BIOMARKER IDENTIFICATION AND EXPOSURE ASSESSMENT OF ENVIRONMENTALLY TOXIC SUBSTANCES IN A POPULATION OF PREGNANT WOMEN AND NEWBORNS

by

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ABSTRACT OF THE DISSERTATION

Biomarker Identification and Exposure Assessment of Environmentally Toxic Substances in a Population of Pregnant Women and Newborns

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Widespread exposure to environmentally toxic chemicals may adversely affect fetal development and birth outcomes. However, data on prenatal exposure and associated health effects in newborns are very limited. A variety of pesticides, phthalates, and their metabolites were measured in maternal urine, maternal serum, cord serum, amniotic fluid, and meconium samples collected at the time of cesarean delivery from 150 women in central New Jersey. Significantly higher concentrations of dacthal ($p=0.007$), diethyltoluamide ($p=0.043$), and phthalimide ($p=0.030$) in cord serum of pesticide users than non-users suggests that residential use of pesticides may contribute to overall exposure. The concentrations of most pesticides in biological matrices of this study population were either comparable to or lower than the levels reported in previous studies and in the US general population, except for orthophenylphenol. The daily intakes of two representative organophosphorus insecticides (chlorpyrifos and diazinon) were

lower than most regulatory protection limits (EPA oral benchmark $dose_{10}$/100, EPA reference oral dose, or ATSDR minimal risk levels). The urinary concentrations of most phthalate metabolites were comparable to or lower than the U.S. general population, except for mono-(2-ethylhexyl) phthalate, mono-(2-ethyl-5-hydroxyhexyl) phthalate, and mono-(2-ethyl-5-oxohexyl) phthalate, three metabolites of di(2-ethylhexyl) phthalate (DEHP). The median urinary concentrations of mono-(2-ethyl-5-hydroxyhexyl) phthalate (109 μg/L) and mono-(2-ethyl-5-oxohexyl) phthalate (95.1 μg/L) were more than 5 times their population-based concentrations, while the median urinary concentration of mono-(2-ethylhexyl) phthalate was over 20 times higher. Calculation of daily phthalate intakes using the urinary biomarker data revealed that none of the pregnant women tested had integrated exposures to DEHP higher than the ATSDR MRLs. High concentrations of DEHP metabolites may indicate a recent exposure to the plastic medical devices containing DEHP in the hospital. However, no abnormal birth outcomes or other adverse clinical reproductive endpoints were noted in those newborns who had higher concentrations of orthophenylphenol and DEHP during the perinatal period. Significantly higher concentrations and detection frequencies in maternal urine than in maternal serum and cord serum suggest that urinary concentrations of the metabolites may be more reliable biomarkers of exposure to the environmental toxicants than the concentrations in other biological specimens.

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INTRODUCTION

ENDOCRINE DISRUPTION

Chemical contamination of the environment is a pervasive, insidious side effect of human population growth and technological development. A wide range of species, from crustaceans, fish, birds through mammals, have been reported to have untoward health effects on their reproduction, growth, and development by man-made chemicals (Crisp *et al.* 1998). The population of bald eagles and other predatory birds has been greatly reduced in North America due to accumulation of the organochlorine pesticides, such as dichlorodiphenyltrichloroethane (DDT) and its metabolites that led to eggshell thinning, and reproductive failure (Waring and Harris 2005). Because these chemicals cause adverse health impacts by interfering with the normal functioning of the endocrine systems, they are defined as endocrine disruptors (EDs) by U.S. Environmental Protection Aagency (EPA). ED can be naturally occurring, such as soy isoflavones, whole grain lignans, microbial products and components from wood, or man-made chemicals, such as plasticizers, and pesticides.

The complexity of the endocrine system provides many potential sites for endocrine disruption to occur. Examples of potential mechanisms include: alterations in receptor-mediated signaling (e.g., agonism and antagonism), alterations in hormone synthesis, alterations in hormone storage and/or release, alterations in hormone transport, alterations in hormone metabolism and clearance, and alterations in post-receptor activation (O'Connor and Chapin 2003).

EDs may cause the toxic effects via genomic or non-genomic mechanisms (Gore 2008). Normally the biological actions of hormones (e.g., estrogen, androgen, thyroxine) are mediated via high affinity protein receptors within the target cells. Man-made estrogen ligands can be found in industrial products, such as alkyl phenols from nonionic detergents, bisphenols from plastics, dye impurities, polymer chemicals, and chlorinated aromatics and pesticides. These chemicals can bind to estrogen receptors and so act as pseudoestrogens *in vivo* (Gore 2008). The alkylphenol polyethoxylate parent compounds are commonly used nonionic surfactants, which undergo degradation to form the alkylphenol, nonylphenol and octylphenol. Octyl-phenol is the most potent of these compounds, but is still 1.5 x 10^3 less potent than 17β-oestradiol (E2), followed by nonylphenol at around 10^4 times less potent than E2 (Johnson and Jurgens 2003). Phthalates are widely used in the manufacture of plastics. The phthalate esters with the most evidence for estrogenic activity *in vitro* are butyl benzyl phthalate (BBzP), dibutyl phthalate (DBP), and di(2-ethyl-hexyl) phthalate (DEHP) with *in vitro* potencies from10^{-5} – 10^{-8} compared to E2 (Johnson and Jurgens 2003). The steroid-receptor complex binds to target regions of DNA termed "response elements", this activating the cascade of responsive reactions (Perera *et al.* 2004b). The potency of an estrogen in inducing a specific biological response is not regulated simply by its binding affinity for the receptor, but can also be modulated by post-receptor interactions or other post-receptor rate-limiting events, which can collectively be considered part of the effector system for the estrogen receptor (Katzenellenbogen and Muthyala 2003). It is this exogenously induced activation or inhibition that, in some cases, might negatively affect the normal

pathways of development or physiological regulation, thereby resulting in endocrine disruption.

EDs may also exert effects on enzymes involved in the synthesis and metabolism of endogenous hormones. De novo synthesis of estrogens starts with the conversion of cholesterol to pregnenolone by CYP11A (cholesterol side-chain cleavage). In the subsequent steps, 3β-HSD (hydroxysteroid dehydrogenase), CYP17 (17α-hydroxylase and 17,20 lyase activity), 17β-HSD and CYP19 (aromatase) are involved (Perkins *et al.,* 2008; Barney *et al.,* 2008). CYP 19 (aromatase) is the rate-limiting enzyme in the formation of estrogens, not only in cells engaged in de novo synthesis of estrogens, such as ovarian granulosa cells and human adrenal cortex, but also in tissues such as the brain, adipose, and placenta, which utilize circulating levels of androstenedione or testosterone as precursors (Katzenellenbogen and Muthyala 2003; Sanderson and van den Berg 2003). Aromatase plays an important role in sexual differentiation, development, reproduction, and behavior, particularly in the gonads and the brain, and also involved in the development and progression of estrogen-dependent tumors (Sanderson and van den Berg 2003). Aromatase inhibitors block the conversion of androgens to estrogens; thus interference with aromatase activity may results in disruption of endocrine-regulated processes such as estrous cycle, sperm production and maturation, development of puberty, masculinization / feminization of sexual behavior, and inhibition or stimulation of the development and growth of hormone-dependent tumors of the breast, ovary, and prostate. Several classes of pesticides, such as DDT, a number of azole fungicides and several 2-chloro-s-triazine herbicides, have been shown to interfere with steroidogenesis (Tiemann 2008).

There are other examples of endocrine disruption that occur at "extra-receptor sites". Steroids such as estrogen normally circulate as their sulphated derivatives which have no hormonal effects; the free steroid is released at the target tissue by the action of tissue-bound sulphatase enzyme. Any compounds that alter the sulphotransferase / sulphatase activity ratio can potentially affect the availability of endogenous estrogen to target tissues. Many phenolic compounds, including the alkylphenol plasticizers, can inhibit SULT 1E1 and SULT 2A1 and cause increase in free estrogen concentration (Waring and Harris 2005).

The environmental hormone mimetic also influences receptor independent cell-signaling pathways. For example, high concentrations of dichlorodiphenyldichloroethane (DDD), a metabolite of DDT, increased the free intracellular calcium concentration in human neuroblastoma cells in vitro (Yu *et al*. 2008). The pesticide lindane, endosulfan blocks γ-amino butyric acid (GABA)-gated chloride ion channels (Law and Lightstone 2008), and the chlorinated insecticide dieldrin down regulates extracellular signal-regulated kinase1/2 (ERK1/2) phosphorylation in cultured human dopaminergic cell line (Cho *et al*. 2008). Collectively these data suggest that chemicals may activate cell-signaling pathways that increase the activity of ER in a ligand-independent manner; thus some chemicals may regulate hormonal responses by directly modulating cell-signaling pathways rather than interacting with hormone receptors.

REPRODUCTIVE AND DEVELOPMENTAL TOXICITIES

In adult animals, EDs typically cause transient adverse effects. Exposure of adult rats to high levels of endogenous estrogens such as 17β-estradiol or estrone, environmental estrogens such as methoxychlor, chlordecone, and octylphenol leads to decreased reproductive capacity in both males and females (O'Connor and Chapin 2003). In males, these effects are characterized by decreased reproductive organ weights and abnormal reproductive tract morphology, often accompanied by impaired spermatogenesis resulting in decreased sperm count, decreased sperm motility, and/or altered sperm morphology (O'Connor and Chapin 2003). In female rats, alterations in estrous cyclicity and evidence of ovarian malfunction (e.g., decreased corporea lutea, decreased ova count) are observed (O'Connor and Chapin 2003). In both cases, reproductive capacity is compromised due to disruption of the hormone feedback loops resulting in decreased gonadotropin release from the pituitary, and ultimately altered function of the male and female gonads.

In mammals the steroid sex hormones (androgens and estrogens) regulate fetal developmental processes such as differentiation and sex determination. Since the developing organism is uniquely sensitive to hormonal perturbations, even small transient alterations or temporal dysregulation in hormonal homeostasis during development can be detrimental. The inherent sensitivity of the fetus is due to the reproductive and behavioral "programming" that occurs during development of the endocrine system in the fetus and neonate (Dohler 1991; Gorski 1996). Even small perturbations in the endocrine axes during this period of development may result in permanent alterations in the way the

affected cells respond to hormones at any time in the future. A variety of pesticides, including vinclozolin, flutamide, procymidone, linuron, and dichlorodiphenyldichloroethylene (DDE), act as AR antagonists (Gray *et al.* 2007; McIntype *et al.* 2001; Andersen *et al.* 2002). The effects induced by vinclozolin exposure include decreased (i.e., female-like) anogenital distance (AGD), delayed puberty (delayed preputial separation), presence of female reproductive tissues (e.g., vaginal pouch), decreased sperm production, and a variety of malformations of the reproductive tract, from small/atrophied to completely absent male reproductive organs (Swan *et al.* 2003). All of the effects observed in the male progeny are the result of insufficient androgen exposure during development as a result of blockage of the AR.

Phthalate esters such as diethylhexylphthalate (DEHP), a major component in the polyvinyl chloride (PVC) plastics, can act as EDs in mammals. The main metabolite, monoethylhexylphthalate (MEHP) acts as an anti-estrogen in rats at low doses but has estrogenic activity at higher levels (Koch *et al.* 2006; Silva *et al.* 2003, 2006). Studies have indicated that DEHP and, more effectively, MEHP interfere with estradiol synthesis, disrupting the estrous cycle in rats. MEHP, but not DEHP, was shown to decrease the activity of aromatase in rat granulosa cells *in vitro* (Sanderson and van den Berg 2003). The mechanism of this decrease did not appear to be catalytic inhibition, but down-regulation of CYP19 mRNA expression (Lovekamp and Davis 2001). Di-n-butyl phthalate (DBP) reduced fertility in rabbits and can bind relatively weakly to the human estrogen receptor (Duty *et al.* 2005). Other plasticisers, such as alkylphenols, nonylphenol, octyl phenol, are weakly estrogenic in reproductive tissues (Calafat *et al.*

2005), but appear to have inhibitory properties toward the 17α-steroid hydroxylase activity of CYP17 (Niwa *et al.* 2002; Murono *et al.* 2000, 2001).

The ecological consequences of widespread use of pesticides are extensive. Because of their insolubility in water and resistance to biodegradation, certain pesticides bioaccumulate in our environment. For example, upon accumulation by vertebrates, DDT is metabolized to dichlorodiphenyldichloroethylene (DDE), which impairs calcium metabolism in the shell gland of adult female birds (Shen and Novak 1997). DDE also acts as a substrate for the CYP11B1 (11β-steroid hydroxylase) in mouse adrenocortical cells, inhibiting glucocorticoid synthesis, ultimately resulting in adrenocortical cytotoxicity (Johansson *et al.* 1998; Lund and Lund 1995). Atrazine herbicide showed estrogenic effects in mammal possibly by their observed ability to induce aromatase *in vitro*. In female Sprague-Dawley rats, atrazine caused lengthening of estrous cycle and a dose-dependent increase in plasma levels of estradiol, which is associated with earlier onset of the incidence of mammary and pituitary tumors (Pinter *et al.* 1990; Witzel *et al.* 1994).

HUMAN EXPOSURE AND HEALTH EFFECTS

While there are a large number of studies in experimental animals that link exposure to environmental chemicals to reproductive, developmental, and increased cancer incidence, the link to human health is still unclear. There is only preliminary but no conclusive evidence that EDs that produce adverse effects in experimental animals will probably do so in humans and/or wildlife.

There is a growing recognition that humans have been exposed to these environmental toxicants, but the full nature of this exposure is poorly appreciated. Though humans have active detoxifying enzyme systems, and are potentially at less risk, however, the metabolites of many contaminants (e.g., phthalates) can give rise to adverse effects (Weisglas-Kuperus *et al.* 2004). Studies have reported such associated adverse effects as altered fertility, immunity, increased cancer rate, adverse growth, developmental effects, and long term health consequences in children and adults (Vassilev *et al.* 2001, Perera *et al.* 2003, 2004a).

A worldwide decline in human sperm production was reported to associate with ED contamination in the environment (Swan *et al.* 2003; Fernandez *et al*. 2007). Increasingly frequent human cancers over the last 20 years include those of the breast, ovaries, testes, and prostate (Crisp *et al.* 1998), all tissues that are sensitive to sex hormones. Raised *in utero* levels of xenoestrogens have been suggested as disregulators of testicular structure and function, with increases in the prevalence of cryptorchidism, hypospadias, and testicular cancer (Colton and Greenberg 1993). Epidemiological studies have associated long-term exposures to triazine herbicides with increased risk of ovarian

cancer in female farm workers in Italy (Dona *et al.* 1989) and increased risk of breast cancer in the general population of Kentucky in US (Kettles *et al.* 1997).

Although there are various sources of bias and controversial indications from these studies, it is certainly possible that sub-sets of the population could have increased susceptibility to EDs, particularly the fetus if the maternal detoxification systems were impaired. Different from adults, infants have smaller size, more rapid growth, and higher caloric consumptions on a per unit body weight basis. Comparison of consumption data for infants and adults on grams per kilogram of body weight basis results in elevated values for infants, and the toxic contaminants found in a mother's blood may have elevated disproportionate adverse health impacts on her infant. Much current evidence points to early development (embryo, fetus) as the most sensitive stages for toxic exposures, although the effects on exposed infants are often not apparent until an organism reaches sexual maturity.

Compounds that can affect any species can potentially affect human beings. What we do not yet know is at what levels. To better understand the risk level of EDs on human population, assessment of the body burden becomes critical as currently there is relatively little information on the body burden of EDs in human populations, and far less is known about the cumulative effects that many chemicals released into the environment have had against the background of human-induced perturbations. Based on the National Health and Nutrition Examination Survey (NHANES 2001-2002), CDC released the third National Report on Human Exposure to Environmental Chemicals (CDC 2005). This report covers the exposure data for the U.S. population for 148 environmental chemicals, including pesticides and phthalates/metabolites in 2,782 participants. This report

identified a number of chemicals that occurred in substantially elevated levels (10 μg/dL or greater) in blood or urine in the population sampled, such as, mono-benzyl phthalate (MBzP), mono-n-butyl phthalate (MBP), and mono-ethyl phthalate (MEP). It indicated that most Americans, particularly women of childbearing age, had higher than expected exposures to phthalates and pesticides. However while so few study locations were sampled no broad generalizations could be made, and lack of absolute evidence of causal relationship between the elevated metabolites levels in blood or urine and the adverse health effects, it is certainly a major wake-up call for more scrutiny and research into the sources of the responsible exposures.

RISK TO VULNERABLE SUBPOPULATION

The identification of environmental agents that are associated with adverse effects on fetal development as a result of endocrine disruptions, as well as the development of predictive biomarkers for exposure assessment, will provide Regulatory Agency with information on hazard identification and risk management, when dealing with vulnerable subpopulations, e.g., pregnant women and new born babies. As chemical contamination of the environment increases worldwide due to human population growth and technological development, this information become increasingly important because the risk assessment approach must be expanded to incorporate a wider range of susceptible populations, exposure assessment using sensitive biomarkers, and consideration of mixtures of toxic substances.

The present study was undertaken to 1) characterize and quantify the environmental toxic chemicals that comprise the major body burden in the vulnerable population of pregnant women and their newborns, 2) identify reliable biomarkers of exposure to those environmental chemicals, and estimate the daily exposure level of the parent compounds using their respective biomarkers, 3) assess the risk level of potential adverse health effects in pregnant women due to exposure to those environmental toxicants, and 4) determine if the subsequent gestational exposure is associated with adverse effects on fetal development. It provided specific data on either the identity or exposure levels of these environmental contaminants in maternal urine, maternal blood, umbilical cord blood, amniotic fluid in a local population of New Jersey mother and

newborns. It might also contribute to the exposure characterization to those contaminants and provide information for risk management process for the sensitive population.

STATEMENT OF HYPOTHESIS

Increasing level of human exposure to environmentally toxicants (phthalates, pesticides, and phenols) is approaching or exceeding the Regulatory exposure limits. This high exposure may contribute to potential adverse health effects, especially on the vulnerable population, e.g., pregnant women and the newborns.

SPECIFIC AIMS

1) To characterize and quantify the environmental toxic chemicals that comprises the major body burden in a local New Jersey population of pregnant women and their newborns

2) To identify reliable biomarkers of human exposure to those environmental chemicals, and estimate the daily exposure level of the parent compounds using their respective biomarkers

3) To assess the risk level of potential adverse health effects in pregnant women and determine if the subsequent gestational exposure is associated with adverse effects on fetal development

METHODS

Our study was a prospective observational analysis of pesticide exposures in maternal and fetal compartments. We recruited 150 mothers and their newborns (Table 3) at a major central New Jersey teaching hospital, Saint Peter's University Hospital in New Brunswick, which averages from 12 to 30 births per day. All subjects provided informed consent prior to participation in the study that was approved by the Institutional Review Board at Rutgers University, UMDNJ-Robert Wood Johnson Medical School, NJ, and Saint Peter's University Hospital, NJ with a collaborative agreement with the Centers for Disease Control and Prevention (CDC). Medical history, information on residential pesticide use, basic demographic and current health information were collected as part of the study. A questionnaire (Table 1) was distributed to the pregnant women to collect information on pest control use, PVC household products and frequency of use. The usage information was gathered by questions such as "Did the mother or anyone else use pesticides in the home while she was pregnant (Never / Sometimes / Often)?" "Did the mother or anyone else use pesticides in the yard while she was pregnant (Never / Sometimes / Often)"? "Did the mother treat the pet(s) for any pest problems (e.g., fleas, ticks, worms)"? "Did the mother microwave food in PVC plastic containers while pregnant (Never/Sometimes/Often)"? "Did the mother use plastic tableware while pregnant (Never/Sometimes/Often)"? "Did the mother save items in plastic containers while pregnant (Never/Sometimes/Often)"? Maternal pregnancy characteristics and neonatal outcome data were recorded following delivery.

Eligible healthy subjects included women with singleton pregnancies scheduled for an elective cesarean delivery at term (≥ 37 weeks) who had blood hemoglobin concentrations ≥ 8 mg/dL. Women were excluded if they took medications that could interfere with the metabolism and/or measurement of environmental chemicals, if they had pregnancy-related complications, or if there was evidence of labor or rupture of membranes at the time of operative delivery. Standard clinical protocols were followed to collect all biological samples. All women were put on intravenous glucose, fluid and electrolyte support after arrival at the hospital. Maternal blood samples (10–30 mL) were obtained on the day of surgery. The maternal urine specimens were collected before delivery, but after the foley catheter and the intravenous lines were placed. The amniotic sac was punctured with a syringe prior to delivery and approximately 10-30 mL of amniotic fluid was withdrawn. After the infant was delivered, the umbilical cord was clamped and 30-60 mL of cord blood was aspirated directly from the umbilical vein. All umbilical vein samples were obtained within 15 minutes of delivery. The whole blood samples were centrifuged to isolate the blood serums in the hospital. Meconium was collected by scraping the solid content (0.5 ~ 2 g) of the diaper into a specimen collection container after the infants had their first bowel movement. An aliquot of each serum, urine, and amniotic fluid sample was stored separately in a glass or Qorpak bottle for pesticide analysis. Ten percent of all samples collected were quality assurance samples.

Prior to initiation of the study, representative samples of all equipment used for either collection or storage of samples were examined by the CDC laboratory to verify the absence of the phthalate metabolites measured for this study. No preservatives were added prior to sampling of the urine, but phosphoric acid was added to maternal and cord

serum samples to quench the esterase activity (Kato *et al.* 2004). Then all samples were stored at -20 °C until transferred to the CDC laboratory for analysis. Specimens were sent overnight express in dry ice to avoid potential freeze-thaw cycles. The samples were maintained at -20 °C until analyzed.

Pesticides and/or their metabolites were measured in urine, serum, amniotic fluid, and meconium using mass spectrometry-based methods to measure a) synthetic pyrethroids, b) chemical-specific metabolites of OP pesticides, such as 3,5,6-trichloropyridinol (TCPy) for chlorpyrifos, c) class-specific metabolites of OP pesticides, such as the six dialkyl phosphate (DAP) metabolites: dimethylphosphate (DMP), dimethylthiophosphate (DMTP), dimethyldithiophosphate (DMDTP), diethylphosphate (DEP), diethylthiophosphate (DETP), and diethyldithiophosphate (DEDTP), and d) other pesticides or metabolites, including carbamates, herbicides, and pesticides with chlorinated phenol metabolites, such as disinfectants (Table 2). Laboratory methods involved a solid-phase or liquid partitioning extraction with analysis using tandem mass spectrometry or high resolution mass spectrometry with isotope dilution calibration. These methods were previously developed and validated at the CDC (Barr *et al.* 2002; Bravo *et al.* 2004, Bravo *et al.* 2005, Olsson *et al.* 2004). Positive and negative control samples were analyzed concurrently with participant samples to ensure quality laboratory measurements.

Phthalate metabolites were measured in maternal urine, maternal serum and cord serum at CDC. The analytical methods involved the enzymatic deconjugation of the phthalate metabolites from their glucuronidated form, automated solid-phase extraction, separation with high performance liquid chromatography, and detection by isotope-

dilution tandem mass spectrometry (Kato *et al.* 2004; Silva *et al.* 2004). The limits of detection (LODs) were in the low ng/ml range; 1 ml of sample was used for the analysis. Each analytical run also included analytical standards, quality control [QC] materials of high concentration, QC materials of low concentration, and reagent blanks. The QC samples were analyzed along with the study samples to monitor for accuracy and precision.

The statistical analysis consisted of summary statistics of each metabolite, such as means, medians, distribution percentiles, ranges, correlation analyses among analytes, and comparisons of the concentrations of the metabolites from each biological matrix. In all analyses, concentration values below the limit of detection (LOD) were assigned a concentration equal to LOD/$\sqrt{2}$ for the significance calculations (Hornung *et al.* 1990). Potential associations of pesticide or phthalate metabolite concentrations in the biological specimens to household product use and clinical birth outcomes were evaluated. All tests were two-tailed and statistical significance was set at $p < 0.05$.

The daily doses of two representative OP insecticides compounds, chlorpyrifos and diazinon were estimated from the urinary metabolite concentration data. Chlorpyrifos and diazinon dose calculations were based upon urinary DEP and DETP concentrations since these are the only two DAP metabolites that can be derived from these pesticides. Given the assumption that metabolite concentrations were the result of exposure to a single pesticide, a mathematical method (Equation 1) was applied to calculate the daily exposures of OP pesticides (Castorina *et al.* 2003; Fenske *et al.* 2000). This method provides a reasonable upper-bound estimate of exposure to specific chemicals, and is consistent with current regulatory methods (Castorina *et al.* 2003).

Equation 1.

$$D_P = [(C_{DEP} / MW_{DEP} + C_{DETP} / MW_{DETP}) \times MW_P \times (Cr_{Ex} / Cr_{Conc})] \div BW$$

Where D_P is the daily dose estimate from the parent OP pesticide (μg/kg/day), C is the urinary dialkyl phosphate metabolite concentration (μg/L), MW_P is the molecular weight of the parent pesticide (grams per mole), Cr_{Ex} is the expected daily urinary creatinine excretion (mg/day) from reference values for pregnant women (Castorina *et al.* 2003), Cr_{Conc} is the creatinine concentration (mg/L). Thus Cr_{Ex}/Cr_{Conc} is the expected 24-hr urine output volume for the pregnant woman (L/day), and BW is the body weight of the pregnant woman.

The daily exposure of the phthalate parent compounds was estimated, assuming steady-state intake of the phthalate diester and metabolic clearance of the metabolite, from the concentrations of the urinary concentrations of the phthalate metabolites using the method proposed by David (2000) as expressed by Koch *et al.* (2003a): $DI = [(ME \times CE) / (\boldsymbol{F_{UE}} \times 1{,}000)] \times MWd / MWm)$ where *DI* is the daily intake in micrograms per kilogram per day; *ME* is the creatinine corrected urinary metabolite concentration in micrograms per gram; *CE* is the creatinine clearance rate, normalized for body weight, in milligrams per kilogram per day; $\boldsymbol{F_{UE}}$ is the molar conversion factor that relates urinary excretion of metabolite to diester ingested; and *MWd* and *MWm* are the molecular weights of diester and metabolite, respectively. For these calculations, we set CE at 20 mg/kg/day for adults (Jacobs *et al.* 2001; Tietz 1990), and used the following $\boldsymbol{F_{UE}}$ values: 0.69 for MEP/DEP and MBP/DBP; 0.059 for MEHP/DEHP, 0.23 for MEHHP/DEHP, 0.15 for MEOHP/DEHP, and 0.73 for MBzP/BBzP (Koch *et al.* 2004a; Anderson *et al.* 2001).

CHAPTER I

Pesticide Concentrations in Matrices Collected in the Perinatal Period in a Population of Pregnant Women and Newborns in New Jersey

ABSTRACT

Gestational exposure to pesticides may adversely affect fetal development and birth outcome. However, data on fetal exposure and associated health effects in newborns remain sparse. We measured a variety of pesticides and metabolites in maternal urine, maternal serum, cord serum, amniotic fluid, and meconium samples collected at the time of cesarean delivery from 150 women in central New Jersey. Women who used pesticides at home had higher concentrations of pesticides or metabolites in cord serum [*e.g.*, dacthal (p=0.007), diethyltoluamide (p=0.043), and phthalimide (p=0.030)] than those who did not use pesticides, suggesting that residential use of pesticides may contribute to overall exposure as assessed by biomonitoring. Except for orthophenylphenol, the concentrations of most pesticides in biological matrices of this study population were either comparable to or lower than the levels reported in previous studies and in the US general population. The daily exposure estimates of two representative organophosphorus insecticides (chlorpyrifos and diazinon) were lower than most regulatory protection limits (EPA oral benchmark $dose_{10}$/100, EPA reference oral dose, or ATSDR minimal risk levels); however, they were near or at the EPA's population adjusted doses for children and women. No abnormal birth outcomes or other clinical endpoints were noted in those

newborns who had higher concentrations of orthophenylphenol during the perinatal period.

Key words: Pesticides; Urine, Blood, Amniotic fluid; Exposure Assessment; Pregnant Women; Children.

INTRODUCTION

Pesticides are a diverse group of chemicals that includes insecticides, fungicides, herbicides, and rodenticides. Among the most widely used pesticides are the organophosphate (OP), carbamate, and pyrethroid insecticides (Landrigan *et al.*, 1999). Approximately 912 million pounds of pesticides are used annually in the United States (Kiely *et al.* 2004). Approximately 80% are used in agriculture, 8% are used in homes and gardens and the remainder is used in governmental, commercial or industrial applications. Herbicides comprise the bulk of pesticides used (42%) while insecticides (10%), fungicides (6%) and other pesticides (fumigants, rodenticides) make up the remainder (Kiely *et al.* 2004). In 1999, approximately 125 million pounds of OP insecticides were used (Donaldson *et al.* 2002). Although some agricultural uses of OP insecticides were restricted in 2001, overall use in this sector has not changed in recent years. However, residential uses of chlorpyrifos and diazinon, two common OP insecticides, were eliminated in 2001 and 2003, respectively (U.S. EPA 2002). Carbamate insecticides are also widely used in both residential and agricultural applications. Synthetic pyrethroids have largely replaced residential uses of OP insecticides and are now the dominant class of insecticides used in homes and gardens (ATSDR 2003).

The current extensive use of pesticides has resulted in widespread exposure to the U.S. population (Barr *et al.* 2004; 2005). For example, insecticide contamination in residential environments, including air, dust, and surfaces, has been documented in a variety of urban and rural environments (Bradman *et al.* 1997; Simcox *et al.* 1995; Whyatt *et al.* 2003). Detectable insecticide residues were found in approximately 47% of

the fruit and vegetable samples tested as part of market-basket surveys by the USDA in 2002 (USDA 2002). Also, pesticide contamination of surface and ground waters has been well-documented (Battaglin *et al.* 2000; Scribner *et al.* 2000).

Dietary exposures likely constitute the largest percentage of general population exposures (MacIntosh *et al.* 2001; Melnyk *et al.* 1997); however, residential use of pesticides is also a potentially important contributor to exposures (CDC 2005; Fenske *et al.* 2000; Lu *et al.* 2000; O'Rourke *et al.* 2000). Approximately 80-90% of American households use pesticides (Landrigan *et al.* 1999). A study of Minnesota households with children found that pesticide products were stored in 97% of the households investigated, and as many as 88% of the households reportedly had used pesticides within the past year (Adgate *et al.* 2000). A New York City study found 85% of minority women had used pest control measures in their home during pregnancy (Whyatt *et al.* 2002), a time during which children are particularly vulnerable to environmental exposures (Eskenazi *et al.* 1999). Commonly detected pesticides in house dust and indoor air of U.S. homes include the OP insecticides chlorpyrifos and diazinon, the pyrethroid insecticides *cis*- and *trans*-permethrin, the carbamates propoxur and bendiocarb, and the fungicide/disinfectant ortho-phenylphenol (Whyatt *et al.* 2003; Eskenazi *et al.* 1999).

Pesticide exposures have been widely studied over the past several decades (Berkowitz *et al.* 2003; Adgate *et al.* 2001; Landrigan *et al.* 1999); however, recent studies are beginning to focus on pregnant women and their fetuses because of the vulnerability of this subpopulation to harmful effects from exposure to environmental contaminants (Eskenazi *et al.* 1999; Slotkin 1999). Human fetuses are particularly sensitive to pesticide exposure because the brain is developing very rapidly and the fetus'

ability to detoxify contaminants is limited. Toxicological studies have shown OP insecticides, along with other insecticides such as p,p'-DDT, hexachlorobenzene and chlordane, can cross the placental and blood-brain barriers (Chanda and Pope 1996; Muto *et al.* 1992; Sala *et al.* 2001). Although more studies are focusing on maternal-fetal pesticide exposures and resulting health outcomes, few have explored biological matrices within the maternal-fetal compartment to determine the relative proportion of pesticides or metabolites in each matrix and the matrix suitability for biomonitoring of in utero exposure to pesticides. The specific aims of this paper were to: a) determine the pesticides to which pregnant women in a New Jersey cohort may have been exposed b) determine the distribution of pesticide and metabolite concentrations in the matrices collected and to compare these concentrations to population-based averages; c) explore the associations between home use and pesticide exposure using the biomarkers in the biological matrices; d) estimate the daily dose of two representative OP compounds relative to regulatory protection limits; and e) assess the birth outcomes associated with pesticide exposure. Our ultimate goal was to provide pesticide exposure and risk information of this vulnerable population to the public health authorities who could plan or conduct interventions, if necessary, to reduce pesticide exposures to pregnant women and their newborns in the community.

METHODS

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RESULTS

Table 3 presents characteristics of the 150 women from central New Jersey area, the number of women who reported using pest control measures in their homes during pregnancy, and the clinical birth outcomes of the 150 newborns. The median age of participants was 33 years. Only 4% of the women smoked sometimes during pregnancy, and none of them reported drinking during pregnancy. The average gestation of the women was 39 weeks (± 0.79); the average birth weight 3775 grams (± 689 SD); and the average Apgar score at 5 minutes was 9 (± 0.09). About a third of the women reported using some form of pest control inside the house and over half reported using pesticides in the yard.

Table 4 shows the concentrations of OP pesticides and their metabolites measured in cord serum, maternal serum, meconium, amniotic fluid, and maternal urine samples. Chlorpyrifos was detected in 37% of the cord serum samples, but it was rarely detected in maternal serum (1%). Diazinon was rarely detected in either cord (1%) or maternal serum (9%). The six DAP metabolites of OP insecticides were infrequently detected in meconium, however, they were detected much more frequently in maternal urine and amniotic fluid. The urinary concentrations of the 6 DAP metabolites were much higher than the concentrations in amniotic fluid and meconium (Table 4). Except for DEDTP and DMDTP, the DAPs were found in 71% to 100% of the samples tested.

Concentrations of phenolic metabolites of multiple pesticides across biological specimens are shown in Table 5. Orthophenylphenol was the most frequently detected phenolic metabolite in both maternal urine (100%) and amniotic fluid (60%). 2,4,5-trichlorophenol, and 2,4,6-trichlorophenol were only found in maternal urine samples,

whereas 2-naphthol, 2, 4-dichlorophenol, 2,5-dichlorophenol, and 4-nitrophenol were also detected in amniotic fluid, albeit with low frequency. 1-naphthol was found in all matrices sampled, but with the exception of cord serum, typically at low frequencies of detection. In general, the urinary concentrations of the metabolites were much higher than the concentrations found in amniotic fluid.

Several pesticides were only measured in maternal and cord serum (Table 6). Diethyltoluamide (DEET) was the most frequently detected pesticide in both maternal (100%) and cord (100%) serum. Phthalimide was found in 76% and 100% of the cord and maternal serum samples, respectively. Similarly, dacthal was found in 71% and 92% cord and maternal samples, respectively. The concentrations of phthalimide and dacthal in maternal serum were 2- to 3-fold higher than the concentrations in cord serum; however, DEET concentrations were fairly consistent among the two matrices. Cis-permethrin, metolachlor, tetrahydrophthalimide, and trans-permethrin were found in cord serum, but were rarely detected in maternal serum.

Table 7 shows the geometric mean and percentile dose estimates of chlorpyrifos and diazinon and the regulatory limits for these pesticides. The median estimated doses of chlorpyrifos and diazinon were 0.124 and 0.108 μg/kg/day, and the 95^{th} percentile estimates were 0.512 and 0.448 μg/kg/day. Figure 1 presents the relative cumulative frequencies of the two representative OP insecticides at different daily doses.

Women who used pesticides in the home had serum concentrations of dacthal, DEET, and phthalimide that tended to be higher, although not significantly so, than serum concentrations in women who did not use pesticides in the home (Figure 2). Cord

serum concentrations of dacthal, DEET, and phthalimide were significantly higher when pesticides were used in the homes (Figure 3).

Regression and correlation analyses were performed among analytes with high detection frequencies in biological matrices using their positive concentrations. Linear correlations were found among maternal serum and cord serum for DEET [Pearson correlation coefficient (r) = 0.3054] and phthalamide (Pearson r = 0.3054) (Figure 4). Similar associations were discovered among chlorpyrifos analytes. TCPy levels in maternal urine correlated well with DETP levels in maternal urine (Pearson r = 0.8613) and chlorpyrifos levels in cord serum were correlated with DEP in amniotic fluid (Pearson r = 0.3940) (Figure 5).

DISCUSSION

To assess exposure to pesticides during pregnancy, biomarkers (i.e., pesticides or their metabolites) have been used as integrative dosimeters that can help to evaluate total exposure regardless of route. Maternal urine and blood are the primary human specimens that have been used for biomonitoring of pesticide exposure during pregnancy; however, meconium and amniotic fluid have also been explored as potential matrices for assessing cumulative *in utero* exposures to the fetus (Whyatt and Barr 2001; Bradman *et al.* 2003).

To our knowledge, our study is the first to measure pesticides and metabolites in multiple matrices collected during the perinatal period. The presence of the pesticides or metabolites in fetal compartment matrices and the linear correlation of their concentrations between maternal and cord serum (Figure 4) is evidence of the transport of these chemicals from the mother to the fetus. Although we cannot discern whether the metabolites were transferred after metabolism in the mother, the relatively undeveloped metabolic capacity of the fetus suggests that most metabolism took place prior to transfer to the fetal compartment. The intact pesticides that were detected in the cord serum or amniotic fluid indicate that some unmetabolized pesticides can also reach the fetal compartment.

We detected DMP and DMTP in the vast majority of the urine samples tested likely indicating some exposure to malathion. Similarly, DEP and DETP were detected in over 70% of the samples tested. Linear correlations existed between the parent compound chlorpyrifos and its metabolite DEP as well as between its metabolites TCPy and DETP (Figure 5). These data coupled with the detection of chlorpyrifos in a reasonable portion of cord serum and TCPy in maternal urine suggest that the pregnant mothers were

exposed to chlorpyrifos and that a portion of the chlorpyrifos was deposited into the fetal compartment.

The urinary metabolite concentrations (geometric means and percentile values) in this study were compared with those found in the general U.S. population (Tables 8 and 9). For most of the metabolites measured, the concentrations were similar to or lower than those found in the general population; however, the frequency of detection of some of the metabolites, particularly the DAP metabolites, in our study were much higher. Interestingly, 2,5-dichlorophenol concentrations were much lower than those typically found in the U.S. population suggesting that the study participants were not in contact with toilet bowl sanitizers/deodorizers or moth balls within the few days prior to sample collection.

The concentrations of orthophenylphenol in maternal urine samples from our study population were about four-fold higher than the 95th percentile concentration found in the U.S. population (Table 9). Orthophenylphenol, an industrial grade disinfectant, is often used in hospital settings. The unusually high concentrations of orthophenylphenol may reflect exposures that occurred in the hospital prior to delivery. Because women are typically in the hospital for many hours prior to parturition, the body burden from exposures they received at home would be declining while exposures potentially received at the hospital setting would likely be increasing the overall body burden during this time. The exposure to orthophenylphenol was either of sufficient magnitude or duration to result in transfer of the pesticide to the fetal compartment as orthophenylphenol was detected in 60% of the amniotic fluid samples tested.

In general, most pesticide exposures are believed to come from the diet. However, residential exposures can still occur and may be a significant source of intermittent exposures (CDC 2005; Fenske *et al.* 2000; Lu *et al.* 2000; O'Rourke *et al.* 2000). We observed significantly higher concentrations of dacthal, diethyltoluamide, and phthalimide in cord serum samples of women who used pesticides during pregnancy which suggests that some portion of pesticide exposure may be derived from home pesticide use.

We compared the maternal serum and cord serum levels of three representative pesticides, e.g., dacthal, diethyltoluamide, and phthalimide, between pesticide users and non-users and found a data trend indicating the pesticide users at home had higher concentrations than the non-users. This finding suggests that residential use of pesticides may contribute to overall pesticide body burden for individuals.

The median concentrations of chlorpyrifos in cord serum and diazinon in maternal serum were 0.0007 (<LOD-0.001 ng/mL) and 0.0004 ng/mL (<LOD-0.0005 ng/mL), respectively, which were lower than those previously reported in maternal and cord plasma samples in a New York city cohort (Whyatt *et al.* 2003, 2004). The lower concentrations found in our study may reflect decreased exposures resulting from the EPA's regulatory action in 2001 to discontinue the residential use of diazinon and chlorpyrifos. The median levels of most other pesticides, such as carbofuran, matalaxyl, chlorothalonil, cis-permethrin, metolachlor and tetrahydrophthalimide, were not detected in the maternal and cord serum of this cohort. The fungicides dacthal, phthalimide and DEET were detected in the maternal and cord serum samples of this cohort with high frequencies. The multiple exposure sources, including diet and residential use, might

account for the fact that levels of dacthal, phthalimide and DEET in maternal and cord serum samples were higher than levels of the other pesticides measured.

The daily exposure estimates for both chlorpyrifos and diazinon were lower than most of the existing exposure guidelines established by the regulatory agencies, such as the EPA oral benchmark dose$_{10}$ (BMD$_{10}$), EPA reference oral dose (RfD), or ATSDR minimal risk level (MRL). However, the median and 95th percentile chlorpyrifos dose estimates were approximately equivalent to the chronic and acute population adjusted doses (cPAD, aPAD), respectively, for children and females. These data suggest that dietary exposures may be close to the regulatory limits. However, enough margin of safety has been built into each of these regulatory limits, and the DAP metabolites can also be derived from exposure to the preformed metabolites; these doses were likely overestimated representing a worst case dose estimate. Currently, no analogous exposure guidelines exist to evaluate the significance of the measured levels of fetal biomarkers exist.

We evaluated whether any birth outcomes were associated with high concentrations of orthophenylphenol. The clinical data of the 5% of newborns who had the highest amniotic fluid orthophenylphenol concentrations were examined. These 7 babies had amniotic fluid orthophenylphenol concentrations ranging from 97.1 to 312.2 ng/mL. No associations with adverse birth outcome (birth weights, APGAR scores, gestational ages) were observed. Similarly, we evaluated the clinical data of the 25% of newborns whose mothers had the highest urinary orthophenylphenol concentrations. No aberrant birth outcomes or abnormal clinical findings were diagnosed by the pediatricians.

Although we detected many of the pesticides in many of the samples collected from the fetal compartment, maternal urine and umbilical cord samples provided the most consistently measurable data. Thus, in studies evaluating *in utero* exposures, cord serum can be analyzed to directly detect the exposure to the fetus, and maternal urine samples can be analyzed as a surrogate exposure measurement.

This study has the advantage of using individual prenatal exposure data from biomonitoring in multiple matrices, as well as concise medical records and questionnaire data. However, it is limited by the modest sample size and comparatively less exposure than many other populations reported. Few of our study participants smoked or drank during the pregnancy based on the questionnaire survey. It is not possible to generalize our findings to other populations. Future studies should involve a larger cohort that includes women from diverse geographic areas, from different social economical classes and who have potential pesticide exposure risk factors in order to better define which chemicals are present in the biological matrices, what parameters may affect the exposure, and whether the gestational exposure is a contributory factor to potential adverse health effects on pregnant women and their newborns.

The doses of some OP insecticides might be overestimated because the subjects could be exposed to the preformed DAP metabolites. OPP was found in higher concentrations in maternal urine from our study cohort than from the U.S. population. The higher OPP concentrations may reflect exposures that occurred in the hospital prior to delivery because OPP is often used in hospital settings as disinfectant; however, we did not collect information from the hospital about OPP usage. Further studies are required to assess exposure to OPP in hospital settings. The results from these studies

could help to determine if any interventions are necessary in the hospital setting to minimize exposure to hospital patients.

CONCLUSION

Biomarkers can be valuable tools to monitor pesticide exposure during fetal development. The parent compounds can be monitored directly in the serum samples instead of measuring their metabolites in urine. Measurements of pesticides in amniotic fluid and meconium produced by the fetus, can be useful biomarkers of direct fetal exposure. Significantly higher concentrations and detection frequencies in maternal urine than in serum and amniotic fluid in this study suggest that the maternal urinary concentrations of the pesticide metabolites may be better and more reliable biomarkers for pesticide exposure assessment.

In this NJ population of pregnant women and newborns, multiple pesticides and metabolites were detected, with varied frequencies, in the biological matrices representing the maternal-fetal compartment. OP insecticide metabolites were identified in more than 76% of the maternal urine samples. OPP was detected in 66% of the amniotic fluid and 100% of the maternal urine samples. Dacthal, DEET, and phthalimide were detected in more than 75% of the cord and maternal serum samples. These data suggest widespread exposure of this study population to those pesticides during pregnancy. The daily exposure estimates from the parent pesticides, e.g., chlorpyrifos and diazinon, were well below many regulatory guidance limits; however, they were near the EPA's PADs for children and women.

The presence of these metabolites reflects exposures to common pesticide products for residential or agricultural use. Our data suggest that residential use of pesticides contributes to human exposure as assessed by biomonitoring. The pesticide concentrations in all of the biological matrices of this study population were typically

lower than those reported in previous studies. However, OPP concentrations in our study participants were higher than general population concentrations, possibly resulting from recent hospital exposures to OPP. No clinical abnormities or aberrant birth outcomes in the newborns were associated with the pesticide exposures.

CHAPTER II

Phthalates Biomarker Identification and Exposure Estimates in a Population of Pregnant Women

ABSTRACT

Phthalates are known reproductive and developmental toxicants in experimental animals. However, in humans, there are few data on the exposure of pregnant women that can be used to assess the potential developmental exposure experienced by the fetus. We measured several phthalate metabolites in maternal urine, maternal serum, and cord serum samples collected at the time of delivery from 150 pregnant women from central New Jersey. The urinary concentrations of most metabolites were comparable to or lower than among the U.S. general population, except for mono(2-ethylhexyl) phthalate (MEHP), mono(2-ethyl-5-hydroxyhexyl) phthalate (MEHHP), and mono(2-ethyl-5-oxohexyl) phthalate (MEOHP), three metabolites of di(2-ethylhexyl) phthalate (DEHP). The median urinary concentrations of MEHHP (109 μg/L) and MEOHP (95.1 μg/L) were more than 5 times their population-based concentrations, while the median urinary concentration of MEHP was over 20 times higher. High concentration of MEHP may indicate a recent exposure to the parent chemical DEHP in the hospital shortly before the collection of the samples. Calculation of daily intakes using the urinary biomarker data reveals that none of the pregnant women tested had integrated exposures to DEHP greater than the Agency for Toxic Substances and Disease Registry's minimal risk levels (MRLs

chronic 60 µg/kg/day, intermediate 100 µg/kg/day). No abnormal birth outcomes (*e.g.,* birth weight, Apgar Score, and gestational age) were noted in those newborns whose mothers had relatively higher exposure to DEHP during the perinatal period than others in this study. Significantly higher concentrations and detection frequencies in maternal urine than in maternal serum and cord serum suggest that the urinary concentrations of the phthalate metabolites may be more reliable biomarkers of exposure than their concentrations in other biological specimens.

Key Words: Phthalates; Metabolites; Daily Intake; Risk Assessment; Women; Children

INTRODUCTION

Phthalates are diesters of 1, 2-benzenedicarboxylic acid (phthalic acid) produced by reaction of phthalic acid with a specific alcohol to form the desired ester with the side chain(s) of interest. They are high production volume chemicals (> 1 million tons produced or imported into the United States per year) and are used in many consumer products, including personal care products (*e.g.,* perfumes, lotions, cosmetics), paints, industrial plastics and pharmaceuticals. Their primary functions in these products are to hold color or fragrance, to provide a film or gloss, to make certain plastics more flexible, or provide timed release for some pharmaceuticals (ATSDR 1995, 1997, 2001, 2002). A large market for several phthalates is as plasticizers for polyvinyl chloride (PVC), and certain types of elastomers. Given their use in a vast range of consumable products and because they are not covalently bound to the other chemicals in the formulations, the potential for human exposure to phthalates is high.

Phthalates can enter the human body by several routes, including ingestion through diet, absorption through skin, and inhalation of indoor and outdoor air. For several phthalates, the principal route of exposure is assumed to be the ingestion of contaminated food products (CERHR 2000, 2005). Phthalates can be absorbed from the intestinal tract and the lung. Dermal absorption is lower with the high molecular weight phthalates (*e.g.,* di(2-ethylhexyl) phthalate (DEHP)) than with the low molecular weight phthalates, such as diethyl phthalate (DEP) and di(*n*-butyl) phthalate (DBP) (ATSDR 1995, 2001, 2002). Metabolism of most phthalates in humans occurs by an initial phase I biotransformation in which the diesters are metabolized into their hydrolytic monoesters.

The monoesters of high molecular weight phthalates (*e.g.,* DEHP) may be further metabolized to produce more hydrophilic oxidative products (Koch et al. 2006; Wilson et al. 2007; ATSDR 1997; Barr *et al.* 2003). Monoesters and the oxidative metabolites of phthalates can be excreted in urine and feces in their free form or as glucuronide conjugates with increased water solubility and increased urinary excretion (ATSDR 1995, 2002).

Phthalates have been reported to be developmental and reproductive toxicants in experimental animals. Developmental anomalies were seen in rodents dosed during gestation and/or lactation with DBP (Mylchreest *et al.* 1999, 2000), DEHP (Gray *et al.* 2000; Koch *et al.* 2006), butylbenzyl phthalate (BBzP) (Tyl *et al.* 2004; Ema *et al.* 2003; Ema and Miyawaki 2002), and diisononyl phthalate (Gray *et al.* 2000). The commonly observed anomalies at experimentally high doses included reductions in androgen-dependent tissue weights (*e.g.*, seminal vesicles, epididymis, and prostate), increased incidence of hypospadias, cryptorchidism, decreased anogenital distance (AGD), delayed preputial separation (pubertal milestone), retention of thoracic nipples, and testicular lesions (*e.g.*, seminiferous tubule atrophy and Leydig cell hyperplasia) (Park JD *et al.* 2002; Ema *et al.* 2003; Foster *et al.* 2002; Gray *et al.* 2000; Mylchreest *et al.* 1998, 2000). Phthalates and their metabolites act functionally as antiandrogens during the prenatal period by interference of normal androgenic signaling, rather than interaction with the androgen receptors (Hotchkiss *et al.* 2004; Mylchreest *et al.* 1998; Parks *et al.* 2000).

There is currently limited or inadequate human data on the relationships between exposure to phthalates and human health effects. In a recent multi-center epidemiologic

study, prenatal exposure to monoethyl phthalate (MEP), monobutyl phthalate (MBP), monobenzyl phthalate (MBzP), and monoisobutyl phthalate (MiBP) was associated with shortened AGD in human male infants (Swan *et al.*, 2005). There was some evidence of associations between environmental exposure to MBP and MBzP and altered semen quality (Duty *et al.* 2003a, 2003b, 2004), MEP and sperm DNA damage (Duty *et al.* 2003a, 2003b), MBP and MBzP and reproductive hormones (Duty *et al.* 2005a, 2005b). An Italian study revealed that newborns who had MEHP in the cord blood (n = 65) had a younger gestational age than MEHP negative newborns (n=19; p = 0.033) (Latini *et al.* 2003).

Just because people have an environmental chemical in their blood or urine does not mean the chemical causes disease. The toxicity of a chemical is related to its dose, time of exposure, frequency and duration, and an individual's susceptibility. The common limitations of the published studies include small human populations, low epidemiologic sensitivity and selectivity, potential preanalytic and analytic contamination, and lack of adequate exposure assessment measurements (either environmental or biological measures). Our study was undertaken to characterize the distribution of pesticides and other EDs in various matrices collected from pregnant women, to identify the predictive biomarkers of exposure, and to assess the exposure and risk levels in this vulnerable population.

METHODS

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RESULTS

Table 3 presents characteristics of the 150 women from central New Jersey area, the number of women who reported using plastic household products in their homes during pregnancy, and the clinical birth outcomes (e.g., birth weight, Apgar score, and gestational age) of the 150 newborns. The median age of participants was 33 years. Only 4% of the women smoked sometimes during pregnancy, and none of them reported drinking during pregnancy. The average gestation of the women was 39 weeks (± 0.79); the average birth weight 3575 grams (± 689 SD); and the average Apgar score at 5 minutes was 9 (± 0.09).

Table 10 exhibits the concentrations and detection frequencies of the nine phthalate metabolites detected in the three biological matrices, *e.g*., cord serum, maternal serum and maternal urine. MMP was detected in 11.8% of the urine samples, mono-3-carboxypropyl phthalate (MCPP) in 70.6%, whereas the rest phthalate metabolites in more than 94% of the urine samples. The arithmetic mean and median levels of the metabolites in maternal urine were higher than the levels in serum samples. For example, the mean of MEHHP in urine was 140.7 ng/ml compared to 1.0 ng/ml in cord serum; so was the mean of MEP (249.8 ng/ml) in urine compared to 4.4 ng/ml in maternal serum. The concentration differences between the urine and serum samples were significant ($p < 0.05$) except for MMP (Figure 6).

The associations between uses of PVC plastic products at home and phthalate exposures were discovered using their respective urinary biomarker data (Table 11). The mean urinary concentration of MBP was 25.4 ng/ml among people who heated food in microwave using plastic containers, which was significant higher than the mean

concentrations among those who do not use plastic containers ($p < 0.05$). Likewise the mean urinary concentration 24.1 ng/ml in people who used plastic containers to store items was significantly higher than those who did not ($p < 0.05$). Same data pattern were also found for MBzP and MCPP (Table 11).

The urinary levels of phthalate metabolites in this study cohort were compared to the levels of the US general and female populations (Table 12). The median and 95th percentile concentrations of MMP, MEP, MBP, MiBP, MBzP and MCPP were lower than or similar to the respective concentrations in the US general population, however, the levels of MEHP, MEHHP and MEOHP in this study cohort were higher than the respective levels in the US general population. For instance, the median urinary concentration of MBP was 14.8 ng/ml compared to 20.4 ng/ml in U.S. general population. However, the median level of MEHHP was 109 ng/ml, which was much higher than the median concentration 20.1 ng/ml in the U.S. general population.

The daily intakes of phthalates were estimated for these NJ pregnant women using the David method (Table 13). The median daily intakes were 4.72, 0.49 and 0.23 μg/kg/day for DEP, DBP/DiBP and BBzP respectively using their monoesters as the biomarkers. In addition, the median DEHP daily intakes were 10.57 and 14.26 μg/kg/day, and the 95th- percentile values of DEHP daily intakes were 30.00 and 34.93 μg/kg/day, when using its two oxidative metabolites MEHHP and MEOHP as the biomarkers respectively.

DISCUSSION

Nine phthalate metabolites were detected in the three biological matrices analyzed. Except for MMP, which was not detectable in most of the subjects (LOD 1 ng/ml), the other phthalate metabolites were found in maternal urine at significantly higher concentrations than in cord serum (CS) and maternal serum (MS). The significantly higher concentration of phthalate metabolites in maternal urine supports the use of urinary metabolites as exposure biomarkers rather than using those in other matrices (*e.g.*, MS, CS), because the latter often contribute to exposure increments that differ by several orders of magnitude. Further, the concentrations of hydrolytic monoesters in MS and CS, even though they can be determined accurately, may include an unknown contribution from hydrolysis of contaminant (exogenous) phthalates by endogenous serum esterases even when phosphoric acid is used to quench the enzymatic activity, and their use as biomarkers of exposure should generally be avoided (Calafat and Needham, 2008). In addition, for the high molecular weight phthalates such as DEHP, the secondary oxidative metabolites (*e.g.*, MEHHP, MEOHP), which can't be formed as a result of contamination during sampling or analysis (Koch *et al.* 2003b), offer more value as biomarkers of exposure than the hydrolytic metabolite MEHP due to their longer $T_{1/2}$ (10 hrs), and recent discovery that these oxidative metabolites might be the ultimate developmental DEHP toxicants (Koch *et al.* 2003b, 2004b).

The usage information of PVC household products were collected in questionnaire to investigate if some products might contribute to phthalate exposure. Since all mothers received an IV injection in the hospital and DEHP is the most

commonly used plasticizer in medical bags and tubes, it is most likely that the dose of DEHP the mothers received through the IV was much higher than the dose they could have received from use of plastic household products. For this reason, the association between use of products and DEHP exposure in this particular population was not examined. Instead, the association between use of the plastic household products and exposure to other phthalates using urinary biomarkers was tested. The data presented in Table 11 suggested that the urinary concentrations of some phthalate metabolites, such as MBP, MBzP, and mono-3-carboxypropyl phthalate (MCPP), were significantly different between plastic product users and non-users suggesting that the use of household products that contain phthalates may contribute to the body burden. As phthalates are not chemically bound to the plastic polymer in these products, under high temperature or as the plastic wears out, phthalates may leach out of the polymer and migrate into the foodstuff, and so add to the total exposure for small molecular phthalates, *e.g.*, DBP, BBzP.

In this group of pregnant women, the urinary monoester concentrations (except for the DEHP metabolites) were lower or similar to the concentrations of the US general population based on the National Health and Nutrition Examination Survey (NHANES) 2001-2002 data (CDC 2005), but DEHP metabolite concentrations were much higher. The major source of DEHP exposure for these women was likely related to their medical interventions in the hospital. DEHP/MEHP could leach out from the IV bags and tubes directly into the women's system circulation, and contribute significantly to their DEHP exposure and body burden. Previous studies showed much lower concentrations in urine of MEHP than of the oxidative metabolites MEHHP and MEOHP (Barr *et al.* 2003; Kato

et al. 2004; Kohn *et al.* 2000; Silva *et al.* 2006a, b). However, in this study, we observed similar urinary concentrations for all three DEHP metabolites measured (Tables 10, 12). This may confirm the recent exposure to DEHP from IV injection during the mothers' stay in the hospital, as the hydrolytic monoester MEHP was not completely metabolized yet at the time point of urine sample collection.

The urinary concentrations of phthalate monoesters were converted to intake levels using the mathematical model described by David (2000) and were related to doses (below which we do not expect to see effects) developed from animal toxicology studies, such as the EPA oral reference doses (RfDs), European Union tolerable daily intakes (TDIs), Agency for Toxic Substances and Disease Registry (ATSDR) minimal risk levels for chronic effects (MRLs) and National Toxicology Program/Center for the Evaluation of Risks to Human Reproduction (NTP/CERHR) no observable adverse effect levels (NOAELs) (CERHR 2000, 2005). The estimated daily exposures to BBzP, DBP, DEP (Table 13) were well below the RfDs, TDIs, or MRLs. Exposures for the general population, estimated by the NTP/CERHR Expert Panels are in good agreement with human daily intake estimates for most of the phthalates from this study.

As discussed above, the higher concentrations of MEHP in maternal urine in this study compared to the urinary concentrations reported in other studies could result from the early transient phase of MEHP metabolism at the time point of urine sample collection as a result of the recent exposure to DEHP from intravenous injection during the mothers' stay in the hospital. Thus using urinary MEHP as the biomarker might not be appropriate to calculate the chronic daily exposure to DEHP, and the dose estimate could easily be exaggerated (Figure 7).

The EPA derived a chronic RfD of 20 μg/kg/day for DEHP based on a low observable adverse effect level of 19 mg/kg/day for hepatic effects in guinea pigs (Carpenter *et al.*, 1953). However, this RfD is outdated, and a reevaluation of DEHP toxicity assessment is underway at EPA. In addition, the hepatic effect is not a known critical endpoint of DEHP toxicity from long duration studies. Considering the male reproductive effects, a known critical endpoint of DEHP based on long duration studies, ATSDR has established a MRL of 60 μg/kg/day for chronic-duration oral exposure to DEHP based on a NOAEL of 5.8 mg/kg/day for testicular pathology in male rats, and a MRL of 100 μg/kg/day intermediate-duration oral exposure based on a NOAEL of 14 mg/kg/day (uncertainty factor 100) for decreased fertility in mice (ATSDR 2002). Using MEHHP and MEOHP as biomarkers, the median DEHP daily intakes were 10.57 and 14.26 μg/kg/day, and the 95^{th}- percentile values 30.00 and 34.93 μg/kg/day respectively (Table 13). None of the pregnant woman in this study had DEHP daily dose higher than the ATSDR exposure guidance limits, and only one woman had a DEHP intake estimate (43.18 or 48.22 μg/kg/day) that was above the TDI of 40 μg/kg/day (Table 13, Figure 7). These data suggest that although these pregnant women had much higher urinary concentrations of MEHP, MEHHP, and MEOHP than the U.S. general and female population, and accordingly a fraction of these pregnant women had estimated daily intakes of DEHP higher than those reported in previous studies, their dose estimates were well below the regulatory exposure limits (*e.g.*, MRLs). In addition, no abnormal birth outcomes (e.g., birth weight, Apgar score, and gestational age) or other clinical reproductive endpoints were noted in those newborns whose mothers had relatively higher exposure estimate to DEHP during the perinatal period than others in this study.

Our data suggest that enough margin of safety may have been built in the various regulatory exposure limits for DEHP. Since we have asserted that the exposure to DEHP could be explained by exposure through the medical devices in the hospital, which was a transient acute exposure, we speculate that we would not see adverse health effects in this pregnant population and their newborns during follow up.

Because of the modest sample size (n = 150) from the local central New Jersey region, it is not possible to generalize our findings to other populations. Future studies should involve a larger cohort that includes women from diverse geographic areas. More importantly, a major limitation of this study was the potential contamination of the biological samples collected in the hospital from these pregnant women with phthalate plasticizers, most notably DEHP, as a result of the medical interventions required for cesarean deliveries. This fact highlights the critical importance of selecting collection protocols and timing for sampling that would avoid such contamination in future studies.

CONCLUSION

In this NJ pregnant population, nine phthalate metabolites were detected in maternal serum, cord serum, and maternal urine samples, indicating widespread exposure to five phthalate parent compounds. Significantly higher concentrations and detection frequencies in maternal urine than in maternal and cord serum suggest that for the non-persistent phthalates, urinary concentrations of phthalate metabolites are better biomarkers for exposure assessment than their respective concentrations in serum. In our study, the urinary concentrations of phthalate metabolites were similar or lower than the concentrations in the US general and female population based on NHANES 2001 – 2002 data, except for the three metabolites of DEHP: MEHP, MEHHP, and MEOHP. The transient exposure to plastic medical devices in the hospital by these women before delivery could explain the high urinary concentrations of DEHP metabolites. Calculation of the daily intakes using the urinary concentrations of the oxidative metabolites (MEHHP and MEOHP) as the biomarkers indicated that the estimated daily dose of DEHP were well below regulatory guidance limits, such as the MRLs. No abnormal birth outcomes or other adverse clinical endpoints were noted in those newborns whose mothers had relatively higher exposure estimate to DEHP during the perinatal period than others in this study.

SUMMARY

A variety of pesticides, phthalates, and their metabolites were measured in maternal urine, maternal serum, cord serum, amniotic fluid, and meconium samples collected at the time of cesarean delivery from 150 women from central New Jersey. For most of the pesticide metabolites measured, the urinary concentrations were similar to or lower than those found in the general population. However, the concentrations of orthophenylphenol in maternal urine samples from this study population were about four-fold higher than the 95th percentile concentration found in the U.S. population. The unusually high concentrations of orthophenylphenol may reflect exposures that occurred in the hospital prior to delivery. Nevertheless, no aberrant birth outcomes or abnormal clinical findings were associated with high concentrations of orthophenylphenol during perinatal period.

There existed linear correlations between the parent compound chlorpyrifos and its metabolite DEP as well as between its metabolites TCPy and DETP. Chlorpyrifos was detected in a reasonable portion of cord serum and TCPy detected in maternal urine. The presence of the pesticides or metabolites in the fetal compartment matrices and the linear correlation of their concentrations between maternal and cord serum is evidence of the transport of these chemicals from the mother to the fetus. The intact pesticides that were detected in the cord serum or amniotic fluid indicate that some unmetabolized pesticides can also reach the fetal compartment.

Significantly higher concentrations of dacthal, diethyltoluamide, and phthalimide in cord serum samples were observed among women who used pesticides during

pregnancy than women who did not. This finding suggests that residential use of pesticides may contribute to overall pesticide body burden for individuals.

The daily exposure estimates for both chlorpyrifos and diazinon were lower than most of the existing exposure guidelines established by the regulatory agencies, such as the EPA oral benchmark dose$_{10}$ (BMD$_{10}$), EPA reference oral dose (RfD), or ATSDR minimal risk level (MRL). However, the median and 95th percentile chlorpyrifos dose estimates were approximately equivalent to the chronic and acute population adjusted doses (cPAD, aPAD), respectively, for children and females. These doses were likely overestimated, representing a "worst case" dose estimate because the DAP metabolites can also be derived from exposure to the preformed metabolites.

Among all the biological matrices where samples were collected from the fetal compartment, maternal urine and umbilical cord samples provided the most consistently measurable data. Thus, in studies evaluating *in utero* exposures, cord serum can be analyzed to directly detect the exposure to the fetus, and maternal urine samples can be analyzed as a surrogate exposure measurement.

The urinary concentrations of some phthalate metabolites, such as mono-n-butyl phthalate (MBP), monobenzyl phthalate (MBzP), and mono-3-carboxypropyl phthalate (MCPP), were significantly different between plastic product users and non-users. This data suggests that the use of household products that contain phthalates contributes to the overall body burden.

The urinary concentrations of most phthalate monoester metabolites were comparable to or lower than the U.S. general population, except for mono-(2-ethylhexyl) phthalate (MEHP), mono-(2-ethyl-5-hydroxyhexyl) phthalate (MEHHP), and mono-(2-

ethyl-5-oxohexyl) phthalate (MEOHP), three metabolites of di(2-ethylhexyl) phthalate (DEHP). The major source of DEHP exposure for these women was likely related to their medical interventions in the hospital. High concentrations of MEHP may indicate a recent exposure to the parent chemical DEHP in the hospital shortly before the collection of the samples.

Calculation of daily phthalate intakes was made using the urinary biomarker data. The estimated daily exposures to BBzP, DBP, and DEP were well below the regulatory protection limits, such as EPA reference oral doses (RfDs), EU total daily intakes (TDIs), or ATSDR minimal risk levels (MRLs). None of the pregnant woman had DEHP daily dose higher than the ATSDR exposure guidance limits (MRLs), and only one woman had a DEHP intake estimate above the European TDI. These high dose estimates were reflected by the higher urinary concentrations of MEHP, MEHHP, and MEOHP than the US general and female population, and could result from contact through the medical devices in the hospital. However, as a transient acute exposure in the hospital, which was well below the ATSDR exposure limits (*e.g.*, MRLs), it is not anticipated to see adverse health effects in this pregnant population and their newborns during follow up.

Significantly higher concentrations in maternal urine than in maternal serum and cord serum suggest that the urinary concentrations of the phthalate metabolites may be more reliable biomarkers of exposure to phthalates than the concentrations in other biological specimens, because the former often contribute to exposure increments that differ for several orders of magnitude. Further, the concentrations of hydrolytic monoesters in maternal serum and cord serum, even though they can be determined accurately, may include an unknown contribution from hydrolysis of contaminant

(exogenous) phthalates by endogenous serum esterase. In addition, the secondary oxidative metabolites, such as MEHHP and MEOHP of the high molecular weight phthalate DEHP, offer more value as biomarkers of exposure than the hydrolytic metabolite MEHP due to their longer $T_{1/2}$ (10 hrs), and ultimate developmental toxicities.

OVERALL CONCLUSION

In this NJ population of pregnant women and newborns, multiple pesticides, their metabolites, and nine phthalate metabolites were detected, with varied frequencies, in the biological matrices representing the maternal-fetal compartment. These data suggest widespread exposure of this study population to those pesticides and phthalates during pregnancy.

Data from this study suggests that residential use of pesticides and household products that contain phthalates contribute to the human exposure and the overall body burden. The pesticide concentrations in all of the biological matrices of these study participants were typically lower than those reported in previous studies, except for OPP. The high exposure to OPP could potentially result from recent exposure in hospital where OPP is commonly used disinfectant. The urinary concentrations of phthalate metabolites were similar or lower than the concentrations in the US general and female population, except for the three metabolites of DEHP: MEHP, MEHHP, and MEOHP. The transient exposure to plastic medical devices in the hospital by these women before delivery and before the collection of the urine samples could explain the higher urinary concentrations of DEHP metabolites than the background. However, no clinical abnormities or aberrant birth outcomes in the newborns were associated with the pesticide and phthalate exposures.

The daily exposure estimates from the parent pesticides, e.g., chlorpyrifos and diazinon, were well below many regulatory guidance limits; however, they were near the EPA's PADs for children and women. The daily intakes of DEHP using the urinary

concentrations of the oxidative metabolites MEHHP and MEOHP as the biomarkers indicated that none of the pregnant women tested had integrated exposures to DEHP that exceeded the Agency for Toxic Substances and Disease Registry's minimal risk levels. No abnormal birth outcomes (*e.g.,* birth weight, Apgar score, and gestational age) were noted in those newborns whose mothers had relatively higher exposure to DEHP during the perinatal period than others, indicating minimal risk of endocrine disruption due to exposure to DEHP at current environmental level in this group.

Significantly higher concentrations and detection frequencies in maternal urine than in serum and amniotic fluid in this study suggest that the maternal urinary concentrations of the pesticide and phthalate metabolites may be better and more reliable biomarkers for exposure assessment.

DISCUSSION AND FUTURE DIRECTIONS

Biomarkers have been used as integrative dosimeters to evaluate total exposure to environmental chemicals (*e.g.,* pesticides, phenols, and phthalates) regardless of the routes. This study discovered significantly higher concentrations and detection frequencies of the metabolites in maternal urine and cord serum than those in other biological specimens. Both maternal urine and umbilical cord samples provided consistently measurable data. Thus, in studies evaluating *in utero* exposures, cord serum can be analyzed to directly detect the exposure to the fetus, and maternal urine samples can be analyzed as a surrogate exposure measurement. Due to low and inconsistent levels detected in meconium and amniotic fluid in this cohort, further studies are needed to explore and validate if meconium and amniotic fluid can be potential matrices for assessing cumulative *in utero* exposures to the fetus.

Because of the modest sample size (n = 150) from the local central New Jersey region, and comparatively less exposure than many other populations reported, it is not possible to generalize our findings to other populations. Future studies should involve a larger cohort that includes women from diverse geographic areas and who have potential pesticide exposure risk factors in order to better define which chemicals are present in the biological matrices, and whether the gestational exposure is a contributory factor to potential adverse health effects on pregnant women and their newborns.

Experimental evidence in laboratory rodents has linked OP exposure during gestation or the early postnatal period to adverse neurodevelopmental sequelae in the offspring (Brimijoin and Koenigsberger 1999; Eskenazi *et al.* 1999). We did not observe

abnormal fetal development or aberrant birth outcomes associated with the current exposure level in this study population, however, we do not know whether the exposures seen in this study, which are lower than doses used in experimental bioassays and other populations reported, are associated with any potential health risks to the developing child later in life. Additional research is needed to better characterize contaminants in fetal compartment and the effects of prenatal exposure on their neurocognitive development.

Each participating subject had multiple environmental toxicants detected in their biological specimens, indicating exposure to complex mixtures rather than single compound, including pesticides and phthalates. Some of the toxicants in the mixture have similar mechanisms of action and/or similar toxicological end points. For example, the organophosphates chlorpyrifos and diazinon are both acetylcholinesterase inhibitors. In this study we calculated single dose estimates for OP pesticides assuming 100% of the exposure was from a single diethyl or dimethyl OP pesticide. Dose estimates were not aggregated across diethyl and dimethyl OP pesticide classes. Further study is necessary to estimate cumulative dose from exposure to mixtures of OP pesticides that are commonly applied in the central New Jersey area. In addition, how the chemicals in this mixture interact, and whether effects are additive, synergistic, or antagonistic, is not known. Additional research is needed to study the effects of pesticides and phthalates at current exposure levels (both singly and in combination) on the developing fetus.

The median and 95th percentile of chlorpyrifos dose estimates were approximately equivalent to the EPA chronic and acute population adjusted doses (cPAD, aPAD), respectively, for children and females. However, because the DAP metabolites can also

be derived from exposure to the preformed metabolites, these doses were likely overestimated representing a worst case dose estimate. Research is needed to identify physical and biological mechanisms of degradation of OPs and fate of metabolites in the environment and in the human body. OPs degrade in foodstuff before ingestion, but the amount of these metabolites and biological effects they might have as they pass through the human body are unknown. Multiroute (e.g., diet, dust, air) environmental exposures, as well as monitoring blood, urine, amniotic fluid, and meconium, need to be measured for both intact pesticides and their degradation products. These data would help better understanding of potential confounding of biomarker data from exposure to the environmental degradates.

Exposure to plastic medical devices before the collection of the urine samples at delivery in the hospital could result in the high urinary concentrations of DEHP metabolites and the high daily dose of DEHP using the urinary concentrations of the oxidative metabolites MEHHP and MEOHP as the biomarkers. Similarly exposure to the disinfectant orthophenylphenol in the hospital could result in high concentrations in maternal urine and amniotic fluid. The potential contamination of the biological samples collected in the hospital from these pregnant women was the major limitation of this study as a result of the medical interventions required for cesarean deliveries or common use of the disinfectant. This fact highlights the critical importance of selecting collection protocols and timing for sampling that would avoid such contamination. Future studies need to control the hospital exposure or take the urine and blood samples before the hospitalization.

Physiologically based pharmacokinetic (PBPK) models have been developed only for some chemicals, such as organophosphates and phthalates. More models need to be developed for other parent compounds measured in this study in order to assess the daily doses of each of them. This study provided preliminary evidence that chemicals (e.g., chlorpyrifos and orthophenylphenol) can transfer from maternal to the fetal compartments. More studies that concurrently measure chemicals in maternal and fetal compartments are required to provide solid data on the interrelationships of maternal and fetal exposures and aid in the development of PBPK models for pregnant women and fetuses. These models may become valuable tools for estimating fetal risk assessment or for epidemiologic studies investigating prenatal exposures where only maternal biologic samples are available. These data can help us better understand the total exposure to each of the pesticides relative to the regulatory protection limits and determine whether intervention is necessary to either minimizing the use of the pesticides in the residential environment or eliminating the possible contact with treated areas or household products.

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LIST OF TABLES

Subject Name
Date
Address
Date of Birth
Present Occupation
Mother's Jobs in the Last 5 Years
Father's Occupation
Father's Jobs in the Last 5 Years
Mother's Hobbies
Any Unique Environmental Exposures in mother's Area (e.g., bus stations, factories, dumps, farms, sewer plants, dry cleaners)?
Did the mother smoke while pregnant (Never/Sometimes/Often)?
Did Anyone Smoke Around the mother while she was pregnant (Never/Sometimes/Often)?
Did the mother or anyone else use pesticides in the home while she was pregnant (Never/Sometimes/Often)?
Did the mother or anyone else use pesticides in the yard while she was pregnant (Never/Sometimes/Often)?
Did the mother live with pets while she was pregnant (Y/N)?
What kind(s) of pets?
Did the mother treat the pet(s) for any pest problems (e.g., fleas, ticks, worms, etc.)?
Did the mother microwave food in plastic containers while pregnant (Never/Sometimes/Often)?
Did the mother use plastic tableware while pregnant (Never/Sometimes/Often)?
Did the mother save items in plastic containers while pregnant (Never/Sometimes/Often)?
Did the mother drink soda or carbonated beverages that came out of plastic containers while pregnancy (Never/Sometimes/Often)?
Did the mother drink bottled water while pregnant (Never/Sometimes/Often)?

Table 2. Pesticides or metabolites measured in the study population and their common insecticidal uses

Metabolites	Parent chemicals	Chemical class	Insecticidal uses
DEP, DETP, DEDTP	O,O-diethyl substituted OPs	Organophosphate	Agriculture, home, graden, veterinary
DMP, DMTP, DMDTP	O,O-dimethyl substituted OPs	Organophosphate	Agriculture, home, graden, veterinary
TCPy, DEP, DETP	Chlorpyrifos	Organophosphate	Termicide, crop, lawn/turf, livestock
IMPY, DEP, DETP	Diazinon	Organophosphate	Commercial, crop, lawn/turf
DMP, DMTP, DMDTP, acid derivatives	Malathion	Organophosphate	Crops, lawn/turf, livestock
4-nitrophenol, DEP, DETP	Parathion	Organophosphate	Fruit, nut and vegetable crops
4-nitrophenol, DMP, DMTP	Methyl parathion	Organophosphate	Cotton, soybean and vegetable fields
1-Naphthal, 2-naphthal	Naphthalene	Repellent/Disinfectant	Moth repellants, toilet deodorant blocks
1-Naphthal	Carbaryl	Carbamate insecticide	Citrus, fruit, cotton, forests, livestock
2,4-Dichlorophenol	Dichlorobenzene	Phenoxy acid herbicide	Broadleaf weed, home and garden use
2,5-Dichlorophenol	p-Dichlorobenzene	Repellant/disinfectant	Moth balls, room deodorizers, fumigant
4-Nitrophenol	Nitrobenzene, Parathion	Fungicide/insecticide	Agriculture, home use
Phenylhydroquinone*, phenylbenzoquinone*	Ortho-phenylphenol	Fungicide/disinfectant	Crops, home disinfectant
2,4,5-trichlorophenol, 2,4,6-trichlorophenol	Chlorinated benzenes	Phenoxy acid herbicide	Broadleaf herbicide, banned in US
Pentachlorophenol	Pentachlorophenol	Fungicide	Wood preservative
Carbofuranphenol	Carbofuran	Carbamate	Agriculture, crops, phasing out
cis-DCCA*	Permethrin	Pyrethroid	Agriculture/crops, insect repellent, home use
trans-DCCA*	Permethrin	Pyrethroid	Agriculture/crops, insect repellent, home use
3,4-Dichloroaniline*, acid derivatives	Dacthal	Terephthalic acid herbicide	Pre-emergence herbicide, broad-leaved weeds
Diethyltoluamide (DEET)	Diethyltoluamide (DEET)	Repellent	Insect repellent, personal use
2,6-Dimethylaniline*	Metalaxyl	Fungicide	Plant, food, crops, residential & greenhouse
Metolachlor mercapturate*	Metolachlor	Chloroacetanilide herbicide	Weedmcontrol, food/feed, non-food crops
Phthalimide	Captan, Captafol, Folpet	Fungicide	Indoor/greenhouse food crops
Tetrahydrophthalimide	Captan, Captafol, Folpet	Fungicide	Indoor/greenhouse food crops
Benzimidazole*	Trifluralin	Dinitroaniline herbicide	Food, feed, & non-food crops

Abbreviations: OPs, organophosphates; DMP, dimethylphosphate; DMTP, dimethylthiophosphate; DMDTP, dimethyldithiophosphate; DEP, diethylphosphate; DETP, diethylthiophosphate; DEDTP, diethyldithiophosphate; IMPY, 2-isopropyl-4-methyl-6-hydroxypyrimidinol; TCPy, 3,5,6-trichloro-2-pyridinol; DCCA, 2,2-(dichloro)-2-dimethylvinylcyclopropane carboxylic acid

*Metabolites not measured

Table 3. Characteristics of study subjects (n = 150)

Characteristic		Value
Age	Median (25%, 75%)	33 (30, 35)
Smoking	Never [n (%)]	144 (96%)
	Sometimes [n (%)]	6 (4%)
Residential use of pesticides	Use pesticides in home	54 (36%)
	Use pesticides in yard	85 (57%)
	Treat pets for pest problems	26 (16%)
Use of plastic products	Microwave food in plastic container	141 (94%)
	Use plastic tableware	111 (74%)
	Save items in plastic container	145 (97%)
Gestational age	Mean (SD) (wks)	39 (±0.79)
Birth outcome	Birth weight (g)	3575 (±689)
	Apgar score (5 min)	9 (±0.092)

Table 4. Concentrations of organophosphorous insecticides and their metabolites in biological specimens

Analytes*	Matrices	Arithemic Mean	Median	95th Percentile	Range	LOD	Sample Size	DF (%)
Chlorpyrifos	CS	0.550	<LOD	1.7376	0.0007 - 1.8413	0.0010	148	37
	MS	0.087	<LOD	<LOD	0.0007 - 10.094	0.0010	138	1
Diazinon	CS	0.026	<LOD	<LOD	0.0004 - 2.8549	0.0005	148	1
	MS	0.276	<LOD	2.8896	0.0004 - 3.1450	0.0005	138	9
3,5,6-Trichloropyridinol (TCPy)	MU	1.515	<LOD	5.7200	0.283 - 10.401	0.4000	34	50
Diethyldithiophosphate (DEDTP)	AF	0.160	<LOD	<LOD	0.1144 - 1.6583	0.2000	128	2
	MU	0.145	<LOD	<LOD	0.1098 - 0.2792	0.2000	34	8
	MC	0.149	<LOD	<LOD	0.1414 - 0.9747	0.2000	147	2
Diethylphosphate (DEP)	AF	0.425	<LOD	1.4966	0.1002 - 2.7185	0.2000	128	40
	MU	5.200	2.5726	12.4942	0.1414 - 68.4266	0.2000	34	76
	MC	0.146	<LOD	<LOD	0.1414 - 0.7542	0.2000	147	1
Diethylthiophosphate (DETP)	AF	0.149	<LOD	<LOD	0.1414 - 1.0557	0.2000	128	1
	MU	1.035	0.3150	3.5932	0.1025 - 13.4863	0.2000	34	71
	MC	0.142	<LOD	<LOD	0.1414 - 0.2387	0.2000	147	1
Dimethyldithiophosphate (DMDTP)	AF	0.142	<LOD	<LOD	0.1414 - 0.2583	0.2000	128	1
	MU	0.766	<LOD	2.5899	0.1414 - 9.0927	0.2000	34	32
	MC	0.144	<LOD	<LOD	0.1414 - 0.5320	0.2000	147	1
Dimethylphosphate (DMP)	AF	0.548	<LOD	1.9388	0.3318 - 3.8565	0.5000	128	20
	MU	1.993	1.7329	5.6306	0.1583 - 7.5350	0.5000	34	91
	MC	0.353	<LOD	<LOD	0.3254 - 0.3536	0.5000	147	1
Dimethylthiophosphate (DMTP)	AF	0.641	<LOD	2.9779	0.1207 - 2.9319	0.5000	128	63
	MU	3.983	2.6884	12.1114	0.4273 - 20.0869	0.5000	34	100
	MC	0.560	<LOD	1.8931	0.1332 - 5.4116	0.5000	147	12

CS: cord serum; MS: maternal serum; AF: amniotic fluid; MU: maternal urine; MC: meconium; DF: detection frequency

*Chlorpyrifus and diazinon are in units of pg/mL whereas others are in ng/mL

Table 5. Levels of detection and concentrations (ng/ml) of phenols detected in biological specimens

Components	Matrices	GM	Arithemic Mean	Standard Error	Range	LOD	Sample Size	DF (%)
1-Naphthol	AF	*	0.575	1.740	0.118 - 27.967	0.40	130	7
	CS	0.448	4.152	3.440	0.007 - 35.627	0.01	148	61
	MS	*	0.418	1.222	0.007 - 10.400	0.01	138	6
	MU	*	1.007	1.424	0.283 - 9.467	0.40	34	21
2,4,-Dichlorophenol	AF	*	0.186	0.360	0.123 - 5.968	0.20	131	2
	MU	*	0.382	0.955	0.141 - 8.024	0.20	34	9
2,5-Dichlorophenol	AF	*	0.192	0.940	0.071 - 15.282	0.10	131	2
	MU	*	19.185	62.055	0.071 - 511.633	0.10	34	26
2-Naphthol	AF	*	0.288	0.796	0.141 - 10.137	0.20	131	2
	MU	*	5.882	9.001	0.141 - 48.569	0.20	34	56
4-Nitrophenol	AF	*	5.713	7.361	0.1173 - 56.987	0.10	130	55
	MU	*	39.127	56.842	0.283 - 395.146	0.40	34	3
Orthophenylphenol	AF	2.489	22.541	28.422	0.141 - 312.204	0.20	131	60
	MU	2.611	2.827	0.801	1.005 - 6.309	0.20	34	100
2,4,5-Trichlorophenol	MU	*	0.438	0.660	0.212 - 5.565	0.30	34	12
2,4,6-Trichlorophenol	MU	*	1.470	1.936	0.354 - 13.782	0.50	34	32

CS: cord serum; MS: maternal serum; AF: amniotic fluid; MU: maternal urine; LOD: limit of detection; DF: detection frequency

*Geometric mean not calculated because detection frequency < 60%

Table 6. Concentrations (pg/ml) of other pesticides detected in the CS and MS

Components	Matrices	GM	Arithmetic Mean	Standard Error	Range	Median	95th Percentile	LOD	Sample Size	DF (%)
Carbofuran	CS	*	4.357	4.588	0.007 - 13.972	<LOD	10.614	0.01	148	49
	MS	*	0.612	2.582	0.007 - 17.632	<LOD	4.732	0.01	138	7
Chlorothalonil	CS	*	1.476	5.795	0.007 - 25.119	<LOD	23.962	0.01	148	6
	MS	*	0.735	4.232	0.007 - 25.313	<LOD	<LOD	0.01	138	3
Cis-permethrin	CS	*	0.246	0.469	0.007 - 1.356	<LOD	1.292	0.01	148	21
	MS	*	0.015	0.090	0.007 - 1.064	<LOD	<LOD	0.01	138	1
Dacthal	CS	0.376	1.460	1.122	0.007 - 3.666	1.429	2.926	0.01	148	71
	MS	1.786	3.430	4.055	0.007 - 35.815	3.193	7.074	0.01	138	92
Dichloran	CS	*	1.697	1.862	0.007 - 9.738	1.619	4.193	0.01	148	53
	MS	*	1.385	2.099	0.007 - 9.834	<LOD	4.766	0.01	138	35
Diethyltoluamide	CS	3.024	3.122	1.064	2.060 - 13.671	2.900	4.155	0.01	148	100
	MS	2.954	3.208	1.950	1.819 - 18.844	2.775	6.313	0.01	138	100
Metalaxyl	CS	*	2.234	2.641	0.007 - 14.605	<LOD	5.752	0.01	148	47
	MS	*	2.013	4.160	0.007 - 14.990	<LOD	14.414	0.01	138	23
Metolachlor	CS	*	0.929	1.068	0.007 - 2.374	<LOD	2.344	0.01	148	43
	MS	*	0.091	0.367	0.007 - 1.958	<LOD	0.229	0.01	138	5
Phthalimide	CS	1.228	5.039	3.224	0.007 - 12.042	5.759	8.885	0.01	148	76
	MS	12.125	14.744	15.699	7.132 - 124.733	10.784	32.210	0.01	138	100
Tetrahydrophthalimide	CS	*	0.953	1.733	0.007 - 13.534	<LOD	3.127	0.01	148	31
	MS	*	0.210	1.128	0.007 - 10.033	<LOD	<LOD	0.01	138	4
Trans-permethrin	CS	*	0.899	0.783	0.007 - 2.078	1.232	1.889	0.01	148	58
	MS	*	0.044	0.257	0.007 - 2.395	<LOD	2.932	0.01	138	2
Trifluralin	CS	0.613	2.163	1.540	0.007 - 4.423	2.106	4.391	0.01	148	75
	MS	*	0.752	1.368	0.007 - 8.529	<LOD	2.932	0.01	138	31

CS: cord serum; MS: maternal serum; DF: detection frequency; LOD: limit of detection

*Geometric mean not calculated because detection frequency < 60%

Table 7. Dose estimates of chlorpyrifos and diazinon relative to the regulatory agencies protection limits (µg/kg/day). GM = geometric mean; EPA = Environmental Protection Agency; ATSDR = Agency for Toxic Substances and Disease Registry; BMD = benchmark dose; RfD = reference dose; PAD = population adjusted dose (acute and chronic) for children and females

	Chlorpyrifos (ug/kg/day)	**Diazinon** (ug/kg/day)
GM	0.089	0.078
50th percentile	0.124	0.108
75th percentile	0.250	0.217
90th percentile	0.373	0.323
95th percentile	0.512	0.448
EPA BMD_{10}/100	14.800	62.400
EPA RfD (Chronic)	3.000	n/a
EPA aPAD	0.5	n/a
EPA cPAD	0.1	n/a
ATSDR MRL (acute)	3.000	6.000
ATSDR MRL (intermediate)	3.000	2.000
ATSDR MRL (chronic)	1.000	0.700

Table 8. Comparison of urinary organophosphorus metabolites (ng/ml) in NJ women to US general and female populations (NHANES 2001-2002 data)

Metabolites	Subjects	GM	50th	75th	90th	95th
3,5,6-Trichloropyridinol	NJ women	*	<LOD	1.65	4.48	5.72
	U.S. total	1.76	2.20	4.95	8.80	12.40
	U.S. female	1.45	1.72	4.38	7.71	10.40
Diethyldithiophosphate	NJ women	*	<LOD	<LOD	<LOD	<LOD
	US total	*	<LOD (0.1)	<LOD	0.61	0.83
	US female	*	<LOD (0.1)	<LOD	0.66	0.99
Diethylphosphate	NJ women	1.70	2.57	4.86	7.18	12.49
	US total	*	<LOD (0.2)	2.76	6.33	11.40
	US female	*	0.26	2.58	5.93	10.40
Diethylthiophosphate	NJ women	0.40	0.32	0.69	1.21	3.59
	US total	0.46	0.57	1.48	2.46	3.94
	US female	0.46	0.55	1.48	2.44	3.91
Dimethyldithiophosphate	NJ women	*	<LOD	0.67	1.85	2.59
	US total	*	<LOD (0.1)	0.89	2.49	4.95
	US female	*	<LOD (0.1)	0.95	2.52	5.10
Dimethylphosphate	NJ women	1.29	1.73	2.52	4.01	5.63
	US total	*	<LOD (0.5)	3.25	8.22	13.40
	US female	*	<LOD (0.5)	3.05	8.34	13.70
Dimethylthiophosphate	NJ women	2.62	2.69	5.26	8.40	12.11
	US total	*	0.45	4.02	16.20	32.60
	US female	*	<LOD (0.4)	3.76	15.90	34.30

*Geometric mean not calculated because detection frequency < 60%

Table 9. Comparison of urinary phenols (ng/ml) in NJ women to US general and female populations (NHANES 2001-2002 data)

Phenols	Subjects	GM	50th	75th	90th	95th
1-Naphthol	NJ women	*	<LOD	<LOD	1.94	5.64
	U.S. total	2.05	1.72	4.76	12.50	22.30
	U.S. female	1.86	1.56	4.12	13.30	22.30
2-Naphthol	NJ women	*	0.62	2.76	23.43	36.52
	U.S. total	2.47	2.28	5.68	14.70	26.00
	U.S. female	2.22	2.06	5.24	13.90	25.10
2,4,5-Trichlorophenol	NJ women	*	<LOD	<LOD	0.63	1.05
	U.S. total	*	<LOD (0.9)	<LOD	<LOD	2.31
	U.S. female	*	<LOD (0.9)	<LOD	<LOD	<LOD
2,4,6-Trichlorophenol	NJ women	*	<LOD	0.83	4.12	6.19
	U.S. total	*	1.68	5.94	10.80	14.90
	U.S. female	*	<LOD (1.0)	4.69	9.71	13.10
2,4-Dichlorophenol	NJ women	*	<LOD	<LOD	<LOD	0.26
	U.S. total	*	<LOD (0.3)	3.43	12.00	23.90
	U.S. female	*	<LOD (0.3)	2.59	9.55	24.50
Orthophenylphenol	**NJ women**	**2.61**	**2.81**	**3.35**	**4.23**	**4.56**
	U.S. total	*	<LOD (0.3)	<LOD	0.57	1.27
	U.S. female	*	<LOD (0.3)	<LOD	0.52	1.22
2,5-Dichlorophenol	NJ women	*	<LOD	0.26	17.36	40.83
	U.S. total	*	2.04	28.80	194.00	657.00
	U.S. female	*	1.41	24.60	194.00	624.00
Pentachlorophenol	NJ women	*	<LOD	<LOD	<LOD	0.56
	U.S. total	*	<LOD (0.5)	<LOD	1.23	1.94
	U.S. female	*	<LOD (0.5)	<LOD	1.10	1.92

*Geometric mean not calculated because detection frequency < 60%

Table 10. Phthalate metabolite concentrations (ng/ml) in biological matrices[a]

Analytes	Matrices	Arithmetic Mean	Standard Error	Median	95th Percentile	Range	LOD (ng/ml)	Sample Size	DF (%)
	CS	2.3	0.4	<LOD	10.9	<LOD - 49.4	1.07	150	28.0
MBP	MS	2.3	0.4	<LOD	12.2	<LOD - 45.7	1.07	148	22.3
	MU	23.3	4.2	14.8	68.9	3.4 - 107.9	1.07	34	100.0
	CS	<LOD	0.1	<LOD	2.1	<LOD - 5.2	1.00	150	8.0
MBzP	MS	<LOD	0.0	<LOD	1.3	<LOD - 3.7	1.00	148	5.4
	MU	20.0	5.5	9.2	52.8	1.0 - 181.3	1.00	34	97.1
	CS	1.3	0.2	<LOD	4.6	<LOD - 16.9	1.00	150	16.0
MCPP	MS	1.4	0.2	<LOD	4.3	<LOD - 19.9	1.00	148	16.2
	MU	2.3	0.4	1.6	7.1	<LOD - 9.3	1.00	34	70.6
	CS	1.0	0.1	<LOD	2.8	<LOD - 10.6	0.95	150	26.0
MEHHP	MS	<LOD	0.1	<LOD	2.9	<LOD - 10.9	0.95	148	14.2
	MU	140.7	16.5	108.9	308.9	21.0 - 444.6	0.95	34	100.0
	CS	8.2	0.3	7.7	14.1	1.0 - 21.8	0.98	150	99.3
MEHP	MS	1.3	0.1	<LOD	3.9	<LOD - 10.3	0.98	148	26.4
	MU	126.9	11.1	114.7	246.2	38.4 - 274.6	0.98	34	100.0
	CS	<LOD	0.1	<LOD	2.3	<LOD - 3.7	1.07	150	13.3
MEOHP	MS	<LOD	0.1	<LOD	2.7	<LOD - 10.1	1.07	148	12.8
	MU	114.8	12.3	95.1	233.0	17.7 - 321.6	1.07	34	100.0
	CS	3.0	0.5	<LOD	12.9	<LOD - 45.3	1.00	150	40.7
MEP	MS	4.4	0.8	<LOD	20.4	<LOD - 89.3	1.00	148	42.6
	MU	249.8	45.7	168.5	749.1	7.7 - 830.2	1.00	29	100.0
	CS	<LOD	0.0	<LOD	<LOD	<LOD - 5.3	1.00	150	2.0
MMP	MS	<LOD	0.0	<LOD	<LOD	<LOD - 1.6	1.00	148	0.7
	MU	1.0	0.2	<LOD	2.5	<LOD - 7.3	1.00	34	11.8
	CS	<LOD	0.0	<LOD	<LOD	<LOD - 5.6	1.04	150	3.3
MiBP	MS	<LOD	0.1	<LOD	1.3	<LOD - 7.6	1.04	148	4.7
	MU	6.4	1.4	4.6	13.5	<LOD - 47.5	1.04	34	94.1

Abbreviations: CS: cord serun; MS: maternal serum; MU: maternal urine

DF: detection frequency; LOD: limit of detection

[a] The concentrations of the hydrolytic monoesters (MBP, MBzP, MEHP, MEP, MMP, and MiBP) in cord and maternal serum, even though they can be determined accurately, may include an unknown contribution from hydrolysis of contaminant (exogenous) phthalates by endogenous esterases (Calafat and Needham, 2008)

Table 11. Association of self-reported use of plastic products and phthalate levels (ng/ml) in maternal urine

Parameters	**Metabolites**	**Non User[a]**	**User[a]**	***P value*[b]**
"Microwave food using plastic container"	**MBP**	11.2 /4.0/ 5	25.4 /25.8 / 29	0.0091
	MBzP	4.9 /3.2 / 5	22.6 /34.3 / 29	0.0112
	MCPP	1.1 /0.7 / 5	2.5 /2.3 / 29	0.0124
"Using plastic tableware"	**MBzP**	8.0 /5.1 / 6	22.5 /35.0 / 28	0.0452
"Save items using plastic container"	**MBP**	10.6 /2.9 / 2	24.1 /24.9 / 32	0.0118
	MBzP	4.2 /1.8 / 2	20.9 /33 / 32	0.0083
	MCPP	0.5 /0.3 / 2	2.4 /2.2 / 32	0.0004

[a] - All values expressed as: mean concentration / standard deviation / subject number.

[b] - Significant differences in urinary concentrations between plastic product users and non-users ($p < 0.05$)

Table 12. Comparison of the urinary levels (ng/ml) of phthalate metabolites in the NJ study population to US general and female population (NHANES 2001-2002 data)

Metabolites	Subjects	GM	50th	75th	90th	95th
MMP	NJ women	*	<LOD[a]	<LOD[a]	1.47	2.48
	US total	1.15	1.50	3.30	6.00	9.80
	US female	1.13	1.30	3.30	6.40	10.3
MEP	NJ women	135	169	400	721	749
	US total	178	169	465	1230	2500
	US female	174	167	427	1050	1840
MBP	NJ women	15.7	14.8	29.6	48.1	68.9
	US total	18.9	20.4	40.4	73.6	108
	US female	20.2	21.6	46.7	85.0	120
MiBP	NJ women	4.05	4.60	7.48	12.6	13.5
	US total	2.71	2.60	5.70	11.9	17.9
	US female	2.68	2.50	5.70	12.6	18.7
MBzP	NJ women	10.0	9.15	21.4	44.3	52.8
	US total	15.1	15.7	38.0	80.8	122
	US female	14.6	15.4	38.1	81.4	122
MCPP	NJ women	1.63	1.55	3.20	4.17	7.09
	US total	2.75	3.00	5.70	10.0	14.6
	US female	2.62	3.00	5.60	10.0	14.7
MEHP	NJ women	**109**	**115**	**155**	**227**	**246**
	US total	4.27	4.10	9.80	22.8	38.9
	US female	4.23	4.10	9.70	23.0	42.5
MEHHP	NJ women	**112**	**109**	**203**	**245**	**309**
	US total	20.0	20.1	43.6	91.3	192
	US female	18.3	18.2	39.8	86.0	170
MEOHP	NJ women	**93.4**	**95.1**	**166**	**213**	**233**
	US total	13.5	14.0	29.6	59.9	120
	US female	12.5	13.0	28.1	57.5	115

* Geometric mean not calculated because detection of frequency < 60%

[a] MMP limit of detection (LOD) = 1.00 ng/ml

Table 13. Estimated daily intake (µg/kg/day) of phthalate for the NJ pregnant women based on the David (2000) method

Metabolites	Diesters (Parent)	25th	Median	75th	95th	Max
MEP	**DEP**	1.98	4.72	16.70	50.08	152.83
MBP + MiBP	**DBP + DiBP**	0.37	0.49	1.08	2.38	2.95
MBzP	**BBzP**	0.11	0.23	0.55	1.35	4.62
MEHP	**DEHP**	30.94	49.87	67.38	107.09	119.45
MEHHP	**DEHP**	6.39	10.57	19.69	30.00	43.18
MEOHP	**DEHP**	8.65	14.26	24.92	34.93	48.22

EPA RfDs (µg/kg/day): DEP = 800, DBP = 100, BBzP = 200

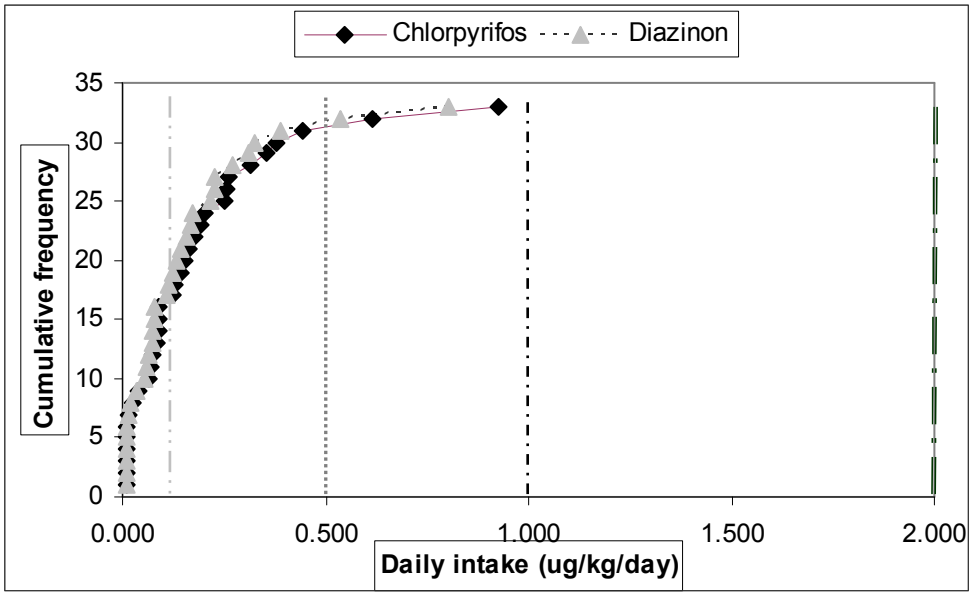
EU TDIs (µg/kg/day): DEP = 8,000; DBP = 10; BBzP = 370; DEHP = 40

ATSDR reproductive MRL (chronic) DEHP = 60 µg/kg/day

ATSDR reproductive MRL (intermediate) DEHP = 100 µg/kg/day

FIGURES

Figure 1. Cumulative frequency of chlorpyrifos and diazinon daily intakes (µg/kg/day)



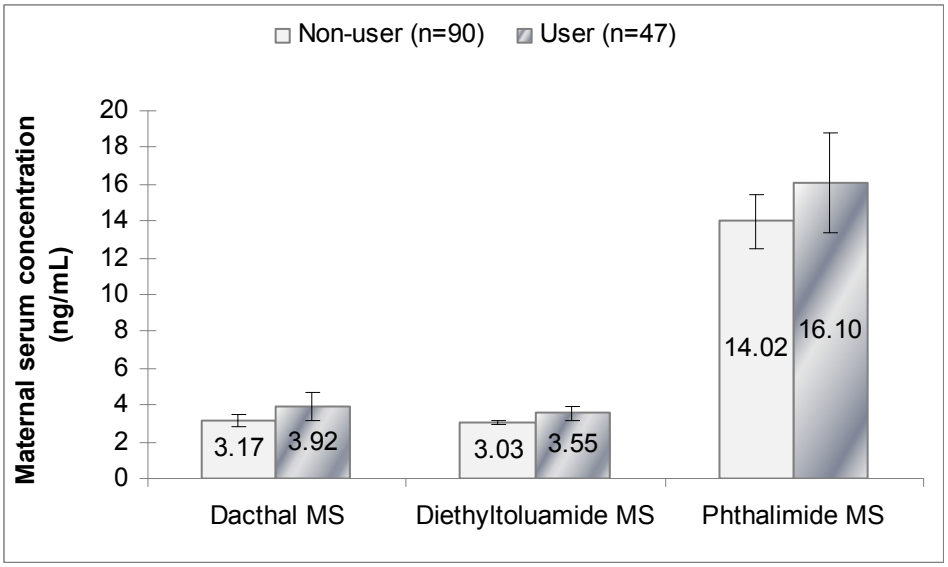
Acute population adjusted dose (aPAD) of chlorpyrifos = 0.5 µg/kg/day

Chronic population adjusted dose (cPAD) of chlorpyrifos = 0.1 µg/kg/day

ATSDR minimal risk level (MRL) of chlorpyrifos = 1.000 µg/kg/day

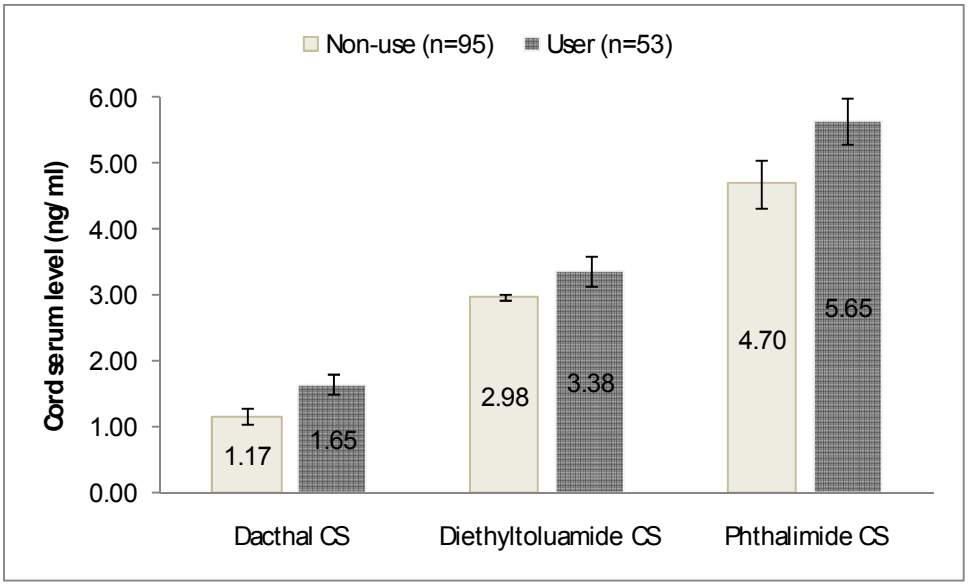
ATSDR minimal risk level (MRL) of diazinon = 2.000 µg/kg/day

Figure 2. Comparison of the concentrations (pg/ml) of representative pesticides in maternal serum between pesticide users and non-users



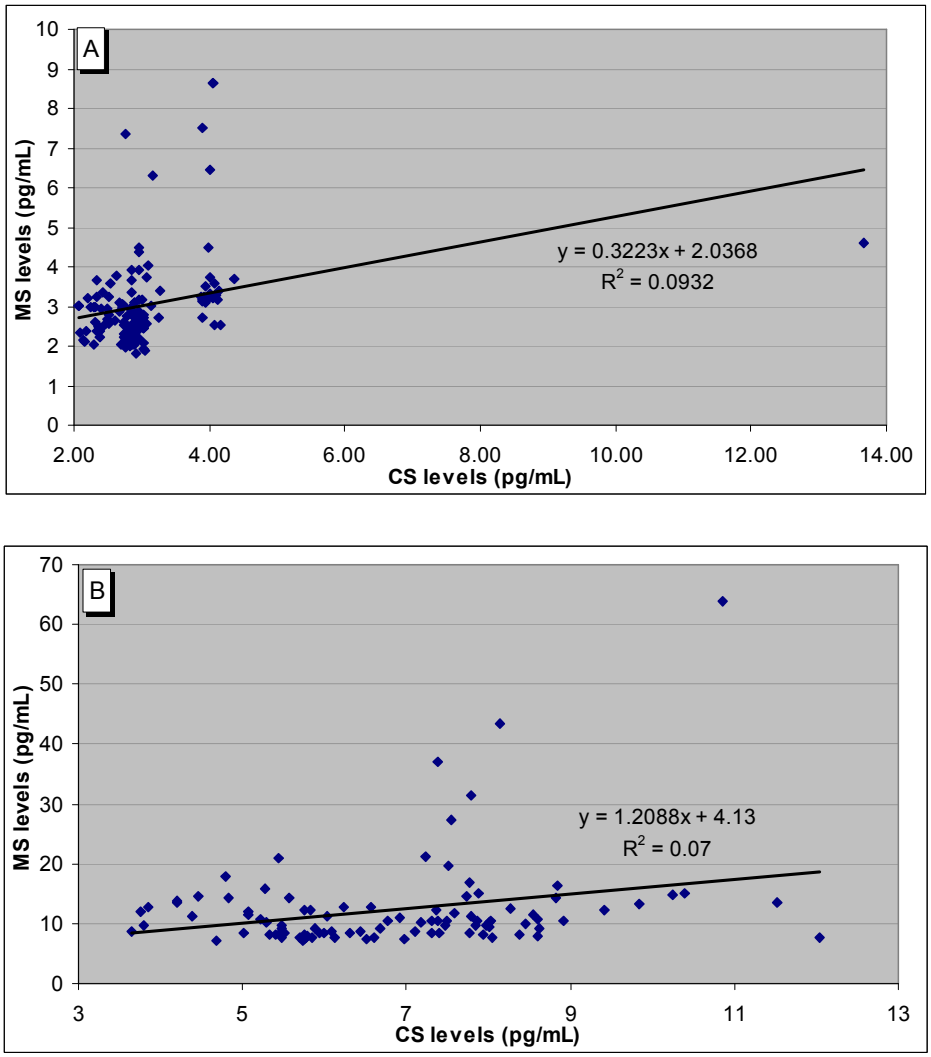
Data trends indicated the pesticides users had higher concentrations of dacthal, diethyltoluamide, and phthalimide in maternal serum than non-users, $p = 0.15$, 0.06, and 0.23 respectively.

Figure 3. Comparison of the concentrations (pg/ml) of representative pesticides in cord serum between pesticide users and non-users



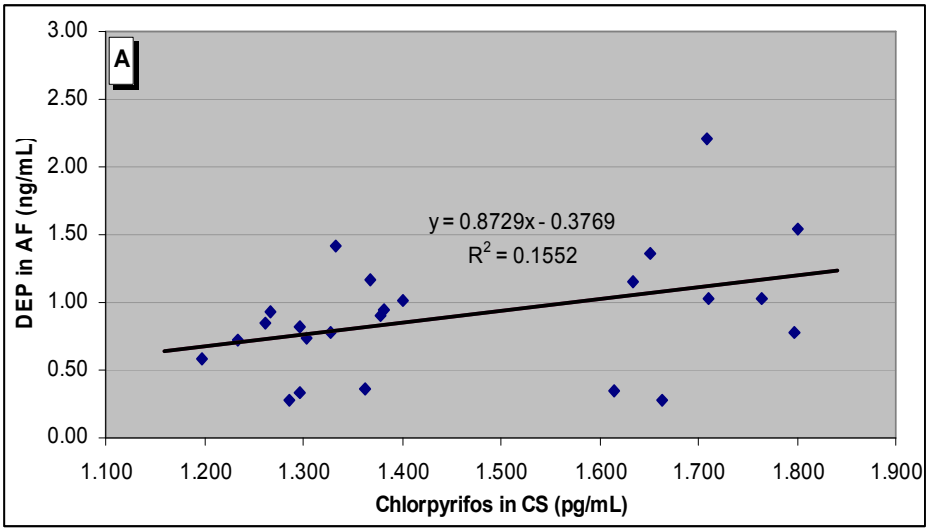
Pesticide users at home had significantly higher concentrations of dacthal, diethyltoluamide, and phthalimide in cord serum than non-users, **$p < 0.05$.**

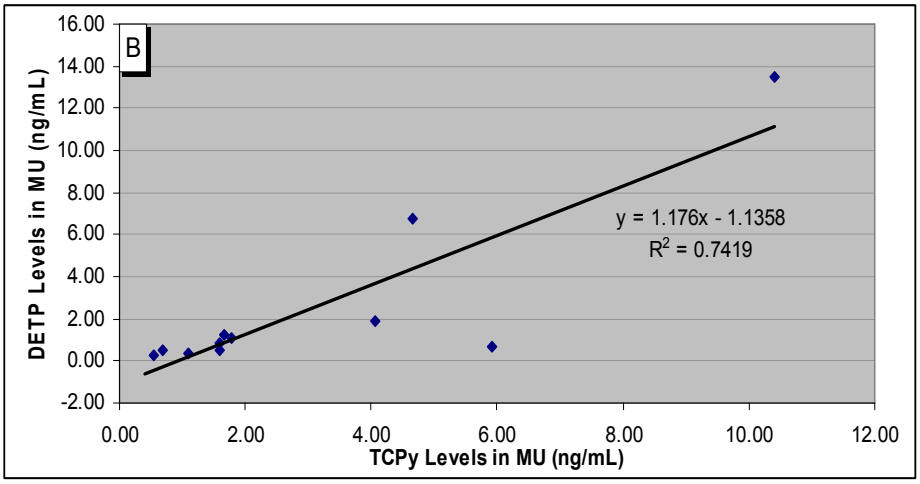
Figure 4. Linear regression and correlation analyses of representative pesticides with high detection of frequency (>50%) among biological matrices



Regression and correlation analyses of positive maternal serum levels vs. positive cord serum levels for (A) diethyltoluamide (DEET) (Pearson r = 0.3054) and (B) phthalamide (Pearson r = 0.2647).

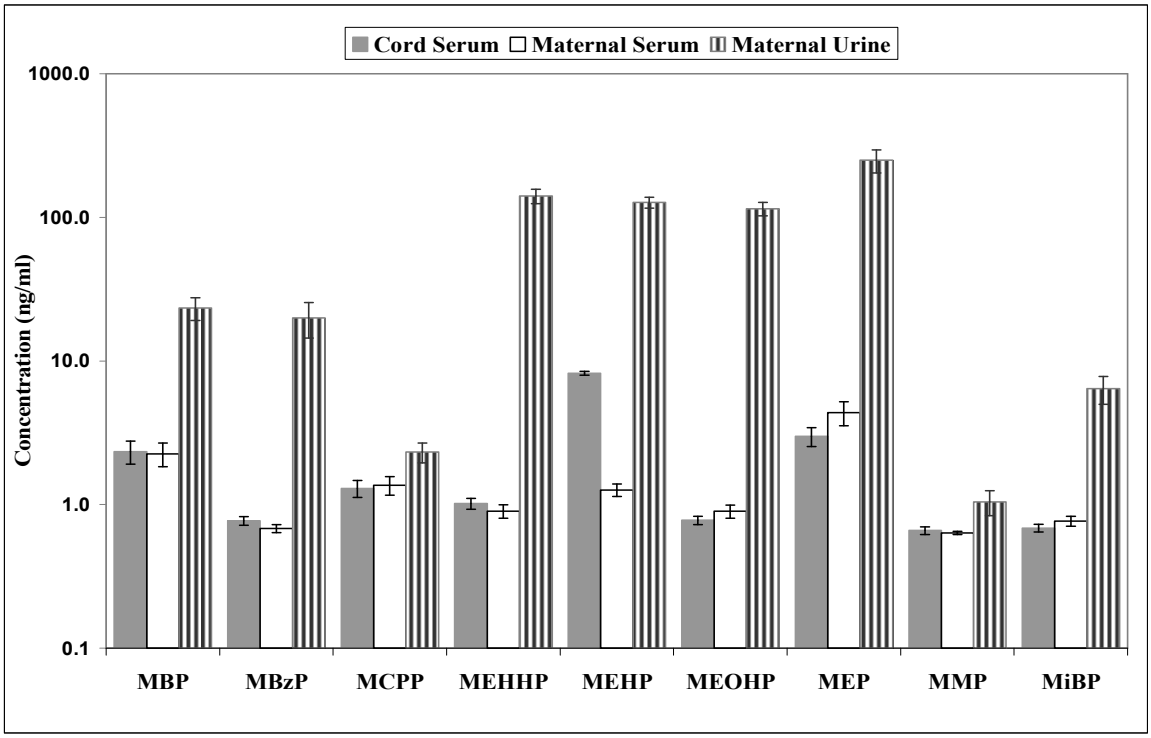
Figure 5. Linear regression and correlation analyses among representative pesticides with detection of frequency more than 35%





Regression and correlation analyses of positive levels among analytes for (A) chlorpyrifos in cord serum (CS) vs. DEP in amniotic fluid (AF; Pearson r = 0.3940) and (B) TCPy vs. DETP in maternal urine (MU; Pearson r = 0.8613).

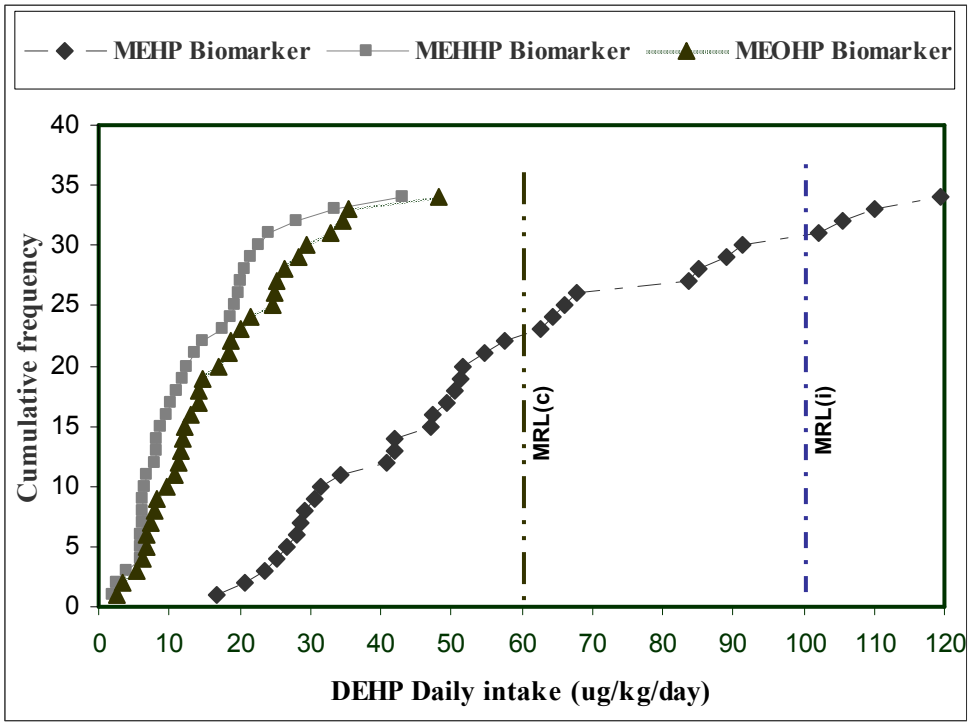
Figure 6. Mean concentrations* of phthalate metabolites in cord serum, maternal serum, and maternal urine



Except for MMP, the phthalate metabolite concentrations in maternal urine are significantly higher than in maternal and cord serum concentrations ($p < 0.05$)

* LOD/SQRT(2) was used for the calculations when cord and maternal serum concentrations were < LOD

Figure 7. Cumulative frequency of DEHP daily intakes



MRL(c): ATSDR reproductive minimal risk level for chronic duration 60 µg/kg/day

MRL(i): ATSDR reproductive minimal risk level for intermediate duration 100 µg/kg/day

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X. Yan, S. Lashley, J. Smulian, C. Ananth, D. Barr, T. Ledoux, P. Hore, M.G. Robson. 2008. Pesticide Concentrations in Matrices Collected in the Perinatal Period in a Population of Pregnant Women and Newborns in New Jersey. Accepted by the *Journal of Human and Ecological Risk Assessment* for publication.

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