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

Karen Huen a,*, Asa Bradman a, Kim Harley a, Paul Yousefi a, Dana Boyd Barr b, Brenda Eskenazi a, Nina Holland a

a Center for Environmental Research and Children’s Health, School of Public Health, University of California, Berkeley, 1995 University Avenue Suite 265, Berkeley CA 94720, USA
b Department of Environmental Health, Rollins School of Public Health, Emory University, 1518 Clifton Road, NE, Claudia Nance Rollins Bldg, Room 2007, Atlanta GA 30322, USA

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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 (PON1192 and PON1107) and PON1 substrate-specific activities in mothers and their newborns to examine whether organophosphate pesticide levels 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), 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 mouse models were associated with changes in...
neuronal cell development (Slotkin et al., 2008), changes in emotional behaviors (Roegee 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; Ekenazi 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 chloryrifos levels in blood and urine from the same subjects (mothers and infants) and reported no association between chloryrifos in maternal or cord blood levels and the chloryrifos 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 activity. 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 allele can hydrolyze the organophosphate oxons chlorpyrifos-oxon and paraxon more efficiently than the PON1_192Q allele, 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 (Ekenazi 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., 2008).

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_192R). 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.
concentration ($\beta$). The limit of quantification was calculated as 3 $\beta$. Concentrations below the limit of quantification can be detected by the instrument but have higher relative standard deviations than those above the limit of quantification (relative standard deviation $=30-40\%$ versus 10-15%, respectively) and should therefore be considered relevant measurements for use in subsequent analyses. The instrument limit of quantifications for individual samples were 21 pg/mL and 16 pg/mL for chlorpyrifos and diazinon, respectively. These quantification limits were higher than limits reported in Whayht et al. (2003), primarily due to (1) inclusion of the pyrhythm target analytes, which reduced sensitivity to chlorpyrifos and diazinon in the elution window and, (2) aging of the equipment.

2.2.2. Urinary metabolites

For analysis of organophosphorous pesticide metabolites, spot urine samples were collected from mothers soon after delivery and stored at $-80 \degree C$ until shipment to the CDC. Urine specimens were analyzed using gas chromatography–tandem mass spectrometry and dialkyl phosphate metabolites were then quantified using isotope dilution calibration (Bravo et al., 2002). Details of laboratory measurements and quality control are described by Bradman et al. (2005).

A total of six dialkyl phosphate metabolites were quantified: three dimethyl phosphate metabolites (dimethylphosphate, dimethyldithiophosphate, dimethyl-thiophosphate) and three diethyl phosphate metabolites (diethyldithiophosphate, diethyl-dithiophosphate, diethylphosphate). Dimethyl phosphates are derived from pesticides such as malathion, oxiemeton-methyl, and dimethoate and diethyl phosphates are derived from pesticides such as diazinon, chlorpyrifos, and disulfoton. This data analysis is limited to dialkyl phosphate metabolites because both of the organophosphates measured in blood, chlorpyrifos and diazinon, devolve into dialkyl phosphates. Dialkyl phosphate concentrations were summed on a molar basis to generate a variable for total dialkyl phosphates. For eight women, levels of one urinary metabolite could not be calculated due to analytic interference. Because metabolites were correlated within the dialkyl phosphate group, regression was used to impute the value of the missing metabolite based on the concentrations of the other two metabolites.

2.3. PON1 genotypes and enzyme activity

PON1 polymorphisms were genotyped as previously described (Holland et al., 2006; Huen et al., 2009a). Briefly, the coding polymorphism PON1 G27Q was analyzed using the Taqman real-time PCR method. Primers for the nucleotide sequence flanking the SNP, and probes specific for the SNPs, were custom-designed by Applied Biosystems, Inc. (Foster City, CA). The promoter SNP, PON1 -238A/G, was genotyped using a fluorogenic allele-specific assay (Amplifluor, Chemicon, Teme-cula, CA). It required a two-part nested PCR strategy, in which the region surrounding the SNP was pre-amplified using non allelic flanking primers and then the amplicon was diluted and used as the template for the Amplifluor assay. Quality assurance procedures for genotyping of these PON1 SNPs included assessment of randomly distributed blank samples in each plate and duplicates of randomly selected samples with independently isolated DNA from the same subjects. Repeated analysis (4% of samples) in several runs showed a high degree (> 99%) of concordance. All discrepancies were resolved with additional genotyping.

We measured PON1 enzyme activity against three different substrates (paraoxon (PO), phenyl acetate (ARY), and chlorpyrifos-oxon (CP0)) in plasma samples using spectrophotometric methods as described previously (Huen et al., 2009b). In this paper, we use these three measurements as markers of PON1 molecular phenotype. The arylesterase assay serves as an indirect measure of PON1 enzyme quantity. ELISA and Western blot based methods have been used in trials to demonstrate a high correlation of PON1 quantity with arylesterase activity ($r > 0.85$) (Connelly et al., 2008; Kujiraiako et al., 2000). In contrast, the paraoxonase and chlorpyrifos-oxon substrate-specific activities reflect both quantity and catalytic efficiency of the enzyme. All assays were performed in triplicate. Quality assurance included assessment of repeat samples (separate aliquots of the same sample run on different days) and internal controls (aliquots of the same sample run on all assay plates). We found a high degree of concordance between repeated samples (3% of samples were repeated). The average relative standard deviation for repeated samples ranged from 6-9% and the correlation coefficients between repeated runs were between 0.91 and 0.98 for all three assays. The same internal control samples were used on every plate for every assay and the inter-assay variability (average relative standard deviation) for these samples ranged from 7% to 9%.

2.4. Statistical analysis

To determine whether there were systematic differences between participants and non-participants in this study, we performed logistic regression to assess whether demographic variables were related to study participation. For non-participants, we performed logistic regression to understand factors that could be modified to increase participation in future studies. To determine the association between the organophosphates in blood and type of participant, we performed logistic regression with multiple imputation to account for non-detectable organophosphate concentrations. Since there were multiple imputations, we used a multiple imputation procedure to estimate our coefficients of interest. We employed a variation of the method described by Rubin (1987) and used Rubin’s rules as described in Schafer and Graham (2002).

We performed 20 imputation cycles, generating 20 individual imputed data sets for which parameter estimates and standard errors were calculated. We combined the 20 individual estimates to obtain the multiple imputation estimate using Rubin’s rules as described in Schafer and Graham (2002). This method has been shown to yield reasonable estimates when detection frequencies are $>70\%$; when detection frequencies are $<70\%$, which is the case for diazinon, this method produces biased parameter estimates but may yield biased variance estimates.

In subsequent analyses, blood organophosphate levels were expressed both continuously using the multiple imputation procedures described above and categorically. They were classified categorically in three ways: (1) as a non-detectable and a detectable (ie, below and above the limit of quantification) value; (2) as a non-detectable, (b) signal detected but < limit of quantification, (c) and > limit of quantification; and (3) as values (a) above and (b) below the limit of quantification (only for chlorpyrifos). Results were similar using all classifications, therefore only the data for non-detectable versus detectable (categorical classification) values are shown below.

Logistic regression models were constructed to examine the association between PON1 genotypes (PON1 192 and PON1 108), enzyme activities (arylesterase, chlorpyrifos-oxonase, and paraoxonase), or PON1 status (arylesterase and PON1 108) and the dependent variable, chlorpyrifos or diazinon detection (non-detected vs. detectable levels). Odds ratios (ORs) for aryelsterase, chlorpyrifos-oxonase, and paraoxonase were expressed as a change in odds of chlorpyrifos or diazinon detection per increase by one standard deviation of enzyme activity (arylesterase, chlorpyrifos-oxonase, or paraoxonase). In models testing for associations with PON1 genotype, odds ratios were expressed as the odds of chlorpyrifos or diazinon detection compared to the reference group (PON1 192 or PON1 108). For the models including PON1 status, PON1 192 genotype was coded 0, 1, or 2, for the number of R alleles only. An interaction term for aryelsterase $\times$ PON1 108 was included, however the likelihood ratio test indicated that this interaction was not significant ($p > 0.20$) and results reported are for models without the interaction terms.

We constructed separate regression models to examine the relationship between PON1 genotypes, enzyme activities or status (e.g., aryelsterase and PON1 192, in the same model) with levels of maternal urinary dialkyl phosphates (dependent variable expressed continuously). Dialkyl phosphate measurements (nmol/L) were log10 transformed to normalize their distribution. We also used linear regression models to determine the relationship between organophosphates in blood and urinary dialkyl phosphates (dependent variable expressed continuously). We included an interaction term between the genotype or enzyme activity and chlorpyrifos or diazinon detection (non-detected vs. detectable levels), and either PON1 genotypes (c) above and (b) below the limit of quantification (for chlorpyrifos).

All statistical analyses were performed using STATA 11.1 (College Station, TX).

3. Results

There were no differences in those women and children who had sufficient plasma available for organophosphate measurements.
3.1. Organophosphate parent compound concentration data

Table 2 presents the means, ranges and percentiles for parent chlorpyrifos and diazinon measured in maternal and umbilical cord blood plasma. The percent of detectable chlorpyrifos and diazinon concentrations was slightly higher in cord blood (87.5% and 47.3%) than in maternal blood (70.5% and 33.3%), whereas the percent > limit of quantification was higher in maternal blood for chlorpyrifos (17.9% and 11.3%, respectively) and identical for maternal and cord blood for diazinon (1.7%). There was a relatively narrow range of diazinon levels in both newborns and mothers from nondetect – 0.5 and nondetect – 0.03 ng/mL in mothers and newborns, respectively. Chlorpyrifos levels in blood ranged from nondetect – 1385 ng/mL in mothers and from nondetect – 1726 ng/mL in newborns. Among mothers, there was one extreme outlier (1385 ng/mL). There was also one extreme outlier among newborns (1726 ng/mL). These levels are more than 100-fold greater than levels measured at the 95th percentile. The mother and newborn were unrelated. The mother with the high level did not work in agriculture but pesticides were found in her home during pregnancy; interestingly her newborn did not have unusually high organophosphate levels in blood. For the newborn with the high level, maternal levels were not measured; pesticides were found in the home and agriculture workers lived in the home. All subsequent analyses do not include these two extreme outliers.

Maternal blood levels of chlorpyrifos were not correlated with levels of diazinon (r(95%CI) = 0.07 (−0.11, 0.24)) nor were they highly correlated in cord blood (r(95%CI) = 0.14 (−0.04, 0.33)). Additionally, organophosphate pesticide levels were not correlated among mother-newborn pairs (n=125; r(95%CI) = 0.05 (−0.16, 0.26) and 0.06 (−0.18, 0.29) for chlorpyrifos and diazinon, respectively).

3.2. Demographic and exposure characteristics

Blood organophosphate levels (nondetectable vs detectable) did not differ significantly by most demographic characteristics including sex of the child and poverty level. Determinants that could potentially affect exposure to pesticides such as whether the mother worked in agriculture, lived with an agriculture worker, or lived near a field during pregnancy were not associated with blood organophosphates in mothers or newborns.

3.3. Organophosphate metabolites (dialkyl phosphates) in urine and comparisons to organophosphate parent compound in blood

Means and ranges for diethyl phosphate metabolites in urine are presented in Table 2. Diethyl phosphate levels ranged from 1.7–596.5 nmol/L with a median of 28.5 nmol/L. While over 99.8% of mothers have diethyl phosphates measurements above the

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**Table 1**

Demographic and exposure characteristics of CHAMACOS participants.

<table>
<thead>
<tr>
<th>Country of birth</th>
<th>N</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mexico</td>
<td>315</td>
<td>84.7</td>
</tr>
<tr>
<td>US</td>
<td>49</td>
<td>13.2</td>
</tr>
<tr>
<td>Other</td>
<td>8</td>
<td>2.2</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Household income</th>
<th>N</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>At or below poverty level</td>
<td>208</td>
<td>59.4</td>
</tr>
<tr>
<td>Within 200% poverty level</td>
<td>131</td>
<td>37.4</td>
</tr>
<tr>
<td>Above 200% poverty level</td>
<td>11</td>
<td>3.1</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Infant sex</th>
<th>N</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Boy</td>
<td>174</td>
<td>46.8</td>
</tr>
<tr>
<td>Girl</td>
<td>198</td>
<td>53.2</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Work status during pregnancy</th>
<th>N</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Did not work</td>
<td>129</td>
<td>35.3</td>
</tr>
<tr>
<td>Some field work</td>
<td>98</td>
<td>26.9</td>
</tr>
<tr>
<td>Some agriculture work</td>
<td>49</td>
<td>13.4</td>
</tr>
<tr>
<td>Other work only</td>
<td>89</td>
<td>24.4</td>
</tr>
<tr>
<td>Lived within 200 ft of a field</td>
<td>45</td>
<td>12.1</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Lived with agriculture worker during pregnancy</th>
<th>N</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes</td>
<td>311</td>
<td>83.8</td>
</tr>
<tr>
<td>No</td>
<td>60</td>
<td>16.2</td>
</tr>
</tbody>
</table>

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**Table 2**

Concentrations of chlorpyrifos and diazinon (ng/mL) in plasma and diethyl phosphates (nmol/L) in urine from mothers and newborns.

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>Percent detectable</th>
<th>Percent &gt; limit of quantification</th>
<th>Range</th>
<th>Percentile 25</th>
<th>50</th>
<th>75</th>
<th>90</th>
<th>95</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mothers at delivery</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chlorpyrifos</td>
<td>234</td>
<td>70.5</td>
<td>17.9</td>
<td>nondetect-1385.1 nondetect</td>
<td>nondetect</td>
<td>&lt; limit of quantification nondetect</td>
<td>&lt; limit of quantification nondetect</td>
<td>0.07</td>
<td>0.40</td>
</tr>
<tr>
<td>Diazinon</td>
<td>234</td>
<td>33.3</td>
<td>1.7</td>
<td>nondetect</td>
<td>0.5 nondetect</td>
<td>&lt; limit of quantification</td>
<td>&lt; limit of quantification</td>
<td>nondetect</td>
<td>&lt; limit of quantification</td>
</tr>
<tr>
<td>Diethyl phosphates</td>
<td>347</td>
<td>99.8</td>
<td>99.8</td>
<td>1.7–596.5</td>
<td>11.8</td>
<td>28.5</td>
<td>61.5</td>
<td>128.8</td>
<td>162.3</td>
</tr>
</tbody>
</table>

| **Newborn children** |    |                    |                                 |       |               |    |    |    |    |
| Chlorpyrifos | 256 | 87.5               | 11.3                            | nondetect-1726.1 nondetect-0.03 | < limit of quantification nondetect | < limit of quantification | < limit of quantification | nondetect | < limit of quantification | < limit of quantification | < limit of quantification |
| Diazinon | 256 | 47.3               | 1.7                             | nondetect-0.03 | nondetect | < limit of quantification | < limit of quantification | < limit of quantification | < limit of quantification | < limit of quantification | < limit of quantification |

* Total number of observations vary due to missing data (table entries do not include imputed values).

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* Detectable values include all non-zero values both above and below the limit of quantification.

* Limit of quantification for the instrument method used to measure chlorpyrifos and diazinon in plasma was 0.021 and 0.016 ng/mL, respectively.

* For descriptive purposes, only values greater than the instrument limit of quantification are reported.
limit of quantification, much fewer mothers and newborns had quantifiable levels of chlorpyrifos and diazinon in their blood. We observed no significant association between detection of chlorpyrifos and diazinon in maternal blood and postpartum diethyl phosphate metabolites in maternal urine (Table 3).

### 3.4. PON1 genotypes and activities

PON1 genotypes and substrate-specific activities in CHAMACOS mothers and their newborns have been described previously (Holland et al., 2006; Huen et al., 2010). For the subset of subjects with measured organophosphate pesticides in blood presented in this paper, all PON1 values were similar to those assessed for the larger subset of the CHAMACOS cohort (n=336 cords and n=382 mothers). Allele frequencies in both mothers and their children were close to 0.5 for both SNPs (PON1_192Q and PON1_192R). Arylesterase ranged from 27–346 U/mL in mothers and 4–98 U/mL in newborns. Chlorpyrifos-oxonase ranged from 1766–14,676 U/L and 154–5255 U/L in mothers and newborns, respectively. Maternal paraoxonase measured ranges from 75 to 3538 U/L and levels in newborns ranged from 7 to 1018 U/L. For all three substrate-specific activities, maternal measures were on average four-fold higher than those in newborns.

### 3.5. Organophosphate pesticides in blood and PON1

Maternal PON1 activity was inversely associated with the odds of organophosphate pesticide detection in cord blood (Table 4). An increase of maternal arylesterase and chlorpyrifos-oxonase by 3.5. Organophosphate pesticides in blood and PON1 sures were on average four-fold higher than those in newborns. 1018 U/L. For all three substrate-specific activities, maternal mea-

### Table 3

<table>
<thead>
<tr>
<th>Maternal diethyl phosphate metabolites (nmol/L)</th>
<th>N</th>
<th>Change in urinary diethyl phosphates for detectable versus nondetectable plasma organophosphate levels (95% confidence interval)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal diazinon (nondetect vs detectable)</td>
<td>221</td>
<td>-0.02 (-0.17,0.13)</td>
</tr>
<tr>
<td>Cord diazinon (nondetect vs detectable)</td>
<td>246</td>
<td>-0.04 (-0.18,0.10)</td>
</tr>
<tr>
<td>Maternal chlorpyrifos (nondetect vs detectable)</td>
<td>220</td>
<td>0.01 (-0.15,0.16)</td>
</tr>
<tr>
<td>Cord chlorpyrifos (nondetect vs detectable)</td>
<td>245</td>
<td>-0.03 (-0.24,0.18)</td>
</tr>
</tbody>
</table>

### Table 4

<table>
<thead>
<tr>
<th>Detectable diazinon</th>
<th>Detectable chlorpyrifos</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal blood odds ratio (95% confidence interval)</td>
<td>Cord blood odds ratio (95% confidence interval)</td>
</tr>
<tr>
<td>Maternal arylesterase activity</td>
<td>0.93(0.70,1.23)</td>
</tr>
<tr>
<td>Cord arylesterase activity</td>
<td>0.97(0.76,1.24)</td>
</tr>
<tr>
<td>Maternal chlorpyrifos-oxonase activity</td>
<td>0.90(0.67,1.20)</td>
</tr>
<tr>
<td>Cord chlorpyrifos-oxonase activity</td>
<td>1.03(0.79,1.29)</td>
</tr>
<tr>
<td>Maternal paraoxonase activity</td>
<td>1.07(0.81,1.43)</td>
</tr>
<tr>
<td>Cord paraoxonase activity</td>
<td>1.03(0.80,1.32)</td>
</tr>
</tbody>
</table>

Odds ratios are for a standard deviation change in PON1 activity. The standard deviation for maternal arylesterase, chlorpyrifos-oxonase, and paraoxonase activities was 44.3 U/mL, 2235.4 U/L, and 621.6 U/L, respectively. The standard deviation for cord arylesterase, chlorpyrifos-oxonase, and paraoxonase activities was 14.5 U/mL, 860.7 U/L, and 162.4 U/L, respectively.
3.6. Maternal urinary organophosphate metabolites, organophosphate pesticides in blood, and PON1

We found no association of maternal PON1 genotypes, activities, or status (arylesterase and PON1<sup>192</sup> genotype) with maternal diethyl phosphates measured around the time of delivery. As mentioned earlier, we observed no association between maternal diethyl phosphates and chlorpyrifos or diazinon detection in blood using crude models; however after adjusting for maternal arylesterase activity, we found a positive association (change in diethyl phosphates(95%CI)=0.61(0.13, 1.10)) between diazinon presence in maternal plasma and postpartum diethyl phosphates (Table 5) and a significant interaction between diazinon and arylesterase activity (change in diethyl phosphates (95%CI)=−0.20(−0.35,−0.05)); a similar trend was found for chlorpyrifos detection however its association with diethyl phosphates was not significant. When we categorized chlorpyrifos levels as above and below the limit of quantification (data not shown), we found similar results (change in diethyl phosphates (95%CI)=0.67(−0.06,1.41)).

We also modeled the association of organophosphate detection in maternal blood with diethyl phosphates stratified by tertile of arylesterase activity to better understand the significant interaction between arylesterase and presence of organophosphates in blood (Fig. 1). The association between diazinon and diethyl phosphates was positive but non-significant for the lowest two tertiles. However, a statistically significant negative association was present for the highest tertile of arylesterase activity (change in diethyl phosphates (95%CI)=−0.36(−0.64,−0.07). These data indicate that for mothers with higher levels of arylesterase, we see lower diethyl phosphate metabolites in urine as diazinon in plasma increases. Similar albeit weaker trends were also seen with detectable chlorpyrifos levels in plasma (change in diethyl phosphates(95%CI)=−0.32(−0.65, 0.01)) for the highest tertile; no significant associations for the lowest and medium tertiles).

### 4. Discussion

In this study, we measured levels of chlorpyrifos and diazinon in maternal and umbilical cord blood collected at the time of delivery from a primarily Mexican–American cohort from the agricultural region of the Salinas Valley, California. To our knowledge, no other

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**Table 5**

Association of chlorpyrifos and diazinon detection in plasma and PON1 activities on maternal urinary diethyl phosphate metabolites (n=206).

<table>
<thead>
<tr>
<th></th>
<th>Change in urinary diethyl phosphates (nmol/L) (95% confidence interval)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Diazinon</strong></td>
<td><strong>Arylesterase</strong></td>
</tr>
<tr>
<td>Arylesterase activity</td>
<td>0.05(−0.05, 0.14)</td>
</tr>
<tr>
<td>Diazinon x arylersterase activity</td>
<td>−0.20(−0.35,−0.05)</td>
</tr>
<tr>
<td>Chlorpyrifos</td>
<td>Maternal chlorpyrifos-oxonase activity</td>
</tr>
<tr>
<td>Arylesterase</td>
<td>Maternal paraoxonase activity</td>
</tr>
</tbody>
</table>

Change in urinary diethyl phosphates for PON1 activity and for interaction terms are expressed per standard deviation change in PON1 activity. The standard deviations for maternal arylesterase, chlorpyrifos-oxonase, and paraoxonase activities were 44.5 U/mL, 2235.4 U/L, and 621.6 U/L, respectively.
studies have examined the association of maternal or newborn PON1 genotype or enzyme activities on levels of organophosphate pesticides in blood and metabolites in urine. We found that elevated maternal PON1 enzyme activities may protect the fetus resulting in lower levels of chlorpyrifos and diazinon measured in umbilical cord blood.

Few studies have measured organophosphate pesticides in maternal or cord blood. Neta et al. (2010) used the same analytical method that we employed in our study to measure chlorpyrifos and diazinon in cord serum in an urban population from Baltimore, Maryland. No newborns had diazinon above the limit of quantification and only 3% had chlorpyrifos levels above the limit of quantification (range: < limit of quantitation – 0.014 ng/mL), indicating lower exposures in this population compared to CHAMACOS newborns. Whyatt et al. (2003), utilizing laboratory methods with lower limits of detection (0.5–1.1 pg/mL and 0.5–1.6 pg/mL for chlorpyrifos and diazinon, respectively) in 211 newborns from a minority cohort in New York City, reported ranges of < limit of quantitation – 67 pg/mL and 14 pg/mL for chlorpyrifos and diazinon, respectively. While the limit of quantification was higher in our study, levels above the 93rd percentile of our distribution exceeded the maximum levels measured in their study, suggesting higher exposures in some CHAMACOS participants; medians levels were also higher in CHAMACOS mothers and newborns than in the NYC study, however the CHAMACOS medians (0.006 and 0.004 pg/mL) were below the instrument limit of quantification. Although Whyatt et al. (2004) reported a strong correlation between maternal and cord blood chlorpyrifos levels, we did not. Neither study – ours or that reported by Whyatt et al. (2009) – observed a significant crude correlation between maternal urinary metabolites with organophosphates in maternal or cord blood. However, after adjusting for maternal arylesterase activity, we did observe a positive association between diazinon presence in maternal plasma and postpartum diethyl phosphates.

Both the Whyatt et al. (2003; 2009) and the Neta et al. studies (2010) were conducted in urban cohorts where exposure was predominantly due to indoor organophosphate pesticide use; this use declined after a voluntary residential restriction in 2000 (Whyatt et al., 2003). However, both pesticides were widely used in agriculture at the time of cord blood collection for this study (1999–2000). In fact, while overall use of organophosphate pesticides has decreased in the state of California, in the Salinas Valley (1999–2000). In fact, while overall use of organophosphate pesticides has decreased in the state of California, in the Salinas Valley (1999–2000).

Only a few studies of pharmacokinetics of chlorpyrifos and/or diazinon are available in humans (Eaton et al., 2008). Two small studies of human volunteers (n=6 and n=12) have measured chlorpyrifos in blood and urine following controlled oral exposures and reported a very rapid initial metabolism of chlorpyrifos (half-life close to 1 h) but much slower elimination of metabolites (measured by appearance in the urine; half-life of 24–27 h) (Nolan et al., 1984; Timchalk et al., 2002). For diazinon, urinary dialkyl phosphate levels were highest between 2 and 12 h after exposure (Garfitt et al., 2002). However since chlorpyrifos is highly lipophilic, there is likely a second compartment whereby a portion of the chlorpyrifos dose which partitions into body fat and/or binds to plasma proteins may be eliminated more slowly and can reach a steady-state level. This may be especially relevant to levels of chlorpyrifos and diazinon in the plasma of CHAMACOS mothers and children who may be chronically exposed to low levels of organophosphate pesticides (Eaton et al., 2008).

Although tissue distribution of chlorpyrifos in humans is not well characterized (Eaton et al., 2008), the tissue distribution of chlorpyrifos in pregnant rats was described in maternal blood, liver, brain, placenta, and the fetus (Abdel-Rahman et al., 2002) providing evidence that indeed maternal exposure and PON1 activities or status could affect the presence of organophosphates in the fetus. This is consistent with our data showing an inverse association between maternal PON1 activity and detectable levels of chlorpyrifos and diazinon in umbilical cord blood, which suggest that maternal PON1 may protect the fetus and result in a lower internal dose. PON1 arylesterase activity may also play an important role in the relationship between organophosphate pesticide levels in blood and urinary dialkyl phosphates. We only observed associations between diethyl phosphates in urine and organophosphate pesticides in blood after adjusting for arylesterase activity and including an interaction term (organophosphate detection × arylesterase). In contrast to our expectation that given the same level of organophosphate detection, we would observe increased diethyl phosphate levels in individuals with higher PON1 arylesterase, our data suggest that at similar levels of blood organophosphate pesticides, those with higher arylesterase may excrete lower levels of dialkyl phosphates. However, it should be noted that these models accounted only for a small portion of the total variability in dialkyl phosphates (r²=0.04).

Other factors that may contribute to urinary diethyl phosphate concentrations include activities of cytochrome P450’s, which are also involved in the organophosphate detoxification pathway and other parts of the oxidative stress network.

This study has several limitations. Mothers who gave birth to girls were more likely to have adequate blood specimens available for use in this study, suggesting a potential for participation bias. However, we did not identify any factors associated with participation rates in children. Furthermore, the association between mother’s participation and child sex may be spurious as biologically, it is unlikely that the sex of the child would affect whether or not blood was collected for a particular mother. Recent research suggests that urinary metabolites may reflect not only an individual’s contact with pesticide parent compounds, but possibly exposure to preformed metabolites present in food or the environment (Lu et al., 2005; Quirós-Alcalá et al., 2012; Weerasekera et al., 2009; Zhang et al., 2008) thus overestimating organophosphate pesticide exposure. This study is also limited by the relatively high limit of quantification for the method we used to measure chlorpyrifos and diazinon in CHAMACOS blood specimens. The available CDC method, which included pyrethoid target analytes, resulted in higher detection limits compared to Whyatt et al. (2003), who utilized a more sensitive analytical method with quantification limits close to 1 pg/mL. Finally, maternal urinary metabolites were measured during the postpartum period; we previously showed urinary dialkyl phosphates to be particularly high during this time (Bradman et al., 2005). Therefore, our findings on the associations of urinary diethyl phosphates and presence of organophosphates in blood in relation to PON1 may not be generalizable to other time periods (e.g., not postpartum).

Measurements of organophosphate pesticides in blood are sometimes considered a better biomarker for organophosphate exposure than urine because they may reflect internal dose more directly (Wessels et al., 2003). However, for many organophosphates that devolve to dialkyl phosphates, such as oxymethonemethyl, there are no published methods to measure the parent compounds in blood. Due to the constraints of the currently available analytical methods, we had limited power to detect chlorpyrifos and diazinon in blood. We chose to include detectable levels of chlorpyrifos and diazinon below the limit of quantification in our statistical analyses even though these concentrations have higher relative standard deviations. Overall, we observed similar results when analyses were repeated with values above the limit of quantification.
5. Conclusions

In summary, our study suggests that PON1 enzyme quantity modifies the fetal dose of chlorpyrifos and diazinon in women exposed to these pesticides. Given that exposures to these organophosphate pesticides has been associated with adverse health effects (Eskenazi et al., 2007; Marks et al., 2010; Whyatt et al., 2004), more research is needed to confirm our findings.

Acknowledgments

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