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ORIGINAL ARTICLE

Organophosphorous pesticide breakdown products in house dust and children's urine

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Human exposure to preformed dialkylphosphates (DAPs) in food or the environment may affect the reliability of DAP urinary metabolites as biomarkers of organophosphate (OP) pesticide exposure. We conducted a study to investigate the presence of DAPs in indoor residential environments and their association with children's urinary DAP levels. We collected dust samples from homes in farmworker and urban communities (40 homes total, $n = 79$ samples) and up to two urine samples from resident children ages 3–6 years. We measured six DAPs in all samples and eight DAP-devolving OP pesticides in a subset of dust samples ($n = 54$). DAPs were detected in dust with diethylphosphate (DEP) being the most frequently detected ($\geq 60\%$); detection frequencies for other DAPs were $\leq 50\%$. DEP dust concentrations did not significantly differ between communities, nor were concentrations significantly correlated with concentrations of chlorpyrifos and diazinon, the most frequently detected diethyl-OP pesticides (Spearman $\rho = -0.41$ to 0.38, $P > 0.05$). Detection of DEP, chlorpyrifos, or diazinon, was not associated with DEP and/or DEP + diethylthiophosphate detection in urine (Kappa coefficients = −0.33 to 0.16). Finally, estimated non-dietary ingestion intake from DEP in dust was found to be $\leq 5\%$ of the dose calculated from DEP levels in urine, suggesting that ingestion of dust is not a significant source of DAPs in urine if they are excreted unchanged.

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INTRODUCTION

Organophosphate (OP) pesticides have been the focus of recent exposure and epidemiologic studies due to their potential adverse health effects, particularly in children. Several of these studies[1–5] have relied on urinary dialkylphosphate (DAP) metabolites as exposure biomarkers.

Although DAP metabolites are class-specific biomarkers, they cannot be used to quantify exposure to individual OP pesticides except in acute exposure settings.[6] Over 70% of the OP pesticides registered for use in the United States by the Environmental Protection Agency (EPA) can metabolize to one or more DAPs in the body,[7] which consist of six individual compounds: three diethyl (DE) phosphate species: diethylphosphate (DEP), diethylthiophosphate (DETP), and diethyldithiophosphate (DEDTP); and three dimethyl (DM) phosphate species: dimethylphosphate (DMP), dimethylthiophosphate (DMTP), and dimethyldithiophosphate (DMDTP). Measurement of these metabolites in urine is often preferred over measurement of parent OP pesticides in other matrices such as blood, because sample collection is simple and non-invasive, concentrations are usually three orders of magnitude higher than in blood thus easier to measure, and laboratory methods are available to measure these metabolites at low detection levels.[8,9] In addition, currently there are no laboratory methods available to measure some commonly used OP pesticides, such as oxydemeton-methyl, in blood.

Several studies have used urinary DAP metabolites to derive biologically-based OP pesticide dose estimates by attributing metabolite levels in urine solely to OP pesticide exposure.[10,11] However, OP pesticides can degrade in the environment or be metabolized by plants, likely leading to the presence of preformed DAPs and other OP pesticide hydrolytic products in food and environmental media.[12–14] Studies have reported the presence of DAPs in fruit juices and produce;[9,15,16] and we previously published a laboratory method to analyze DAPs in dust and documented the existence of DAPs in house dust samples from urban and agricultural homes in California.[9] Thus, urinary DAP levels may represent exposure to parent OP pesticides and to the preformed degradation products (i.e., DAPs) present in food and environmental media. Two recent studies on rodents suggest that ingested DAPs are excreted unchanged in urine.[17,18] If similar metabolism of DAPs occurs in humans, attributing urinary DAP metabolite levels solely to OP pesticide exposure could lead to overestimation and potentially misclassification of exposure for epidemiologic studies and risk assessments, particularly in non-acute exposure settings.

Here, we report the concentrations of DAPs in house dust and determine whether DAP concentrations differ between homes

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located in urban and agricultural communities, evaluate the association between OP pesticide and DAP residues in dust, and determine the relationship between DAP levels present in house dust and urinary DAP metabolite levels in young children residing in these homes.

METHODS

Study Population

Participants for this study were selected from families of children participating in a study evaluating dietary pesticide exposure to young children. Children followed a conventional diet for 4 days, then an organic diet for 7 days, and returned to a conventional diet for 5 days. The study was conducted between July and September of 2006. We recruited a convenience sample of 20 families residing in a predominantly urban community (Oakland, CA, USA; located in Alameda County) and 20 families residing in an agricultural community with intense agricultural OP pesticide use (Salinas, CA, USA; located in Monterey County) from local community clinics and organizations serving low-income populations. Urban homes consisted of inner city dwellings located more than 25 km from the nearest field where agricultural pesticide applications were reported. Only families that did not habitually consume organic foods were selected for the study. Ethnicity was restricted to Mexican immigrants or Mexican-American families to minimize cultural disparities between the populations. All Salinas households included at least one farmworker resident. Eligible children were toilet-trained and were between 3 and 6 years old. All study procedures were reviewed and approved by the University of California, Berkeley Committee for the Protection of Human Subjects and written informed consent was obtained from parents for themselves and their children upon enrollment in the study.

Data Collection

Before sample collection, bilingual staff administered a questionnaire to collect demographic information on children and household members and information on factors potentially related to indoor pesticide contamination including occupation of household residents and storage and residential use of OP pesticides. We also conducted a home inspection to ascertain general housing quality and proximity of the homes to the nearest agricultural field, orchard, or golf course where OP pesticide applications may have occurred. Daily questionnaires were administered during the study to ensure that recent exposure information was captured (e.g., use of pesticides at home or at work on the previous day).

Dust Sample Collection

We collected up to two dust samples in each home with a High Volume Small Surface Sampler (HVS3; Envirometrics, Seattle, WA, USA). The HVS3 was developed for the US EPA for sampling house dust (a complex mixture of biologically derived material, particulate matter deposited from indoor aerosol, and soil particles brought in by foot traffic) to be analyzed for pesticides and other toxics from carpets and bare floors. This sampling equipment is capable of collecting sufficient dust for pesticide residue analysis, at a constant sampling rate, and in a highly reproducible manner.[19–21] The first dust sample was collected during the first conventional diet phase (i.e., days 3 or 4 of the study) and the second sample was collected toward the end of the organic diet phase (5–8 days after the first sample collection). One participant was lost to follow-up before collection of their second sample yielding a total of 79 dust samples (40 samples from 20 farmworker homes and 39 samples from 20 urban homes). Dust samples were collected from an area 1–2 m^2 using a standardized collection procedure.[22] Collection of dust samples involved marking off a designated area with tape and then making eight passes (four in each direction).[22] Collection equipment was thoroughly cleaned and allowed to dry completely between sample collections to avoid cross contamination of samples. The majority of samples were collected from carpets in areas where children spent time playing. For three agricultural homes with no carpeted areas, we collected samples from upholstered furniture using a furniture attachment on the HVS3. Samples were collected from the same general area during both collections. Dust samples were sieved to obtain the fine fraction ($<150\,\mu$m) more likely to adhere to human skin[20,21] and were stored in freezers at -80°C until shipped on dry ice for laboratory analysis. All 79 dust samples were analyzed individually for DAPs at the Centers for Disease Control and Prevention (CDC), National Center for Environmental Health in Atlanta, GA, USA. Samples with ≥ 0.5 g of dust remaining ($n = 54$) were analyzed for OP pesticides at Battelle Memorial Institute in Columbus, OH, USA.

Urine Sample Collection

Parents were instructed to collect children's first morning voids over 15 consecutive days. If parents were not able to collect the child's first morning void then a spot sample was collected. Children voided directly into a collection jar or into a clean, sterile Specipan™ (Baxter Scientific, McGaw Park, IL, USA). If a Specipan™ was used for collection, parents transferred the sample into a collection jar. For all specimens, parents recorded the collection time and stored the sample in a cooler with ice packs. Study staff collected urine samples from parents on each collection day and provided them with fresh ice packs and materials to collect the next day's specimen. In total, 148 first morning voids and 9 random spot samples were collected for the analysis presented herein. Urine specimens were aliquoted at the field laboratory and stored at -80°C. For quality control (QC) purposes, frozen field blanks and spikes, previously prepared by CDC, were defrosted and then re-packaged in the field according to collection procedures used for study samples. All samples were shipped on dry ice to CDC for laboratory analysis of DAPs.

Laboratory Analysis

DAPs in dust (n = 79). All six DAPs (DEP, DETP, DEDTP, DMP, DMTP, and DMDTP) were measured in dust samples using a previously validated laboratory method.[9] Briefly, dust samples were aliquoted into 1-g units and fortified with an isotopically labeled internal standard solution consisting of DEP (diethyl-$^2H_{10}$), DETP (diethyl-$^2H_{10}$), DEDTP (diethyl-$^{13}C_4$), DMP (dimethyl-$^2H_6$), DMTP (dimethyl-$^2H_6$), and DMDTP (dimethyl-$^2H_6$). DAPs were extracted using a phosphate buffer and sample cleanup was done via solid phase extraction. DAPs were then derivatized and analyzed by isotope dilution gas chromatography-tandem mass spectrometry (GC-MS/MS) according to the method of Bravo et al.[8] Each analytical run consisted of seven calibration standards, two QC samples (20 ng/g and 100 ng/g), one blank, and study samples. No DAPs were present in any blank dust samples indicating contamination during laboratory sample processing did not occur. The relative standard deviation for DE DAPs ranged from 5.9% to 14.4% for QC high samples and from 0.6% to 21.1% for QC low samples. For DM DAPs, relative standard deviations ranged from 5.0% to 8.8% for QC high spike samples and from 9.3% to 17.1% for QC low samples. The limits of detection (LOD) were 10.4, 5.8, and 5.2 ng/g for DEP, DETP, and DEDTP, respectively, and 4.8, 2.8, and 9.9 ng/g for DMP, DMTP, and DMDTP, respectively.

OP pesticides in dust (n = 54). Of the 40 households sampled, 15 agricultural homes and 13 urban homes had adequate dust sample volumes (≥ 0.5 g) for analysis of OP pesticides after initial analysis of DAPs. There were no demographic or housing differences in those homes with adequate *vs* inadequate volume of remaining dust. Laboratory methods for OP pesticides in dust have been described previously.[23] Target OP pesticides and respective LODs included four DM-devolving OP pesticides: malathion (10 ng/g), methidathion (10 ng/g), methyl parathion (10 ng/g), and tetrachlorvinphos (10 ng/g) and four DE-devolving OP pesticides: chlorpyrifos (10 ng/g), diazinon (4 ng/g), diazinon-oxon (4 ng/g), and phorate (10 ng/g). Diazinon-oxon is not used as a pesticide, but is an oxidative product of the insecticide diazinon; it is also a precursor of DEP. Selection of target analytes was based on active ingredients in household products stored or used indoors, compatibility with a single analytical method, and county-level agricultural and non-agricultural pesticide use in both study locations as reported in the California Pesticide Use Reporting Database (http://www.cdpr.ca.gov/docs/pur/purmain.htm) Table 1 presents DAP-devolving precursor OP pesticides and respective degradation

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Table 1. DAP-devolving OP pesticide compounds, respective DAP degradation products, and amount of OP pesticide applied at the county level in the two study locations during the year in which samples were collected.

OP precursor compound	*Potential DAP environmental degradates and/or metabolites*	*Amount applied at the county level in 2006 (kg) in the locations sampled*[a]	
		Monterey county (predominantly agricultural region; location of Salinas homes) Ag use (non-ag use)[b]	*Alameda county (predominantly urban region; location of Oakland homes) Ag use (non-ag use)*[b]
Azinphos-methyl	DMP, DMTP, DMDTP	2 (0)	—
Chlorethoxyphos	DEP, DETP	—	—
Chlorpyrifos	DEP, DETP	27,959 (126)	62 (6)
Chlorpyrifos-methyl	DMP, DMTP	—	—
Coumafos	DEP, DETP	—	—
Diazinon	DEP, DETP	65,268 (24)	0 (5)
Dichlorvos[c]	DMP	—	—
Dicrotophos[d]	DMP	—	—
Dimethoate	DMP, DMTP, DMDTP	16,024 (8)	36 (0)
Disulfoton	DEP, DETP, DEDTP	2161 (0)	0 (<1)
Ethion[c]	DEP, DETP, DEDTP	—	—
Fenitrothion	DMP, DMTP	—	—
Fenthion[c]	DMP, DMTP	—	—
Isazofos-methyl[c]	DMP, DMTP	—	—
Malathion	DMP, DMTP, DMDTP	16,686 (120)	0 (577)
Methidathion	DMP, DMTP, DMDTP	3287 (0)	NA
Methyl Parathion	DMP, DMTP	93 (0)	0 (11)
Naled	DMP	6968 (12)	—
Oxydemeton-methyl	DMP, DMTP	32,215 (0)	—
Parathion	DEP, DETP	—	—
Phorate	DEP, DETP, DEDTP	274 (0)	—
Phosmet	DMP, DMTP, DMDTP	32 (0)	—
Pirimiphos-methyl	DMP, DMTP	—	—
Sulfotepp[c]	DEP, DETP	—	—
Temephos	DMP, DMTP	—	—
Terbufos	DEP, DETP, DEDTP	—	—
Tetrachlorvinphos	DMP	—	—
Trichlorfon[c]	DMP	—	—
Total amount of diethyl-devolving OP pesticides applied (kg)		95,812	73
Total amount of dimethyl-devolving OP pesticides applied (kg)		75,447	624
Total amount of OP pesticides applied (kg)		171,259	697

Abbreviations: DEP, diethyphosphate; DETP, diethylthiophosphate; DEDTP, diethyldithiophosphate; DMP, dimethyphosphate; DMTP, dimethylthiophosphate; DMDTP, dimethyldithiophosphate.
[a]Source: California Department of Pesticide Regulation Pesticide Use Reporting Database. Available at: http://www.cdpr.ca.gov/docs/pur/-pur06rep/06_pur.htm.
[b]Non-agricultural (non-ag) uses refers to applications for landscape maintenance, public health, commodity fumigation, rights-of-way, and structural pest control applications by licensed applicators reported to the state of California.
[c]Uses for this OP pesticide were canceled in the United States by the EPA. Source: http://www.epa.gov/pesticides/reregistration/status_op.htm.
[d]Registered for use in the United States but not for use in California. Source: http://www.epa.gov/pesticides/reregistration/status_op.htm.

products and/or metabolites along with general usage at the county-level where our study homes were located.

Briefly, dust aliquots were fortified with 250 ng of two surrogate recovery standards — fenchlorphos and $^{13}C_{12}$-trans-permethrin (the former for OPs and the latter for other pesticides reported elsewhere[23]). OP pesticides were extracted using ultrasonication in 1:1 hexane:acetone and cleaned-up using an aminopropyl solid phase extraction cartridge. Extracts were then concentrated, fortified with an internal standard (dibromobiphenyl), and analyzed using electron impact GC/MS in the multiple ion detection mode. For QA/QC purposes, we included a solvent method blank, matrix spike sample, and duplicate study sample in each analytical set. No analytes were detected in the four solvent method blanks. Analyte recoveries in the four matrix spike samples averaged $117 \pm 19\%$ for OP pesticides. The average relative percent difference in concentration for two OP analytes detected in duplicate samples (chlorpyrifos and diazinon) was $8 \pm 10\%$ indicating good analytical precision.

DAP concentrations in urine ($n = 157$). We measured all six DAP metabolites (DEP, DETP, DEDTP, DMP, DMTP, and DMDTP) in children's urine samples using a previously validated method.[8] Briefly, urine specimens were lyophilized to remove water and the remaining residue redissolved in acetonitrile:diethyl ether. DAPs were then derivatized and concentrated extracts were analyzed by isotope dilution GC-MS/MS. Analytical QC procedures included repeat analysis of three in-house urine pools enriched with known amounts of DAP residues whose target values and confidence limits were previously determined. Westgard rules for QC were used to validate each analytical run.[24] LODs for DAP metabolites were as follows: 0.2, 0.1, and 0.1 μg/l for DEP, DETP, and DEDTP, respectively; and 0.6, 0.2, and 0.1 μg/l for DMP, DMTP, and DMDTP, respectively. Creatinine concentration (mg/dl) in each specimen was determined with a commercially available method (Vitros CREA slides, Ortho Clinical Diagnostics, Raritan, NJ, USA). In addition to the use of field QC samples (blank and spiked samples), we also analyzed duplicate urine samples to assess the precision of our analytical runs. No DAP metabolites were present in any blank samples indicating that no contamination occurred in the field, during sample processing, or during shipment to the laboratory.

Data Analysis

We used Fisher's exact tests to determine whether there were any differences in demographic characteristics between participants from

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urban and agricultural homes. We calculated detection frequencies (DFs) and descriptive statistics for each analyte in both dust and urine samples stratified by location and collection time point (i.e., conventional and organic diet phase). For subsequent statistical analyses, we focused on the DAPs and OP pesticides with DFs >50% in dust in at least one location. Analyte concentrations below the LOD were assigned a value of LOD/$\sqrt{2}$ for statistical analyses and results were considered statistically significant at $P<0.05$.

DAP dust concentrations within and between homes. We computed Spearman rank-order correlations to evaluate the association of individual DAP concentrations within homes (i.e., between collections). To determine whether individual DAP dust concentrations significantly differed between agricultural and urban homes at each collection, we performed Wilcoxon rank-sum tests. Only those DAPs frequently detected (DF ≥50%) at each collection were considered in these analyses.

Relationship between OP pesticide and DAP residues concentrations in dust. We computed Spearman rank-order correlations to assess the association between OP pesticide and DAP residue concentrations in dust at each collection. We also computed the molar ratio of total moles of DAPs to the total moles of respective OP pesticides in each dust sample. Both Spearman correlations and molar ratios were calculated for the most frequently detected DAPs and OP pesticides in dust (DF >50%).

Association between residue concentrations in dust (OPs and DAPs) and DAPs in children's urine. Because OP pesticides are rapidly metabolized and excreted[25] and no human data are available on the metabolism of preformed DAPs, we examined the relationships between frequently detected analyte dust residues (OP pesticides and DAPs) and DAP concentrations in children's urine using voids collected on the day of dust sample collection ($n=79$) and on the day after dust sample collection ($n=78$). To evaluate the correspondence between analyte dust residue concentrations and respective DAP concentrations in children's urine without making any assumptions about the distribution of concentrations less than LOD for which we have no data, we calculated Kappa statistics by categorizing detection (i.e., ≥LOD = 1 *vs* <LOD = 0) for each of the frequently detected analytes in dust (individually and collectively) and respective urinary DAP metabolites as LOD or <LOD. We then determined the level of agreement of detection of each of these compounds in dust (individually and collectively) and detection of respective metabolites in children's urine at each collection time point, by calculating Cohen's Kappa coefficients (where 1 = perfect agreement, 0 = no agreement above that expected by chance, and 1 = perfect disagreement). We also computed the Spearman rank-order correlation between the concentration for the frequently detected DAPs in dust and frequently detected precursor OP pesticides in dust (individually and collectively as the molar sum) with respective DAP concentrations in urine. We evaluated correlations using both unadjusted and creatinine-adjusted urinary DAP concentrations (nmol metabolite per grams of creatinine).

Contribution of DEP in dust to DEP concentrations in urine. We estimated the potential contribution of the most frequently detected DAP in dust, DEP, via the non-dietary ingestion pathway to the estimated DEP dose predicted from the individual DEP urine concentrations by calculating the ratio of estimated intake to the dose predicted from urine. To estimate children's non-dietary intake, we used individual DEP dust concentrations (ng/g) observed in homes, assuming a dust ingestion rate of 0.100 g/day according to US EPA,[26] and 100% absorption of the dose based on animal data.[18] We calculated intake by multiplying the DEP concentration in dust by the dust ingestion rate and then dividing by the child's body weight, which was measured at the time of the interview. Intake was calculated for those children with detectable levels of DEP in urine samples (31 children with 46 urine samples collected on the day of dust sample collection and 32 children with 47 urine samples collected on the day after dust sample collection). To estimate the children's DEP dose (D_{Urine_DEP}, ng/kg/day), we used the following equation:

$$D_{Urine_DEP}(ng/kg/day) = C_{urine}(nmol/l) \times \frac{Cre_{24}(mg/day)}{Cre_i(mg/l)} \times MW(ng/nmol) \times \frac{1}{BW(kg)}$$

Table 2. Select demographic and household characteristics for participants living in Salinas agricultural and Oakland urban homes sampled in 2006.

	Salinas, CA (agricultural homes; n = 20) n *(%)*	*Oakland, CA (urban homes; n = 20)* n *(%)*
Family income relative to federal poverty level[a]		
At or below poverty level	13 (65.0)	13 (65.0)
Above poverty but ≤200% of poverty level	7 (35.0)	7 (35.0)
Maternal education (highest grade completed)		
Completed 9th grade or lower	12 (60.0)	12 (60.0)
Grades 10–12 (no diploma)	3 (15.0)	3 (15.0)
High school diploma/GED or technical school	3 (15.0)	5 (25.0)
College graduate	2 (10.0)	0 (0.0)
Paternal education (highest grade completed)[b]		
Completed 9th grade or lower	15 (79.0)	13 (72.2)
Grades 10–12 (no diploma)	1 (5.3)	1 (5.6)
High school diploma/GED or technical school	3 (15.8)	4 (22.2)
College graduate	0 (0.0)	0 (0.0)
Number of household members		
3–5	12 (60.0)	10 (50.0)
>6	8 (40.0)	10 (50.0)
Reported OP pesticide application in the 3 months preceding sample collection		
Yes	1 (5.0)[c]	—
No	19 (95.0)	20 (100.0)
Farmworkers living in the home (past 3 months)		
0	1 (5.0)[d]	18 (90.0)
1–3	15 (75.0)	2 (10.0)[e]
4–7	4 (20.0)	—
Farmworkers currently living in the home		
0	1 (5.0)[d]	20 (100.0)
1–3	15 (75.0)	—
4–7	4 (20.0)	—
Farmworkers wore work clothing indoors[f]		
Yes	17 (10.5)	—
No	2 (89.5)	—
Farmworkers wore work shoes indoors[f]		
Yes	10 (52.6)	—
No	9 (47.4)	—
Distance of home to nearest field/orchard		
50–200 feet	1 (5.0)	—
>200 feet-1/4 mile	3 (15.0)	—
>1/4 mile	16 (80.0)	—

[a]Families' poverty levels were based on US Department of Health and Human Services thresholds for 2006.
[b]Information was not available for one father living in an agricultural home and two fathers living in urban homes.
[c]One participant reported applying tetrachlorvinphos 7–30 days before the study for flea treatment on their pet dog.
[d]One participant in the agricultural group reported that the father was a farmworker during eligibility screening; however, the father was not living in the home during the sample collection period.
[e]Two participants reported having a parent or parent's sibling working in a field/golf course doing maintenance/landscaping work, which may have involved pesticide use in the 3 months preceding the study; however, they were not doing this work at the time of the study.
[f]Information not available for one father living in an agricultural home.

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where, C_{urine} is the DEP concentration in urine in nmol/l, Cre_{24} is the estimated daily creatinine excretion in mg/day based on the child's sex and age, Cre_i is the creatinine concentration in the child's urine sample in mg/l, MW is the molecular weight for DEP in ng/nmol (154 ng/nmol), and BW is the child-specific body weight in kg. To estimate daily creatinine excretion (Cre_{24}), we used 24-h creatinine excretion data obtained from a study we conducted on low-income Mexican-American children between 3 and 6 years of age ($n = 25$; data not shown). Based on that study, we assigned the mean daily age- and sex-specific creatinine excretion rate to the children in our study: 293.2, 331.6, 390.4, and 737.9 mg/day for 3-, 4-, 5-, and 6-year-old girls, respectively, and 193.8 and 344.4 mg/day for 3- and 4-year-old boys and 504.5 mg/day for 5- and 6-year-old boys. Because no data on creatinine excretion were available for 6-year-old boys, we assigned 6-year-old boys the same excretion rate as 5-year-old boys. Our estimates were based on the following assumptions: (1) DEP metabolite concentrations in urine voids were representative of steady state conditions; and (2) 100% of the absorbed DEP dose from DE-devolving parent OP pesticides and DE-DAPs was expressed in urine as DEP.

Lastly, we examined the distribution of total DEs (molar sum of DEP + DETP + DEDTP), total DMs (molar sum of DMP + DMTP + DMDTP), and total DAPs (molar sum of total DEs + total DMs) in dust and urine samples to determine which species (i.e., DEs or DMs) contributed most to total DAPs in each media.

We performed all statistical analyses using Stata 10 for Windows (StataCorp LP, College Station, TX, USA).

RESULTS

Population and Household Characteristics

Table 2 summarizes demographic and household characteristics for study participants. All households were low-income; most of them were below the poverty level based on the US Census data for 2006.[27] The majority of the children's parents had completed <10 years of education. Most participants reported applying a pesticide in the home sometime in the last 3 months before sample collection (~60% of agricultural households and ~80% of urban households, not shown). One agricultural participant reported applying the insecticide tetrachlorvinphos (a DMP-devolving OP pesticide) for flea treatment on the house pet 7–30 days preceding sample collection. No other home OP pesticide use was reported up to 3 months preceding the study. The majority of agricultural households had one to three farmworkers living in the home and lived more than 1/4 mile from the nearest agricultural field or orchard. Other than farmworker status and proximity of the home to the nearest agricultural field or orchard, demographic characteristics were similar in the two study locations of Salinas and Oakland ($P > 0.05$).

Detection and concentrations of DAPs in dust for agricultural and urban homes. DEP and DMP were the most frequently detected DAPs in dust samples in both locations (Table 3). The overall DF for DEP in dust was 65% for agricultural homes and 67% for urban homes. Among all samples in each location, median DEP concentrations were slightly higher in the urban homes (47 ng/g) compared with agricultural homes (35 ng/g). However, the maximum DEP concentrations were higher in agricultural homes (859 ng/g *vs* 316 ng/g in urban homes). The overall DF for DMP was 48% among agricultural homes and 33% among urban homes. Median DMP concentrations were below the LOD in both locations, but maximum concentrations were higher in urban (1588 ng/g) than in agricultural (806 ng/g) homes. Other DAPs were not detected or detected at much lower frequencies. For example, DETP and DEDTP were not detected in any urban homes while DMTP was not detected in any agricultural homes. For the participant in the agricultural home who reported applying tetrachlorvinphos on their pet, we detected DMP in only the sample obtained at the second collection; the DMP concentration (44 ng/g) was above the 95th percentile concentration observed among all samples in agricultural homes (not shown).

We report subsequent dust results solely for DEP, as it was the only DAP with a frequency of detection >50% in both locations. DEP dust concentrations were moderately correlated between collections in agricultural homes (Spearman $\rho = 0.49$, $P = 0.03$); however, when we removed one influential point, the correlation became weaker (Spearman $\rho = 0.39$, $P = 0.09$). In urban homes, we observed a weak correlation of DEP concentrations between collections (Spearman $\rho = 0.28$, $P = 0.25$). We also found that DEP dust concentrations did not significantly differ between urban and agricultural homes at each collection (Wilcoxon rank-sum tests $P > 0.05$, not shown).

Association between OP pesticides and DAP concentrations in dust. We detected several OP pesticides in dust samples from both locations, including two DE-devolving OP pesticides: diazinon and chlorpyrifos, one DE-devolving OP intermediary product, diazinon-oxon, and two DM-devolving OP pesticides: tetrachlorvinphos and malathion (Table 4). (For the participant who applied tetrachlorvinphos on their house pet, we were not able to analyze their dust samples for OP pesticides due to insufficient sample mass after analysis of DAPs.) Diazinon and chlorpyrifos were the only OP pesticides with DFs ≥50% in at least one location. Environmental degradation of these DE-devolving OP pesticides could result in the presence of two DE-DAPs: DEP and DETP. Because we did not detect DETP in the majority of dust samples (i.e., DETP was only detected in two agricultural homes), we restricted subsequent analyses and comparisons between concentrations of diazinon and chlorpyrifos to DEP residues in dust.

Diazinon concentrations in dust were not significantly correlated with DEP dust concentrations among urban and agricultural homes or overall (i.e., for all homes regardless of location) at each collection (Spearman $\rho = -0.02$ to 0.07, $P > 0.05$, not shown). Similarly, chlorpyrifos concentrations in dust were not significantly correlated with DEP dust concentrations among homes or overall at each collection (Spearman $\rho = -0.41$ to 0.38, $P > 0.05$, not shown). Furthermore, the molar sum of diazinon and chlorpyrifos dust concentrations was not correlated with DEP dust concentrations in any homes (Spearman $\rho = -0.14$ to 0.14, $P > 0.05$, not shown) at either collection. We also observed wide variation in the mole ratio of DEP to the molar sum of chlorpyrifos and diazinon. Median and maximum DEP mole ratios were 2.2 and 24.1, respectively, for samples from agricultural homes and 1.6 and 70.0, respectively, for samples from urban homes. In all, 66% and 76% of the dust samples analyzed in agricultural and urban homes, respectively, had mole ratios >1 indicating that DEP dust concentrations were generally greater than the combined diazinon and chlorpyrifos dust concentrations.

Association between OP pesticides and DAP concentrations in dust and DAP concentrations in children's urine. Because our findings did not differ based on whether we examined the association of analytes in dust and respective DAP concentrations in urine samples collected on the same day or the day after dust collection, we present results related to urine samples collected on the same day as dust collection. Table 5 presents summary statistics for DAP concentrations in children's urine by location and collection time point. Overall, the most frequently detected diethyls in children's urine were DEP and DETP. The DEP detection frequency was somewhat higher in urine samples from children residing in the urban *vs* agricultural communities (≥65% *vs* <50%, respectively) at both collections. Although we observed comparable detection frequencies of DETP in urine samples from children living in the agricultural and urban communities during the first collection (65% and 60%, respectively), we found higher detection frequency in samples from children residing in the agricultural community

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Table 3. Limits of detection and summary statistics for DAP compounds in house dust for 20 Salinas agricultural homes and 20 Oakland urban homes by collection time point (ng/g).[a,b]

DAPs	*LOD (ng/g)*	*Homes*	*Collection time point*	*% DF*	*Concentration in dust (ng/g)*			
					p50	*p75*	*p95*	*Max*
DEP	10.4	Agricultural	1	60	30	57	246	386
			2	70	43	66	484	859
		Urban	1	70	50	79	246	316
			2	63	47	64	245	245
DETP	5.8	Agricultural	1	10	<LOD	<LOD	154	183
			2	0	—	—	—	—
		Urban	1	0	—	—	—	—
			2	0	—	—	—	—
DEDTP	5.2	Agricultural	1	5	<LOD	<LOD	<LOD	31
			2	5	<LOD	<LOD	<LOD	11
		Urban	1	0	—	—	—	—
			2	0	—	—	—	—
DMP	4.8	Agricultural	1	45	<LOD	8	28	29
			2	50	<LOD	20	425	806
		Urban	1	40	<LOD	9	822	1588
			2	26	<LOD	8	79	79
DMTP	2.8	Agricultural	1	0	—	—	—	—
			2	0	—	—	—	—
		Urban	1	5	<LOD	<LOD	<LOD	17
			2	5	<LOD	<LOD	<LOD	20
DMDTP	9.9	Agricultural	1	20	<LOD	<LOD	70	98
			2	10	<LOD	<LOD	20	22
		Urban	1	5	<LOD	<LOD	<LOD	17
			2	11	<LOD	<LOD	18	18

Abbreviations: DF, detection frequency; LOD, limit of detection; "—", statistic not reported when analyte was not detected.
[a]One participant in the urban cohort (Oakland, CA) was lost to follow-up before collection of the second dust sample; thus, statistics reported for the second collection time point are for 19 urban homes.
[b]Samples 1 and 2 were collected 5–8 days apart from the same general location in the home.

compared with children in the urban community (50% *vs* 32%, respectively) during the second collection. Median urinary DEP concentrations for children in the agricultural community were <LOD at both collections, whereas for children in the urban community they were 16 and 20 nmol/l at the first and second collections, respectively. Median concentrations for DETP were <LOD for all children at both collections. Maximum urinary DEP concentrations were higher in samples from children residing in the agricultural community (401 nmol/l) compared with children residing in the urban community (181 nmol/l); whereas maximum DETP concentrations were higher in children from the urban community (169 nmol/l) compared with children from the agricultural community (104 nmol/l).

The most frequently detected dimethyls in urine included DMTP followed by DMP. DMP was detected more frequently in urine samples from urban than agricultural community children ($\geq$60% *vs* <50%, detection frequencies respectively), whereas DMTP detection frequencies were similar in urine samples from children in both locations (DF >80%). Median urinary DMP concentrations were lower in the agricultural, rather than urban, community children (<LOD) at both collections. Similarly, DMTP median concentrations were higher in urine samples from agricultural, rather than urban, community children at both collections. Maximum urinary DMP concentrations were comparable for children in both communities (227 and 223 nmol/l, respectively), whereas maximum DMTP concentrations were higher in children from the agricultural community (993 nmol/l) compared to children in the urban community (777 nmol/l). We also found that higher-molecular-weight (sulfur containing) DEs and DMs, such as DETP and DMTP, were more frequently detected in urine than in dust.

Detection of DEP in dust and same day urine samples was observed for 13 and 17 children during the first and second collections, respectively. We did not observe agreement in detection for frequently detected DEP sources in dust (chlorpyrifos, diazinon, and DEP) and respective DE metabolites in urine (Kappa coefficients ranged from −0.33 to 0.16, $P>0.10$; Table 6). Results were similar when we looked at the level of agreement within location (not shown). In addition, concentrations for DEP sources in dust (singly or collectively) were not significantly correlated with respective DE metabolites at either collection by location or overall (Spearman $\rho=-0.38$ to 0.22, $P>0.05$ not shown). We also observed that the distribution of concentrations for total diethyls (molar sum of DEP, DETP, and DEDTP) and total DMs (molar sum of DMP, DMTP, and DMDTP) differed between media (Figure 1). For example, total DAPs in children's urine consisted mostly of total dimethyls, whereas total DAPs in dust samples consisted mostly of total diethyls. Finally, the estimated potential non-dietary ingestion intake of DEP ranged from 0.02 to 3 ng/kg/day, accounting for 0% to 5% of the overall dose estimated from urinary DEP concentrations.

DISCUSSION

Urinary DAP metabolite concentrations have been widely used to assess human OP pesticide exposure in epidemiologic and biomonitoring studies. However, the validity of their use as biomarkers of exposure has been questioned, particularly in non-acute, non-occupational exposure settings, due to recent studies reporting detection of preformed DAPs in fruit juices, produce, and house dust samples.[15,9,16] The present study is the first to conduct an in-depth analysis of DAP concentrations in house dust from homes in urban and agricultural communities including an estimate of their potential contribution to children's urinary DAP levels.

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Table 4. Limits of detection and summary statistics for detected OP pesticides in house dust from 15 Salinas agricultural homes and 13 Oakland urban homes by collection time point (ng/g).[a,b]

Precursor OP compound	*Dialkylphosphate breakdown product(s)*	*LOD (ng/g)*	*Homes*	*Collection time point*	*No. of homes sampled*	*% DF*	*Concentration in dust (ng/g)*			
							p50	*p75*	*p95*	*Max*
Diazinon	DEP, DETP	4	Agricultural	1	14	86	15	19	56	56
				2	15	73	14	17	36	36
			Urban	1	13	54	9	16	139	139
				2	12	50	5	21	133	133
Chlorpyrifos	DEP, DETP	10	Agricultural	1	14	57	22	32	200	200
				2	15	53	20	26	135	135
			Urban	1	13	39	<LOD	35	56	56
				2	12	33	<LOD	33	43	43
Tetrachlorvinphos	DMP	10	Agricultural	1	14	14	<LOD	<LOD	252	252
				2	15	7	<LOD	<LOD	271	271
			Urban	1	13	8	<LOD	<LOD	16	16
				2	12	0	—	—	—	—
Malathion	DMP, DMTP, DMDTP	10	Agricultural	1	14	7	<LOD	<LOD	52	52
				2	15	7	<LOD	<LOD	71	71
			Urban	1	13	15	<LOD	<LOD	877	877
				2	12	8	<LOD	<LOD	1,158	1,158
Diazinon-oxon[c]	DEP	4	Agricultural	1	14	0	—	—	—	—
				2	15	0	—	—	—	—
			Urban	1	13	8	<LOD	<LOD	5	5
				2	12	0	—	—	—	—

Abbreviations: LOD, limit of detection; DF, detection frequency; "—", statistic not reported when analyte was not detected.
[a]Organophosphate (OP) pesticides not detected in any samples included: methidathion, methyl parathion, and phorate.
[b]Number of samples analyzed for OP pesticides in each location was restricted to samples with adequate amount of dust volume remaining after initial analyses for DAPs: 29 samples from 15 Salinas agricultural homes and 25 samples from 13 urban homes. Summary statistics reported are for the number of samples at each collection.
[c]One molecule of the OP pesticide diazinon can either breakdown into DETP and the specific metabolite, isopropyl-methyl-hydroxypyrimidine (IMHP), or it may undergo activation into diazinon-oxon subsequently breaking down into DEP and IMHP.

DEP was the most frequently detected DAP in dust samples from both the urban and agricultural homes. Other DAPs were not detected or had much lower detection frequencies. It is not likely that degradation of higher-molecular-weight DAPs occurred during sample storage, processing, or laboratory analysis as preanalytic and analytic conversion was previously determined to be minimal using this method.[9] The low detection frequency of higher molecular-weight DAPs in dust suggests that these compounds may have degraded to the lower molecular weight species (e.g., DEP) in the environment. Consistent with this finding, Zhang et al. reported an increase in DMP residues and a decrease in DMTP residues over time in strawberries treated with malathion.[16] Information on the environmental fate of OP pesticide degradation products is scarce; research in this area could help inform future pesticide exposure studies.

Given the higher usage of OP pesticides at the county level in Salinas compared to Oakland, we hypothesized that OP pesticide degradation in the environment would result in higher DAP concentrations in dust from agricultural homes compared to urban homes. However, we did not observe significant differences in detection frequencies or median concentrations for DEP in dust between locations despite intense agricultural OP pesticide use in the agricultural community. Comparable detection frequencies and median concentrations for DEP in dust in homes from both locations suggest that other sources may also be responsible for its presence indoors. One possible source could be historical residential use of DE-devolving OP pesticides. Home pesticide use was common in both locations and while no participants reported residential applications of DE-devolving OP pesticides up to three months preceding the study, we do not have information on prior use or on the persistence of DAPs in dust after home application of OP pesticides. Although chlorpyrifos and diazinon were voluntarily phased-out for residential uses by the end of 2001 and 2004,[28,29] respectively, these DE-devolving OP pesticides were the most frequently detected in house dust from participants' homes. If DAPs persist in the environment then application of these DE-devolving OP pesticides before our study could explain detection of DEP indoors. We have no information on whether DEP or other preformed DAPs could devolve from other household or industrial chemicals.

We found that detection of DEP or frequently detected precursor OP pesticides in dust was not associated with detection of respective urinary DAP metabolites, and that, based on our stated assumptions, DEP in dust may contribute up to 5% of the DEP excreted in urine. These findings suggest that this non-dietary ingestion of DAPs in dust does not significantly impact urinary DAP metabolite levels. In addition, as observed in previous studies,[30,31] we found that total DAPs in urine consisted mostly of total DMs; however, this was not the case in our dust samples where total DAPs consisted mostly of total DEs. Similarly, DMTP was frequently detected in urine samples in our study population, but not in our dust samples. Our findings suggest that DAPs might break down differently in the body than in the environment and that other sources and routes of exposure (e.g., ingestion of produce and juices treated with organophosphorous pesticides) may be more important contributors of DAPs in urine.

Currently, it is not known whether a person who is exposed to preformed DAPs will excrete them unchanged or if further metabolism occurs. A recent study by Timchalk et al.[18] reported that oral doses of DEP and DETP to rats were well absorbed and excreted unchanged in the urine. Another study by Forsberg et al.[17] assessed the metabolic stability of DMP using pooled human and rat hepatic microsomes and evaluated the amount of DMP recovered in urine after oral administration of this analyte.

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Table 5. Summary statistics for DAP metabolites in children's urine samples collected on the same day as dust samples by location and collection time point (nmol/l).[a,b,c]

DAPs	*Community*	*Collection time point*	n[c]	*% DF*	*Metabolite concentrations in children's urine (nmol/l)*				
					p25	*p50*	*p75*	*p95*	*Max*
DEP	Agricultural	1	20	45	<LOD	<LOD	32	142	205
		2	20	45	<LOD	<LOD	121	307	401
	Urban	1	20	65	<LOD	16	47	121	181
		2	19	79	7	20	33	118	119
DETP	Agricultural	1	20	65	<LOD	19	36	103	104
		2	20	50	<LOD	4	42	78	81
	Urban	1	20	60	<LOD	6	13	139	169
		2	19	32	<LOD	<LOD	12	49	49
DEDTP	Agricultural	1	20	5	<LOD	<LOD	<LOD	<LOD	4
		2	20	0	—	—	—	—	—
	Urban	1	20	15	<LOD	<LOD	<LOD	37	63
		2	19	5	<LOD	<LOD	<LOD	90	90
DMP	Agricultural	1	20	40	<LOD	<LOD	62	192	206
		2	20	45	<LOD	<LOD	34	188	227
	Urban	1	20	80	8	20	46	149	223
		2	19	63	<LOD	15	40	57	57
DMTP	Agricultural	1	20	100	47	119	199	426	525
		2	20	90	24	54	151	669	993
	Urban	1	20	95	24	51	134	527	777
		2	19	84	9	19	98	259	259
DMDTP	Agricultural	1	20	25	<LOD	<LOD	2	36	39
		2	20	20	<LOD	<LOD	<LOD	57	69
	Urban	1	20	40	<LOD	<LOD	11	97	98
		2	19	11	<LOD	<LOD	<LOD	10	10

Abbreviations: LOD, limit of detection; DF, detection frequency; "—", statistic not reported when analyte was not detected.
[a]Detection limits: DEP = 0.2 μg/l (1.3 nmol/l), DETP and DEDTP = 0.1 μg/l (0.6 nmol/l), DMP = 0.6 μg/l (4.8 nmol/l), DMTP = 0.2 μg/l (1.4 nmol/l), and DMDTP = 0.1 μg/l (0.6 nmol/l).
[b]Concentrations reported are not creatinine adjusted.
[c]One participant in the urban cohort (Oakland, CA) was lost to follow-up before collection of the second urine sample.

Table 6. Level of detection agreement between frequently detected analyte residues in dust and respective urinary metabolites by collection time point.

Analyte(s) detected in dust	*Agreement with detection of DEP in urine*				*Agreement with detection of DEP+DETP in urine*			
	Collection time point	n[a]	*Kappa coefficient*[b]	P-*value*[c]	*Collection time point*	n[a]	*Kappa coefficient*[b]	P-*value*[c]
DEP	1	40	−0.13	0.81	1	—	—	—
	2	39	0.11	0.24	2	—	—	—
Chlorpyrifos	1	27	−0.33	0.96	1	27	−0.27	0.93
	2	27	0.05	0.40	2	27	0.05	0.38
Diazinon	1	27	−0.24	0.91	1	27	−0.01	0.51
	2	27	−0.07	0.64	2	27	0.16	0.12
DEP+chlorpyrifos+diazinon	1	27	−0.27	0.95	1	27	−0.27	0.93
	2	27	−0.19	0.89	2	27	−0.07	0.64

[a]Indicates number of samples included in the calculation; children contributed up to two dust samples.
[b]Kappa coefficient: 1 = perfect agreement, 0 = no agreement above that expected by chance, 1 = perfect disagreement.
[c]High *P*-values ($P > 0.05$) indicate that Kappa coefficients are not significantly greater than 0 (i.e., no agreement in detection between media above that expected by chance). Notation: "—" Because DEP in dust could not lead to detection of DETP in urine no kappa statistic is provided.

Researchers reported that DMP was not metabolized by rat or pooled hepatic microsomes, that DMP oral bioavailability was found to be 107 ± 39% and that the amount of orally administered DMP dose recovered in urine was 30 ± 9.9% by 48 h. The authors concluded that the *in vitro* metabolic stability, high bioavailability, and extent of DMP urinary excretion following oral exposure in a rat model suggests that measurement of DMP as a biomarker of OP exposure may lead to overestimation of human exposure. More research is needed on the pharmacokinetics and toxicodynamics of preformed DAPs and other specific OP pesticide metabolites to determine the extent of their contribution to urinary biomarkers in humans.

This study has several limitations. First, our sample size was small, limiting statistical power. Another limitation is that we were not able to measure every DAP-devolving OP pesticide in dust that could have led to detection of DAPs in dust and/or urine. For example, we were not able to measure oxydemeton-methyl, a DM-devolving OP pesticide, which was heavily used in 2006

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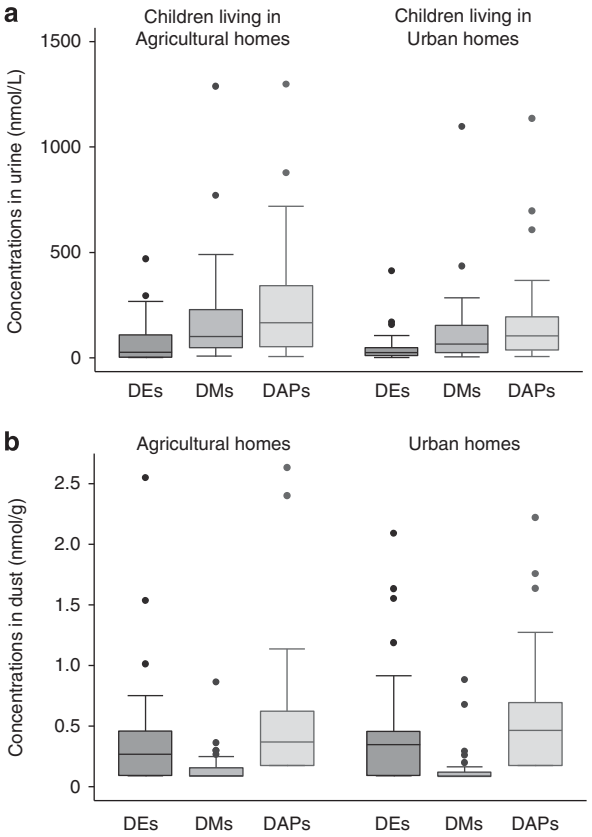

Figure 1. Distribution of total DE, DM, and DAP levels in children's urine samples collected on the same day as dust collection (a) and in dust samples (b) by location. Data presented are for 20 children and 20 homes in each location. Each participant contributed up to two urine and two house dust samples, except for two agricultural homes and one urban home, for which we did not include their dust concentrations for one collection for graphical representation (dust concentration, >6 nmol/g). Results and interpretation of data remain with or without omission of these data points. Total DE (molar sum of DEP + DETP + DEDTP), DM (molar sum of DMP + DMTP + DMDTP), and DAP (total DE + total DM) concentrations; values less than LOD were imputed to LOD/$\sqrt{2}$ to get a molar sum.

($>30{,}000$ kg applied for crops) for agricultural purposes in Monterey County where the Salinas farmworker homes were located. However, we did measure chlorpyrifos and diazinon, which together made up the majority (97% and 100% in the urban and agricultural community, respectively) of all of the DE-devolving OP pesticides used in each county for agriculture or structural pest control purposes. We also did not measure precursor OP pesticides and DAPs in food or other environmental media (e.g., soil and air) through which children may have been exposed[13] nor did we consider dermal or inhalation exposure to DEP.

Our exposure estimates were based on several assumptions that may have under- or overestimated true exposure to DAPs. For instance, if a child were to ingest more than 0.100 g/day of dust, our potential daily intakes would underestimate exposure through the non-dietary ingestion pathway. Similarly, daily creatinine excretion is also a source of variability in our exposure calculations. Nevertheless, intake of DEP from food is likely to be a more significant contributor to urinary DAP concentrations than from dust ingestion (if they are excreted unchanged). For example, assuming that the DEP concentration in orange juice is 8 μg/l (based on Lu et al.[15]), a median body weight of 20 kg (based on children in our study) and an ingestion rate of 312 ml/day (based on daily orange juice intake reported for five random children in our study), the estimated potential daily intake of DEP from orange juice would be 125 ng/kg/day. By contrast, assuming the median DEP dust concentration from our population (43 ng/g) and an average 20-kg child, the estimated intake of DEP from dust would be 0.22 ng/kg/day. Based on these estimates, intake of DEP from juice alone would be more than 500 times that calculated via the non-dietary ingestion route emphasizing the importance of other routes of exposure, particularly diet, to preformed DAPs.

In summary, we have documented the presence of preformed DAPs in house dust from agricultural and urban homes. However, our findings provide supporting evidence that preformed DAPs in dust (via ingestion) are not a significant contributor to DAP concentrations in urine in our study population, although it is still possible that other pathways of exposure to preformed DAPs (e.g., diet) may contribute, to a larger extent, to urinary DAP concentrations.[12,15] Future studies are needed to apportion the contribution of other preformed DAP sources, such as diet, relative to precursor pesticide exposures.

ABBREVIATIONS

CDC, Centers for Disease Control and Prevention; DAP, dialkylphosphate; DE, diethyl; DEP, diethylphosphate; DEDTP, diethyldithiophosphate; DETP, diethylthiophosphate; DF, detection frequency; DM, dimethyl; DMP, dimethylphosphate; DMDTP, dimethyldithiophosphate; DMTP, dimethylthiophosphate; EPA, Environmental Protection Agency; GC-MS/MS, gas chromatography-tandem mass spectrometry; LOD, limits of detection; OP, organophosphate; QC, quality control

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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