Determinants of Organophosphorus Pesticide Urinary Metabolite Levels in 42-Month-Old Children Participating in the CHAMACOS Birth Cohort Study

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1. ABSTRACT

Young children living in agricultural communities may be exposed to organophosphorus (OP) pesticides through several pathways including drift from applications near their homes, para-occupational exposures from their farmworker parents, and diet. We measured six OP dialkyl phosphate (DAP) metabolites (three dimethyl alkylphosphates (DMAP) and three diethyl alkylphosphates (DEAP)) in urine samples collected from 253 42-month-old children living in an agricultural community. We also collected information on diet and potential exposure determinants including parental occupation and exposure-related behaviors such as wearing work clothes and shoes into the home, residential and childcare proximity to agricultural fields and home pesticide use. We constructed separate bivariate and multivariate models for child DMAP and DEAP metabolite levels. Results from multivariate models showed that both DMAP and DEAP metabolite levels were higher when urine samples were collected in the spring and summer versus the fall and winter months, albeit not significantly ($p<0.1$). Higher DMAP levels were significantly associated with having an agricultural worker living in the home ($p<0.01$) and urinary creatinine levels ($p<0.01$). Higher DEAP levels were associated with increasing daily servings of fruits and vegetables, albeit not significantly ($p<0.1$). Overall, our findings indicate that diet and take-home exposures are key determinants of OP pesticide exposure in this population.

2. INTRODUCTION

Concerns about children’s pesticide exposure resulted in the passage of the U.S. Food Quality Protection Act (FQPA) in 1996. The FQPA required the U.S. Environmental Protection Agency (U.S. EPA 2010) to set dietary food tolerances that account for non-dietary pesticide exposures (U.S. EPA 2015). The FQPA also required that the U.S. EPA consider the special susceptibility of children to pesticide exposure when assessing tolerances to better protect this sensitive population (U.S. EPA 2015). Young children are more vulnerable to pesticides because they have higher exposures per unit of body weight compared with adults (Aprea et al. 2000) and because their rapidly developing body systems may be disrupted by xenobiotic insults (Morgan et al. 2005; Moya et al. 2004) resulting in greater risk of neurodevelopmental health outcomes such as attention-deficit/hyperactivity disorder (ADHD), learning deficits, and impaired memory and motor function (Bouchard et al. 2010; Butler-Dawson et al. 2016; Marks et al. 2010; Ruckart et al. 2004). Postnatal OP pesticide exposure is also associated with decreased lung function (Raanan et al. 2016).

Children may be exposed to organophosphorus (OP) pesticides through several pathways. For example, several organic diet intervention studies indicate that dietary ingestion is an important route of exposure in children (Bradman et al. 2015; Curl et al. 2003; Hyland et al. 2019; Lu et al. 2006). Drift from pesticide applications near homes may also be an important exposure pathway. Studies conducted in Washington State reported decreasing urinary metabolites in children with increasing distance of their homes from pesticide application sites (Coronado et al. 2011; Lu et al. 2000), suggesting that residential proximity to agricultural pesticide use is an important determinant of exposure. Exposure to OP pesticides may also occur through para-occupational or “take-home” pathways. Several studies indicate that farmworkers can unintentionally carry pesticides into their homes from residues on their clothing, skin, shoes, or hair (Curl et al. 2002; Fenske et al. 2002; McCauley et al. 2001; Thompson et al. 2014). With pesticide residue transferred into the home, children are at greater risk of exposure to these chemicals. In a prior study of the 6-, 12- and 24-month-old children participating in the CHAMACOS (Center for the Health Assessment of Mothers and Children of Salinas) birth cohort study, Bradman et al. reported that important OP exposure determinants included child age, diet, and regional pesticide use (Bradman et al. 2011), consistent with other studies. In the present study, we evaluated determinants of OP pesticide exposure among children participating in the CHAMACOS cohort at 42-months old (N=253).

3. METHODS

3.1 Participants and Recruitment

CHAMACOS is a longitudinal birth cohort study investigating environmental exposures and the health of pregnant women and their children living in the Salinas Valley, Monterey County, California (Eskenazi et al. 2003). Between October 1999 and November 2000, 601 pregnant women were enrolled in the CHAMACOS birth cohort study, resulting in 538 live births. Eligible women were older than 18 years of age, less than 20 weeks gestation, Spanish-or English-speaking, eligible for Medi-Cal, receiving perinatal care at local community clinics, and planning to deliver at the county hospital in Salinas, California (Bradman et al. 2011). We collected urine samples from 253 participating children at 42-months of age. Written informed consent was obtained from all participants and the study was approved by the Committee for the Protection of Human Subjects at the University of California Berkeley.

3.2 Interviews and Home Assessments

Mothers were interviewed when the children were 42-months old. Interviews were conducted in Spanish or English by bilingual interviewers. Information collected included demographics, household enumeration, occupational status, whether work clothes or work shoes were worn in the home, daily servings of child fruit and vegetable consumption based on a modified food frequency questionnaire, and time spent in child care. Shortly after each interview, study staff conducted a home inspection. Among other information, study staff recorded the distance between the home and the nearest agricultural field. For 51% (129/253) of the participating children, study staff also recorded the distance between the participant's child care center and the nearest agricultural field.

3.3 Meteorological Data

We obtained meteorological data for the Salinas Valley region from the California Climate Data Archive (CalClim: California Climate Data Archive). Using these data we examined the effects of evapotranspiration, total precipitation, solar radiation, vapor pressure, air temperature, relative humidity, dew point, average wind speed, wind run, and average soil temperature on DAP metabolite levels. We also examined season of urine collection (Spring/Summer versus Winter/Fall) as a potential determinant of exposure since most agricultural pesticide use in Monterey County occurs during the spring and summer (DPR 2004).

3.4 Child Urine Sample Collection

Random spot urine samples were collected from each child at 42-months of age (N=253). Samples were collected in a sterile urine cup. If the child could not provide a urine sample during the visit, a spot sample was collected on the next day at the child's home. Samples were collected between October 2003 and May 2005. Specimens were aliquoted into precleaned glass containers with Teflon-lined caps, bar-coded, and stored at –80°C until shipment. Samples were shipped on dry ice to the CDC and stored at –70°C until analysis (Bradman et al. 2005).

3.5 Laboratory Analysis

Urine samples were analyzed by the Centers for Disease Control and Prevention (CDC) in Atlanta, Georgia. We measured six non-specific dialkyl phosphate (DAP) metabolites of organophosphorus (OP) pesticides. Three measured metabolites were classified as dimethyl alkylphosphate (DMAP) metabolites: dimethylphosphate (DMP), dimethyl-thiophosphate (DMTP), and dimethyl-dithiophosphate (DMDTP). Three other measured metabolites were classified as diethyl alkylphosphate (DEAP) metabolites: diethylphosphate (DEP), diethyl-thiophosphate (DETP), and diethyl-dithiophosphate (DEDTP). We analyzed levels of DAP metabolites by isotope dilution gas chromatography-tandem mass spectrometry (GC-MS/MS) (Bravo et al. 2004). We measured DAP metabolites, rather than pesticide-specific metabolites, because there are no laboratory methods to measure specific metabolites of several OP pesticides used in the study area, such as oxydemeton-methyl. Approximately 80% of the OP pesticides used in the Salinas Valley devolve to a DAP metabolite. OP pesticide usage in Monterey County in 2004 and nonspecific DAP metabolites are presented in Supplementary Material (SM), Table S1. Creatinine concentrations were determined using a commercially available diagnostic enzyme method (Vitro CREA slides, Ortho Clinical Diagnostics, Raritan, NJ).

Laboratory quality control included repeat analysis of three in-house urine pools enriched with known amounts of pesticide residues whose target values and confidence limits were previously determined. Further details of the laboratory analytical methods are described elsewhere (Bradman et al. 2011). The limits of detection (LODs) were calculated at 0.1 µg/L for DMDTP, DEP, and DEDTP, 0.2 µg/L for DETP, and 0.5 µg/L for DMP and DMTP. Metabolite levels below the LOD were randomly imputed based on a log-normal probability distribution. Because individual pesticides can devolve to more than one DAP metabolite, we conducted separate summations for DMAP and DEAP metabolites on a molar basis to reflect total DMAP or total DEAP metabolite levels. Frozen field blanks, prepared earlier by CDC, were defrosted, re-packaged in the field in a manner identical to collection procedures for actual samples, then shipped blinded to CDC. The mean levels of individual DAP metabolites in blank field samples were <2 µg/L (Bradman et al. 2011). The median values of the DAP metabolites in the field blanks were all below the detection limit.

3.6 Data Analysis

Data analyses were performed in Stata Version 15.1 (StataCorp LP, College Station, TX). Additional calculations were performed in Microsoft Excel (Microsoft Office 365 ProPlus, Redmond, WA). We first summarized demographic characteristics of participating families. We then computed descriptive statistics and percentiles for individual and total DMAP and DEAP metabolites at the 42-month sampling time point. Urinary metabolite levels were $\log_{10}$-transformed for further statistical analysis. We used Pearson correlations and ANOVA to assess bivariate associations between the $\log_{10}$-transformed metabolite levels and potential exposure determinants. We selected a list of *a priori* potential exposure determinants including sex, daily consumption of fruits and vegetables, season, home distance to nearest agricultural field, wearing work clothes or shoes into the home, mother's occupational status, and urinary creatinine levels.

We examined *post facto* additional determinants which may be related to drift of pesticides from fields including daily rainfall, air temperature, wind speed, time spent in child care, and proximity of child care to agricultural fields (Harnly et al. 2005; Lambert et al. 2005; Lu et al. 2000, 2004; McCauley et al. 2001). For statistical analysis, we present results that were not adjusted for urine dilution. Analyses were repeated with creatinine-adjusted values to confirm our bivariate results.

We then constructed multivariate regression models with $\log_{10}$-transformed DMAP and DEAP metabolite levels and potential exposure determinants. Initial models were constructed with the *a priori* variables. *Post facto* determinants found to have significant ($p<0.1$) bivariate relationships with either DMAP or DEAP metabolite levels were added to the appropriate *post facto* model. For ease of interpretation, we converted beta (β) coefficients and their 95% confidence intervals into measurements of percent change in DMAP or DEAP urinary metabolite levels associated with a one-unit increase in the predictor variable using the formula: percent change = 100 x (antilog(β)-1) (Castorina et al. 2017; Ruotsalainen et al. 1993).

4. RESULTS

4.1 Demographic Characteristics

Table 1 presents the demographic characteristics of participating CHAMACOS mothers (N=253) when their children were 42-months old. At the time of the 42-month interview, 18% of mothers were employed in either fieldwork or agricultural work. Seventy-two percent of mothers shared a home with at least one agricultural worker. Sixty-six percent of mothers were living at or below the U.S. federal poverty threshold. Ninety-three percent of mothers responded that Spanish was the primary language spoken at home and only 19% of mothers had completed a high school education (through 12th grade) at the time of their baseline interview. The average age (SD) of participating children at the 42-month sampling time point was 43.2 (2.2) months.

4.2 Urinary Metabolite Concentration Data

Table 2 presents descriptive statistics for the total DMAP, DEAP, and DAP molar concentrations at the 42-month sampling time point. Results presented in Table 2 are not adjusted for urine dilution. Measurements for DMAP metabolites were mainly comprised of DMTP and to a lesser extent, DMP (90.5% and 49.8% detection frequency, respectively). DMDTP was detected in only 32.4% of samples. Malathion, oxydemeton-methyl, and dimethoate were the most commonly used dimethyl OP pesticides used in Monterey County in 2004 (SM, Table S1). Measurements for DEAP were mainly comprised of DEP and DETP (44.3% and 34.0% detection frequency, respectively at 42-months). DEDTP was detected in 0.0% of the samples. Diazinon was the most common diethyl OP pesticide used in Monterey County in 2004 and devolves to both DEP and DETP, but not DEDTP (SM, Table S1). Descriptive urinary metabolite levels adjusted for creatinine are presented in SM, Table 2.

4.3 Determinants of DMAP Metabolites

The bivariate relationships between potential exposure determinants and $\log_{10}$-transformed DMAP metabolite levels at 42-months are shown in SM, Table S3. DMAP metabolites were shown to be strongly and positively associated with creatinine levels (Pearson $r = 0.32$ ($p<0.01$)). At 42-months DMAP metabolites were higher in children that shared a home with an agricultural worker versus those that did not share a home with an agricultural worker (geometric mean = 70.6 vs. 42.4 nmol/L ($p<0.05$)). The season of urine sample collection was associated with DMAP metabolite levels, albeit not significantly ($p<0.1$). The DMAP metabolite levels were higher when urine sample collection occurred in the spring and summer versus the fall and winter (geometric mean = 72.8 vs. 51.2 nmol/L ($p<0.1$)). Lastly, the distance between the location of child care and the nearest agricultural field were also shown to be associated, albeit not significantly ($p<0.1$). Children whose primary child care location was less than 60 meters (200 feet) from the closest agricultural field had higher DMAP metabolite levels than children whose primary child care location was farther than 60 meters (200 feet) (geometric mean = 130.0 vs. 61.2 nmol/L ($p<0.1$)). In the bivariate analysis, DMAP metabolite levels were not found to be significant with other factors such as daily servings of fruits and vegetables, total precipitation, nor mother's occupational status.

Table 3 presents results from the multivariate regression model for log10-transformed DMAP metabolite levels. Urinary DMAP metabolite levels were 93.1% (95% CI: 16.9, 219.0) higher when an agricultural worker lived in the home compared to when no agricultural worker lived in the home ($p<0.01$). Additionally, DMAP metabolite levels were 45.5% (95% CI: -3.0, 118.4) higher when urine sample collection occurred during the spring and summer versus the fall and winter months, albeit not significantly ($p<0.1$). Urinary creatinine levels were significantly associated with increasing DMAP metabolite levels. For every unit increase in urinary creatinine level (mg/dL), there was a 1.7% (95% CI: 1.0, 2.3) increase in DMAP metabolite levels ($p<0.01$). No other determinant variables reached statistical significance in the DMAP model. In constructing the multivariate regression model for DMAP metabolites, we did not include the distance between child care and agricultural field because ~40 percent of observations are missing for this variable.

4.4 Determinants of DEAP Metabolites

The bivariate relationships between potential exposure determinants and $\log_{10}$-transformed DEAP metabolite levels at 42-months are shown in the SM, Table S4. Urinary DEAP metabolite levels were significantly higher in children for which urine samples were collected in the spring and summer versus the fall and winter (geometric mean = 4.3 vs. 1.3 nmol/L ($p<0.01$)). DEAP metabolites were positively associated with daily servings of fruits and vegetables (Pearson r = 0.13 ($p<0.05$)), mean average air temperature (Pearson r = 0.14 ($p<0.05$)), and mean average wind speed (Pearson r = 0.15 ($p<0.05$)). DEAP metabolite levels were also found to be higher in girls versus boys, albeit not significantly (geometric mean = 3.3 vs. 1.8 nmol/L ($p<0.1$)). Urinary DEAP metabolite levels were not found to be significant with other factors such as total precipitation, the presence of an agricultural worker living in the home, nor urinary creatinine levels.

Table 3 presents results from the multivariate regression model for $\log_{10}$-transformed DEAP metabolites. While no determinant variables reached statistical significance in the DEAP metabolite model, we found that DEAP metabolites were higher when more daily servings of fruits and vegetables were consumed and when samples were collected in the spring and summer months. Per unit increase in daily servings of fruits and vegetables, there was a 10.3% (95% CI: -1.0, 22.8) increase in DEAP metabolite levels ($p<0.1$). Additionally, DEAP metabolite levels increased by 150.8% (95% CI: -0.6, 532.8) when urine samples were collected in the spring and summer versus the fall and winter months ($p<0.1$).

5. DISCUSSION

We investigated the relationships between potential exposure determinants of OP pesticides and urinary DAP metabolites in 253 42-month-old children. Consistent with previous studies, urinary DMAP metabolites in this population were higher than DEAP metabolite levels (Barr et al. 2004; Bradman et al. 2005, 2007). We found that both DMAP and DEAP metabolite levels were associated with the season of urine collection, with higher levels in children with urine samples collected during the spring and summer versus the fall and winter months. The greater exposure to OP pesticides during the spring and summer is consistent with pesticide use data showing that the majority of agricultural OP pesticide use in Monterey County occurred between March and September in 2004 (DPR 2004). In addition, OP pesticides are more likely to volatilize in the warmer temperatures that occur during the spring and summer.

Airborne particles pose an inhalation hazard to those in proximity to pesticide application sites. This is especially true for children who are more vulnerable than adults to pesticide exposure due to their increased air intake relative to their body weight (Moya et al. 2004). Increased wind speed can also contribute to pesticide drift because higher wind speeds can carry airborne particles for farther distances from pesticide application sites, potentially exposing more individuals beyond the immediate vicinity to OP pesticides. In bivariate analyses, the effects of air temperature and wind speed were consistently and significantly associated with DEAP metabolites levels. However, the effects of air temperature and wind speed on DEAP metabolite levels did not remain statistically significant in the multivariate model.

DMAP metabolite levels in the children were found to be strongly and positively associated with the presence of an agricultural worker living in the home. This suggests that OP pesticides that devolve to DMAP metabolites could be brought home through the take-home exposure pathway. Upon entering the home, OP pesticide residue that had been carried on the farmworker’s body and garments could deposit elsewhere in the residence. Because of low moisture, lack of biological activity, and little to no exposure to sunlight, pesticides deposited in the residence could persist for longer periods of time compared with residues in fields (Simcox et al. 1995; Starr HG Jr et al. 1974). A recent review of the take-home pesticide exposure pathway in children concluded that children of farmworkers are exposed to pesticides at higher levels than children from non-agricultural backgrounds (Hyland and Laribi 2017). A study from Washington State found that urine samples from farmworker children had median pesticide concentrations five times higher than samples from non-farmworker children (Lu et al. 2000). Fenske et al. also conducted a study in Washington State that reported urinary OP metabolite levels three to six times higher in agricultural children than for the reference population (Fenske et al. 2000).

Children’s produce consumption, measured by daily servings of fruits and vegetables, was associated with urinary DEAP metabolite levels. These results confirm that diet remains an important exposure pathway for OP pesticides as children continue in their growth and development. Our conclusion regarding diet confirms the findings of other studies. Bradman et al. concluded that an organic diet was significantly associated with reduced urinary concentrations of nonspecific DMAP metabolites in children 3 to 6 years of age, living in California urban and agricultural communities (Bradman et al. 2015). Another study by Curl et al. assessed OP pesticide exposure from diet by comparing urinary DAP metabolite levels between children who consumed either an organic or conventional diet. Participants for this study were between 2 and 5 years of age and resided in a suburban setting in Washington State. Since this study was conducted in a more suburban area of Washington State, researchers were able to control for the effects of other exposure determinants related to proximal agricultural activity. Curl et al. concluded that children who consumed primarily organic diets had significantly lower levels of total DMAP metabolites in their urine than did children with conventional diets (Curl et al. 2003).

Children are more physiologically vulnerable to pesticide exposure than adults because they have a lessened ability to metabolize and eliminate chemicals. For example, children below the age of seven have a lower capacity to detoxify OP pesticides in their bodies compared with older children and adults due to significantly lower levels of the enzyme paraoxonase 1 (PON1) (Eskenazi et al. 2010; Roberts and Karr 2012). Additional research is needed to better understand pesticide exposure, absorption and detoxification in children. This is of particular interest among children living in agricultural communities as they likely experience higher exposure to pesticides than children that live in non-agricultural settings. With increased susceptibility to these hazardous exposures, recent policy proposals have sought to define farmworker children as a vulnerable population that require additional protection on the grounds of environmental justice (U.S. EPA 2010).

This study has several limitations. When multiple OP pesticides are applied to the same tract of land, measurement of the non-specific DAP metabolites does not provide information on the identity of the specific parent OP compound (Needham 2005). Many OP pesticides used in the Salinas Valley vary widely in usage, environmental persistence, and physical-chemical properties. This lack of consistency in properties across OP pesticides adds variability to biomonitoring measurements and potentially biases statistical models towards null results. Additionally, current pesticide use patterns have changed over time and may not reflect pesticide use as it was in Monterey County in 2004. However, many of the pesticides used in Monterey County in 2004 are still in widespread use in other regions of California. The contributions of preformed DAPs in the environment signify another limitation in our study. DAPs in urine may reflect exposure to preformed DAPs in the environment or food rather than exposure to the parent OP compound and thus overestimate OP pesticide exposure (Lu et al. 2005; Weerasekera et al. 2009). Furthermore, maternal reports of the child's fruit and vegetable intake from the modified food frequency questionnaire were not calibrated to specific portion sizes. This may have introduced variability into the intake measurements.

Using additional data from the CHAMACOS study cohort, we will extend this analysis to perform a longitudinal assessment of the predictors of urinary DAP metabolite levels in these children at 42- and 60-months. Our future research approaches will include multivariate longitudinal models using 42- and 60-month data from this study as well as 6-, 12-, and 24-month data from the preceding study (Bradman et al. 2011). With this information, we could further refine our understanding of exposure determinants of OP pesticides for children at multiple points of growth and development. We recommend that further research also be conducted on the potential behavioral determinants of OP pesticide exposure including but not limited to physical activity and exertion. These findings and others could be used to modify existing interventions to reduce children's exposure to OP pesticides.

6. CONCLUSION

In this study, we found that 42-month-old children living in an agricultural setting are likely exposed to OP pesticides through diet and take-home exposures. Given the health benefits of fresh fruit and vegetable consumption, we do not suggest that children limit intake of these foods but encourage the thorough washing of all produce prior to consumption. Postnatal OP pesticide exposures have been associated with poorer child neurodevelopmental outcomes

(Bouchard et al. 2010; Butler-Dawson et al. 2016; Marks et al. 2010; Ruckart et al. 2004). Additional research is needed to clarify the potential exposure determinants pathways and to inform policy to reduce children's exposure to OP pesticides.

Table 1. Demographic characteristics of participating families (N=253).

Characteristic	**n**	**(%)**
Mother's age (mean (SD) = 26.1 (5.3) years)[a]		
18-24	106	(41.9)
25-29	87	(34.4)
30-35	38	(15.0)
35+	22	(8.7)
Number of years residing in the US at baseline[b]		
Less than 5 years	124	(49.0)
5 or more years	129	(51.0)
Mother's country of birth		
US	27	(10.7)
Mexico	224	(88.5)
Other	2	(0.8)
Language spoken at home		
Spanish mostly	234	(92.5)
Both equally	9	(3.6)
English mostly	6	(2.4)
Other	4	(1.6)
Mother's level of education at baseline[b]		
Some elementary school (grades 1 to 6)	110	(43.5)
Grades 7 to 12	94	(37.2)
High school graduate	49	(19.4)
Baseline poverty level categories[c]		
At or below poverty threshold	158	(66.4)
Above poverty threshold but below 200% of poverty	71	(29.8)
200% poverty threshold or greater	9	(3.8)
Number of people living in household at 42m		
2 to 3	37	(10.8)
4 to 5	116	(33.7)
6 to 9	153	(44.5)
10+	38	(11.1)
Mother's current work status at 42m		
Not working	154	(60.9)
Fieldwork	36	(14.2)
Ag work	9	(3.6)
Other work	54	(21.3)
Number of agricultural workers currently living in home at 42m		
0	72	(28.5)
1 to 3	155	(61.3)
4 to 9	26	(10.3)

[a] At time of child's birth.
[b] During pregnancy (i.e. at time of entry into the CHAMACOS project).
[c] Poverty thresholds were calculated using the U.S. Department of Health and Human Services' level for 2000.

Table 2. Urinary DAP metabolite levels at 42 months (nmol/L)[a]

	Detection Frequency (%)	**Geometric Mean**	**Percentiles**				
42 months (N=253)			25	50	75	90	Max
DMP	49.8	5.3	<LOD	<LOD	55.6	120.5	988.8
DMTP	90.5	44.6	16.5	41.9	135.7	312.5	3,723.2
DMDTP	32.4	<LOD	<LOD	<LOD	10.1	31.3	434.2
Total DMAP	91.7	62.3	21.4	61.3	198.7	459.3	4,581.9
DEP	44.3	0.6	<LOD	<LOD	15.1	53.8	278.2
DETP	34.0	<LOD	<LOD	<LOD	9.1	24.5	122.5
DEDTP	0.0	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Total DEAP	58.5	2.5	<LOD	4.6	22.3	88.6	351.2
Total DAP	94.1	76.0	25.1	77.7	241.6	546.4	4,751.2

[a] Detection limits from multiple batches of urinary metabolite data: DMP = 0.5 μg/L; DMTP = 0.5 μg/L; DMDTP = 0.1 μg/L; DEP = 0.1 μg/L; DETP = 0.2 μg/L; DEDTP = 0.1 μg/L.

Table 3. Percent change in urinary DMAP and DEAP metabolite levels ($\log_{10}$-transformed) from *post facto* multivariate regression models of exposure predictors at 42-months (N = 251).

	DMAP at 42 months, %[a] (95% CI)
Child's sex (Girl = 1)	1.1 (-31.4, 49.0)
Daily servings of fruits and vegetables	3.0 (-3.0, 9.5)
Spring/summer (*vs.* winter/fall)	45.5* (-3.0, 118.4)
Distance between home and fields (>60 m = 1)	22.7 (-35.8, 134.4)
Mother's occupational status	45.3 (-15.3, 149.0)
Farmworkers wearing work clothes inside (Y/N)	33.2 (-37.4, 183.4)
Farmworkers wearing work shoes inside (Y/N)	13.1 (-29.3, 81.2)
Creatinine (mg/dL)	1.7*** (1.0, 2.3)
Agricultural worker living in the home (Y/N)	93.1*** (16.9, 219.0)
	DEAP at 42 months, %[a] (95% CI)
Child's sex (Girl = 1)	69.1 (-15.9, 240.0)
Daily servings of fruits and vegetables	10.3* (-1.0, 22.8)
Spring/summer (*vs.* winter/fall)	150.8* (-0.6, 532.8)
Distance between home and fields (>60 m = 1)	13.5 (-64.5, 263.0)
Mother's occupational status	18.1 (-55.6, 213.6)
Farmworkers wearing work clothes inside (Y/N)	-36.9 (-83.7, 144.2)
Farmworkers wearing work shoes inside (Y/N)	2.7 (-53.4, 126.5)
Creatinine (mg/dL)	0.4 (-0.6, 1.6)
Mean average air temperature (F)[b]	2.0 (-6.3, 11.1)
Mean average wind speed (mph)[b]	11.8 (-16.0, 48.8)

* p-value < 0.1; ** p-value < 0.05; *** p-value < 0.01 (For the multivariate model).

[a] Percent change in DMAP or DEAP metabolite levels associated with 1-unit increase or a yes/no difference in exposure characteristic. Calculated with formula: 100 x (antilog(β)-1).

[b] Meteorological data sourced from California Climate Data Archive. Data reflects meteorological conditions measured in Salinas, CA during the 1 week period prior to urine collection.

Table S1. 2004 organophosphorus (OP) pesticide usage in Monterey County[a,b,c].

Pesticide[d]	**Kilograms applied in 2004**	**% applied in 2004**	**Metabolites**
Azinphos-methyl	193	0.2	DMP, DMTP, DMDTP
Dimethoate	20,042	19.0	DMP, DMTP, DMDTP
Malathion	39,636	37.5	DMP, DMTP, DMDTP
Methidathion	5,363	5.1	DMP, DMTP, DMDTP
Methyl parathion	47	0.0	DMP, DMTP
Naled	11,174	10.6	DMP
Oxydemeton-methyl	29,140	27.6	DMP, DMTP
Phosmet	0	0.0	DMP, DMTP, DMDTP
Total dimethyls	105,595	100.0	
Chlorpyrifos	27,622	24.8	DEP, DETP
Diazinon	78,202	70.2	DEP, DETP
Disulfoton	5,605	5.0	DEP, DETP, DEDTP
Total diethyls	111,428	100.0	DMP, DMTP, DMDTP

[a] Pesticide use data sourced from California Pesticide Information Portal (CalPIP) managed by California Department of Pesticide Regulation (DPR 2004). Accessed 26 Apr 2019.

[b] Includes agricultural, landscape maintenance, structural pest control, and right-of-way pesticide usage (DPR 2004).

[c] Pesticide usage is reported in kilograms of active ingredient.

[d] OP pesticides that do not metabolize to dialkyl phosphate compounds (e.g. bensulide, acephate, etc.) are not listed.

Table S2. Creatinine-adjusted urinary DAP metabolite levels at 42-months (nmol/gcre)[a].

	Detection Frequency (%)	**Geometric Mean**	**Percentiles**				
42 months (N=253)			25	50	75	90	Max
DMP	49.8	10.1	<LOD	12.0	102.3	197.8	1,142.2
DMTP	90.5	84.9	30.9	98.6	216.4	566.7	7,182.8
DMDTP	32.4	<LOD	<LOD	<LOD	12.1	47.8	536.9
Total DMAP	91.7	118.6	41.0	139.8	332.3	707.1	8,694.3
DEP	44.3	<LOD	<LOD	<LOD	32.5	95.2	263.7
DETP	34.0	<LOD	<LOD	<LOD	12.5	40.2	526.2
DEDTP	0.0	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Total DEAP	58.5	4.7	<LOD	11.2	47.9	152.8	545.9
Total DAP	94.1	144.7	49.1	170.7	422.5	866	9,240.2

[a] Detection limits from multiple batches of urinary metabolite data: DMP = 0.5 μg/L; DMTP = 0.5 μg/L; DMDTP = 0.1 μg/L; DEP = 0.1 μg/L; DETP = 0.2 μg/L; DEDTP = 0.1 μg/L.

Table S3. Summary of geometric mean DMAP urinary metabolite levels (nmol/L) at 42-months by exposure determinants[a].

		n (%)	**GM (95% CI) or Correlation[b]**
Child's Sex			
	Boy	119 (47.0)	62.9 (46.0, 86.0)
	Girl	134 (53.0)	61.8 (47.2, 80.9)
Daily servings of fruits and vegetables			
	Pearson r	253 (100)	0.062
Season of urine collection			
	Winter/Fall	112 (44.3)	51.2 (37.6, 69.8)
	Spring/Summer	141 (55.7)	72.8* (55.5, 95.4)
Total precipitation			
	Pearson r	253 (100)	0.066
Mean average air temperature (F)			
	Pearson r	253 (100)	-0.015
Mean average wind speed (mph)			
	Pearson r	253 (100)	0.06
Distance between home and fields			
	≤ 60 m (200 ft)	27 (10.8)	61.9 (35.8, 107.0)
	> 60 m (200 ft)	224 (89.2)	62.8 (50.3, 78.3)
Agricultural worker living in home			
	No	62 (24.5)	42.4 (28.5, 63.0)
	Yes	191 (75.5)	70.6** (55.7, 89.4)
Mom currently works in agriculture			
	Works in agriculture	45 (17.8)	51.1 (31.9, 82.0)
	Other job or not working	208 (82.2)	65.0 (51.8, 81.6)
Farmworkers wearing work clothes inside			
	No	20 (7.9)	51.1 (31.9, 82.0)
	Yes	233 (92.1)	65.0 (51.8, 81.6)
Farmworkers wearing work shoes inside			
	No	98 (38.7)	64.5 (45.7, 91.2)
	Yes	155 (61.3)	60.9 (47.3, 78.5)
Child spends > 15 hrs/wk in child care			
	No	124 (49.4)	56.9 (42.4, 76.4)
	Yes	127 (50.6)	67.1 (50.3, 89.4)
Distance between child care and ag field			
	≤ 60 m (200 ft)	18 (14.0)	130.0* (58.6, 288.4)
	> 60 m (200 ft)	111 (86.0)	61.2 (45.1, 83.0)
Creatinine levels			
	Pearson r	253 (100)	0.316***

* p-value < 0.1; ** p-value < 0.05; *** p-value < 0.01 (For the bivariate analyses).

[a] P-values are from Pearson correlations or ANOVA of log transformed DMAP metabolite levels.

[b] Geometric means and 95% confidence intervals except when Pearson coefficient (r) is presented.

Table S4. Summary of geometric mean DEAP urinary metabolite levels (nmol/L) at 42-months by exposure determinants[a].

		n (%)	**GM (95% CI) or Correlation[b]**
Child's Sex			
	Boy	119 (47.0)	1.8 (1.1, 3.1)
	Girl	134 (53.0)	3.3* (2.1, 5.2)
Daily servings of fruits and vegetables			
	Pearson r	253 (100)	0.1304**
Season of urine collection			
	Winter/Fall	112 (44.3)	1.3 (0.7, 2.1)
	Spring/Summer	141 (55.7)	4.3*** (2.7, 6.7)
Total precipitation			
	Pearson r	253 (100)	-0.068
Mean average air temperature (F)			
	Pearson r	253 (100)	0.142**
Mean average wind speed (mph)			
	Pearson r	253 (100)	0.155**
Distance between home and fields			
	≤ 60 m (200 ft)	27 (10.8)	1.9 (0.6, 6.6)
	> 60 m (200 ft)	224 (89.2)	2.6 (1.8, 3.7)
Agricultural worker living in home			
	No	62 (24.5)	1.8 (0.9, 3.7)
	Yes	191 (75.5)	2.8 (1.8, 4.1)
Mom currently works in agriculture			
	Works in agriculture	45 (17.8)	3.0 (1.4, 6.4)
	Other job or not working	208 (82.2)	2.4 (1.6, 3.5)
Farmworkers wearing work clothes inside			
	No	20 (7.9)	3.8 (0.9, 15.6)
	Yes	233 (92.1)	2.4 (1.7, 3.4)
Farmworkers wearing work shoes inside			
	No	98 (38.7)	3.0 (1.8, 5.2)
	Yes	155 (61.3)	2.2 (1.4, 3.5)
Child spends > 15 hrs/wk in child care			
	No	124 (49.4)	2.2 (1.3,3.8)
	Yes	127 (50.6)	2.8 (1.7, 4.4)
Distance between child care and ag field			
	≤ 60 m (200 ft)	18 (14.0)	4.1 (1.4, 12.2)
	> 60 m (200 ft)	111 (86.0)	2.6 (1.5, 4.4)
Creatinine levels			
	Pearson r	253 (100)	0.032

* p-value < 0.1; ** p-value < 0.05; *** p-value < 0.01 (For the bivariate analyses).

[a] P-values are from Pearson correlations or ANOVA of log transformed DEAP metabolite levels.

[b] Geometric means and 95% confidence intervals except when Pearson coefficient (r) is presented.

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