



HPLC-MS/MS Method for the Measurement of Insecticide Degradates in Baby Food

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Supporting Information

ABSTRACT: A solid phase extraction method was developed to isolate four insecticide degradates from baby food that were measured subsequently using high-performance liquid chromatography–tandem mass spectrometry. The degradates [parent insecticide] measured were malathion dicarboxylic acid [malathion], 3,5,6-trichloro-2-pyridinol [chlorpyrifos, chlorpyrifos methyl] (TCPy), *cis/trans*-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane-1-carboxylic acid [permethrin, cypermethrin, cyfluthrin], and 3-phenoxybenzoic acid [general pyrethroid]. All degradates produced recoveries between 80 and 120% except TCPy in fruit (122% recovery), and all relative standard deviations were <16%. Use of this method demonstrated that insecticide degradates were found in baby foods frequently purchased in the United States, supporting the need for this method. These data will assist in differentiating whether biomarker levels of insecticide metabolites are the result of exposures to the toxic insecticide or its preformed degrade.

KEYWORDS: insecticide, degradation, dietary intake, method development, solid phase extraction

■ INTRODUCTION

Insecticides are used worldwide in both agricultural and residential settings.^{1,2} In particular, insecticides are widely used in the United States, where \$4.3 billion was spent on 93 million pounds of insecticide active ingredients in 2007.^{1,3,4} Insecticides are used largely in the agricultural sector; in 2007, 65 million pounds of active insecticide ingredients was used on agriculture alone in the United States.³ Two of the currently most-used insecticide classes in the United States are organophosphorus (OP) and pyrethroid insecticides.³

Urinary metabolites of OP and pyrethroid insecticides are commonly used as biomarkers of insecticide exposure.^{5–11} Some of these metabolites indicate exposure to a class of insecticides rather than a specific insecticide. An example of such are dialkyl phosphate (DAPs) metabolites of OP insecticides.¹² Other metabolites are specific to particular pesticides, such as malathion dicarboxylic acid (MDA), a metabolite of malathion.¹³ The use of these metabolites as biomarkers of exposure assumes that the output of urinary biomarker reflects the intake of the parent insecticide, but often, mass balance cannot be achieved.⁵

Degradates of some insecticides have been found in food.^{9,12,14,15} Because these preformed metabolites, also called degradates, are identical to the urinary insecticide metabolites, use of these compounds as biomarkers of exposure may lead to overestimation of insecticide exposure.¹⁶ Furthermore, pyrethroid and OP insecticides are used on crops in the United States and abroad.³ Both classes may be used on the same crop, which increases the likelihood that degradates from both classes may be present in a single food item.

Few studies of the degradation of insecticides in food have been conducted. Those that have been evaluated the degradates by either examining the kinetics of insecticide degradation in food matrices or detecting the degradates themselves present in food.^{9,14,17–19} Some of these examinations considered only loss of the parent compound.^{18,19} Other studies of insecticide degradation measured only the insecticide degradates.¹² Studies that evaluated only either the loss of the parent compound or the presence of degradates do not provide complete information on insecticide loss. Observation of the decrease in parent compound alone does not provide information as to whether the insecticide degraded through hydrolysis, photolysis, or other means or if they instead dissipated by volatilization. Similarly, observation of only the formation of degradates does not allow comparison between parent and degrade compounds in instances when the degrade may be derived from multiple insecticides.

A few studies have been reported in which both an insecticide and its degrade were analyzed simultaneously in food.^{14,15,17} However, none of these studies account nor correct for analytic degradation, which is the formation of the degrade during the extraction, cleanup, and/or chromatographic separation or detection process, which could falsely elevate the degrade concentrations. Thus, the results of these studies are limited.

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To perform complete analyses of both OP and pyrethroid insecticide degradation, methods for the extraction and analysis of both parent insecticide compounds and their degradates are required. A suitable method for the extraction of insecticides from foods and the subsequent analysis of these analytes by gas chromatography (GC) with electron capture detection has previously been developed.²⁰ However, few methods have been developed to analyze insecticide degradates in food.^{9,12,14,21} None of the previously reported extraction methods combine analysis of pyrethroid and OP insecticide degradation products. Because both classes of insecticides may be used on a single crop, it is possible for degradates of both classes to be present in food.

As recognized in the Food Quality Protection Act of 1996,²² risk factors unique to children make understanding their insecticide exposure particularly important. Children's physiological traits put them at higher risk for long-term damage from insecticide exposure.⁹ For example, their high metabolic rate causes them to eat more food per kilogram of body weight than adults, and their lower detoxifying enzymatic activity and immature neurological system make this potentially heightened pesticide exposure even more hazardous.^{9,23} Their behavioral tendencies, such as preference for foods that tend to contain higher amounts of insecticides and greater tendency to place fingers and objects in their mouths, also put them at risk for higher insecticide exposure.^{9,24} Assuming insecticide degradates are present in food and other environmental matrices such as dust, the same risk factors that make children more likely to be exposed to insecticides also make them likely to be exposed to degradates. Hence, it is particularly important to understand children's exposure to insecticide degradates, especially in the case when measurement of such compounds as biomarkers of exposure is anticipated.

There are currently few data on the adsorption, further metabolism, and potential toxicity of many insecticide degradates.^{9,12} Consequently, it is difficult to separate the health effects of insecticide degradates from those of the parent compounds. The ability to extract these compounds from food would allow research separating the health effects of the parent and degrade compounds.

For these reasons, a method for the extraction of insecticide degradates in baby food was developed. Degradates analyzed include malathion dicarboxylic acid (MDA), a hydrolysis degradation product of the OP insecticide malathion; 3,5,6-trichloro-2-pyridinol (TCPy), a hydrolysis degrade of the OP insecticides chlorpyrifos and chlorpyrifos-methyl; *cis/trans*-2,2-(dichloro)-2-dimethylvinylcyclopropane carboxylic acid (DCCA), a hydrolysis product of several pyrethroid insecticides; and 3-phenoxybenzoic acid (3-PBA), a nonspecific hydrolysis/oxidation product of several pyrethroid insecticides resulting from the hydrolysis of the insecticide and then subsequent oxidation of the degrade.

MATERIALS AND METHODS

Reagents and Materials. Methanol (HPLC grade), acetonitrile (HPLC grade), and glacial acetic acid were purchased from Sigma-Aldrich, Inc. (St. Louis, MO, USA). Water was purified in-house to 18.2 M Ω -cm with a Milli-Q water system (Millipore, Billerica, MA, USA). Oasis HLB mixed phase extraction sorbent extraction cartridges (200 mg, 6 mL) were purchased from Waters Corp. (Milford, MA, USA). Bond Elut reservoir cartridges were purchased from Agilent Technologies (Santa Clara, CA, USA).

Standards. MDA was purchased from Crescent Chemical Co. (Islandia, NY, USA). TCPy was purchased from Sigma-Aldrich (St.

Louis, MO, USA). DCCA was purchased from Cambridge Isotope Laboratories (Tewksbury, MA, USA), and 3-PBA was purchased from Acros Organics (Pittsburgh, PA, USA). All standard purities were >95%. A stock solution containing 10 μ g/g MDA, TCPy, DCCA, and 3-PBA in acetonitrile (ACN) was used to create standard dilutions from 5 to 2000 ng/mL. These dilutions were used to create matrix-based calibration curves ranging from 0.25 to 100 ng/g of matrix. This range was based on amounts of insecticide degradation products found previously in foods and juices.^{9,12}

Isotopically labeled standards (MDA-D₆, DCCA-¹³C₃, and 3-PBA-¹³C₆) were purchased from Cambridge Isotope Laboratories. These standards were diluted to 1000 ng/mL made in ACN and used as internal standards (ISTDs).

Baby Foods. We have elected to use baby food in this work to avoid the typical heterogeneity associated with standard food samples. Numerous methods can be found in the literature to produce homogeneous food samples. The use of baby food, which has been processed into a uniform consistency, eliminates most of the heterogeneity associated with processing raw samples.

Baby foods were purchased from local grocery stores in Atlanta, GA, USA. Green beans, carrots, peas, apples, bananas, and pears were chosen on the basis of the vegetable and fruit baby foods most frequently purchased in the United States.²⁵ Two popular conventional brands of each were chosen, as well as an organic version of one of these conventional brands. Only organic baby food was used for method validation to minimize degrade contamination from the food itself.

Sample Preparation. The method developed here was based on an extraction of urinary insecticide metabolites previously reported but with substantial modification.^{11,26} One gram (1.0 g) of baby food, 50 μ L of labeled ISTD, and 2 mL of water were added to a trace-cleaned conical centrifuge tube. The tube was vortex mixed for 4 min at 1000 rpm and centrifuged for 7 min at 150g. An Oasis HLB cartridge was placed on a vacuum manifold and preconditioned with 3 mL of methanol (MeOH) and 3 mL of 1% acetic acid in water. The water extract (supernatant) was filtered through a Bond Elut Reservoir cartridge and then loaded onto the OASIS cartridge. After the cartridge was loaded, it was washed twice with 2 mL of 1:5:94 (v/v/v) acetic acid/MeOH/water. The cartridge was then dried under vacuum. A test tube was placed under the cartridge in the vacuum manifold, and the sample was eluted twice with 2.5 mL of methanol. Because the cartridge had been dried completely, vacuum was required to start the elution process. Once the eluate began to flow through the cartridge, the vacuum was broken and the eluate was allowed to flow out using gravity only. The eluate was evaporated to dryness using a Turbopap LV evaporator (Zymark Corp., Hopkinton, MA, USA) under air at 15 psi and 45 °C and was then reconstituted with 100 μ L of 30:70 (v/v) MeOH/water. The sample was then vortex mixed briefly and centrifuged for 3 min before being transferred to an HPLC vial for analysis. Any residual particulate matter was left behind.

Analysis by LC-MS/MS. An Agilent 6460 triple-quadrupole HPLC-MS/MS (Agilent, Santa Clara, CA, USA) equipped with a negative mode electrospray ionization (ESI) interface was used to analyze samples. A Zorbax Eclipse Plus Phenyl-hexyl column (3.0 \times 100 mm, 3.5 μ m particle size, Agilent) was used for separation and kept at 45 °C. Solvent A was water with 1% acetic acid, and solvent B was MeOH with 1% acetic acid. The following parameters were used: the source temperature was 250 °C, the vaporizer gas flow (N₂) was 5 L/min, the nebulizer gas flow pressure was set to 35 psi, and the corona voltage was 3500 V. Each metabolite was matched to its own isotopically labeled internal standard with the exception of TCPy, for which no isotopically labeled standard was available. The best results were found when isotopically labeled DCCA was used as an internal standard for TCPy. Ions were analyzed in selected reaction monitoring (SRM) mode; their optimized precursor and product ions along with their optimized fragmentation and collision energies are shown in Table 1. Mass Hunter Quantitative software (Agilent Technologies) and Microsoft Excel (Redmond, WA, USA) were used for data analysis.

Table 1. Instrument Parameters for Optimized Quantification and Confirmation Ions of Insecticide Degradation Products with Fragmentation Energies (FE), Collision Energies (CE), and Retention Times (RT)^a

compound	precursor ion	product ion	FE (V)	CE (V)	RT (min)
MDA-ISTD	280	147	80	1	3.3
MDA	273	141	80	1	3.9
MDA	273	157	80	12	3.9
TCPy	198	198	96	0	7.3
TCPy	196	196	96	0	7.3
DCCA-ISTD	210	210	90	0	6.7
DCCA	207	207	90	0	8.2
DCCA	209	209	90	0	8.2
3-PBA-ISTD	219	99	98	20	7.4
3-PBA	213	93	122	16	9.0
3-PBA	213	169	122	8	9.0

^aISTD, internal standard; MDA, malathion dicarboxylic acid; TCPy, 3,5,6-trichloro-2-pyridinol; DCCA, *cis/trans*-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane-1-carboxylic acid; 3-PBA, 3-phenoxybenzoic acid.

During compound identification and optimization, by infusing the solution containing standard chemicals into the LC-MS/MS system, a number of precursor/product ion pairs were identified for each analyte according to their fragmentation patterns. A multiple time segment containing a specific multiple reaction monitoring (MRM) experiment was created and used during MS/MS acquisition. We selected both the quantification and confirmation ions by monitoring the intensity, peak shape, background level, and potential interferences in different urine samples. The same procedure was also applied to the isotopically labeled internal standard.

Method Validation. *Limits of Detection (LODs).* LODs were calculated using replicates of blanks and low-spike (10 ng/g) samples as the lowest analyte concentration at which reliable detection was feasible. If analyte was detected in the blank samples, the following procedure was used to determine the LOD. Ten replicate samples were injected, and the mean blank concentration was determined. The standard deviation (SD_{blank}) of the blank concentrations was also determined. The LOD, defined here as the lowest concentration acceptable as a measured quantity, was defined by the equation

$$\text{LOD} = \text{mean}_{\text{blank}} + 3(SD_{\text{blank}})$$

If no analyte was detected in the sample, a different procedure was used to determine the LOD. MassHunter software afforded an estimate of the signal-to-noise ratio (S/N) for each sample taken. The reported LOD was determined by noting when S/N drops below 3 among the standards. If the S/N for the lowest standard was >3, we reported the LOD as the concentration that would have produced S/N = 3 based on extrapolation. If the extrapolated LOD falls below the lowest standard, the lowest standard was used as the LOD instead.

Accuracy and Precision. Method accuracy and precision were determined by measurements of spiked samples at two concentrations (10 and 25 ng/g). For each matrix, 1.0 g of each type of food was fortified with either parent or metabolite compounds to either 10 or 25 ng/g. These quality control (QC) samples were then extracted and analyzed alongside other samples. Percent spiked recovery (relative recovery; Table 2) was calculated on the basis of the percentage of observed concentrations of the nominal spiked concentrations. Within-run precision, presented as relative standard deviation (RSD), was also calculated for fruits and vegetables on the basis of these fortified samples.

Test for Analytic Degradation. To test for analytic degradation in baby food fruits and vegetables, six samples of 1.0 g of carrots and six samples of 1.0 g of apples were obtained. For each matrix, three of the samples were fortified with 25 ng/g parent insecticide (malathion, permethrin, and chlorpyrifos) per sample, and all samples were fortified with degradate ISTD at a final concentration of 50 ng/g. The

Table 2. Relative Recoveries for Degradation Products in Baby Food Expressed as Percentages^a

	MDA	TCPy	DCCA	3-PBA
green beans				
QCL	85.8	119.5	100.0	94.5
QCH	93.1	95.4	93.4	97.8
peas				
QCL	109.8	93.0	104.6	91.0
QCH	106.8	84.5	110.2	94.1
carrots				
QCL	79.6	116.6	104.1	92.9
QCH	80.2	108.2	97.7	88.1
apples				
QCL	76.6	133.1	103.1	114.5
QCH	86.1	123.7	102.3	112.6
banana				
QCL	107.3	109.3	109.7	98.3
QCH	127.1	110.2	106.0	93.3
pears				
QCL	92.0	123.5	93.2	98.9
QCH	109.1	132.3	100.5	101.5

^aQCL, low (10 ng/(g)) quality control sample; QCH, high (25 ng/(g)) quality control sample; MDA, malathion dicarboxylic acid; TCPy, 3,5,6-trichloro-2-pyridinol; DCCA, *cis/trans*-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane-1-carboxylic acid; 3-PBA, 3-phenoxybenzoic acid. *N* = 1 in all cases.

samples were then immediately extracted and analyzed according to the insecticide metabolite procedure. To test for analytic degradation, we examined the concentration of analytes from fortified and unfortified samples. A two-tailed *t* test (*p* = 0.05) was used as the test criterion to ascertain that the degradate concentrations were identical regardless of parent insecticide spiking.

Matrix Effects. Matrix effects were accounted for by developing calibration curves in a surrogate matrix. The surrogate matrices included one for vegetables and one for fruits consisting of an equal contribution of each individual food for the two classes.

Method Implementation. The method was used to measure pesticide degradates in several baby food samples on the basis of the most frequently purchased baby foods in the United States: green beans, carrots, peas, apples, bananas, and pears.²⁵ For each food, there were three samples: two different conventional brands and one organic brand.

RESULTS

Method Performance. The extraction and cleanup method produced mass chromatograms free of interfering peaks. A typical QC mass chromatogram is shown in Figure 1. Two sets of matrix-based calibration curves were created, one in vegetables and one in fruits. Calibration curves were linear and resulted in $R^2 > 0.98$ for all compounds. A typical matrix calibration plot is shown in Figure 2. Calibration curves for the other analytes can be found in the Supporting Information.

As shown in Table 3, relative recoveries were between 80 and 120% for all analyte/matrix combinations except TCPy in fruit. Table 3 summarizes method performance by showing average relative recovery, RSD, and LODs stratified by fruits and vegetables. All RSDs were under 15% with the exception of MDA.

Test for Analytic Degradation. Using a two-tailed *t* test (*p* = 0.05), we determined that there was no significant difference between insecticide degradate concentrations between identical food samples regardless of parent compound

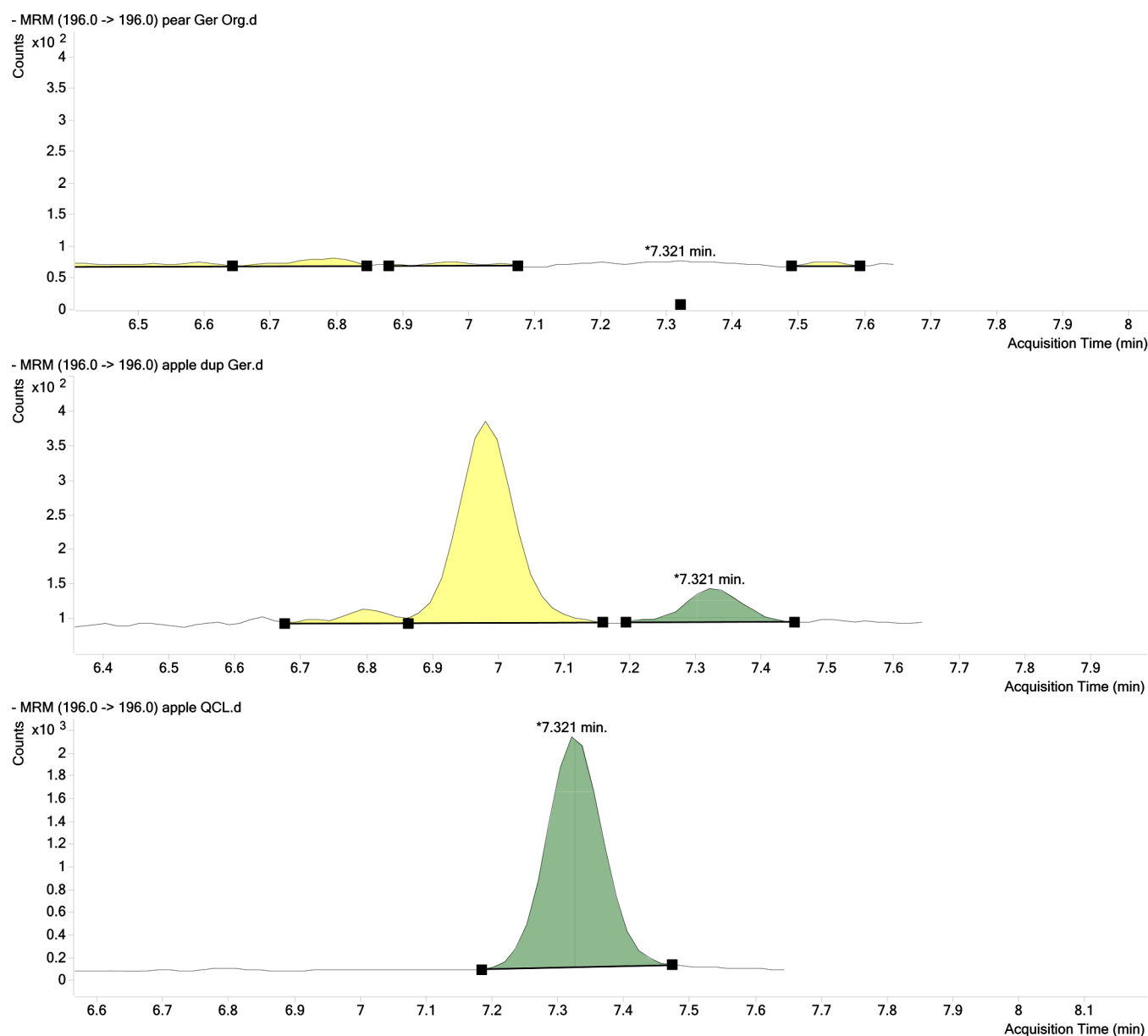


Figure 1. Comparison of chromatographic peaks for chromatogram of TCPy: (top) sample with no detection; (middle) peak detected at 7.321 min in an apple juice sample with a concentration below the LOQ (scale identical to top); (bottom) spiked quality control materials at 10 ng/g equivalent (note factor of 10 change in scale).

fortification. Table 4 presents average concentrations and standard deviations for spiked and nonspiked samples.

Method Implementation. Degradates were found in three samples. One conventional sample of pears contained TCPy at a concentration of 1.81 ng/g, the organic banana sample contained DCCA at a concentration of 1.64 ng/g, and the organic pea sample contained MDA at 3.63 ng/g. 3-PBA was not detected in any samples.

DISCUSSION

Method Performance. All degradate recoveries were between 80 and 120% except TCPy in fruit (122%), which is likely the result of the lack of an isotopically labeled analogue for TCPy. If DCCA, the labeled standard used as a surrogate standard for TCPy, was preferentially adsorbed by proteins or other matrix surfaces or interacted differently with the matrix from TCPy, falsely high recoveries (from calculations) could

have resulted. Ion abundances in the mass spectrometer may have changed because of solvent and eluted matrix component composition changes resulting from retention time shifts. Such changes that are not matched by ISTD may also have led to less accurate recoveries.

Blanks were developed from matrix-matched samples with no detectable concentration of degradate under most circumstances. Each type of sample had numerous “blank” values associated with it. For example, blank “vegetable” samples included a measure of organic peas that displayed no measurable concentration of degradate. We choose foods labeled “organic” for our blank samples under the supposition that they would likely contain lower levels of any parent pesticide or degradate. This supposition was borne out as measurable concentrations of degradate in such systems were <LOD. For a small number of cases, we detected the presence of degradate in these “blank” samples. Under such a scenario,

3-PBA-Q - 9 Levels, 9 Levels Used, 27 Points, 27 Points Used, 0 QCs

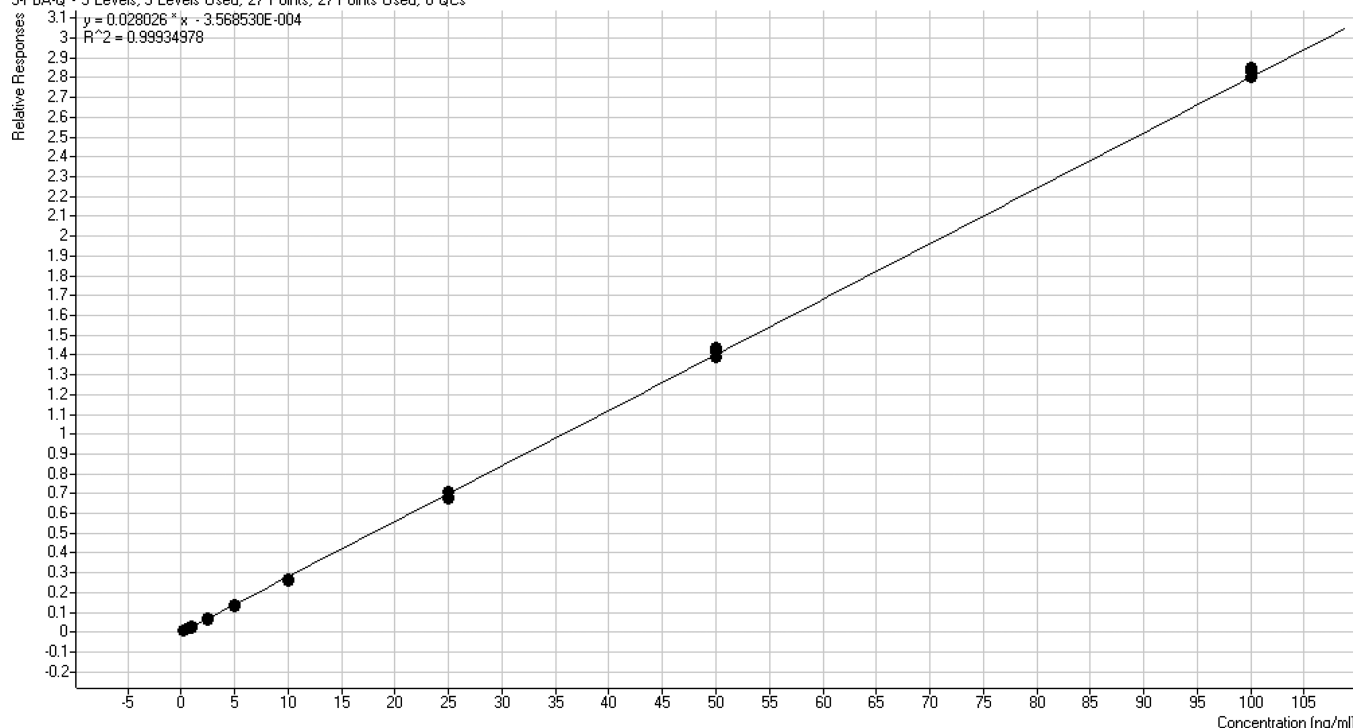


Figure 2. Calibration curve for 3-phenoxybenzoic acid, a degradate of many pyrethroid insecticides, in vegetables. All calibration points were injected in triplicate, and all points are present on the graph.

Table 3. Results for Recovery of Degradates from Fruits and Vegetables^a

	QCL	QCH	LOD (ng/g)
vegetables			
MDA	91.7 ± 15.9	93.4 ± 13.3	2.3
TCPy	109.7 ± 14.5	96.0 ± 11.9	2.7
DCCA	102.9 ± 2.6	100.4 ± 8.7	0.87
3-PBA	92.8 ± 1.7	93.3 ± 4.9	0.25
fruits			
MDA	92.0 ± 15.4	107.5 ± 20.6	0.18
TCPy	122.0 ± 12.0	122.1 ± 11.1	0.76
DCCA	102.0 ± 8.3	102.0 ± 8.3	0.87
3-PBA	103.9 ± 9.2	102.5 ± 9.7	0.25

^aRelative recoveries (±SDs) and limits of detection (LODs) are presented. QCL, low quality control material fortified to 10 ng/g; QCH, high quality control material fortified to 25 ng/g; MDA, malathion dicarboxylic acid; TCPy, 3,5,6-trichloro-2-pyridinol; DCCA, *cis/trans*-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane-1-carboxylic acid; 3-PBA, 3-phenoxybenzoic acid. *N* = 3 in all cases.

the reported concentration after spiking had to be greater than the mean blank value plus 3 times the standard deviation of the blank value (mean + 3σ(blank)) to be considered as >LOD.

RSDs were <15% for all analytes except MDA. RSDs under 15% are preferred for analytical analyses.²⁷ MDA may have had less precise results because a deuterated MDA was used for an ISTD. Deuterium may have been exchanged much more easily during solvent interactions than ¹³C or other isotopes, causing greater imprecision. Alternatively, the fact that MDA has two ionizable groups, thereby causing the possibility of four forms to be present at a given time, may contribute to its high RSD.

During compound optimization, no fragmentation was observed for both TCPY and DCCA. Although we could induce the “loss of chlorine” and use *m/z* of 35 as a product

Table 4. Test for Analytic Degradation in Vegetables and Fruits^a

	spiked samples	nonspiked samples
carrots		
MDA	1.44 ± 0.01	1.43 ± 0.02
TCPy	0.26 ± 0.38	0.31 ± 0.12
DCCA	0.22 ± 0.38	nd
3-PBA	0.09 ± 0.01	0.08 ± 0.01
apples		
MDA	nd	nd
TCPy	nd	nd
DCCA	1.05 ± 0.26	0.46 ± 0.42
3-PBA	0.14 ± 0.01	0.13 ± 0.01

^aSpiked samples were fortified with 25 ng/g malathion, chlorpyrifos, and permethrin, precursors to the four analytes. There was no significant difference found between sample concentrations using a two-tailed *t* test (*p* = 0.05). MDA, malathion dicarboxylic acid; TCPy, 3,5,6-trichloro-2-pyridinol; DCCA, *cis/trans*-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane-1-carboxylic acid; 3-PBA, 3-phenoxybenzoic acid; nd, not detected. *N* = 3 in all cases.

ion, it could come with higher background noises due to its low mass value. We thus decided to employ pseudotransitions for these compounds, which means the same *m/z* was detected in both Q1 and Q3 of the mass spectrometer.

Analytic Degradation. High concentrations of insecticides that could degrade into the target analytes were spiked into baby foods before extraction of degradates to test for analytic degradation. Even when using as much as 25 ng insecticide/g matrix, which is 100 times the lowest degradate calibration point used, statistically significant amounts of degradation products were not produced. This observation suggests that the use of this method to analyze the degradation of insecticides in

food without creating false-positive detection of insecticide degradate is acceptable.

Although not statistically significant, there was a larger difference in DCCA concentrations in spiked versus unspiked samples of apples than of carrots. There is the potential that the lower pH of apples (pH 3.0–4.0) versus carrots (pH 4.5–6.0) contributes to the degradation of permethrin, the parent of DCCA, during extraction, thereby causing the larger analyte difference in spiked versus unspiked samples.²⁸

Matrix Effects. Matrix effects are common in analyses of food samples and should be accounted for. In principle, there may well be a different matrix effect for each food analyzed. For single food analyses, this presents only minor problems as a matrix calibration curve can easily be constructed. However, for more complex samples, such as a duplicate diet sample, establishing separate calibration curves for each food is not practical. We made the compromise in our analysis to construct calibration curves separately for vegetables and fruits. These two food groups are likely to have different matrix characteristics with, for example, fruits being more acidic. We have included all foods of these two classes for which we performed analysis into two matrices and developed curves using these mixtures. All vegetable samples were analyzed using the curve developed using the vegetable mixture with similar protocols followed for fruit samples. We offer this as a compromise solution to the likely matrix effects problem.

Method Implementation. MDA, TCPy, and DCCA were all found in baby food, including some organic foods. 3-PBA was not found in any baby food. It is not certain whether 3-PBA, 3-phenoxybenzyl alcohol (3-PBAIc), or both compounds would be produced from the degradation of pyrethroids. Hydrolysis of these compounds should lead to 3-PBAIc, not 3-PBA. However, in the mammalian body, the alcohol moiety is transformed to the acid by an oxidase.²⁹ This oxidation may not happen in fruits and vegetables. Previous studies have demonstrated that the presence of antioxidants, such as those found in fruits and fruit juices, suppresses the production of pesticide oxidation degradates, so perhaps oxidation of 3-PBAIc to 3-PBA is suppressed by these compounds.^{16,17}

Although the detection frequency of these three compounds may not seem very high (17% for MDA, TCPy, and DCCA) compared to insecticide degradate concentrations found in other studies, there are a few possible explanations. First of all, other studies in which pesticide degradate concentrations were found in food observed DAPs,^{12,14,15} which are nonspecific degradates formed from 75% of the EPA-registered OP insecticides.³⁰ However, MDA is formed from only malathion, TCPy is formed from only chlorpyrifos and chlorpyrifos-methyl, and DCCA is formed from the three pyrethroids permethrin, cypermethrin, and cyfluthrin.³¹ Because DAPs are formed by so many more insecticides than these three compounds, it makes sense that their detection frequency was higher than that of MDA, TCPy, and DCCA. Furthermore, two of the studies cited analyzed for DAPs and/or 3-PBA only in produce already known to contain the parent insecticide.^{14,15} This sample preselection likely led to a larger degradate detection frequency than would happen if produce was analyzed regardless of parent insecticide content. Although 3-PBA, which is also a broad-range insecticide degradate, was not found in any of our baby food samples, this is possibly due to suppression of 3-PBAIc oxidation.

One may inquire as to the optimization of the final elution program of the SPE by comparing our work with normal-phase

SPE or ion exchange SPE. However, in the case of a reversed phase SPE, a generic method provided by the manufacturer can be followed with minimum optimization process, as long as the isotopic analogues are used as internal standards and the target analytes are polar compounds. Our analytes are quite polar, and on the basis of their Log *P* values they can be dissolved in polar solvents such as acetonitrile, methanol, ethyl acetate, and acetone. We chose not to use acetonitrile to reduce the laboratory cost, and acetone is not recommended for use due to high chemical backgrounds. Using the same elution solvent, Olsson et al.²⁶ reported their extraction recoveries of >80% for all analytes except MDA. Although we are satisfied with our method LODs for most of the compound measured, we do recommend further optimization to increase the LOD of MDA if such analysis is deemed critical for a given study.

This method could be expanded to analyze produce or other foods for insecticide degradation products. If other foods were analyzed, prehomogenization using a blender, food processor, or other homogenization system before the extraction step would be required to ensure a true understanding of analyte content in the sample.

This method is suitable for the analysis of insecticide degradates in baby food. The presence of degradates in baby food discovered during method implementation supports the need for this method. Furthermore, the widespread measurement of these urinary metabolites for exposure assessment purposes highlights the need for a better understanding of the contribution of preformed environmental metabolites to urinary concentrations. Use of this method in food samples will allow a more complete understanding of insecticide degradation product concentrations in such samples, and this knowledge in turn will allow more accurate estimation of human insecticide exposure.

■ ASSOCIATED CONTENT

§ Supporting Information

Structures, calibration curves, and matrix effects. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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Notes

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