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A mass spectrometry-based method to measure dialkylphosphate degradation products of organophosphorous insecticides in dust and orange juice†

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Dialkylphosphates (DAPs) are urinary metabolites and breakdown products of organophosphorous (OP) pesticides. Urinary DAPs are widely used to assess exposure to OP pesticides in epidemiologic studies. Recent evidence suggests that preformed DAPs are present in food and that they may also be present in other parts of the environment. Thus, DAP concentrations observed in urine may reflect a person's exposure to both parent OP pesticides and preformed DAPs in food and other environmental media. The presence of preformed DAPs in multiple media may indicate that previous studies have overestimated exposure to OP pesticides and that the use of urinary DAPs as biomarkers of exposure for OP pesticides may not accurately characterize exposure in non-acute settings. To establish the presence of DAPs in environmental and food media, we developed analytical methods to measure six DAPs in dust and orange juice. The limits of detection (LOD) for the dimethyl phosphates (dimethylphosphate (DMP), dimethylthiophosphate, and dimethyldithiophosphate) ranged from 2.8–9.9 ng g^{-1} and 0.2–0.4 ng mL^{-1} in dust and juice, respectively. The LODs for the diethyl phosphates (diethylphosphate (DEP), diethylthiophosphate, diethyldithiophosphate) ranged from 5.2–10.4 ng g^{-1} and 0.5–3.0 ng mL^{-1} in dust and juice, respectively. The extraction efficiencies for the analytes ranged from 23% to 91% and from 41% to 85% in dust and orange juice, respectively. DMP was detected in about half of the dust samples whereas DEP was detected in 80% of the dust samples tested. Other DAPs were less frequently detected in dust. Less than 3% of intact pesticide present in the matrices was converted to their respective DAPs during the pre-analytic and analytic process. Evaluation of the conversion of intact pesticides in the samples to DAPs will help us to better understand the contribution of preformed DAPs to urinary DAP concentrations.

Introduction

Urinary dialkylphosphates (DAPs) have been widely used to assess human exposure to organophosphorus (OP) pesticides, both in the general population[1–7] and in occupational settings.[3,8–11] Approximately 75% of the OP pesticides (*O*,*O*-dimethyl and *O*,*O*-diethyl substituted OP pesticides) registered with the United States Environmental Protection Agency (EPA) can be metabolized to form one or more DAPs that are excreted in urine.[12] DAPs consist of three diethyl (DE) and three dimethyl (DM) alkylphosphate species: diethylphosphate (DEP), diethylthiophosphate (DETP), diethyldithiophosphate (DEDTP), dimethylphosphate, dimethylthiophosphate (DMTP), and dimethyldithiophosphate (DMDTP). DAPs are class-specific metabolites, and urinary DAP concentrations reflect cumulative exposure to OP pesticides and potentially the preformed metabolites present in the environment, rather than to specific OP pesticides. These biomarkers of exposure are commonly used because they integrate exposure to OP pesticides from multiple routes,[2,13–16] there are existing validated laboratory methods to measure DAPs in urine at low levels of detection,[2,17] and collection of urine is relatively simple and noninvasive, especially when one is assessing OP pesticide exposure in children.[10,12]

Historically, urinary DAP concentrations have been attributed solely to OP pesticide exposure.[10,16] However, recent evidence suggests that people may be exposed to preformed DAPs present in their diet and environment.[18–21] Rodent studies have shown that DAPs are excreted unchanged after oral exposure,[22] thus urinary DAPs would represent exposure to both OP pesticides and preformed DAPs. Lu *et al.*[19] reported concentrations of DAPs in both organic and conventional fresh fruit juices purchased from local grocery stores, with conventional juices having higher DAP concentrations than organic juices. The authors also found that OP pesticides degraded in refrigerated juices to form DAPs after only 72 hours of storage. The authors concluded that the presence of DAPs in fresh fruit juices casts doubts on the usefulness of urinary DAPs as biomarkers of

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exposure for OP pesticides, and that it overestimates exposure to parent OP pesticides. In another study by Zhang *et al.*, 61% of the produce samples analyzed contained higher concentrations of preformed DAPs than OP pesticides.[21]

Another possible route of exposure to preformed DAPs is through environmental media, such as soil and dust. Studies of multiple environmental media have found the specific urinary metabolite of chlorpyrifos, 3,5,6-tricholoro-2-pyridinol (TCPY), in air, dust, and soil, as well as in dietary samples.[20,23] The hydrolytic products of chlorpyrifos are TCPY, DEP and DETP; therefore, the presence of TCPY in environmental media suggests that the other hydrolytic products (*i.e.* DAPs), would also be present in these other media.[19,20,23]

The presence of DAPs in environmental and dietary media may indicate that previous epidemiological studies that used DAPs in urine as a measure of exposure may have overestimated exposure to OP pesticides.[16,24] This possible overestimation may indicate that DAPs may not be the most accurate biological exposure metric in non-acute exposure settings. Although DAPs are presumably reliable biomarkers in acute exposure settings, they may be present as contaminants in pesticide formulations so exposure to DAPs is still plausible. Currently, these exposures metrics are the mostly widely available, and suitable alternatives may not exist. To analyze for the presence of DAPs in the environment and foods, we developed and validated an extraction method (with minor changes to account for matrix differences) for measuring DAPs in dust and orange juice, common exposure matrices used for children.[15,19]

Experimental

Materials

All solvents used were analytical grade with purity greater than 98%. We obtained 1-chloro-3-iodopropane (CIP) from Aldrich Chemical Co. (Milwaukee, WI, USA). Ten sets of calibration spiking standards in acetonitrile with analyte concentrations ranging from 0.4 to 600 ng mL^{-1} were prepared in-house and validated against standards prepared under contract by Battelle Memorial Institute (Bel Air, MD, USA). Native standards of DMP (98% purity) and DEP (95% purity) were purchased from Acros (New Jersey, USA). DETP (K^+ salt) (98%) and DEDTP (90%) were both purchased from Aldrich Chemical Co. (Milwaukee, WI, USA). Native standards of DMTP (Na^+ salt) (98%) and DMDTP (98%) were purchased from Cambridge Isotope Laboratories (Andover, MA, USA) with purities greater than 99%. No further purifications were performed for the native analytes, but the purities (as provided by the manufacturers) were used to calculate the final concentration of the native analytes. Isotopically labeled internal standards (ISTD) of DMP (dimethyl-$^2H_6$), DEP (diethyl-$^2H_{10}$), DMTP (dimethyl-$^2H_6$), DMDTP (dimethyl-$^2H_6$), DETP (diethyl-$^2H_{10}$), DEDTP (diethyl-$^{13}C_4$) were custom-synthesized by Cambridge Isotope Laboratories (Andover, MA, USA) with isotopic purities greater than 99%. Acetonitrile and methanol were purchased from Tedia Company Inc. (Fairfield OH, USA); formic acid (99%), sodium phosphate monobasic, and sodium phosphate dibasic were purchased from Fisher Scientific (Fair Town, NJ, USA); triethylamine (labeled 100% GC corrected assay; TEA) was purchased from Mallinckrodt Baker, Inc. (Phillipsburg, NJ, USA). Water was purified with a Nanopure Infinity system (Barnstead International, Dubuque, Iowa, USA). Strata™ X-AW 6 mL solid phase extraction (SPE) cartridges were purchased from Phenomenex (Torrance, CA, USA). Anhydrous K_2CO_3 was purchased from J. T. Baker (Phillipsburg, NJ, USA).

Preparation of standards

Isotopically labeled internal standards. A 10 μg mL^{-1} solution of the isotopically labeled internal standards (ISTD) was prepared in acetonitrile. A 5 mL aliquot of ISTD was diluted to 50 mL, using a Gilson 215 Liquid Handler (Gilson, Inc. Middleton, WI, USA), and 125 μL spiked into unknown samples, quality control (QC) materials, and calibration standards for all the orange juice samples analyzed. For dust, we manually spiked 62.5 μL of internal standard into all samples. The calculated concentration of each ISTD analyte was 62.5 ng g^{-1} in the dust and 62.5 ng mL^{-1} for the orange juice.

Calibration standards

Dust. Calibration samples were prepared daily by spiking 1.0 g of "blank" dust with 62.5 μL of the ISTD solution (1 μL mL^{-1}) and with 62.5 μL of the appropriate calibration spiking standard solution to produce concentrations ranging from 1 to 600 ng g^{-1}.

Orange juice. Calibration standards were prepared daily by spiking 2.0 ml of "blank" orange juice with 125 μl of the ISTD solution (1 μL mL^{-1}) and with 125 μL of the appropriate calibration spiking standard solution to produce concentrations ranging from 0.4 to 100 ng mL^{-1}.

Blank sample matrix

Dust. We collected vacuum cleaner bags from urban households where no pesticide applications had taken place for at least one year. We also collected dust from random households from which pesticide applications were unknown. The dust was then sieved by hand with a No.100 WS Tyler USA Standard Sieve.[25] The sieve was used to collect particles less than 150 μm and to remove large non-dust debris (*e.g.*, hair, carpet fibers). The smaller-diameter particles are known to adhere to hands and are a potential pathway of childhood exposure to pesticides.[26–28] Sieved samples yielded approximately 250 g dust for method development. Each dust sample was pre-screened for DAPs. Only dust samples that had DAP concentrations lower than our lowest calibration standard concentration were used. Initial pre-screening revealed that some of the dust samples had DAP concentrations greater than 600 ng g^{-1}.

The dust was cleaned to lower any endogenous DAP concentrations present. Briefly, the cleaning method consisted of aliquoting approximately 10 g dust samples into 50 mL centrifuge tubes (Whatman® Vecta Spin 20 Polypropylene Mesh 50 mL), rinsing the dust with deionized water (pH 7.0), and centrifuging each 10 g sample for 10 min at 2500 × *g*. This procedure was repeated four times for each sample. The dust was then transferred onto an aluminium pan, placed in an oven at 125 °C to dry overnight, homogenized in a blender, and pre-screened again. Dust samples were pooled to control for

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differences in dust composition from household to household. Pooled dust samples were stored in a −20 °C freezer. The cleaning procedure did not appreciably alter the dust characteristics, including extraction efficiency.

Orange juice. Several conventional and organic brands of orange juice were analyzed to prescreen for the presence of preformed DAPs. A common store-brand organic juice, with non-detectable levels of endogenous DAPs, was used as a blank matrix.

Quality control (QC) materials

Dust. Blank pooled dust was divided into three separate pools. The first pool (QC low pool) was spiked with the native standard stock solution to yield an approximate concentration of 20 ng g^{-1} for all analytes. The second pool (QC high pool) was spiked with the native standard stock solution to yield an approximate concentration of 100 ng g^{-1}, and the third pool was not spiked. The third pool was used as matrix material for calibration standards and blanks.

Orange juice. Blank orange juice was divided into three pools. The first pool (QC low pool) was spiked with the native standard stock solution to yield an approximate concentration of 5 ng mL^{-1} for all analytes. The second pool (QC high pool) was spiked with the native standard stock solution to yield an approximate concentration of 20 ng mL^{-1}, and the third pool was not spiked. After being screened for possible endogenous analytes, the third pool was used as matrix material for calibration standards and blanks.

All aliquots for QC pools for dust and orange juice were transferred into vials and stored at −20 °C until used. Each pool was characterized to determine the mean and the 95th and 99th control limits by consecutive analysis of at least 20 samples from each QC pool. After establishment of the control limits of the pools, individual QC samples contained within each analytical run were evaluated for validity by use of Westgard multirules.[29]

Sample preparation methods

All samples, reagents, and standards were brought to room temperature. Each analytical run consisted of seven calibration standards, two QC samples, one blank, and 36 unknown samples. Dust and orange juice samples as well were prepared according to the following procedures:

Dust. A 1 g aliquot of each dust sample was added to the top portion of the centrifuge filter, then we added 62.5 μL of the ISTD to all dust samples, including unknown samples, QC samples, and calibration standards. We added 3 mL phosphate buffer (pH 7.0) to each sample. Samples were then shaken to homogenize the contents and centrifuged at 2500 × *g* for 5 min. A second phosphate buffer extraction was performed repeating this process. After each extraction, the phosphate buffer solution was present in the bottom of the centrifuge tube, while the dust remained atop the filter. The extracts were then prepared according to the procedure described below.

Orange juice. We added 125 μL of ISTD to a 2 mL aliquot of each juice sample, including unknown samples, QC samples, and calibration standards. The samples were vortex-mixed to ensure the homogeneity of the solution.

Extraction and sample cleanup. The pH of each sample was adjusted to 2 by adding 100 μL formic acid to orange juice samples and 400 μL formic acid to dust buffer extracts. For sample cleanup, SPE cartridges were conditioned with 3 mL of 1% formic acid in methanol, followed by 3 mL of 1% formic acid in water. Samples were loaded into the cartridges and a vacuum was applied for 30 s. Cartridges were washed with 1% formic acid in methanol and a vacuum was applied for 5 min. The cartridges were eluted twice with a 2 mL 20% TEA in acetonitrile solution into clean centrifuge tubes. Samples were then concentrated to dryness by use of a TurboVap evaporator (Zymark Corporation, Hopkinton, MA) set at 40 °C and 15 psi. The dried residue was reconstituted in 1 mL of acetonitrile vortex mixed for 15 sec. K_2CO_3 (10 mg) and 50 μL 1-chloro-3-iodopropane were added to each sample to form chloropropyl esters of each DAP, and they were placed in a dry bath set at 60 °C for three hours. Samples were centrifuged for 5 minutes, decanted into new 15 mL centrifuge tubes, placed in the TurboVap (40 °C and 15 psi), and concentrated to dryness. The dried residue was reconstituted in toluene (100 μL), vortex-mixed, and transferred to autosampler vials. Derivatized extracts were analyzed by gas chromatography-tandem mass spectrometry (GC-MS/MS) according to the method of Bravo *et al.*[2]

Method validation

Limits of detection (LOD). A method LOD, based on the precision of measured values ($n = 10$), was calculated for each analyte as $3s_0$, where s_0 is the estimated standard deviation of measured concentration values as the concentration approaches zero. The s_0 was estimated as the *y*-intercept of a linear regression analysis of a plot of the standard deviation (in units of concentration) *versus* the concentrations of the three lowest standards.[30] We verified each analyte at its calculated LOD by extracting dust and juice samples that were spiked at the calculated concentration levels, injecting them into the instrument, and ensuring that the resulting peak signal-to-noise ratio was greater than three.

Extraction efficiencies. The extraction efficiency in each matrix was determined by spiking ten blank samples with a known amount of native standard spiking solution and extracting according to the method developed. A second set of ten blank samples was left unspiked and extracted concurrently and used as reference samples. We then spiked all 20 extracts with the same amount of ISTD prior to the derivitization step to correct for instrument variation and to yield a more accurate recovery calculation. The reference samples were then spiked with a known amount of the appropriate native standard solution, to serve as representative of 100% recovery. The response factors (area of the native analyte divided by the native of the labeled ISTD) of the recovery samples were divided by the response factors of the reference samples and reported as a percentage, to assess the extraction efficiencies of each analyte for each matrix. Because the use of an ISTD accounts for any losses during

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extraction, extraction efficiency is not critical for the final method, but it served as a guide for method optimization.

Accuracy. The percent deviation from the mean value of the six samples, compared to the mean of the QC pool ($n = 30$) concentration, described the accuracy of the method.[6] The accuracy for both dust and juice was tested at two different concentrations (20 ng g^{-1} and 100 ng g^{-1} for dust and 5 ng mL^{-1} and 20 ng mL^{-1} for juice).

Precision. The precision of the method was determined by calculating the percent relative standard deviation (%RSD) of repeat measurements ($n = 30$) of quality control materials at different concentrations (20 and 100 ng g^{-1} for dust and 5 and 20 ng mL^{-1} for juice). Six samples were freshly prepared each day for five different days and analyzed, according to the method described, to evaluate the within-day and between-day variation.

Conversion of parent pesticide to DAPs during pre-analytic and analytic processes. To assess any analytic or pre-analytic hydrolysis of intact pesticides in the samples to their corresponding hydrolytic DAPs, we spiked matrix samples with concentrations of pesticides that may be encountered in environmental matrices (125 and 250 ng g^{-1}). Azinphosmethyl and chlorpyrifos were spiked into the matrix samples and were used as surrogates for all dimethyl-substituted and diethyl-substituted OP insecticides, respectively. An aliquot of each sample was analyzed immediately after spiking and mixing, and other aliquots were stored under three different conditions (−70 °C, 4 °C and room temperature) and reanalyzed after 1 day, 1 week and 1 month. The percentage of hydrolysis encountered in both the analytic and pre-analytic (storage) processes were calculated as the concentration of DAPs formed divided by the concentration of pesticide spiked (in molar units) multiplied by 100.

Results

Method LODs for dust and orange juice are displayed in Table 1. For dust, the LODs ranged from 4.1–10.4 ng g^{-1} while for orange juice, LODs ranged from 0.2–3.0 ng mL^{-1}.

Extraction efficiencies for both media are presented in Tables 2a and 2b. For dust, extraction recoveries ranged from 23–91%, with higher recoveries for the diethyl substituted DAPs. Total recoveries for dust were similar for DMP, DMTP, and DEP at low and high concentrations, while better recoveries were obtained at the lower concentrations for DMDTP and DEDTP. Analyte recovery did not decrease at lower concentrations; in fact, several DAPs (DEP, DMTP, DETP, DEDTP) had higher recovery at the lower concentration than at the higher concentration. For orange juice, the recoveries for all six DAPs were above 40%. DMP had lower recoveries of 43% (at 5 ng mL^{-1}) and 41% (at 20 ng mL^{-1}).

Table 1 Limits of detection for dust and orange juice

Analyte	Dust/ng g^{-1}	Orange juice/ng mL^{-1}
DMP	4.1	0.2
DMTP	2.8	0.4
DMDTP	9.9	0.4
DEP	10.4	3.0
DETP	5.8	0.9
DEDTP	5.2	0.5

Table 2 a. Extraction efficiencies for dust ($n = 10$). b. Extraction efficiencies for orange juice ($n = 10$)

	%	
Analyte	20 ng g^{-1} (mean ± SD)	100 ng g^{-1} (mean ± SD)
DMP	25 ± 5	23 ± 5
DMTP	65 ± 8	69 ± 1
DMDTP	78 ± 28	51 ± 2
DEP	89 ± 9	89 ± 1
DETP	79 ± 11	91 ± 1
DEDTP	63 ± 9	46 ± 1
	%	
Analyte	5 ng mL^{-1} (mean ± SD)	20 ng mL^{-1} (mean ± SD)
DMP	43 ± 9	41 ± 3
DMTP	73 ± 12	65 ± 3
DMDTP	52 ± 17	59 ± 3
DEP	85 ± 13	72 ± 3
DETP	61 ± 8	44 ± 2
DEDTP	53 ± 21	52 ± 3

The accuracy for both matrices is shown in Table 3. Overall, the percent deviation from the spiked concentrations was less than 15%. Tables 4a and 4b display the precision of our method. In general, within-day and between-day variations were reasonably comparable for dust. Overall RSDs for DM DAPs ranged from 12–59% and 17–22% at low and high concentrations, respectively. For DE DAPs, overall RSDs ranged from 14–33% and 14–22% at the low and high concentrations, respectively. There were two instances in which overall variation was lower than within-day variation, suggesting that between-day variation was close to zero. In these two cases, the between-day mean square was less than the within-day mean square, and between-day variance was set to zero; thus, the standard deviation for between-day was also set to zero.[31] For orange juice at the lower concentration (5 ng mL^{-1}), only one of the six DAPs (DEP) had a total RSD that exceeded 15%. All analytes had total RSD values less than 15% at the higher concentration analyzed (20 ng mL^{-1}). The within-day variation ranged from 4–19% and 4–12% for 5 ng mL^{-1} and 20 ng mL^{-1}, respectively. The between-day variation ranged from 6–24% and 2–15% for 5 ng mL^{-1} and 20 ng mL^{-1}, respectively. The between-day variation for DMDTP at the lower concentration was set at zero for dust for the same reasons previously mentioned.[31]

Azinphos methyl did not appear to hydrolyze to DMP or DMTP during the analytic process in either dust or orange juice samples. In orange juice, 2.5–13.3% of the spiked chlorpyrifos was hydrolyzed to DETP during the analytic and pre-analytic (storage) process. In dust, <1% of the total spiked concentration of chlorpyrifos was hydrolyzed to DETP during the analysis. We did not detect any DEP resulting from hydrolysis of chlorpyrifos.

Household dust samples were collected from urban and agricultural homes in northern and central California, respectively, and analyzed for DAPs using the method described herein. DEP was detected in 80% of the samples tested while DMP was

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Table 3 Percent deviations from spiked concentrations in dust and orange juice

	Dust samples % deviation (mean ± SD) $n = 6$		Juice samples % deviation (mean ± SD) $n = 6$	
Analyte	20 ng g^{-1} pool	100 ng g^{-1} pool	5 ng mL^{-1} pool	20 ng mL^{-1} pool
DMP	3 ± 32	15 ± 7	3 ± 8	3 ± 3
DMTP	9 ± 15	8 ± 6	5 ± 8	2 ± 3
DMDTP	19 ± 28	1 ± 17	13 ± 8	2 ± 5
DEP	1 ± 15	1 ± 12	2 ± 16	10 ± 12
DETP	6 ± 14	5 ± 8	5 ± 5	2 ± 2
DEDTP	5 ± 22	1 ± 13	1 ± 10	3 ± 4

detected in 60% and 45% of the agricultural and urban samples, respectively. Other DAPs were less frequently detected.

Discussion

Table 5 shows the LODs for DAPs in various media, including our method for dust and orange juice. Overall, our method LODs for dust were lower than those reported for produce[21] (except for DEP and DETP) and higher than those reported for urine and apple juice.[2,19] LODs for DM DAPs were noticeably lower than those for DE DAPs. Because LODs are influenced by volume or mass of media analyzed, our low dust sample mass of 1 g may account for the higher LODs compared with other media. For orange juice, although the recovery for DMP was relatively low, the LOD determined for DMP was the lowest, at 0.2 ng mL^{-1}, of all six DAPs, demonstrating that the instrument may be particularly sensitive for DMP. All other DAPs had LODs ranging from 0.4 ng mL^{-1} (DMTP, DMDTP) to 3.0 ng mL^{-1} (DEP). Similarly, orange juice DM DAP LODs were noticeably lower than those for the DE DAPs, which may also be an indicator of instrument sensitivity. All LODs were lower than the LODs found for apple juice, using the method described in Lu *et al.*,[19] excluding DEP, which had the same LOD at 3.0 ng mL^{-1}. Given that both labeled ISTDs and GC-MS/MS instrumentation were used in this method and in the method developed by Lu *et al.*,[19] we conclude that the SPE method used for this method for DAP extraction may be a more selective preparative method than liquid–liquid extraction.

For dust samples, the evaporation times were lengthy, in excess of 2 hours, after derivatization. This long evaporation step may result in a greater percentage of the more volatile DAPs chloropropyl esters evaporating from the sample before analysis, although this error should be minimized by using labeled internal standards. For the dust method, precision was poorer at the lower concentration, perhaps because the concentrations of these DAPs were near their respective LODs. In addition, obtaining a homogenous, representative sample of dust is a difficult process. Since dust is a solid, relatively heterogeneous matrix, great care was taken to ensure that the sample taken was as representative as possible of the mass of matrix available. However, the smaller the mass of the dust sample analyzed, the less homogeneous the sample becomes. The lack of homogeneity may have a significant impact on the method's precision and accuracy among discrete samples. The aliquoted sample (1 g samples) may not be representative of the larger dust batch, and it is possible that some of the analytes may have preferentially adhered onto certain dust particles and hence made the sample less homogeneous.

Only DMTP and DETP resulted in overall RSDs less than 15%; all other DAPs ranged from RSD of 23% to 59%. At the higher QC concentration (100 ng g^{-1}), the overall, calculated RSDs were very similar for all DAPs, ranging from 14% to 22%. The higher RSDs determined at the QC low concentration may be partially attributable to the difficulty in homogenizing the spiked dust pools. Between-day and within-day variation for dust mirrored overall variation, showing higher RSDs at the lower QC concentration over the higher QC concentration analyzed. According to the QC results of orange juice, the variation decreased at the higher concentrations for all analytes except DEDTP, which remained at 14%. The higher variation seen at the lower concentration can be most likely attributed to the concentrations of these DAPs near their respective LODs. Between-day and within-day variation was similar for all DAPs at the lower level, excluding DMDTP, which had a between-day variation of near zero compared to its within-day variation at 10%. Between-day and within-day variation at the higher end was very similar for all analytes with the exception of DEDTP (15% for between-day and 5% for within-day). The similar RSDs obtained for these analytes indicate a similar behavior pattern of the respective DAPs with sample preparation performed either on the same day or over a period of several days.

One of the biggest limitations of many methods in which environmental degradates are measured is that the hydrolysis of the parent chemical to the degradate during storage or analysis is not evaluated, and the resulting data, if warranted, are not appropriately corrected for inadvertent hydrolysis. While validating our method, we carefully evaluated this potential hydrolysis. We found no detectable levels of hydrolysis products of azinphos methyl, a dimethyl substituted OP pesticide. For the diethyl substituted pesticide, chlorpyrifos, the stability was good in dust samples with less than 1% hydrolyzing; however, chlorpyrifos appeared to be more labile in the acidic juice matrix. Thus, for dust and for dimethyl substituted pesticides in orange juice, our data indicate that we are not overestimating the concentrations of DAPs present in the environmental matrices tested.

Because we frequently detected DEP and DMP in dust samples tested, our method is suitable for measuring DAPs in dust samples collected in the United States. A more detailed description of the dust data and its relation to urinary DAPs from the California exposure pilot study will be published separately.

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Table 4 a. Relative standard deviations for dust measurements. b. Relative standard deviations for orange juice measurements

	QC Low (20 ng mL^{-1})				QC High (100 ng mL^{-1})			
		% RSD				% RSD		
Analyte	Mean concentration ($n = 30$)	Overall ($n = 30$)	Between day ($n = 5$)	Within day ($n = 6$)	Mean Concentration ($n = 30$)	Overall ($n = 30$)	Between day ($n = 5$)	Within day ($n = 6$)
DMP	18.19	23	18	17	97.41	20	19	11
DMTP	25.14	12	8	9	141.33	17	14	12
DMDTP	4.79	59	46	42	30.03	22	0[a]	22
DEP	36.44	23	18	16	158.16	20	19	10
DETP	18.86	14	11	10	100.63	14	10	10
DEDTP	7.68	33	20	28	29.19	22	0[a]	23

[a] Within-day variation was higher than overall variation; hence between-day variation was minimal and set to zero.

	QC Low (5 ng mL^{-1})				QC High (20 ng mL^{-1})			
		% RSD				% RSD		
Analyte	Mean concentration ($n = 30$)	Overall ($n = 30$)	Between day ($n = 5$)	Within day ($n = 6$)	Mean concentration ($n = 30$)	Overall ($n = 30$)	Between day ($n = 5$)	Within day ($n = 6$)
DMP	4.80	12	9	9	16.74	5	4	4
DMTP	6.66	7	6	5	30.91	6	4	4
DMDTP	0.85	10	0[b]	10	3.27	6	2	6
DEP	6.84	29	24	19	13.88	13	7	12
DETP	8.10	7	6	4	32.07	7	6	4
DEDTP	1.37	14	11	9	5.43	14	15	5

[b] Within-day variation was higher than overall variation; hence between-day variation was minimal and set to zero.

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Table 5 Limits of Detection for dialkylphosphates in various matrices.[2,19,21]

	Matrix				
Analyte	Urine/ng mL^{-1}	Produce/ng g^{-1}	Dust/ng g^{-1}	Apple juice/ng mL^{-1}	Orange juice ng mL^{-1}
DMP	0.6	5	4.1	1	0.2
DMTP	0.2	5	2.8	1	0.4
DMDTP	0.1	10	9.9	1	0.4
DEP	0.2	5	10.4	3	3.0
DETP	0.1	5	5.8	1	0.9
DEDTP	0.1	10	5.2	1	0.5

Conclusion

In order to assess the contribution of preformed DAP exposure to humans to urinary excretion of DAPs, a variety of matrices should be considered. We have found DAPs in both household dust and juices (when screening for blank dust and orange juices); thus, these methods can be used to evaluate the potential exposure to preformed DAPs and its possible relation to the DAP concentrations observed in urine when assessing exposure to OP pesticides. Assuming that exposure to preformed DAPs occurs and that they are excreted unchanged, we should be able to more accurately estimate exposure to OP pesticides by understanding the total contribution to urinary DAP concentrations by exposure to the preformed DAPs. Although the acute affects of exposures to OP pesticides are well understood, we have yet to understand any toxicity related to preformed DAP exposures; however, we expect the toxicity of DAPs to be less than their parent pesticides. Thus, differentiating urinary contributions from preformed DAP and OP pesticides is critical for epidemiologic studies.

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