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Dialkylphosphates (DAPs) in Fruits and Vegetables May Confound Biomonitoring in Organophosphorus Insecticide Exposure and Risk Assessment

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Trace residues of organophosphorus (OP) pesticides are associated with fruits and vegetables that have been sprayed with those OP pesticides to guard against insect pests. Human dietary exposure to these OP pesticides is commonly estimated by measuring the amount of OP metabolites in urine, assuming a stoichiometric relationship between a metabolite and its parent insecticide. Dialkylphosphates (DAPs) are the OP metabolites that are most often used as markers in such biomonitoring studies. However, abiotic hydrolysis, photolysis, and plant metabolism can convert OP chemicals (OP residues) to DAP residues on or in the fruits and vegetables. To evaluate the extent of these conversions, OPs and DAPs were measured in 153 produce samples. These samples from 2 lots were known to contain OP insecticide residues based on routine monitoring by California producers and shippers. A total of 12 OPs were quantified, including mevinphos, naled, acephate, methamidophos, oxidemeton-methyl, azinphos-methyl, dimethoate, malathion, methidathion, phosmet, chlorpyrifos, and diazinon. All OP insecticide residues were below their respective residue tolerances in 2002−2004. A total of 91 of 153 samples (60%) contained more DAP residues than parent OPs. The mean mole fractions [DAPs/(DAPs + OPs)] for the first and second lots of produce were 0.62 and 0.50, respectively, and the corresponding geometric means were 0.55 and 0.34. The corresponding mean mole ratios (DAPs/OP) were 7.1 and 3.4, with geometric means of 2.1 and 0.9. Any preformed DAPs ingested in the diet that are excreted in urine may inflate the estimated absorbed OP insecticide doses in occupational and environmental studies. In subsequent prospective studies, time-dependent production of dimethylphosphate (DMP) and dimethylthiophosphate (DMTP) in strawberries and leaves following malathion sprays occurred concomitant with the disappearance of the parent insecticide and its oxon. DAPs are more persistent in plants and produce at routinely measured levels than their parent OP insecticides.

KEYWORDS: Dialkylphosphates; biomarker; organophosphorus insecticide; exposure assessment; risk assessment; food safety

INTRODUCTION

Organophosphorus (OP) insecticides are widely used in the United States and the rest of the world (*1*). Approximately 60 million pounds of OP insecticides are applied to approximately 60 million acres of U.S. agricultural crops annually (*2*). California Pesticide Use Reports provide current estimates of OP insecticide use and evidence of the continuing utility of this important class of pesticides (*3*). In 2001, about 6.3 million pounds were reportedly used (primarily in crop protection). In 2006, the use rate was 5.4 million pounds (*3*). The general population is principally exposed to OP insecticides and dialkylphosphates (DAPs) (*4*) through consuming foods containing trace levels of OP insecticide residues (*5*). Residential uses of indoor and garden products, most notably chlorpyrifos, have been reduced as an agreement between the U.S. Environmental Protection Agency (EPA) and Dow AgroSciences (*6*).

The exposure of the general population to low levels of OP insecticide residues has been amply demonstrated through a number of biomonitoring studies (*7*−*13*). These studies provide

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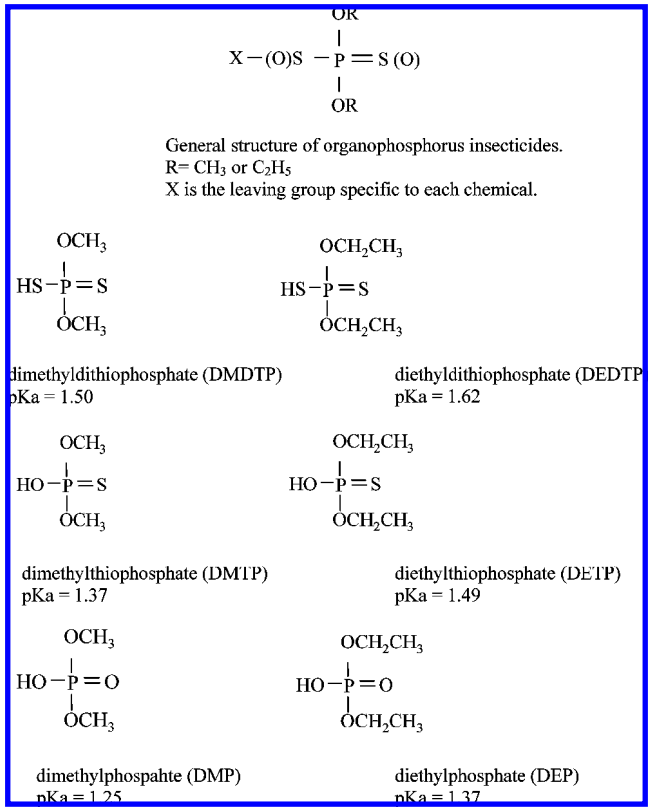

Figure 1. Structures of OP insecticides and DAPs and DAP ionization constants.

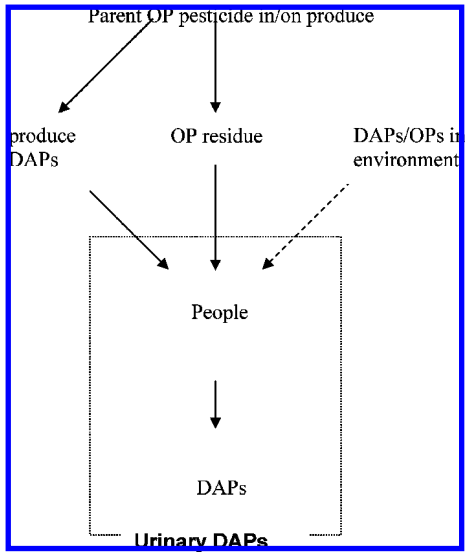

Figure 2. Source of urinary DAPs.

the present basis for estimating the potential aggregate and cumulative OP insecticide exposure. Validation of the accuracy of such data is important for the responsible administration of the aggregate and cumulative exposure criteria of the Food Quality Protection Act (FQPA) of 1996.

All OP insecticides share a similar general structure (**Figure 1**). Hydrolysis and oxidation in the environment (*14*) or in plants (*14*−*19*) and animals (*20*−*22*) are well-established transformations of OP insecticides. Most hydrolysis products include *O*,*O*-dimethyl- or *O*,*O*-diethylphosphorus derivatives, i.e., DAPs, including dimethylphosphate (DMP), dimethylthiophosphate (DMTP), dimethyldithiophosphate (DMDTP), diethylphosphate (DEP), diethylthiophosphate (DETP), and diethyldithiophosphate (DEDTP). The most prominent metabolites

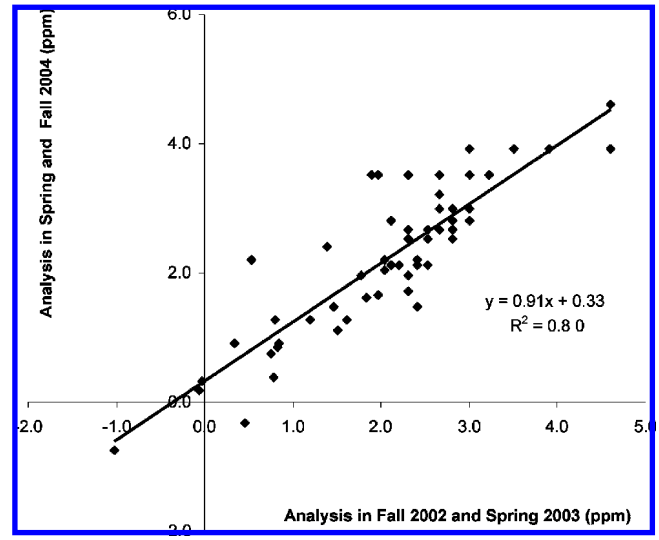

Figure 3. Stability of OP insecticides during frozen storage.

in human urine are DMTP and DMP from dimethyl-substituted OP insecticides and DETP and DEP from diethyl OP-substituted pesticides (*9*−*13, 23*−*25*).

Although there are data indicating that OP residues break down on fruits and vegetables (*26*), there are no published data available on the degree to which DAPs coexist with pesticides on contaminated produce (**Figure 2**). This research reports levels of DAPs in produce associated with their parent OP insecticides in produce known to contain an OP residue. Additional studies of malathion degradation and the formation of biomarkers in strawberries are included to support the residue survey.

MATERIALS AND METHODS

Materials. Produce samples (N = 153) representing 44 different kinds of produce were obtained from two similar lots at primuslabs.com, Santa Maria, CA. All samples had been previously tested for pesticide residues by growers or shippers prior to entering the channels of trade. Each produce sample contained at least one OP residue. Samples without OP residues were excluded from the study. The specific crops are not reported because pesticide use data for each type of sample were not available (application date, rate, lapsed time, weather, etc.). The data represent premarket produce OP residues and corresponding DAP levels. The samples were stored frozen at primuslabs.com for up to 6 months before analysis. The first lot of 77 samples was analyzed between September 2002 and March 2003. The second lot of 76 samples from the next growing season was analyzed from March to October 2004.

Fresh strawberries were collected in Irvine, CA in May 2003 and in Santa Maria, CA in April 2004. The Irvine samples were collected 1 and 2 weeks after routine malathion applications (2 lb of active ingredient/acre). Each collection consisted of six samples. The Santa Maria strawberry samples were picked 1, 3, 6, and 9 days after malathion application (1 lb of active ingredient/acre). Samples were collected in triplicate and stored forzen. Frozen strawberry samples were sent to cooperating laboratories for malathion (and malaoxon) and DAPs analysis.

An additional series of fresh strawberries and strawberry leaves were collected at darensberries.com, Santa Maria, CA, following application of malathion (1 lb of active ingredient/acre) in August 2007. Berries (1 lb boxes) were randomly sampled from the commercial harvest on days 3 and 21. Leaves were collected for residue analysis on days 2, 8, and 20. Malathion and its oxon, malathion mono- (MMA) and diacid (MDA), and DMP, DMTP, and DMDTP were measured in acetone/water (2:1) extracts of tissue homogenates (2.6 ± 0.1 mL of extract/g of berries and 5.1 ± 1.0 mL of extract/g of leaves) (**Table 5**).

Analysis of OP Insecticides. OP residues in produce were measured at primuslabs.com. Standard Food and Drug Administration Pesticide

Table 1. DAP Residue in the 153 Fruit and Vegetable Samples Measured in 2003 and 2004[a]

		first batch samples	second batch samples	all samples
DAPs/OPs (mole ratio)	range	0.1−73	0.02−33	0.02−73
	mean	7.1	3.4	5.2
	geomean	2.1	0.9	1.4
	median	1.8	1.5	1.7
	percent of ratio ≥1 (%)	68	53	60
preformed DAPs	range (μmol/g)	1.0×10^{-4}−0.022	8.0×10^{-5}−0.022	8.0×10^{-5}−0.022
	mean (μmol/g)	2.2×10^{-3}	1.8×10^{-3}	2.0×10^{-3}
	geomean (μmol/g)	8.3×10^{-4}	7×10^{-4}	7.4×10^{-4}
OPs	range (μmol/g)	3.0×10^{-5}−8.2×10^{-3}	9×10^{-5}−8.9×10^{-3}	3×10^{-5}−8.9×10^{-3}
	mean (μmol/g)	9.0×10^{-4}	1.5×10^{-3}	1.2×10^{-3}
	geomean (μmol/g)	3.9×10^{-4}	7.0×10^{-4}	5.3×10^{-4}

[a] Mole ratio of DAPs to parent OPs = ∑DAPs (μmol/g of produce) ÷ ∑parent OPs (μmol/g of produce). Mole fraction of preformed DAPs = ∑measured DAPs (μmol/g) ÷ [∑DAPs (μmol/g) + ∑parent OPs (μmol/g)].

Table 2. OP Insecticides and Corresponding DAPs Metabolites in Produce

			mole fraction of preformed DAP[a]		mole ratio of DAP/OP[b]	
OP insecticide[c]	$t_{1/2}$ in water at pH 7 (day)[d]	n	arithmetic mean	geometric mean	arithmetic mean	geometric mean
acephate, methamidophos	NA[e]	6	0.14	0.07	0.27	0.09
dimethoate/omethoate	NA	16	0.30	0.17	1.02	0.27
oxydemeton-methyl	−	7	0.35	0.28	7.12	0.61
acephate	46	3	0.54	0.53	1.41	1.21
diazinon	70	6	0.54	0.41	5.09	1.30
phosmet	0.5	16	0.58	0.52	1.89	1.37
chlorpyrifos	35	22	0.60	0.53	3.54	1.67
dimethoate	0.5[f]	12	0.62	0.55	4.97	2.05
azinphos-methyl	10	9	0.81	0.81	7.00	4.94
malathion	3	23	0.79	0.76	11.06	5.61

[a] Mole fraction of preformed DAP = moles of DAP ÷ (moles of OP + moles of DAPs). [b] Mole ratio of DAP/OP = moles of DAPs ÷ moles of OP. [c] Those samples with three or more OP residues are not included because the DAPs could not be assigned to certain OP. [d] Half-lives of each OP in water at pH of 7 (*35*). [e] NA, not applicable; −, data is not available. [f] Half-life for dimethoate was measured at pH of 6 (*35*).

Analytical Manual (*27*) residue methods (PAM 242.1) were used. To evaluate the stability of OPs in produce samples during storage and handling, 65 samples (27 from the first batch and 38 from the second) were re-analyzed for OP insecticides about 6 months after the initial analysis.

Analysis of DAPs. Approximately 100 g of each frozen produce sample was thawed and weighed before transfer to a blender (Oster, Sunbeam Products, Inc., Boca Raton, FL). A total of 200 mL of deionized water at room temperature was added. The mixture was blended for 15 s at high speed, followed by a settling period of 15 s. The blending process was repeated 2 more times. The mixture was then transferred to a 500 mL centrifuge bottle and centrifuged at 10 000 g/min (8600 rpm) for 30 min at 4 °C (Sorvall Instrument, Model RC5C). The supernatant was decanted and weighed. A 20 mL aliquot of supernatant was removed, frozen, and later shipped on dry ice to Pacific Toxicology Laboratories, Chatsworth, CA, for analysis of the DAPs. Randomized sets of samples were submitted, and analysts were blinded to sample identification and study objectives.

DAPs (DMP, DMTP, DMDTP, DEP, DETP, and DEDTP) were determined using techniques similar to those developed for analysis of human urine specimens (*28*−*33*). A total of 1 mL of supernatant was freeze-dried, resuspended in acetone containing 3-benzyl-1-*p*-tolyltriazine, and held overnight at room temperature. The benzyl derivatives with an internal standard (fenthion) were extracted from an aqueous salt solution using cyclohexane. All derivatives were analyzed using a gas chromatography−flame photometric detector (GC−FPD). DAPs were reported as ppb (μg/kg wet weight produce). The limits of quantification (LOQ) for DMP, DMTP, and DMDTP were about 5, 5, and 10 ppb. Recoveries from spiked specimens were about 99% (DMP), 106% (DMTP), and 112% (DMDTP). The LOQs for DEP, DETP, and DEDTP were also about 5, 5, and 10 ppb. The corresponding recovery rates were approximately 108, 90, and 108%. All produce known to have been treated with an OP insecticide contained DAP residues. Fortification of untreated macerated strawberries with malathion and chlorpyrifos did not yield detectable oxons or corresponding DAPs.

Additional spiked samples were prepared from fresh, pesticide-free strawberries (Driscoll's, Watsonville, CA). These samples consisted of (a) unfortified controls, (b) low and high spike levels of DMP, DMTP, and DMDTP, and (c) low and high spike levels of DEP, DETP, and DEDTP. Recoveries of DMP, DMTP, and DMDTP from fresh strawberries were 76.4 ± 3.1, 109.6 ± 6.1, and 96.5 ± 4.1%, respectively. DEP, DETP, and DEDTP recovery was 69.3 ± 5.4, 106.9 ± 8.0, and 87.2 ± 24.4, respectively. Analytical samples were not corrected for recovery.

The 2007 analyses of leaves and berries followed a revised procedure. Acetone/water (2:1) extracts of tissue homogenates were processed without further freeze-drying or concentration because sufficiently large amounts of analyte(s) permitted direct analysis (**Table 5**). The derivatization scheme for analysis of MMA and MDA was as published previously with minor modification (*34*).

Calculations. The algorithms used for determination of total DAPs in produce, the mole ratio of total DAPs to the parent OPs, and the mole fraction of preformed DAPs were as follows:

$$\text{measured DAP } (\mu\text{g/g of produce}) = \text{DAP concentration } (\mu\text{g/mL}) \times \text{supernatant volume (mL)} \div \text{weight of produce (g)}$$

$$\text{measured DAP } (\mu\text{mol/g of produce}) = \text{measured DAP } (\mu\text{g/g of produce}) \div \text{formula weight of DAP}$$

$$\text{mole ratio of DAPs to parent OPs} = \sum\text{DAPs } (\mu\text{mol/g of produce}) \div \sum\text{parent OPs } (\mu\text{mol/g of produce})$$

$$\text{mole fraction of preformed DAPs} = \sum\text{measured DAPs } (\mu\text{mol/g}) \div [\sum\text{measured DAPs } (\mu\text{mol/g}) + \sum\text{parent OPs } (\mu\text{mol/g})]$$

where ∑DAPs represents either measured dimethyl or diethyl phosphates corresponding to the parent OP insecticide residue.

Statistical Analysis. Arithmetic averages, medians, and geometric means of the samples were calculated. The correlation between the results of the OP analysis performed initially and after 6 months being

stored frozen was examined by linear regression analysis. Differences were analyzed using a paired *t* test. Regression analysis was also used to examine the relationship of OP half-lives in water, as well as the corresponding geometric means of the mole ratio of DAPs/OPs. Table Curve 2D (version 5.0; www.systat.com) was used to explore best fitting regression functions describing the relationship of OP half-life and DAP/OP mole ratio.

RESULTS

OP Insecticides and DAPs in Fruits and Vegetables. The results of produce analysis for trace residual OP pesticides and DAPs are summarized in **Table 1**. A total of 12 insecticides were identified from the 153 produce samples known to contain OP residue (**Table 2**). The insecticides were acephate, azinphos-methyl, chlorpyrifos, diazinon, dimethoate/omethoate, malathion, methamidophos, methidathion, mevinphos, naled, oxidemeton-methyl, and phosmet. At least 1 of the 12 OP insecticides was known to occur in each sample. Because the produce had been frozen for extended periods (up to 6 months), the stability of the residue was examined by correlating the initial OP pesticide level with the pesticide level when the samples were prepared for DAP analysis. The resulting R^2 was 0.80 (**Figure 3**). A paired *t* test showed that there was no significant difference between the two separate OP insecticide residue analyses ($p > 0.05$), indicating good stability of the OP insecticides in the frozen produce.

By inspection, the concentrations of the OP insecticide and DAP residues on the produce did not appear to be normally distributed. Consequently, geometric means or medians were reported. The OP insecticide residue levels in the first batch of produce ranged from 3.0×10^{-5} to 8.2×10^{-3} μmol/g. The arithmetic mean was 9.0×10^{-4} μmol/g, with a geometric mean of 3.9×10^{-4} μmol/g. The range for the sum of the corresponding DAP concentrations was 1.0×10^{-4} to 2.2×10^{-2} μmol/g. The arithmetic mean was 2.2×10^{-3} μmol/g, with a geometric mean of 8.3×10^{-4} μmol/g. The mole fraction of preformed DAPs/(OPs + DAPs) on produce thus ranged from 0.06 to 0.99, with a mean of 0.62 and geometric mean of 0.55. The mole ratio of DAPs to OP insecticides on the produce in the first batch of samples ranged from 0.1 to 73, with a mean of 7.1 and geometric mean of 2.1. A total of 51 of the 77 produce samples (68%) contained more DAP residues than parent OPs.

The second batch of produce samples contained OP residues ranging in concentration from 9.0×10^{-5} to 8.9×10^{-3} μmol/g, with an arithmetic mean of 1.5×10^{-3} μmol/g and a geometric mean of 7.0×10^{-4} μmol/g. The DAP concentrations ranged from 8.0×10^{-5} to 2.2×10^{-2} μmol/g, with an arithmetic mean of 1.8×10^{-3} μmol/g and geometric mean of 7.0×10^{-4} μmol/g. The mole fraction of preformed DAPs/(OPs + DAPs) on these produce samples ranged from 0.03 to 0.97, with a mean of 0.50 and geometric mean of 0.34. The mole ratio of DAPs to OPs in the second batch of samples ranged from 0.02 to 33, with a mean of 3.4 and geometric mean of 0.9.

The retrospective residue survey lacks data concerning formulations, application rates, preharvest intervals, conditions at harvest, handling, and storage conditions that could promote hydrolysis prior to the analysis of the produce. The two batches of produce that were obtained from frozen storage represented successive growing seasons in southern California. If the results are combined, 91 of 153 samples (60%) contained more DAP residues than parent OP insecticides. The mole fraction of preformed DAPs ranged from 0.02 to 0.99, with an arithmetic mean of 0.56 and geometric mean of 0.43. The mean concentrations of OP insecticide residues and DAPs in the produce were 0.0012 and 0.0020 μmol/g, respectively. The geometric means for concentrations of OPs and DAPs in produce were 0.000 53 and 0.000 74 μmol/g, respectively.

Table 3. Mole Fraction of DAP and Mole Ratio of DAP/OP in the Produce with ≥3 Samples

		mole fraction of preformed DAP[a]		mole ratio of DAP/OP[b]	
crop[c]	*n*	arithmetic mean	geometric mean	arithmetic mean	geometric mean
1	3	0.06	0.06	0.06	0.06
2	4	0.22	0.16	0.38	0.21
3	8	0.26	0.20	0.56	0.29
4	3	0.34	0.26	0.98	0.44
5	16	0.39	0.23	1.89	0.46
6	5	0.43	0.33	0.98	0.62
7	4	0.47	0.34	1.29	0.71
8	7	0.54	0.45	2.30	1.18
9	4	0.58	0.55	1.92	1.44
10	6	0.51	0.42	12.94	1.44
11	5	0.52	0.40	10.80	1.51
12	8	0.57	0.52	3.07	1.51
13	5	0.60	0.59	1.71	1.54
14	7	0.62	0.59	2.37	1.76
15	5	0.67	0.66	2.61	2.19
16	8	0.69	0.68	2.37	2.23
17	6	0.79	0.78	9.63	5.15
18	12	0.81	0.74	11.49	6.17
19	7	0.84	0.84	9.34	6.55
20	5	0.87	0.86	10.12	7.50

[a] Mole fraction of preformed DAP = moles of DAP ÷ (moles of OP + moles of DAPs). [b] Mole ratio of DAP/OP = moles of DAPs ÷ moles of OP. [c] Those crop categories refer to specific types of produce; those with less than three samples are not listed.

The mole fraction of preformed DAPs and mole ratio of DAPs to OPs seemed to be chemically specific. The produce treated with diazinon, phosmet, chlorpyrifos, azinphos-methyl, and malathion always had higher concentrations of DAPs than OP insecticides (geometric mean of the mole ratio of DAPs/OPs > 1). Similarly, some produce (**Table 3**), regardless of pesticide treatment, always had higher concentrations of DAPs than OP insecticides (geometric mean of mole ratio of DAPs/OPs > 1).

The regression analysis of hydrolysis half-lives (*35*) and the corresponding geometric means of the mole ratios shown in **Figure 4** is consistent with hydrolysis being the most important transformation of the OP insecticides. Because preharvest intervals and laboratory storage times were both unknown, this measure of stability cannot be reliably related to particular produce or pesticides. However, storage stability as measured by the parent OP supports the concept that DAPs did not occur as a result of frozen storage.

Malathion and DAP Residues in Fresh Strawberries. We prospectively studied the fate of malathion, malaoxon, and the DAPs in fresh strawberries from two California farms in 2003 and 2004. We measured analytes without the uncertainty introduced by frozen storage. At the Santa Maria site in 2004, DMTP was the major DAP immediately following the malathion application (1 lb/acre). When the strawberries could be picked for consumption at the end of preharvest interval on day 3, the DAP/malathion mole ratio was more than 3. By day 9, the ratio was 8.7 at Santa Maria. Higher residues were measured in 2003 at Irvine. Malathion residues on strawberries at Irvine in 2003 decreased from 0.17 nmol/g at 1 week to 0.07 nmol/g at 2 weeks ($p < 0.05$). The molar sum of malathion (malaoxon was not quantifiable; LOQ = 0.01 ppm) and DAPs was not changed ($p > 0.05$) during that period (**Table 4**). After 2 weeks, the mole ratio of DAPs to malathion was nearly 50. The DMDTP residue

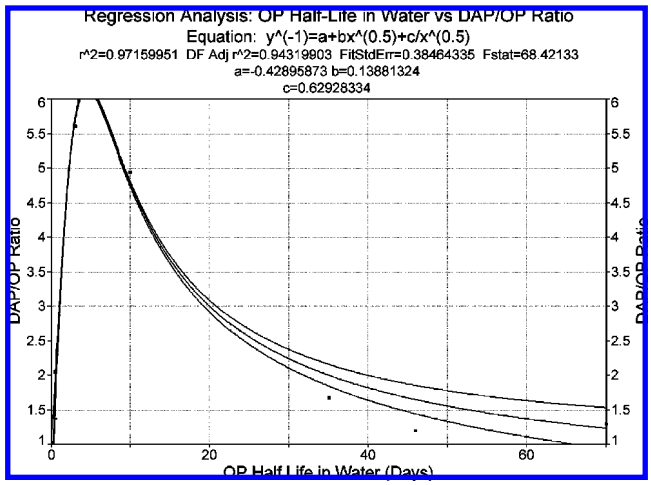

Figure 4. Nonlinear regression of OP half-life in water versus geometric mean of DAP/OP mole ratio in produce. The 95% confidence intervals are shown. The figure includes paired half-life and mole ratio values from **Table 2** for acephate, diazinon, phosmet, chlorpyrifos, dimethoate, azinphos-methyl, and malation.

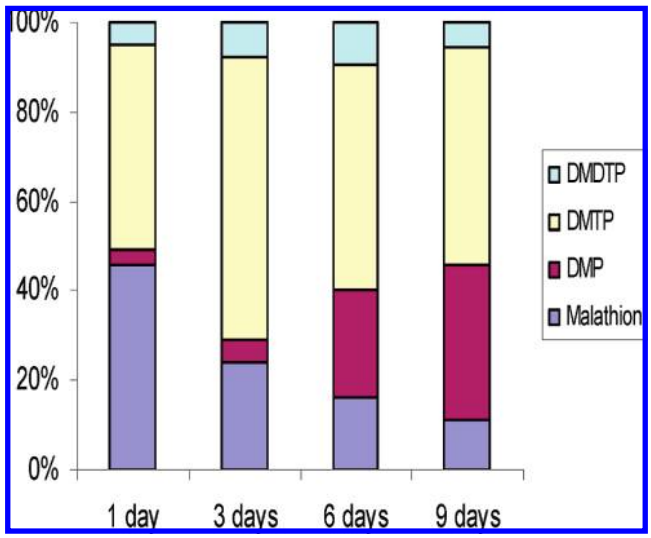

Figure 5. Percent contribution of malathion and DAP residues to the total residues in strawberries collected from Santa Maria, CA, 1−9 days after routine malathion treatment. Fruit residues were converted into nmol/g fruit. Each segment represents the percentage of each individual compound in the sum of all four residues (including $^1/_2$ LOQ for ND).

was either low or nonquantifiable in both the Irvine 2003 and the Santa Maria 2004 strawberry collections (**Table 4**). The changes in DMTP and DMP residues were inconsistent in the two collections during 2003 and 2004 (**Table 4**). The farming operations at the two sites were seemingly the same, but very different amounts of DAPs were present in the produce. Malaoxon was unquantifiable in both lots of strawberries. No explanation is offered for the different DAP/malathion ratios at Irvine and Santa Maria. Both sets of field studies clearly demonstrate important time-dependent changes of malathion biomarkers in fruit that could confound exposure biomonitoring (*4*).

When the more complete set of 2004 Santa Maria residue data are plotted as a percent contribution of malathion and DAPs, a more likely product−precursor relationship between DMTP and DMP was evident (**Figure 5**). This important issue is the subject of continuing study. Malathion residue decayed from 0.48 to 0.10 nmol/g (not significant with sample size, $n = 3$ in each group, $p = 0.07$). The sum of malathion and DAPs were still the same ($p > 0.05$), indicating no loss of mass. DMDTP was detected at trace levels, while DMTP was the most abundant DAP. The mole ratio of ∑DAPs to OPs ranged from 1.4 ± 0.5 to 8.7 ± 2.8.

Current research concerning the fate of OP insecticides and preformed biomarkers contributes additional important data (**Table 5**). In this case malathion, malaoxon, DMP, DMTP, and DMDTP, and the mono- and diacids were measured in both strawberry leaves and berries during a period of about 3 weeks. The leaf/berry ratio of malathion on day 2−3 was about 55. The total nanomoles of malathion in leaves were 11.45 ± 2.59 nmol/g. Derivatives (maloxon, DAPs, and malathion acids) accounted for 18.00 ± 5.15 nmol/g in the same samples. For reference purposes, the more familiar dislodgeable foliar residue of malathion was 0.04 ± 0.01 μg/cm^2. Low levels of malaoxon (0.19 nmol/g) were present on leaves at 2 days, and the residue declined to 0.09 on day 20. Malaoxon was not quantifiable in strawberries at 0.01 ppm (LOQ) at any time.

The other malathion-derived analytes were in berries in much lower amounts than in leaves. On day 2, leaves contained malathion, malaoxon, DMP, DMTP, DMDTP, and both malathion monoacid (MMA) and malathion diacid (MDA). When the relative biomass of leaves and fruit are considered, the leaves can be appreciated as a much greater source of OP insecticide-derived residues than berries. Quantitative comparative data are lacking at this time on additional crops. Understanding the dynamic relationship between residues in leaves and the growth and development of fruit is likely to reveal insight about the duration and extent of occurrence of preformed biomarkers in leaves and berries.

On day 20, DMP accounted for 87 mol % of the DAP residue in berries and 56 mol % of the DAP residue in leaves. MMA was more predominant than MDA at each interval, and both were more prominent in leaves than in berries. The DAPs and malathion acids in **Table 5** are each potential malathion biomarkers that may be present in produce treated with this OP insecticide.

DISCUSSION

Our retrospective residue analysis with frozen produce samples treated with OPs revealed that at least 1 DAP was found in each of the 153 produce samples known to contain at least one OP insecticide. A total of 91 samples (60%) had more DAPs than parent insecticide. OP insecticides are hydrolyzed in plants to produce DAPs (*14*−*19*). Prospective studies of the transformation of an organophosphorpous insecticide, malathion, to greater amounts of DMP and DMTP is presented in **Figure 5** and **Tables 4** and **5**. Because samples that lacked an OP residue were not included in our initial surveys, it is likely that some sprayed produce would have contained infinitely greater amounts of DAPs because some DAP was always present. These findings may have profound implications for the practice of relating urine DAPs to low-level OP exposures, particularly in dietary, residential, and bystander research.

There is no doubt that DAPs are present in OP-treated produce, and their formation has been demonstrated in strawberries (**Table 5**). Produce samples, collected at unknown times after pesticide application and stored for varying periods (up to 6 months), had measurable residues of OPs and DAPs. There was no means to directly determine possible degradation of the parent OP insecticide residues during frozen storage and analysis. This uncertainty was indirectly addressed by re-analysis of a portion of the produce. A linear regression analysis of the

Table 4. Malathion and DAPs in Strawberries from Santa Maria and Irvine, CA

study and application	residue	time after application	DMP (nmol/g)	DMTP (nmol/g)	DMDTP (nmol/g)	malathion (nmol/g)	sum of malathion and DAP (nmol/g)[a]	mole ratio of DAP/malathion[b]	mole fraction of DAP[c]
Irvine, 2003	fruit residue	7 days	1.70 ± 0.27	0.59 ± 0.61	0.23 ± 0.36	0.17 ± 0.04[d]	2.7 ± 0.9[e]	16.6 ± 10.1	0.9 ± 0.03
2 lb/acre		14 days	1.77 ± 0.59	0.96 ± 0.46	0.08[f]	0.07 ± 0.03[d]	2.9 ± 1.1[e]	47.8 ± 2.7	1.0 ± 0.03
	DAP percentage	7 days	73.1 ± 21.9%	19.8 ± 19.4%	7.1 ± 7.4%				
		14 days	65.5 ± 8.6%	30.6 ± 12.0%	3.9 ± 3.5%				
this study[g]	fruit residue	not known	3.71 ± 7.15	1.60 ± 1.06	0.71 ± 0.94	0.53 ± 0.40	6.55 ± 7.38	15.5 ± 11.0	0.9 ± 0.1
	DAP percentage	not known	42.3 ± 23.8%	41.6 ± 18.6%	16.0 ± 19.0%				
Santa Maria, 2004	fruit residue	1 day	0.03[f]	0.48 ± 0.12	0.05[f]	0.48 ± 0.30[e]	1.1 ± 0.4[e]	1.4 ± 0.5	0.6 ± 0.1
1 lb/acre		3 days	0.03[f]	0.42 ± 0.12	0.05[f]	0.16 ± 0.02[e]	0.7 ± 0.1[e]	3.1 ± 0.4	0.8 ± 0.02
		6 days[h]	0.13	0.28	0.05	0.09	0.6	5.1	0.8
		9 days	0.31 ± 0.06	0.43 ± 0.07	0.05[f]	0.10 ± 0.05[e]	0.9 ± 0.2[e]	8.7 ± 2.8	0.9 ± 0.04
	DAP percentage[i]	1 day	6.1%	84.1 ± 3.3%	9.8%				
		3 days	6.9%	82.1 ± 3.9%	11.0%				
		6 days	24.5%	63.1%	12.5%				
		9 days	38.7 ± 4.0%	54.8 ± 3.9%	6.5%				

[a] Sum of malathion and DAP (nmol/g) = malathion + DMP + DMTP + DMDTP. [b] Mole ratio of DAP/malathion = (DMP + DMTP + DMDTP) ÷ malathion. [c] Mole fraction of DAP = (DMP + DMTP + DMDTP) ÷ (malathion + DMP + DMTP + DMDTP). [d] Difference is significant ($p < 0.05$). [e] There is no significant difference ($p > 0.05$). [f] Residues were not quantifiable. Half of the quantification limit value was given in the calculation. [g] In this study, those strawberry samples in which only malathion was found ($n = 7$) were included. [h] One of the triplicate samples was nonquantifiable; therefore, SD was not calculated. [i] DMP % = DMP ÷ (DMP + DMTP + DMDTP) × 100%; for DMTP and DMDTP.

Table 5. Malathion and Metabolites on Strawberry Leaves and Berries (nmol/g,[a] Mean ± SD), 2007

				dialkyl phosphates				malathion acids	
days	*n*	malathion	malaoxon	*n*	DMP	DMTP	DMDTP	*n*	MMA	MDA
Leaves										
2	10	11.45 ± 2.59	0.19 ± 0.04	10	3.25 ± 2.98	5.73 ± 4.24	4.80 ± 3.25	8	6.80 ± 3.99	1.42 ± 0.68
8	10	2.34 ± 1.07	0.17 ± 0.05	10	2.92 ± 1.43	5.55 ± 2.75	2.57 ± 1.91	10	7.59 ± 3.43	1.64 ± 1.20
20	10	0.43 ± 0.14	0.09 ± 0.02	10	7.88 ± 4.91	3.69 ± 2.42	1.20 ± 1.06[b1]	8	5.02 ± 2.51	1.42 ± 1.13
Berries										
3	10	0.21 ± 0.05	0.02 ± 0.00[c]	6	0.70 ± 0.28	0.60 ± 0.40	0.18 ± 0.17	4	1.72 ± 1.48	0.20 ± 0.31[d1]
21	10	0.04 ± 0.02	0.02 ± 0.00[c]	10	4.24 ± 1.75	0.58 ± 0.25	0.08 ± 0.07[b2]	10	2.29 ± 1.52	0.31 ± 0.40[d2]

[a] nmol/g = (μg/g × 1000)/molecular weight. The molecular weights for malathion, malaoxon, DMP, DMTP, DMDTP, MMA, and DMA are 330, 314, 126, 142, 158, 302, and 274, respectively. [b] $^1/_{10}$ and $^6/_{10}$ were not quantifiable at the LOQ of 0.01 ppm (μg/g) for b1 and b2, respectively. LDL/2 was calculated. [c] All samples were not quantifiable at the LOQ of 0.01 ppm (μg/g). LOQ/2 was calculated. [d] $^2/_4$ and $^4/_{10}$ were not quantifiable at the LOQ of 0.005 ppm (μg/g) for c1 and c2, respectively. LOQ/2 was calculated.

initial OP residue concentration plotted value against remeasured OP residues was performed (**Figure 3**). The OP residues were stable during storage period as indicated by points falling about equally above and below the line and a slope near 1. Bias was minimized by use of randomized, coded samples and the practice of blinding analysts to all experimental objectives.

Some crops consistently had larger DAP/OP mole ratios than others; similarly, some OP insecticides were present at consistently lower concentrations than DAPs (**Tables 2** and **3**).

The OP insecticide half-life in water (*35*) was associated with the DAP/OP ratio. When the half-lives were regressed against geometric means of the mole ratio of DAPs/OPs, the R^2 was 0.9, demonstrating that shorter half-lives resulted in degradation of OP insecticide residue in produce producing higher amounts of DAPs. Although the interval between OP application and harvesting of the produce could not be established, the relationship among OP, its derivative DAPs, and handling deserves further study.

Although our study clearly demonstrates the presence of DAPs in fresh produce, it has several limitations. First, we were unable to control for potential degradation of OP insecticides to their respective DAP hydrolysis products during storage. We did, however, demonstrate that degradation of OP insecticides was minimal (**Figure 3**), thus having little real impact on our observations. Second, we were unable to control for the possible conversion of residual OP insecticides to DAPs during the sample preparation and analysis process. The time series of sampling and analysis has produced consistent results. Analysts were given randomized sets of samples and were blinded to experimental objectives of our residue and biomonitoring research to promote objectivity and minimize analyst bias. These measures are not perfect but give us strong confidence in the analytical work of our cooperators. These same considerations have been in place during ongoing prospective studies of malathion metabolism in strawberries from Santa Maria (**Table 5**) during the past 3 growing seasons. Our measures of stability of the DAPs as trace produce residues are indirect, but under the conditions that prevail, these analytes are stable.

The retrospective survey over two growing seasons included a large number of samples that shared only the characteristic of positive OP insecticide residue analysis. Analysis of multiple produce samples treated in the field with a single OP insecticide was expected to yield less variable data. Malathion residues in strawberry leaves ($n = 10$) collected at 8 days corresponding to the second pick after an application under field conditions were 2.34 ± 1.07 (**Table 5**). The corresponding coefficients of variability were 49−74% for the DAPs and 45 and 74% for the malathion mono- and diacids, respectively. Similar levels of derivatives that have traditionally been used as biomarkers of exposure were present at 21 days. Malathion was barely detectable (~0.01 ppm), and malaoxon was absent. Derivatives are more persistent than their parent insecticides in produce.

Of special interest are the relationships among different types of treated produce, time since pesticide application, and specific OP insecticides and corresponding DAP residue concentrations. Other agronomic factors related to the vigor and productivity

of plants are also likely determinants of potential DAP levels in or on produce.

Our study has demonstrated that DAP residues are present on produce at concentrations near and frequently exceeding intact OP pesticide residues. Given the variety of produce and the large number of samples tested, we can reasonably assume that produce treated with OP insecticides for protection will likely contain OP and DAP residues at some level. Following our earlier report (*4*), a similar study (*12*) evaluating the decomposition of intact OP insecticides in commercial juice samples found measurable DAP concentrations in unspiked juice samples, demonstrating the presence of DAPs in a different commodity than those evaluated in the present study (*12*). However, that study did not evaluate the mole fraction of the DAPs. Regardless, both studies demonstrate that humans can potentially be exposed to low levels of DAPs along with low levels of OP insecticides in fresh produce or processed commodities. This conclusion is supported by the observation that trichloropyridinol, another persistent OP biomarker, is frequently found at higher concentrations in food than the parent OP, chlorpyrifos (*26*).

In conclusion, in produce samples when OPs were present, DAPs were invariably found, sometimes in greater amounts than the parent insecticide. If the bioavailability of DAPs and other urinary biomarkers of OP exposure is significant, excretion of these preformed OP derivatives may rival or exceed the amount attributable to OP insecticide exposure. Under this condition preformed, DAPs can confound the interpretation of urinary DAP data (*4*).

Pharmacokinetic studies concerning the disposition of ingested DAPs can clarify the rate and extent of DAP absorption and elimination following ingestion in produce. DAP elimination in urine potentially results from absorption of preformed DAPs in addition to the hydrolysis and excretion of OP insecticides and other chemicals. OP insecticide residue studies with malathion have shown that water-soluble DAPs are more persistent in produce than either their parent insecticide or its oxon (**Table 5**).

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