Monitoring of Total Type II Pyrethroid Pesticides in Citrus Oils and Water by Converting to a Common Product 3-Phenoxybenzoic Acid

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Ⓢ Supporting Information

ABSTRACT: Pyrethroids are a class of insecticides that are becoming increasingly popular in agricultural and home use applications. Sensitive assays for pyrethroid insecticides in complex matrices are difficult with both instrumental and immunochemical methods. Environmental analysis of the pyrethroids by immunoassay requires either knowing which pyrethroids contaminate the source or the use of nonspecific antibodies with cross-reactivities to a class of compounds. We describe an alternative method that converts the type II pyrethroids to a common chemical product, 3-phenoxybenzoic acid (3-PBA), prior to analysis. This method is much more sensitive than detecting the parent compound, and it is much easier to detect a single compound rather than an entire class of compounds. This is useful in screening for pyrethroids as a class or in situations where a single type of pyrethroid is used. We demonstrated this technique in both citrus oils and environmental water samples with conversion rates of the pyrethroid to 3-PBA that range from 45 to 75% and methods that require no extraction steps for either the immunoassay or the liquid chromatography−tandem mass spectrometry (LC-MS/MS) techniques. Limits of detection for this technique applied to orange oil are 5 nM, 2 μM, and 0.8 μM when detected by LC-MS/MS, gas chromatography−mass spectrometry, and immunoassay, respectively. The limit of detection for pyrethroids in water when detected by immunoassay was 2 nM.

KEYWORDS: immunoassay, 3-phenoxybenzoic acid, pyrethroid, citrus oil, orange oil

■ INTRODUCTION

Pyrethroids are a class of synthetic insecticides similar to the natural chemical pyrethrins found in chrysanthemum flowers (*Chrysanthemum cinerariaefolium* and *C. coccineum*). Because pyrethroids and pyrethrins are effective broad-spectrum insecticides with low acute toxicity to birds and mammals, they have been gradually replacing organophosphate insecticides for pest control in urban environments. In 2007, 258 tons of pyrethroids was used for nonagricultural pest control in California alone. Meanwhile, their usage for agriculture has also steadily increased, with 160 tons used during the same period in California.[1] As a result, pyrethroids are increasingly found in California's water sources from either residential or agricultural runoff.[2,3] Pyrethroids enter human and animals via the skin, by inhalation, and from the gastrointestinal tract. Although human health effects from pyrethroid pesticides at low-dose environmental exposures are unknown, studies have concluded that some pyrethroids are neurological toxins, causing prolonged nervous system depolarization and hyperexcitation.[4] It is thought that the adverse effects from large dose exposures in humans are associated with this nervous system toxicity.[5,6] Concomitant exposure to organophosphorus insecticides and pyrethroids may increase the latter's toxicity by slowing the metabolic clearance of the pyrethroids. A state-wide investigation in California discovered that cyfluthrin was primarily associated with respiratory irritation, dermal effects, and paresthesias, which were reported in agricultural workers from 1996 to 2002.[7]

Determination of pyrethroids by immunoassay has been well established in the literature with immunoassays reported for many of the individual type II pyrethroids (e.g., deltamethrin,[8] cypermethrin,[9] flucythrinate,[10] cyhalothrin,[11] fenpropathrin,[12] and esfenvalerate[13]). Many of these assays have also been demonstrated to work in environmental matrices such as soil extracts,[8] grain extracts,[8] milk,[14] fruit juices,[10] plant and fruit extracts,[15] wine,[15] and orange oil.[16] In most cases, especially in more complex matrices, extensive cleanup procedures are required. Class determination of the pyrethroids is problematic, since immunoassay analysis is usually highly selective for its target analyte. To have an immunoassay that detects the entire range of pyrethroids, it must be based on an antibody that has a natural cross-reactivity for the analytes of interest. Many immunoassays have been reported with cross-reactivities that range across the type II pyrethroids;[17−19] however, most often, sensitivities to the different pyrethroids range across several orders of magnitude. Usually, such method bias will result in assays that are more qualitative in nature. Also, because
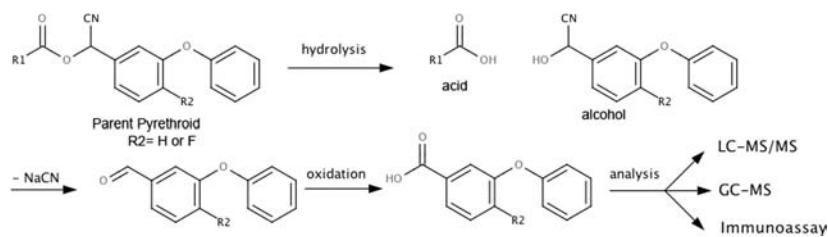
Figure 1. Chemical conversion of type II pyrethroids to 3-PBA.

antibodies are selected for their broad selectivity instead of their sensitivity, the resulting assay will likely result in higher limits of quantitation.

Instrumental methods for the pyrethroid class of compounds have their own set of challenges. Mass spectrometry (MS)-based multiresidue methods are most widely used for the determination of multiple individual pyrethroids that are separated by either gas chromatography (GC) or liquid chromatography (LC) in a variety of matrices. However, apparent analytical difficulties have been reported for deltamethrin, cyhalothrin, other pyrethroids,[20] and pyrethrins.[21] Most of the chromatography mass spectrometry-based methods offered limits of detection (LODs) for pyrethroids in the upper ppb (part per billion) or low ppm (part per million) level in complex matrices, such as vegetable oils,[22] soybean oils,[23] and blood,[24] even after multistep sample cleanup and concentration. Experiments conducted in our laboratory also found that the sensitivity for some pyrethroids, especially cyhalothrin and β-cyfluthrin, are much worse than other types of pesticides, by using either a GC−tandem mass spectrometry (MS/MS) or a LC-MS/MS technique. For cypermethrin, the LOD obtained in our laboratory was 40 ppb in orange oil by using a very sensitive tandem mass spectrometer,[16] while an entry level tandem mass spectrometer did not have enough sensitivity to measure cypermethrin under 500 ppb. In other investigations, LODs ranging from 13 to 49 ppb in tomato extracts for nine pyrethroids were achieved by using LC postcolumn photo-derivatization and chemiluminescence detection followed by vigorous sample extraction and a preconcentration step.[25] Considering that the IC$_{50}$ values of pyrethroids to aquatic organisms, such as fish, are less than 1.0 ppb,[26] these methods lack adequate sensitivity to assess the safety of the water environment.

Type II pyrethroids are those that contain an α-cyano substituent in the alcohol moiety (Figure 1). In our approach to the analysis of type II pyrethroid insecticides in environmental samples, rather than analyzing for individual pesticides, type II pyrethroids were chemically converted to a common product, 3-phenoxybenzoic acid (3-PBA). To our knowledge, this is the first study to use such an approach to determine a total level of type II pyrethroid contamination. Flumethrin and cyfluthrin, which are converted to 4-F-3-PBA, were also detected by instrumental methods and by immunoassay since 4-F-3-PBA cross-reacts in the assay by 72% of 3-PBA.[27] We chose citrus oils as the study samples, because they represent one of the most difficult matrices due to their hydrophobic nature and matrix complexity. Pyrethroids have been reported to be difficult to separate from oily matrices,[28] and the cleanup of the matrix or preconcentration of pyrethroids to improve sensitivity is challenging. One advantage to converting the parent compound to 3-PBA is that the 3-PBA is more hydrophilic and thus easier to separate from the matrix should future

cleanup or preconcentration be needed. A drawback is that the results may be confounded by 3-PBA existing in the sample. Indeed, preliminary analysis of randomly selected citrus oil samples found levels of 3-PBA or 4-F-3-PBA from non-detectable to as high as 50 ppb. Analysis of the sample before and after hydrolysis should address this issue. The major goal of the method development reported here was to find an appropriate method to convert pyrethroids to 3-PBA in citrus oils.

■ MATERIALS AND METHODS

Eighteen MΩ water was produced by a Barnstead Easypure Rodi water purification system (Thermo-Fisher, San Jose, CA). Acetonitrile (99.5+%), 3-PBA (98%), ammonium acetate (99.99+%), dimethyl sulfoxide (DMSO), *N,O*-bis(trimethylsilyl) trifluoroacetamide (BSTFA), goat antirabbit-horseradish peroxidase, 3,3',5,5'-tetramethyl-benzidine (TMB), bovine serum albumin (BSA), and 80% puriss grade sodium chlorite were purchased from Sigma-Aldrich (St. Louis, MO). ACS-certified methanol, L-ascorbic acid (99+%), sodium acetate, ethyl acetate, hexane, 30% hydrogen peroxide, 0.5% platinum on alumina pellets, and sodium hydroxide were obtained from Thermo-Fisher, and glacial acetic acid was from Mallinckrodt Baker (Phillipsburg, NJ). Mixed mode anion exchange C8 SPE cartridges (Strata-Screen A) were purchased from Phenomenex (Torrance, CA). Nitrogen gas used by the AB Sciex mass spectrometer was generated from a Parker LCMS5000 high-purity nitrogen and zero air gas generator (Parker Hannifin Corp., Haverhill, MA).

Immunoassays were performed with 96-well Nunc microtiter plates (MaxiSorp surface, Roskilde, Denmark). Normal strength phosphate-buffered saline (PBS) ($1 \times$ PBS; 8 g/L of NaCl, 0.2 g/L of Na$_2$HPO$_4$, and 0.2 g/L of KCl, pH 7.5), PBST (PBS containing 0.05% Tween 20), 0.05 M citrate-acetate buffer (14.71 g/L Na$_3$C$_6$H$_5$O$_7$·2H$_2$O, pH to 5.5 using glacial acetic acid) were used for immunoassay. Substrate buffer was prepared by adding 400 μL of 0.6% TMB in DMSO and 100 μL of 1% H$_2$O$_2$ into 25 mL of citrate-acetate buffer.

Individual pyrethroid pesticide stock solutions (100 or 2000 ppm in acetonitrile) for deltamethrin, cypermethrin, acrinathrin, λ-cyhalothrin, and β-cyfluthrin were obtained from either Chem Service (West Chester, PA) or Resteck (Bellefonte, PA). The polyclonal anti-3-PBA antibody was developed as previously described.[29]

Instruments. The LC/ESI-MS/MS system used was an Agilent 1200 rapid resolution HPLC (Agilent, Palo Alto, CA) coupled with an API 5000 tandem mass spectrometer (AB Sciex, Foster City, CA) with an electrospray interface (ESI). Both instruments were controlled by the Analyst 1.5 software. Detailed mass spectral optimized settings are located in the Supporting Information.

The GC-MS used was a Hewlett-Packard 6890 gas chromatograph equipped with a Hewlett-Packard 5873 Mass Spectral Detector. The column was a DB5MS L-30 m 0.25 mm i.d. film 0.25 mm from Agilent (Santa Clara, CA).

LC-MS/MS Performance and Recovery Studies. The method LOD was calculated as three times the standard deviation of 10 replicates of 5 ppb 3-PBA spiked in 5% citrus oils. The determined LOD for 3-PBA in this study was 1 ppb according to LC-MS/MS analysis. The calibration was linear from 1 ppb to 5 ppm.

GC-MS Performance and Recovery Studies. The method LOD was calculated as three times the standard deviation of 10 replicates of

500 ppb 3-PBA suspended in BSTFA. The determined LOD for 3-PBA in this study was 400 ppb according to GC-MS analysis. The calibration was linear from 400 ppb to 10 ppm.

Immunoassay Performance and Recovery Studies. The method LOD was defined as the IC10 value of the 3-PBA standard curve produced from a dilution series made in the blank citrus oil following oxidation. Because the calibration curve was linear from the IC_{20} to the IC_{80}, the limit of quantification was defined by the IC_{20} value. Method detection limits were determined by the average of five standard curves that were performed on separate days with separate oxidations.

Volatilization of Citrus Oil. Pyrethroid-spiked orange oil was prepared by spiking the sample from a 1000 ppm stock solution prepared in isopropanol. Citrus oil (100 μL) was pipeted into a 2 dram vial, which was then heated at 70 °C on a heat block under a steady stream of nitrogen gas for 30 min, to remove the volatile components.

Converting Pyrethroids to 3-PBA in Volatilized Citrus Oils Using Sodium Chlorite. The volatilized oil residue was resuspended by adding DMSO (200 μL) and 10% sodium chlorite in 0.1 N NaOH (800 μL) to the sample vial. Samples in vials were incubated for 1 h at 70 °C with constant stirring.

After they were cooled, the vials were neutralized with 1 M HCl and cleaned up by SPE for instrument analysis. For immunoassay analysis, 1.0 mL of 1.0 M ascorbic acid with 0.3 N NaOH was added to neutralize the oxidant followed by a 20-fold dilution with PBS to bring the final dilution from the original citrus oil to 400×.

Converting Pyrethroids to 3-PBA in Volatilized Citrus Oils Using Hydrogen Peroxide. The volatilized oil residue was resuspended by adding dioxane (200 μL), and 15% hydrogen peroxide in 0.1 N NaOH (800 μL) was added to the samples. Samples were incubated for 1 h at 70 °C with constant stirring.

After they were cooled, the vials were neutralized with 1 M HCl and cleaned up by SPE for instrument analysis. For immunoassay, one 0.5% (w/w) platinum on alumina pellet was added while still hot to neutralize the excess oxidant. The pellets were allowed to incubate for 1 h before the sample was neutralized with 6 M HCl and diluted an additional 10-fold with PBS to bring the final dilution from the original citrus oil to 100-fold.

Converting Pyrethroids to 3-PBA in Water Samples Using Hydrogen Peroxide for Immunoassay Analysis. The conversion process was accomplished in a one pot as follows: 30% H_2O_2 in 0.8 N NaOH (250 μL) was added to 250 μL of the water sample, and the mixture was kept at 70 °C for 30 min. The sample was then removed from the heat source and neutralized by addition of a 0.5% platinum on alumina pellet until bubbles stopped forming (usually <1 h). The pellet was removed, and 0.5 M phosphate buffer (500 μL, initial pH 7.4) in 0.4 M acetic acid was added. Samples were then run by enzyme-linked immunosorbent assay (ELISA) without further treatment. For GC-MS analysis, a 1.0 mL sample size was used, and 1.0 mL of the hydrogen peroxide NaOH mixture was added. Before they were derivatized and analyzed, the samples were neutralized and cleaned up using the mixed mode SPE column after neutralization before being derivatized and analyzed. For other LC-MS, GC-MS, and immunoassay methods, see the Supporting Information.

■ RESULTS AND DISCUSSION

In this study, several procedures were tested to convert pyrethroids to the intermediate alcohol and aldehyde. The procedures tested were based on chemical hydrolysis[30] or photolysis.[31,32] The conversion scheme of pyrethroid hydrolysis and oxidation is illustrated in Figure 1. Photolysis resulted in uncontrollable conversion to other unknown products (data not shown), so chemical hydrolysis and oxidation was the preferred method to produce 3-PBA from the type II pyrethroids. Because citrus oils have high levels of volatile components, the matrix was initially simplified by volatilization of 95% of the mass under a steady stream of nitrogen. Analysis confirmed that there was no significant loss of the pyrethroid

during this process (Supporting Information). Hydrolysis was accomplished with sodium hydroxide, which yields an α-cyano alcohol. The alcohol rearranges rapidly in the presence of water to the corresponding aldehyde. The main challenge was to select the proper oxidant that could convert the aldehyde to the carboxylic acid in the presence of other functional groups that could compete for the oxidant, including competing aldehydes.[33] Because the concentration of total aldehyde in the oils ranged from 1 to 5 wt %, the selected method should oxidize all aldehydes in the citrus oil selectively over the other functional groups. The oxidation of the other aldehydes has another benefit by creating natural surfactants to help solubilize the remaining citrus oil components. Of the oxidants that were evaluated, household bleach, sodium chlorite, and hydrogen peroxide all demonstrated the ability to perform the oxidation in the presence of citrus oil (Figures S2–S4 in the Supporting Information).

Optimization of the chemical conversion was critical to the success of this oxidation method. We varied the concentrations of oxidants and sodium hydroxide in a test mixture of 10 μM deltamethrin and 10 μM C^{13}-3-PBA. We discovered that with increasing concentrations of household bleach and sodium chlorite, the efficiency of the conversion of pyrethroid to 3-PBA also increased. However, if the oxidant concentration was too high, then chlorination occurred, resulting in a loss of the product and the C^{13}-3-PBA internal standard. On the basis of these data, hydrogen peroxide and 10% chlorite were the most effective oxidants to use for the process. Household bleach was not studied further because it is difficult to accurately assess the concentration of oxidant and thus would be hard to prevent chlorination.

Because the method would be most useful without cleanup steps, we developed methods that would allow for immunoassay analysis after the oxidation with limited sample workup. Options for neutralization of the large excess of oxidant prior to analysis that would not require extraction were explored. Many different antioxidants were considered and tested with the discovery that ascorbic acid was of sufficient reactivity to neutralize the chlorite, and a solid phase platinum catalyst could neutralize the hydrogen peroxide. The solid phase catalyst was preferred in this case, because it could be easily removed before analysis. After neutralization, the mixture was diluted in PBS prior to analysis by immunoassay or cleaned up using a mixed mode SPE prior to instrument analysis. Matrix effects were observed at dilutions lower than 400-fold, with less of a matrix effect observed for the samples that were oxidized by hydrogen peroxide (Supporting Information).

Samples of orange oil were then spiked with a 10 μM concentration of different type II pyrethroids (deltamethrin, cypermethrin, λ-cyhalothrin, acrinathrin, and fenpropathrin) to determine if the conversion rate was similar for the different pyrethroids. All were oxidized with 10% sodium chlorite and were spiked with C^{13}-3-PBA as a recovery standard. Following the oxidation, the samples were cleaned up with a mixed mode SPE and spiked with 2-PBA as an internal standard prior to GC-MS analysis. The selected pyrethroids ranged from 40 to 60% conversion to 3-PBA with cypermethrin being the most efficient and acrinathrin being the least when using chlorite oxidation, hydrogen peroxide ranged from 50 to 70% conversion, and recovery of the difference between the two oxidation methods was not significant (Figure 2). This method was also applied to 10-fold concentrated orange oil, lemon, and grapefruit oils (Figure 3); overall, the conversion ranged
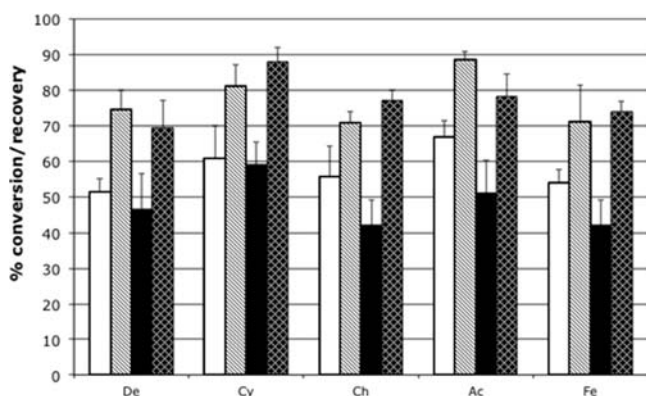
Figure 2. Percent conversion and recovery of 5 ppm of deltamethrin (De), cypermethrin (Cy), lambda-cyhalothrin (Ch), acrinathrin (Ac), and fenpropathrin (Fe) spiked into orange oil. A recovery standard of ^{13}C-3-PBA was spiked at 2.5 ppm. Samples were oxidized with 15% hydrogen peroxide (white bars, pyrethroid; with dashes, ^{13}C-3-PBA) or with 10% sodium chlorite (black bars, pyrethroid; white checkerboard, ^{13}C-3-PBA). Both oxidation methods yielded about 50% conversion and recovery from the orange oil for all pyrethroids. Recoveries of the standard were about 70%. Samples were run in quadruplicate and analyzed by GC-MS, with the standard deviation being shown as the error bars.
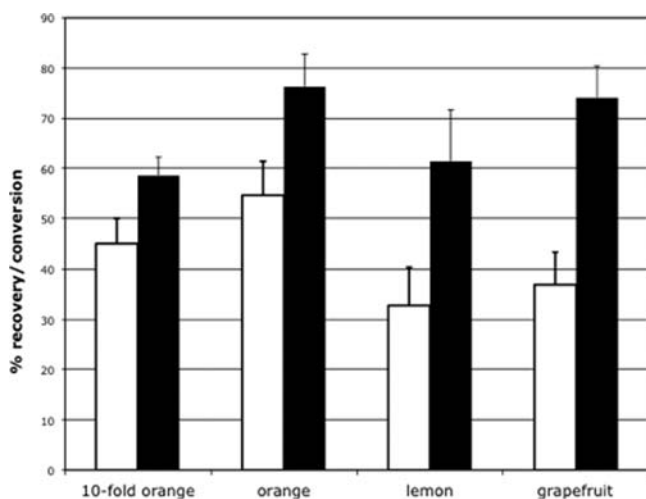

Figure 3. Conversion and recovery of 5 ppm of deltamethrin (white bars) and recovery of 2.5 ppm of ^{13}C-3-PBA (black bars) in other citrus oils as analyzed by GC-MS. Oils were oxidized using hydrogen peroxide and cleaned up using the mixed mode SPE. Samples were run in quadruplicate with the error bars being the standard deviation of the replicates.

between 30 and 50% in other citrus oil matrices with a decrease in conversion to the 3-PBA in lemon, grapefruit, and 10× orange oil as compared to the conversion efficiency in orange oil.

A comparison of the recovery of the various methods of analysis was determined at two concentrations by spiking orange oil samples with different combinations of pyrethroids. Four sets of samples, labeled A−D, were created with each set containing one sample with low and one sample with high pyrethroid concentration. Set A was spiked with deltamethrin, set B contained β-cyfluthrin for 4-F-3-PBA, set C had a combination of deltamethrin and acrinathrin, and set D was spiked with a combination of deltamethrin, acrinathrin, and λ-cyhalothrin. The individual pyrethroids were converted to 3-

PBA or 4-F-PBA for analysis by using either sodium chlorite for instrumental analysis or sodium chlorite and hydrogen peroxide for the immunoassay (Table 1). Each sample was analyzed in triplicate by the different quantitation methods. Overall, the GC-MS had the lowest sensitivity to the lower concentration of pyrethroid with samples A2 and B2 being lower than the limit of quantitation and samples C2 and D2 showing an artificially high recovery because they were very close to the limit of quantitation. Both ELISA and the LC-MS/MS methods were able to reliably detect the 3-PBA in the samples, although some of the lower concentration samples were very close to the LOD with the ELISA method. The relatively low recovery at high concentrations and high recovery at low concentrations for sets C and D may reflect the variation among individual pyrethroids and the effect of the concentration on the conversation rate. Of note is that the spiked orange oil was assayed by LC-MS/MS to determine that the spiking concentration was accurate; yet, three of the four low concentration samples were below the detectable limit when assayed for individual pyrethroids by this instrumental method; however, when those samples were oxidized and converted to 3-PBA, the LC-MS/MS was able to detect them easily.

This method described above was also applied to a water matrix by addition of hydrogen peroxide to laboratory and environmental waters samples. Hydrogen peroxide was selected because it eliminated the loss of 3-PBA to chlorination unlike the sodium chlorite-oxidized samples. Much lower dilution (4-fold dilution overall from the original sample) was needed for the immunoassay since there was little matrix effect observed for the oxidation in water. Nanopure water was spiked with a 20 nM concentration of several individual pyrethroids, which was then hydrolyzed and oxidized prior to analysis by GC-MS and immunoassay (Figure 4). Overall, conversion efficiencies of each of the individual pyrethroids were above 50% in all cases.

An environmental water sample was collected from Putah Creek (a local creek that flows through agricultural lands) and analyzed before and after oxidation with hydrogen peroxide. No contamination from type II pyrethroids or 3-PBA was detected by the immunoassay method using either oxidized or nonoxidized Putah Creek water at a limit of quantification, determined using the IC_{80} of the immunoassay standard curve, of 1 nM for 3-PBA in nonoxidized samples, and using a lower threshold of 50% conversion, we estimate a 2 nM LOD for type II pyrethroid determination in oxidized water samples. To see the effectiveness of this procedure in environmental water, it was spiked with different pyrethroids at 20 and 100 nM, and the recovery was determined (Table 2). The average conversion and recovery rate from all replicates was 67% of the pyrethroids in water. This was similar in efficiency to the conversion observed earlier with citrus oils where we saw an average conversion and recovery of 58%. However, LODs using this method with immunoassay are much lower than citrus oils due to the lower matrix effect, which requires less dilution.

Here, we have described a new approach for type II pyrethroid analysis and demonstrated its feasibility for use in both environmental water and citrus oils. This approach allows for quantitation with LC-MS/MS, GC-MS, or immunoassay. This method provides a semiquantitative analysis of the total amount of type II pyrethroids and is relatively quick, requires no extraction steps (except for GC-MS analysis), and is fairly robust. The authors acknowledge that a limiting factor is the endogenous 3-PBA, which in the samples would give a higher value for pyrethroid contamination, but this can be corrected by

Table 1. Analysis of Individual Pyrethroids and Pyrethroid Mixtures in Orange Oil[a]

| | | | LC-MS/MS | | | GC-MS | | | ELISA | | | | | |
| | | | | | | | | | chlorite | | | hydrogen peroxide | | |
pyrethroid	theor concn (μM)	concn (LC-MS/MS)	av	% RSD	recovery (%)	av	% RSD	recovery (%)	av	% RSD	recovery (%)	av	STD %	recovery (%)
						high								
De	9.9	11	4.9	15	46	11	10	107	5.8	9	55	5.9	6	55
Cf[b]	12	11	4.7	8	42	6.0	12	53	5.8	16	52	4.7	13	41
De,Ac	9.6	9.5	3.3	18	35	7.3	11	77	5.6	13	59	5.6	9	59
De,Ac,Ch	12	11	4.1	19	36	7.1	14	63	6.6	14	58	5.6	5	49
						low								
De	2.0	2.3	1.2	8	51	<LOQ			0.90	11	39	1.4	11	62
Cf[b]	2.4	2.3[c]	0.89	7	39	<LOQ			1.0	20	43	1.6	4	69
De,Ac	1.9	1.9[c]	1.4	7	75	4.1	3	215	1.5	20	79	1.8	12	94
De,Ac,Ch	2.4	2.3[c]	1.8	6	77	5.0	8	218	1.5	13	65	2.6	11	112

[a]Samples contained deltamethrin, β-cyfluthrin, a 1:1 mixture of deltamethrin and acrinathrin, or a 1:1:1 mixture of deltamethrin, acrinathrin, and λ-cyhalothrin. The lower concentration samples were prepared by diluting the first set of samples 5-fold. Results are shown following oxidation by sodium chlorite and analysis by LC-MS/MS, GC-MS, and ELISA and oxidation with hydrogen peroxide with analysis by ELISA. All oxidations were run in triplicate with the percent relative standard deviation shown. The spiked orange oil was analyzed for individual pyrethroids by LC-MS/MS to determine the pyrethroid content more accurately, and LODs were 5 nM (1 ppb), 2 μM, and 0.8 μM when detected by LC-MS/MS, GC-MS, and immunoassay, respectively. De, deltaemethrin; Cf, cyfluthrin; Ac, acrinathrin; and Ch, λ-cyhalothrin. [b]The concentrations of 4-F-3PBA were calculated against the 3-PBA calibration. [c]These samples were below the detectable limit for the LC-MS/MS so the reported values are the values from the corresponding high concentration sample divided by 5.
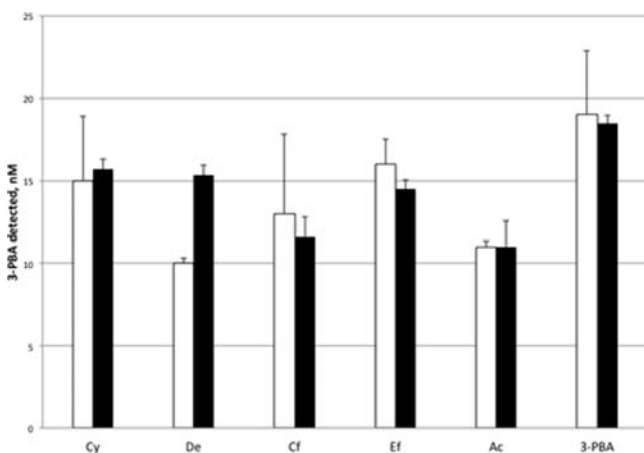

Figure 4. Conversion of 20 nM of cypermethrin (Cy), deltamethrin (De), cyfluthrin (Cf), esfenvalerate (Ef), and acrinathrin (Ac) and recovery of 20 nM 3-PBA in water using hydrogen peroxide oxidation. All samples were run in triplicate. Detection was performed using ELISA (white bars) and GC-MS (black bars). Error bars are the standard deviation from the three replicates.

analysis of the sample before and after performing the oxidation procedure. In many cases, the 3-PBA would be from the breakdown of pyrethroids in the sample and would be indicative of pyrethroid use. Another limiting problem is the inherent variability of the pyrethroid conversion, but the

benefits in the time and sensitivity of analysis may outweigh this drawback. This method is likely most useful for the immunoassay determination of pyrethroid levels and is a good method for first pass screening of samples before more detailed quantitation as would be the case in quality control screening. Finally, the sensitivity achieved using the LC-MS/MS technique for 3-PBA in the citrus oil matrix is lower than the LOD when assaying individual pyrethroids in the citrus oil.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Detailed instrumental settings, methods for extraction, ELISA analysis of 3-PBA, optimization of the oxidants, and preparation of standards. This material is available free of charge via the Internet at http://pubs.acs.org.

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Table 2. Conversion of Cypermethrin and Deltamethrin in Spiked Putah Creek Water after Conversion to 3-PBA by Hydrogen Peroxide Oxidation Followed by Immunoassay Detection[a]

spiked concn	rep 1	rep 2	rep 3	rep 4	mean	% RSD	% conversion
100 nM cypermethrin	67.6	68.1	73.2	70.5	69.8	3.7	70
20 nM cypermethrin	12.8	11.9	16.3	15.9	14.2	15.5	71
100 nM deltamethrin	58.3	74.0	55.2	75.0	67.1	15.4	67
20 nM deltamethin	11.0	13.6	9.2	14.3	12.0	19.4	60

[a]All results are reported as nanomolar.

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