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# Organophosphate pesticide dose estimation from spot and 24-hr urine samples collected from children in an agricultural community

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## Abstract

**Background:** Spot urine samples are often used to assess exposure to organophosphate (OP) pesticides in place of "gold standard" 24-hr samples, which are cumbersome to collect. Assessment of non-persistent chemicals using spot urine samples may result in exposure misclassification that could bias epidemiological analyses towards the null. Few studies have examined the validity of measurements of urinary metabolites in spot samples to estimate daily OP dose or the potential implications of reliance on spot samples for risk assessments.

**Objective:** Examine the validity of using first morning void (FMV) and random non-FMV urine samples to estimate cumulative 24-hr OP pesticide dose among children living in an agricultural region.

**Methods:** We collected urine samples over 7 consecutive days, including two 24-hr samples, from 25 children living in an agricultural community. We used measurements of urinary

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dialkylphosphate (DAP) metabolites, data on nearby agricultural pesticide applications, and daily dietary intake data to estimate internal dose from exposure to a mixture of OP pesticides according to the U.S. Environmental Protection Agency Cumulative Risk Assessment guidelines. Dose estimates from volume- and creatinine-adjusted same-day FMV and non-FMV spot urine samples were compared to the "gold standard" estimates from 24-hr samples.

**Results:** Non-FMV samples had relatively weak ability to predict 24-hr dose ($R^2 = 0.09$–$0.38$ for total DAPs) and tended to underestimate the percentage of samples exceeding regulatory guidelines. Models with FMV samples or the average of an FMV and non-FMV sample were similarly predictive of 24-hr estimates ($R^2$ for DAPs = 0.40–0.68 and 0.40–0.80, respectively, depending on volume adjustment method).

**Conclusion:** Reliance on non-FMV samples for risk assessments may underestimate daily OP dose and the percentage of children with dose estimates exceeding regulatory guidelines. If 24-hr urine sample collection is infeasible, we recommend future studies prioritize the collection of FMV samples to most accurately characterize OP dose in children.

## 1. Introduction

Organophosphate (OP) pesticides are commonly used insecticides that inhibit acetylcholinesterase (AChE) enzyme function and have been associated with poorer neurodevelopment in children (Bouchard et al., 2010, 2011; Engel et al., 2011; Eskenazi et al., 2007; Marks et al., 2010; Rauh et al., 2011, 2006). Children are particularly susceptible to the adverse impacts of pesticides (Bradman et al., 2011; Eskenazi et al., 2007; Rauh and Margolis, 2016) and those living in agricultural areas may be exposed via multiple pathways, including diet, drinking water, residential use, drift from agricultural applications, and take-home exposures (Curl et al., 2002; Fenske et al., 2002; Harnly et al., 2009; Hyland and Laribi, 2017; Koch et al., 2002; Lambert et al., 2005; Lu et al., 2000, 2004; Simcox et al., 1995). Assessing exposure to OP pesticides is difficult due to their short biologic half-lives and rapid excretion from the body (Barr and Angerer, 2006; Barr, 2008). Dialkylphosphate (DAP) metabolites, the most commonly used biomarker to characterize OP exposure in epidemiologic studies (Kavvalakis and Tsatsakis, 2012), have biological half-lives of less than 30 min to >24 h, depending on the parent OP and route of exposure (Bouvier et al., 2006).

Measurements of metabolites or parent chemicals in 24-hr urine samples are considered the "gold standard" for assessing daily exposure to pesticides and other environmental chemicals that are excreted in urine (Lermen et al., 2019; Scher et al., 2007). However, factors such as cost and participant burden make it difficult to collect 24-hr samples (Barr et al., 2005). While collection of spot urine samples is a convenient alternative, research suggests that analysis of biomarkers with short half-lives, including DAPs, in spot samples may result in exposure misclassification due to higher inter- and intra-individual variability (Bradman et al., 2013; Calafat et al., 2015; Meeker et al., 2005). First morning void (FMV)

urine samples may reduce exposure misclassification, as they are more concentrated and reflect a longer period of accumulation (Bradman et al., 2013; Kissel et al., 2005). Few studies have assessed how well either random spot or FMV urine samples approximate internal pesticide dose estimated from 24-hr samples, information that is critical for risk assessment and pesticide regulation.

Estimating dose based on metabolite concentrations from spot samples also requires an accurate measure of urinary dilution and total daily urinary output volume (Harris et al., 2000). In adults, 24-hr urinary metabolite excretion has been estimated from spot urine samples by adjusting for creatinine excretion as an index of total daily urinary output volume (Barr et al., 2005; Castorina et al., 2003; Harris et al., 2000; Lermen et al., 2019). However, few studies have evaluated the validity of this approach in children. Due to likely differences in children's urinary creatinine excretion from factors including age, sex, muscle mass, body mass index (BMI), diet, and fluid intake (Barr et al., 2005; Boeniger et al., 1993; Mage et al., 2004), adjusting for creatinine to estimate toxicant doses in children may introduce unknown sources of variability (Bradman et al., 2013). Although not used as widely as creatinine correction, some evidence suggests that adjusting for specific gravity may be a more robust method to account for urinary output among children (Pearson et al., 2009; Wang et al., 2015).

The US Environmental Protection Agency (EPA) is mandated by the 1996 Food Quality Protection Act (FQPA) to review and establish health-based standards for pesticide residues in foods and examine the cumulative health effects of exposure to mixtures of pesticides that share a common mechanism of toxicity, with prioritization of pesticides that may pose the greatest risk, such as OPs (U.S. EPA. 2006). The U.S. EPA has selected the Relative Potency Factor (RPF) method to conduct hazard and dose-response assessments. RPFs are calculated as the ratio of the toxic potency of a given chemical, determined by the oral benchmark dose$_{10}$ (BMD$_{10}$) value based on a 10% brain cholinesterase inhibition, to that of an index chemical. Individual OP doses derived from index chemical toxicity equivalent doses can be summed to create cumulative OP dose equivalents (Castorina et al., 2003).

In this study, we measured DAP metabolites in spot and 24-hr void urine samples collected from 25 preschool-aged children over 7 consecutive days. The objective of this analysis was to evaluate the validity of using volume- and creatinine-adjusted FMV and non-FMV spot urine samples to estimate total 24-hr OP dose in children according to the 2006 US EPA Organophosphorus Cumulative Risk Assessment guidelines. The results of these analyses have implications for policy and risk assessments and could serve as a case study for other non-persistent toxicants measured in urine.

## 2. Methods

### 2.1. Study population

Subject recruitment and procedures have been described previously (Bradman et al., 2013). Briefly, we enrolled a convenience sample of 25 children (10 boys, 15 girls) recruited from clinics serving low-income families in the Salinas Valley, California. Eligible children were 3–6 years old, in good health with no history of diabetes or renal disease, toilet trained, and

free of enuresis, and had English- or Spanish-speaking mothers who were    18 years old. Sampling occurred in March and April 2004. The University of California at Berkeley Committee for the Protection of Human Subjects approved all study procedures and parents provided written informed consent.

## 2.2. Data collection

Each family participated in the study over 7 consecutive days. On the first day, study staff measured the participating child's height and weight, provided the supplies needed to collect urine samples, including specimen trays and jars, gloves, collection jars with blank labels, a small refrigerator, and two 24-hr sampling record forms, and instructed the parents and child on how to collect, record, and store samples. Urine voids were collected either directly into a collection jar or into a sterile pre-cleaned specimen tray placed over the toilet, which was then transferred by parents into the collection jar.

Fig. 1 shows the timing of study activities. On spot-sampling days (1, 3, 4, 6, and 7), families collected a single void at their convenience, recording the time of collection on the jar labels and identifying the sample as an FMV or non-FMV spot sample. On 24-hr sampling days (2 and 5), families were instructed to collect all urine voids from the 24-hr period as separate specimens, including the child's FMV, all daytime and evening spot voids, and the FMV of the following day (i.e., study days 3 and 6), if it occurred within the 24-hr sampling period. Participants were instructed to record the timing of all voids, including missed voids, on the 24-hr sampling record form. We limited the current analyses to samples collected on 24-hr sampling days (referred to henceforth as 24-hr composites or same-day FMV and non-FMV samples).

Research staff reviewed the 24-hr sampling record with the parents to ensure accuracy and completeness. Urine samples were stored in the sample refrigerator until daily collection by research staff. Trained, bilingual study staff administered daily questionnaires that assessed the child's exposure to pesticides, including questions regarding dietary intake of fruits, vegetables, and juices; time spent indoors/outdoors; parental occupational exposures; and residential pesticide use over the previous 24-hr period.

## 2.3. Sample processing and analysis

Study staff processed the samples at the study field office, recording the weight (grams) and volume (milliliters). On 24-hr sampling days, staff were instructed to select the first FMV sample plus one to three randomly selected additional spot samples for individual analysis. All remaining voids from the sampling period were pooled prior to analysis. The total volume of the 24-hr composite sample was based on the volume of the individually analyzed samples plus the volume of all samples that were included in the pooled sample. The DAP concentrations were based on volume-weighted averages of concentrations in the individually analyzed samples plus the pooled sample. Samples were stored at −80 °C until shipment on dry ice to the Centers for Disease Control and Prevention for analysis in August and September 2004.

Laboratory methods and quality control procedures have previously been described in detail (Bravo et al., 2004) and are available in the Supplementary Materials. Limits of detection

(LODs) were 0.2 μg/L for all diethyls (DEs), 0.5 μg/L for dimethylphosphate (DMP), 0.4 μg/L for dimethylthiophosphate (DMTP), and 0.1 μg/L for dimethyldithiophosphate (DMDTP). Values below the LOD were assigned a value of LOD/ 2 (Hornung and Reed, 1990). Total dimethyl (DM), total DE, and total DAP concentrations were calculated within each sample by summing molar concentrations. We computed metabolite levels in 24-hr samples using the volume-weighted average of concentrations in all samples collected in that 24-hr sampling period (which included the FMV sample from the following day for 9 "participant-days" in which the FMV on the mornings of study days 3 and 6 occurred within the 24-hr sampling period).

### 2.4. Data analysis

Statistical analyses were performed using Stata 14 for Windows (StataCorp LP, College Station, TX). We characterized the mixture of OPs that participants were potentially exposed to based on: 1) nearby pesticide applications, and 2) diet (described in detail below).

**Pesticide use data:** In California, all agricultural pesticide use, including crop, active ingredient, date, pounds applied, and location of use within one square mile (1.6 × 1.6 km) sections defined by the Public Lands Survey System (PLSS) are recorded in pesticide use reports (PUR) by the California Department of Pesticide Regulation (DPR; Sacramento, CA). We used the latitude and longitude of the participant's home, geocoded from their street address, to map pesticide applications. We considered pesticide use within three kilometers of the home in the six months prior to each of the two 24-hr urine sampling days for each study participant, as these are within the range of distances and time periods that have been mostly strongly associated with OP concentrations in samples from this region (Harnly et al., 2009). We included 11 OPs that devolve into DAPs that are used in the Salinas Valley, which is representative of the most commonly used OPs nationally in the same time period (Atwood and Paisley-Jones, 2016). These 11 OPs include eight DM (azinphos-methyl, dimethoate, malathion, methidathion, methyl parathion, naled, oxydemeton-methyl, phosmet) and three DE (chlorpyrifos, diazinon, disulfoton) pesticides. All estimates were adjusted for the proportion of time the residence was downwind of each pesticide application (Nuckols et al., 2008).

**Dietary exposure assessment.—**At each study visit, study staff asked parents to report (yes/no) whether their child had consumed fresh fruits or vegetables from a 21-item list since the previous visit. Parents were also asked to report their child's consumption of any fruits or vegetables that were not on the list; canned, jarred, or frozen fruits and vegetables; and orange, apple, or other 100% fruit juice (Table S1).

Each year since 1991, the United States Department of Agriculture (USDA) Pesticide Data Program (PDP) has tested food commodities, including fruits and vegetables, for approximately 450 pesticides and their breakdown products (USDA, 2014). Using a food consumption-chemical residue (FCCR) approach described previously (Curl et al., 2015; MacIntosh et al., 2001), we used these publicly available data to calculate the mean concentration of the 11 OPs of interest (μg OP/g food) for each of the food items reported in our study.

To estimate dietary OP exposure, we multiplied the estimated concentration of the 11 OPs in each food item by the estimated intake of that food item. Per the US EPA Cumulative Organophosphorus Risk Assessment guidelines, we also included omethoate, the dimethoate oxon, in our dietary assessment, however it was not detected on any of the food commodities of interest in 2004. We made the assumption that each reported consumption of a particular fruit or vegetable was equal to one serving and used data for children ages 3–6 years from the 2003–2004 National Health and Nutrition Examination Survey (NHANES) "What we Eat in America" study (U.S. Department of Agriculture, 2006) linked to Food Commodity Intake Database (FCID) (U.S. Environmental Protection Agency – Office of Pesticide Programs) codes to estimate the weight of each food item. We estimated total exposure for each OP by summing estimated intake (μg) across all food items. We included reported food consumption that we were certain had preceded the urine void. For 24-hr samples, we considered the average exposure from all produce reported on the current day and previous day (i.e., produce consumed on days 1 and 2 for 24-hour sampling on day 2). For spot samples, we considered all produce reported on the day prior to sampling in order to ensure the produce was consumed before the sample was collected. We used USDA pesticide residue data from 2004 (the year of urine sample collection), when available. For commodities not analyzed in 2004, we used data from the most proximate year (Table S1). PDP samples with values <LOD were set to 0.

**Dose calculations:** We used the 2006 U.S. EPA OP Cumulative Risk Assessment guidelines to estimate total OP pesticide dose (U.S. EPA. 2006). These guidelines consider the effects of exposures to mixtures of pesticides and assume that OPs share a common mechanism of toxicity (i.e., the inhibition of cholinesterase activity). We used the approach outlined by Castorina et al. (2003) to calculate cumulative OP dose in units of chlorpyrifos equivalents (μg /kg/day) from nearby agricultural pesticide use, based on PUR data, using the following equation:

$$D_{cum} = \frac{\mu Mol_{Diethyl} \sum P_i MW_i RPF_i}{BW} + \frac{\mu Mol_{Dimethyl} \sum P_i MW_i RPF_i}{BW} \qquad (1)$$

where $D_{cum}$ is the cumulative dose equivalent (μg/kg/day), $\mu Mol_{Diethyl}$ is total micromoles of DE metabolites (DEP, DETP, DEDTP), $\mu Mol_{Dimethyl}$ is total micromoles of DM metabolites (DMP, DMTP, DMDTP) excreted over a 24-hr period, $P_i$ is the proportion of pesticide $i$ in the mixtures calculated from PUR data for each participant, $MW_i$ is the molecular weight of the $i$th pesticide in micrograms per micromole, and $RPF_i$ is the relative potency factor of the ith pesticide in the cumulative assessment group, and BW is the body weight of the child at the time of urine sample collection.

Using the FCCR approach outlined by Curl et al. (2015), we adapted Eq. (1) to estimate cumulative OP dose in units of chlorpyrifos equivalents (μg/kg/day) from diet. After calculating the intake of each of the 11 OPs in μg as described above, we estimated the proportion of each pesticide ($P_i$) by dividing the estimated dietary concentration of that pesticide by the total concentration of DMs or DEs estimated from diet.

Based on results in a similar population of 40 children ages 3–6 years living in Salinas Valley and Oakland, CA in which investigators observed that Salinas area children's total urinary DAPs decreased by about 40% following an organic diet intervention (Bradman et al., 2015), we estimated that diet contributed approximately 40% of overall OP exposure to the children in the current study. We assumed the additional 60% of pesticide exposure was derived from nearby pesticide use, represented by PUR data. Total OP exposure in chlorpyrifos equivalents were calculated for total DAPs, DMs, and DEs separately using Eq. (2):

$$Total\ dose\ (\mu g\ chlorpyrifos\ equivalents\ /\ kg\ /\ day) = (Dose_{PUR}*0.60) + (Dose_{Diet}*0.40) \tag{2}$$

Underlying our dose estimation models are the following assumptions, adapted from Castorina et al. (2003): (1) urinary concentrations represent steady state conditions over a 24-hr period; (2) 100% of absorbed OP pesticide dose is expressed as urinary diethyl and dimethyl phosphate metabolites; (3) the estimated proportion of pesticides from the PUR and dietary assessments is a reasonable surrogate for the mixture of OPs to which participants were exposed from all sources; and (4) OP metabolite concentrations are equivalent to internal doses on a molar basis.

**Volume adjustment:** In order to estimate the micromoles of each of the six DAPs excreted over a 24-hr period based on spot samples (Eq. (1)), we multiplied the observed urinary metabolite concentration in that spot sample by an estimate of the 24-hr urinary output volume (L/day) using four distinct volume-adjustment approaches. First, we used expected 24-hr child urinary output based on reference values (henceforth referred to as volume-adjusted dose estimates based on *expected* daily urinary volume). Previous literature estimates that children have a urinary output of 1–2 mL/kg/hr (Aust, 2012); we used the average output to estimate each child's urinary output in L/day. Second, we used the mean volume of each individual's two 24-hr composite urine samples (henceforth referred to as volume-adjusted dose estimates based on *observed* daily urine volume). Third, we estimated expected 24-hr urine output based on expected creatinine excretion using the following equation (henceforth referred to as creatinine-adjusted dose estimates based on *expected* daily creatinine excretion):

$$Vi = \frac{Ccr_i}{Cc_i} \tag{3}$$

where $Vi$ is the expected 24-hr urine output for the $i$th participant (L/day), $Ccr_i$ is the expected daily creatinine excretion (mg/day) based on Eqs. (4) and (5) for the $i$th participant, and $Cc_i$ is the observed creatinine concentration in the $i$th participant's urine sample (mg/L). Expected creatinine excretion was calculated based on the following equations (Mage et al., 2008), where Ht = height in centimeters:

$$Expected\ creatinine\ (mg/day)\ for\ males = Ht \times [6.265 + 0.0564(Ht - 168)] \tag{4}$$

$$Expected\ creatinine\ (\text{mg/day})\ for\ females = 2.045 \times Ht^{[0.01552(Ht - 90)]} \qquad (5)$$

Finally, we estimated 24-hr urine output based on the mean observed 24-hr creatinine excretion from each individual's 24-hr composite samples (henceforth referred to as creatinine-adjusted dose estimates based on *observed* daily creatinine excretion).

We chose to use Eqs. (3)-(5) to estimate expected 24-hr urinary output volume based on observed and reference creatinine excretion values because these would be the only methods available for use in many epidemiologic studies and risk assessments that make inferences based on the collection of spot samples alone. Dose estimates from 24-hr composites were not corrected for urinary volume, as they already reflected the actual 24-hr urine output.

**Comparing spot, FMV, and 24-hr samples:** We used generalized estimating equation (GEE) models using DAP, DM, and DE dose estimates from each 24-hr composite as the outcome variable and dose estimates from same-day spot (FMV and non-FMV) as the predictor variable. We also used the combination of each same-day FMV and non-FMV spot sample as a predictor variable by computing the arithmetic average of the dose estimate from the individual samples. Missing voids from 24-hr samples were excluded from the analysis, as both the volume of the sample and DAP concentrations were unknown. Analyses were conducted for volume- and creatinine-adjusted dose estimates. All dose estimates were $\log_{10}$-transformed. We assessed the performance of the models for each predictor variable using the predictive power of the model defined as the coefficient of determination ($R^2$); the root mean squared error (RMSE), which is a measure of both precision and accuracy of the model; and the intraclass correlation (ICC) (Fisher, 1992), which measures agreement between the dose estimates.

### 2.5. Sensitivity analyses

We conducted several sensitivity analyses to examine the robustness of our results: (1) we excluded participants with >1 FMV sample collected during a 24-hr sampling period; (2) we limited analyses to participants with complete collection of all spot samples within a 24-hr urine sampling period; and (3) we varied the proportion of OP exposure from diet and nearby agricultural pesticide use. Based on the results from a recent study that found that DAPs decreased by approximately 70% among nine children ages 4–15 years living in four U.S. urban areas following an organic diet intervention (Hyland et al., 2019), we attributed 70% of exposure to diet and 30% of exposure to nearby agricultural pesticide use.

## 3. Results

All children were Mexican American and ranged in age from 3 to 6.5 years (mean ± SD = 4.5 ± 0.93 years). We included 69 same-day non-FMV spot samples and 54 same-day FMV spot samples (including FMV samples collected on mornings 1 and 2 of 24-hr sampling periods) from 50 "child-days" (*n* = 25 children over two 24-hr sampling periods) in the analysis. Nine participant-days had 24-hr composites that included two FMV samples (2 FMV samples collected from morning of study day 2 to morning study day 3 and 7 FMV

samples collected from morning of study day 5 to morning of study day 6). Participants collected 89% (range = 50–100%) of reported voids during 24-hr sampling (range = 4–12 voids; mean = 7.4 voids).

Twenty-two (44%) of 24-hr samples were based on 100% collection of all voids. The maximum number of missed voids for a single participant for a 24-hr sample was 3 (out of 6 total voids reported). Seven participants missed two or more voids during one of the 24-hr sampling periods. Reasons for missed voids included out-of-home bathroom use and toileting accidents. We collected entire urine voids. The volume of individual spot samples collected during 24-hr sampling periods ranged from 4.8 to 642.2 mL (mean, 157.5 mL) for FMV samples and 16.4 to 238.3 mL (mean, 73.0 mL) for non-FMV samples.

Tables S2 and S3 present estimated cumulative OP dose for 24-hr, non-FMV spots, and FMV spot samples assuming that that the exclusive source of OP exposure was either nearby agricultural pesticide use or diet, respectively. Dose estimates were significantly higher and a greater percentage of samples exceeded the benchmark dose in models in which all OP exposure was attributed to nearby agricultural pesticide use.

We observed high detection frequencies of >90% for DEs, DMs, and total DAPs (Table 1). Total DAP levels were driven primarily by DM metabolites.

Table 2 reflects the total estimated cumulative OP dose, assuming that nearby agricultural pesticide use and diet contributed to 60% and 40% of total OP exposure, respectively. We observed that both volume- and creatinine-adjusted non-FMV spot samples tended to underestimate dose relative to 24-hour composites (median dose for DAPs from 24-hr composites = 3.18 μg/kg/day; from volume-adjusted estimates based on *expected daily urine volume* = 1.55 μg/kg/day; from volume-adjusted estimates based on *observed daily urine volume* = 2.22 μg/kg/day; from creatinine-adjusted estimates based on *expected daily creatinine excretion* = 3.01 μg/kg/day), and likewise underestimated the percentage of children exceeding the daily benchmark dose relative to estimates based on 24-hr samples. Of the non-FMV samples, those adjusted for *observed daily creatinine excretion* were most similar to estimates from 24-hr composites (median dose for DAPs = 3.20 μg/kg/day), but still tended to underestimate dose at higher percentiles (e.g., dose estimates at 90th percentile for non-FMV and 24-hr composites = 14.76 μg/kg/day and 19.91 μg/kg/day, respectively). Total DAP doses based on the average of a non-FMV and FMV spot sample most closely approximated dose from 24-hr samples.

Table 3 presents results of GEE models examining how well dose estimated from same-day FMV and non-FMV spot samples predicted 24-hr OP dose after excluding one participant-day with abnormally high urinary DAP concentrations (>3 SD from mean). For models estimating the association between a single volume- or creatinine-adjusted spot sample and its respective 24-hr composite, the $R^2$ was highest for FMVs ($R^2$ for total DAPs = 0.40–0.68 for FMVs and 0.09–0.38 for non-FMVs, depending on method of volume adjustment). While the predictive power tended to be slightly greater for estimates adjusted for observed 24-hr urine volume or observed 24-hr creatinine, the $R^2$ and RMSE values indicated that models adjusted for expected 24-hr creatinine excretion also had relatively high ability to

predict 24-hr dose ($R^2$ for total DAPs for FMVs and average of non-FMV and FMV = 0.65 and 0.72, respectively). ICC values indicated poor reproducibility for non-FMV samples (ICC for total DAPs = 0.14–0.59 for non-FMV and 0.63–0.82 for FMV samples, depending on the volume-adjustment method).

The best-fitting models were obtained when either an FMV sample or the arithmetic mean of an FMV and non-FMV sample was used to predict the 24-hr dose, depending on the metabolite type and volume-adjustment method ($R^2$ = 0.40–0.68 for FMV samples and 0.40–0.80 for average of FMV and non-FMV samples for total DAPs; Table 2). Similarly, RMSE values indicated that models with either FMV samples or the average of an FMV and non-FMV samples were the most accurate predictors of 24-hr dose (RMSE = 0.28–0.38 for FMV samples and 0.22–0.37 for average of FMV and non-FMV samples for total DAPs). The best model fit for total DAPs was observed for the mean of an FMV and non-FMV sample adjusted for observed 24-hr creatinine excretion ($R^2$ = 0.80; RMSE = 0.22). Model fit was strongest for total DAPs and DMs and considerably weaker for DE metabolites.

Results from sensitivity analyses in which we (1) excluded participants with > 1 FMV sample during a 24-hr sampling period (Tables S4-S5); (2) excluded participants with less than 100% collection of urine samples during a 24-hr sampling period (Tables S6-S7); and (3) varied the proportion of estimated OP exposure from diet and nearby agricultural pesticide use (Tables S8-S9) were largely consistent with findings from our main analyses. Dose estimates from sensitivity analyses in which 70% of OP exposure was attributed to diet were considerably lower than dose estimates from main analyses (Table S8). Additionally, model fit was slightly better for non-FMV samples in sensitivity analyses in which we limited to participants with 1 FMV sample (Table S5) or complete collection of all urine samples during a 24-hr sampling period (Table S7). Consistent with results from the main analyses, the best model fit for total DAPs in each sensitivity analysis was observed for the mean of an FMV and non-FMV sample adjusted for observed 24-hr creatinine excretion.

## 4. Discussion

In this study of 25 children living in an agricultural region, we found that volume- and creatinine-adjusted non-FMV spot urine samples had relatively weak ability to predict 24-hr cumulative OP dose. Moreover, our results indicate that reliance on non-FMV spot samples may underestimate daily cumulative OP dose and the percentage of samples exceeding regulatory guidelines, regardless of the method used to account for expected daily urinary excretion. Models including the average of an FMV and non-FMV spot had the greatest ability to predict 24-hr dose, however models containing just an FMV sample were often similarly predictive of daily dose. Our findings are consistent with previous analyses in this population in which we found that spot urine samples had relatively weak ability to predict cumulative exposure over one week and that reliance on spot samples to reflect chronic OP pesticide exposure may result in exposure misclassification that could bias effect estimates towards null findings (Bradman et al., 2013). Because 24-hr sampling, considered the "gold standard", or the collection of multiple daily spot samples is infeasible in most epidemiologic studies, we recommend that future studies prioritize the collection of FMV samples to most accurately characterize OP dose.

To our knowledge, only two other studies have examined the ability of same-day spot urine samples to predict 24-hr OP pesticide exposure or dose (Kissel et al., 2005; Scher et al., 2007). In a study of 13 2–5 year old children, Kissel et al., analyzed OP metabolite concentrations from urine samples collected during each of two 24-hr sampling cycles in two different seasons and found that FMV samples were the best predictor of weighted-average daily metabolite concentration in both creatinine-adjusted and unadjusted analyses (Kissel et al., 2005). They also observed high intra-child variability in metabolite levels from urine samples collected on the same day (Kissel et al., 2005). Their findings indicate that full 24-hr sampling may reduce measurement error due to within-person variability, however if spot sampling is to be conducted, collection of FMV samples are preferable for analytes with short half-lives (Kissel et al., 2005).

In another analysis of 20 farmers and their children, Scher et al., analyzed agreement between two OP parent compounds/metabolites (2,4-dichlorophenoxy acetic acid (2,4-D) and 3,5,6-trichloro-2-pyridinol (TCP)) in morning void samples with 24-hr composite exposure and dose estimates from urine collected between 24 h before through 96 h after pesticide application (Scher et al., 2007). Compared to estimates based on 24-hr samples, investigators found that single morning void urine samples tended to overestimate daily exposure and dose estimates of 2,4-D and chlorpyrifos (the parent compound of the metabolite TCP) (Scher et al., 2007). More specifically, four children had chlorpyrifos dose estimates above the acute population adjusted dose (aPAD) regulatory level of 0.5 μg/kg/day based on morning void samples, whereas no 24-hr dose estimates exceeded EPA safety thresholds (Scher et al., 2007). Taken together with our results, these findings suggest that reliance solely on non-FMV spot samples may underestimate OP dose, whereas analysis of FMV samples alone may overestimate dose.

Previous epidemiologic analyses among children living in the Salinas Valley have found DMs to drive associations between urinary DAPs and adverse child neurodevelopment (Bouchard et al., 2011; Eskenazi et al., 2007; Marks et al., 2010). We observed that DMs had a substantial influence on OP dose estimates and ability of spot samples to predict 24-hr dose. There are a few possible explanations for this. First, of the 11 OPs examined in this analysis, 8 are DMs and only 3 are DEs. These eight DMs had a much higher total molar mass (2,387 g/mol) than the three DEs (929 g/mol). Second, oxydemeton methyl, a highly toxic DM with a large RPF (16.4 for the index chemical chlorpyrifos), increased in use in the Salinas Valley shortly after our study started (California Departement of Pesticide Regulation, 2004)) and may be influencing the associations observed in our study and previous epidemiologic analyses from this region. Pesticide use trends have shifted drastically since we conducted this study and some of the most toxic OPs have largely been phased out of agricultural use in the Salinas Valley and across the United States. Additional investigations are needed to examine cumulative OP dose estimates and potential contributions from DEs and DMs with the current mixture of OPs being applied. In addition to the potential influence of specific OPs, it's possible that DEs are chemically less stable and have higher intrinsic variability than DMs (Bradman et al., 2007).

We found that estimates adjusted for expected 24-hr creatinine had similar ability to predict daily OP dose as estimates adjusted for observed 24-hr creatinine excretion or urine volume.

Conversely, in a study of 109 children living in an agricultural area in Washington State, investigators found that creatinine-adjusted doses tended to be lower than those calculated with daily urine volume (Fenske et al., 2000). Previous studies have found that creatinine concentrations may be highly variable due to factors such as age, sex, BMI, diet, and fluid intake (Barr et al., 2005; Boeniger et al., 1993; Mage et al., 2004) and that correcting for specific gravity may introduce less variability and may be a more robust method in studies focusing on children (Pearson et al., 2009; Wang et al., 2015). Additional research may be needed to evaluate the validity of creatinine correction in children. Furthermore, we recommend that future studies collect urine specific gravity information, particularly given the ease of measuring this metric (Pearson et al., 2009).

This study has multiple strengths and implications for future risk assessments and epidemiologic studies. We extended previous examinations that estimated cumulative OP dose from diet (Curl et al., 2015) and nearby agricultural pesticide use (using PUR data) (Castorina et al., 2003) separately by considering these exposures in conjunction. Additionally, this is one of only a few studies to examine cumulative OP pesticide dose among children living in an agricultural area and to examine the ability of spot samples to predict 24-hr dose. These results have important implications for risk assessments and could be applied to other non-persistent environmental chemicals.

This study also has limitations. We did not have specific gravity measurements and could not compare adjustment for urinary dilution using specific gravity. Notably, while DAPs represent exposure to approximately 80% of the OPs used in the Salinas Valley (Castorina et al., 2003), children may have been exposed to other OPs that do not devolve into DAPs.

While California's unique and comprehensive PUR database allowed us to estimate the mix of pesticides participants may have been exposed to from nearby agricultural pesticide use, relying solely on these data to estimate all non-dietary exposures may not adequately account for all sources and pathways of exposure. We examined agricultural pesticide applications near participants' residences in the six months prior to each 24-hr sampling in order to tiy to account for exposures from multiple sources, including agricultural drift and accumulation of pesticides in the home (i.e., in carpets, household surfaces, and dust), however participants may have also been exposed to pesticides via the take-home exposure pathway, particularly if they lived with farmworkers (Hyland and Laribi, 2017; Lopez-Galvez et al., 2019). However, because the dose calculations incorporate the proportion of potential exposure to each pesticide in relation to total DEs and DMs applied, rather than a sum of each pesticide, and because we anticipate that children living with farmworkers were likely exposed to a similar mixture of OPs from para-occupational exposures, we do not believe that this impacted our results substantially. No residential use of OPs was reported by participants.

Our assumption that 100% of absorbed OP dose was excreted as urinary diethyl and dimethyl metabolites may underestimate dose, as approximately 20% of the OPs used in the study area do not metabolize to any of the DAP metabolites (Castorina et al., 2003). Furthermore, the OPs that do devolve into DAP metabolites are not excreted entirely as DAP metabolites within 24 h, as they may be excreted in other biological media (Bouchard et al.,

2003) and as non-DAP urinary metabolites (Barr and Angerer, 2006; Bouchard et al., 2003). Factors such as the route of exposure may also impact the proportion of parent OPs excreted as DAPs, with previous studies finding a higher recovery for oral versus dermal exposures (Bouchard et al., 2003; Griffin et al., 1999).

Another limitation is that we did not administer a comprehensive dietary assessment. We asked mothers to state whether their child had consumed any fruits or vegetables in the previous day. Compared to a more rigorous Food Frequency Questionnaires (FFQs), our assessment may have underestimated dietary exposures. Moreover, the USDA PDP program publishes food residue data from food samples acquired from across the country without regard to region of origin. Employing these data inherently assumes participants consumed fruits and vegetables with similar exposure profiles of produce sold throughout the U.S. It is possible that participants from an agriculture region are more likely to consume locally grown produce, resulting in exposure profiles that may or may not reflect those sold nationwide. For example, we observed that dose estimates based solely on nearby agricultural pesticide use were significantly higher than dietary dose estimates, in part due to the higher proportion of exposure from more toxic pesticides such as oxydemeton methyl and disulfoton in PUR dose estimates. If specific OPs that were sprayed locally in this timeframe were also present to a higher degree on locally consumed produce, our use of national food residue data may have underestimated dietary dose estimates.

When determining the proportion of exposure to attribute to diet, we chose to incorporate data from an organic diet intervention study in a similar population of children living in Salinas and Oakland, CA in 2006 (Bradman et al., 2015). Various studies, including other intervention studies that have observed decreases in DAP concentrations from 70 to 89% among children and adults following an organic diet intervention (Göen et al., 2017; Hyland et al., 2019; Oates et al., 2014), suggest that diet is the primary source of OP exposure among children in non-agricultural areas (Curl et al., 2015; Lu et al., 2006, 2008; Morgan et al., 2005). It is possible that diet accounted for a greater proportion of exposure than we attributed to it in this analysis. However, longitudinal studies of children living in agricultural and suburban areas in Washington State suggest that DAP concentrations may vary temporally and that diet may not necessarily be the primary source of OP exposure among agricultural children during spray seasons (Fenske Richard et al., 2005; Koch et al., 2002). Furthermore, the overall interpretation regarding the predictive power of FMV and non-FMV spots remained consistent between main analyses and sensitivity analyses in which we varied the proportion of exposure from diet. Additional studies are needed to disentangle the proportion of exposure from diet, nearby agricultural pesticide use, and other sources among children living both in agricultural and non-agricultural regions. Regardless of the proportion of exposure assigned to each source, our overall conclusions that non-FMV spots may underestimate exposure remain the same.

## 5.  Conclusion

Because collection of 24-hr urine samples is cumbersome and often cost prohibitive, many risk assessments and pesticide regulations have been informed from studies that rely on one or two random spot samples to approximate chronic OP exposure and internal dose. Our

results suggest that non-FMV spot samples tend to underestimate daily OP dose and may underestimate the percentage of children with dose estimates exceeding regulatory guidelines, which could impact regulatory decision-making. If 24-hr sampling is infeasible, we recommend that future studies prioritize the collection of FMV samples to most accurately characterize OP dose in children. The results of these analyses may help inform future epidemiologic study design and risk assessments and could be extended beyond OPs to other non-persistent chemicals.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

## Acknowledgements

## Abbreviations:

| | |
|---|---|
| **AChE** | Acetylcholinesterase |
| **DAP** | Dialkylphosphate metabolite |
| **DE** | Diethyl phosphate |
| **DEDTP** | Diethyldithiophosphate |
| **DEP** | Diethylphosphate |
| **DETP** | Diethylthiophosphate |
| **DF** | Detection Frequency |
| **DMDTP** | Dimethyldithiophosphate |
| **DMP** | Dimethylphosphate |
| **DMTP** | Dimethylthiophosphate |
| **FCCR** | Food Consumption-Chemical Residue |
| **FCID** | Food Commodity Intake Database |
| **FQPA** | Food Quality Protection Act |
| **FMV** | First Morning Void |
| **ICC** | Intraclass Correlation Coefficient |
| **LOD** | Limit of Detection |

| **OP** | Organophosphorous |
| **PDP** | Pesticide Data Program |
| **RMSE** | Root Mean Square Error |
| **USDA** | United States Department of Agriculture |

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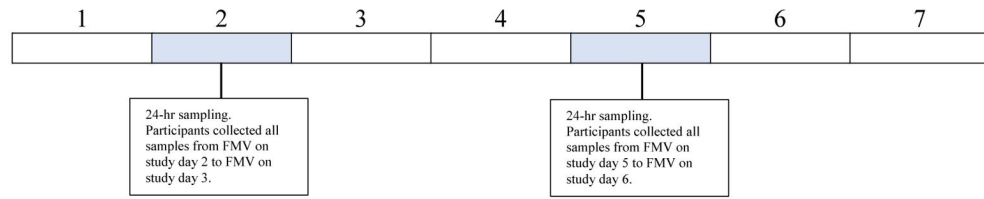
**Fig. 1.**
Study activities by day. Participants collected all urine voids for a 24-hr period on study days 2 and 5, including the FMV, all daytime and evening spot voids, and the FMV of the following day (study days 3 and 6). The current analyses were limited to samples collected during the 24-hr sampling periods.

**Table 1**

Unadjusted and creatinine-adjusted DAP concentrations in urine samples collected from 2 24-hr sampling periods.

| Type of sample | DF (%) | Unadjusted (nmol/L) | | | | Creatinine adjusted (nmol/g creatinine) | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | GM | Mean | Median | Range | GM | Mean | Median | Range |
| 24-hr composite samples (*n* = 50) | | | | | | | | | |
| Total DAPs | – | 158.0 | 295.5 | 144.3 | 34.7–3,698.9 | 274.5 | 620.5 | 244.8 | 47.5–10,144.5 |
| Total DMs | – | 94.6 | 230.4 | 89.9 | 11.8–3,593.0 | 166.1 | 507.8 | 138.5 | 15.3–9,923.1 |
| Total DEs | – | 45.9 | 65.0 | 53.3 | 4.8–248.3 | 78.9 | 112.8 | 93.6 | 8.6–609.7 |
| Non-FMV spot (*n* = 69) | | | | | | | | | |
| Total DAPs | 98.6 | 92.5 | 225.6 | 87.16 | 7.8–4,823.8 | 193.5 | 692.7 | 190.9 | 9.2–20,614.6 |
| Total DMs | 92.8 | 54.3 | 176.8 | 50.4 | 5.2–4,788.9 | 113.7 | 602.3 | 101.8 | 5.6–20,465.5 |
| Total DEs | 94.2 | 20.7 | 48.8 | 22.3 | 2.5–474.6 | 43.4 | 90.5 | 66.8 | 3.0–463.2 |
| FMV spot (*n* = 54) | | | | | | | | | |
| Total DAPs | 98.2 | 177.4 | 307.7 | 146.8 | 7.8–1,617.1 | 218.8 | 404.5 | 212.6 | 13.1–2,472.7 |
| Total DMs | 98.2 | 99.0 | 228.9 | 94.4 | 5.2–1,519.8 | 122.0 | 308.2 | 122.3 | 6.2–2,323.8 |
| Total DEs | 98.2 | 50.3 | 78.8 | 57.6 | 2.5–267.9 | 62.0 | 96.3 | 79.2 | 4.0–355.2 |

Abbreviations: DF, detection frequency; GM, geometric mean.

**Table 2**

Estimated cumulative OP chlorpyrifos equivalent dose (µg/kg/day) based on nearby agricultural pesticide use and diet [a] ($n = 25$ children [b]).

| Type of spot sample/metabolite excretion units | Metabolite type | n | Percentiles | | | | | Range | Estimates exceeding index chemical's $BMD_{10}/100$ (%) [d] |
|---|---|---|---|---|---|---|---|---|---|
| | | | 10th | 25th | 50th | 75th | 90th | | |
| 24-hr composite samples [c] | Total DAPs | 50 | 1.16 | 1.60 | 3.18 | 10.06 | 19.91 | 0.76–146.81 | 9 (18.0) |
| | Total DMs | 50 | 0.79 | 1.11 | 2.77 | 10.00 | 19.07 | 0.60–146.29 | 7 (14.0) |
| | Total DEs | 50 | 0.10 | 0.30 | 0.43 | 0.85 | 1.01 | 0.01–2.94 | 0 (0.0) |
| Non-FMV spot | | | | | | | | | |
| Expected 24-hr urine volume [e] | Total DAPs | 69 | 0.24 | 0.79 | 1.55 | 4.92 | 10.14 | 0.12–30.96 | 4 (5.8) |
| | Total DMs | 69 | 0.20 | 0.67 | 1.29 | 4.27 | 8.19 | 0.07–26.97 | 3 (4.3) |
| | Total DEs | 69 | 0.02 | 0.07 | 0.26 | 0.63 | 1.87 | 0.01–5.72 | 0 (0.0) |
| Observed 24-hr urine volume [f] | Total DAPs | 69 | 0.45 | 1.17 | 2.22 | 6.97 | 15.17 | 0.21–195.15 | 7 (10.1) |
| | Total DMs | 69 | 0.40 | 0.80 | 1.99 | 6.03 | 13.06 | 0.16–194.97 | 5 (7.2) |
| | Total DEs | 69 | 0.02 | 0.05 | 0.22 | 0.61 | 1.25 | 0.01–3.72 | 0 (0.0) |
| Expected 24-hr creatinine excretion [g] | Total DAPs | 69 | 0.51 | 1.26 | 3.01 | 6.92 | 15.45 | 0.11–555.50 | 8 (11.6) |
| | Total DMs | 69 | 0.35 | 1.04 | 2.55 | 6.56 | 14.67 | 0.09–554.99 | 6 (8.7) |
| | Total DEs | 69 | 0.03 | 0.11 | 0.33 | 0.72 | 1.39 | 0.01–3.04 | 0 (0.0) |
| Observed 24-hr creatinine excretion [h] | Total DAPs | 69 | 0.51 | 1.59 | 3.20 | 6.86 | 14.76 | 0.19–381.92 | 6 (8.7) |
| | Total DMs | 69 | 0.43 | 1.27 | 2.33 | 5.48 | 13.52 | 0.14–381.57 | 6 (8.7) |
| | Total DEs | 69 | 0.02 | 0.08 | 0.33 | 0.79 | 1.14 | 0.01–4.31 | 0 (0.0) |
| FMV spot | | | | | | | | | |
| Expected 24-hr urine volume [e] | Total DAPs | 54 | 0.69 | 2.22 | 4.06 | 6.32 | 13.59 | 0.22–25.28 | 3 (5.6) |
| | Total DMs | 54 | 0.53 | 1.65 | 3.27 | 5.95 | 11.91 | 0.17–23.48 | 1 (1.9) |
| | Total DEs | 54 | 0.08 | 0.37 | 0.61 | 1.08 | 1.63 | 0.02–2.59 | 0 (0.0) |
| Observed 24-hr urine volume [f] | Total DAPs | 54 | 0.79 | 1.59 | 4.33 | 11.99 | 24.45 | 0.18–53.37 | 11 (20.4) |
| | Total DMs | 54 | 0.44 | 1.10 | 3.61 | 10.81 | 23.71 | 0.15–52.98 | 11 (20.4) |
| | Total DEs | 54 | 0.09 | 0.29 | 0.51 | 0.85 | 1.24 | 0.01–2.73 | 0 (0.0) |
| Expected 24-hr creatinine excretion [g] | Total DAPs | 54 | 0.76 | 1.11 | 3.21 | 7.95 | 18.75 | 0.18–80.46 | 8 (14.8) |
| | Total DMs | 54 | 0.34 | 0.78 | 2.86 | 7.64 | 18.20 | 0.12–79.87 | 8 (14.8) |

| Type of spot sample/metabolite excretion units | Metabolite type | n | Percentiles | | | | | Range | Estimates exceeding index chemical's $BMD_{10}/100$ (%)[d] |
|---|---|---|---|---|---|---|---|---|---|
| | | | 10th | 25th | 50th | 75th | 90th | | |
| | Total DEs | 54 | 0.08 | 0.24 | 0.38 | 0.65 | 0.93 | 0.01–1.41 | 0 (0.0) |
| bserved 24-hr creatinine excretion [h] | Total DAPs | 54 | 0.74 | 1.47 | 3.15 | 7.17 | 18.81 | 0.11–48.75 | 9 (16.7) |
| | Total DMs | 54 | 0.34 | 0.98 | 2.74 | 6.75 | 18.38 | 0.09–47.02 | 8 (14.8) |
| | Total DEs | 54 | 0.10 | 0.24 | 0.37 | 0.63 | 1.01 | 0.01–1.74 | 0 (0.0) |
| Average of non-FMV and FMV spots [i] | | | | | | | | | |
| Expected 24-hr urine volume [e] | Total DAPs | 68 | 0.75 | 1.67 | 3.53 | 5.77 | 8.03 | 0.22–21.18 | 2 (2.9) |
| | Total DMs | 68 | 0.58 | 1.39 | 3.14 | 4.93 | 7.17 | 0.13–18.96 | 2 (2.9) |
| | Total DEs | 68 | 0.14 | 0.32 | 0.54 | 0.84 | 1.27 | 0.02–3.52 | 0 (0.0) |
| Observed 24-hr urine volume [f] | Total DAPs | 68 | 1.01 | 1.55 | 4.63 | 9.59 | 19.93 | 0.49–109.80 | 8 (11.8) |
| | Total DMs | 68 | 0.86 | 1.25 | 4.37 | 8.95 | 18.67 | 0.32–109.34 | 8 (11.8) |
| | Total DEs | 68 | 0.13 | 0.22 | 0.45 | 0.73 | 0.99 | 0.01–2.27 | 0 (0.0) |
| Expected 24-hr creatinine excretion [g] | Total DAPs | 68 | 0.82 | 1.29 | 3.67 | 8.10 | 19.93 | 0.24–291.70 | 9 (13.2) |
| | Total DMs | 68 | 0.65 | 1.05 | 3.30 | 7.31 | 19.21 | 0.16–291.02 | 9 (13.2) |
| | Total DEs | 68 | 0.09 | 0.19 | 0.41 | 0.66 | 0.91 | 0.01–1.88 | 0 (0.0) |
| Observed 24-hr creatinine excretion [h] | Total DAPs | 68 | 1.00 | 1.50 | 3.79 | 8.63 | 18.80 | 0.47–200.54 | 9 (13.2) |
| | Total DMs | 68 | 0.78 | 1.26 | 3.20 | 8.09 | 18.38 | 0.31–200.08 | 9 (13.2) |
| | Total DEs | 68 | 0.13 | 0.24 | 0.39 | 0.70 | 0.91 | 0.01–2.67 | 0 (0.0) |

[a] n = 50 child-days with 24-hour samples; 69 non-FMV and 54 FMV spot samples from either 24-hour sampling period.

[b] 60% of estimated OP exposure attributed to nearby agricultural use and 40% of estimated OP exposure attributed to diet.

[c] 24-hour samples reflect collection of all non-FMV and FMV spot samples for that 24-hour period (4 samples lacked non-FMV spot and 5 samples lacked FMV spot).

[d] $BMD10/100$ of index chemical (chlorpyrifos) = 14.8 ug/kg/day. 100-fold uncertainty factor applied to account for intra- and interspecies variability.

[e] Dose estimates from spot samples multiplied by expected 24-hr urine output volume based on reference values.

[f] Dose estimates from spot pot samples multiplied by observed 24-hr urine output volume (from mean volume of 24-hr urine samples from that participant across the two sampling periods.)

[g] Dose estimates from spot pot samples multiplied by expected 24-hr urine output volume based on observed and reference creatinine excretion in spot samples.

[h] Dose estimates from spot pot samples multiplied by observed 24-hr creatinine excretion.

[i] Average samples reflect collection of 69 non-FMV and 54 FMV spot samples from 41 child-days that provided both a non-FMV and FMV spot sample in the same 24-hour period (n = 68 samples with average of non-FMV and FMV spot samples collected in the same 24-hour period).

**Table 3**

Modeling of 24-hour dose using same-day spot urine samples as predictors ($\log_{10}$-transformmed) ($n = 25$ children[a]).

| Type of spot sample/metabolite excretion units | Metabolite type | n | β (95% CI) | Intercept | Model $R^2$ | RMSE | ICC |
|---|---|---|---|---|---|---|---|
| **Non-FMV spot** | | | | | | | |
| Expected 24-hr urine volume[b] | Total DAPs | 68 | 0.23 (−0.02, 0.47) | 0.53 | 0.09 | 0.43 | 0.14 |
| | Total DMs | 68 | 0.25 (0.00, 0.49) | 0.47 | 0.09 | 0.46 | 0.16 |
| | Total DEs | 68 | 0.31 (0.15, 0.47) | −0.21 | 0.20 | 0.40 | 0.36 |
| Observed 24-hr urine volume[c] | Total DAPs | 68 | 0.43 (0.21, 0.65) | 0.41 | 0.25 | 0.39 | 0.45 |
| | Total DMs | 68 | 0.47 (0.28, 0.65) | 0.36 | 0.28 | 0.41 | 0.48 |
| | Total DEs | 68 | 0.35 (0.17, 0.54) | −0.16 | 0.28 | 0.38 | 0.40 |
| Expected 24-hr creatinine excretion[d] | Total DAPs | 68 | 0.43 (0.23, 0.63) | 0.38 | 0.33 | 0.37 | 0.54 |
| | Total DMs | 68 | 0.46 (0.29, 0.63) | 0.33 | 0.36 | 0.39 | 0.57 |
| | Total DEs | 68 | 0.28 (0.11, 0.46) | −0.23 | 0.18 | 0.41 | 0.35 |
| bserved 24-hr creatinine excretion[e] | Total DAPs | 68 | 0.51 (0.30, 0.73) | 0.34 | 0.38 | 0.35 | 0.59 |
| | Total DMs | 68 | 0.54 (0.36, 0.72) | 0.30 | 0.41 | 0.37 | 0.62 |
| | Total DEs | 68 | 0.37 (0.17, 0.57) | −0.18 | 0.38 | 0.38 | 0.44 |
| **FMV spot** | | | | | | | |
| Expected 24-hr urine volume[b] | Total DAPs | 53 | 0.64 (0.28, 0.91) | 0.26 | 0.40 | 0.38 | 0.63 |
| | Total DMs | 53 | 0.71 (0.46, 0.95) | 0.21 | 0.43 | 0.41 | 0.65 |
| | Total DEs | 53 | 0.42 (0.23, 0.62) | −0.26 | 0.29 | 0.33 | 0.51 |
| Observed 24-hr urine volume[c] | Total DAPs | 53 | 0.70 (0.56, 0.84) | 0.16 | 0.68 | 0.28 | 0.82 |
| | Total DMs | 53 | 0.66 (0.51, 0.81) | 0.17 | 0.66 | 0.32 | 0.80 |
| | Total DEs | 53 | 0.44 (0.24, 0.63) | −0.22 | 0.32 | 0.32 | 0.55 |
| Expected 24-hr creatinine excretion[d] | Total DAPs | 53 | 0.69 (0.56, 0.82) | 0.25 | 0.65 | 0.29 | 0.79 |
| | Total DMs | 53 | 0.65 (0.51, 0.79) | 0.26 | 0.63 | 0.33 | 0.76 |
| | Total DEs | 53 | 0.43 (0.22, 0.64) | −0.16 | 0.28 | 0.33 | 0.50 |
| Observed 24-hr creatinine excretion[e] | Total DAPs | 53 | 0.73 (0.56, 0.89) | 0.23 | 0.68 | 0.28 | 0.81 |
| | Total DMs | 53 | 0.68 (0.51, 0.85) | 0.24 | 0.65 | 0.32 | 0.78 |
| | Total DEs | 53 | 0.44 (0.23, 0.64) | −0.16 | 0.30 | 0.32 | 0.51 |

| Type of spot sample/metabolite excretion units | Metabolite type | n | β (95% CI) | Intercept | Model $R^2$ | RMSE | ICC |
|---|---|---|---|---|---|---|---|
| Average of non-FMV and FMV spot[f] | | | | | | | |
| Expected 24-hr urine volume[b] | Total DAPs | 67 | 0.73 (0.43, 1.03) | 0.23 | 0.40 | 0.37 | 0.47 |
| | Total DMs | 67 | 0.76 (0.43, 1.10) | 0.21 | 0.41 | 0.41 | 0.49 |
| | Total DEs | 67 | 0.65 (0.42, 0.88) | −0.20 | 0.48 | 0.29 | 0.54 |
| Observed 24-hr urine volume[c] | Total DAPs | 67 | 0.92 (0.77, 1.06) | 0.02 | 0.78 | 0.23 | 0.60 |
| | Total DMs | 67 | 0.91 (0.76, 1.05) | 0.02 | 0.78 | 0.25 | 0.80 |
| | Total DEs | 67 | 0.69 (0.46, 0.92) | −0.14 | 0.53 | 0.27 | 0.78 |
| Expected 24-hr creatinine excretion[d] | Total DAPs | 67 | 0.78 (0.60, 0.96) | 0.15 | 0.72 | 0.26 | 0.73 |
| | Total DMs | 67 | 0.79 (0.63, 0.96) | 0.12 | 0.74 | 0.28 | 0.79 |
| | Total DEs | 67 | 0.62 (0.34, 0.90) | −0.12 | 0.44 | 0.31 | 0.64 |
| Observed 24-hr creatinine excretion[e] | Total DAPs | 67 | 0.89 (0.75, 1.03) | 0.07 | 0.80 | 0.22 | 0.79 |
| | Total DMs | 67 | 0.89 (0.76, 1.02) | 0.06 | 0.81 | 0.23 | 0.81 |
| | Total DEs | 67 | 0.44 (0.17, 0.71) | −0.20 | 0.29 | 0.35 | 0.48 |

[a] n = 49 child-days with 24-hour samples; 68 non-FMV and 53 FMV spot samples from either 24-hour sampling period.

[b] Spot samples multiplied by expected 24-hr urine output volume based on reference values.

[c] Spot samples multiplied by observed 24-hr urine output volume (from mean volume of 24-hr urine samples from that participant across the two sampling periods.)

[d] Spot samples multiplied by expected 24-hr urine output volume based on observed and reference creatinine excretion in spot samples.

[e] Spot samples multiplied by expected 24-hr urine output volume based on observed creatinine excretion (from mean 24-hr creatinine from that participant across the two sampling periods).

[f] Average samples reflect collection of 68 non-FMV and 53 FMV spot samples from 41 child-days that provided both a non-FMV and FMV spot sample in the same 24-hour period (n = 67 samples with average of non-FMV and FMV spot samples collected in the same 24-hour period).