



Published in final edited form as:

J Agric Food Chem. 2012 May 23; 60(20): 5065–5070. doi:10.1021/jf2051653.

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

Mark R. McCoy¹, Zheng Yang², Xun Fu³, Ki Chang Ahn¹, Shirley J. Gee⁴, David C. Bom³, Ping Zhong³, Dan Chang⁴, and Bruce D. Hammock^{1,*}

¹Department of Entomology and UCD Cancer Center, University of California Davis, Davis, CA 95616

²Givaudan Flavors Corp., 4705 U.S. Highway 92 East, Lakeland, FL 33801

³Givaudan Flavors Corp., 1199 Edison Drive, Cincinnati, OH 45216

⁴Synthia-LLC, P.O. Box 1238, Gualala, CA 95445

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 both with instrumental and immunochemical methods. Environmental analysis of the pyrethroids by immunoassay requires either knowing which pyrethroids contaminate the source or the use of non-specific 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%-75% and methods that require no extraction steps for either the immunoassay or LC-MS/MS techniques. Limits of detection for this technique applied to orange oil are 5 nM, 2 μ M, and 0.8 μ M when detected by LC-MS/MS, GC-MS, 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*). Since pyrethroids and pyrethrins are effective broad-spectrum insecticides with low acute

*CORRESPONDING AUTHOR: bdhammock@ucdavis.edu, (530)752-8465 .
mrmccoy@ucdavis.edu

Supporting Information. Detailed instrumental settings, methods for extraction, ELISA analysis of 3-PBA, optimization of the oxidants, and preparation of standards can all be found in the supporting information. This information is available free of charge via the internet at <http://pubs.acs.org>.

toxicity to birds and mammals, they have been gradually replacing organophosphate insecticides for pest control in urban environments. In 2007, 258 tons of pyrethroids were used for non-agricultural 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 run-off.(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 the 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. In order 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, since 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 multi-residue 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 for 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 multi-step sample cleanup and concentration. Experiments conducted in our laboratory also found that the sensitivity for some pyrethroids, especially cyhalothrin and beta-cyfluthrin, are much worse than other types of pesticides, by using either a GC-MS/MS or 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 post-column photoderivatization and chemiluminescence detection followed by vigorous sample extraction and a pre-concentration step.(25) Considering the IC₅₀ 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 alpha 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-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, that 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 pre-concentration 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

18 MΩ Water was produced by a Barnstead Easypure Rodi water purification system (Thermo-Fisher, San Jose, CA). Acetonitrile (99.5+%), 3-phenoxybenzoic acid (3-PBA, 98%), ammonium acetate (99.99+%), dimethyl sulfoxide (DMSO), N,O-bis(trimethylsilyl) trifluoroacetamide (BSTFA), goat anti-rabbit-horseradish peroxidase, 3,3',5,5'-tetramethylbenzidine (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 Corporation, Haverhill, MA).

Immunoassays were performed with 96-well Nunc microtiter plates (MaxiSorp surface, Roskilde, Denmark). Normal strength phosphate-buffered saline (PBS) (1 × PBS; 8 g/L of NaCl, 0.2 g/L of Na₂HPO₄, 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 Na₃C₆H₅O₇·2H₂O, pH to 5.5 using glacial acetic acid) were used for immunoassay. Substrate buffer was prepared by adding 400 l of 0.6% TMB in DMSO and 100 l of 1% H₂O₂ into 25 mL of citrate-acetate buffer.

Individual pyrethroid pesticide stock solutions (100 or 2000 ppm in acetonitrile) for deltamethrin, cypermethrin, acrinathrin, lambda-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-30m 0.25 mm I.D. film 0.25 mm from Agilent (Santa Clara, CA).

LC-MS/MS performance and recovery studies

The method limit of detection (LOD) was calculated as three times the standard deviation of 10 replicates of 5ppb 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 limit of detection (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 limit of detection (LOD) was defined as the IC₁₀ value of the 3-PBA standard curve produced from a dilution series made in the blank citrus oil oxidation. Since the calibration curve was linear from the IC₂₀-IC₈₀ the limit of quantification was defined by the IC₂₀ value. Method detection limits were determined by the average of 5 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 μ L) 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 μ L) and 10% sodium chlorite in 0.1 N NaOH (800 μ L) to the sample vial. Samples in vials were incubated for 1 h at 70°C with constant stirring.

After cooling, the vials were neutralized with 1M 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 400x.

Converting pyrethroids to 3-PBA in volatilized citrus oils using hydrogen peroxide

The volatilized oil residue was resuspended by adding dioxane (200 μ L) and 15% hydrogen peroxide in 0.1 N NaOH (800 μ L) was added to the samples. Samples were incubated for 1 h at 70°C with constant stirring.

After cooling, the vials were neutralized with 1M HCl and cleaned up by SPE for instrument analysis. For immunoassay, one 0.5% (wt/wt) platinum on alumina pellet was added while still hot to neutralize the excess oxidant. The pellets were allowed to incubate for one hour before the sample was neutralized with 6M 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% H₂O₂ in 0.8 N NaOH (250 μ L) was added to 250 μ L 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 μ L, initial pH 7.4) in 0.4 M acetic acid was added. Samples were then run by 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 being 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 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. Since 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 alpha-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) Since, 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).

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 μ M deltamethrin and 10 μ M C¹³-3-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 C¹³-3-PBA internal standard. Based on 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.

Since the method would be most useful without clean-up 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 10 μM of different type-II-pyrethroids (deltamethrin, cypermethrin, lambda-cyhalothrin, acrinathrin, 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 40-60% conversion to 3-PBA with cypermethrin being the most efficient and acrinathrin being the least when using chlorite oxidation, hydrogen peroxide ranged from 50-70% conversion and recovery 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 between 30-50% in other citrus oil matrices with a decrease in conversion to the 3-PBA in lemon, grapefruit, and 10x orange oil 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 through 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 lambda-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 sample A2 and B2 being lower than the limit of quantitation and sample 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 limit of detection 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 conversion 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 20nM 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 were detected by immunoassay method using either oxidized or non oxidized Putah Creek water at a limit of quantification, determined using the IC80 of the immunoassay standard curve, of 1 nM for 3-PBA in non-oxidized samples and using a lower threshold of 50% conversion we estimate a 2 nM limit of detection 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, limits of detection 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 semi quantitative 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 in the samples would give a higher value for pyrethroid contamination, but this can be corrected by 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 limit of detection when assaying individual pyrethroids in the citrus oil.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was supported in part by the following: The National Institute of Environmental Health Sciences (NIEHS) Superfund Basic Research Program [P42 ES004699]; Research agreement S08-004603 with Synthia-LLC; and Mark McCoy was supported by a National Institute of Health National Research Service Award [F32 ES018039]. The support of the Western Center for Agricultural Health and Safety at the University of California Davis [PHS OH07550] is also acknowledged.

REFERENCES

1. Weston DP, Lydy MJ. Urban and agricultural sources of pyrethroid insecticides to the Sacramento-San Joaquin Delta of California. *Environ. Sci. Technol.* 2010; 44(5):1833–1840. [PubMed: 20121184]
2. Bacey J, Spurlock F, Starnes K, Feng H, Hsu J, White J, Tran DM. Residues and toxicity of esfenvalerate and permethrin in water and sediment, in tributaries of the Sacramento and San Joaquin rivers, California, USA. *Bull. Environ. Contam. Toxicol.* 2005; 74(5):864–871. [PubMed: 16097319]

3. Lubick N. Scaling peaks: the life and science of Walter Giger. *Environ. Sci. Technol.* 2008; 42(17): 6313–6317. [PubMed: 18800495]
4. Tabarean IV, Narahashi T. Kinetics of modulation of tetrodotoxin-sensitive and tetrodotoxin-resistant sodium channels by tetramethrin and deltamethrin. *J. Pharmacol. Exp. Ther.* 2001; 299(3): 988–997. [PubMed: 11714887]
5. Shafer TJ, Meyer DA, Crofton KM. Developmental neurotoxicity of pyrethroid insecticides: critical review and future research needs. *Environ. Health Perspect.* 2005; 113(2):123–136. [PubMed: 15687048]
6. Soderlund DM, Clark JM, Sheets LP, Mullin LS, Piccirillo VJ, Sargent D, Stevens JT, Weiner ML. Mechanisms of pyrethroid neurotoxicity: implications for cumulative risk assessment. *Toxicology.* 2002; 171(1):3–59. [PubMed: 11812616]
7. Spencer J, O'Malley M. Pyrethroid illnesses in California, 1996–2002. *Rev Environ Contam Toxicol.* 2006; 186:57–72. [PubMed: 16676901]
8. Lee N, Beasley HL, Skerritt JH. Development of Immunoassays for Type II Synthetic Pyrethroids. 2. Assay Specificity and Application to Water, Soil, and Grain. *J. Agric. Food Chem.* 1998; 46(2): 535–546. [PubMed: 10554274]
9. Lee HJ, Shan G, Ahn KC, Park EK, Watanabe T, Gee SJ, Hammock BD. Development of an enzyme-linked immunosorbent assay for the pyrethroid cypermethrin. *J. Agric. Food Chem.* 2004; 52(5):1039–1043. [PubMed: 14995094]
10. Nakata M, Fukushima A, Ohkawa H. A monoclonal antibody-based ELISA for the analysis of the insecticide flucythrinate in environmental and crop samples. *Pest Manag. Sci.* 2001; 57(3):269–277. [PubMed: 11455657]
11. Gao H, Ling Y, Xu T, Zhu W, Jing H, Sheng W, Li QX, Li J. Development of an enzyme-linked immunosorbent assay for the pyrethroid insecticide cyhalothrin. *J Agric Food Chem.* 2006; 54(15): 5284–5291. [PubMed: 16848507]
12. Hammock BD, Wengatz I, Stoutamire DW, Gee SJ. Development of an enzyme-linked immunosorbent assay for the detection of the pyrethroid insecticide fenprothrin. *J. Agr. Food Chem.* 1998; 46(6):2211–2221.
13. Shan G, Stoutamire DW, Wengatz I, Gee SJ, Hammock BD. Development of an immunoassay for the pyrethroid insecticide esfenvalerate. *J. Agric. Food Chem.* 1999; 47(5):2145–2155. [PubMed: 10552511]
14. Lee HJ, Watanabe T, Gee SJ, Hammock BD. Application of an enzyme-linked immunosorbent assay (ELISA) to determine deltamethrin residues in milk. *Bull. Environ. Contam. Toxicol.* 2003; 71(1):14–20. [PubMed: 12945836]
15. Park EK, Kim JH, Gee SJ, Watanabe T, Ahn KC, Hammock BD. Determination of pyrethroid residues in agricultural products by an enzyme-linked immunosorbent assay. *J. Agric. Food Chem.* 2004; 52(18):5572–5576. [PubMed: 15373394]
16. Nichkova M, Fu X, Yang Z, Zhong P, Sanborn JR, Chang D, Gee SJ, Hammock BD. Immunochemical screening of pesticides (simazine and cypermethrin) in orange oil. *J. Agric. Food Chem.* 2009; 57(13):5673–5679. [PubMed: 19526986]
17. Gee SJ, Mak SK, Shan G, Lee HJ, Watanabe T, Stoutamire DW, Hammock BD. Development of a class selective immunoassay for the type II pyrethroid insecticides. *Anal. Chim. Acta.* 2005; 534(1):109–120.
18. Hao XL, Kuang H, Li YL, Yuan Y, Peng CF, Chen W, Wang LB, Xu CL. Development of an enzyme-linked immunosorbent assay for the alpha-cyano pyrethroids multiresidue in Tai lake water. *J. Agric. Food Chem.* 2009; 57(8):3033–3039. [PubMed: 19368347]
19. Zhang Q, Zhang W, Wang X, Li P. Immunoassay development for the class-specific assay for types I and II pyrethroid insecticides in water samples. *Molecules.* 2010; 15(1):164–177. [PubMed: 20110881]
20. Lehotay SJ, de Kok A, Hiemstra M, van Bodegraven P. Validation of a fast and easy method for the determination of residues from 229 pesticides in fruits and vegetables using gas and liquid chromatography and mass spectrometric detection. *J. Aoac Int.* 2005; 88(2):595–614. [PubMed: 15859089]

21. Pang GF, Fan CL, Liu YM, Cao YZ, Zhang JJ, Fu BL, Li XM, Li ZY, Wu YP. Multi-residue method for the determination of 450 pesticide residues in honey, fruit juice and wine by double-cartridge solid-phase extraction/gas chromatography-mass spectrometry and liquid chromatography-tandem mass spectrometry. *Food Addit. Contam.* 2006; 23(8):777–810. [PubMed: 16807205]
22. Pastor A, Esteve-Turrillas FA, de la Guardia M. Determination of pyrethroid insecticide residues in vegetable oils by using combined solid-phases extraction and tandem mass spectrometry detection. *Anal. Chim. Acta.* 2005; 553(1-2):50–57.
23. Lee GH, Nguyen TD, Lee MH. Rapid determination of 95 pesticides in soybean oil using liquid-liquid extraction followed by centrifugation, freezing and dispersive solid phase extraction as cleanup steps and gas chromatography with mass spectrometric detection. *Microchem. J.* 2010; 95(1):113–119.
24. Corrion ML, Ostrea EM Jr, Bielawski DM, Posecion NC Jr, Seagraves JJ. Detection of prenatal exposure to several classes of environmental toxicants and their metabolites by gas chromatography-mass spectrometry in maternal and umbilical cord blood. *J. Chromatogr. B Analyt. Technol. Biomed Life Sci.* 2005; 822(1-2):221–229.
25. Galera MM, Garcia MD, Valverde RS. Determination of nine pyrethroid insecticides by high-performance liquid chromatography with post-column photoderivatization and detection based on acetonitrile chemiluminescence. *J Chromatogr A.* 2006; 1113(1-2):191–197. [PubMed: 16516905]
26. Werner, I.; Moran, K. *Synthetic Pyrethroids*. American Chemical Society; 2008. *Effects of Pyrethroid Insecticides on Aquatic Organisms*; p. 310-334.
27. Munoz B, Almodovar A, Arrebola JR. *Ganula gadirana* n. sp., a new Hygromiidae from southern Spain (Pulmonata : Helicoidea). *Malacologia.* 1999; 41(1):163–174.
28. Pastor A, Esteve-Turrillas FA, Aman CS, de la Guardia A. Microwave-assisted extraction of pyrethroid insecticides from soil. *Anal. Chim. Acta.* 2004; 522(1):73–78.
29. Shan G, Huang H, Stoutamire DW, Gee SJ, Leng G, Hammock BD. A sensitive class specific immunoassay for the detection of pyrethroid metabolites in human urine. *Chem. Res. Toxicol.* 2004; 17(2):218–225. [PubMed: 14967009]
30. Master HE, Khan SI, Poojari KA. Synthesis of low molecular weight compounds with complement inhibition activity. *Bioorg. Med. Chem. Lett.* 2003; 13(7):1249–1251. [PubMed: 12657256]
31. Ruzo LO, Holmstead RL, Casida JE. Solution Photochemistry of Potent Pyrethroid Insecticide Alpha-Cyano-3-Phenoxybenzyl Cis-2,2-Dimethyl-3-(2,2-Dibromovinyl)Cyclopropanecarboxylate. *Tetrahedron Lett.* 1976; 35:3045–3048.
32. Lores M, Fernandez-Alvarez M, Sanchez-Prado L, Llompert M, Garcia-Jares C, Cela R. Alternative sample preparation method for photochemical studies based on solid phase microextraction: Synthetic pyrethroid photochemistry. *Journal of Chromatography A.* 2007; 1152(1-2):156–167. [PubMed: 17234200]
33. Wilson CW, Shaw PE. Quantitation of Individual and Total Aldehydes in Citrus Cold-Pressed Oils by Fused-Silica Capillary Gas-Chromatography. *J Agr Food Chem.* 1984; 32(2):399–401.

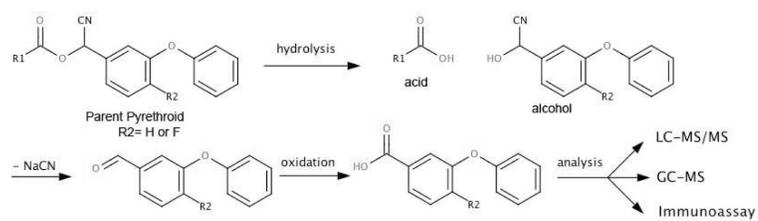


Figure 1.
Chemical conversion of type II pyrethroids to 3-PBA

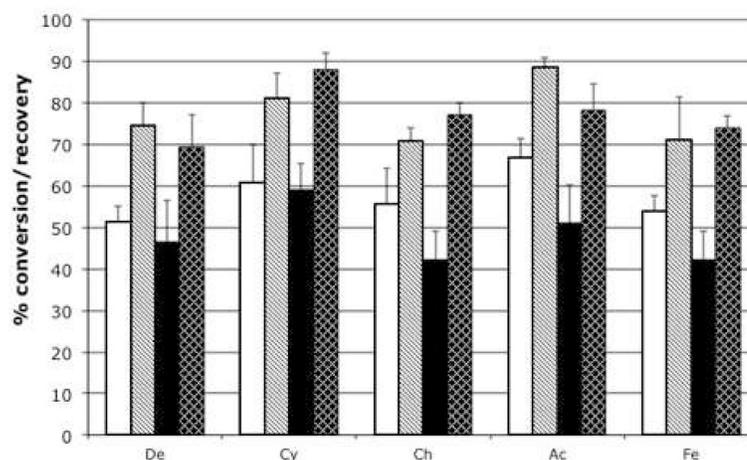


Figure 2.

Percent conversion and recovery of 5ppm of deltamethrin (De), cypermethrin (Cy), lambda-cyhalothrin (Ch), acrinathrin (Ac), and fenpropathrin (Fe) spiked into orange oil. A recovery standard of ¹³C-3-PBA was spiked at 2.5 ppm. Samples were oxidized with 15% hydrogen peroxide (white bars pyrethroid, with dashes C13-3-PBA) or with 10% sodium chlorite (black bars pyrethroid, white checkerboard C13-3-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.

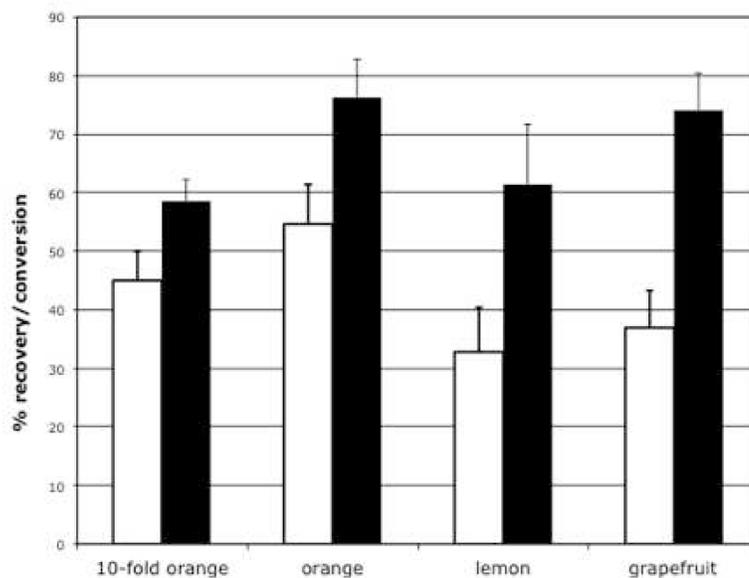


Figure 3. Conversion and recovery of 5 ppm deltamethrin (white bars) and recovery of 2.5 ppm C13-3-PBA (black bars) in other citrus oils as analyzed by GC-MS. Oils were oxidized using hydrogen peroxide and cleaned up using the mixed mode SPE. Samples were run in quadruplicate with the error bars being the standard deviation of the replicates.

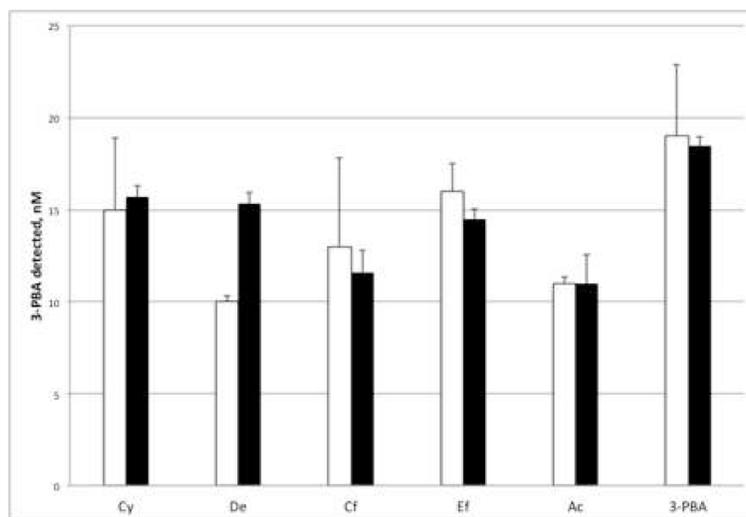


Figure 4. Conversion of 20 nM of cypermethrin (Cy), deltamethrin (De), cyfluthrin (Cf), esfenvalerate (Ef), and acrinathrin (Ac) and recovery of 20 nM 3-PBA in water using hydrogen peroxide oxidation. All samples were run in triplicate. Detection was performed using ELISA (white bars) and GC-MS (black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

Table 1

Analysis of individual pyrethroids and pyrethroid mixtures in orange oil. Samples contained deltamethrin; beta-cyfluthrin; 1:1 mixture of deltamethrin and acrinathrin; or 1:1:1 mixture of deltamethrin, acrinathrin, and lambda-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 pyrethroid content more accurately, limits of detection were 5 nM (1 ppb), 2 μ M, and 0.8 μ M when detected by LC-MS/MS, GC-MS, and immunoassay respectively.

Pyrethroid	Theoretical Concentration (uM)	Concentration (LC-MS/MS)	LC-MS/MS			GC-MS			ELISA chlorite			ELISA Hydrogen Peroxide		
			Average	%RSD	Recovery (%)	Average	%RSD	Recovery (%)	Average	%RSD	Recovery (%)	Average	STD%	Recovery (%)
De	9.9	10.6	4.9	15	46	11.39	10	107	5.8	9	55	5.9	6	55
Cf*	12	11.3	4.7	8	42	5.99	12	53	5.8	16	52	4.7	13	41
De, Ac	9.6	9.5	3.3	18	35	7.29	11	77	5.6	13	59	5.6	9	59
De, Ac, Ch	12	11.3	4.1	19	36	7.11	14	63	6.6	14	58	5.6	5	49
De	2.0	2.3	1.2	8	51	<LOQ			0.9	11	39	1.4	11	62
Cf*	2.4	2.3 ⁺	0.9	7	39	<LOQ			1.0	20	43	1.6	4	69
De, Ac	1.9	1.9 ⁺	1.4	7	75	4.09	3	215	1.5	20	79	1.8	12	94
De, Ac, Ch	2.4	2.3 ⁺	1.8	6	77	5.01	8	218	1.5	13	65	2.6	11	112

The concentrations of 4-F-3PBA were calculated against the 3-PBA calibration.

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 De = Deltamethrin, Cf = Cyfluthrin, Ac = Acrinathrin, Ch = lambda-cyhalothrin

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. All results are reported as nM.

Spiked concentration	Rep 1	Rep 2	Rep 3	Rep 4	Mean	%RSD	Percent 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 Deltamethrin	11.0	13.6	9.2	14.3	12.0	19.4	60