%; 12.96 μg/ml), DDT (26.5%; 12.56 μg/ml), lindane (73.5%; 2.0 μg/ml), malathion (53.0; 6.80 μg/ml), parathion (32.0%; 2.30 μg/ml) and pentachlorphenol (16.1%; 90.00 μg/ml). Some maternal and neonatal factors that were significantly associated with the presence of environmental toxins in meconium included multigravidity, multiparity, multiple gestation, meconium stained fluid, smoking, gestational age, low birth weight and infant gender. Conclusion: Meconium analysis is a new and sensitive tool to detect fetal exposure to environmental toxins and its clinical use awaits further investigation.

Keywords: Meconium; Drugs; Environmental pollutants; Neonate; Pesticide; Heavy metals; Lead; Mercury

INTRODUCTION

Environmental pollutants are a constant threat to human health. These agents are ubiquitous and are found in food, water, air and other parts of the environment. Maternal exposure to environmental toxins can have significant effects on the fetus. The range of their exposure to these toxins varies from subclinical to severe cases. In the latter, such as has been reported in accidental poisonings with mercury and PCB, severe immediate and long term effects on the infant and child were observed (Chen et al., 1992, 1994; Bakir et al., 1980; Amin-Zaki et al., 1976). However, even at subclinical exposure of the mother to these toxins, e.g. from the consumption of fish in the Great Lakes area that were contaminated with PCB, adverse immediate and long term outcome in the infants have been observed (Schantz, 1996; Jacobson et al., 1985; Jacobson and Jacobson, 1996a,b; Safe, 1993).

Abbreviations: Pb, lead; Cd, cadmium; Hg, mercury; As, arsenic; GC/MS, gas chromatography/mass spectrometry; DDT, dichloro,diphenyl,trichloroethane; PCB, polychlorinated biphenyls; PCP, pentachlorphenol; ppb, parts per billion (ng/ml); ppm, parts per million (μg/ml); FIRST Hospital, First Integrated Hospitals; UST Hospital, University of Santo Tomas Hospital; PCMC Hospital, Philippine Children's Medical Center Hospital

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The detection of maternal or fetal exposure to environmental toxins have so far utilized the examination of maternal and cord blood, and in some cases, maternal hair. Meconium is a matrix that has been previously analyzed to detect fetal exposure to a number of xenobiotic agents, including illicit drugs (Ostrea et al., 1992, 1993, Maynard et al., 1991; Callahan et al., 1992), licit drugs (Ostrea et al., 1998; Alano et al., 2001), alcohol metabolites (Mac et al., 1994; Bearer et al., 1999; Klein et al., 1999) and nicotine metabolites (Ostrea et al., 1994). The objective of this study was to determine whether environmental toxins, specifically heavy metals (lead, cadmium, mercury and arsenic) and organochlorine and organophosphate pesticides could also be detected in meconium. Since this study was an exploratory test of a new matrix (meconium), there was no attempt to compare meconium to blood or hair analyses.

METHODS

Infants were recruited from the nurseries of three perinatal centers in Manila, Philippines. We selected Manila for the study, because of its high environmental pollution index. The nurseries that were selected represented a socioeconomic cross-section of hospitals in Manila which ranged from charity to private pay hospitals. A total of 150–160 mother/infant dyads were sampled per center and a sequence of prospective enrollment of infants was adopted which was calculated based on the annual birth rate in each hospital. For example, if a center has 2400 livebirths per year, there will be approximately 200 livebirths per month. For the random sampling of 13 infants per month, approximately every 15th live birth will be recruited. The subjects in the study were anonymous and no consent was obtained from the mother. Meconium was collected directly from the infant's diaper and the sample was identified only through an assigned number code. Demographic information on the mother and infant were obtained from their charts. There was no examination of the infant or mother for this study. The study was approved by the human investigation committee of each participating institution.

Meconium was collected during the first two postnatal days and were pooled into one sample. The meconium samples were stored in a freezer at −18 °C and sent in batches, under refrigeration, to the appropriate laboratories. Batches of the meconium container were tested for heavy metal contamination which were negative.

Meconium was analyzed for heavy metals (mercury, lead, cadmium and arsenic) by atomic absorption spectrophotometry. Mercury was analyzed as total mercury by cold vapor atomic absorption spectrophotometry. Standard and blank solutions were prepared using aliquots of 0.5, 1.5, 2.5, 5 and 10 ml of 0.1 ppm standard mercury solution. Meconium was weighed (0.2 g) and digested in 5 ml of aqua regia (one part HNO_3:three parts HCl) in a water bath at 90 °C for 5 min and then cooled. Deionized water (40 ml) and 15 ml of 5% $KMnO_4$ were added. The mixtures were returned to the water bath for further digestion for 30 min. The samples were cooled and alkalinized with 6 ml of 12% NaCl–NH_4Cl. Five milliliters of 10% stannous chloride was added and the samples were aerated. Absorbance was read after 30 s. For the analysis of lead, cadmium and arsenic, blank solutions and standards were prepared at 1.0, 2.0 and 3.0 ppm of lead, 0.1, 0.3 and 0.5 ppm of cadmium and 25, 50 and 100 ppb of arsenic. Meconium was weighed (0.1 g) and digested with 30 ml of concentrated HNO_3 and heated near dryness. Thereafter, 25 ml of deionized water was added and the solution heated to about 10 ml. The sample was cooled and volume was reconstituted to 50 ml with deionized water. Absorbance for lead, cadmium and arsenic were read at maximum readings. The minimum detection level was 10 ppm for lead, 1.5 ppm for cadmium, 0.2 ppm for arsenic and 0.4 ppb for mercury. The recovery of heavy metals (lead, cadmium, arsenic and mercury) spiked in meconium ranged between 100 and 102%. Interassay coefficient of variability ranged between 0.34 and 8.9%; intraassay coefficient of variability ranged between 0.44 and 8.44%.

Meconium was analyzed for chlordane, chloropyfiros, diazinon, DDT (p, p'DDT), lindane, pentachlorphenol, malathion and parathion by GC/MS. One half (0.5) gram of meconium was added to a meconium processor tube (Mectest, Meco Industries, Walnut, Ca) which contained 5 ml of buffered methanol (pH = 7.4). Suspension was mixed and centrifuged for 1 h at 4600 rpm. The supernatant was pipetted into Millipore tube and centrifuged for 1–2 h at 4600 rpm. A C18 extraction column (Alltech, Ill) was initially conditioned by adding in sequence, 3 ml of acetonitrile-deionized water (70:30 v/v), 3 ml methanol and 3 ml deionized water. The ultrafiltrate was added to the column and the pesticides were subsequently eluted with 6 ml of acetonitrile:deionized water (70:30 v/v) and the eluate collected, dried, and resuspended in 100 μl of methanol for GC/MS analysis. Four microliters of an internal standard solution (1,4 dichlorobenzene-d4) were added to

100 μl of the eluate in a reactivial. Four microliter of the eluate was then injected into an HP5890 series II gas chromatograph with electronic pressure control and an HP5971A mass spectrometer. The column used was a DB-5 ms (30 m, 0.25 mm i.d., 1 μm film, J&W Scientific P/N 122–5533). Analysis was performed in a scan mode and the compounds were identified through their specific target and qualifying ions at known ratios. The concentration of each compound was calculated based on the known concentration of the internal standard. Calibration curves of pesticides at a range of 50–400 μg/ml were linear. Interassay and intraassay coefficients of variability of the assays ranged between 0.8 and 8.5%. Recovery studies on meconium spiked with pesticides at concentrations of 150 and 300 μg/ml ranged between 71.3 and 96.7%. The limits of detection (μg/ml) for the different pesticides were: chlordane (0.186), chlorpyrifos (1.232), DDT (0.322), diazinon (0.173), lindane (0.546), malathion (1.048), parathion (1.168) and pentachlorphenol (6.616).

The concentrations of the toxins in meconium are expressed in micro- or nanogram per milliliter (ppm or ppb). Recalculation of the concentrations in micro- or nanogram per gram of meconium can be done since the amount (g) of meconium and the solvent volume used to resuspend meconium are known.

Statistical Analysis

Descriptive statistics were performed on the data and in skewed distributions, median and percentiles were used. Exposure was defined as the presence or detection of a toxin in meconium and was analyzed as a categorical ''yes or no'' variable. Preliminary association between maternal and infant conditions and fetal exposure to heavy metals or pesticides were done by Chi-square analysis with fetal exposure expressed as a dichotomous, yes/no outcome. Hospital site and maternal or infant conditions were also expressed as dichotomous, categorical groups. Maternal and fetal factors that were significantly ($P < 0.05$) associated with fetal exposure to pollutants were entered into a logistic regression model to determine the interaction and independent effects of the covariates on the dependent variable. A P-value of <0.05 was taken as the level of statistical significance.

RESULTS

A total of 442 mother per infant dyads were initially enrolled in the study but only 426 subjects were studied since 16 were excluded due to insufficient meconium obtained from the infants. Most of the mothers were married (91.3%) and had prenatal care (92.5%). The percentage of mothers who were gravida and parity <3 were 82.3 and 90.5%, respectively. There was a high incidence of cesarean section delivery (42%). The incidence of abnormal amniotic fluid (polyhydramnios, oligohydramnios) was 12%. Abnormalities in the placenta (5.7%) consisted primarily of abruptio placenta or placenta praevia. Cord abnormality (8.8%) consisted of nuchal cord, true knot or cord prolapse.

In the infants, 49.2% were male, with mean (S.D.) gestational age of 38.5 ± 2.1 weeks, birth weight of 3.03 ± 0.53 kg, length of 48.9 ± 3.0 cm and head circumference of 33.2 ± 2.2 cm. Sixteen percent of the infants were $\leq$37 weeks in gestation and 11.4% had a birth weight <2500 g.

Lead, mercury, cadmium were found in 26.5, 83.9 and 8.5% of the samples, respectively. Arsenic was not detected. Mercury was found in a high percentage of the samples analyzed, although its concentration was a thousand-fold lower (ng/ml or ppb) compared to lead or cadmium (μg/ml or ppm). A number of samples also had more that one heavy metal present: 19% had two heavy metals and 7% had three heavy metals. A wide concentration range of heavy metals was found in the samples and the concentrations were highest for lead: 50% of the positive samples had lead concentration ranging from 18.11 to 177.06 μg/ml.

Two hundred meconium samples were analyzed for pesticides and the results are shown in Table 1. The exposure rate ranged from 12.7% (chlordane) to 73.5% (lindane). Comparatively high exposure rates were also observed for diazinon (34.3%), parathion (32%) and malathion (53%). Despite the ban two decades ago on DDT use, the exposure rate to DDT in the study was 26.5%. One infant with a very high concentration of DDT in meconium (10,406 ppm) also had a high concentration of parathion (559 ppm). Pentachlorphenol was found in a small percentage of the samples analyzed (16.1%). However, the concentration of pentachlorphenol was high in the positive samples: 50% had concentration ranging from 22.96 to 250.32 μg/ml. Many meconium samples were positive for more thanone pesticide: 28.6% had two pesticides, 14.3% had three pesticides, 12.6% had four pesticides, 4.9% had five pesticides, 3.8% had six pesticides and 4.9% had seven pesticides.

The exposure to heavy metals was related to the hospital site of recruitment and a number of maternal or infant conditions (Table 2). By hospital site, exposure was significantly high among infants who were born at

Table 1
Prevalence and concentrations of environmental pollutants in meconium of neonates in Manila, Philippines

	Positive percentage	Concentration in positive samples (ppm or ppb)[a]					
		Mean	Median	Range	Percentile		
					25th	50th	75th
(A) Heavy metals ($N = 426$)							
Lead (ppm)	26.5	120.56	35.77	8.23–603.04	18.11	35.77	177.06
Mercury (ppb)	83.9	6.59	3.17	0.43–71.58	1.78	3.17	6.82
Cadmium (ppm)	8.5	13.00	13.37	2.09–27.39	5.33	13.37	7.81
Arsenic	0.0	0.00	0.00	0.00	0.00	0.00	0.00
(B) Pesticides (ppm) ($N = 200$)							
Chlordane	12.7	282.80	22.48	0.48–2237.80	2.76	22.48	139.48
Chlorpyrifos	11.0	53.46	8.26	0.40–458.04	1.15	8.26	54.84
DDT	26.5	373.70	12.56	0.16–10406.40	1.22	12.56	63.77
Diazinon	34.3	226.46	12.96	0.24–6027.00	10.28	12.96	48.48
Lindane	73.5	37.16	2.00	0.04–539.44	0.32	2.00	37.40
Malathion	53.0	71.69	6.80	0.04–771.28	0.85	6.80	43.86
Parathion	32.0	86.03	2.30	0.04–2116.36	1.31	2.30	16.98
Pentachlorphenol	16.1	195.68	90.00	3.08–1762.32	22.96	90.00	250.32

[a] ppm (parts per million or μg/ml), ppb (parts per billion or ng/ml).

Table 2
Exposure to heavy metals: prevalence according to hospital site and association with maternal and infant factors

	Exposure to heavy metals					
	Lead		Cadmium		Mercury	
	Positive	Negative	Positive	Negative	Positive	Negative
(I) Prevalence of exposure[a]						
FIRST (%)	28.6		11.7**		84.3	
UST (%)	13.7		5.1		79.5	
PCMC (%)	65***		0.0		100*	
(II) Maternal factors[b]						
Age ≤25 years (%)	20.0	28.1	17.2	27	16.0	5.6
Gravida >3 (%)	27.8**	14.9	27.6	17.4	18.9	14.5
Parity >3 (%)	15.5*	7.3	22.2*	8.2	9.0	5.7
No pre-natal care (%)	8.1	4.5	0.0	5.9	5.3	5.7
Multiple gestation (%)	10*	4.0	10.3	5.1	5.9	5.5
Meconium stained fluid (%)	14.3*	6.4	13.8	8.2	9.8	3.7
Illicit drug use (%)	18.8	23.3	3.4*	24	22.9	17.6
Smoking (%)	3.9	3.2	4.0	3.3	2.8	6.4
Alcohol use (%)	2.3	0.8	0.0	1.3	1.4	0
Abnormal placenta (%)	5.6	2.8	0.0	3.9	3.2	5.5
(III) Infant factors[b]						
Pre-maturity (%)	15.5	14.0	17.2	27	16*	5.6
Male gender (%)	48.2	47.6	37.9	48.9	51.4***	30.9
Birth weight <2500 g (%)	14*	7.3	10.3	8.8	10.3*	3.6

[a] Comparison between hospital sites (Chi-square analysis).
[b] Comparison between positive and negative groups (Chi-square analysis).
* $P < 0.05$.
** $P < 0.01$.
*** $P < 0.001$.

the FIRST and PCMC Hospitals (65% for lead and 100% for mercury at PCMC and 11.7% for cadmium at the FIRST Hospital). Univariate analysis showed that exposure to lead was significantly increased in mothers who were >3 in gravidity (27.8 versus 14.9%, $P < 0.001$) and parity (15.5 versus 7.3%, $P < 0.03$), in multiple gestation (10 versus 4%, $P < 0.05$) and meconium stained fluid (14.3 versus 6.4%, $P < 0.04$). Exposure to cadmium was significantly increased in mothers with parity >3 (22.2 versus 8.2%, $P < 0.03$) and exposure to mercury was significantly increased with the male gender (51.4 versus 30.9%, $P < 0.01$).

For the pesticides, exposure to lindane and parathion were significantly high among the infants born at the FIRST Hospital (79.1 and 35.8%, respectively) and to malathion, among infants born at the UST Hospital (75.8%)—Table 3. Exposure to diazinon was significantly increased with gravida >3 (30.6 versus 16%; $P < 0.03$) and multiple gestation (14.5 versus 5.0%, $P < 0.04$); exposure to lindane was associated with female gender (54.9 versus 36.2%, $P < 0.04$), exposure to parathion was associated with multiple gestation (15.5 versus 4.9%, $P < 0.02$) and exposure to pentachlorphenol was associated with smoking (11.1 versus 0.8%, $P < 0.01$). Maternal and infant risk factors that were significantly ($P < 0.05$) associated with exposure to the individual heavy metals or pesticides by univariate analysis, were included and analyzed in a stepwise, logistic regression model. The exposure to lead was significantly associated with parity >3, ($P < 0.02$), birth weight <2500 g ($P < 0.025$), and meconium staining of the amniotic fluid ($P < 0.016$) and exposure to mercury was significantly associated with infant gender ($P < 0.01$) and gestational age ≤37 weeks ($P < 0.05$).

The correlation between the concentrations of the various pollutants (heavy metals and pesticides) in meconium are shown in Table 4. The following pollutants had significant intercorrelations to one another either in single or in multiple analyses: lindane, malathion, parathion, chlordane, chlorpyrifos, lead and cadmium. Very high correlation coefficients were particularly observed between parathion and DDT ($r = 0.925$) and between cadmium and lead ($r = 0.873$).

DISCUSSION

Environmental pollution is a global, public health problem. Because of industrialization and widespread use of pesticides, vast quantities of chemicals are released and dispersed into the environment each year. As a consequence, these pollutants are ubiquitous and are found in air, water, soil, food sources and other biologic materials. Of specific concern is the exposure of the pregnant woman to environmental toxins since the fetus is highly vulnerable to these agents owing to the state of rapid growth and differentiation of its nervous system. Many of the heavy metals, organochlorine and organophosphate pesticides are neurotoxicants (Schettler et al., 2000; US EPA, 1998a,b) and have direct effects on neuronal proliferation, migration, differentiation, synaptogenesis, myelination and apoptosis (Rodier et al., 1984; Choi, 1986; Graff et al., 1997; Barone et al., 1998; Eriksson, 1997; Liu et al., 1997; Bailey and Chen, 1991; Monnet-Tschudi, 1998). Others interfere with hormones (endocrine disruptors), neurotransmitters, or neurotrophic growth factors that effect brain development (Cameron et al., 1998; Owen and Bird, 1995; Sternfeld et al., 1998; Grifman et al., 1998; Lauder, 1990; Larkfors et al., 1991). The devastating effects of environmental toxins on the fetus were seen in the severe cases of poisonings that occurred in mothers who had accidentally ingested food contaminated with mercury (Bakir et al., 1980; Harada, 1978; Amin-Zaki et al., 1976; Ehassani et al., 1976) or polychlorinated biphenyls (Chen et al., 1992, 1994; Rogan et al., 1988). The infants suffered from gross neurologic damages, at birth, which persisted into their later life. However, the majority of maternal exposure to environmental toxins does not lead to observable adverse effects in the infants at birth. However, an increasing number of children with developmental, learning and behavioral difficulties have been noted recently, ranging from mental retardation, learning disability, attention deficit hyperactivity disorders and autism and it has been suggested, based on animal and human data, that a variety of environmental toxins can contribute to these disorders even at low levels of exposure (US Census, 1994; Parrill, 1996; Kavale and Forness, 1992; Boyle et al., 1994; Goldman et al., 1998; Rowland, 1999; Safer et al., 1996; Gillberg and Wing, 1999; California Health, 1999). For instance, increases in blood lead levels during infancy and childhood are associated with attention deficits, increased impulsiveness, reduced school performance, aggression, and delinquent behavior (Needleman et al., 1985; Bellinger et al., 1987). Small fetal exposures to methylmercury, such as those resulting from regular maternal fish consumption, have been implicated in language, attention, and memory impairments that appear to be permanent (Davidson et al., 1995; Rice and Gilbert, 1990; Crump et al., 1998). Monkeys

Table 3
Exposure to pesticides: prevalence according to hospital site and association with maternal and infant factors

	Diazinon		Lindane		Malathion		Parathion		PCP		Chlordane		DDT		Chlorpyrifos	
	Positive	Negative	Positive	Negative	Positive	Negative	Positive	Negative	Positive	Negative	Positive	Negative	Positive	Negative	Positive	Negative
(I) Hospital site[a]																
FIRST (%)	32.4		79.1***		48.0		35.8**		19.7		11.5		25.7		10.8	
UST (%)	42.4		48.5		75.8***		15.2		0.0		18.2		30.3		12.1	
(II) Maternal factors[b]																
Age ≤25 years (%)	22.6	23.5	21.2	29.2	21.9	24.7	19.0	25.2	27.6	22.5	17.4	24.1	20.8	23.3	10.0	24.2
Gravida >3 (%)	30.6**	16	22.6	16.7	25.0	16.5	25.9	18.7	20.7	20.5	21.7	20.9	22.9	20.3	20.0	21.1
Parity >3 (%)	18.3	8.5	14.5*	4.3	12.9	10.6	14.3	10.7	17.2	10.1	13.6	11.5	15.2	10.6	5.6	12.5
No pre-natal care (%)	3.3	1.7	3.1	1.0	3.2	1.2	3.5	1.6	0.0	2.7	4.5	1.9	4.3	0.8	5.3	1.3
Multiple gestation (%)	14.5**	5.0	10.5**	2.1	10.4	5.9	15.5**	4.9	0.0	9.9	4.3	8.9	6.3	9.0	5.0	8.7
Meconium stained fluid (%)	15.3	12.3	12.4	15.9	15.1	11.3	16.4	11.9	3.6	15.3	19.0	12.5	17.4	11.0	22.3	11.6
Smoking (%)	1.7	2.9	2.5	2.6	3.4	1.4	5.9	0.9	11.1**	0.8	0.0	2.8	4.7	1.7	0.0	2.8
Alcohol use (%)	1.6	0	0.8	0	0.0	1.2	0.0	0.8	0.0	0.7	0.0	0.6	0.0	0.8	0.0	0.6
Abnormal placenta (%)	3.2	0	0.8	2.1	2.1	0	3.4	0	0.0	1.3	4.3	0.6	2.1	0.8	5.0	0.6
(III) Infant factors[b]																
Pre-maturity (%) (≤37 weeks)	20.0	14.0	16.3	15.6	14.1	18.3	18.2	15.1	17.2	16.0	8.7	17.2	14.9	16.5	15.8	16.1
Female gender (%)	50.8	36.2	54.9*	36.2	48.4	51.8	46.6	51.6	48.3	50.7	39.1	51.6	52.1	49.2	55.0	49.4
Birth weight <2500 g (%)	6.6	7.6	7.5	6.4	8.4	5.9	6.9	7.4	10.3	6.7	8.7	7.0	10.4	6.8	10.0	7.5

[a] Comparison between FIRST and UST (Chi-square analysis).
[b] Comparison between positive and negative groups (Chi-square analysis).
* $P < 0.05$.
** $P < 0.01$.
*** $P < 0.001$.

Table 4
Correlation between the concentrations of heavy metals and pesticides in meconium

	Diazinon	Lindane	Malathion	Parathion	PCP	Chlordane	DDT	Chlorpyr	Lead	Cadmium	Mercury
Diazinon	1.000	−0.045	−0.012	−0.019	−0.027	−0.018	−0.019	−0.022	−0.070	−0.059	−0.049
Lindane	−0.045	1.000	0.126	0.110	0.135	0.367**	0.017	0.354**	0.417**	0.447**	0.130
Malathion	−0.012	0.126	1.000	0.170*	0.133	0.300**	0.169*	0.483**	0.142	0.155*	−0.032
Parathion	−0.019	0.110	0.170*	1.000	−0.005	0.399**	0.925**	0.261**	0.021	0.027	−0.028
PCP	−0.027	0.135	0.133	−0.005	1.000	−0.029	0.066	−0.030	0.088	0.123	0.009
Chlordane	−0.018	0.367**	0.300**	0.399**	−0.029	1.000	0.150*	0.422**	0.104	0.090	0.017
DDT	−0.019	0.017	0.169*	0.925**	0.066	0.150*	1.000	0.300**	0.056	0.065	−0.028
Chlorpyrifos	−0.022	0.354**	0.483**	0.261**	−0.030	0.422**	0.300**	1.000	0.293**	0.310**	0.090
Lead	−0.070	0.417**	0.142	0.021	0.088	0.104	0.056	0.293**	1.000	0.873**	0.016
Cadmium	−0.059	0.447**	0.155*	0.027	0.123	0.090	0.065	0.310**	0.873**	1.000	0.043
Mercury	−0.049	0.130	−0.032	−0.028	0.009	0.017	−0.028	0.090	0.016	0.043	1.000

* Correlation is significant at the 0.05 level (two-tailed).
** Correlation is significant at the 0.01 level (two-tailed).

exposed to dioxin as fetuses show evidence of learning disabilities (Schantz and Bowman, 1989). Humans and animals exposed to low levels of PCBs as fetuses have IQ deficits, learning disabilities, hyperactivity, and attention deficits when tested years later (Rogan et al., 1988; Chen et al., 1992; Holene et al., 1995; Gladen and Rogan, 1991; Lonky et al., 1996). Animals that are exposed to organophosphates show that small single doses on a critical day of development can cause hyperactivity and permanent changes in neurotransmitter receptor levels in the brain (Ahlbom et al., 1995). A common pesticide, chlorpyrifos, decreases DNA synthesis in the developing brain, resulting in deficits in cell numbers and decrease in brain weight (Chanda and Pope, 1996; US EPA, 1998a,b). Some pyrethroids cause permanent hyperactivity in animals exposed to small doses on a single critical day of development (Eriksson and Fredriksson, 1991; Maiaviya et al., 1993). In an agricultural community in Mexico, children exposed to a variety of pesticides show impaired stamina, coordination, memory, and capacity to represent familiar subjects in drawings (Guillette et al., 1998).

A sensitive method of detecting fetal exposure to environmental toxins is, therefore, important in our understanding of their potential toxicity. Unfortunately, this is not an easy task. Maternal interview to detect environmental exposure is of limited use. The toxins in blood are in a constant dynamic state of metabolism, tissue distribution and excretion and their measurement in the blood may not represent the true extent of exposure (Drasch et al., 2001). A number of pesticides deposit in adipose tissues, because of their high lipid solubility. Toxins have been measured in subcutaneous fat, but obtaining the sample is invasive. More readily available tissues such as the placenta have not been satisfactory due to poor deposition of toxicants in these organ (Schramel et al., 1988; Truska et al., 1989). In some instances, neonatal hair has been analyzed, but hair sampling is invasive and some infants are born with sparse amount of hair. The ideal matrix to analyze for fetal exposure to environmental toxins is a substrate that can be obtained easily and noninvasively and is representative of a wide period of exposure in the fetus during gestation. We propose that meconium is ideal for this purpose.

Based on a number of studies, we have demonstrated that meconium analysis is useful for detecting fetal exposure to a number of xenobiotic agents, since (1) meconium is a repository of many xenobiotic substances and their metabolites that are deposited early in gestation, i.e. from the 12th week of pregnancy (Ostrea et al., 1997), through bile secretion and fetal swallowing of amniotic fluid which contains the xenobiotic agents that are excreted in the fetal urine, and (2) meconium is not normally excreted by the fetus until after birth; thus, meconium provides a wider window to detect exposure in the fetus. To date, meconium analysis has been used to detect fetal exposure to illicit drugs (Ostrea et al., 1992, 1993; Maynard et al., 1991; Callahan et al., 1992), nicotine metabolites (Ostrea et al., 1994), alcohol metabolites (Mac et al., 1994; Bearer et al., 1999; Klein et al., 1999), a number of prescribed and over the counter medications and food additives (Ostrea et al., 1998) and environmental toxins (Ramirez et al., 2000; Whitehall et al., 2000; Whyatt and Barr, 2001).

Maternal exposure to the environmental toxins may have occurred through the common routes of inhalation (of polluted air), absorption through direct skin

contact and the ingestion of contaminated food and water. The air pollution index in the study area (Metropolitan Manila) is high particularly for lead since leaded gasoline is still widely used in motor vehicles (Philippine Environment, 1996; Philippine Environment Health, 1996). Significant contamination of fruits, vegetables, fish and shellfish with mercury and pesticides have been reported in certain areas of the Philippines and food from these places may be brought to Manila for sale and human consumption (Philippine Environment, 1996; Philippine Environment Health, 1996). The antitermite pesticides, pentachlorphenol and chlordane, are commonly used to treat wood that is used in house construction or are applied directly on the ground, around the house, as an alternate means of protecting the house from termite. Lindane is commonly used for the treatment of lice and scabies and is applied like a shampoo. Maternal exposure to lindane can occur through direct contact with the pesticide from failure to use protective gloves or ineffective hand washing. The mother/infant dyads in the study were representative of the different socioeconomic classes in Manila and the observed similar exposure rates in such a diverse socioeconomic population underscores the ubiquitous distribution of environmental toxins.

The exposure of the mother and her fetus to heavy metals and pesticides was significantly associated with a number of factors which included site of subject recruitment and some maternal and neonatal conditions. The infants born at the PCMC Hospital had a higher rate of exposure to lead and mercury most likely because the hospital serves a population where a lead battery recycling plant, which has been repeatedly cited for environmental violations, is located. It is interesting to note that among the maternal risk factors, increased pregnancy rate as evidenced by multigravida, multiparity and multiple gestation was significantly associated with increased exposure to heavy metals (lead and cadmium) and pesticides (diazinon and parathion). We speculate that the efficiency of the placental barrier to these toxins may be compromised in conditions of increased frequency of pregnancy or multiple gestation. A significant association between smoking and the exposure to pentachlorphenol was also noted. It is likely that the tobacco leaves that are used to make the cigarettes can become contaminated with the pesticide since pentachlorphenol is a common antitermite agent for farm and household use. The association of mercury and lead exposure to prematurity and low birth weight may be based on a cause and effect relationship as has been suggested by earlier studies (Recknor et al., 1997; Andrews et al., 1994; Sikorski et al., 1986). Lastly, the high intercorrelation between a number of pesticides, e.g. lindane, malathion, parathion, chlordane and chlorpyrifos may be related to the collective use of these common pesticides in the farms and homes. We recently conducted a survey of an agricultural town outside of Manila and have found that 75% of the pesticides used in the farms and households included the pyrethroids (cyfluthrin, cypermethrin, bioallethrin and transfluthrin), carbamate (propoxur) and organophosphates (chlorpyrifos, malathion)—unpublished observations.

There are some limitations in this study. First, an arbitrary decision was made to limit the analysis of the meconium samples for pesticides to the first 200 samples. The decision was made because the primary objective of the study to detect environmental toxins in meconium was satisfactorily achieved in the first 200 meconium samples analyzed; further analysis of the remaining samples was also costly and time consuming. Secondly, since this study was an exploratory investigation of environmental pollutants in meconium, the toxins that were analyzed were restricted to some of the major heavy metals and commonly used pesticides and that the latter was further restricted to the parent compound only. For the same reason, meconium analysis was also not compared to blood or hair analyses. However, a recent study comparing mercury exposure during pregnancy by the analysis of mercury in meconium, maternal or cord blood, breast milk or newborn hair showed that mercury detection was highest in meconium (Ramirez et al., 2000). Lastly, the study was not designed to specifically address the association between environmental toxins in meconium and maternal and newborn outcomes. Although this study observed some associations between some toxins and maternal or infant outcome, these analyses did not take into account other confounding factors that may have been responsible for such effects, due to the small size of the population, especially for the pesticide data. Furthermore, approximately 20 outcome variables or associations were analyzed in the study; thus, spurious results based on chance alone was likely. Thus, these observations, albeit interesting, should only be regarded as preliminary and will require further investigations with the use of a more specifically designed and adequately powered study.

We conclude that analysis of meconium can detect antenatal fetal exposure to environmental toxins, specifically, heavy metals and pesticides. We propose that this method is an important tool to investigate on the

exposure of pregnant woman to environmental pollutants and their potential adverse effects on the mother and infant.

REFERENCES

Ahlbom J, Fredriksson A, Eriksson P. Exposure to an organophosphate (DEP) during a defined period in neonatal life induces permanent changes in brain muscarinic receptors and behavior in adult mice. Brain Res 1995;677:13–9.

Alano MA, Ngougmna E, Ostrea Jr, EM, Konduri GG. Analysis of nonsteroidal anti-inflammatory drugs in meconium and its relation to persistent pulmonary hypertension of the newborn. Pediatrics 2001;107:519–23.

Amin-Zaki L, Elhassani S, Majeed MA, Clarkson TW, Doherty RA, Greenwood MR, Giovanoli-Jakubczak T. Perinatal methylmercury poisoning in Iraq. Am J Dis Child 1976;130: 1070–6.

Andrews KW, Savitz DA, Hertz-Picciotto. Pre-natal lead exposure in relation to gestational age and birth weight: a review of epidemiologic studies. Am J Ind Med 1994;26:13–32.

Bailey CH, Chen M. Morphological aspects of synaptic plasticity in Aplysia. An anatomical substrate for long term memory. Ann New York Acad Sci 1991;627:181–96.

Bakir F, Rustam H, Tikriti S, Al-Damluji SF, Shihristani H. Clinical and epidemiological aspects of methylmercury poisoning. Postgrad Med J 1980;56:1–10.

Barone Jr, S, Haykal-Coates N, Parran DK, Tilson HA. Gestational exposure to methylmercury alters the developmental pattern of trk-like immunoreactivity in the rat brain and results in cortical dysmorphology. Dev Brain Res 1998;109:13–31.

Bearer CF, Lee S, Salvator AE, Minnes S, Swick A, Yamashita T, Singer LT. Ethyl linoleate in meconium: a biomarker for prenatal ethanol exposure. Alcohol Clin Exp Res 1999;23:487–93.

Bellinger O, Leviton A, Waternaux C. Longitudinal analysis of pre-natal and post-natal lead exposure and early cognitive development. N Engl J Med 1987;16:1037–43.

Boyle CA, Decoufle P, Yeargin-Allsopp M. Prevalence and health impact of developmental disabilities in US children. Pediatrics 1994;93:399–403.

California Health and Human Services. Department of Development Services, Changes in the Population of Persons with Autism and Pervasive Developmental Disorders in California's Developmental Services System: 1987–1998. A Report to the Legislature, Mar 1999.

Callahan CM, Grant TM, Phipps P, Clark G, Novack AH, Streissguth AP, Raisys VA. Measurement of gestational cocaine exposure: sensitivity of newborn hair, meconium, and urine. J Pediatr 1992;120:763–78.

Cameron HA, Hazel TG, McKay RD. Regulation of neurogenesis by growth factors and neurotransmitters. J Neurobiol 1998;36: 287–306.

Chanda SM, Pope ON. Neurochemical and neurobehavioral effects of repeated gestational exposure to chlorpyrifos in maternal and developing rats. Pharmacol Biochem Behav 1996;53:771–6.

Chen YC, Cuo YL, Hsu CC, Rogan WI. Cognitive development of Yu-Cheng (oil disease) children pre-natally exposed to heat-degraded PCBs. JAMA 1992;268:3213–8.

Chen YC, Yu ML, Rogan WJ, Gladen BC, Hsu CC. A 6-year follow-up of behavior and activity disorders in the Taiwan Yu-Cheng children. Am J Publ Health 1994;84:415–21.

Choi BH. Methylmercury poisoning of the developing nervous system: I. Pattern of neuronal migration in the cerebral cortex. Neurotoxicology 1986;7:591–600.

Crump KS, Kjellstrom T, Shipp AM. Iinfluence of pre-natal mercury exposure upon scholastic and psychological test performance: benchmark analysis of a New Zealand cohort. Risk Anal 1998;6:701–13.

Davidson PW, Myers C, Cox C, Shamlaye CF, Marsch DO. Longitudinal neurodevelopmental study of Seychellois children following in utero exposure to methylmercury from maternal fish ingestion outcomes at 19 and 29 months. Neurotoxicology 1995;118:677–88.

Drasch G, Bose-O'Reilly S, Beinhoff C, Roider G, Maydl S. The Mt. Diwata study on the Philippines 1999—assessing mercury intoxication of the population by small scale gold mining. Sci Total Environ 2001;267:151–68.

Ehassani S, Majeed MA, Clarkson TW, Dohgerty RA, Greenwood MR, Giovanoli-Jakubczak T. Perinatal methylmercury poisoning in Iraq. Am J Dis Child 1976;130:1070–6.

Eriksson P. Developmental neurotoxicity of environmental agents in the neonate. Neurotoxicology 1997;18:719–26.

Eriksson P, Fredriksson A. Neurotoxic effects of two different pyrethroids, bioallethrin and deltamethrin, on immature and adult mice: changes in behavioral and muscarinic receptor variables. Toxicol Appl Pharmacol 1991;108:65–76.

Gillberg C, Wing L. Autism: not an extremely rare disorder. Acta Psychiatr Scand 1999;99:339–406.

Gladen BC, Rogan WJ. Effects of perinatal polychlorinated biphenyls and dichlorodiphenyl dichloroethene on later development. J Pediatr 1991;119:56–63.

Goldman L, Genel M, Bezman RJ, Slanetz PJ. Diagnosis and treatment of attention deficit hyperactivity disorder in children and adolescents. JAMA 1998;279(14):1100–7.

Graff RD, Falconer MM, Brown DL, Reuhl KR. Altered sensitivity of post-translationally modified microtubules to methylmercury in differentiating embryonal carcinoma-derived neurons. Toxicol Appl Pharmacol 1997;144:215–24.

Grifman M, Galyam N, Seidman S, Soreq H. Functional redundancy of acetylcholinesterase and neuroligin in mammalian neuritogenesis. Proc Natl Acad Sci USA 1998;95:13935–40.

Guillette EA, Maca MM, Aquilar MG, Soto AD. An anthropological approach to the evaluation of pre-school children exposed to pesticides in Mexico. Environ Health Perspect 1998; 106:347–53.

Harada H. Congenital Minamata disease: intrauterine methylmercury poisoning. Teratology 1978;18:285–8.

Holene E, Nafstad I, Skaare JU. Behavioral effects of pre- and post-natal exposure to individual polychlorinated biphenyl congeners in rats. Environ Toxicol Chem 1995;14:957–76.

Jacobson JL, Jacobson SW. Dose-response in perinatal exposure to polychlorinated biphenyls (PCBs): the Michigan and North Carolina cohort studies. Toxicol Ind Health 1996a;12: 435–45.

Jacobson JL, Jacobson SW. Intellectual impairment in children exposed to polychlorinated biphenyls in utero. N Engl J Med 1996b;335:783–9.

Jacobson SW, Fein GG, Jacobson JL, Schwartz PM, Dowler JK. The effect of intrauterine PCB exposure on visual recognition memory. Child Dev 1985;56:853–60.

Kavale NA, Forness RR. Co-variants in learning disability and behavior disorders: an examination of classification and placement issues. Adv Learn Behav Disab 1992;12:1–42.

Klein J, Karaskov T, Koren G. Fatty acid ethyl esters: a novel biologic marker for heavy in utero ethanol exposure: a case report. Ther Drug Monit 1999;21:644–6.

Larkfors L, Oskarsson A, Sundberg J, Ebendal T. Methylmercury induced alterations in the nerve growth factor level in the developing brain. Dev Brain Res 1991;62:287–91.

Lauder JM. Ontogeny of the serotonergic system in the rat: serotonin as a developmental signal. Ann New York Acad Sci 1990;600:297–313.

Liu J, Morrow AL, Oevaud LL, Grayson OR, Lauder JM. Regulation of GABA(A) receptor subunit mRNA expression by the pesticide dieldrin in embryonic brainstem cultures: a quantitative, competitive reverse transcription-polymerase chain reaction study. J Neurosci Res 1997;49:645–53.

Lonky E, Reihman J, Darvill T. Neonatal behavioral assessment scale performance in humans influenced by maternal consumption of environmentally contaminated Lake Ontario fish. J Great Lakes Res 1996;22:198–212.

Mac E, Pacis M, Garcia G, Ostrea EM. A marker of fetal exposure to alcohol by meconium analysis. Pediatr Res 1994;35: 238A.

Maiaviya M, Husain R, Seth PK, Husain P. Perinatal effects of two pyrethroid insecticides on brain neurotransmitter function in the neonatal rat. Vet Human Toxicol 1993;35:199–212.

Maynard E, Amoroso LP, Oh W. Meconium for drug testing. Am J Dis Child 1991;45:650–3.

Monnet-Tschudi F. Induction of apoptosis by mercury compounds depends on maturation and is not associated with microglial activation. J Neurosci Res 1998;53:361–7.

Needleman HL, Geiger S, Erank R. Lead and 10 scores: a reanalysis. Science 1985;227:701–4.

Ostrea EM, Brady M, Gause S, Raymundo A. Drug screening of newborn infants by meconium analysis: a large scale prospective, epidemiologic study. Pediatrics 1992;89:107–13.

Ostrea Jr, EM, Romero A, Yee H. Adaptation of the meconium drug test for mass screening. J Pediatr 1993;122:152–4.

Ostrea EM, Knapp DK, Romero A, Montes M, Ostrea AR. Meconium analysis to assess fetal exposure to active and passive maternal smoking. J Pediatr 1994;15:471–6.

Ostrea EM, Romero A, Knapp DK, Ostrea AR, Utarnachitt RB. Post-mortem analysis of meconium in early gestation human fetuses exposed to cocaine: clinical implications. J Pediatr 1994;124:477–9.

Ostrea EM, Prescilla R, Morales V, Go J, Tan E, Hernandez E, Ramirez G, Cifra H, Manlapaz M. Significant fetal exposure to heavy metals as detected by meconium analysis. Pediatr Res 1997;41:168A.

Ostrea Jr, EM, Matias O, Keane C, Mac E, Utarnachitt R, Ostrea A, Mazhar M. Spectrum of gestational exposure to illicit drugs and other xenobiotic agents in newborn infants by meconium analysis. J Pediatr 1998;133:513–5.

Ostrea Jr, EM, Ngoumgna E, Alano M, Tan E, Hernandez E, Ramirez GB, Manlapaz M. Biomarkers of fetal exposure to pesticides and their correlates to outcome. Pediatr Res 1998; 43:224A.

Owen A, Bird M. Acetylcholine as a regulator of neurite outgrowth and motility in cultured embryonic mouse spinal cord. Neuroreport 1995;6:2269–72.

Parrill M. Research implications for health and human services. In: Learning Disabilities, Lifelong Issues. Cramer and Ellis, 1996. p. 227–95.

Philippine Environmental Health Assessment, vol. 1. Environmental Health Service, Department of Health Publication, Government of the Philippines, 1996. p. 15–29.

Philippine Environmental Quality Report 1990–1995. Publication of the Environmental Management Bureau, Department of Environmental and Natural Resources, Government of the Philippines, 1996. p. 12–26.

Ramirez GB, Cruz CV, Pagulayan O, Ostrea EM, Dalisay C. The Tagum study I: analysis and clinical correlates of mercury in maternal and cord blood, breast milk, meconium and infants' hair. Pediatrics 2000;106:774–81.

Recknor JC, Reigart JR, Darden PM, Goyer RA, Olden K, Richardson MC. Pre-natal care and infant lead exposure. J Pediatr 1997;130:123–7.

Rice DC, Gilbert SC. Effects of developmental exposure to methylmercury on spatial and temporal visual function in monkeys. Toxicol Appl Pharmacol 1990;102:151–63.

Rodier PM, Aschner M, Sager PR. Mitotic arrest in the developing CNS after pre-natal exposure to methylmercury. Neurobehav Toxicol Teratol 1984;6:379–85.

Rogan WJ, Gladen BC, Hung KL, Koong SL, Shih LY. Congenital poisoning by polychlorinated biphenyls and their contaminants in Taiwan. Science 1988;241:334–8.

Rowland A. Prevalence and risk factors for ADHD in a North Carolina County: study design and preliminary results. In: Proceedings of the Conference on Environmental Influences on Children. New York Acad Med, 1999.

Safe S. Toxicology, structure-function relationship, and human and environmental health impacts of polychlorinated biphenyls: progress and problems. Environ Health Persp 1993;100:259–68.

Safer O, Zito I, Fine E. Increased methylphenidate usage for attention deficit disorder in 1990. Pediatrics 1996;98:1084–8.

Schantz SL. Developmental neurotoxicity of PCBs in humans: what do we know and where do we go from here? Neurotoxicol Teratol 1996;18:217–27.

Schantz S, Bowman RE. Learning in monkeys exposed perinatally to 2.3.7.8-tetrachlorodibenzo-*p*-dioxin (TODD). Neurotoxicol Teratol 1989;11:13–9.

Schettler T, Stein J, Reich F, Valenti M, Walinga D. In: Harm's Way: Toxic Threats to Child Development. A report by Greater Boston Physicians for Social Responsibility. MA, Cambridge: 2000. p. 9–22.

Schramel P, Hasse S, Ovcar-Pavlu J. Selenium, cadmium, lead, and mercury concentrations in human breast milk, in placenta, maternal blood, and the blood of the newborn. Biol Trace Element Res 1988;15:111–24.

Sikorski R, Paszkowski T, Szprengier-Juszkiewicz T. Mercury in neonatal scalp hair. Sci Total Environ 1986;57:105–10.

Sternfeld M, Ming G, Song H, Sela K. Acetylcholine-sterase enhances neurite growth and synapse development through alternative contributions of its hydrolytic capacity, core protein, and variable C termini. J Neurosci 1998;18: 1240–9.

Truska P, Rosival L, Balazova G, Hinst J, Rippel A, Palusova O, Grunt J. Blood and placental concentrations of cadmium, lead, and mercury in mothers and their newborns. J Hygiene Epidemiol Microbiol Immunol 1989;33:141–7.

US Census Bureau Population Estimates Program. American Psychiatric Association, Diagnostic and Statistical Manual of Mental Disorders, 4th ed. Washington, DC, 1994.

US EPA. Office of Prevention, Pesticides and Toxic Substances. Endocrine Disruptor Screening and Testing Advisory Committee, Final Report. Washington, DC, 1998a.

US EPA. A retrospective analysis of 12 developmental neurotoxicity studies submitted to the US EPA OPPTS. November 1998b.

Whitehall J, Ostrea EM, Patole S. Fetal exposure to pollutants in Townesville, Australia as detected in meconium. Pediatr Res 2000;47:299A.

Whyatt RM, Barr DB. Measurement of organophosphate metabolites in post-partum meconium as a potential biomarker of pre-natal exposure: a validation study. Environ Health Perspect 2001;109:417–20.