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Organophosphate pesticide levels in blood and urine of women and newborns living in an agricultural community ☆

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ABSTRACT

Organophosphate pesticides are widely used and recent studies suggest associations of in utero exposures with adverse birth outcomes and neurodevelopment. Few studies have characterized organophosphate pesticides in human plasma or established how these levels correlate to urinary measurements. We measured organophosphate pesticide metabolites in maternal urine and chlorpyrifos and diazinon in maternal and cord plasma of subjects living in an agricultural area to compare levels in two different biological matrices. We also determined paraoxonase 1 (*PON1*) genotypes ($PON1_{192}$ and $PON1_{-108}$) and *PON1* substrate-specific activities in mothers and their newborns to examine whether *PON1* may affect organophosphate pesticide measurements in blood and urine.

Chlorpyrifos levels in plasma ranged from 0–1726 ng/mL and non-zero levels were measured in 70.5% and 87.5% of maternal and cord samples, respectively. Diazinon levels were lower (0–0.5 ng/mL); non-zero levels were found in 33.3% of maternal plasma and 47.3% of cord plasma. Significant associations between organophosphate pesticide levels in blood and metabolite levels in urine were limited to models adjusting for *PON1* levels. Increased maternal *PON1* levels were associated with decreased odds of chlorpyrifos and diazinon detection (odds ratio(OR): 0.56 and 0.75, respectively). Blood organophosphate pesticide levels of study participants were similar in mothers and newborns and slightly higher than those reported in other populations. However, compared to their mothers, newborns have much lower quantities of the detoxifying *PON1* enzyme suggesting that infants may be especially vulnerable to organophosphate pesticide exposures.

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1. Introduction

Organophosphorous pesticides are widely used in agriculture in the United States (DPR, 2008); despite the voluntary phase out of residential uses of chlorpyrifos and diazinon between 2000 and 2004 (U.S. EPA, 2000, 2001), some organophosphate pesticides are still registered for home garden use (U.S. EPA, 2006). Acute exposure to organophosphate pesticides can lead to neurotoxic effects through inhibition of the enzyme acetylcholinesterase (Costa et al., 2008). Recent epidemiologic studies suggest associations of low dose chronic prenatal exposure to organophosphate pesticides with adverse birth and neurodevelopmental outcomes including reduced birth weight and length (Whyatt et al., 2004), shorter gestational duration (Eskenazi et al., 2004), increased number of abnormal reflexes in neonates (Engel et al., 2007; Eskenazi et al., 2008), higher risk of reported attention problems (Marks et al., 2010), and lower intelligence in 7-year olds (Bouchard et al., 2011).

Although the majority of animal data provide evidence of organophosphate toxicity through cholinergic pathways, some studies suggest potential mechanisms for the adverse effects of organophosphate pesticide exposures, even at dose levels below the threshold for acetylcholinesterase inhibition (Costa, 2006). For instance, exposures to low doses of diazinon and/or chlorpyrifos in rat and or mouse models were associated with changes in

Abbreviations: CHAMACOS, Center for Health Assessment of Mothers and Children of the Salinas Valley; CI, confidence interval; EPA, Environmental Protection Agency; NHANES, National Health and Nutrition Examination Survey; OR, odds ratio; *PON1*, paraoxonase 1

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neuronal cell development (Slotkin et al., 2008), changes in emotional behaviors (Roegge et al., 2008), up regulation of serotonin neurotransmitters (Aldridge et al., 2003; Slotkin et al., 2006), and changes in thyroid hormone levels and the reproductive system (Buratti et al., 2006; De Angelis et al., 2009; Haviland et al., 2010). Recent studies also provide evidence that organophosphate pesticide exposure induces oxidative stress (Samarawickrema et al., 2008; Slotkin and Seidler, 2009), a condition associated with common diseases like cardiovascular disease and diabetes (Bhattacharyya et al., 2008; Li et al., 2003).

Estimating the internal dose of organophosphate pesticide exposure in biological specimens is particularly challenging because organophosphate pesticides have relatively short half-lives and are quickly metabolized and excreted from the body (Wessels et al., 2003). Organophosphate metabolites, including dialkyl phosphates, in urine have been used as biomarkers of organophosphate pesticide exposure in many studies (Bouchard et al., 2010; Eskenazi et al., 2004; Fenske et al., 2002; Grandjean et al., 2006; Lacasana et al., 2010; Ye et al., 2009). Collection of urine specimens from study participants is relatively noninvasive and methods for analyzing organophosphate pesticide metabolites are well established (Bradman and Whyatt, 2005). Analysis of organophosphate pesticide levels in blood allows for direct measurement of parent compounds rather than metabolites and may more accurately represent the dose that reaches the target tissue (Bradman and Whyatt, 2005). Although the rate of clearance from the blood is initially quite rapid, chlorpyrifos and diazinon are lipophilic so the portion of compound that partitions into body fat may be eliminated more slowly (Eaton et al., 2008). Therefore, levels in blood may represent a steady state concentration (Needham, 2005). However, since concentrations of organophosphate pesticides in blood are much lower (by orders of magnitude) than metabolite levels in urine, very sensitive analytical methods are required to measure them (Perez et al., 2010). Thus far, only a small number of studies have measured prenatal organophosphate pesticide exposure in maternal or umbilical cord blood (Neta et al., 2010; Whyatt et al., 2003). Only one study has compared chlorpyrifos levels in blood and urine from the same subjects (mothers and infants) and reported no association between chlorpyrifos in maternal or cord blood and levels of the chlorpyrifos metabolite 3,5,6-trichloro-2-pyridinol in urine (Whyatt et al., 2009). Additionally, there are no published analytical methods for some organophosphate pesticides in blood, such as oxydemeton methyl and thus, blood measures may not fully capture exposure especially in populations exposed to multiple organophosphate pesticides. As there are strengths and weaknesses in using either of the two biological matrices, it remains unclear which measures will be more useful in epidemiological studies of prenatal organophosphate pesticide exposures and adverse health effects.

The *PON1* enzyme can detoxify the oxon derivatives of some organophosphate pesticides and also acts as an antioxidant (James, 2006; Li et al., 2003). Individuals with low *PON1* activity may be more susceptible to organophosphate pesticide exposures due to both decreased metabolic capacity towards organophosphate oxons and lower antioxidant defenses in comparison to those with average or high *PON1* activities. In humans, *PON1* enzymatic activities vary widely among adults (Deakin and James, 2004) and children (Chen et al., 2003; Huen et al., 2010), due in part to genetics (Costa et al., 2005; Deakin and James, 2004). For instance, a single nucleotide polymorphism (SNP) at position −108 in the promoter region of the *PON1* gene is associated with two-fold higher levels of *PON1* quantity for the $PON1_{-108C}$ allele compared to the $PON1_{-108T}$ allele (Deakin et al., 2003). The nonsynonymous coding SNP, $PON1_{192}$, strongly affects substrate-specific catalytic efficiency. In vitro and in vivo studies have demonstrated that the $PON1_{192R}$ alloform can hydrolyze the organophosphate oxons chlorpyrifos-oxon and paraoxon more efficiently than the $PON1_{192Q}$ alloform, conferring a greater degree of protection from organophosphate pesticide exposures (Costa et al., 2003).

Previously, we measured urinary dialkyl phosphate metabolites of organophosphate pesticides in pregnant mothers from the Center for Health Assessment of Mothers and Children of Salinas (CHAMACOS) study and found that prenatal and postpartum metabolite levels were higher in these women than in women of childbearing age who participated in the National Health and Nutrition Examination Survey (NHANES) study (Bradman et al., 2005). In the present study, we measured levels of chlorpyrifos and diazinon in maternal and umbilical cord blood of CHAMACOS participants. Our primary aims were to describe the distribution of these measurements and compare them with urinary dialkyl phosphates in the same subjects. Since *PON1* may affect an individual's ability to metabolize and excrete oxon derivatives of organophosphates, we also determined whether *PON1* genotype and enzyme activity affect the levels measured in both biological matrices.

2. Materials and methods

2.1. Study population

The CHAMACOS Study is a longitudinal birth cohort study examining the effects of pesticide and other environmental exposures on children's neurodevelopment, growth, and respiratory disease (Eskenazi et al., 2003). The study is located in the Salinas Valley in Monterey County, CA, an intensively farmed region with approximately 200,000 kg of organophosphates applied annually (DPR, 2007). Women eligible to participate in the study were at least 18 years of age, spoke English or Spanish, qualified for medicaid, were less than 20 weeks gestation, and were receiving prenatal care in one of six clinics serving the community. Participants were primarily Mexican-American, many of whom were born in Mexico. Six hundred and one pregnant women were enrolled in 1999–2000 and 526 delivered liveborn singleton newborns.

Organophosphate pesticides were measured in blood collected from mothers at the hospital shortly before delivery and in umbilical cord blood (n=234 and 256, respectively). Measurements were only made in those participants with sufficient blood volumes for the analysis. Heparinized whole blood was collected in BD Vacutainers® (Becton, Dickinson and Company, Franklin Lakes, NJ), centrifuged, divided into plasma, buffy coats and red blood cells, and stored at −80 °C. Serum and blood clots were collected in vacutainers containing no anticoagulant. DNA was isolated from clots as described previously (Holland et al., 2006).

Most of the mothers also provided urine specimens in the peripartum period after delivery, which were used to measure urinary dialkyl phosphate metabolites (n=221). *PON1* genotypes were ascertained in 221 mothers and 244 children ($PON1_{192}$ and $PON1_{-108}$). In addition, 219 of these women and 236 of the newborns had adequate samples available for *PON1* enzyme activity measurements in maternal and umbilical cord blood.

Study protocols were approved by the University of California, Berkeley Committee for Protection of Human Subjects. Written informed consent was obtained from all mothers.

2.2. Pesticide exposure measurement

2.2.1. Organophosphate pesticide parent compound in blood

Frozen aliquots of heparinized plasma were shipped to the Center for Disease Control and Prevention (CDC) for analysis of parent organophosphate pesticides in maternal and umbilical cord blood on dry ice. Chlorpyrifos, diazinon, and several pyrethroid pesticides were measured in plasma specimens using solid phase extraction and gas chromatography-high resolution mass spectrometry as described previously (Perez et al., 2010). (This paper focuses solely on measurements of the organophosphorous pesticides diazinon and chlorpyrifos). Pesticide measurement results were reported for all samples and fell into three categories: 1) non-detect for which no signal was detected, 2) detectable concentrations that were below the instrument limit of quantification (Armbruster and Pry, 2008) and 3) quantifiable concentrations above the limit of quantification. Data were reported as detectable only when a peak was visibly detectable and the signal to noise ratio was greater than three. The limit of quantification for chlorpyrifos and diazinon were determined by regression techniques. The standard deviation of known concentrations was plotted against their measured values and the y-intercept of the line was extrapolated to derive the estimated standard deviation at zero