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Analytical Methods for Pesticides in Food and Residential Dust

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Analytical Methods for Pesticides in Food and Residential Dust

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B.A., Mercer University, 2004

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An abstract of
A dissertation submitted to the Faculty of the Graduate School of Emory University
in partial fulfillment of the requirements for the degree of
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Abstract

Analytical Methods for Pesticides in Food and Residential Dust
By Ronald E. Hunter, Jr.

Due to widespread use of agrochemicals, such as organophosphorus (OP) and pyrethroid pesticides, Americans are exposed to insecticides via food and dust ingestion daily. Upon the extraction of house dust and commonly consumed foods, researchers have demonstrated the prevalence of OP and pyrethroid pesticides in these matrices. Despite advancements in pesticide residue analysis of food and residential dust, there is still a need for the further development of economical, high-throughput, rapid, multi-residue methods. Via these methods, researchers can investigate insecticidal dietary exposure of small populations by introducing innovative sample compositing techniques, such as categorizing and compositing food samples by food type (*e.g.* fruit and above- or below-ground vegetables). Consequently, we recognized that regularly consumed foods and house dust contain measurable quantities of OP and pyrethroid pesticides, developed analytical methods for quantifying amounts of these pesticide residues in food and residential dust, and applied methodologies to relative samples collected from a population of 12 in a pilot study endeavoring to assess persons' total pesticide exposure to three OP and four pyrethroid pesticides. This is important because there is (1) a shortage of multi-residue methods for food and house dust, (2) an increased consumption of imported food in the U.S., (3) a harmful effect of insecticides at all stages of life, and (4) a need to obtain limits of detection ≤ those used in Food and Drug Administration surveillance programs because researchers are observing pesticide metabolites in part per trillion levels in urine.

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LIST OF ACRONYMS

2,4-D	2,4-Dichlorophenoxyacetic Acid
3-PBA	3-phenoxybenzoic acid
4-F-3-PBA	4-fluoro-3-phenoxybenzoic acid
ACh	Acetylcholine
AChE	Acetylcholinesterase
AOAC	Association of Official Analytical Chemists
ATSDR	Agency for Toxic Substance and Disease Registry
BHC	Benzene Hexachloride
CFSAN	Center for Food Safety and Applied Nutrition
CN	Cyano
CSFII	Continuing Survey of Food Intake by Individuals
DAPs	Dialkylphosphates
DB-5	Durabond-5
DCCA	3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane-1-carboxylic acid
DDT	Dichloro-diphenyl-trichloroethane
DSPE	Dispersive Solid-phase Extraction
ECD	Electron-capture Detector
EPA	Environmental Protection Agency
ESI	Electrospray Ionization
FAO	Food and Agriculture Organization
FDA	Food and Drug Administration
FFDCA	Federal, Food, Drug, and Cosmetic Act
FID	Flame Ionization Detector
FIFRA	Federal Insecticide, Fungicide and Rodenticide Act
FQPA	Food Quality Protection Act
GC	Gas Chromatography
GPC	Gel Permeation Chromatography
HPLC	High-pressure Liquid Chromatography
HVS3	High Volume Small Surface Sampler
LOD	Limit of Detection
LOG D	Distribution Coefficient
LOQ	Limit of Quantification
LVI	Large Volume Injector
MASE	Microwave-assisted Solvent Extraction
MDL	Method Detection Limit
MRL	Maximum Residue Level
MRM	Multi-residue Method
MS	Mass Spectrometer
MSPD	Matrix Solid-phase Dispersion
NAFTA	North American Free Trade Agreement
NFCS	National Food Consumption Survey
NH_2	Aminopropyl
OP	Organophosphorus
PDP	Pesticide Data Program

PLE	Pressurized Liquid Extraction
PRA	Pesticide Residue Analysis
PSA	Primary-secondary Amine
QqQ	Triple Quadrupole
QuEChERS	Quick, Easy, Cheap, Effective, Rugged, and Safe
RfD	Oral Reference Dose
SAS	Statistical Analysis System
SAX	Strong Anion Exchange
SBSE	Stir Bar Sorption Extraction
SFE	Supercritical Fluid Extraction
SIM	Selected Ion Monitoring
SPE	Solid-phase Extraction
SPME	Solid-phase Microextraction
TCPy	3,5,6-trichloro-2-pyridinol
TDS	Total Diet Study
TOF	Time of Flight
TWG	Technical Working Group
UPLC	Ultra-performance Liquid Chromatography
USDA	U.S. Department of Agriculture
VSSC	Voltage-sensitive Sodium Channels
WHO	World Health Organization
WWII	World War II

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CHAPTER 1: INTRODUCTION AND BACKGROUND

Ronald E. Hunter, Jr.

A Short History of Pesticide Use in the U.S.

Mankind began using pesticides against pests as early as 2500 BC. For example, the ancient Samarians used sulfur to control insects. In 1000 BC Homer suggested using sulfur to fumigate homes, and the Chinese used arsenic to control garden insects as early as 900 BC (CIPM 2003). For thousands of years, humans have been battling pests, any plants or animals that threaten our food supply, health or comfort (Delaplane 1996).

Until World War II (WWII), the United States used primarily inorganic and biological substances, such as petroleum, turpentine, Paris Green (copper (II) acetoarsenite), and pyrethrum to combat pests, particularly insects and plant diseases, such as fungi (CIPM 2003; Delaplane 1996; Regenstein 1982). In recent years, the majority of pest control was carried out non-chemically, with methods such as crop rotations, cultivation, and meticulous regulation of sowing dates. However, pesticides have also been used for agricultural purposes, as farmers focus on protecting valuable, small acreage crops, such as produce and cotton. Copper sulfate and hydrated lime called Bordeaux mixture proved to be an effective fungicide and became widely used between 1860 and 1942. Alongside the development of inorganic and biological pesticides, the technology of pesticide dispersion has also seen advances. Between 1860 and1942, the hand and power driven pressure sprayer were invented and aerial application implemented. As a result, the application of pesticides became easier and more economical and applicable to large scale use in agriculture (CIPM 2003). Pest control became more efficient with the advent of new pesticides that resulted from chemical warfare used in WWII (CIPM 2003; Delaplane 1996).

With the introduction of the new chemicals dichloro-diphenyl-trichloroethane (DDT), lindane or benzene hexachloride (BHC), aldrin, dieldrin, endrin, and 2,4-dichlorophenoxyacetic acid (2,4-D), a new period of inexpensive, effective, and popular pest control began (CIPM 2003; Delaplane 1996). The introduction of these pesticides reduced labor needs, since previously widespread labor-intensive weed and insect control methods and pest-reducing practices were no longer needed. Because of its broad applicability against insects affecting both human health and agriculture, DDT became the most standard pesticide to use in the 1940's (Delaplane 1996). For example, in the 1940's extensive spraying of DDT financed by the local and federal governments in metropolitan communities decimated the population of mosquitoes, fire ants, the Japanese beetle, and other insects (Smeltzer 2003). As a result of the increased effectiveness of pesticides, an increase in crop yields shortly followed (CAEPA 2006). Moreover, the popularity and efficacy of these new chemicals led to the development of other synthetic insecticides, such as organophosphorus (OP) pesticides (CIPM 2003).

This new era of OP pesticides saw a shift from protecting exclusive, small acreage crops to safeguarding major field crops, such as grains and corn, where weeds were easily controlled with 2,4-D (CIPM 2003; Delaplane 1996). Again, new inventions, such as the development of granular pesticide formulations, helped to make the widespread use of pesticides more economical and practical for major field crops (CIPM 2003). Farmers profusely began applying pesticides to fields, a practice that perpetuated the creation of pest-free surroundings (Delaplane 1996). Many persons predicted pesticides coupled with high-yield plant types, chemical fertilizers, irrigation technology, and mechanization

would be a “Green Revolution” that would create an abundance of food for the world (CAEPA 2006; Smeltzer 2003). Society began to view pesticides as miracle chemicals in the years following WWII. Ironically, this meaning is contrary of the connotation of “Green Revolution” today. Still, during the past 60 years, agricultural production around the world has increased noticeably partly because of pesticide usage. Many global health concerns have also been addressed through the use of these chemicals. For instance, we have seen the eradication of malaria-carrying mosquitoes in many parts of the world (Smeltzer 2003).

As pesticide use increased substantially in the late 1940s, farmers began to experiment with them in diverse ways on all classes of crops. However, little was known about the toxicity of pesticides, and few persons were concerned about the negative effects of pesticides because apparent, immediate benefits, such as hearty produce and improved crop yields, overshadowed these concerns (CAEPA 2006). Constantly attacked by chemical reagents, pests became genetically resistant to pesticides (Delaplane 1996). Harmless plants, animals, and insects, such as non-target crops, farm animals, and honeybees, began to endure the repercussions of widespread, lackadaisical pesticide use (CAEPA 2006; Delaplane 1996). More importantly, occupational injury and death resulted from incorrect pesticide applications (CAEPA 2006).

At the peak of the great “Green Revolution” and use of “miracle” chemicals like the exalted DDT in the 1960s, the warning of Edwin Way Teale, a nature writer, began to resound loud and clear. He stated, “’A spray as indiscriminate as DDT can upset the

economy of nature as much as a revolution upsets social economy. Ninety percent of all insects are good, and if they are killed, things go out of kilter right away'" (NRDC 1997). Eventually, the publication of *Silent Spring* by Rachel Carson in 1962 would spark the modern environmental movement (CAEPA 2006; CIPM 2003; Delaplane 1996; NRDC 1997; Smeltzer 2003).

In *Silent Spring* Carson describes how DDT can enter the food chain, accumulate in the fatty tissue of all animals, including humans, and cause cancer and genetic damage. She concluded that DDT and other pesticides had irreversibly harmed birds and animals and negatively affected the world's food supply (NRDC 1997). When *Silent Spring* was published, the book received much criticism, specifically from pesticide companies, such as the American Cyanamid Company and Monsanto, who simultaneously published refutations portraying a world devastated by famine and disease because chemical pesticides. Eminent scientists encouraged President John F. Kennedy via the President's Science Advisory Committee to examine issues raised by the book. Consequently, the government began to monitor closely the use of DDT and ultimately banned it. At this point, manufacturers (*e.g.* Monsanto) were now challenged to prove that the chemicals they were producing were not harmful (NRDC 1997).

No one more than Carson accentuated pesticide risks raising questions about the concrete benefits of pesticides (Delaplane 1996). *Silent Spring* sparked widespread awareness that nature is susceptible to human intervention. Persons saw the need to regulate industry to

facilitate the protection of the environment for the first time (NRDC 1997), and many federal and state laws have been changed since the publishing of *Silent Spring*.

Pesticide Regulation in the U.S.

Regulation of the development, production, and use of pesticides has become a focus of the status quo of pesticides. However, modest interest in pesticide regulation persisted until about the 20th century. People were using chemicals, such as sulfur, petroleum, and arsenicals, they had used for decades, and there did not appear to be a need for chemical regulation. For example, directly after the Post-Civil War era the equivalent of today's Secretary of Agriculture, the Commissioner of Agriculture, reported only inspecting pesticides for chemical composition and the further development of their proposed uses in pest control (CIPM 2003).

Awakening the public's awareness of quality of life in regards to the food supply, Upton Sinclair, a predecessor of Carson, published *The Jungle* in which he challenged the healthiness of the U.S. food supply. Sinclair emphasized that the meat packing industry is the major culprit negatively influencing the food supply (Sinclair 2008). *The Jungle* led to the passage of the Food and Drug Act of 1906, which gave the government authority over food treated with pesticides and food traded in interstate commerce (CIPM 2003; NAS 1975).

At the end of the 19th century, laws began to emerge protecting growers from ineffective insecticides. At this time, the pesticide industry did not exist, and farmers made insecticide formulations. Congress recognized that insecticide dealers could exploit farmers and send imitation products as most insecticides were sent via mail or sold by traveling salesmen. As a result, they passed the first serious regulation of insecticides in

the U.S., the Insecticide Act of 1910, which protected farmers against insecticide fraud (CIPM 2003). The Insecticide Act of 1910 also created tolerances for specific insecticides (CIPM 2003; Odom 1991). This Act covered only insecticides while commonly used pesticides, such as fungicides, were not included in the Act (CIPM 2003).

Another pre-WWII regulation was the enactment of the Federal Food, Drug, and Cosmetic Act (FFDCA) in 1938. This Act provided that tolerances be set for chemicals (including pesticides) and established tolerances for residues in food where select chemicals were necessary for the production of the food supply. The main chemicals regulated were Paris Green, pyrethrin, and Bordeaux mixture (Kenaga 1989). The Act also require the addition of color to insecticide preparations as a preventive measure of misuse (Grodner 1997).

Pre-WWII pesticide regulations had little effect although the increase in the production of DDT, BHC, dithiocarbamate fungicides and 2,4-D created the need for better pesticide reform at that time. Previous regulations and the inception of pesticide industry led to the passage of the 1947 Federal Insecticide, Fungicide and Rodenticide Act (FIFRA), which superseded the Federal Insecticide Act of 1910. FIFRA covered all pesticides and required the registration of all pesticides through the U.S. Department of Agriculture (USDA) (CIPM 2003).

FIFRA maintained the measures set out by the Federal Insecticide Act of 1910. FIFRA continued to protect farmers and others against harmful, ineffective products, and the USDA had to approve product labels prior to vending (CIPM 2003). Appropriately termed a "labeling act", FIFRA did not sanction pesticide abuse, stop orders of harmful pesticides, or expand penalties for companies that sold unsafe products (Briggs 1992). Companies rarely adhered to FIFRA since they could obtain a "protest registration" and sell dangerous products despite denial of registration by the USDA. Subsequent amendments to FIFRA corrected these critical flaws and (1) registration of pesticides began to include a federal registration number; (2) warning labels were added where appropriate; and (3) false declarations of safety were removed from labels (CIPM 2003).

Similarly, amendments regarding pesticides were also made to the FFDCA during the 1950s. The Miller Act of 1954 revised the FFDCA to allow new means for establishing tolerances of pesticide residues in food and to give the job of screening food for pesticide residues to the Food and Drug Administration (FDA) (CIPM 2003). Then Congress passed the Delaney Clause governing regulation of pesticide residues in processed foods as part of the 1958 Food Additives Amendment to the FFDCA. The Delaney Clause established that no pesticide residues ever attributed to cause cancer in animals would be permissible as a food additive. This ignored any benefits of pesticides and explicitly conveyed that tolerance levels must be based on the risk of carcinogenicity only (Vogt 1992). The primary purpose of the Miller Act and the Food Additives Amendment of 1958 was to allow the FDA to denounce raw agricultural commodities, processed foods, and animal feed found to contain any pesticides not approved for use or pesticide levels

exceeding preset tolerances. By creating a no tolerance for any carcinogenic chemical, the Amendment of 1958, particularly the Delaney Clause, did not coincide with other amendments of the FFDCA (CIPM 2003). Governing the regulation of residues in raw commodities, the Pesticide Residue Amendment of the FFDCA called for a balancing act between the necessity of consumer health protection and the basic requirement of a wholesome, inexpensive food supply (Holloway and Rowell 1992).

A decade after Carson's book, *Silent Spring*, Congress pressured by increased concerns about pesticides amended FIFRA in 1972. DDT was actually removed from the market a few months prior to the Amendments of1972. The extreme editing of FIFRA by the Amendments of 1972 made it more of an environmental protection act. The major modification of FIFRA addressed human health and environmental protection while maintaining original consumer protections outlined in the Act of 1910. Other changes included enforcement provisions, additional flexibility in harmful chemical regulation, the extension of federal law to include intrastate registrations, the establishment of different types of registrations (*e.g.* general, restricted use), the reformation of governmental appeals processes, dealing with trademark/copyright issues, and reregistering old pesticides (CIPM 2003). Because of the Amendment of 1972, many pesticides were removed from the market during the 1970s and 1980s to include organochlorine pesticides, such as aldrin, dieldrin, chlordane, heptachlor, and kepone (CIPM 2003).

Of all of the new tasks the government mandated the Environmental Protection Agency

(EPA) to perform, the EPA struggled most with the call to reregister all existing pesticides by specific target dates. Although amendments in 1988 provided revenue to increase the availability of EPA resources for the Pesticide Program and relative sectors, re-registration continued to be a gradual process (CIPM 2003). Because of this, the federal government introduced the Food Quality Protection Act (FQPA) of 1996 dramatically changing pesticide regulation in the United States (Lu, *et al.* 2004). The FQPA of 1996, an amendment to both FIFRA and the FFDCA, expedited the re-registration process (CIPM 2003). For example, FQPA of 1996 required EPA to review the safety of all existing tolerances that were in use as of August 1996. Of the 9,721 existing tolerances, EPA was required to reassess 33% by August 3, 1999, 66% by August 3, 2002, and 100% by August 3, 2006 (USEPA 2008). Figure 1.1 below shows how well this was accomplished after the enactment of the FQPA of 1996.

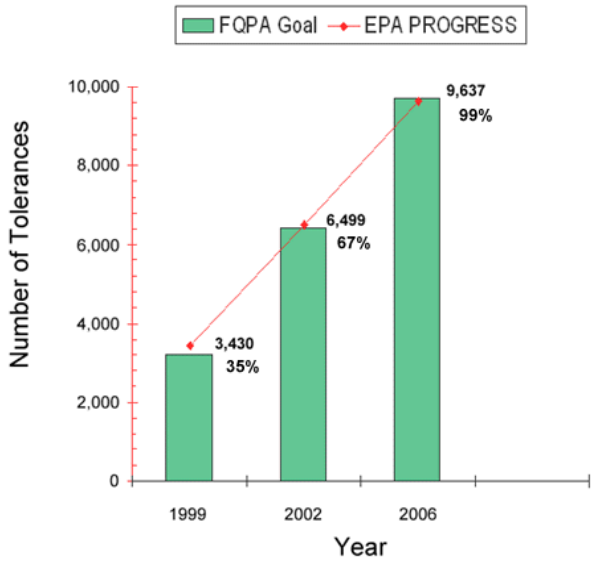

Figure. 1.1. U.S. EPA completed 9,637, or over 99% of the 9,721 tolerance reassessment decisions required by FQPA, meeting mandated reassessment percentages by each deadline (USEPA 2008).

The FQPA of 1996 also obligated the EPA to consider a variety of facets of pesticide use (Kieszak, *et al.* 2002). These factors, which also incorporated the overall effects of exposure to pesticides with a common mechanism of toxicity (Kieszak, *et al.* 2002), included

- authorizing a single, health-based standard for all pesticides in all foods and eliminating the issues with the Delaney Clause by replacing it with a "de minimis" risk policy (CIPM 2003; Holloway and Rowell 1992);
- providing special protections for infants and children and special attention to chemicals that disrupt the endocrine system (CIPM 2003; USEPA 2008);
- accelerating the approval of safer pesticides (USEPA 2008);
- generating incentives for the expansion and maintenance of effective crop protection tools for U.S. farmers (USEPA 2008);
- and emphasizing the consumers' right to know about pesticides (CIPM 2003).

Since domestic pesticide matters may be affected by global pesticide use, the U.S. must work with other nations and international organizations in regulating pesticide use. In 1996 the U.S. and Canada included Mexico in pesticide regulatory efforts via the North American Free Trade Agreement's (NAFTA's) Technical Working Group (TWG) on Pesticides. The goals of the TWG is to standardize pesticide regulations among NAFTA members by tackling trade irritants, developing national regulatory and scientific capacity, sharing the review burden, and reaching similar regulatory and scientific resolutions on pesticides (CIPM 2003). The Codex Alimentarius, another example of collaborative pesticide regulatory efforts, is a respected guide of the global food market

composed by members of the Food and Agriculture Organization (FAO) and the World Health Organization (WHO). The Codex Alimentarius Commission sets maximum residue levels in food to protect consumers and avert foreign trade interruptions (FAO/WHO 2009).

Types of Pesticide and Use

Table 1.1 elaborates on the several types of pesticides, target pests, and usage benefits from pest control. In the U.S. in 2000 and 2001 pesticide use exceeded 1.2 billion pounds, in proportions similar to those of world pesticide use that exceeded 5.0 billion pounds (Figure 1.2), with a larger portion of total U.S. pesticide use of chlorine/hypochlorite (Figure 1.3) and OP pesticides (Figure 1.4).

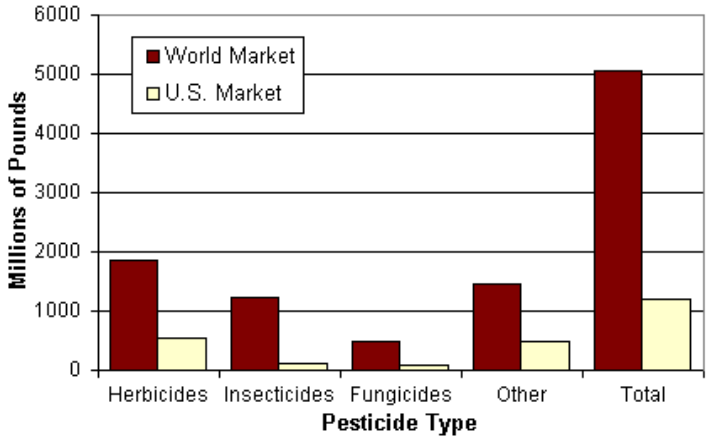

Figure 1.2. 2001 estimates of world and U.S. pesticide amounts of active ingredient at user level by pesticide type (USEPA 2009a)

Type of Pesticide	Target Pest Group
Acaricide	Mites, ticks, spiders
Antimicrobial	Bacteria, viruses, other microbes
Attractant	Attracts pests for monitoring or killing
Avicide	Birds
Fungicide	Fungi
Herbicide	Weeds
Insecticide	Insects
Molluscicide	Snails and slugs
Nematicide	Nematodes
Piscicide	Fish
Predacide	Vertebrate predators
Repellant	Repels pests
Rodenticide	Rodents
Synergist	Improves performance of another pesticide

Table 1.1. Major classes of pesticides and target pest group (Delaplane 1996).

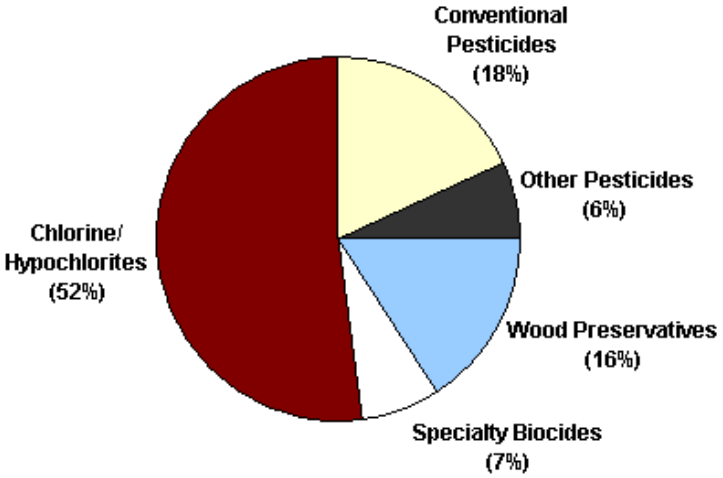

Figure 1.3. 2001 estimates of amount of pesticide used in the U.S. by pesticide group (USEPA 2009a).

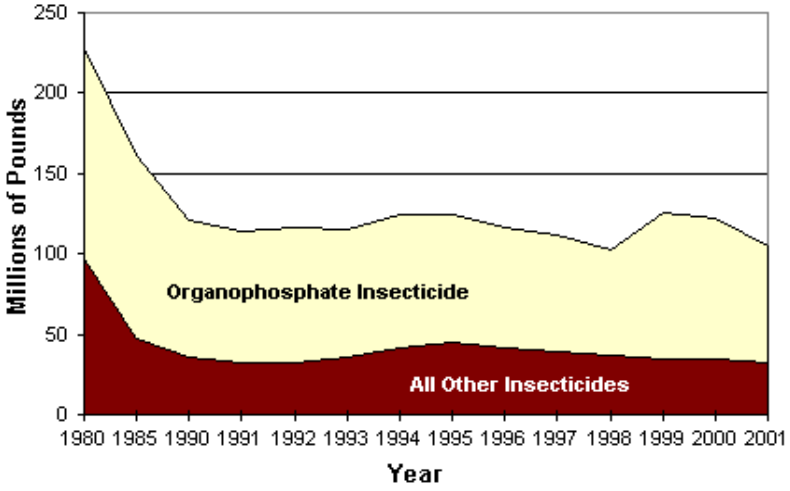

Figure 1.4. Total amount of OP insecticide and all other insecticides by active ingredients used in the U.S., all market sectors, 1980 – 2001 (USEPA 2009b).

In this work, the main pesticide types include OP and pyrethroid pesticides. OP pesticides lack environmental persistence, which was a major problem with organochlorine pesticides. Moreover, OP pesticides are relatively inexpensive, are broad-spectrum pesticides, and have not led to many OP pesticide resistant insects. Unfortunately, OP pesticides have serious acute toxicity health effects (Mills and Zahm 2001).

OP pesticides have become the most commonly applied insecticide due to market forces and regulation. Possessing four atoms directly attached to a phosphorous atom, the

majority of OP pesticides may be regarded as derivatives of phosphoric acid. Three of these bonds are usually single bonds while the other, commonly shown as a double bond, is a coordinate, covalent bond (Chambers and Levi 1992).

Prototypes of the entire OP pesticide family, true phosphates have oxygen as all four atoms surrounding the phosphorous. These compounds are highly reactive and are used only where short residual activity is desirable, such as on or near livestock or on the verge of the harvest of produce (Chambers and Levi 1992).

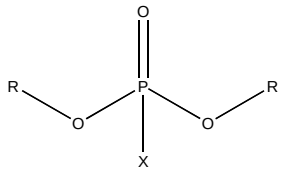

Figure 1.5. The general structure of an OP pesticide.

More numerous are the OP pesticides containing sulfur in a P=S moiety. These phosphorothionates include important insecticides, such as parathion, methyl parathion, diazinon, and chlorpyrifos. Often used as soil insecticides, this type of OP pesticide possesses a marked increase in toxicity (Chambers and Levi 1992).

Another large subclass of OP pesticides is phosphorothionothiolates in which most phosphorodithioates belong. In this case, one sulfur atom is in the P=S moiety while the other is a thioester. This group of OP pesticides includes the highly toxic compounds, phorate and terbufos, and relatively “safe” compounds, such as malathion and dimethoate. Only two phosphorodithioates, ethoprop and ebufos, are produced in the U.S. Surprisingly, they are only used outside of the U.S. (Chambers and Levi 1992).

Upon human exposure to OP pesticides, toxic effects of OP pesticide exposure can be noted primarily due to acetylcholinesterase (AChE) inhibition. Critical to the normal functioning of the nervous system, AChE is inhibited by OP pesticides similarly to the processes in a transphosphorylation reaction (Chambers and Levi 1992). Due to this, acetylcholine (ACh), the normal AChE substrate, accumulates at peripheral and central cholinergic synapses and signs of cholinergic overload are observed (Ward*, et al.* 1993). Moreover, the intact OP pesticide is not recovered upon the recovery of enzyme activity even though enzyme inhibition is reversible (Chambers and Levi 1992). On many occasions death can result in mammals due to respiratory arrest and cardiac failure (Ward*, et al.* 1993). Normal hydrolysis of ACh involves the acetylation of the serine-hydroxyl group at the catalytic center of the enzyme allowing release of the choline moiety. Further hydrolysis of acetyl-AChE completes the reaction (Chambers and Levi 1992).

During OP pesticide inhibition the converse occurs, the OP pesticide mimics ACh, and phosphorylation of the serine-hydroxyl group occurs. The hydrolysis of the phosphoryl-AChE is slow, and the catalytic activity of the enzyme is lost. The potency of anti-AChE OP pesticides depends on the degree to which phosphorylation of the serine-hydroxyl occurs. The substituents on the phosphorous atom determine potency. Thus, electron-donating atoms or groups significantly reduce anti-AChE activity while electron-withdrawing atoms or groups increase anti-AChE activity (Chambers and Levi 1992). The same mechanism that causes toxicity in insects also causes toxicity in mammals.

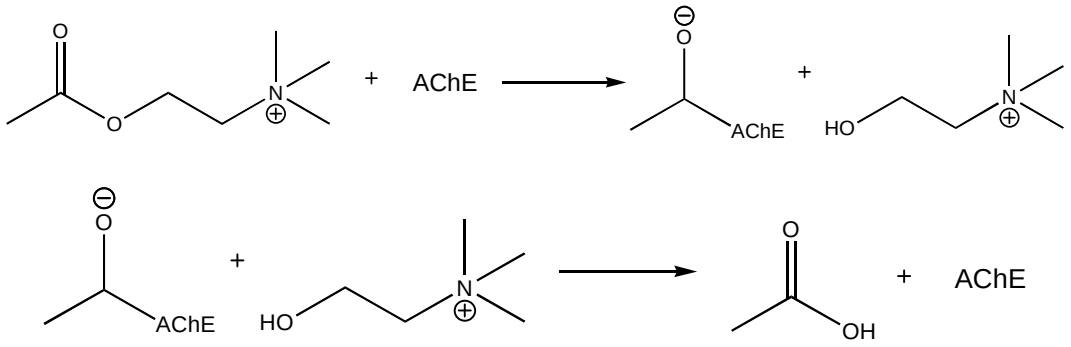

Figure 1.6. The normal reaction scheme of AChE.

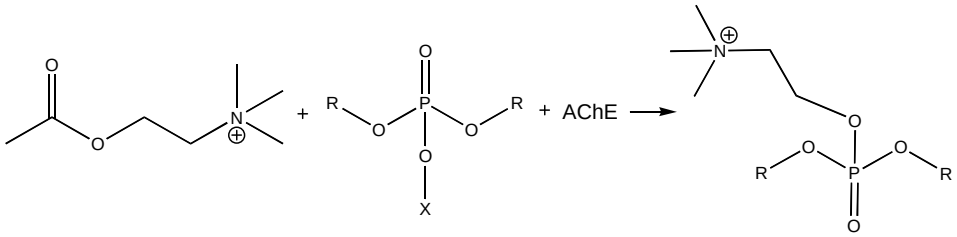

Figure 1.7. The reaction scheme of AChE in the presence of an OP pesticide.

The other category of pesticide of interest is pyrethroid pesticides. Pyrethroid pesticides are liberally used in agricultural and indoor pest control (Schettgen, *et al.* 2002). Accounting for more than 25% of the global insecticide market, pyrethroid pesticides have been increasingly used over the last 30 years. Currently, 16 pyrethroids are registered for use in the U.S. in a variety of consumer and agricultural products. They are often sold and used as mixtures containing a combination of two or more pyrethroid pesticides (Shafer, *et al.* 2005).

Pyrethrin pesticides are derived from natural compounds isolated from the Chrysanthemum genus of plants. Natural pyrethrin pesticides consist of an acid moiety, ester linkage, and an alcohol moiety. The acid moiety contains two chiral carbons and most pyrethrin pesticides exist as stereoisomeric compounds (Shafer, *et al.* 2005). Pyrethrin pesticides are photo-labile; thus, many researchers have synthesized their more

photo-stable analogs, pyrethroid pesticides (Katsuda 1999). There are two types of pyrethroid pesticides – type I, such as allethrin and tetramethrin, and type II pyrethroids, such as cypermethrin and deltamethrin (Soderlund, *et al.* 2002). The different types of pyrethroid pesticides are recognized based upon the structural presence or absence of a cyano (-CN) group in the alpha position of the 3-phenoxybenzyl alcohol moiety of the compound. Type I and II pyrethroid pesticides can also be differentiated based upon the symptoms of pyrethroid pesticide poisoning seen in rodents.

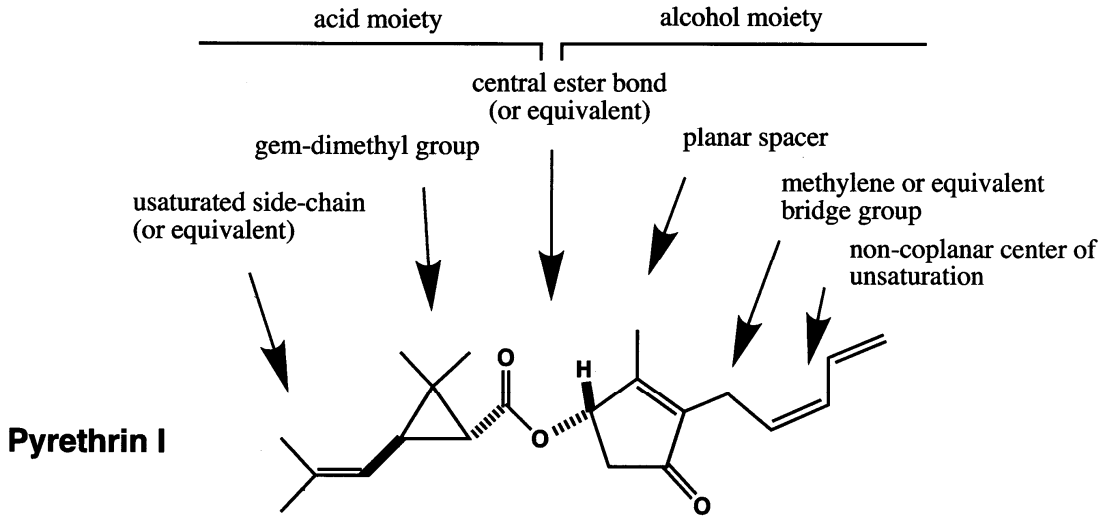

Figure 1.8. Example of a type I pyrethroid structure (Soderlund, *et al.* 2002).

Type I pyrethroid pesticides do not include a cyano group, and their toxicity symptoms, collectively known as T-syndrome, include signs of aggressive behavior, increased sensitivity to external stimuli, and tremors. Possessing a cyano group, Type II poisoning symptoms comprise salivation and choreoathetosis or sinuous writhing and are jointly called CS-syndrome. T- and CS-syndromes may exist in mammals, but pyrethroid pesticide levels high enough to trigger these symptoms in mammals are a rare occurrence (Soderlund, *et al.* 2002).

The primary mode of pyrethroid pesticide action in both insects and mammals is the disruption of voltage-sensitive sodium channels (VSSC). Perturbation of sodium channel function by pyrethroid pesticides occurs stereospecifically. Stereoisomers that disrupt VSSC function the most have the greatest toxicological effect and are the most effective insecticides. Pyrethroid pesticides slow the activation or opening and inactivation or closing of VSSC's. Thus, "sodium channels opened at more hyperpolarized potentials, stay open longer and allow more sodium ions to cross and depolarize the neuronal membrane" (Shafer*, et al.* 2005).

Short tail sodium currents are associated with type I pyrethroid pesticides exposure while long tail sodium currents are observed with exposure to type II pyrethroid pesticides (Soderlund*, et al.* 2002). Although much is already known about the mechanism of action of pyrethroid pesticides in insects, more information is needed concerning mammalian toxicological effects. Since pyrethroid pesticide and OP pesticide exposure occurs every day, exposure assessment of both is needed.

General Pesticide Biomarker Information

Ryan and coworkers define biomarkers as the results of interactions of a biological, chemical, or physical environment with a biological system (Ryan*, et al.* 2007). When discussing biomarkers, researchers focus primarily on chemical agents, and investigators typically discuss biomarkers according to three types – biomarkers of exposure, biomarkers of effect, and biomarkers of susceptibility (NRC 1987). Biomarkers of exposure and biomarkers of effect establish relationships regarding health effects and serve as the foundation for relating exposure to a causative agent and resulting biological outcome. For example, a biomarker of exposure can be parent compounds or metabolites or their DNA or protein adducts that are directly attributed to the causative agent of interest. Biomarkers of effect imply changes to the biological system of interest (*i.e.* the inhibitory effects of OP pesticides by acetylcholinesterase). Biomarkers of susceptibility affect the degree of the effect or generate this effect based upon a set dosage. Polymorphism of certain genes relative to metabolism of toxicants is how a biomarker of susceptibility may express itself. The trilogy of the biomarkers of exposure, effect, and susceptibility is usually the needed array to make a complete assessment of risk and exposure (Ryan*, et al.* 2007).

Currently, the literature has defined an ideal biomarker as fitting seven standards to include

- long-lasting
- non-invasively collected with minor technical complexity
- attributive to disease

- large sample size
- widespread distribution coupled with sequential occurrence
- sensitive
- benefits measuring parent compounds more than metabolites (Groopman and Kensler 2005; Metcalf and Orloff 2004; Schulte and Mazzuckelli 1991).

Ryan and coworkers applied the biomarker array concept to OP pesticides (Ryan, *et al.* 2007), and we will also briefly review this concept in the context of pyrethroid pesticides. OP and pyrethroid pesticides each have a common mechanism of toxicity although they may be broadly different in chemical structure (Ryan, *et al.* 2007; Starr, *et al.* 2008). The primary mechanism of action of OP pesticides is acetylcholinesterase inhibition while the principal mode of toxicity of pyrethroid pesticides is disruption of VSSC function (Ryan, *et al.* 2007; Starr, *et al.* 2008). The biomarkers of exposure to OP pesticides consist of dialkylphosphates (DAPs) and biomarkers specific or closely specific to different OP pesticides, such as 3,5,6-trichloro-2-pyridinol (TCPy) (Barr, *et al.* 2005; Barr, *et al.* 2004). The relative general pyrethroid biomarkers are 3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane-1-carboxylic acid (DCCA) and 3-phenoxybenzoic acid (3-PBA) whereas 4-fluoro-3-phenoxybenzoic acid (4-F-3-PBA) represents a more specific biomarker (Starr, *et al.* 2008). DAPs, DCCA, and 3-PBA provide information on generalized exposure to OP or pyrethroid pesticides and overall risk assessment (Ryan, *et al.* 2007; Starr, *et al.* 2008). On the other hand, TCPy, 4-F-3-PBA, and other OP- or pyrethroid-specific biomarkers, provide insight on exposure to specific OP or pyrethroid pesticides, such as chlorpyrifos (OP pesticide) or cyfluthrin (pyrethroid pesticide), the

parent compounds from which TCPy and 4-F-3-PBA originate, respectively (Ryan*, et al.* 2007; Starr*, et al.* 2008). For OP pesticides the biomarker of effect is acetylcholinesterase inhibition and for pyrethroid pesticides the biomarker of effect is VSSC disturbance (Ryan*, et al.* 2007; Starr*, et al.* 2008). This information may reveal something about susceptibility but not cause of inhibition or disruption. Moreover, overestimation of parent compound intake can result if premature degradation of the parent compound occurs in the environmental matrix, such as food. Consequently, inference of the biomarker of exposure is reduced (Ryan*, et al.* 2007). Nevertheless, the array concept provides appreciably more information than accumulating individual biomarker information.

Pesticide Exposure from Beverages, Food, Soil, and Residential Dust

Paustenbach (Paustenbach 2000) describes exposure as a term that is ambiguous due to inconsistency among the definitions found throughout literature.

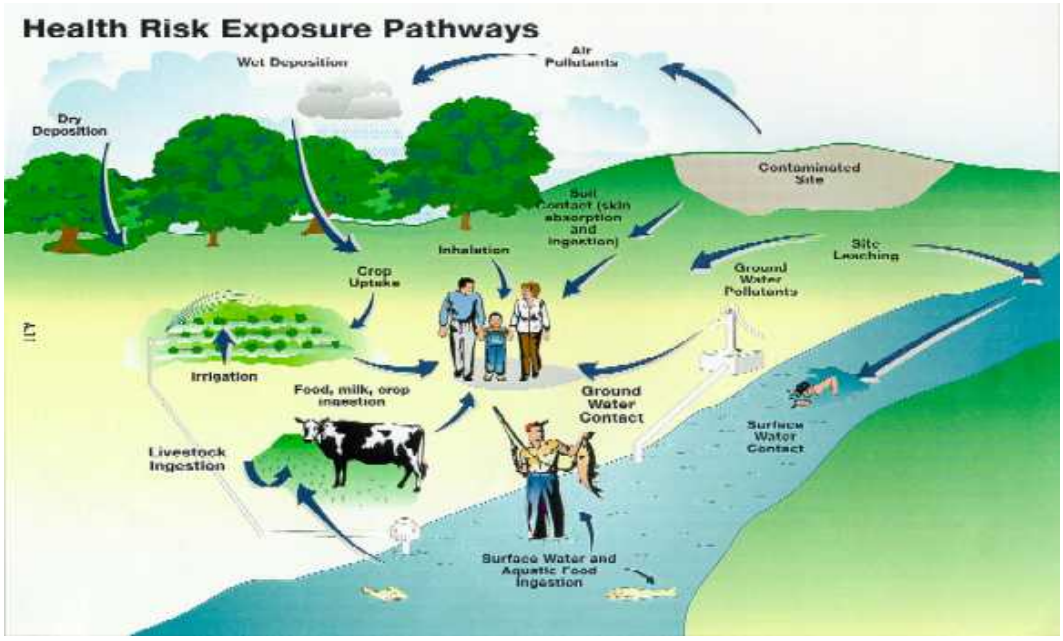

Figure 1.9. Exposure pathways (Kim Smith).

Most researchers agree that human exposure means contact with a chemical or a toxic agent (Allaby 1983). In this case, the definition of contact is unclear – does this mean visible contact or contact via exchange boundaries like the lungs and gastrointestinal tract? Many scientists set a hypothetical outer boundary of the body, which consists of the skin and openings of the body. These openings include body orifices, such as the mouth, nose, and cuts in one's skin. Exposure is then defined as the contact of a toxicant with part of this boundary (Paustenbach 2000). Figure 1.9 depicts pathways of exposure.

Many key aspects must be considered in order to determine the degree of exposure an individual has had. Exposure assessment is the quantitative or qualitative evaluation of

this contact. Qualitatively, exposure assessment describes the intensity, frequency, and duration of contact, the exposure route (dermal, oral, or respiratory) of the chemical, the dose, and the internal dose or the actual amount of chemical absorbed (Paustenbach and Leung 1994). A quantitative definition of exposure is the product of concentration, time, and duration, or rate of transport of the toxicant (mg/cm^2-min) (Paustenbach 2000). For instance, a homeowner who sprays his yard with pesticides bi-weekly and a pesticide applicator who sprays pesticides daily will have different degrees of exposure. The source, duration, magnitude, frequency, and transport medium will all greatly influence their degrees of exposure (Paustenbach and Leung 1994). In short, researchers defined exposure as "'the product of concentration, time, and duration, or rate of transport of toxicant (mg/cm^2-min)'" (Paustenbach 2000). This definition has become the general, practical and measurable definition of exposure.

Almost all food in society contains a great number of intentionally and unintentionally added chemicals, including pesticide residues, food additives to preserve taste and enhance the aesthetic value of food, and naturally occurring chemicals (Paustenbach 2000). Researchers estimate dietary exposure by considering the concentration of a residue in or on a food, the bioavailability of the contaminant in the media, and the amount of the food consumed (Paustenbach 2000; Suhre 2000). Generally, ingestion concerns the intake of one of the following matrices – drinking water, other liquids, food, soil, and house dust.

In order to estimate the degree of exposure from drinking water, one needs to know the quantity of water consumed, what concentrations of chemicals were present in the water, and how bioavailable that person's gastrointestinal tract is to the chemical. Soil contamination via landfills, hazardous waste sites, or discharges from water transport systems may cause drinking-water contamination (Paustenbach 2000). Total fluid intake is defined as the drinking of a variety of fluids, such as tap water, milk, soda, alcohol, and the inherent water in foods. Water drank from the tap as a drink or used in preparation of foods or drinks, such as coffee or tea, is defined as total tap water. All of these factors are variable per person and dependent upon factors like physical activity (Paustenbach 2000). As of 1997, the U.S. EPA said that a value of 2L/day for adults and 1L/day for infants is the assumed value of total tap water consumption when minimal information is known about the details of a chemical exposure to drinking water (USEPA 1997). Finally, ingestion of polluted drinking water may not always be the principal exposure route to toxicants in drinking water. Persons may be exposed to these chemicals via breathing them in as the chemical is released into air during showering, dishwasher processes, and other methods of chemical volatilization (Jo, *et al.* 1990; Kerger, *et al.* 2000; Kezic, *et al.* 1997; USEPA 1997).

Another potential route of exposure to toxic agents is via soil and house dust ingestion (Roberts and Dickey 1995). Soil and house dust exposure happens indirectly via several routes, such as (1) soil becomes house dust by local dust deposition and mud and dirt carried in by shoes and pets; (2) house dust and other fine particles remain on objects and children's hands; and (3) children ingest dust particles via hand-to-mouth contact

(Paustenbach, *et al.* 1997a; Paustenbach, *et al.* 1997b). Children possess the greater exposure potential for soil ingestion because the nature of being a child causes direct ingestion of soil and greater quantities of it. Moreover, mouthing behavior by children is normal and common (Barltrop 1966). Approximately 80% of all one-year old children display mouthing behavior. Mouthing behavior and the range of articles ingested generally decreases with age (Barltrop 1966). Children and adults alike ingest soil while eating incompletely washed produce, during hand-to-mouth contact, and through direct ingestion (primarily children). When particles too large to reach the lower respiratory tract are inhaled, they are swallowed and soil is ingested yet again. House dust contaminated with a number of chemicals can be ingested as well due to contact with foods and hand-to-mouth activities (Paustenbach, *et al.* 1997a; Paustenbach, *et al.* 1997b).

Although rare, the disease pica or the deliberate ingestion of soil, may occur in children and adults (Paustenbach 2000). Many researchers do not differentiate between small amounts of soil ingested because of mouthing behavior and pica (Kimbrough, *et al.* 1984). Those that blatantly eat large amounts of dirt, plaster, or paint chips (1-10 g/day) are at great risk for health problems and suffer from pica. Others suffer from geophagia, the craving of eating only dirt. Nevertheless, pica is usually considered typical behavior in young children (Paustenbach 2000).

Other causes of pica do exist as well. Culturally, pica for soil is prevalent because of the medicinal effects it is thought to have. For example, Aborigines use it to relieve diarrhea

and some women in the Southeastern U.S. crave soil and clay during gestation (Paustenbach 2000). Some have also reported pica to be associated with physical disorders like iron deficiencies or mental illness. In either case, unintentional soil ingestion resulting from mouthing behavior or accidental hand-to-mouth activity is the focus of most exposure because pica behavior is atypical (Kimbrough*, et al.* 1984).

Ingestion of contaminated food is another potential exposure route to chemicals. Knowing the percentage of foods grown above and below ground is valuable when estimating the toxicant concentrations in food from those concentrations in soil, water, and air. For instance, below-ground vegetables are more likely to be tainted with chemicals applied to soil while vegetables grown above ground would be more likely polluted by toxicants sprayed into the air. Table 2.1 gives a more complete picture of exposure factors and confidence ratings for liquids and food (USEPA 1997).

In order to assess the population's pesticide residue exposure from food ingestion, the FDA initiated the Total Diet Study (TDS), also known as the "market basket study" (Egan*, et al.* 2002; Pennington 1996). In the TDS the collection of food to be analyzed for pesticide residue is based on food consumption surveys, particularly the Continuing Survey of Food Intake by Individuals (CSFII), a national food consumption survey (NFCS) conducted by the USDA (Jacobs*, et al.* 1998). Intake data from the individual NFCS and CSFII components are based on "as eaten" (*i.e.* cooked or prepared) forms of the food items or groups. In this case, there is no need to account for distortions that occur during the cooking process (USDA 1987). From these surveys, researchers chose

the basis for the selection of the diets and helped to reflect the current food supply and consumption patterns of the population.

Exposure Factor	Recommendation	Confidence Rating
Drinking-water intake rate	21 ml/kg-d or 1.4 L/d (average) 34 ml/kg-d or 2.3 L/d (90th percentile) Percentiles and distribution also included Means and percentiles also included for pregnant and lactating women	Medium Medium
Total fruit intake rate	3.4 g/kg-d (per capita average) 12.4 g/kg-d (per capita 95th percentile) Percentiles also included Means presented for individual fruits	Medium Low
Total vegetable intake rate	4.3 g/kg-d (per capita average) 10 g/kg-d (per capita 95th percentile) Percentiles also included Means presented for individual vegetables	Medium Low
Total meat intake rate	2.1 g/kg-d (per capita average) 5.1 g/kg-d (per capita 95th percentile) Percentiles also included Percentiles also presented for individual meats	Medium Low
Total dairy intake rate	8.0 kg-d (per capita average) 29.7 g/kg-d (per capita 95th percentile) Percentiles also included Means presented for individual dairy products	Medium Low
Grain intake	4.1 g/kg-d (per capita average) 10.8 g/kg-d (per capita 95th percentile) Percentiles also included	High Low in long-term upper percentiles
Breast-milk intake rate	742 ml/d (average) 1,033 ml/d (upper percentile)	Medium Medium
Fish intake rate	*General population* 20.1 g/d (total fish) average 14.1 g/d (marine) average 6.0 g/d (freshwater/estuarine) average 63 g/d (total fish) 95th percentile long-term Percentiles also included *Serving size* 129 g (average) 326 g (95th percentile) *Recreational marine anglers* 2–7 g/d (finfish only) *Recreational freshwater* 8 g/d (average) 25 g/d (95th percentile) *Native American subsistence population* 70 g/d (average) 170 g/d (95th percentile)	 High High High Medium High High Medium Medium Medium Low

Table 1.2. Summary of default exposure factor recommendations and confidence ratings for citizens of U.S. (USEPA 1997).

Reasons to Care about Exposure

Beginning with foods from the TDS, researchers calculated the levels of different pesticides in food items representative of the U.S. diet. Then researchers endeavored to determine pesticide intake using multi-residue analysis methods (MRMs) developed by the FDA in collaboration with the Association of Official Analytical Chemists (AOAC) based upon the intake of food items derived from food consumption surveys (Sawaya*, et al.* 2000). For example, the FDA and Center for Food Safety and Applied Nutrition (CFSAN) has determined that 50% of all coffee/tea/wine samples evaluated via the Pesticide Program Residue Monitoring in 2003 were in violation of previous maximum residue limits set. This was also the case for two-thirds of the baby foods/formula and 80% of peanuts and peanut products examined (FDA/CFSAN). This delineates the need for further study of pesticide exposure via food ingestion and the effects of this exposure on the population. Additionally, the established maximum residue limits are based on animal data, such as mice models; therefore, relatively little information is actually known about maximum residue limits relative to humans.

In the U.S., there is a great consumption of imported foods, such as fish, fresh produce, and grains (Sawaya*, et al.* 2000). Many of the countries that we import food from are third-world countries where there is little to no control over the use of pesticide residues in foods. In these countries, data baselines on the status of the pesticide levels in foods eaten and their dietary intake by different groups of the population are nonexistent (Sawaya*, et al.* 2000). Pesticides banned or withdrawn from our market, such as the environmentally-persistent DDT organochlorine pesticide, are still produced, used, and

sold in markets of developing countries. Approximately 200 million pounds of compounds banned from use in the U.S. were applied in developing countries in 1995. OP and carbamate pesticides are also used significantly in the Third World. This makes the problem of food contamination more critical. In fact, most of the growth in the world pesticide market is concentrated in developing nations. Globally, OP pesticides account for nearly 40% of total insecticide sales by value, followed by carbamate pesticides (20.4%), pyrethroid pesticides (18.4%), and organochlorine pesticides (6.1%) (Mansour 2004).

Another reason to assess exposure to pesticides in food is the harmful effects pesticides may have at all of stages of life, particularly the developing fetus. For example, many OP pesticides are lipophilic and easily cross the placenta (Richardson 1995). Much experimental evidence links OP pesticide exposure during gestation or the early post-natal period to adverse neurodevelopmental effects of progeny (Eskenazi*, et al.* 1999). Exposure during the third-trimester when fetal brain growth is at its peak may be even more harmful. A dosimeter of prenatal exposure may be the measurement of OP pesticide in the complex matrix, meconium, since many have shown that OP pesticides exist in meconium obtained at birth (Whyatt and Barr 2001). A mother feeding her child via the nutrients from food she consumes could be inadvertently exposing her unborn child to OP pesticides and other toxicants. Any high-level OP pesticide exposures have both acute and long-term neurological effects. These effects are also known to last for 10 years after poisoning, suggesting permanent residual damage and the persistence of the pesticide in the body. Moreover, less severe poisoning can have long-term consequences (Kamel*, et*

al. 2005). In either case, we may be exposed to pesticides and endure their adverse effects before we are even born and years after birth.

Children exposure to pesticides is a current public health concern (Olden and Guthrie 2000). Diet is one of the significant sources of pesticide exposure considered in many child risk models (Akland*, et al.* 2000). Eating different foods than adults, including fresh produce, juice and processed foods, baby foods and fruit juices, children eat more food per body mass (Fenske*, et al.* 2002). These foods may contain higher levels of pesticide residues than foods commonly consumed by adults (Paustenbach 2000). In addition to pesticides ingested directly from food, there is also the potential for excess pesticide dietary intake caused by food-to-surface-to-mouth or surface-to-hand-to-food activities (Melnyk*, et al.* 2000). Children exposure to pesticides occurs while they are rapidly developing, and their bodies may be more susceptible to the effects of pesticides. Moreover, the amount of chemicals absorbed in the body by adults and children are influenced by the physiological differences between the two groups. In comparison to adults, children have a greater surface area to body weight ratio. This may lead to greater amounts of chemical absorption by the body. Children also have greater circulatory flow rates that can impact the distribution of pesticides in the body. During key developmental years, changes occur in liver enzyme systems that can increase or decrease the toxicity of pesticides in children. This causes the same amount of chemical exposure in a child to have a greater impact proportionally than in an adult. Furthermore, the immature immune system of a child may be less effective than that of adults for detoxification and elimination of toxicants (Bearer 1995; Mills and Zahm 2001). For example, during a case

study in McFarland, a farming town in the central valley of California, researchers observed that the incident rate of childhood cancer was twice as high as expected (Moses 1989). Many suspected this unusual rate was due to pesticide exposure. The magnitude of the risks associated with pesticides is greater in children than adults, and this suggests greater child susceptibility to pesticides (Mills and Zahm 2001).

Analytical Chemistry of Pesticide Monitoring of Food

Many employ chromatographic methods coupled with a variety of sample preparation techniques when endeavoring to determine pesticide residue prevalence in food samples (Anastassiades*, et al.* 2003; Torres*, et al.* 1996). Recently, researchers have focused more on chromatographic separation or detection procedures and less on sample preparation (Smith 2003). According to Hercegová *et al*. (Hercegova*, et al.* 2007), MRMs have become the most effective tactic in pesticide residue analysis (PRA). In the 1960s, the first MRM, called the Mills method, gained attention as it was used to determine non-polar organochlorine pesticides in non-fatty foods (Mills*, et al.* 1963). This method used acetonitrile to extract samples followed by a dilution of the extract in water and a partitioning of the pesticides into a non-polar solvent. In attempts to make the Mills method better, other researchers tried to apply the method to polar pesticides by using different solvents for the initial extraction and adding NaCl to the partitioning step (Luke*, et al.* 1975). The 1980's called for the avoidance of dangerous solvents and the creation of more environmental and health friendly methods. From these needs, solid-phase extraction (SPE) was developed as a cleanup step to circumvent the use of harmful substances during liquid-liquid partitioning (Hercegova*, et al.* 2007). To reduce not only solvent use but also manual labor, others developed a number of extraction approaches including matrix solid-phase dispersion (MSPD), supercritical fluid extraction (SFE), solid-phase microextraction (SPME), and the quick, easy, cheap, effective, rugged and safe (QuEChERS) method (Anastassiades*, et al.* 2003; Hercegova*, et al.* 2007).

Ultimately, researchers want a variety of desirable properties to be an inherent part of the

sample preparation method for PRA. They are

- singular, multi-residue procedure
- recoveries approaching 100%
- sufficient cleanup of sample
- increased concentration of analytes
- superior precision
- ruggedness
- economical
- rapid
- easy
- environmental-friendly (safe).

Recent trends have also added to this list the use of minimal amounts of sample, a continuance of making methods safer and more "green", and a decrease of time and manual labor without sacrificing recovery or precision (Hercegova*, et al.* 2007). Similarly, current trends have also involved the monitoring of pesticide residues, specifically in the food of children. Thus, PRA of baby food has become a central focus (Hercegova*, et al.* 2007). Hercegova *et al*. (Hercegova*, et al.* 2007) defined baby food as food made explicitly for infants, ages 6-24 months. One of the commonalities of baby food that make it ideal for PRA is the fact that it is intended to be eaten easily and constitutes a soft, fluid and/or easily chewable paste. A variety of matrices encompass what we know as baby food. For example, non-fatty matrices (< 2% fat composition), such as produce-based baby food, and fatty matrices (> 2% fat content), such as meat/eggs/cheese baby food, exists (USDA 1999). Grain matrices, such as cereal-based

baby food, with a range of fat content are also a staple in baby food diets. More importantly, breast and/or infant formula milk constitutes the brunt of any baby's primary nutrition. Thus, employing baby food during method development of any method for PRA in food is a satisfactory option.

Some food samples require pre-treatment, specifically solid samples (Cajka and Hajslova 2004). Here, a homogenization process is used. For example, many studies have cut solid samples into smaller pieces and mixed them in a mixer at room temperature (Lehotay, *et al.* 2005b). Fussel *et al*. (Fussell, *et al.* 2002) showed that pesticides were lost when produce samples were pulverized at room temperature. Others solved this problem via cryogenic milling where they homogenized samples in the presence of dry ice. Anastassiades *et al*. (Anastassiades, *et al.* 2003) makes a comparison between shaking and blending in the presence of dry ice as the first step of extraction to ascertain the extent to which pesticide residues could be extracted from produce. Shaking proved best for extraction in their study. Sample extraction efficacy is increased when an adequate homogenization method is used, such as homogenization with the Ultra Turax (Lehotay, *et al.* 2005b). Also, the less the researcher manipulates the sample, the less uncertainty exists regarding pesticide residue concentrations, particularly at low levels (Hercegova, *et al.* 2007).

Another pre-treatment factor is the sample size. The inclination to minimize sample size persists in PRA due to the desire to increase efficiency, trim down costs, and use less solvent (Hercegova, *et al.* 2007). Various sample size amounts have been used in PRA of

food and evaluations of quality of the data performed in laboratories. The majority of MRMs used 50-100g of sample taken from a larger sample set that has been previously homogenized with chopping devices (Anastassiades, *et al.* 2003). Baby food analysis sample sizes have been smaller (2-25g) (Halvorsen, *et al.* 2000; Hercegova, *et al.* 2007).

Liquid extraction is the primary method for the removal of pesticide residues from baby food and other food samples (Hercegova, *et al.* 2007). Affecting the ability to extract non-polar and polar pesticides, cleanup steps, and other factors, solvent selection is the most important choice in developing MRMs. One of the most popular extraction procedures of the past for PRA of produce was the Luke method, which involves an acetone extraction followed by separation with a mixture of dichloromethane and light petroleum (Luke, *et al.* 1975). Another popular method used an ethyl acetate extraction in the presence of Na_2SO_4 (Bicchi, *et al.* 1996). Both methods have been modified in many regards to fit the trends of today.

Over the last decade, acetone, the combination of acetone and dichloromethane, ethyl acetate, acetonitrile, and methanol have been commonly used solvents in MRMs for PRA of produce and baby food (Hercegova, *et al.* 2007). Three solvents have been utilized the most – acetone, ethyl acetate, and acetonitrile. Methanol is used more often for the extraction of pesticide residues in produce in conjunction with high-pressure liquid chromatography with mass spectrometer (MS)/MS (HPLC-MS/MS) (Hercegova, *et al.* 2005; Hernandez, *et al.* 2006). Acetone allows efficient contact with aqueous parts of produce. However, there was difficulty partitioning acetone from the aqueous phase since

it is wholly water-miscible. Moreover, acetone's volatility during the extraction process caused an increased evaporation during sample handling (Hercegova*, et al.* 2007). Conversely, ethyl acetate is not 100% miscible with water; thus, water was easily removed via an excess of drying agent, such as Na_2SO_4 (van der Hoff and van Zoonen 1999). Acetonitrile gained popularity after the advent of the QuEChERS method (Anastassiades*, et al.* 2003). Acetonitrile easily partitions in the presence of water with NaCl and leads to increased recoveries of polar compounds (Anastassiades*, et al.* 2003). Acetonitrile also removes fewer lipophilic compounds from samples compared to acetone and ethyl acetate (Mastovska and Lehotay 2004). Another benefit of acetonitrile is the fact that it is compatible with reverse phase liquid chromatography (Leandro*, et al.* 2006). Solvent exchange is not necessary because it can serve as a medium for gas chromatography (GC) injection. Unlike acetonitrile, ethyl acetate and acetone do not have such a large solvent expansion volume during vaporization in GC and are volatile and less toxic (Anastassiades*, et al.* 2003). Still, all three solvents provide acceptable recoveries (70-120%) for a variety of pesticides, and Maštovká and Lehotay ranked the solvents according to use for sample preparation for PRA of produce denoting acetonitrile as most useful and acetone as least useful (Mastovska and Lehotay 2004).

PRA of food becomes more complex as the fat content increases in the food sample. Lipids need to be removed during the sample preparation process or they can reap havoc on chromatographic instrumentation, ruining the column and GC sensitivity. Inherent differences exist in the analysis of low-fat foods, such as milk with < 3% fat content, and high-fat foods, such as animal fat or butter with > 20% fat content. Low-fat foods

introduce the occurrence of both lipophilic and hydrophilic pesticides, and MRMs have to cater to a wide polarity range of pesticides. We only expect lipophilic analytes in high fatty matrices and utilize non-polar solvents to dissolve the fat and extract the pesticides (Lehotay, *et al.* 2005c).

In the analysis of most milk samples, sample preparation has involved the de-proteinization of the sample followed by the extraction of fats and relative pesticide residues from the milk's aqueous phase (Burke, *et al.* 2003; Campoy, *et al.* 2001). Subsequently, a cleanup step is used to partition interferences of the sample matrix from the pesticide residues (Hercegova, *et al.* 2007). Others have lyophilized milk prior to extraction, separated the fat via centrifugation or performed extraction of liquid milk (Chao, *et al.* 2006; Lehotay, *et al.* 2005c; Newsome, *et al.* 1995). In this case, solvent systems used have involved extraction with ethyl ether/hexane, diethyl ether/petroleum ether, and acetone/hexane solvent mixtures (Burke, *et al.* 2003; Campoy, *et al.* 2001).

Most notably, Moore *et al*. determined eight organochlorine pesticides and OP pesticides at 10 ng/g in baby formula using a multi-residue extraction method with an acetone/hexane/water extraction solvent mixture (Moore, *et al.* 2000). Recoveries ranged from 66-121%. All pesticides were determined by high-resolution GC with electron-capture detector (ECD) (Cressey and Vannoort 2003). Using this technology, the limit of detection (LOD) was 0.2 ng/g for organochlorine pesticides and 10 ng/g for OP pesticides. Wang and Chueng also created a sensitive multi-residue liquid chromatography electrospray ionization (ESI)-MS/MS method with LODs < 0.6 ng/g for

carbamate pesticides (Wang and Cheung 2006). Finally, a buffered QuEChERS method used for PRA of produce was also used in PRA of fatty food matrices (Lehotay, *et al.* 2005b). Researchers conducted PRA of milk, eggs, and avocado at a fortification level of 50 ng/g and observed the fat content's effect on recoveries of a wide range of polarity of pesticides. They noticed that as the fat content of the sample increased, the recovery of non-polar pesticides decreased. The limit of quantification (LOQ) was extrapolated to be < 10 ng/g (Lehotay, *et al.* 2005c).

Other extraction techniques, such as SFE, SPME, stir bar sorption extraction (SBSE), and MSPD, are also used (Hercegova, *et al.* 2007). SFE resulted from the analytical community's challenge to minimize solvent use and manual labor (Lehotay 1997). SFE techniques demand the optimization of several parameters for each matrix and increase the cost of instrumentation use. The principal advantages of SFE are the fact that high selectivity exists and that the obtained extract is pure and pre-concentrated (Hercegova, *et al.* 2007). This methodology has been used in produce and nonfat baby food. For example, Halvorsen *et al*. used it to determine fenpyroximate (0.1-1.0 ng/g) in apples (Halvorsen, *et al.* 2000).

SPME is usually used in conjunction with GC and is able to extract and detect volatile residues easily, rapidly, and economically. However, these luxuries come at the expense of mediocre quantification, definite matrix dependence, and other practicalities. Because SPME in PRA usually necessitates more than one extraction step, SPME is best used when one wants to obtain qualitative information, such as transformational changes.

SBSE has also been used in PRA of baby food and is similar to SPME (Hercegova*, et al.* 2007). However, SBSE can be used to extract less polar pesticide residues quantitatively. SBSE with LODs of 2.0 and 5.0 ng/g was used to extract organochlorine pesticides from strawberries (Wennrich*, et al.* 2001).

The majority of MSPD has used reversed-phase materials, such as C_{18}, C_8, and Florisil sorbents (Navarro*, et al.* 2002). Reversed-phase materials were used more for the removal of lipophilic interferences while others have employed more polar phases to remove polar residues. Minimal solvent (10-15mL) is used in MSPD, and primary solvents used are ethyl acetate and dichloromethane. Moreover, MSPD involves a pre-concentration of the final extracts on many occasions (Navarro*, et al.* 2002). Kristenson *et al*. demonstrated and confirmed the automation of a miniaturized MSPD technique with subsequent GC-MS analysis for PRA in fruits (Kristenson*, et al.* 2001). They were able to obtain LODs between 10-50 ng/g and utilized the reversed-phase C_8 sorbent (Kristenson*, et al.* 2001). Of the other available techniques, MSPD is the only one that has been used in the PRA of baby food fatty matrices and used to extract a wide variety of pesticide residues, such as organochlorine, OP, and pyrethroid pesticides. In comparison to the buffered QuEChERS method, MSPD has been shown to give 50% higher recoveries in MRMs with lipophilic pesticides in milk and eggs (Lehotay*, et al.* 2005c).

After the appropriate extraction technique is chosen, most of the resulting extracts need to be cleaned via SPE, dispersive solid-phase extraction (DSPE), or gel permeation

chromatography (GPC). Although rarely used for MRMs due to their strong affinity to polar OP pesticides, Florisil and silica are some of the first sorbents applied and still used during SPE (Hercegova*, et al.* 2007). Subsequently, researchers began using a variety of types of carbon sorbents, particularly graphitized carbon black (GCB), for the cleanup of pesticide extracts from produce matrices (Schenck*, et al.* 2002). This type of SPE sorbent strongly absorbs planar molecules, such as pigments, and isolates them from the sample extract. However, carbon sorbents did not get rid of confounders, such as matrix co-eluants or response augmentation (Hercegova*, et al.* 2007).

Other types of SPE columns applied to MRMs of produce and baby food have been the reverse phase C_{18} sorbent and chemical bonded stationary phases, such as aminopropyl (-NH_2), primary-secondary amine (PSA), and strong anion exchange (-SAX) (Leandro*, et al.* 2005). Comparing the cleaning efficiency of these columns on acetone and acetonitrile sample extracts from a variety of produce, Schenck and coworkers determined that -NH_2 and PSA normal phase SPE columns were most effective (Schenck*, et al.* 2002). Aminopropyl and PSA columns removed hexadecanoic and octadecanoic acids, fatty acids present in many green vegetables, while C_{18} and SAX did not eliminate the majority of matrix co-eluants in the sample (Schenck*, et al.* 2002). Moreover, PSA can also remove sugars and other interferences that are capable of forming hydrogen bonds. Fatty matrices have also been cleaned up using Alumina, Florisil, C_{18}, -NH_2, Oasis Hydrophilic-Lipophilic Balance, and Bond Elut Polychlorinated Biphenyl SPE cartridges (Hercegova*, et al.* 2007). As GCB removes pigments and -NH_2 and PSA are effective at removing fatty acids, many researchers have been using a tandem system of a

combination of two or three of GCB, C_{18}, -SAX, PSA, and/or -NH_2 columns in MRMs to make cleanup more efficient (Schenck, *et al.* 2002).

With the introduction of QuEChERS by Anastassiades, *et al*., the popularity of using DSPE cleanup techniques increased (Anastassiades, *et al.* 2003). SPE sorbents are combined with the crude extract after extraction and liquid-liquid separation in DSPE (Anastassiades, *et al.* 2003). Via gravimetric analyses, researchers determined the majority of what matrix confounders were removed. Using GC-MS they established what different sorbents (GCB, PSA, -NH_2, and alumina-N) were retained. Due to the fact that PSA has both a primary and secondary amine, gravimetric analysis showed that PSA removed more matrix co-eluants than -NH_2 and Alumina. Samples with higher pigment percentages, such as carrots (carotenoids) and spinach (chlorophyll), used a combination of GCB and PSA DSPE (Hercegova, *et al.* 2007). QuEChERS has used DSPE with a variety of food commodities, such as lettuce and oranges. They have validated the method for use with 229 pesticide residues using large volume injector (LVI) GC-MS and HPLC-MS/MS at 10 ng/g fortification levels (Lehotay, *et al.* 2005a). Fatty samples are effectively cleaned up using a combo of C_{18}, GCB and PSA in the presence of $MgSO_4$ in DSPE. For example, in the QuEChERS method, DSPE gave higher, more reproducible recoveries than using regular SPE. Although SPE does provide a better cleanup, the practicalities and overall higher recoveries make DSPE a better candidate for fatty matrices (Lehotay, *et al.* 2005c).

Polystyrenedivinylbenzene type gel is mixed with designated elution solvents, ethyl

acetate or cyclohexane, and used in the PRA of baby food (peaches, apples, and strawberries) (Cajka and Hajslova 2004). Known as GPC, this technique has been used to remove pesticides ranging in size from approximately 200-400 g/mol from samples having a molecular weight (600-1500 g/mol). This technique is time-consuming, maximizes solvent use, uses huge columns, and has low flow-rates. Likewise, pyrethroid pesticides cannot be separated efficiently via GPC due to their high molecular mass. Nevertheless, GPC can be used to partition pesticides from lipids and waxes. The most reported PRA of milk utilizes GPC and SPE (Hercegova*, et al.* 2007). In this instance, Pereira and Cass use a HPLC method to determine sulfamethoxazole and trimethoprim in cow's milk via a restricted access media (RAM) cleanup column (C_8) coupled online to an analytical column partitioning the desired analytes from other milk components (Pereira and Cass 2005).

Sample preparation, extraction, and cleanup should be carefully considered in conjunction with applied chromatographic techniques and detection. The leading approaches in PRA of baby food and food – GC and HPLC combined with MS, MS/MS – are used to give the best LOQ and selectivity.

In analytical chemistry, GC has been the foundation of methodologies for over 60 years (Hercegova*, et al.* 2007). Commercially available fused-silica capillary columns also made it more advantageous for GC use for PRA of food. Ultra-trace concentration levels require splitless injection methods (Cajka and Hajslova 2004). Injecting larger volumes leads to lower LOQs if matrix noise is not a confounding factor, but additional care needs

to be taken to ensure adequate cleanup with large volume injection to avoid contaminating the injection liner, capillary column, and/or detector. MRMs usually use a 5% diphenyl 95% dimethylpolysiloxane stationary phase capillary column with normal (20-30 m × 0.25 mm × 0.25 μm) or narrow bore (15 m × 0.15 mm) in PRA of baby food (Hercegova*, et al.* 2007).

Detectors most widely used with GC techniques are ECD/MSD. ECD allows researchers to analyze halogenated pesticides at lower detection levels than flame ionization detectors (FID) (Hercegova*, et al.* 2007). Cressey *et al.* analyzed organochlorine pesticides in baby formula at a LOD of 0.2 ng/g (Cressey and Vannoort 2003). Nevertheless, if a sample exceeds the set maximum residue limit value subsequent confirmation is needed. Although mass spectrometers vary according to LODs, resolution, acquisition rates, and quality of mass spectra, MSD provides structural explication allowing for more precise analyte detection and confirmation. More accurate than GC-MS is GC-MS/MS because this technique further reduces matrix effects and lowers LODs (Hercegova*, et al.* 2007). Routine GC-MS/MS can be achieved economically via bench-top ion traps. Researchers have applied GC-MS/MS (ion trap) to PRA of orange extract obtaining an LOD of 0.5 ng/g (Schachterle*, et al.* 1994). Currently, LOQs ranging from 0.04-9.64 ng/g are achievable for 130 different classes of pesticides using GC-MS/MS (Hercegova*, et al.* 2007).

Another detector used often with capillary GC is quadrupole MSD. Applied to apple matrices, researchers have used quadrupole MSD in the determination of 19 pesticides at

a concentration level of 5.0 ng/g (Hercegova*, et al.* 2005). Obtaining concentrations acceptable for regulatory purposes (< 10 ng/g), one has to use selected ion monitoring (SIM) at the sacrifice of spectral information (Hercegova*, et al.* 2007). Principal detection systems of PRA at ultra-low levels in baby food are triple quadrupole (QqQ) and ion trap working in selected reaction monitoring mode or multiple reactions monitoring mode (Leandro*, et al.* 2005). Spectra can be made clearer via using time-of-flight (TOF) MSD to achieve high data points across a focused peak in full scan operation. Researchers demonstrated TOF MSD in blank matrix obtaining most LOQs between (0.5-2.5 ng/g) (Cajka and Hajslova 2004).

While HPLC is effective in the partitioning of non-volatile and thermally unstable compounds, its application to PRA was only when a GC analytical method was not available for the compounds of interest. This is due to newer classes of pesticides that possess a medium to high polarity and are thermally labile and relatively non-volatile. Still, HPLC is able to encompass analytes not applicable to GC methodologies due to its capability to cover pesticides having a wide array of physical and chemical properties. To cause partitioning in HPLC, C_{18} stationary phases are usually used with the addition of a guard column. The mobile phase plays a great role in obtaining adequate chromatographic separation and affecting analyte ionization and mass spectrometer sensitivity. Past HPLC-based methodologies employed UV-Vis and diode array detectors for PRA of food. Both of these detectors are not selective or sensitive enough to deal with complex matrices, such as food, or ultra-trace level residues present in such samples (Hercegova*, et al.* 2007). For instance, Bicchi, *et al*. determined five pesticide residues at

10 ng/g using HPLC-UV-Vis but had to pre-concentrate 6.25 fold to obtain data (Bicchi, *et al.* 1996). This changed when HPLC was coupled with MS. However, a major deficiency in HPLC-MS still precluded this detection system from reaching its full potential. The inherent characteristics of the mass spectrum of pesticides often provides only a molecule adduct or weak fragmentation ions. Application of enhanced collision-induced dissociation causes co-eluting compounds to form ions with the same *m/z* values as the analyte rendering the spectrum useless (Hercegova, *et al.* 2007).

Recently, LC-MS/MS has become the most powerful instrument for PRA of all types of matrices superseding most other chromatographic techniques because LC-MS/MS has dramatically increased selectivity and sensitivity, minimized sample pre-treatment, and increased dependability of quantification and confirmation at ultra-low concentrations. The LC-MS/MS interface was designed to provide a soft-ionization process that leads to mass spectra with only a few ions (Hercegova, *et al.* 2007). As evidence of the benefits of LC-MS/MS use, LC-MS/MS has been used in PRA to determine > 50 pesticides in produce in the last two years (Lehotay, *et al.* 2005a). LC-MS/MS has superseded QqQ mass analyzer as the most applied technique for PRA in baby food as well (Hercegova, *et al.* 2007).

As researchers continued to improve upon chromatographic methods, they developed and introduced ultra-performance liquid chromatography (UPLC). UPLC is based upon a reduction of particle size to < 2.5 μm. Due to this size reduction, efficiency is significantly increased and maintained even at increased flow rates or linear velocities

(Hercegova*, et al.* 2007). Comparing UPLC to HPLC, Leandro and coworkers used 1.7 µm particles with HPLC to quantify and confirm 16 pesticides in a variety of baby foods at 1.0 ng/g (2006). UPLC proved to be 2.5 times faster and gave better resolution, signal-to-noise ratios, and confirmation of the target analytes (Leandro*, et al.* 2006). While LC-MS/MS and/or GC-MS still provide the most efficient and effective analysis of pesticide residues in food to-date, other new techniques may surpass them in application of PRA of food.

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CHAPTER 2: METHOD DEVELOPMENT FOR MULTI-RESIDUE PESTICIDE EXTRACTION FROM FOOD

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Introduction

Regulatory agencies and contract, industrial, and academic laboratories often conduct global surveillance of pesticides in food. Utilizing a variety of methods, researchers analyze thousands of samples annually for a variety of purposes including regulatory enforcement and surveillance monitoring (Ridgway, *et al.* 2007). Many researchers are focused on investigating and developing MRMs with optimal recovery for tens or hundreds of pesticides for only one food (Avramides and Gkatsos 2007; Cho, *et al.* 2008; Fenoll, *et al.* 2007; Li, *et al.* 2007). In addition, many MRMs are currently focused on fruits and vegetables (Fernandez-Moreno, *et al.* 2008; Romero-Gonzalez, *et al.* 2008; Schenck, *et al.* 2008b; Takatori, *et al.* 2008). Pesticides are not only found in fruits and vegetables but also in grain products, dairy, some meats, and beans/legumes (Osteen, *et al.* 2008; USFDA 2001). In the United States, pesticides are regularly monitored in domestically-grown foods to ensure compliance with residue limits or tolerances set by the U.S. EPA. The USDA's International Maximum Residue Limit Database includes U.S. tolerance limits for various foods as well as maximum acceptable levels in 70 other countries for a variety of pesticides (USEPA 2009). There are still countries with limited or no control over pesticide residues in food, and the U.S. increasingly imports food from these countries (Sawaya, *et al.* 2000). Pesticides in food are potentially harmful to the developing fetus (Eskenazi, *et al.* 1999; Kamel, *et al.* 2005; Richardson 1995; Whyatt and Barr 2001) and to children (Bearer 1995). These factors warrant further development of methods to assess dietary exposure and the need for a quick, high-throughput, low-cost MRM able to quantify pesticide residues in various types of food products at low ng/g levels.

Historically, two extraction methods have been used for pesticide residue analyses in fruit and vegetables (Hercegová, *et al.* 2007) – the Luke method, involving acetone extraction followed by partitioning with a mixture of dichloromethane and light petroleum (Luke, *et al.* 1975), and a method involving ethyl acetate extraction in the presence of Na_2SO_4 as a drying agent (Bicchi, *et al.* 1996). Both methods have been modified in recent years to be less labor and time-intensive and less environmentally hazardous (Hercegová, *et al.* 2007). For example, Anastassiades, *et al*., (Anastassiades, *et al.* 2003) developed QuEChERS, a method using acetonitrile extraction with NaCl as a salting-out agent and $MgSO_4$ as a drying agent followed by dispersive PSA sorbent SPE cleanup instead of a SPE column elution of the extract. In this case and many others, instrumental analysis was carried out via GC (Barbini, *et al.* 2007; Fenoll, *et al.* 2007). A number of published methods utilize GC-ECD to investigate pesticide residues (Barbini, *et al.* 2007; Cao, *et al.* 2008; Khay, *et al.* 2009; Valsamaki, *et al.* 2007) and GC-MS to confirm pesticide identity (Barbini, *et al.* 2007; Khay, *et al.* 2009; Valsamaki, *et al.* 2007).

Acetonitrile has become a favored extraction solvent because it: (a) is easily separated from water upon salt addition; (b) leads to increased recovery of polar compounds like OP pesticides; and (c) minimizes the number of co-extractives, such as lipids and wax materials (Hercegová, *et al.* 2007). The Dutch Inspectorate for Health Protection validated the QuEChERS method for 400 pesticides in produce (Hercegová, *et al.* 2007) and Lehotay, *et al*., validated the QuEChERS method for the determination of more than 200 pesticides in produce (Lehotay, *et al.* 2005). Lightfield, *et al*., modified it to improve extraction and the stability of fungicides via using a 1% acetic acid to protonate any

deprotonated compounds in the acetonitrile extraction (Lightfield, *et al.* 2005). Moreover, the QuEChERS method has been used successfully with a combination C_{18} and PSA sorbent in a variety of food matrices (Leandro, *et al.* 2006). Investigators at Agriculture and Agri-Food Canada used the QuEChERS method coupled with Supelclean™ ENVI-CARB-II SPE (Sigma-Aldrich, Inc., Bellefonte, PA) cartridges to reduce background interference (Fillion, *et al.* 2000). Recent methods for determining pesticide residues in produce use a tandem configuration of two or three SPE columns for cleanup of raw extract (GCB, C_{18}, aminopropyl bonded silica, PSA, strong anion exchange (Hercegová, *et al.* 2007)).

In spite of these developments, there is a need for improvement of MRMs for an assortment of foods. QuEChERS has primarily been used and validated only in the analysis of fruit and vegetables (*22-24*, *26*). Moreover, Anastassiades, *et al*., assert that the QuEChERS method preferentially removes many polar matrix components like organic acids, certain pigments, and sugars, to some extent (*22*). This may lead to the accumulation of deposit in the instrumentation used, possibly resulting in a decrease in analytical sensitivity with increasing sample size and an increase in time needed for instrument maintenance. Lightfield, *et al*., altered the QuEChERS methods to include a buffering step to obtain specific pesticides (*24*). Moreover, Anastassiades, *et al*., Lehotay, *et al*., and Fillion, *et al*., used large sample sizes (10-50g) and such a sample size may not be readily available (*22*, *23*, *26*). In this work, we present a customized procedure based upon QuEChERS-type methods developed by Anastassiades, *et al*., (Anastassiades, *et al.* 2003) and Fillion, *et al*., (Fillion, *et al.* 2000) for the rapid, high-throughput, inexpensive

multi-residue determination of OP and pyrethroid pesticides in 16 different types of foods requiring only a one gram sample. Quantitation was carried out by GC-ECD and confirmatory analysis was carried out by GC-MS in SIM mode.

Materials and Methods

Reagents and Materials. Acetonitrile (HPLC grade), toluene (HPLC grade), and Na_2SO_4 (ACS grade) were purchased from Sigma-Aldrich, Inc. (St. Louis, MO). NaCl (ACS grade) was purchased from J. T. Baker (Phillipsburg, NJ). The Supelclean™ ENVI-CARB-II/PSA SPE cartridges (Bed A: 500 mg ENVI-CARB-II; Bed B: 300 mg PSA) were purchased from Sigma-Aldrich, Inc. (Bellefonte, PA). Helium (zero grade) and nitrogen (zero grade) gas were 99.999% ultra high purity obtained from Specialty Gases Southeast, Inc. (Suwanee, GA). The water used was obtained from an ultrapure 18.2 MΩ·cm Milli-Q® water (Millipore, Billerica, MA) system.

The TurboVap LV, an evaporative concentrator, was obtained from Zymark (Hopkinton, MA). The 15-mL glass centrifuge tubes and snap caps were purchased from VWR (Suwanee, GA). Adjustable single-channel pipetters were obtained from Eppendorf North America (Westbury, NY; Calibrated Nov 2007). The Vortex-Genie® 2 was purchased from Scientific Industries, Inc. (Bohemia, NY). The centrifuge used was obtained from International Equipment Co. (Needham Heights, MA).

Standards. Pesticide reference standards were obtained from the National Center for Environmental Health, Center for Disease Control and Prevention (CDC; Atlanta, GA) or Chem Service, Inc. (West Chester, PA). Stock solutions and working standard solutions were prepared in acetonitrile. Mixed fortification standards, each containing 3 OP (diazinon, malathion, and chlorpyrifos) and 4 pyrethroid (permethrin, cyfluthrin,

cypermethrin, and deltamethrin) pesticides at 5.0 μg/mL were prepared in acetonitrile from stock standard solutions.

Food Samples. For fortification recovery studies, foods were obtained from the local grocery store. We purchased the baby food form of green beans, butternut squash, carrots, sweet potatoes, apple sauce, bananas, beef, and chicken. Baby food was selected, as it is pre-homogenized, minimizing the variability associated with the heterogeneity of these foods. We also bought apple juice, beer, breadcrumbs, oats, skim milk, plain yogurt, black beans, and soy milk. All foods were used as purchased and none of the foods was labeled organic. Some of the foods, such as apple juice, bananas, carrots, apple sauce, and green beans, were chosen based upon the fact that they are consumed in large amounts by children and/or are important parts of their diets (USEPA 2008). All fortified samples were analyzed for background pesticide concentrations as well as in fortified form. With the exception of baby food carrots, no detectable background levels were noted. For baby food carrots, a background malathion mean concentration of 36.0 ± 6.6 ng/g (n = 3) was measured.

Instrumental Analysis. A Hewlett-Packard Model 5890A Series II GC equipped with an Agilent Technologies (Santa Clara, CA) model electron-capture detector (ECD) and a 7683B Series Injector autosampler (Agilent Technologies, Inc., Santa Clara, CA) was used. The Durabond-5 (DB-5) (Agilent Technologies, Inc., Santa Clara, CA) GC column used was 30 m, 0.25 mm i.d., 0.25 μm film thickness [5% phenyl, 95% dimethylpolysiloxane]. The temperature programming began at 80°C, held 2 minutes, 80-

280°C at 10°C/min to 280°C then held for 13 minutes. The helium carrier gas was at a constant flow of 2 mL/min and nitrogen makeup gas flow was 60 mL/min. The injection was 1.0 μL (splitless). Other relevant analytical parameters included: 2 mm i.d. single taper injection liner, injection port temperature 240°C, detector temperature 280°C.

To confirm the identities of the pesticide residues in all matrices, a Model 6890 GC (Agilent Technologies, Inc. Santa Clara, CA) equipped with a MAT 95XL (ThermoFinnigan, Bremen, Germany; 5kV) magnetic sector mass spectrometer was used. The GC column was a 30m (0.25mm i.d. by 0.25 μm film thickness) DB-5MS column (J&W Scientific, Folsom, CA). The initial column temperature was 100°C and was held for 1 min. Then the oven was heated to 320°C at 10°C/min. The temperature was then held at 320°C for 5 min. The GC system was operated in splitless injection mode with a 1.0 μL injection and a constant flow of 1 mL/min of helium. The screening analysis was performed in the SIM mode, monitoring at least two characteristic ions for each pesticide compound (Table 2.1). For diazinon, malathion, and chlorpyrifos, we observed only one peak while multiple peaks were observed for permethrin, cyfluthrin, cypermethrin, and deltamethrin.

Table 2.1. GC-ECD and GC-MS mean percent recovery ranges, molecular masses, and selected ions of extracted baby food samples fortified at 50 ng/g.

Pesticide	GC-ECD % Recovery Range	Molecular Weight	Selected Ion (m/z) Range	GC-MS % Recovery Range
diazinon	83.0 - 98.4	304	303.6005-304.6005	18.0 - 71.4
malathion	62.8 - 135.5	330	172.5808-173.5808	47.0 - 103.5
chlorpyrifos	82.0 - 122.6	351	313.4569-314.4569	32.8 - 74.4
cis/trans-permethrin	86.1 - 107.7	391	182.5804-183.5804	48.3 - 100.1
cyfluthrin	78.8 - 104.1	434	205.5600-206.5600	50.5 - 100.0
cypermethrin	83.4 - 122.0	416	180.5648-181.5648	54.3 - 135.9
deltamethrin	81.1 - 113.8	505	252.4045-253.4045	44.5 - 110.7

Procedure. Samples were handled with trace-cleaned glass or metal equipment. Trace-cleaning consisted of washing with warm tap water and a 1% Alconox solution (Alconox, Inc., White Plains, NY) followed by thorough rinsing with tap water, then three times with de-ionized water and a final time with ultrapure Milli-Q® water. Equipment was left to dry in an oven at 150°C, then rinsed once with HPLC-grade acetonitrile (Sigma Aldrich, St. Louis, MO).

Briefly, the food matrix (1 g for solid food or 1 mL for liquid) was placed in a 15-mL disposable glass centrifuge tube to which 5 mL acetonitrile and 1 g NaCl was added. The mixture was vortexed for 3 minutes, then centrifuged for 5 minutes. The ENVI-CARB-II/PSA cartridges were conditioned with 5 mL of 25% v/v toluene in acetonitrile. Na_2SO_4 was added on top of each SPE cartridge to a depth of ~2 mm. A 2 mL aliquot of the organic extract was loaded onto the cartridge, which was then eluted with 10 mL of 25% v/v toluene in acetonitrile. The eluant was collected in a 15-mL disposable glass centrifuge tube and placed in a TurboVap LV and evaporated under a stream of air at 10 psi and 35°C for 15 minutes and again at 25 psi and 35°C for 30 minutes to an approximate volume of 800 µL. We eluted the cartridge once more with 10 mL of 25% v/v toluene in acetonitrile, adding it to the reduced volume of the first eluant. The combined eluants were then evaporated to dryness using the TurboVap LV first at 10 psi and 35°C for 15 minutes and then at 25 psi and 35°C. Samples were reconstituted in 1 mL of acetonitrile and stored at -20°C.

Fortification. Twelve 1 g samples of each food type were weighed into 15-mL disposable glass centrifuge tubes. Nine of theses samples (n = 3 for each fortification level) were fortified with fortification standard solutions and vortexed for three minutes to achieve final concentrations of 50, 100, and 200 ng/g, respectively. The nine samples were extracted and the extracts transferred to GC sample vials. Three blank (unfortified) samples for each food type were prepared by adding 1 g samples of each food type to 15-mL disposable glass centrifuge tubes.

Identification, Quantification, and Confirmation of Pesticides in Food Samples. Solvent standards were prepared from more concentrated standards at various concentrations (1, 5, 10, 25, 50, 100, 150, 200, 250, 500, and 1000 ng/g) and used to create an 11-point calibration curve for quantification. Method detection limits (MDLs) were calculated for each analyte using a power regression model. We defined the lowest concentration used in the calibration curve, 1 ng/g, to be the LOD for each pesticide. Detection limits were verified by injection of the samples prepared at 1 ng/g to ensure that discernible peaks had a signal-to-noise ratio > 3.

Sample extracts and standards were injected on the GC-ECD. Comparing their retention times to the retention times of the standards, we identified peaks. The method of standard addition was employed to account for any matrix effects. We also used the method of standard addition to determine the concentration of unfortified and fortified samples based on the standard calibration curve (Skoog, *et al.* 1997). We conducted a confirmation analysis of the baby food samples fortified at 50 ng/g using GC-MS.

Confirmation analysis was carried out using an internal standard, PCB-156, at a single concentration.

Results

Recoveries. Tables 2.2 and 2.3 summarize mean percent recoveries by food types and fortification levels. Mean percent recoveries ranged from 49%-146% across all foods and replicates, with 80% of mean percent recoveries between 80-120%. The values for the coefficients of variance ranged from 0 to 37% across all foods, pesticides, and replicates with the majority of coefficients of variance below 10%. All mean percent recoveries were < 80% for the more polar OP pesticide, diazinon, in apple juice samples. Mean percent recoveries in black beans were also < 80% for chlorpyrifos, permethrin, and cyfluthrin, and generally lower for all fortified pesticides in comparison to the other food samples. Also, the widest range of mean percent recovery, 49.1-84.9%, occurred with black beans. In general, malathion mean percent recoveries were higher than 120% across most foods and most replicate samples.

Table 2.2. Mean percent recoveries of OP and pyrethroid pesticides extracted from baby foods fortified at 50, 100, and 200 ng/g.[a] Values in parentheses are coefficients of variation.

	Apple Sauce						Bananas						Butternut Squash					
	50 ng/g		100 ng/g		200 ng/g		50 ng/g		100 ng/g		200 ng/g		50 ng/g		100 ng/g		200 ng/g	
diazinon	92.3	(2)	97.1	(8)	97.2	(1)	89.8	(5)	100.5	(1)	98.4	(1)	91.5	(2)	95.0	(8)	94.2	(6)
malathion	125.5	(8)	131.7	(6)	128.9	(4)	131.9	(7)	138.3	(3)	130.7	(6)	126.8	(2)	130.7	(5)	126.1	(7)
chlorpyrifos	112.5	(4)	108.0	(6)	107.4	(5)	121.1	(11)	116.0	(7)	109.1	(6)	122.6	(1)	116.9	(7)	110.9	(6)
permethrin	101.4	(5)	102.4	(4)	99.1	(1)	107.4	(9)	106.7	(1)	103.5	(1)	107.3	(6)	105.1	(6)	94.0	(3)
cyfluthrin	102.4	(8)	104.6	(4)	96.6	(4)	104.1	(5)	109.2	(1)	102.0	(9)	104.0	(4)	98.0	(2)	102.8	(5)
cypermethrin	102.8	(3)	96.8	(3)	97.5	(0)	112.9	(22)	100.3	(1)	98.0	(7)	119.9	(7)	101.2	(3)	100.3	(5)
deltamethrin	91.0	(18)	107.6	(7)	82.5	(11)	108.5	(5)	132.9	(2)	122.6	(8)	113.8	(2)	103.1	(4)	104.6	(10)

	Carrot						Sweet Potatoes						Beef					
	50 ng/g		100 ng/g		200 ng/g		50 ng/g		100 ng/g		200 ng/g		50 ng/g		100 ng/g		200 ng/g	
diazinon	89.6	(7)	98.2	(2)	93.9	(3)	87.2	(4)	93.3	(9)	77.4	(7)	83.0	(5)	91.2	(12)	91.3	(17)
malathion	62.8	(24)	107.6	(3)	125.1	(4)	85.4	(8)	93.9	(1)	92.1	(9)	108.4	(17)	134.7	(11)	129.9	(14)
chlorpyrifos	97.1	(6)	104.0	(4)	104.9	(3)	82.0	(15)	92.2	(13)	90.0	(3)	93.1	(11)	104.4	(12)	106.2	(10)
permethrin	107.7	(15)	109.3	(2)	100.4	(2)	86.1	(10)	100.6	(8)	88.9	(6)	94.0	(7)	101.7	(8)	98.5	(5)
cyfluthrin	85.9	(17)	90.4	(5)	93.1	(4)	78.8	(13)	91.5	(7)	93.3	(2)	78.9	(18)	98.8	(7)	99.9	(8)
cypermethrin	87.3	(15)	102.4	(3)	98.9	(8)	83.4	(8)	92.5	(3)	94.4	(4)	85.6	(14)	97.3	(3)	101.6	(8)
deltamethrin	98.8	(8)	100.7	(15)	99.1	(2)	81.1	(14)	94.0	(7)	94.5	(7)	95.3	(17)	113.5	(3)	108.9	(13)

	Green Beans						Chicken					
	50 ng/g		100 ng/g		200 ng/g		50 ng/g		100 ng/g		200 ng/g	
diazinon	93.7	(1)	97.4	(6)	80.6	(9)	98.4	(4)	103.6	(5)	99.3	(4)
malathion	97.6	(6)	124.3	(6)	118.6	(9)	135.5	(0)	146.0	(6)	138.6	(4)
chlorpyrifos	91.6	(4)	103.3	(9)	95.0	(10)	107.6	(3)	110.8	(3)	108.2	(5)
permethrin	93.8	(5)	103.3	(4)	99.9	(1)	95.3	(5)	91.9	(7)	98.3	(12)
cyfluthrin	81.1	(13)	96.7	(3)	93.0	(6)	91.3	(8)	93.5	(15)	99.9	(10)
cypermethrin	122.0	(16)	120.6	(5)	101.7	(6)	95.6	(7)	97.2	(14)	104.8	(9)
deltamethrin	83.6	(13)	115.4	(11)	97.2	(7)	89.7	(3)	110.2	(18)	110.8	(9)

[a]n=3; three samples were fortified at each of the fortification levels

Table 2.3. Mean percent recoveries of OP and pyrethroid pesticides extracted from foods fortified at 50, 100, and 200 ng/g.[a] Values in parentheses are coefficients of variation.

	Apple Juice						Beer						Black Beans					
	50 ng/g		100 ng/g		200 ng/g		50 ng/g		100 ng/g		200 ng/g		50 ng/g		100 ng/g		200 ng/g	
diazinon	70.1	(17)	75.5	(2)	71.3	(4)	91.5	(3)	89.2	(5)	89.9	(2)	49.1	(24)	84.9	(22)	64.0	(18)
malathion	87.6	(12)	92.1	(3)	88.9	(7)	90.2	(7)	107.1	(4)	111.3	(5)	77.5	(8)	85.4	(2)	89.1	(4)
chlorpyrifos	86.5	(7)	78.5	(1)	73.7	(7)	77.0	(19)	84.3	(3)	86.3	(4)	54.7	(4)	52.9	(5)	55.6	(6)
permethrin	105.5	(8)	115.6	(7)	102.3	(2)	106.6	(9)	102.9	(5)	101.6	(7)	77.0	(6)	75.5	(6)	75.3	(7)
cyfluthrin	89.5	(10)	98.4	(4)	95.1	(4)	92.6	(8)	94.0	(5)	95.7	(3)	74.8	(9)	79.8	(4)	75.9	(9)
cypermethrin	91.1	(7)	103.7	(1)	95.8	(3)	99.8	(12)	98.4	(5)	97.4	(1)	80.6	(6)	79.8	(1)	78.1	(10)
deltamethrin	107.2	(8)	99.7	(5)	101.8	(7)	108.7	(21)	107.6	(3)	98.4	(8)	80.4	(8)	82.3	(5)	84.7	(10)

	Bread Crumbs						Oats						Milk					
	50 ng/g		100 ng/g		200 ng/g		50 ng/g		100 ng/g		200 ng/g		50 ng/g		100 ng/g		200 ng/g	
diazinon	80.2	(10)	111.4	(7)	99.6	(10)	94.5	(10)	103.9	(6)	102.0	(2)	77.1	(3)	76.6	(3)	81.3	(4)
malathion	135.2	(18)	96.6	(13)	101.3	(5)	116.4	(13)	123.0	(2)	115.8	(3)	101.3	(8)	112.0	(5)	115.1	(7)
chlorpyrifos	82.3	(2)	101.3	(3)	103.8	(5)	116.0	(8)	117.2	(2)	111.6	(3)	94.2	(4)	95.2	(5)	95.6	(0)
permethrin	112.4	(25)	109.1	(14)	101.0	(4)	97.2	(4)	100.8	(5)	96.2	(3)	91.4	(4)	97.2	(7)	97.9	(5)
cyfluthrin	100.3	(8)	93.9	(10)	85.3	(8)	75.5	(37)	94.8	(4)	101.1	(1)	81.2	(6)	90.4	(8)	92.4	(2)
cypermethrin	135.5	(7)	102.2	(6)	78.6	(3)	92.9	(1)	99.5	(3)	101.4	(1)	93.2	(12)	96.9	(3)	97.4	(4)
deltamethrin	110.5	(12)	111.4	(15)	101.2	(7)	83.6	(5)	94.7	(4)	96.1	(10)	79.8	(3)	97.2	(6)	96.1	(3)

	Soy Milk						Yogurt					
	50 ng/g		100 ng/g		200 ng/g		50 ng/g		100 ng/g		200 ng/g	
diazinon	88.6	(7)	89.9	(6)	83.1	(4)	106.4	(10)	95.4	(4)	97.2	(7)
malathion	114.6	(11)	129.0	(4)	121.0	0	112.3	(6)	119.4	(3)	129.1	(8)
chlorpyrifos	105.3	(9)	117.3	(7)	108.9	(4)	112.4	(6)	104.3	(2)	106.7	(4)
permethrin	117.7	(9)	106.0	(9)	100.6	(6)	94.5	(1)	102.6	(3)	97.8	(2)
cyfluthrin	97.8	(11)	95.6	(9)	97.3	(2)	91.7	(6)	92.8	(5)	96.5	(5)
cypermethrin	119.0	(12)	106.6	(7)	105.8	(1)	93.7	(5)	95.9	(5)	99.1	(3)
deltamethrin	98.0	(19)	97.7	(12)	98.6	(4)	100.2	(16)	100.0	(1)	96.7	(9)

[a]n=3; three samples were fortified at each of the fortification levels

Chromatography. Most of the GC chromatograms showed little interference from the sample matrix. Figures 2.1 and 2.2 show the chromatograms of the 7 pesticides in black beans and baby food beef, respectively. These two chromatograms are indicative of the extremes in interference observed during study in which both show multiple peaks of interference. None of the multiple peaks co-eluted or interfered with peaks of target analytes. Stable chromatographic retention times allowed for reliable identification of unknown peaks. For example, the retention time of diazinon (~ 16.544 min) did not vary by more than ± 0.004 min during the course of a 28-hour analytical run. Minimal peak broadening, tailing, and peak matrix interference were observed. Baseline resolution was achieved the majority of the time affording separation of peaks differing in retention times by < 0.3 min. Consequently, retention times were used to estimate accurately the identity of unknown peaks.

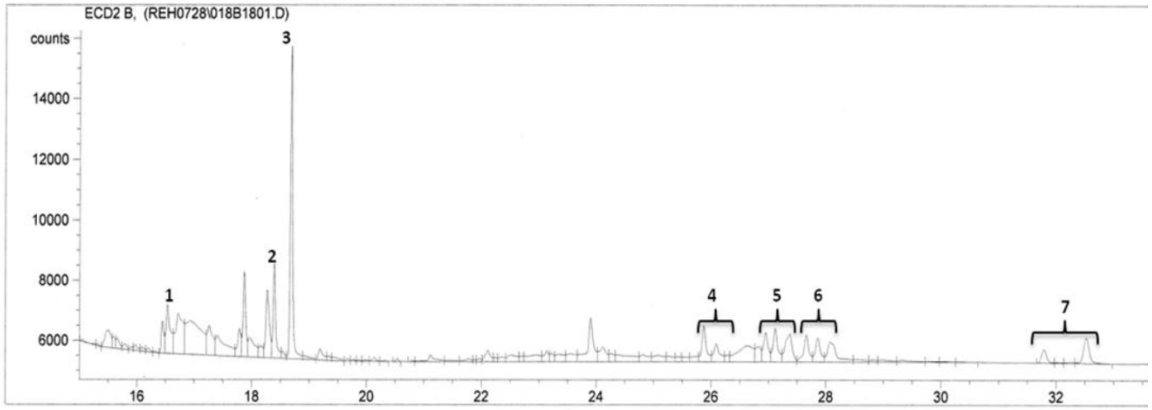

Figure 2.1. GC-ECD chromatogram of a black beans extract fortified with 50 ng/g OP and pyrethroid pesticides. X-axis = time in min. Y-axis = area counts. **1**: diazinon; **2**: malathion; **3**: chlorpyrifos; **4**: permethrin; **5**: cyfluthrin; **6**: cypermethrin; **7**: deltamethrin.

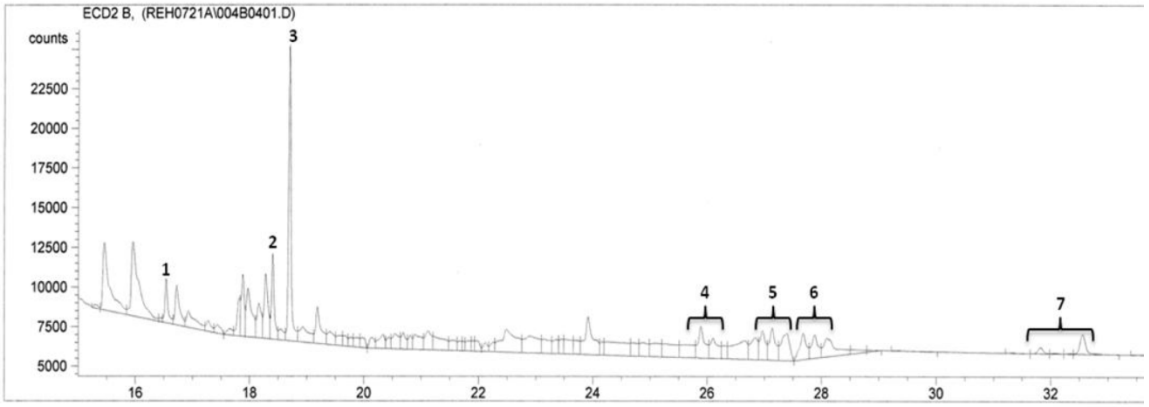

Figure 2.2 GC-ECD chromatogram of a baby food beef extract fortified with 50 ng/g OP and pyrethroid pesticides. X-axis = time in min. Y-axis = area counts. **1**: diazinon; **2**: malathion; **3**: chlorpyrifos; **4**: permethrin; **5**: cyfluthrin; **6**: cypermethrin; **7**: deltamethrin.

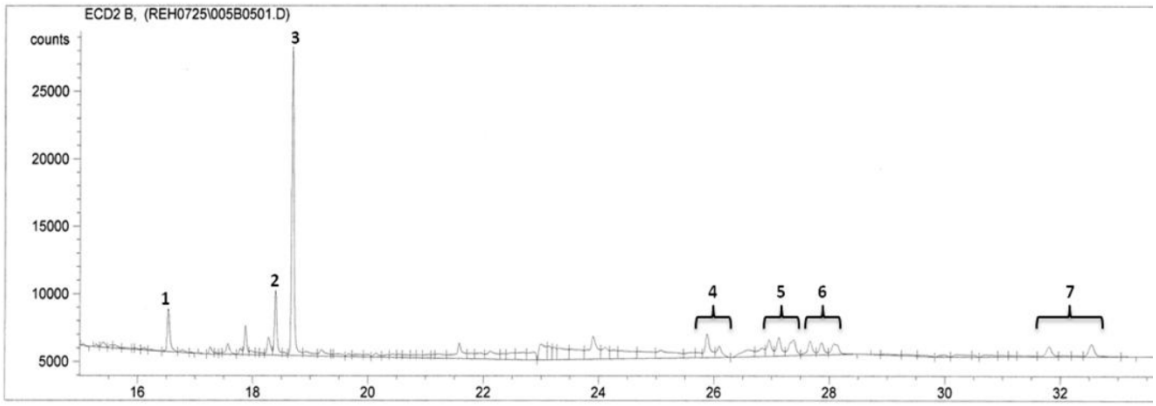

Figure 2.3. GC-ECD chromatogram of a plain yogurt extract fortified with 50 ng/g OP and pyrethroid pesticides. X-axis = time in min. Y-axis = area counts. **1**: diazinon; **2**: malathion; **3**: chlorpyrifos; **4**: permethrin; **5**: cyfluthrin; **6**: cypermethrin; **7**: deltamethrin.

Confirmation Analysis. Selected ions, molecular weight, and recovery range for GC-ECD and GC-MS are shown in Table 2.1. Recoveries were averaged for each pesticide and the relative standard deviations calculated. Across all baby food matrices and pesticides fortified at 50 ng/g, overall GC-ECD mean percent recovery ranged from 62.8-135.5% while the same range obtained during confirmation analysis was 18.0-135.9%.

Discussion

Extraction Procedure. Fillion, *et al*., used an acetonitrile extraction with a first cleanup with a C_{18} cartridge followed by an additional cleanup with carbon SPE cartridge coupled to an aminopropyl cartridge cleanup to remove co-extractives. Determination of pesticides was by GC with mass-selective detection in the selected-ion monitoring mode and liquid chromatography with post-column reaction and fluorescence detection for *N*-methyl carbamates (Fillion*, et al.* 2000). We used a 1 g sample versus the 50 g sample used by Fillion and co-workers, thus our method required less solvent (30 mL vs. 105 mL) and a smaller sample size. We modified the QuEChERS and Fillion methods (*22, 30*) to include a NaCl partitioning step during the acetonitrile extraction. We found the salting-out of the aqueous phase in the sample and acetonitrile to be efficient, eliminating the need for a drying agent such as $MgSO_4$. Moreover, we were able to analyze OP and pyrethroid pesticides via GC-ECD without further workup (*i.e*. solvent exchange or internal standard addition) after reconstitution.

Co-extracted sample matrix components frequently produce co-eluting chromatographic peaks that preclude the accurate detection of low (< 20 ng/g) pesticide residue levels in the sample extract (Schenck*, et al.* 2008a). The removal of interferences, such as pigments and fats, with the ENVI-CARB-II/PSA cartridge reduced the occurrence of co-extractives and matrix enhancement effects in our method (Supelco 2005). Coupling a rapid, economical, high-throughput sample cleanup with a selective and sensitive detector aids in trace-level analysis (Schenck*, et al.* 2008a). The instrumentation run becomes more reliable since multiple samples can be analyzed without constant instrument

maintenance. We also reduced our waste by streamlining the Fillion, *et al*. method to use only one SPE cartridge instead of three. Finally, Fillion, *et al*., stated that generally they can prepare 42 samples for analysis each week (Fillion, *et al.* 2000). Using the method we present allows for 24-30 samples to be extracted per day or a throughput of in excess of 100 samples per week. Moreover, the typical material costs for this multi-residue method was approximately $6 per sample while other MRMs can cost up to twice that amount per sample (Ahmed 2001).

Recoveries. Mean percent recoveries of fortified levels were generally accurate for the majority of samples analyzed. Apple juice, however, gave lower overall recoveries for OPs in comparison to the other foods. For example, whereas the peak height was between 22000-27000 units for yogurt and beef samples, the peak height for the apple juice sample was a little over 19000 units for an identical spiking concentration. Mean percent recoveries were lower for the more polar OP pesticides in apple juice samples likely due to the propensity of these molecules to undergo acid hydrolysis causing the formation of OP degradation products of the parent compound under acidic conditions. As we were evaluating only the parent compound, we can only speculate that degradation may account for the lower mean percent recovery.

The black beans chromatogram (Figure 2.1) displayed peak heights < 16000 units. Standard addition analysis of the fortified samples suggests residual matrix effects for this food. The mean percent recovery was < 80% for most of the OP and pyrethroid pesticides in black beans. We hypothesize that this is due to the heterogeneity of the

sample. We also observed lower mean percent recovery of the OP versus the pyrethroid pesticides in black beans. The mean percent recovery of malathion from black beans was 84.0 ± 5.9%. In the homogeneous foods, the mean percent recovery was generally higher, sometimes exceeding 100%. Also, black beans gave the widest range of mean percent recovery for the target pesticides.

Heterogeneous distribution of pesticide residues within a particular food sample may be due to uneven application of pesticides on the original crop, uneven uptake into the plant matrix, or other factors. We purchased black beans as canned, whole beans. In this case, heterogeneity in the sample may have resulted from application/uptake heterogeneities and/or to our fortification method. We fortified samples by adding pesticide and vortexing for three minutes. The fortified pesticide may have bound heterogeneously to the different components of the bean matrix, i.e. to pieces of waxy skin instead of starchy flesh. This in turn may have precluded the extraction from occurring uniformly, resulting in widely varied recoveries among the fortified black beans samples. When analyzing black beans and similar samples in the future, we recommend they be homogenized prior to fortification.

Method Advantages and Limitations. This procedure was applied to 16 foods collected from local grocery stores. Unfortified samples were analyzed via injection on the GC-ECD for pesticides. Unfortified-sample chromatograms had few matrix interference peaks. Consequently, we could determine if the pesticides used to fortify samples were present in unfortified-sample chromatograms via visual inspection; qualitatively, the

presence of a peak at the appropriate retention time indicates the presence of the spiked pesticide.

The main disadvantage of the standard QuEChERS method was its lack of utility in analyzing matrices with moderate fat content such as some dairy products or meats (Anastassiades*, et al.* 2003). Some dairy products, including milk, exist as an emulsion, and have often proven to be difficult to extract due to the fact that organic extraction breaks down the emulsion resulting in a heterogeneous sample. Using our method on fortified yogurt samples resulted in a chromatogram with easily discernible peaks and few matrix interferences, confirming the utility of our cleanup procedure. Similarly, many researchers are reluctant to test red meat due to its high fat content. Unlike the yogurt chromatogram (Figure 2.3), the baby food beef chromatogram (Figure 2.2) showed more matrix interference in the early retention time region. Nonetheless, the chromatogram is relatively clean and the peaks of interest are clearly discernible. During our experimentation, we also tried to apply this method to 100% fat and oil matrices (*e.g.* canola oil) without success.

In general, our clean up method was effective since the chromatograms displayed little matrix effects in the region of interest. An alternative strategy would involve the use of an internal standard, preferably an isotopically-labeled version of one or more of the measured pesticides. This would be considerably more expensive, however. Given our goals of low-cost and rapid-throughput, we opted not to use labeled standards.

Malathion generally showed an augmented percent recovery (i.e. > 120%) in many of the samples. This did not diminish with concentration and the co-eluting peaks did not change in size across the fortification levels. In confirmatory analysis, we did not observe a similar enhancement. Thus, we attribute the increased recovery to an interference caused by an enhancement of electron-capture detector response and/or co-elution of another compound with similar retention time to malathion during GC-ECD analysis.

We chose a routine confirmatory technique that used a different detection system (Arndt and Kropf 2002). Since GC-MS is regarded as the recommended reference method due to its accurate sensitivity and specificity (Arndt and Kropf 2002), we used it to confirm peak identities in baby food samples fortified at 50 ng/g. Although we achieved adequate separation and detection in minimal time using a capillary column and an ECD, we wanted to preclude any significant probability of false positive results from potential interference. The confirmation analysis ruled out this probability since peaks apparent in the ECD chromatograms also appeared in the MS chromatograms.

We demonstrated successful application of our method to a variety of food matrices. Future studies may extend the method to increase the number of pesticides analyzed, evaluate additional classes of pesticides, or investigate additional food matrices. Continued work focusing on high-throughput, low-cost methods will be of importance in assessing the public health impact of pesticides.

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APPENDIX 2A: METHODOLOGIES ASSESSED FOR PESTICIDE RESIDUE ANALYSIS

All chemicals used in the methodologies below were ACS grade or better. All SPE cartridges used had bed weight/volume of 500 mg/6 mL, unless otherwise specified.

Method A1

One milliliter aliquots of red wine fortified at 100 ng/g was placed in a 15-mL disposable glass centrifuge tube to which ~ 2 g NaCl was added. The mixture was vortexed for 1 minute. A C_{18} cartridge was conditioned with 3 mL of ethyl acetate followed by 96% v/v ethanol in Milli-Q H_2O and once with 3 mL 10% v/v ethanol in Milli-Q H_2O. Na_2SO_4 was added on top of each SPE cartridge to a depth of ~2 mm. The wine and salt mixture was loaded onto the cartridge and the cartridge was not disturbed for 20 minutes. The cartridge was then eluted with 4 mL of acetonitrile, ethyl acetate, methanol, dichloromethane, toluene, hexane, or diethyl ether and the eluant was collected in a 15-mL disposable glass centrifuge tube. Then a Florisil cartridge with Na_2SO_4 added on top to a depth of ~2 mm was conditioned with 5 mL ethyl acetate. The eluant from the C_{18} cartridge was loaded onto the Florisil cartridge. The Florisil cartridge was eluted with 4 mL ethyl acetate and the eluant was collected in a 15-mL disposable glass centrifuge tube. The eluant was then placed in a TurboVap LV and evaporated to dryness at 10 psi and 35°C for 15 minutes and then at 25 psi and 35°C. Samples were reconstituted in 1 mL of acetonitrile and stored at -20°C until ready for analysis via GC-ECD.

Method A1 Mean Percent Recoveries

Table A1. Percent recoveries of OP and pyrethroid pesticides extracted via Method A1 from wine fortified at 100 ng/g (n=3) with varying C_{18} cartridge elution solvent. Values in parentheses are coefficients of variation.

	Acetonitrile	CH_3OH	Ethyl Acetate	CH_2Cl_2	Diethyl Ether	Toluene	Hexane
diazinon	0.0	0.0	0.0	0.0	0.0	0.0	0.0
malathion	0.0	0.0	0.0	0.0	0.0	0.0	0.0
chlorpyrifos	0.0	0.0	0.0	32.4 (1)	0.0	39.6 (11)	49.8 (34)
permethrin	0.0	0.0	0.0	36.2 (2)	0.0	45.0 (13)	44.1 (34)
cyfluthrin	0.0	0.0	0.0	62.8 (3)	0.0	73.2 (9)	79.1 (12)
cypermethrin	0.0	0.0	0.0	64.6 (3)	0.0	76.0 (8)	76.3 (14)
deltamethrin	0.0	0.0	0.0	67.7 (2)	0.0	75.7 (7)	77.2 (8)

Method A2

One milliliter aliquots of red wine fortified at 100 ng/g was placed in a 15-mL disposable glass centrifuge tube to which ~ 2 g NaCl was added. The mixture was vortexed for 1 minute. A C_{18} cartridge was conditioned with 3 mL of ethyl acetate followed by 96% v/v ethanol in Milli-Q H_2O and once with 3 mL 10% v/v ethanol in Milli-Q H_2O. Na_2SO_4 was added on top of each SPE cartridge to a depth of ~2 mm. The wine and salt mixture was loaded onto the cartridge and the cartridge was not disturbed for 20 minutes. The cartridge was then eluted with 4 mL of acetonitrile, ethyl acetate, methanol, dichloromethane, toluene, hexane, or diethyl ether and the eluant was collected in a 15-mL disposable glass centrifuge tube. Then a Florisil cartridge with Na_2SO_4 added on top to a depth of ~2 mm was conditioned with 5 mL ethyl acetate. The eluant from the C_{18} cartridge was loaded onto the Florisil cartridge. The Florisil cartridge was eluted with 4 mL acetonitrile and the eluant was collected in a 15-mL disposable glass centrifuge tube. The eluant was then placed in a TurboVap LV and evaporated to dryness at 10 psi and 35°C for 15 minutes and then at 25 psi and 35°C. Samples were reconstituted in 1 mL of acetonitrile and stored at -20°C until ready for analysis via GC-ECD.

Method A2 Mean Percent Recoveries

Table A2. Percent recoveries of OP and pyrethroid pesticides extracted via Method A2 from wine fortified at 100 ng/g (n=3) with varying C_{18} cartridge elution solvent. Values in parentheses are coefficients of variation.

	Acetonitrile	CH_3OH		Ethyl Acetate		CH_2Cl_2		Diethyl Ether	Toluene	Hexane	
diazinon	0.0	0.0		0.0		0.0		0.0	0.0	0.0	
malathion	0.0	0.0		0.0		0.0		0.0	0.0	0.0	
chlorpyrifos	0.0	6.3	(36)	22.4	(5)	33.0	(7)	0.0	0.0	108.1	(7)
permethrin	0.0	13.9	(30)	19.9	(14)	20.0	(10)	0.0	0.0	43.2	(19)
cyfluthrin	0.0	42.1	(9)	44.9	(2)	42.2	(1)	0.0	0.0	37.3	(16)
cypermethrin	0.0	38.6	(11)	42.2	(5)	41.1	(3)	0.0	0.0	43.8	(25)
deltamethrin	0.0	40.5	(7)	46.3	(7)	43.2	(11)	0.0	0.0	44.5	(23)

Method A3

One milliliter aliquots of red wine fortified at 100 ng/g was placed in a 15-mL disposable glass centrifuge tube to which ~ 2 g NaCl was added. The mixture was vortexed for 1 minute. A C_2 cartridge was conditioned with 3 mL of ethyl acetate followed by 96% v/v ethanol in Milli-Q H_2O and once with 3 mL 10% v/v ethanol in Milli-Q H_2O. Na_2SO_4 was added on top of each SPE cartridge to a depth of ~2 mm. The wine and salt mixture was loaded onto the cartridge and the cartridge was not disturbed for 20 minutes. The cartridge was then eluted with 4 mL of acetonitrile, ethyl acetate, methanol, dichloromethane, toluene, hexane, or diethyl ether and the eluant was collected in a 15-mL disposable glass centrifuge tube. Then a Florisil cartridge with Na_2SO_4 added on top to a depth of ~2 mm was conditioned with 5 mL ethyl acetate. The eluant from the C_2 cartridge was loaded onto the Florisil cartridge. The Florisil cartridge was eluted with 4 mL acetonitrile and the eluant was collected in a 15-mL disposable glass centrifuge tube. The eluant was then placed in a TurboVap LV and evaporated to dryness at 10 psi and 35°C for 15 minutes and then at 25 psi and 35°C. Samples were reconstituted in 1 mL of acetonitrile and stored at -20°C until ready for analysis via GC-ECD.

Method A3 Mean Percent Recoveries

Table A3. Percent recoveries of OP and pyrethroid pesticides extracted via Method A3 from wine fortified at 100 ng/g (n=3) with varying C_2 cartridge elution solvent. Values in parentheses are coefficients of variation.

	Acetonitrile		CH_3OH		Ethyl Acetate		CH_2Cl_2		Diethyl Ether	Toluene		Hexane
diazinon	0.0		0.0		0.0		0.0		0.0	0.0		0.0
malathion	0.0		0.0		0.0		0.0		0.0	0.0		0.0
chlorpyrifos	21.4	(21)	10.3	(20)	24.3	(7)	60.2	(18)	0.0	24.0	(79)	0.0
permethrin	49.7	(122)	74.7	(64)	27.9	(7)	38.8	(19)	0.0	15.5	(16)	0.0
cyfluthrin	41.5	(5)	56.3	(18)	47.2	(9)	66.8	(15)	0.0	48.7	(37)	0.0
cypermethrin	40.7	(8)	63.1	(27)	52.0	(11)	69.7	(10)	0.0	46.6	(41)	0.0
deltamethrin	40.2	(1)	45.8	(6)	49.1	(12)	66.3	(13)	0.0	47.3	(35)	0.0

Method A4

One milliliter aliquots of red wine fortified at 100 ng/g was placed in a 15-mL disposable glass centrifuge tube to which ~ 2 g NaCl was added. The mixture was vortexed for 1 minute. A phenyl cartridge was conditioned with 3 mL of ethyl acetate followed by 96% v/v ethanol in Milli-Q H_2O and once with 3 mL 10% v/v ethanol in Milli-Q H_2O. Na_2SO_4 was added on top of each SPE cartridge to a depth of ~2 mm. The wine and salt mixture was loaded onto the cartridge and the cartridge was not disturbed for 20 minutes. The cartridge was then eluted with 4 mL of acetonitrile, ethyl acetate, methanol, dichloromethane, toluene, hexane, or diethyl ether and the eluant was collected in a 15-mL disposable glass centrifuge tube. Then a Florisil cartridge with Na_2SO_4 added on top to a depth of ~2 mm was conditioned with 5 mL ethyl acetate. The eluant from the phenyl cartridge was loaded onto the Florisil cartridge. The Florisil cartridge was eluted with 4 mL acetonitrile and the eluant was collected in a 15-mL disposable glass centrifuge tube. The eluant was then placed in a TurboVap LV and evaporated to dryness at 10 psi and 35°C for 15 minutes and then at 25 psi and 35°C. Samples were reconstituted in 1 mL of acetonitrile and stored at -20°C until ready for analysis via GC-ECD.

Method A4 Mean Percent Recoveries

Table A4. Percent recoveries of OP and pyrethroid pesticides extracted via Method A4 from wine fortified at 100 ng/g (n=3) with varying Phenyl cartridge elution solvent. Values in parentheses are coefficients of variation.

	Acetonitrile		CH_3OH		Ethyl Acetate		CH_2Cl_2		Diethyl Ether		Toluene		Hexane	
diazinon	0.0		0.0		0.0		0.0		0.0		0.0		0.0	
malathion	0.0		0.0		0.0		0.0		0.0		0.0		0.0	
chlorpyrifos	19.5	(9)	0.0		23.1	(8)	31.1	(9)	0.0		29.1	(5)	58.9	(9)
permethrin	15.6	(6)	11.3	(4)	15.7	(13)	20.5	(14)	0.0		24.3	(6)	35.2	(11)
cyfluthrin	44.4	(2)	37.5	(5)	41.1	(1)	49.5	(3)	0.0		57.1	(2)	56.1	(14)
cypermethrin	43.7	(3)	34.1	(4)	41.7	(3)	46.2	(6)	0.0		54.2	(7)	56.0	(14)
deltamethrin	42.6	(4)	35.9	(2)	41.7	(2)	46.7	(4)	0.0		53.9	(2)	54.3	(13)

Method A5

One milliliter aliquots of grape juice fortified at 100 ng/g was placed in a 15-mL disposable glass centrifuge tube to which ~ 2 g NaCl was added. The mixture was vortexed for 1 minute. A C_{18} cartridge was conditioned with 3 mL of ethyl acetate followed by 96% v/v ethanol in Milli-Q H_2O and once with 3 mL 10% v/v ethanol in Milli-Q H_2O. Na_2SO_4 was added on top of each SPE cartridge to a depth of ~2 mm. The grape juice and salt mixture was loaded onto the cartridge and the cartridge was not disturbed for 20 minutes. The cartridge was then eluted with 4 mL of acetonitrile, ethyl acetate, methanol, dichloromethane, toluene, hexane, or diethyl ether and the eluant was collected in a 15-mL disposable glass centrifuge tube. Then a Florisil cartridge with Na_2SO_4 added on top to a depth of ~2 mm was conditioned with 5 mL ethyl acetate. The eluant from the C_{18} cartridge was loaded onto the Florisil cartridge. The Florisil cartridge was eluted with 4 mL acetonitrile and the eluant was collected in a 15-mL disposable glass centrifuge tube. The eluant was then placed in a TurboVap LV and evaporated to dryness at 10 psi and 35°C for 15 minutes and then at 25 psi and 35°C. Samples were reconstituted in 1 mL of acetonitrile and stored at -20°C until ready for analysis via GC-ECD.

Method A5 Mean Percent Recoveries

Table A5. Percent recoveries of OP and pyrethroid pesticides extracted via Method A5 from grape juice fortified at 100 ng/g (n=3) with varying C_{18} cartridge elution solvent. Values in parentheses are coefficients of variation.

	Acetonitrile		CH_3OH		Ethyl Acetate		CH_2Cl_2		Diethyl Ether		Toluene		Hexane	
diazinon	0.0		0.0		0.0		0.0		0.0		0.0		0.0	
malathion	0.0		0.0		0.0		0.0		0.0		0.0		0.0	
chlorpyrifos	19.1	(14)	0.0		18.1	(32)	27.5	(22)	0.0		50.1	(10	45.0	(59)
permethrin	14.0	(60)	8.3	(69)	10.9	(6)	19.5	(17)	0.0		25.8	(11)	18.4	(42)
cyfluthrin	45.1	(16)	42.2	(12)	44.8	(7)	65.3	(28)	0.0		66.8	(3)	41.5	(28)
cypermethrin	42.8	(19)	39.6	(16)	42.4	(4)	53.1	(3)	0.0		64.8	(6)	38.4	(16)
deltamethrin	43.8	(18)	40.2	(12)	41.7	(1)	62.2	(25)	0.0		66.3	(5)	39.4	(13)

Method A6

One milliliter aliquots of grape juice fortified at 100 ng/g was placed in a 15-mL disposable glass centrifuge tube to which ~ 2 g NaCl was added. The mixture was vortexed for 1 minute. A C_2 cartridge was conditioned with 3 mL of ethyl acetate followed by 96% v/v ethanol in Milli-Q H_2O and once with 3 mL 10% v/v ethanol in Milli-Q H_2O. Na_2SO_4 was added on top of each SPE cartridge to a depth of ~2 mm. The grape juice and salt mixture was loaded onto the cartridge and the cartridge was not disturbed for 20 minutes. The cartridge was then eluted with 4 mL of acetonitrile, ethyl acetate, methanol, dichloromethane, toluene, hexane, or diethyl ether and the eluant was collected in a 15-mL disposable glass centrifuge tube. Then a Florisil cartridge with Na_2SO_4 added on top to a depth of ~2 mm was conditioned with 5 mL ethyl acetate. The eluant from the C_2 cartridge was loaded onto the Florisil cartridge. The Florisil cartridge was eluted with 4 mL acetonitrile and the eluant was collected in a 15-mL disposable glass centrifuge tube. The eluant was then placed in a TurboVap LV and evaporated to dryness at 10 psi and 35°C for 15 minutes and then at 25 psi and 35°C. Samples were reconstituted in 1 mL of acetonitrile and stored at -20°C until ready for analysis via GC-ECD.

Method A6 Mean Percent Recoveries

Table A6. Percent recoveries of OP and pyrethroid pesticides extracted via Method A6 from grape juice fortified at 100 ng/g (n=3) with varying C_2 cartridge elution solvent. Values in parentheses are coefficients of variation.

	Acetonitrile		CH_3OH	Ethyl Acetate		CH_2Cl_2		Diethyl Ether	Toluene		Hexane	
diazinon	0.0		0.0	0.0		0.0		0.0	0.0		0.0	
malathion	0.0		0.0	0.0		0.0		0.0	0.0		0.0	
chlorpyrifos	22.4	(18)	0.0	47.3	(11)	49.9	(102)	0.0	48.8	(69)	36.5	(82)
permethrin	13.6	(29)	0.0	24.1	(18)	14.1	(11)	0.0	28.8	(38)	17.5	(106)
cyfluthrin	49.5	(14)	0.0	69.1	(8)	46.1	(10)	0.0	48.2	(3)	54.3	(43)
cypermethrin	46.9	(15)	0.0	66.6	(10)	42.4	(12)	0.0	45.8	(7)	50.5	(45)
deltamethrin	44.9	(16)	0.0	65.6	(8)	43.5	(10)	0.0	45.3	(6)	51.9	(41)

Method A7

One milliliter aliquots of grape juice fortified at 100 ng/g was placed in a 15-mL disposable glass centrifuge tube to which ~ 2 g NaCl was added. The mixture was vortexed for 1 minute. A phenyl cartridge was conditioned with 3 mL of ethyl acetate followed by 96% v/v ethanol in Milli-Q H_2O and once with 3 mL 10% v/v ethanol in Milli-Q H_2O. Na_2SO_4 was added on top of each SPE cartridge to a depth of ~2 mm. The grape juice and salt mixture was loaded onto the cartridge and the cartridge was not disturbed for 20 minutes. The cartridge was then eluted with 4 mL of acetonitrile, ethyl acetate, methanol, dichloromethane, toluene, hexane, or diethyl ether and the eluant was collected in a 15-mL disposable glass centrifuge tube. Then a Florisil cartridge with Na_2SO_4 added on top to a depth of ~2 mm was conditioned with 5 mL ethyl acetate. The eluant from the phenyl cartridge was loaded onto the Florisil cartridge. The Florisil cartridge was eluted with 4 mL acetonitrile and the eluant was collected in a 15-mL disposable glass centrifuge tube. The eluant was then placed in a TurboVap LV and evaporated to dryness at 10 psi and 35°C for 15 minutes and then at 25 psi and 35°C. Samples were reconstituted in 1 mL of acetonitrile and stored at -20°C until ready for analysis via GC-ECD.

Method A7 Mean Percent Recoveries

Table A7. Percent recoveries of OP and pyrethroid pesticides extracted via Method A7 from grape juice fortified at 100 ng/g (n=3) with varying Phenyl cartridge elution solvent. Values in parentheses are coefficients of variation.

	Acetonitrile		CH_3OH	Ethyl Acetate	CH_2Cl_2		Diethyl Ether	Toluene		Hexane	
diazinon	0.0		0.0	0.0	0.0		0.0	0.0		0.0	
malathion	0.0		0.0	0.0	0.0		0.0	0.0		0.0	
chlorpyrifos	22.4	(18)	0.0	0.0	47.0	(35)	0.0	51.5	(12)	52.2	(84)
permethrin	8.8	(76)	0.0	0.0	20.5	(24)	0.0	30.5	(45)	25.5	(4)
cyfluthrin	43.9	(24)	0.0	0.0	69.4	(10)	0.0	61.6	(8)	52.1	(41)
cypermethrin	40.0	(24)	0.0	0.0	66.8	(10)	0.0	58.0	(8)	51.2	(45)
deltamethrin	40.7	(19)	0.0	0.0	67.3	(8)	0.0	56.4	(8)	48.1	(36)

Method A8

One milliliter aliquots of Milli-Q H_2O fortified at 175 ng/g was placed in a 15-mL disposable glass centrifuge tube to which ~ 2 g NaCl was added. The mixture was vortexed for 1 minute. A C_{18} cartridge was conditioned with 3 mL of ethyl acetate followed by 96% v/v ethanol in Milli-Q H_2O and once with 3 mL 10% v/v ethanol in Milli-Q H_2O. Na_2SO_4 was added on top of each SPE cartridge to a depth of ~2 mm. The Milli-Q H_2O and salt mixture was loaded onto the cartridge and the cartridge was not disturbed for 20 minutes. The cartridge was then eluted with 4 mL of ethyl acetate, dichloromethane, toluene, or hexane and the eluant was collected in a 15-mL disposable glass centrifuge tube. Then a Florisil cartridge with Na_2SO_4 added on top to a depth of ~2 mm was conditioned with 5 mL ethyl acetate. The eluant from the C_{18} cartridge was loaded onto the Florisil cartridge. The Florisil cartridge was eluted with 4 mL acetonitrile and the eluant was collected in a 15-mL disposable glass centrifuge tube. The eluant was then placed in a TurboVap LV and evaporated to dryness at 10 psi and 35°C for 15 minutes and then at 25 psi and 35°C. Samples were reconstituted in 1 mL of acetonitrile and stored at -20°C until ready for analysis via GC-ECD.

Method A8 Mean Percent Recoveries

Table A8. Percent recoveries of OP and pyrethroid pesticides extracted via Method A8 from Milli-Q H_2O fortified at 100 ng/g (n=3) with varying C_{18} cartridge elution solvent. Values in parentheses are coefficients of variation.

	Ethyl Acetate		CH_2Cl_2		Toluene		Hexane	
diazinon	0.0		0.0		0.0		0.0	
malathion	35.8	(6)	41.3	(32)	49.9	(21)	116.0	(14)
chlorpyrifos	29.7	(9)	33.8	(13)	34.0	(8)	40.1	(6)
permethrin	15.8	(6)	13.6	(14)	17.9	(32)	16.5	(25)
cyfluthrin	28.6	(9)	31.6	(14)	35.5	(20)	24.8	(24)
cypermethrin	26.4	(9)	28.9	(15)	29.7	(21)	18.6	(17)
deltamethrin	26.7	(8)	24.7	(17)	26.3	(6)	17.6	(13)

Method A9

Five milliliter aliquots of Milli-Q H_2O fortified at 200 ng/g was placed in a 15-mL disposable glass centrifuge tube. A C_{18} cartridge was conditioned with 15 mL of methanol followed 10 mL Milli-Q H_2O. The 5 mL aliquot of fortified Milli-Q H_2O was loaded onto the cartridge and the cartridge was washed with 2.5 mL of a 9:1 Milli-Q H_2O to 2-propanol. The cartridge was then allowed to air-dry for 45 minutes via blowing air through it. The cartridge was then eluted with 3 mL of ethyl acetate, after soaking for 5 minutes with ethyl acetate and the eluant was collected in a 15-mL disposable glass centrifuge tube. Then a Florisil cartridge was conditioned with 5 mL ethyl acetate and allowed to air-dry for 5 minutes. The eluant from the C_{18} cartridge was loaded onto the Florisil cartridge and the eluant was collected in a 15-mL disposable glass centrifuge tube. The eluant was then placed in a TurboVap LV and evaporated to dryness at 10 psi and 35°C for 15 minutes and then at 25 psi and 35°C. Samples were reconstituted in 1 mL of acetonitrile and stored at -20°C until ready for analysis via GC-ECD.

Method A9 Mean Percent Recoveries

Table A9. Percent recoveries of OP and pyrethroid pesticides extracted via Method A9 from Milli-Q H_2O fortified at 200 ng/g (n=3). Values in parentheses are coefficients of variation.

diazinon	65.1	(6)
malathion	35.0	(8)
chlorpyrifos	67.3	(13)
permethrin	18.4	(32)
cyfluthrin	36.4	(7)
cypermethrin	35.5	(5)
deltamethrin	31.1	(11)

Method A10

Five milliliters acetonitrile, ethyl acetate, CH_2Cl_2, toluene, or hexane, 2.5 mL Milli-Q H_2O fortified at 200 ng/g, and ~ 5.0 g Na_2SO_4 was placed in a 15-mL disposable glass centrifuge tube and vortexed for 3 minutes. The mixture was centrifuged for 3 minutes at ~ 4000 rpm. The sample was then placed in the freezer over night to freeze the aqueous layer. Subsequently, a 2-mL aliquot of the organic layer was placed in a 15-mL disposable glass centrifuge tube and evaporated to dryness at 10 psi and 35°C for 15 minutes and then at 25 psi and 35°C and reconstituted in 1 mL toluene. A C_{18} cartridge was conditioned with 3 mL of toluene. The 1-mL toluene sample was loaded onto the cartridge. The cartridge was then eluted with 3 mL of 95% v/v toluene in ethyl acetate followed by 3 mL ethyl acetate and the eluant was collected in a 15-mL disposable glass centrifuge tube. The eluant was then placed in a TurboVap LV and evaporated to dryness at 10 psi and 35°C for 15 minutes and then at 25 psi and 35°C. Samples were reconstituted in 1 mL of acetonitrile and stored at -20°C until ready for analysis via GC-ECD.

Method A10 Mean Percent Recoveries

Table A10. Percent recoveries of OP and pyrethroid pesticides extracted via Method A10 from Milli-q H2O fortified at 200 ng/g (n=3) with varying extraction solvent. Values in parentheses are coefficients of variation.

	Acetonitrile		Ethyl Acetate		CH_2Cl_2		Toluene		Hexane	
diazinon	77.0	(1)	157.3	(29)	96.7	(22)	76.3	(18)	69.5	(8)
malathion	151.4	(14)	108.8	(27)	140.7	(24)	113.9	(9)	135.1	(27)
chlorpyrifos	73.9	(2)	53.1	(7)	41.8	(32)	77.0	(20)	64.3	(8)
permethrin	39.7	(10)	36.3	(5)	32.4	(13)	40.1	(15)	35.2	(9)
cyfluthrin	73.2	(15)	70.3	(2)	50.6	(9)	71.8	(8)	66.2	(2)
cypermethrin	78.9	(11)	68.9	(3)	52.7	(10)	80.0	(14)	68.6	(9)
deltamethrin	91.0	(17)	65.6	(10)	57.3	(10)	92.5	(13)	77.6	(13)

Method A11

Five milliliters acetonitrile, ethyl acetate, diethyl ether, CH_2Cl_2, toluene, or hexane, 2.5 mL Milli-Q H_2O fortified at 200 ng/g, and ~ 5.0 g Na_2SO_4 was placed in a 15-mL glass disposable centrifuge tube and vortexed for 3 minutes. The mixture was then sonicated for one hour followed by centrifugation for 10 minutes at ~ 4000 rpm. The organic phase was pipetted into another 15-mL glass disposable centrifuge tube that contained ~ 0.5 g Na_2SO_4. Subsequently, a 2-mL aliquot of the organic layer was placed in a another 15-mL disposable glass centrifuge tube and evaporated to dryness at 10 psi and 35°C for 15 minutes and then at 25 psi and 35°C and reconstituted in 1 mL toluene. A Florisil cartridge was conditioned with 3 mL of toluene. The 1-mL toluene sample was loaded onto the cartridge. The cartridge was then eluted with 3 mL of 95% v/v toluene in ethyl acetate followed by 3 mL ethyl acetate and the eluant was collected in a 15-mL disposable glass centrifuge tube. The eluant was then placed in a TurboVap LV and evaporated to dryness at 10 psi and 35°C for 15 minutes and then at 25 psi and 35°C. Samples were reconstituted in 1 mL of acetonitrile and stored at -20°C until ready for analysis via GC-ECD.

Method A11 Mean Percent Recoveries

Table A11. Percent recoveries of OP and pyrethroid pesticides extracted via Method A11 from Milli-Q H_2O fortified at 200 ng/g (n=3) with varying extraction solvent. Values in parentheses are coefficients of variation.

	Acetonitrile		Ethyl Acetate		CH_2Cl_2		Diethyl Ether		Toluene		Hexane	
diazinon	107.2	(9)	87.6	(9)	68.5	(18)	66.9	(9)	87.4	(13)	88.9	(7)
malathion	73.7	(23)	104.5	(10)	91.1	(16)	77.8	(12)	85.6	(19)	79.2	(15)
chlorpyrifos	103.0	(10)	104.1	(4)	88.5	(8)	80.1	(7)	92.4	(12)	96.2	(6)
permethrin	60.9	(6)	51.1	(6)	70.4	(1)	62.6	(2)	51.6	(23)	54.8	(15)
cyfluthrin	113.8	(9)	94.0	(7)	95.9	(5)	79.1	(3)	86.8	(26)	97.9	(13)
cypermethrin	133.9	(5)	92.7	(4)	101.7	(7)	91.1	(3)	86.5	(21)	91.9	(14)
deltamethrin	148.1	(16)	129.7	(5)	126.9	(12)	112.0	(6)	104.7	(27)	120.5	(21)

Method A12

Five milliliters acetonitrile or ethyl acetate, 2.5 mL Milli-Q H_2O fortified at 175 ng/g, and ~ 1.0 g NaCl was placed in a 15-mL glass disposable centrifuge tube and vortexed for 3 minutes. The mixture was then sonicated for one hour followed by centrifugation for 10 minutes at ~ 4000 rpm. The organic phase was pipetted into another 15-mL glass disposable centrifuge tube that contained ~ 0.5 g Na_2SO_4. Subsequently, a 2-mL aliquot of the organic layer was placed in a another 15-mL disposable glass centrifuge tube and evaporated to dryness at 10 psi and 35°C for 15 minutes and then at 25 psi and 35°C and reconstituted in 1 mL toluene. A Florisil cartridge was conditioned with 3 mL of toluene. The 1-mL toluene sample was loaded onto the cartridge. The cartridge was then eluted with 3 mL of 95% v/v toluene in acetonitrile followed by 3 mL acetonitrile and the eluant was collected in a 15-mL disposable glass centrifuge tube. The eluant was then placed in a TurboVap LV and evaporated to dryness at 10 psi and 35°C for 15 minutes and then at 25 psi and 35°C. Samples were reconstituted in 1 mL of acetonitrile and stored at -20°C until ready for analysis via GC-ECD.

Method A12 Mean Percent Recoveries

Table A12. Percent recoveries of OP and pyrethroid pesticides extracted via Method A12 from Milli-Q H_2O fortified at 175 ng/g (n=3) with varying extraction solvent. Values in parentheses are coefficients of variation.

	Acetonitrile		Ethyl Acetate	
diazinon	69.5	(13)	143.8	(13)
malathion	49.8	(21)	55.4	(15)
chlorpyrifos	94.3	(9)	104.3	(5)
permethrin	97.0	(3)	107.6	(4)
cyfluthrin	89.7	(9)	106.1	(13)
cypermethrin	88.1	(8)	110.0	(13)
deltamethrin	100.4	(7)	142.6	(9)

Method A13

Five milliliters acetonitrile or ethyl acetate, 2.5 mL Milli-Q H_2O fortified at 200 ng/g, and ~ 1.0 g NaCl was placed in a 15-mL glass disposable centrifuge tube and vortexed for 3 minutes. The mixture was then centrifuged for 5 minutes at ~ 4000 rpm. The organic phase was pipetted into another 15-mL glass disposable centrifuge tube that contained ~ 0.5 g Na_2SO_4. Subsequently, a 2-mL aliquot of the organic layer was placed in a another 15-mL disposable glass centrifuge tube and evaporated to dryness at 10 psi and 35°C for 15 minutes and then at 25 psi and 35°C and reconstituted in 1 mL toluene. A Florisil cartridge was conditioned with 3 mL of toluene. The 1-mL toluene sample was loaded onto the cartridge. The cartridge was then eluted with 3 mL acetonitrile, acetone, ethyl acetate, 95% v/v toluene in acetonitrile, or 95% v/v toluene in ethyl acetate and the eluant was collected in a 15-mL disposable glass centrifuge tube. The eluant was then placed in a TurboVap LV and evaporated to dryness at 10 psi and 35°C for 15 minutes and then at 25 psi and 35°C. Samples were reconstituted in 1 mL of acetonitrile and stored at -20°C until ready for analysis via GC-ECD.

Method A13 Mean Percent Recoveries

Table A13. Percent recoveries of OP and pyrethroid pesticides extracted via Method A13 from Milli-Q H_2O fortified at 200 ng/g (n=3) with varying elution solvent. Values in parentheses are coefficients of variation.

	Acetonitrile		Acetone		Ethyl Acetate		95:5 Tol:MeCN		95:5 Tol:EtOAc	
diazinon	53.0	(23)	60.7	(14)	63.4	(24)	42.8	(17)	60.2	(15)
malathion	184.1	(6)	94.6	(11)	170.6	(1)	115.7	(32)	152.6	(10)
chlorpyrifos	101.3	(1)	73.9	(13)	91.8	(2)	85.5	(23)	97.6	(6)
permethrin	87.8	(8)	78.3	(7)	81.5	(6)	106.1	(5)	98.4	(7)
cyfluthrin	115.6	(14)	76.1	(8)	103.8	(3)	91.1	(28)	95.4	(4)
cypermethrin	110.7	(14)	74.4	(11)	97.3	(4)	91.3	(25)	94.1	(5)
deltamethrin	147.6	(15)	95.1	(6)	119.7	(3)	112.1	(31)	117.8	(8)

Method A14

Five milliliters ethyl acetate, 2.5 mL Milli-Q H_2O fortified at 200 ng/g, and ~ 1.0 g NaCl was placed in a 15-mL glass disposable centrifuge tube and vortexed for 3 minutes. The mixture was then sonicated for one hour followed by centrifugation for 5 minutes at ~ 4000 rpm. The organic phase was pipetted into another 15-mL glass disposable centrifuge tube that contained ~ 0.5 g Na_2SO_4. Subsequently, a 2-mL aliquot of the organic layer was placed in a another 15-mL disposable glass centrifuge tube and evaporated to dryness at 10 psi and 35°C for 15 minutes and then at 25 psi and 35°C and reconstituted in 1 mL toluene. A Florisil cartridge was conditioned with 3 mL of toluene. The 1-mL toluene sample was loaded onto the cartridge. The cartridge was then eluted with 3 mL of 95% v/v toluene in acetonitrile followed by 3 mL acetonitrile (elution 1) or 3 mL 95% v/v toluene in acetone followed by 3 mL acetone (elution 2) and the eluant was collected in a 15-mL disposable glass centrifuge tube. The eluant was then placed in a TurboVap LV and evaporated to dryness at 10 psi and 35°C for 15 minutes and then at 25 psi and 35°C. Samples were reconstituted in 1 mL of acetonitrile and stored at -20°C until ready for analysis via GC-ECD.

Method A14 Mean Percent Recoveries

Table A14. Percent recoveries of OP and pyrethroid pesticides extracted via Method A14 from Milli-Q H_2O fortified at 175 ng/g (n=3) with varying extraction solvent. Values in parentheses are coefficients of variation.

	Elution 1		Elution 2	
diazinon	67.9	(17)	54.5	(13)
malathion	213.3	(3)	115.2	(12)
chlorpyrifos	95.4	(18)	89.0	(8)
permethrin	107.1	(8)	108.5	(11)
cyfluthrin	146.0	(1)	138.7	(11)
cypermethrin	143.3	(1)	138.7	(13)
deltamethrin	192.9	(4)	184.7	(20)

Method A15

Five milliliters ethyl acetate, 2.5 mL Milli-Q H_2O fortified at 200 ng/g, and ~ 1.0 g NaCl was placed in a 15-mL glass disposable centrifuge tube and vortexed for 3 minutes. The mixture was then sonicated for 20 minutes followed by centrifugation for 5 minutes at ~ 4000 rpm. The organic phase was pipetted into another 15-mL glass disposable centrifuge tube that contained ~ 0.5 g Na_2SO_4. Subsequently, a 2-mL aliquot of the organic layer was placed in a another 15-mL disposable glass centrifuge tube and evaporated to dryness at 10 psi and 35°C for 15 minutes and then at 25 psi and 35°C and reconstituted in 1 mL toluene. A Florisil cartridge was conditioned with 3 mL of toluene. The 1-mL toluene sample was loaded onto the cartridge. The cartridge was then eluted with 3 mL of 95% v/v toluene in acetonitrile followed by 3 mL acetonitrile (elution 1) or 3 mL 95% v/v toluene in acetone followed by 3 mL acetone (elution 2) and the eluant was collected in a 15-mL disposable glass centrifuge tube. The eluant was then placed in a TurboVap LV and evaporated to dryness at 10 psi and 35°C for 15 minutes and then at 25 psi and 35°C. Samples were reconstituted in 1 mL of acetonitrile and stored at -20°C until ready for analysis via GC-ECD.

Method A15 Mean Percent Recoveries

Table A15. Percent recoveries of OP and pyrethroid pesticides extracted via Method A15 from Milli-Q H_2O fortified at 200 ng/g (n=3) with varying elution systems. Values in parentheses are coefficients of variation.

	Elution 1		Elution 2	
diazinon	57.3	(1)	41.5	(12)
malathion	86.0	(27)	114.3	(9)
chlorpyrifos	51.3	(17)	48.7	(10)
permethrin	143.9	(1)	148.0	(8)
cyfluthrin	84.1	(3)	82.1	(11)
cypermethrin	85.5	(2)	84.2	(14)
deltamethrin	89.7	(4)	86.7	(14)

Method A16

Five milliliters ethyl acetate, 2.5 mL Milli-Q H_2O fortified at 200 ng/g, and ~ 1.0 g NaCl was placed in a 15-mL glass disposable centrifuge tube and vortexed for 3 minutes. The mixture was then centrifuged for 5 minutes at ~ 4000 rpm. The organic phase was pipetted into another 15-mL glass disposable centrifuge tube that contained ~ 0.5 g Na_2SO_4. Subsequently, a 2-mL aliquot of the organic layer was placed in a another 15-mL disposable glass centrifuge tube and evaporated to dryness at 10 psi and 35°C for 15 minutes and then at 25 psi and 35°C and reconstituted in 1 mL toluene. A Florisil cartridge was conditioned with 3 mL of toluene. The 1-mL toluene sample was loaded onto the cartridge. The cartridge was then eluted with 3 mL of 95% v/v toluene in acetonitrile followed by 3 mL acetonitrile (elution 1) or 3 mL 95% v/v toluene in acetone followed by 3 mL acetone (elution 2) and the eluant was collected in a 15-mL disposable glass centrifuge tube. The eluant was then placed in a TurboVap LV and evaporated to dryness at 10 psi and 35°C for 15 minutes and then at 25 psi and 35°C. Samples were reconstituted in 1 mL of acetonitrile and stored at -20°C until ready for analysis via GC-ECD.

Method A16 Mean Percent Recoveries

Table A16. Percent recoveries of OP and pyrethroid pesticides extracted via Method A16 from Milli-Q H_2O fortified at 200 ng/g (n=3) with varying elution systems. Values in parentheses are coefficients of variation.

	Elution 1		Elution 2	
diazinon	66.4	(10)	44.3	(8)
malathion	96.9	(1)	128.9	(6)
chlorpyrifos	65.1	(14)	62.7	(8)
permethrin	133.2	(2)	137.9	(6)
cyfluthrin	71.4	(2)	70.2	(9)
cypermethrin	83.2	(3)	80.1	(9)
deltamethrin	85.9	(2)	81.7	(12)

Method A17

Five milliliters ethyl acetate, 2.5 mL Milli-Q H_2O fortified at 200 ng/g, and ~ 1.0 g NaCl was placed in a 15-mL glass disposable centrifuge tube and vortexed for 3 minutes. The mixture was then centrifuged for 5 minutes at ~ 4000 rpm. The organic phase was pipetted into another 15-mL glass disposable centrifuge tube that contained ~ 0.5 g Na_2SO_4. Subsequently, a 2-mL aliquot of the organic layer was placed in a another 15-mL disposable glass centrifuge tube and evaporated to dryness at 10 psi and 35°C for 15 minutes and then at 25 psi and 35°C and reconstituted in 1 mL toluene. A Florisil cartridge was conditioned with 3 mL of toluene. The 1-mL toluene sample was loaded onto the cartridge. The cartridge was then eluted with 3 mL hexane or toluene and the eluant was collected in a 15-mL disposable glass centrifuge tube. The eluant was then placed in a TurboVap LV and evaporated to dryness at 10 psi and 35°C for 15 minutes and then at 25 psi and 35°C. Samples were reconstituted in 1 mL of acetonitrile and stored at -20°C until ready for analysis via GC-ECD.

Method A17 Mean Percent Recoveries

Table A17. Percent recoveries of OP and pyrethroid pesticides extracted via Method A17 from Milli-Q H_2O fortified at 250 ng/g (n=3) with varying elution solvent. Values in parentheses are coefficients of variation.

	Toluene		Hexane	
diazinon	75.3	(1)	77.5	(28)
malathion	73.2	(8)	89.7	(9)
chlorpyrifos	49.4	(7)	61.5	(11)
permethrin	106.2	(11)	161.8	(2)
cyfluthrin	92.4	(8)	96.1	(7)
cypermethrin	91.6	(11)	97.4	(7)
deltamethrin	96.8	(14)	100.6	(4)

Method A18

Five milliliters ethyl acetate, 2.5 mL Milli-Q H_2O fortified at 200 ng/g, and ~ 5.0 g $MgSO_4$ was placed in a 15-mL glass disposable centrifuge tube and vortexed for 3 minutes. For salting out, ~ 1.0 g NaCl was also added to the mixture. The mixture was then centrifuged for 5 minutes at ~ 4000 rpm. The organic phase was pipetted into another 15-mL glass disposable centrifuge tube that contained ~ 0.5 g $MgSO_4$. For double extraction, to the remaining aqueous layer was added another 5 mL ethyl acetate. The mixture was vortexed again for 3 minutes and centrifuged again for 5 minutes at ~ 4000 rpm. Subsequently, a 2-mL aliquot of the organic layer was placed in a another 15-mL disposable glass centrifuge tube and evaporated to dryness at 10 psi and 35°C for 15 minutes and then at 25 psi and 35°C and reconstituted in 1 mL toluene. A Florisil cartridge was conditioned with 3 mL of toluene. The 1-mL toluene sample was loaded onto the cartridge. The cartridge was then eluted with 3 mL 95% v/v toluene in ethyl acetate followed by 3 mL ethyl acetate and the eluant was collected in a 15-mL disposable glass centrifuge tube. The eluant was then placed in a TurboVap LV and evaporated to dryness at 10 psi and 35°C for 15 minutes and then at 25 psi and 35°C. Samples were reconstituted in 1 mL of acetonitrile and stored at -20°C until ready for analysis via GC-ECD.

Method A18 Mean Percent Recoveries

Table A18. Percent recoveries of OP and pyrethroid pesticides extracted via Method A18 from Milli-Q H_2O fortified at 200 ng/g (n=3) with single or doube extraction with or without salting out. Values in parentheses are coefficients of variation.

	Single		Double		Single w/ Salting		Double w/ Salting	
diazinon	50.5	(12)	57.6	(17)	65.0	(18)	71.9	(8)
malathion	71.2	(9)	77.7	(11)	83.7	(6)	83.1	(8)
chlorpyrifos	52.7	(7)	51.9	(13)	58.8	(3)	56.7	(14)
permethrin	144.0	(6)	131.8	(4)	154.3	(5)	148.7	(5)
cyfluthrin	78.9	(19)	81.9	(5)	93.7	(5)	97.9	(6)
cypermethrin	79.0	(19)	80.1	(5)	90.8	(6)	92.9	(5)
deltamethrin	82.0	(26)	81.4	(8)	93.4	(6)	95.3	(9)

Method A19

Five milliliters acetonitrile, 2.5 mL Milli-Q H_2O fortified at 150 ng/g, and ~ 2.0 g NaCl was placed in a 15-mL glass disposable centrifuge tube and vortexed for 3 minutes. The mixture was then centrifuged for 5 minutes at ~ 4000 rpm. The organic phase was pipetted into another 15-mL glass disposable centrifuge tube that contained ~ 2.0 g $MgSO_4$. Subsequently, a 2-mL aliquot of the organic layer was placed in a another 15-mL disposable glass centrifuge tube and evaporated to dryness at 10 psi and 35°C for 15 minutes and then at 25 psi and 35°C and reconstituted in 1 mL acetonitrile. An ENVI-CARB-II/PSA or PSA cartridge was conditioned with 5 mL of 25% v/v toluene in acetonitrile. The 1-mL acetonitrile sample was loaded onto the cartridge. The cartridge was then eluted with 10 mL of 25% v/v toluene in acetonitrile. The eluant was collected in a 15-mL disposable glass centrifuge tube and placed in a TurboVap LV and evaporated under a stream of air at 10 psi and 35°C for 15 minutes and again at 25 psi and 35°C for 30 minutes to dryness. In the case of the 20 mL elution, we eluted the cartridge once more with 10 mL of 25% v/v toluene in acetonitrile. Here, the combined eluants were then evaporated to dryness using the TurboVap LV first at 10 psi and 35°C for 15 minutes and then at 25 psi and 35°C. Samples were reconstituted in 1 mL of 50% v/v hexane in acetone and stored at -20°C until ready for analysis via GC-ECD.

Method A19 Mean Percent Recoveries

Table A19. Percent recoveries of OP and pyrethroid pesticides extracted via Method A19 from Milli-Q H_2O fortified at 150 ng/g (n=3) with varying SPE cartridge and solvent elution volume. Values in parentheses are coefficients of variation.

	ENVI-Carb/PSA - 20 mL		PSA - 20 mL		ENVI-Carb/PSA - 10 mL		PSA - 10 mL	
diazinon	59.5	(18)	78.1	(16)	82.7	(18)	91.7	(17)
malathion	68.8	(5)	82.4	(12)	85.9	(14)	89.9	(32)
chlorpyrifos	32.8	(22)	55.3	(26)	51.1	(30)	62.5	(23)
permethrin	194.4	(23)	166.0	(2)	129.0	(7)	126.0	(5)
cyfluthrin	104.3	(11)	85.6	(9)	99.1	(11)	97.7	(28)
cypermethrin	101.3	(8)	88.9	(14)	99.2	(13)	101.5	(25)
deltamethrin	77.0	(1)	78.4	(11)	81.3	(10)	87.7	(31)

Method A20

Five milliliters acetonitrile, 2.5 mL Milli-Q H_2O fortified at 200 ng/g, and ~ 2.0 g NaCl was placed in a 15-mL glass disposable centrifuge tube and vortexed for 3 minutes. The mixture was then centrifuged for 5 minutes at ~ 4000 rpm. The organic phase was pipetted into another 15-mL glass disposable centrifuge tube that contained ~ 5.0 g $MgSO_4$. Subsequently, a 2-mL aliquot of the organic layer was placed in a another 15-mL disposable glass centrifuge tube and evaporated to dryness at 10 psi and 35°C for 15 minutes and then at 25 psi and 35°C and reconstituted in 1 mL acetonitrile. A Florisil or PSA cartridge was conditioned with 5 mL of 25% v/v toluene in acetonitrile. The 1-mL acetonitrile sample was loaded onto the cartridge. The cartridge was then eluted with 10 mL of 25% v/v toluene in acetonitrile. The eluant was collected in a 15-mL disposable glass centrifuge tube and placed in a TurboVap LV and evaporated under a stream of air at 10 psi and 35°C for 15 minutes and again at 25 psi and 35°C for 30 minutes to dryness. Samples were reconstituted in 1 mL of 50% v/v hexane in acetone and stored at -20°C until ready for analysis via GC-ECD.

Method A20 Mean Percent Recoveries

Table A20. Percent recoveries of OP and pyrethroid pesticides extracted via Method A20 from Milli-Q H_2O fortified at 200 ng/g (n=3) with varying SPE cartridges. Values in parentheses are coefficients of variation.

	Florisil		PSA	
diazinon	52.9	(1)	42.2	(44)
malathion	61.6	(16)	74.4	(22)
chlorpyrifos	16.3	(62)	28.7	(31)
permethrin	140.9	(11)	151.7	(9)
cyfluthrin	135.3	(20)	132.9	(15)
cypermethrin	135.0	(20)	136.7	(14)
deltamethrin	121.1	(26)	121.3	(14)

Method A21

Five milliliters acetonitrile, 2.5 mL Milli-Q H_2O fortified at 428.5 ng/g, and ~ 2.0 g NaCl was placed in a 15-mL glass disposable centrifuge tube and vortexed for 3 minutes. The mixture was then centrifuged for 5 minutes at ~ 4000 rpm. The organic phase was pipetted into another 15-mL glass disposable centrifuge tube that contained ~ 2.0 g Na_2SO_4. A Florisil cartridge was conditioned with 5 mL hexane. A 2-mL aliquot of the organic layer was loaded onto the cartridge. The cartridge was then eluted with 10 mL of 30% v/v diethyl ether in hexane. The eluant was collected in a 15-mL disposable glass centrifuge tube and placed in a TurboVap LV and evaporated under a stream of air at 10 psi and 35°C for 15 minutes and again at 25 psi and 35°C for 30 minutes to dryness. The cartridge was eluted once more with 10 mL of 30% v/v diethyl ether in hexane. The eluant was then evaporated to dryness again using the TurboVap LV first at 10 psi and 35°C for 15 minutes and then at 25 psi and 35°C. Samples were reconstituted in 1 mL of acetonitrile and stored at -20°C until ready for analysis via GC-ECD.

Method A21 Mean Percent Recoveries

Table A21. Percent recoveries of OP and pyrethroid pesticides extracted via Method A21 from Milli-Q H_2O fortified at 428.5 ng/g (n=3) with varying SPE cartridges. Values in parentheses are coefficients of variation.

	Florisil	
diazinon	29.8	(16)
malathion	35.3	(13)
chlorpyrifos	29.7	(6)
permethrin	52.9	(16)
cyfluthrin	46.3	(4)
cypermethrin	44.8	(5)
deltamethrin	38.8	(7)

Method A22

Three milliliters CH_2Cl_2 and 2 mL acetone, 2.5 mL Milli-Q H_2O fortified at 428.5 ng/g, and ~ 2.0 g NaCl was placed in a 15-mL glass disposable centrifuge tube and vortexed for 3 minutes. The mixture was then centrifuged for 5 minutes at ~ 4000 rpm. The organic phase was pipetted into another 15-mL glass disposable centrifuge tube that contained ~ 2.0 g Na_2SO_4. A C_{18} cartridge was conditioned with 10 mL hexane. A 2-mL aliquot of the organic layer was loaded onto the cartridge. The cartridge was then eluted with 10 mL hexane. The eluant was collected in a 15-mL disposable glass centrifuge tube and placed in a TurboVap LV and evaporated under a stream of air at 10 psi and 35°C for 15 minutes and again at 25 psi and 35°C for 30 minutes to dryness. The cartridge was then eluted with 10 mL acetone. The eluant was then evaporated to dryness again using the TurboVap LV first at 10 psi and 35°C for 15 minutes and then at 25 psi and 35°C. Samples were reconstituted in 1 mL of acetonitrile and stored at -20°C until ready for analysis via GC-ECD.

Method A22 Mean Percent Recoveries

Table A22. Percent recoveries of OP and pyrethroid pesticides extracted via Method A22 from Milli-Q H_2O fortified at 428.5 ng/g (n=3) with varying SPE cartridges. Values in parentheses are coefficients of variation.

	C_{18}	
diazinon	28.8	(12)
malathion	16.8	(4)
chlorpyrifos	15.3	(6)
permethrin	33.1	(28)
cyfluthrin	41.7	(7)
cypermethrin	41.0	(12)
deltamethrin	35.2	(10)

Method A23

Five milliliters acetonitrile, 2.5 mL Milli-Q H_2O fortified at 500 ng/g, and ~ 2.0 g NaCl was placed in a 15-mL glass disposable centrifuge tube and vortexed for 3 minutes. The mixture was then centrifuged for 5 minutes at ~ 4000 rpm. The organic phase was pipetted into another 15-mL glass disposable centrifuge tube that contained ~ 5.0 g Na_2SO_4. An ENVl-CARB-II/PSA (500 mg/500 mg/20 mL) cartridge was conditioned with 5 mL of acetonitrile or 25% v/v toluene in acetonitrile. A 2-mL aliquot of the organic extract was loaded onto the cartridge. The cartridge was then eluted with 10 mL of acetonitrile or 25% v/v toluene in acetonitrile, respectively, dependent upon which was used to condition the cartridge. The eluant was collected in a 15-mL disposable glass centrifuge tube and placed in a TurboVap LV and evaporated under a stream of air at 10 psi and 35°C for 15 minutes and again at 25 psi and 35°C for 30 minutes to dryness. We eluted the cartridge once more with 10 mL of acetonitrile or 25% v/v toluene in acetonitrile. Again, the eluant was then evaporated to dryness using the TurboVap LV first at 10 psi and 35°C for 15 minutes and then at 25 psi and 35°C. Samples were reconstituted in 1 mL of acetonitrile and stored at -20°C until ready for analysis via GC-ECD.

Method A23 Mean Percent Recoveries

Table A23. Percent recoveries of OP and pyrethroid pesticides extracted via Method A23 from Milli-Q H_2O fortified at 500 ng/g (n=3) with varying elution solvent. Values in parentheses are coefficients of variation.

	Acetonitrile		3:1 MeCN:Toluene	
diazinon	50.2	(10)	32.2	(32)
malathion	11.4	(11)	72.1	(2)
chlorpyrifos	11.4	(10)	46.5	(10)
permethrin	12.6	(20)	102.8	(3)
cyfluthrin	7.2	(6)	106.9	(6)
cypermethrin	0.0		107.2	(6)
deltamethrin	0.0		93.8	(7)

Method A24

Five milliliters acetonitrile, 2.5 mL Milli-Q H_2O fortified at 500 ng/g, and ~ 2.0 g NaCl was placed in a 15-mL glass disposable centrifuge tube and vortexed for 3 minutes. The mixture was then centrifuged for 5 minutes at ~ 4000 rpm. The organic phase was pipetted into another 15-mL glass disposable centrifuge tube that contained ~ 5.0 g Na_2SO_4. An ENVl-CARB-II/PSA (500 mg/300 mg/6 mL) cartridge was conditioned with 5 mL of acetonitrile or 25% v/v toluene in acetonitrile. A 2-mL aliquot of the organic extract was loaded onto the cartridge. The cartridge was then eluted with 5, 10, 15, 20, or 30 mL 25% v/v toluene in acetonitrile. The eluant was collected in a 15-mL disposable glass centrifuge tube and placed in a TurboVap LV and evaporated under a stream of air at 10 psi and 35°C for 15 minutes and again at 25 psi and 35°C for 30 minutes to dryness. For volumes > 10 mL, this elution and turbovapping process was repeated until the entire volume was eluted through the cartridge. Samples were reconstituted in 1 mL of acetonitrile and stored at -20°C until ready for analysis via GC-ECD.

Method A24 Mean Percent Recoveries

Table A24. Percent recoveries of OP and pyrethroid pesticides extracted via Method A24 from Milli-Q H_2O fortified at 500 ng/g (n=3) with varying elution solvent volume. Values in parentheses are coefficients of variation.

	5 mL		10 mL		15 mL		20 mL		30 mL	
diazinon	55.9	(12)	61.1	(34)	77.5	(4)	64.0	(6)	82.9	(15)
malathion	66.2	(2)	69.8	(7)	72.5	(1)	96.0	(4)	103.1	(7)
chlorpyrifos	46.2	(4)	64.9	(17)	76.7	(5)	64.2	(5)	78.5	(9)
permethrin	79.7	(7)	82.9	(6)	79.5	(5)	128.8	(2)	139.9	(8)
cyfluthrin	93.2	(11)	90.3	(10)	85.3	(7)	90.8	(4)	76.7	(20)
cypermethrin	93.5	(10)	89.2	(11)	85.5	(6)	95.6	(6)	80.9	(21)
deltamethrin	85.7	(13)	82.4	(11)	75.5	(6)	80.5	(7)	64.6	(23)

Method A25

Five milliliters acetonitrile, 2.5 mL Milli-Q H_2O fortified at 500 ng/g, and ~ 2.0 g NaCl was placed in a 15-mL glass disposable centrifuge tube and vortexed for 3 minutes. The mixture was then centrifuged for 5 minutes at ~ 4000 rpm. The organic phase was pipetted into another 15-mL glass disposable centrifuge tube that contained ~ 5.0 g Na_2SO_4. An ENVl-CARB-II/PSA (500 mg/300 mg/6 mL) cartridge was conditioned with 5 mL of acetonitrile or 25% v/v toluene in acetonitrile. A 2-mL aliquot of the organic extract was loaded onto the cartridge. The cartridge was then eluted with 5, 10, 15, 20, or 30 mL 50% v/v toluene in acetonitrile. The eluant was collected in a 15-mL disposable glass centrifuge tube and placed in a TurboVap LV and evaporated under a stream of air at 10 psi and 35°C for 15 minutes and again at 25 psi and 35°C for 30 minutes to dryness. For volumes > 10 mL, this elution and turbovapping process was repeated until the entire volume was eluted through the cartridge. Samples were reconstituted in 1 mL of acetonitrile and stored at -20°C until ready for analysis via GC-ECD.

Method A25 Mean Percent Recoveries

Table A25. Percent recoveries of OP and pyrethroid pesticides extracted via Method A25 from Milli-Q H_2O fortified at 500 ng/g (n=3) with varying elution solvent volume. Values in parentheses are coefficients of variation.

	20 mL		30 mL	
diazinon	94.9	(8)	117.1	(5)
malathion	117.3	(1)	128.7	(5)
chlorpyrifos	90.4	(3)	105.2	(6)
permethrin	150.7	(7)	192.0	(2)
cyfluthrin	86.6	(13)	103.3	(11)
cypermethrin	92.4	(12)	107.2	(8)
deltamethrin	70.3	(14)	89.3	(12)

Method A26

Five milliliters acetonitrile, 2.5 mL Milli-Q H_2O fortified at 200 ng/g, and ~ 2.0 g NaCl was placed in a 15-mL glass disposable centrifuge tube and vortexed for 3 minutes. The mixture was then centrifuged for 5 minutes at ~ 4000 rpm. The organic phase was pipetted into another 15-mL glass disposable centrifuge tube that contained ~ 5.0 g Na_2SO_4. An ENVl-CARB-II/PSA (500 mg/300 mg/6 mL) cartridge was conditioned with 5 mL of acetonitrile or 25% v/v toluene in acetonitrile. A 2-mL aliquot of the organic extract was loaded onto the cartridge. The cartridge was then eluted with 15 or 20 mL using 1 or 2% v/v methanol solution made in 25% v/v toluene in acetonitrile. The eluant was collected in a 15-mL disposable glass centrifuge tube and placed in a TurboVap LV and evaporated under a stream of air at 10 psi and 35°C for 15 minutes and again at 25 psi and 35°C for 30 minutes to dryness. For volumes > 10 mL, this elution and turbovapping process was repeated until the entire volume was eluted through the cartridge. Samples were reconstituted in 1 mL of acetonitrile and stored at -20°C until ready for analysis via GC-ECD.

Method A26 Mean Percent Recoveries

Table A26. Percent recoveries of OP and pyrethroid pesticides extracted via Method A26 from Milli-Q H_2O fortified at 200 ng/g (n=3) with varying elution solvent and elution solvent volume. Values in parentheses are coefficients of variation.

	15 mL - 2% CH_3OH		20 mL - 2% CH_3OH		15 mL - 1% CH_3OH		20 mL - 1% CH_3OH	
diazinon	82.4	(3)	91.9	(8)	74.6	(6)	96.8	(1)
malathion	75.4	(12)	86.9	(12)	71.2	(8)	89.6	(1)
chlorpyrifos	77.0	(6)	84.1	(6)	69.3	(5)	81.9	(1)
permethrin	106.7	(13)	103.9	(15)	108.2	(12)	101.1	(5)
cyfluthrin	83.2	(13)	105.4	(10)	80.8	(14)	94.3	(10)
cypermethrin	88.5	(13)	110.4	(9)	86.9	(11)	100.0	(10)
deltamethrin	69.4	(20)	96.2	(9)	70.6	(17)	82.1	(10)

Method A27

Five milliliters acetonitrile, 2.5 mL red wine or black coffee fortified at 200 ng/g, and ~ 2.0 g NaCl was placed in a 15-mL glass disposable centrifuge tube and vortexed for 3 minutes. The mixture was then centrifuged for 5 minutes at ~ 4000 rpm. The organic phase was pipetted into another 15-mL glass disposable centrifuge tube that contained ~ 5.0 g Na_2SO_4. An ENVl-CARB-II/PSA (500 mg/300 mg/6 mL) cartridge was conditioned with 5 mL of acetonitrile or 25% v/v toluene in acetonitrile. A 2-mL aliquot of the organic extract was loaded onto the cartridge. The cartridge was then eluted with 20 mL using 2% v/v ethyl acetate solution made with 25% v/v toluene in acetonitrile. The eluant was collected in a 15-mL disposable glass centrifuge tube and placed in a TurboVap LV and evaporated under a stream of air at 10 psi and 35°C for 15 minutes and again at 25 psi and 35°C for 30 minutes to dryness. We eluted the cartridge once more with 10 mL of 2% v/v ethyl acetate solution made with 25% v/v toluene in acetonitrile. Again, the eluant was then evaporated to dryness using the TurboVap LV first at 10 psi and 35°C for 15 minutes and then at 25 psi and 35°C. Samples were reconstituted in 1 mL of acetonitrile and stored at -20°C until ready for analysis via GC-ECD.

Method A27 Mean Percent Recoveries

Table A27. Percent recoveries of OP and pyrethroid pesticides extracted via Method A27 from red wine and coffee fortified at 200 ng/g (n=3). Values in parentheses are coefficients of variation.

	Wine		Coffee	
diazinon	45.3	(13)	42.8	(3)
malathion	65.8	(6)	53.2	(2)
chlorpyrifos	65.9	(8)	68.7	(6)
permethrin	104.8	(14)	113.0	(14)
cyfluthrin	68.6	(14)	82.5	(15)
cypermethrin	67.6	(14)	81.4	(14)
deltamethrin	55.1	(19)	70.5	(18)

Method A28

Five milliliters acetonitrile, 2.5 mL each red wine or black coffee fortified at 200 ng/g, and ~ 2.0 g NaCl was placed in a 15-mL glass disposable centrifuge tube and vortexed for 3 minutes. The mixture was then centrifuged for 5 minutes at ~ 4000 rpm. The organic phase was pipetted into another 15-mL glass disposable centrifuge tube that contained ~ 5.0 g Na_2SO_4. An ENVl-CARB-II/PSA (500 mg/300 mg/6 mL) cartridge was conditioned with 5 mL of acetonitrile or 25% v/v toluene in acetonitrile. A 2-mL aliquot of the organic extract was loaded onto the cartridge. The cartridge was then eluted with 20 mL using 25% v/v toluene in acetonitrile. The eluant was collected in a 15-mL disposable glass centrifuge tube and placed in a TurboVap LV and evaporated under a stream of air at 10 psi and 35°C for 15 minutes and again at 25 psi and 35°C for 30 minutes to dryness. We eluted the cartridge once more with 10 mL of 25% v/v toluene in acetonitrile. Again, the eluant was then evaporated to dryness using the TurboVap LV first at 10 psi and 35°C for 15 minutes and then at 25 psi and 35°C. Samples were reconstituted in 1 mL of acetonitrile and stored at -20°C until ready for analysis via GC-ECD.

Method A28 Mean Percent Recoveries

Table A28. Percent recoveries of OP and pyrethroid pesticides extracted via Method A28 from red wine and coffee fortified at 200 ng/g (n=3). Values in parentheses are coefficients of variation.

	Wine		Coffee	
diazinon	89.8	(12)	92.9	(8)
malathion	79.0	(3)	71.1	(13)
chlorpyrifos	74.0	(4)	70.0	(4)
permethrin	101.1	(7)	111.2	(10)
cyfluthrin	88.0	(16)	103.3	(7)
cypermethrin	94.9	(16)	110.0	(11)
deltamethrin	74.7	(22)	87.3	(8)

Method A29

Five milliliters acetonitrile, 2.5 mL each red wine or black coffee fortified at 25 and 50 ng/g, and ~ 2.0 g NaCl was placed in a 15-mL glass disposable centrifuge tube and vortexed for 3 minutes. The mixture was then centrifuged for 5 minutes at ~ 4000 rpm. The organic phase was pipetted into another 15-mL glass disposable centrifuge tube that contained ~ 5.0 g Na_2SO_4. An ENVl-CARB-II/PSA (500 mg/300 mg/6 mL) cartridge was conditioned with 5 mL of acetonitrile or 25% v/v toluene in acetonitrile. A 2-mL aliquot of the organic extract was loaded onto the cartridge. The cartridge was then eluted with 20 mL using 2% v/v ethyl acetate solution made with 25% v/v toluene in acetonitrile. The eluant was collected in a 15-mL disposable glass centrifuge tube and placed in a TurboVap LV and evaporated under a stream of air at 10 psi and 35°C for 15 minutes and again at 25 psi and 35°C for 30 minutes to dryness. We eluted the cartridge once more with 10 mL of 2% v/v ethyl acetate solution made with 25% v/v toluene in acetonitrile. Again, the eluant was then evaporated to dryness using the TurboVap LV first at 10 psi and 35°C for 15 minutes and then at 25 psi and 35°C. Samples were reconstituted in 1 mL of acetonitrile and stored at -20°C until ready for analysis via GC-ECD.

Method A29 Mean Percent Recoveries

Table A29. Percent recoveries of OP and pyrethroid pesticides extracted via Method A29 from red wine and coffee fortified at 25 and 50 ng/g (n=3). Values in parentheses are coefficients of variation.

	Wine - 25 ppb		Coffee - 25 ppb		Wine - 50 ppb		Coffee - 50 ppb	
diazinon	92.6	(6)	111.6	(7)	87.1	(26)	89.6	(10)
malathion	97.1	(10)	100.9	(16)	97.7	(10)	85.6	(7)
chlorpyrifo	56.6	(9)	51.1	(8)	87.1	(9)	65.1	(7)
permethrir	267.3	(12)	226.7	(13)	146.5	(1)	135.2	(1)
cyfluthrin	120.2	(5)	147.6	(5)	91.7	(2)	111.1	(4)
cypermeth	118.9	(6)	149.5	(5)	86.7	(14)	116.8	(5)
deltamethı	112.3	(7)	132.8	(5)	81.0	(4)	96.9	(7)

Method A30

Five milliliters acetonitrile, 1.25 mL each red wine and black coffee or 0.83 mL each red wine, black coffee, and beer for a total of 2.5 mL fortified at 200 ng/g, and ~ 2.0 g NaCl was placed in a 15-mL glass disposable centrifuge tube and vortexed for 3 minutes. The mixture was vortexed for 3 minutes, then centrifuged for 5 minutes. The ENVI-CARB-II/PSA cartridges were conditioned with 5 mL of 25% v/v toluene in acetonitrile. Na_2SO_4 was added on top of each SPE cartridge to a depth of ~2 mm. A 2 mL aliquot of the organic extract was loaded onto the cartridge, which was then eluted with 10 mL of 25% v/v toluene in acetonitrile. The eluant was collected in a 15-mL disposable glass centrifuge tube and placed in a TurboVap LV and evaporated under a stream of air at 10 psi and 35°C for 15 minutes and again at 25 psi and 35°C for 30 minutes to an approximate volume of 800 µL. We eluted the cartridge once more with 10 mL of 25% v/v toluene in acetonitrile, adding it to the reduced volume of the first eluant. The combined eluants were then evaporated to dryness using the TurboVap LV first at 10 psi and 35°C for 15 minutes and then at 25 psi and 35°C. Samples were reconstituted in 1 mL of acetonitrile and stored at -20°C.

Method A30 Mean Percent Recoveries

Table A30. Percent recoveries of OP and pyrethroid pesticides extracted via Method A30 from red wine and black coffee mixture and red wine, black coffee, and beer mixture fortified at 200 ng/g (n=3). Values in parentheses are coefficients of variation.

	Wine & Coffee		Wine, Coffee, & Beer	
diazinon	107.7	(4)	94.5	(4)
malathion	107.3	(6)	120.5	(4)
chlorpyrifos	123.3	(7)	140.3	(3)
permethrin	119.0	(7)	139.5	(2)
cyfluthrin	97.1	(11)	110.7	(2)
cypermethrin	95.2	(9)	113.9	(4)
deltamethrin	69.7	(13)	88.9	(4)

Method A31

The food matrix (2.5 g for solid food or 2.5 mL for liquid) was placed in a 15-mL disposable glass centrifuge tube to which 5 mL acetonitrile and 1 g NaCl was added. The mixture was vortexed for 3 minutes, then centrifuged for 5 minutes. The ENVI-CARB-II/PSA cartridges were conditioned with 5 mL of 25% v/v toluene in acetonitrile. Na_2SO_4 was added on top of each SPE cartridge to a depth of ~2 mm. A 2 mL aliquot of the organic extract was loaded onto the cartridge, which was then eluted with 10 mL of 25% v/v toluene in acetonitrile. The eluant was collected in a 15-mL disposable glass centrifuge tube and placed in a TurboVap LV and evaporated under a stream of air at 10 psi and 35°C for 15 minutes and again at 25 psi and 35°C for 30 minutes to an approximate volume of 800 µL. We eluted the cartridge once more with 10 mL of 25% v/v toluene in acetonitrile, adding it to the reduced volume of the first eluant. The combined eluants were then evaporated to dryness using the TurboVap LV first at 10 psi and 35°C for 15 minutes and then at 25 psi and 35°C. Samples were reconstituted in 1 mL of acetonitrile and stored at -20°C.

Method A31 Mean Percent Recoveries

Table A31. Percent recoveries of OP and pyrethroid pesticides extracted via Method A31 from 13 different foods at 200 ng/g (n=3). Values in parentheses are coefficients of variation.

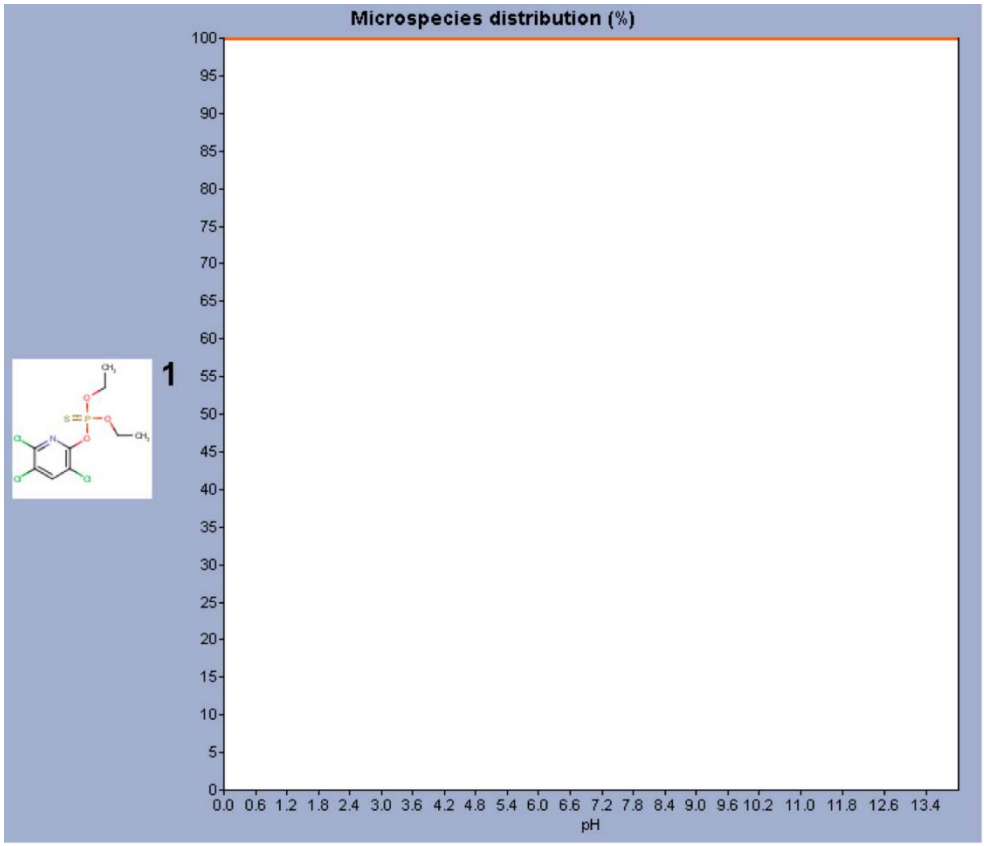
	Banana		Beef		Beer		Carrot		Chicken		Grains		Ham	
diazinon	99.4	(8)	58.8	(12)	114.0	(25)	117.9	(7)	76.6	(7)	86.5	(14)	54.5	(24)
malathion	98.9	(3)	79.3	(3)	82.2	(3)	88.7	(5)	69.2	(12)	71.6	(5)	75.7	(15)
chlorpyrifos	105.5	(3)	70.1	(5)	101.0	(2)	114.5	(4)	73.0	(4)	75.2	(3)	67.6	(19)
permethrin	122.3	(3)	131.6	(4)	126.5	(9)	131.3	(7)	109.6	(7)	95.8	(6)	123.1	(11)
cyfluthrin	83.8	(6)	86.5	(9)	83.0	(16)	109.0	(21)	80.5	(6)	80.4	(3)	92.7	(16)
cypermethrin	82.9	(6)	86.0	(6)	86.0	(14)	111.7	(14)	78.8	(4)	82.0	(2)	91.5	(15)
deltamethrin	70.7	(12)	89.3	(5)	59.1	(11)	125.9	(14)	81.0	(5)	88.0	(5)	97.8	(18)

	Oil		Orange		Soy Milk		Spinach		Sweet Potatoes		Whole Milk	
diazinon	71.6	(9)	86.7	(5)	85.1	(19)	48.7	(4)	82.7	(4)	98.9	(7)
malathion	73.1	(7)	69.6	(18)	60.4	(30)	68.6	(4)	58.1	(2)	69.6	(7)
chlorpyrifos	47.7	(5)	85.1	(7)	75.8	(13)	52.9	(20)	70.0	(3)	96.1	(13)
permethrin	66.7	(3)	111.8	(8)	130.0	(15)	91.0	(5)	108.8	(11)	115.7	(34)
cyfluthrin	60.6	(4)	84.9	(11)	115.5	(21)	93.6	(8)	79.6	(4)	87.9	(37)
cypermethrin	59.2	(3)	85.0	(13)	110.7	(23)	101.1	(2)	79.2	(2)	88.2	(40)
deltamethrin	68.1	(5)	70.5	(17)	112.6	(22)	108.5	(11)	83.8	(5)	86.8	(24)

APPENDIX 2B: PHYSICAL AND CHEMICAL PROPERTIES OF PESTICIDES

The following figures detail physiochemical details of target analytes (generated from ChemAxon© MarvinSketch version 1.4.6):

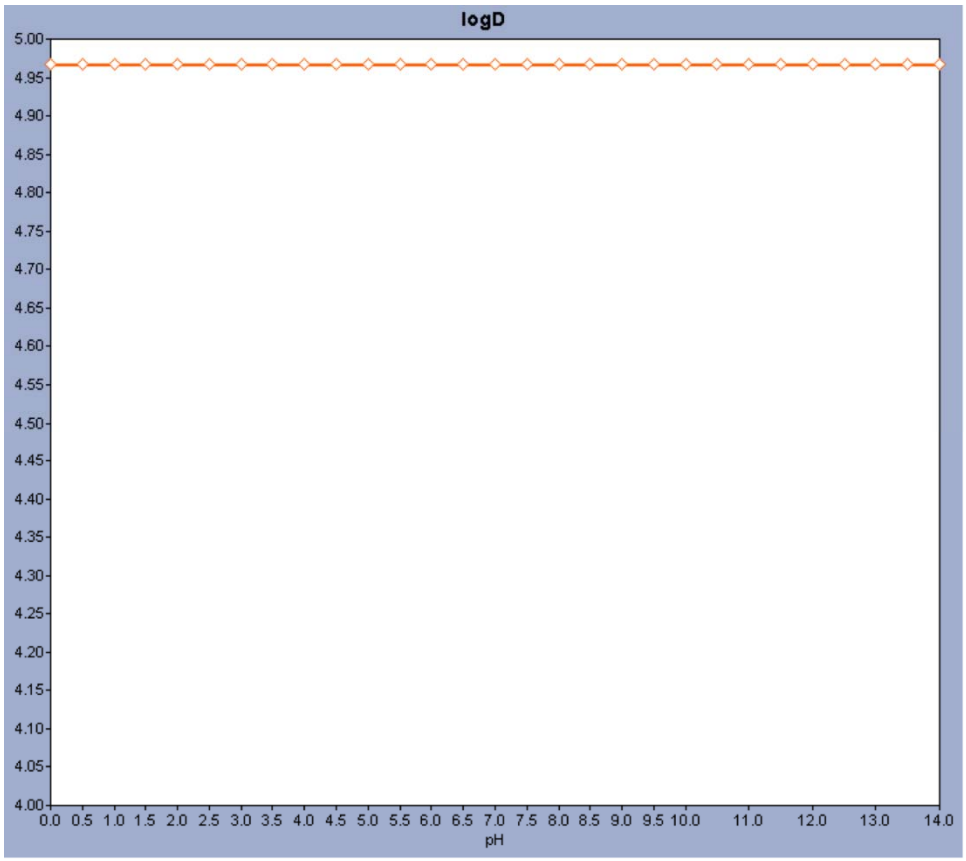
Chlorpyrifos pKa Determination – No ionizable atoms between pH 1-14



Active State

pH

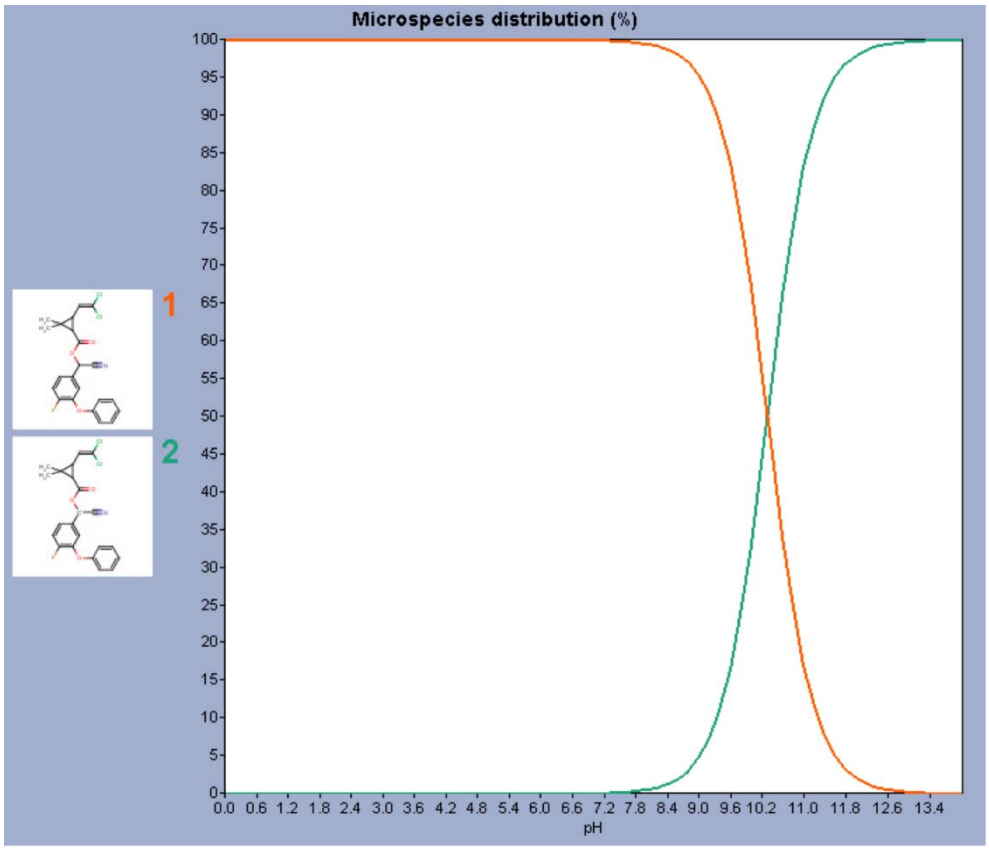
Chlorpyrifos – logD Determination



logD

pH

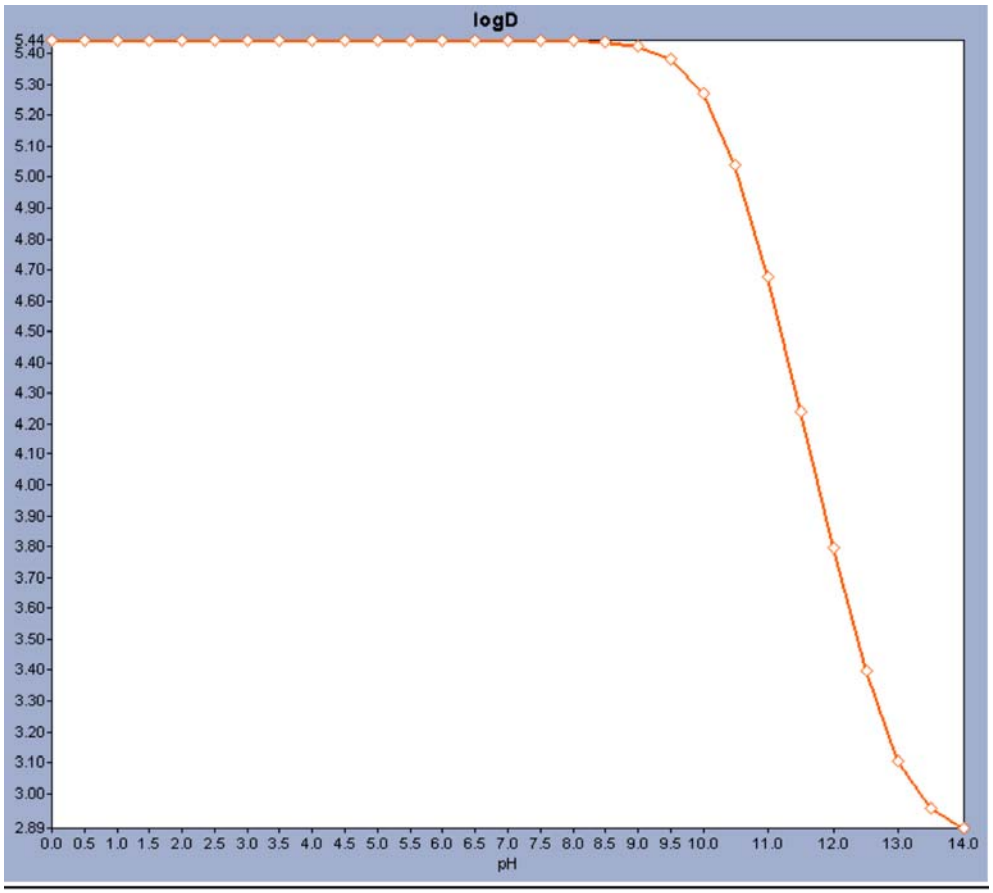
Cyfluthrin pKa Determination – One ionizable atoms between pH 1-14



Active State

pH

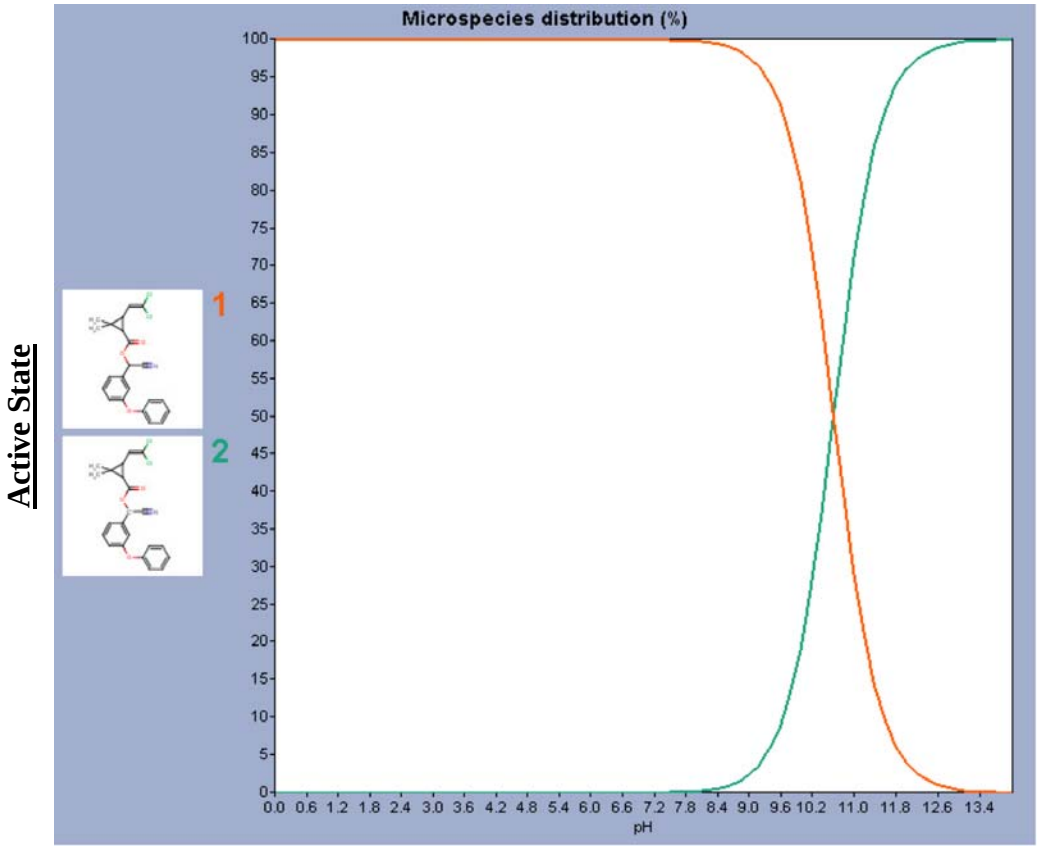
Cyfluthrin – logD determination



logD

pH

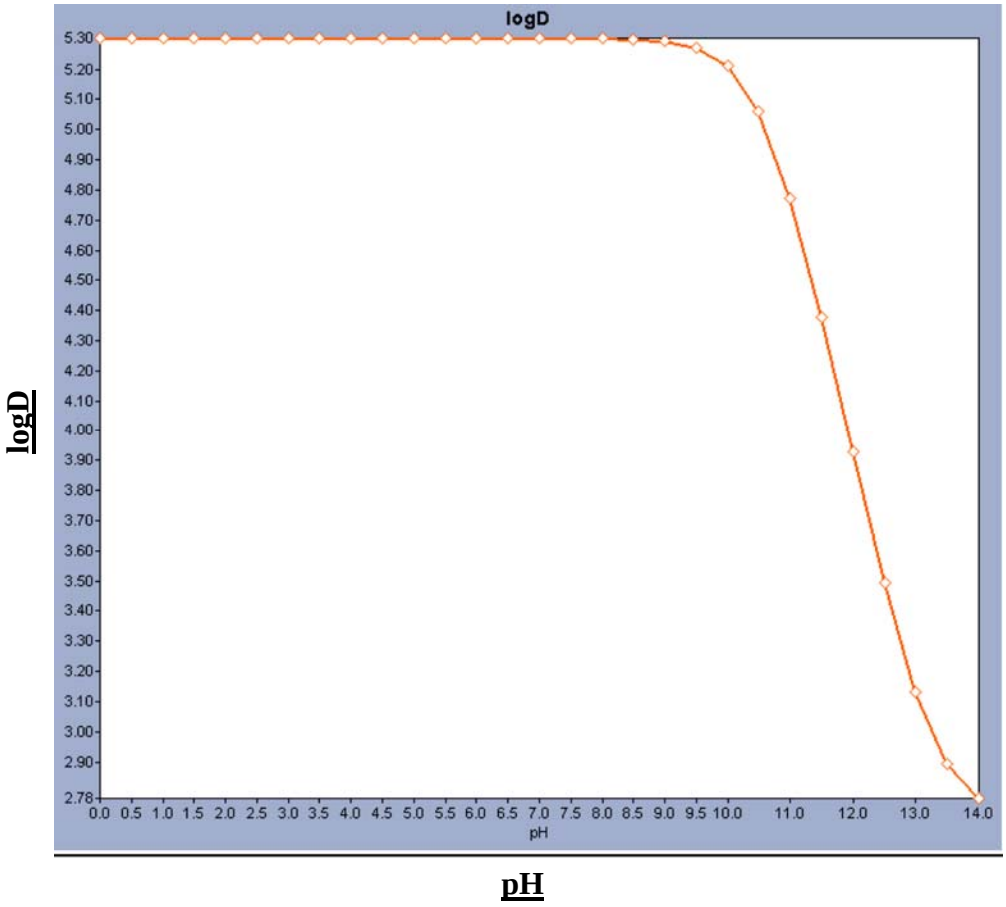
Cypermethrin pKa Determination – One ionizable atome between pH 1-14



Active State

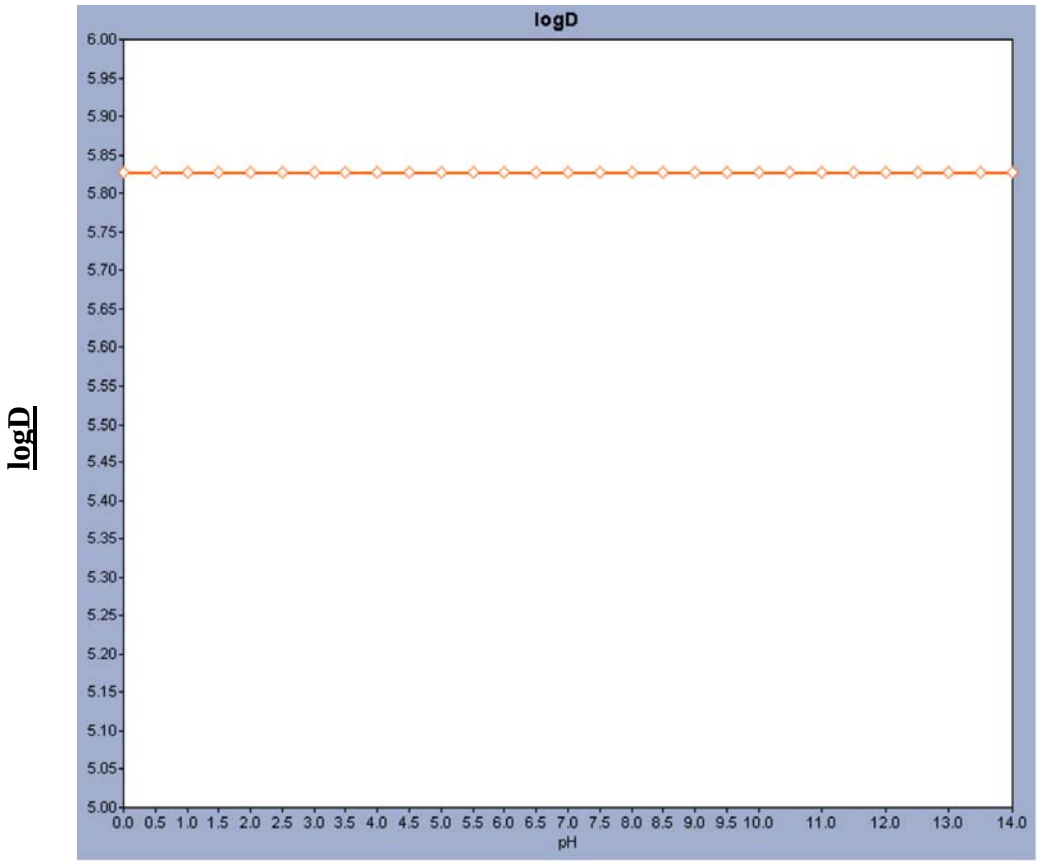
pH

Cypermethrin – logD Determination



Deltamethrin pKa Determination – No ionizable atoms between pH 1-14 (no figure generated)

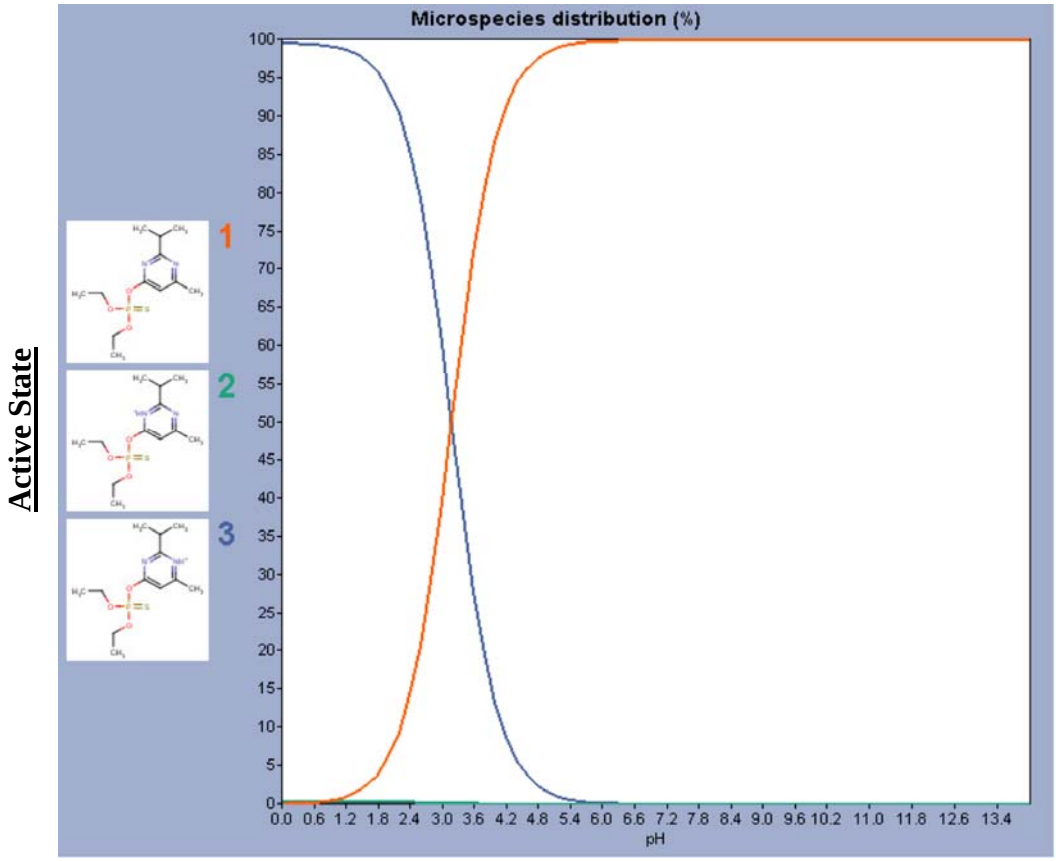
Deltamethrin – logD Determination



logD

pH

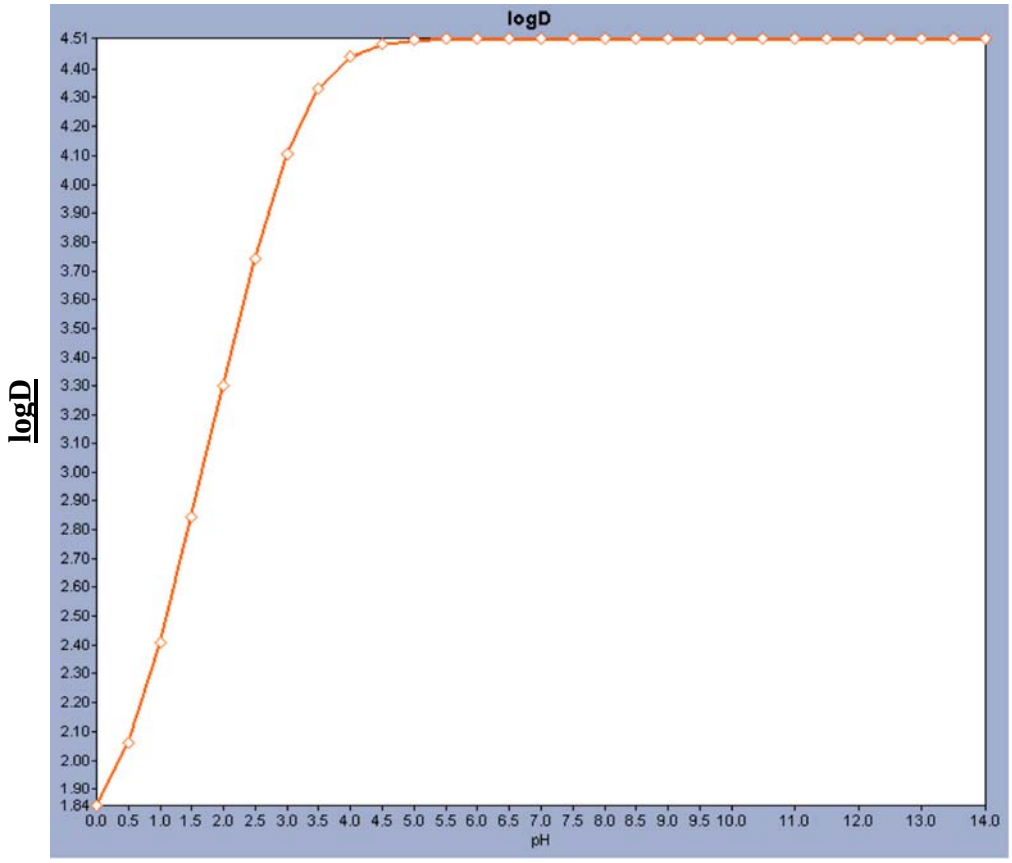
Diazinon pKa Determination – One ionizable atom between pH 1-14



Active State

pH

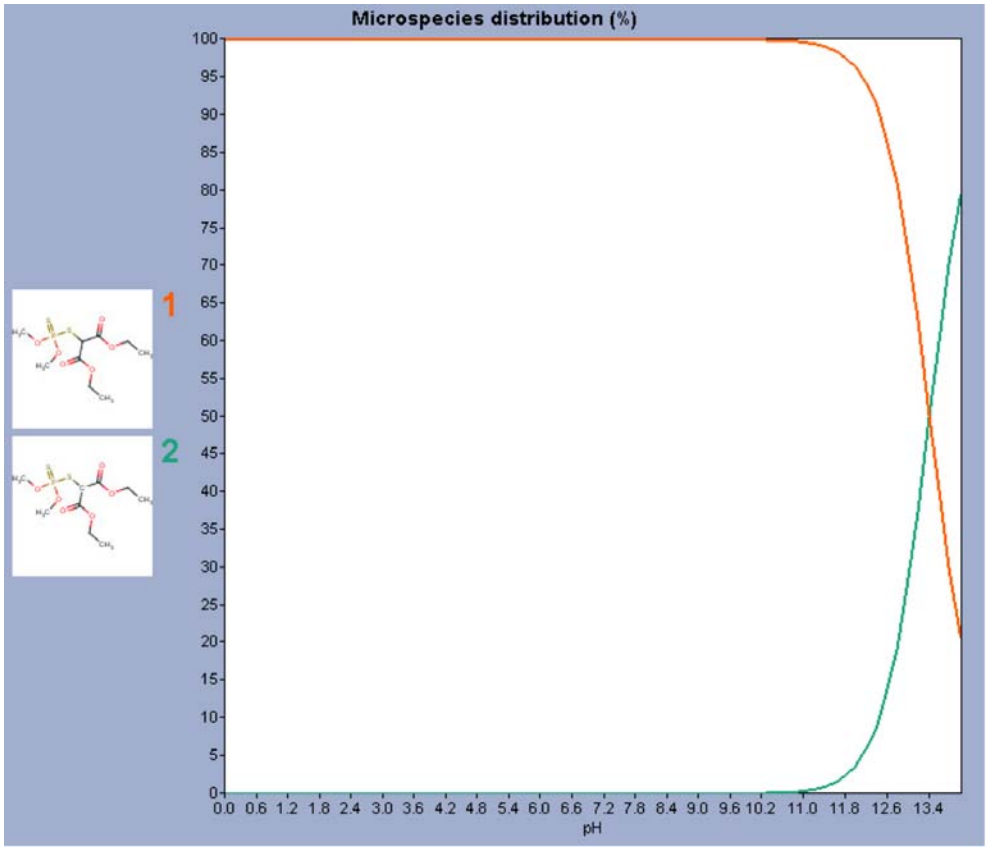
Diazinon – logD Determination



logD

pH

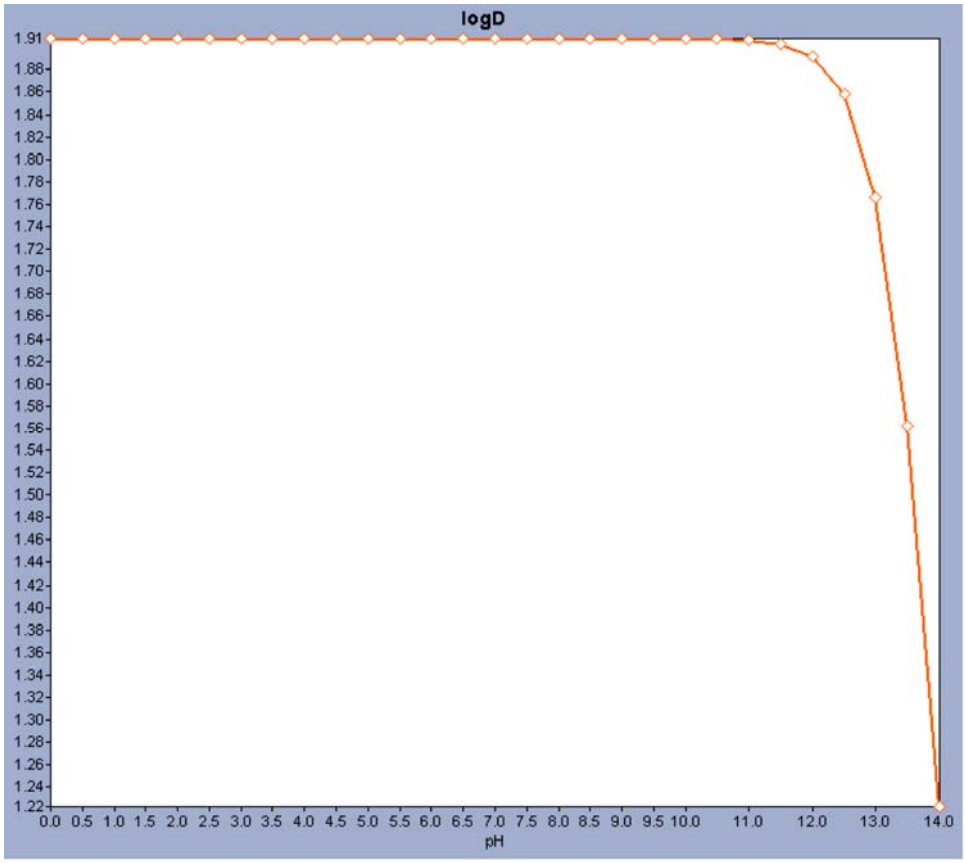
Malathion pKa Determination – One ionizable atom between pH 1-14



Active State

pH

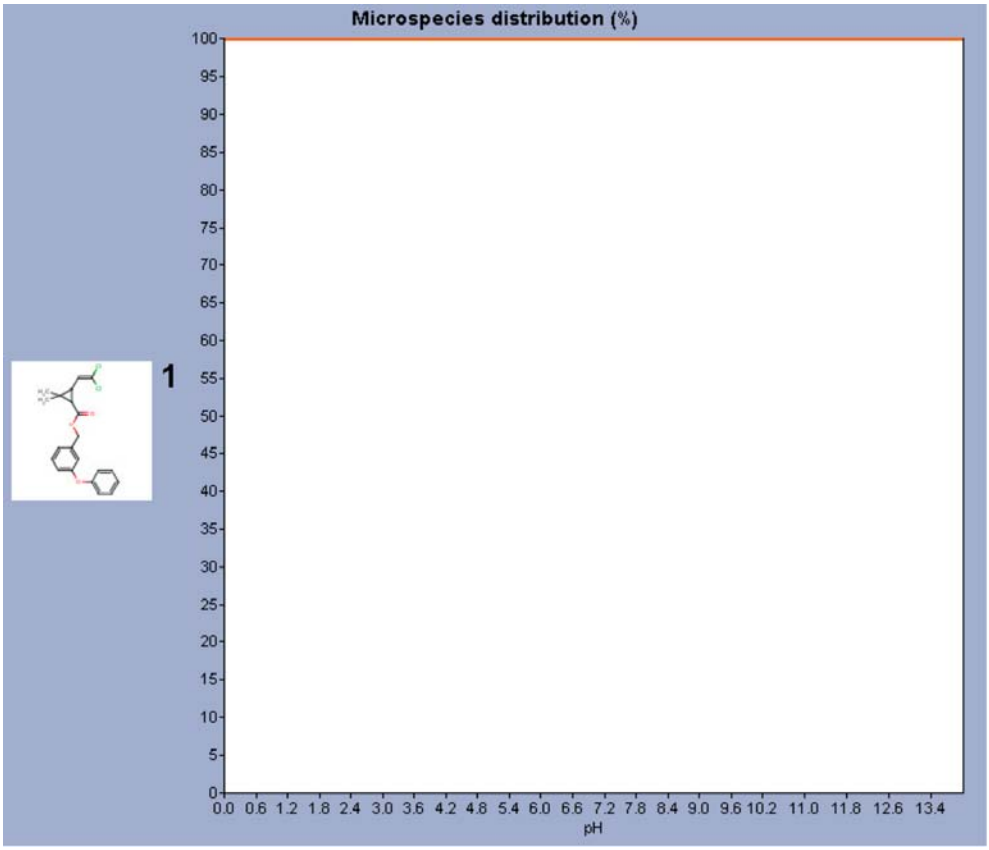
Malathion – logD Determination



logD

pH

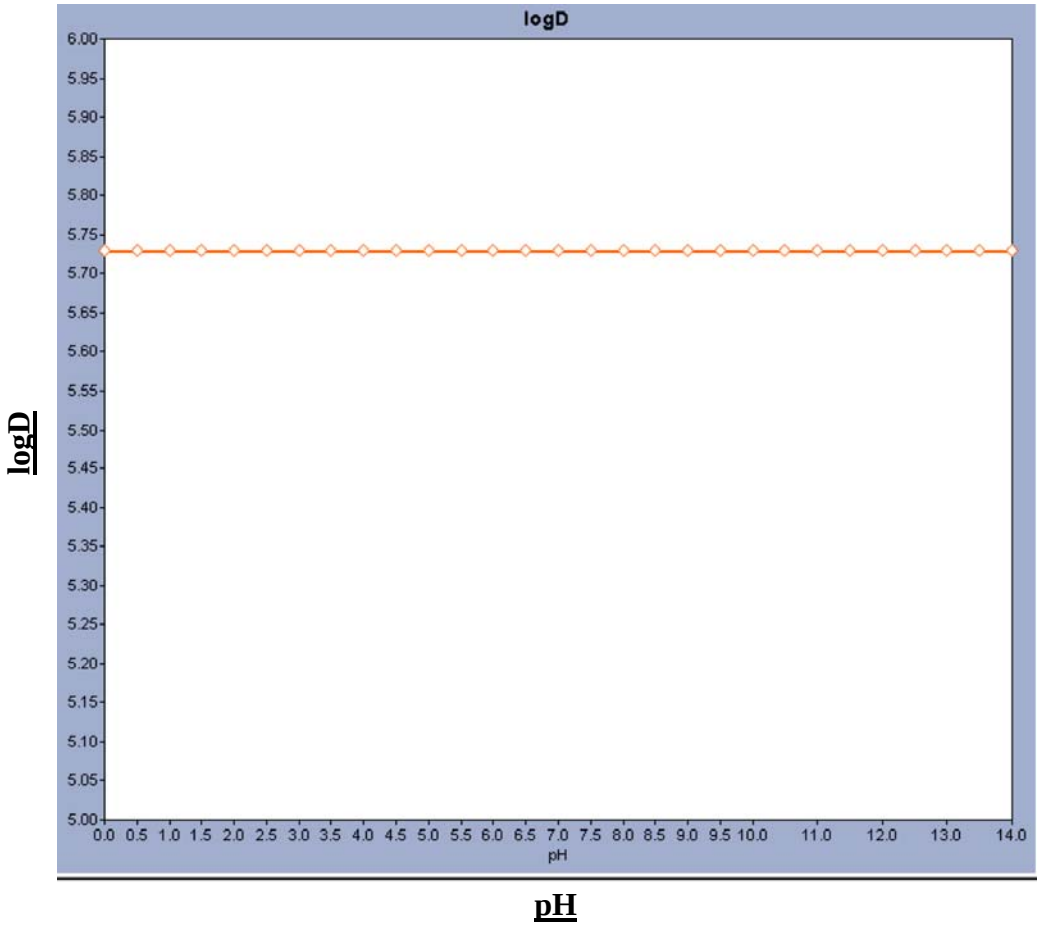
Permethrin pKa Determination – No ionizable atoms between pH 1-14



Active State

pH

Permethrin – logD Determination



CHAPTER 3: ORGANOPHOSPHORUS AND PYRETHROID PESTICIDE RESIDUES IN COMPOSITE DIET SAMPLES FROM ATLANTA, USA ADULTS

Anne M. Riederer, Ronald E. Hunter, Jr., P. Barry Ryan

Introduction

Dietary intake has been identified as an important source of non-occupational pesticide exposure among U.S. adults, particularly for OP and pyrethroid pesticides (Buck, *et al.* 2001; Dougherty, *et al.* 2000; MacIntosh, *et al.* 1996; Meeker, *et al.* 2005; Pang, *et al.* 2002; Riederer, *et al.* 2008). These are registered for a wide range of agricultural and livestock applications in the United States and other countries. Data from the 2005-2007 Pesticide Data Program (PDP) of the USDA showed detectable OP and pyrethroid residues in 35 and 44, respectively, different commodities including fruits, vegetables, nuts, dairy, grains, and meats (USDA 2006; 2007; 2008). Commodities with the highest OP or pyrethroid pesticide detection frequencies were wheat (grain), almonds, honey, spinach, celery, and cherries. The OP pesticide, malathion, for example, was detected in 65% of wheat samples while the pyrethroid pesticide, permethrin, was detected in 56% of spinach samples. Certain commodities, including blueberries, cherries, grapes, green beans, collard greens, kale, lettuce, and peaches, contained > 10 different pyrethroids/OP pesticides during these PDP years.

While the PDP reports pesticide residues in raw commodities, the U.S. FDA TDS examines levels in foods that are table-ready (*i.e.* ready to be eaten) (Pennington, *et al.* 1996). Among the most commonly detected pesticides in the 2004-2006 TDS were the OP pesticides, malathion (18% of samples), chlorpyrifos-methyl (16% of samples), and chlorpyrifos (8% of samples), while the pyrethroid pesticide, permethrin, was detected in 6% of samples (USFDA 2004; 2005; 2006). In our own previous study of OP pesticides in 379 duplicate four-day diet samples (solid foods only) collected from 75 adults in

Maryland during 1995-1996, we detected malathion and chlorpyrifos in 75% and 38% of samples, respectively (Macintosh*, et al.* 2001). A similar study of the Arizona population in the late 1990s found the OP pesticides, chlorpyrifos and diazinon, in 22% and 8%, respectively, of 24-hr duplicate solid diet samples (Moschandreas*, et al.* 2002).

The objective of the present study was to measure concentrations of OP and pyrethroid pesticides in 24-hr duplicate diet samples collected from 12 adult volunteers in Atlanta, Georgia, USA. These data are being used to aid the design of a larger study of multimedia pesticide exposures among preschoolers (*i.e.* 3-5 years old) in Atlanta. In the adult study, we collected 8 days of samples from each participant in two cycles of four consecutive days each to evaluate potential seasonal differences in pesticide residue concentrations (Cycle 1: July-October 2005; Cycle 2: January-April 2006). We focused on a subset of four pyrethroid pesticides (permethrin, cyfluthrin, cypermethrin, deltamethrin) and three OP pesticides (chlorpyrifos, diazinon, malathion) to represent those commonly used in U.S. agriculture.

Materials and Methods

Sample Collection and Processing. Samples were handled with only glass or metal equipment that was trace-cleaned prior to use. Trace-cleaning consisted of washing and rinsing with warm tap water and a 1% Alconox solution (Alconox, Inc., White Plains, NY) followed by three rinses with de-ionized water then one rinse with ultrapure Milli-Q® water (Millipore, Billerica, MA). Equipment was left to air-dry overnight in a chemical fume hood then rinsed once with HPLC-grade hexane (Sigma Aldrich, St. Louis, MO) and stored until use wrapped in hexane-rinsed aluminum foil.

We trained participants and provided written instructions on how to collect their own duplicate diet samples. We asked them to separate food items into nine composite categories: above-ground vegetables, beans/nuts/legumes/miscellaneous, below-ground vegetables, dairy, fats/oils, fruit/fruit juices, grains, meat/fish/eggs, and non-dairy beverages. We classified vegetables as above- (*e.g*. lettuce) or below-ground (*e.g.* carrots) depending on where the bulk of the edible portion grows. We defined fruit juices as those containing ≥ 10% juice; we asked participants to place fruit juices/drinks with < 10% juice in the non-dairy beverages jar. We developed these categories in particular because we expected to find similar analytes in each based on their U.S. registration status and pesticide residue data from the 2003 PDP (USDA 2005). We did not ask participants to save salt, pepper, or other spices. We asked them to put sugar, sugar substitutes, jams/jellies and candy into the beans/nuts/legumes/miscellaneous jar.

Participants composited samples in clear glass jars that we provided each day. We considered one sampling day to be the 24-hr period from midnight the night before to midnight of the sampling day. We asked participants to prepare duplicate portions of all foods and beverages (except water) that they consumed that day. We also asked those who usually prepared snacks and lunch at home before leaving for work to composite these items before leaving home. We asked others to composite items purchased and eaten away from the home when they returned home from work in the evening. We provided detailed instructions (including a compositing key that included several thousand foods) on which jars to put specific foods in along with a 24-hr telephone number to call with questions. We asked participants to keep the samples in their own refrigerators, or in coolers with ice packs that we provided, while they were at work and overnight before we picked them up the next morning. Participants were compensated for the cost of duplicate groceries.

Before compositing each portion, we asked participants to weigh it to the nearest 1 g using an Ohaus CS2000 scale (Ohaus Corporation, Pine Brook, New Jersey) that we provided. We asked them to prepare the duplicate portion identically to the portion they ate, *e.g*. remove peels, pits, and green leafy tops, and choose duplicate items of similar ripeness/maturity/size. For each item composited, we asked them to record the following information on a standardized log (food log): description, weight (g), organic label, recipe, fresh/frozen/can/jar/other, brand, pre-packaged/homemade, country/state of origin, prewashed/washed myself/unwashed, peeled/unpeeled, composite jar, time composited, time eaten, label saved, and comments/unusual circumstances. To minimize

sample contamination, we advised participants and family members to avoid touching the inside of the jars or lids, opening them when not in use, or touching/handling the contents once inside the jar. On the third sampling day, we gave participants an empty jar labeled "field blank," and asked them to leave this jar open for the same amount of time it took them to composite the samples.

We picked up the samples each morning and brought them to the laboratory in coolers containing frozen ice packs. Samples were either processed within several hours, or stored at 4ºC until they could be processed the following day. Each composite jar was weighed to the nearest 1 g and a stainless steel spatula was used to transfer the contents to a stainless steel container for the blender (Model 7010, Waring Laboratory and Science, Inc., Torrington, CT). We blended the sample at high speed for 1 min using the spatula to break up chunks and ensure the sample was homogenized. To all but non-dairy beverages samples, we added 50 mL of HPLC-grade acetonitrile (Sigma-Aldrich, Inc., St. Louis, MO) to the blender. We added 200 mL instead of 50 mL to grains samples since pilot work revealed that 50 mL did not wet the typically-sized grains sample appreciably. We blended the sample at high speed for another minute or until visibly homogenous, then transferred the homogenate into pre-weighed amber glass jars and weighed them. With the lids partially on, we left the jars in a chemical fume hood overnight to evaporate the solvent. We weighed them again, screwed the lids on, and stored them at -20°C until extraction.

Field blanks were processed by swirling 50 mL of acetonitrile inside the field blank jar ensuring all sides were coated evenly. The acetonitrile was decanted into an amber glass jar and stored with the other samples. A random set of 10-30% of homogenized samples from each composite group was split into laboratory duplicates to assess analytical precision. Duplicate aliquots were removed after the final blending step and stored in amber glass jars with the other samples. Precision was evaluated using the mean absolute difference as well as the mean relative % difference between duplicates x1 and x2, where relative % difference = (absolute difference x1, x2)/(mean x1, x2) x 100.

Sample Extraction and Analysis. We developed a multi-residue method using liquid-liquid extraction and a secondary SPE cleanup step to quantify our target analytes in GC-ECD. Briefly, 1 g of homogenized sample was placed in a 15-mL disposable glass centrifuge tube and combined with 5 mL acetonitrile and ~1 g NaCl (J.T. Baker®, Phillipsburg, NJ). The mixture was vortexed for 3 min using a Vortex-Genie® 2 (Scientific Industries, Inc., Bohemia, NY) then centrifuged for 5 min using a MediSpin centrifuge (Krackeler Scientific, Inc., Albany, NY). ENVI-CARB-II/PSA cartridges (Sigma-Aldrich, Inc., St. Louis, MO) were conditioned with 5 mL of 25% v/v HPLC-grade toluene (Sigma-Aldrich, Inc., St. Louis, MO) in acetonitrile. Na_2SO_4 (ACS grade, Sigma-Aldrich, Inc., St. Louis, MO) was added on top of each cartridge to a depth of ~2 mm. A 2 mL aliquot of the organic extract was loaded onto the cartridge. For each field blank, the entire 50 mL was loaded onto the cartridge in 5-mL increments. Each cartridge was eluted with 10 mL of 25% v/v toluene in acetonitrile. The eluant was collected in a 5-mL disposable glass centrifuge tube then placed in a TurboVap LV

(Zymark, Hopkinton, MA) and evaporated under a stream of air at 10 PSI and 35°C for 15 minutes and again at 25 PSI and 35°C for 30 minutes to an approximate volume of 500 µL. We eluted the cartridge once more with 10 mL of 25% v/v toluene in acetonitrile adding it to the reduced volume of the first eluant. The combined eluants were evaporated to dryness using the TurboVap LV at 10 PSI and 35°C for 15 minutes then at 25 PSI and 35°C. Laboratory blank samples were prepared by conducting the cleanup steps on unloaded cartridges. Samples were reconstituted in 1 mL of acetonitrile and stored at -20°C until ready for analysis.

Samples were injected by Agilent 7683B Automatic Liquid Sampler (Agilent Technologies, Inc., Santa Clara, CA) into a HP 5890A Series II GC (Hewlett Packard, Co., Palo Alto, CA). The capillary column used was a high-resolution, low-bleed 30-m DB-5 (5% phenyl, 95% dimethylpolysiloxane) column with 0.25 µm film thickness and 0.25 mm internal diameter (Agilent Technologies, Inc., Santa Clara, CA). The GC program began at 80°C for 2 minutes, ramping at 10°C/min to 280°C then held for 13 minutes for a 35-minute total run time. The helium carrier gas was at a constant flow of 2 mL/min and nitrogen makeup gas flow was 60 mL/min. Detection was made by an Agilent Technologies (Santa Clara, CA) ECD. The injection was 1.0 µL (splitless). Other relevant analytical parameters included: 2 mm i.d. single taper injection liner, injection port temperature 240°C and detector temperature 280°C. Peaks were identified by comparing their retention times to the retention times of the standards. Solvent standards were prepared at various concentrations from 1-1000 ng/g and used to create an 11-point calibration curve for quantification. LODs were calculated for each analyte

using a power regression model derived from solvent standards. We determined the target analyte concentration based on the calibration curve and the area counts provided by the GC-ECD. Analytes were quantified using Agilent ChemStation® rev. A. 10.02 software (Agilent Technologies, Inc., Santa Clara, CA). To account for mass lost during the overnight drying step, the extracted food weight was multiplied by the ratio of the original sample mass to the overnight-dried sample mass. The final concentration was computed by dividing the analyte mass by this adjusted weight. To confirm peak identities, we analyzed 21 samples (three randomly selected from each composite type) using GC-MS in the SIM mode.

Statistical Analysis. Food logs were entered verbatim into MS Access (Microsoft Corporation, Redmond, WA) then exported to Statistical Analysis System (SAS) 9.2 (SAS Institute, Cary, NC) while analytical results were entered into MS Excel then exported to SAS. The food log and analytical databases were combined for statistical analysis. All database entries were checked once against hard copy records by one analyst and a second time by another to detect and fix coding errors. Descriptive statistics were calculated using SAS.

Total diet concentrations were calculated by summing the analyte mass (ng) for all composite types then dividing by the sum of the mass (g) of all composite types collected from a participant on a particular cycle and day. Total daily intakes (mg/kg-d) of each pesticide were calculated for each participant by summing the mass of the selected pesticide in all composites collected that day and dividing by the participant's body

weight. One-half the LOD was substituted for concentration values below the LOD. We used the Wilcoxon Rank Sum test to test whether or not mean total daily intakes by pesticide differed by sampling cycle. For each pesticide, we compared intakes to the oral reference dose (RfD), the U.S EPA's health based guideline for pesticide ingestion. We did not find current RfDs for deltamethrin or diazinon. For diazinon, we used the Maximum Residue Levels (MRLs) from the Agency for Toxic Substances and Disease Registry (ATSDR) (CDC 2008). MRLs were not available for deltamethrin. Following the U.S. EPA recommended guidelines for cumulative risk assessment of the OP pesticides (USEPA 2006a), we converted chlorpyrifos, diazinon and malathion concentrations to methamidophos-equivalents, then calculated the methamidophos-equivalent intakes and compared them to the methamidophos RfD.

Results and Discussion

Data Completeness and Analytical Quality Control. Of the 12 volunteers, one participated in Cycle 1 only while another participated in Cycle 2 only, resulting in 11 participants per sampling cycle. We collected a total of 42 and 43 food logs in Cycles 1 and 2, respectively. We collected 220 composite samples in Cycle 1 and 217 in Cycle 2.

We did not detect the target pesticides in any of the field blank or non-dairy beverages samples. GC-MS confirmatory analysis excluded the probability of false positive results since the target peaks appeared in both ECD and MS chromatograms. We were not able to analyze fats/oils because our method generally gave < 60% recovery of fortified fats/oils samples. Excluding non-dairy beverages samples, we analyzed 86 duplicate pairs, comprising 602 pesticide-composite combinations. Of these combinations, both samples of the pair were < LOD for 80% of cases, while one sample was < LOD and the other ≥ LOD in 13% of cases. Of the latter, 15 pairs appeared problematic, *i.e*. one duplicate was < LOD and the other > 100 ng/g for one or more analytes. For the remaining pairs, with both samples ≥ LOD, the mean relative % difference by composite type generally fell in the range 0-50%. An exception was diazinon in three duplicate grains pairs. Low precision in some of the duplicates may be explained partly by incomplete sample homogenization. These were comprised of foods, such as beans, mixed vegetables, cereal bars and similar items. Our blender process may not have sufficiently homogenized the skins and starchy components of the beans, for example, as we occasionally observed bean skins in the homogenate as well as whole beans trapped under the blender blades.

Table 3.1. Frequency of detection of target pesticides in food composite samples by sampling cycle

Sampling cycle/ composite type	# samples analyzed[a]	% of samples >LOD[b]						
		permethrin	cyper-methrin	cyfluthrin	delta-methrin	chlor-pyrifos	diazinon	malathion
Cycle 1 (July - October 2005)								
Above ground vegetables	35 (35)	14	11	6	0	46	43	9
Beans/nuts/legumes/misc.	37 (37)	11	8	14	8	16	5	14
Below ground vegetables	13 (15)	8	31	0	0	46	38	8
Dairy	31 (31)	6	19	0	3	42	13	0
Fruits and fruit juices	33 (34)	12	18	9	9	36	6	0
Grains	42 (42)	7	29	10	5	17	14	5
Meat, fish, eggs	29 (29)	17	14	10	3	41	3	0
Non-dairy beverages	31 (31)	0	0	0	0	0	0	0
Total diet[c]	41 (43)	46	63	37	20	80	66	24
Cycle 2 (January - April 2006)								
Above ground vegetables	32 (32)	13	13	28	19	0	9	3
Beans/nuts/legumes/misc.	33 (33)	3	45	18	0	18	27	9
Below ground vegetables	13 (13)	8	46	69	15	8	8	0
Dairy	31 (31)	16	23	35	6	3	13	3
Fruits and fruit juices	33 (33)	9	21	30	0	0	6	0
Grains	44 (44)	5	9	16	20	43	52	0
Meat, fish, eggs	31 (31)	26	19	16	3	13	10	0
Non-dairy beverages	37 (37)	0	0	0	0	0	0	0
Total diet[c]	43 (44)	40	70	81	35	56	65	12

[a] Number of samples collected in parentheses. [b]LOD = limit of detection: permethrin 0.38 ng/g; cypermethrin 0.79 ng/g; cyfluthrin 0.72 ng/g; deltamethrin 0.84 n/g; chlorpyrifos 0.88 ng/g, diazinon 0.66 ng/g; malathion 0.76 ng/g. [c] Total diet = sum of analyte mass in eight composite groups divided by sum of weight (g) of eight composite groups, with 0 substituted for values < LOD.

Pesticide Detection Frequencies. Table 3.1 summarizes analyte detection frequencies by food composite type and sampling cycle. In Cycle 1, chlorpyrifos and diazinon were the most frequently detected analytes, with chlorpyrifos found in over 30% of above- and below-ground vegetables, dairy, fruit and fruit juices, and meat/fish/eggs samples, and diazinon found in over 30% of above- and below-ground vegetables samples. Cypermethrin was the most commonly detected pyrethroid, found in over 30% of below-ground vegetables samples. Malathion was the least commonly detected analyte in both cycles. In Cycle 2, chlorpyrifos and diazinon were detected in over 30% of grains samples, cypermethrin in over 30% of beans/nuts/legumes/miscellaneous and below-ground vegetables samples, and cyfluthrin in over 30% of below-ground vegetables and dairy samples. With respect to total diet, chlorpyrifos and cyfluthrin were the most frequently detected analytes in Cycles 1 and 2, respectively. These results provide

evidence of frequent dietary exposure of the participants to one or more of the target pesticides.

Pesticide Concentrations by Composite Type. Figure 3.1 presents box plots of target analyte concentrations (ng/g) by composite type and sampling cycle for samples > LOD. Median permethrin concentrations > LOD ranged from 21 ng/g (n=3, Cycle 2) in fruit/fruit juices to 460 ng/g, the highest detected permethrin concentration, in one Cycle 2 beans/nuts/legumes/miscellaneous sample comprised of a veggie burrito, peanut butter and raspberry jelly. Permethrin is currently registered in the United States for use on corn, livestock, and variety of fruits and vegetables (*e.g*. apples, cherries, peaches, broccoli, lettuce, onions, potatoes, spinach, tomatoes); there are no current tolerances for beans, peanut butter or raspberries (USEPA 2006b).

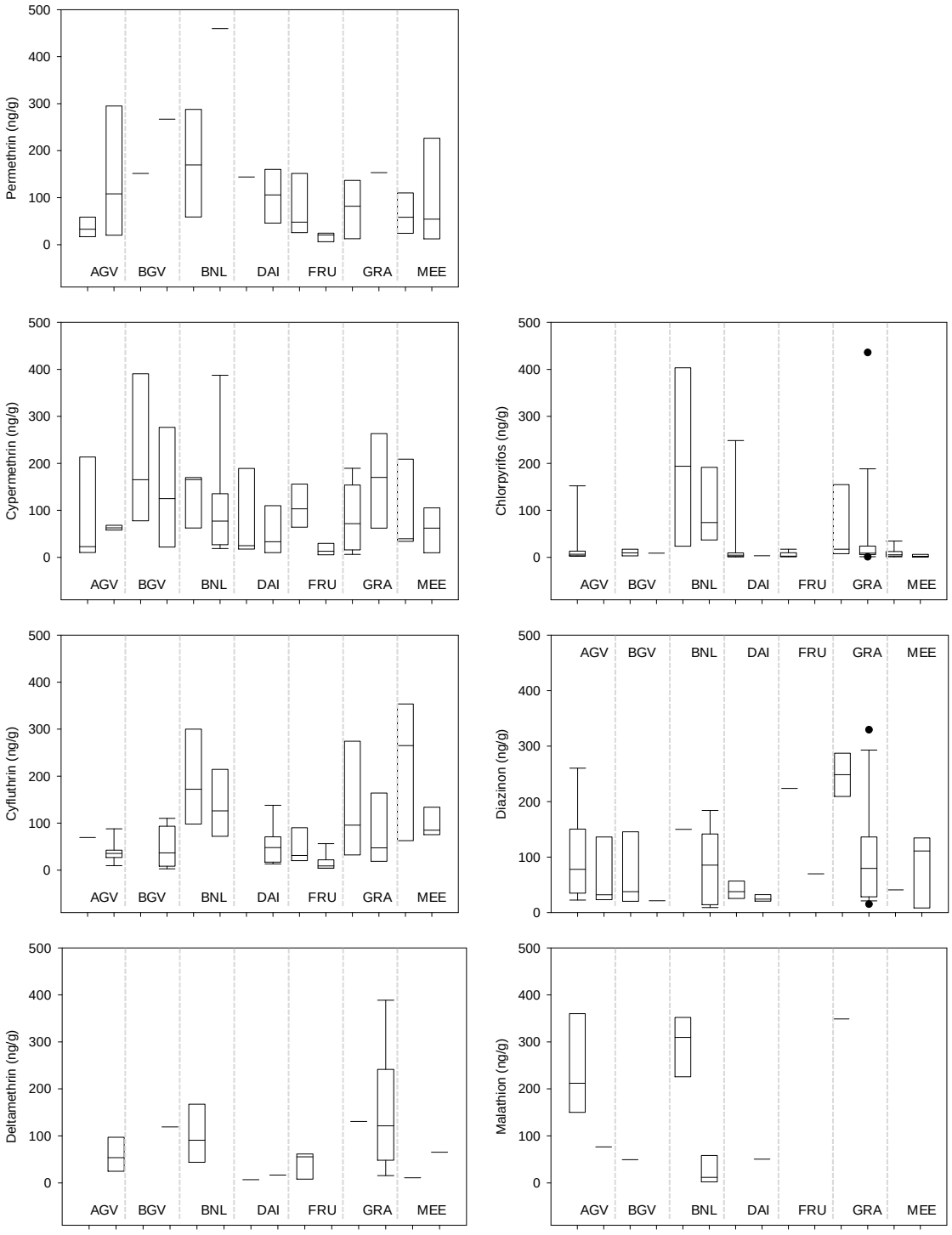


Figure 3.1 Box plots of target analyte concentrations (ng/g, detects only) by composite type (AGV = above-ground vegetables, BGV = below-ground vegetables, BNL = beans/nuts/legumes/miscellaneous, DAI = dairy, FRU = fruit/fruit juice, GRA = grains, MEE = meat/fish/eggs); left-hand plot within dashed grey lines = Cycle 1 data; right-hand plot within dashed grey lines = Cycle 2 data; lower box boundary = 25th percentile, line within box = median, upper box boundary = 75th percentile, whiskers = 10th and 90th percentiles, • = observation outside 10th or 90th percentile.

Median cypermethrin concentrations > LOD ranged from 13 ng/g in fruit/fruit juices (n=7, Cycle 2) to 170 ng/g in grains (n=4, Cycle 2). We measured the highest cypermethrin level (468 ng/g) in a Cycle 2 beans/nuts/legumes/miscellaneous sample comprised of peanut butter bars, chutney and orzo pasta. Registered uses for cypermethrin are similar to those for permethrin and cyfluthrin (described below) (CYPERMETHRIN 2007a).

Median cyfluthrin concentrations > LOD ranged from 9.1 ng/g in fruit/fruit juices (n=10, Cycle 2) to 264.9 ng/g in meat/fish/eggs (n=3, Cycle 1). The highest level (397.0 ng/g) was found in a Cycle 1 beans/nuts/legumes/miscellaneous sample consisting of humus. Current U.S. registrations for cyfluthrin include a wide range of items, including carrots, corn, lettuce, and tomatoes as well as citrus, pomes and stone fruits, cereal grains, and dried/shelled peas and beans, among others (CYFLUTHRIN 2008b). The current tolerance for dried/shelled peas and beans is 150 ng/g (CYFLUTHRIN 2008b).

Median deltamethrin concentrations > LOD ranged from 6.9 ng/g in a single Cycle 1 dairy sample to 130.7 ng/g in grains (n=2, Cycle 2). The highest level (388.7 ng/g) was found in a Cycle 2 grains sample consisting of bread, bagel, chocolate muffin and pineapple cake. Deltamethrin is registered for similar uses as the other pyrethroids, although for a more restricted set of fruit and vegetable crops (DELTAMETHRIN 2007b). The current tolerances for cereal grains and wheat bran are 1,000 ng/g and 5,000 ng/g, respectively (DELTAMETHRIN 2007b).

Median chlorpyrifos concentrations > LOD ranged from 2.4 ng/g for meat/fish/eggs (n=4, Cycle 2) to 193.7 ng/g for beans/nuts/legumes/miscellaneous (n= 6, Cycle 1). The highest level (435.8 ng/g) was found in the Cycle 2 grains sample described above with the highest deltamethrin concentration. Chlorpyrifos is registered for use on a wide range of commodities; the current tolerance for wheat grain is 500 ng/g (CHLORPYRIFOS 2008a). Chlorpyrifos was detected in < 1% of wheat samples analyzed in the 2005 and 2006 PDP, at concentrations ranging from 10-42 ng/g (USDA 2007; 2008).

Median diazinon concentrations in samples > LOD ranged from 21.2 ng/g in a Cycle 1 sample of below-ground vegetables to 248.5 ng/g in grains samples (n=6, Cycle 1). The highest level (380.8 ng/g) was found in a grains sample of whole-wheat sourdough bread. Diazinon is registered for use on a range of crops but not wheat (DIAZINON 2008c). Diazinon was detected in only 1 of 1,361 of wheat samples analyzed in the 2005 and 2006 PDP, at 5 ng/g (USDA 2007; 2008).

Median malathion concentrations > LOD ranged from 11.6 ng/g in beans/nuts/legumes/miscellaneous (n=3, Cycle 2) to 348.8 ng/g in grains (n=2, Cycle 1). The highest level (377.9 ng/g) was found in a Cycle 1 beans/nuts/legumes/miscellaneous sample consisting of beans, a sandwich, energy bars, and peanut butter. Malathion is currently registered for use on a wide range of U.S. crops; the current tolerance for beans, soybeans and peanuts is 8 ng/g (MALATHION 2008d).

Detection Differences Between Sampling Cycles. The number of samples analyzed in each composite group was similar in Cycles 1 and 2. Table 3.1 illustrates differences in detection frequencies by cycle. Detection frequencies increased from Cycle 1 to Cycle 2 for cypermethrin in beans/nuts/legumes/miscellaneous, diazinon in grains, and cyfluthrin in below-ground vegetables, dairy and fruit/fruit juices, while chlorpyrifos detections decreased in above- and below-ground vegetables, dairy, fruit/fruit juices, and meat/fish/eggs. Figure 3.1 shows no clear pattern of difference in median concentrations by cycle.

The differences between cycles are not likely due to field or laboratory contamination, since no analytes were detected in the blanks from either cycle. Further, after we extracted samples from each cycle, we analyzed them together, thus our detection limits did not change. Likewise, the differences are not likely due to changes in regulatory status, since most of the registered uses of our target pesticides did not change within the study timeframe. The differences between cycles are likely due to changes in the types of foods in the composite samples, seasonal changes in residue levels or other unknown factors. Regardless, these findings underscore the need for seasonal sampling of pesticides in duplicate diet samples.

Pesticides in foods labeled "organic." Of the 1000 individual foods (excluding fats/oils) recorded in the food logs, 18% had an "organic" label. We detected one or more target analytes (except malathion) in half of the 47 composite samples comprised only of organic foods. For example, we measured 457 ng/g of cypermethrin in a sample

comprised of raw, domestic (California), pre-cut, "organic" carrots; this is five times the current cypermethrin tolerance for carrots (100 ng/g) (CYPERMETHRIN 2007a). For comparison, the 2006 PDP reported no cypermethrin detects in 744 carrot samples at analytical detection limits of 30-60 ng/g (USDA 2008). The participant who submitted this sample reported never using pesticides in her rented apartment since moving there in 2004; thus, it is unlikely that the cypermethrin measured represents household contamination.

Four other all-"organic" samples contained pesticide concentrations > 100 ng/g. An "organic" 14-grain bread sample contained 330 ng/g of diazinon; this participant reported using no pesticides in her apartment since moving there in 2004. A sample of carrots, yellow onions, potato and sweet potato, all "organic," cooked with olive oil, pepper and rosemary contained 180 ng/g of cypermethrin and 267 ng/g of permethrin; this participant reported using no pesticides in her apartment the month before both samples were collected. A sample of "organic" sprouted grain tortilla from the same participant contained 133 ng/g of diazinon. A sample of "organic" homemade chicken soup (chicken, carrots, potatoes) collected from another participant contained 109 ng/g cyfluthrin; this participant reported using no pesticides in her apartment the month before this sample was collected, although she reported using several during the previous sampling cycle, including a cyfluthrin crack-and-crevice treatment. Notably, we detected 40 ng/g of deltamethrin and 38 ng/g of cyfluthrin in an "organic" lettuce sample of U.S. origin. Deltamethrin is not currently registered for use on U.S. lettuce

(DELTAMETHRIN 2007b) and this participant reported using no pesticides in her rented townhome the month before sampling.

Table 3.2. Total daily intakes (mg/kg body weight-d) versus oral reference dose (RfD) by pesticide (< LOD samples set to 1/2 LOD)

		Daily intake (mg/kg-d) (n = 84)			
Pesticide	Oral RfD[a]	Mean	Median	Max	% > RfD
Permethrin	5.0E-02	1.1E-04	4.0E-06	1.5E-03	0
Cypermethrin	1.0E-02	1.7E-04	5.8E-05	1.4E-03	0
Cyfluthrin	2.5E-02	1.6E-04	6.0E-05	1.7E-03	0
Deltamethrin	n/a	1.1E-04	6.2E-06	2.7E-03	-
Chlorpyrifos	3.0E-03	2.1E-04	1.7E-05	3.3E-03	1
Diazinon	7.0E-04[b]	2.8E-04	7.5E-05	3.0E-03	10
	2.0E-03[c]				1
Malathion	2.0E-02	1.5E-04	5.0E-06	3.8E-03	0
Chlorpyrifos/diazinon/ malathion - methamidophos equivalents[d]	5.0E-05	1.5E-05	3.2E-06	2.1E-04	6

[a] Source of oral reference doses, unless otherwise noted: www.epa.gov/iris [accessed 2 Feb 2009]. [b] Agency for Toxic Substances and Disease Registry Maximum Residue Level (MRL) for chronic duration (= 365 days) oral exposure (Available: www.atsdr.cdc.gov/toxprofiles/tp86.html, accessed 2 Feb 2009]. [c] MRL for intermediate duration (15-364 days) oral exposure. [d] Chlorpyrifos, diazinon and malathion concentrations each converted to methamidophos equivalents following U.S. EPA's Organophosphorus Cumulative Risk Assessment guidelines (www.epa.gov/pesticides/cumulative/2006-op/op_cra_main.pdf) and summed; RfD shown is for methamidophos.

Total daily pesticide intakes. Table 3.2 presents descriptive statistics of participants' total daily intakes by pesticide and compares them to the oral RfDs. There was no significant difference in mean daily intakes by sampling cycle for most analytes except cyfluthrin ($p < 0.05$); thus, Table 3.2 presents data for both cycles combined. Intakes of permethrin, cypermethrin, cyfluthrin and malathion were all below the RfDs. One intake exceeded the oral RfD for chlorpyrifos. On that day, the participant's chlorpyrifos intake came exclusively from a beans/nuts/legumes/miscellaneous sample comprised of cereal,

soy milk, cookies, peanut butter and a granola bar. Another participant's intake exceeded the intermediate duration MRL for diazinon; this intake came from a beans/nuts/legumes/miscellaneous sample containing 214 ng/g of diazinon and comprised of an energy bar, and a fruit/fruit juices sample containing 362 ng/g of diazinon and comprised of grapes and a smoothie.

Six percent of methamidophos-equivalent OP intakes exceeded the methamidophos RfD. This illustrates how combining OP residues using the U.S. EPA's cumulative approach can produce an exceedance of the health guidelines in certain cases where individual OP pesticide intakes do not. Although most of our participants' intakes were below current reference values, we only measured seven pesticides. The presence of additional OP pesticides in the samples may lead to intakes exceeding the methamidophos RfD. Further, we only measured dietary intakes. Other ingestion exposure pathways, such as inadvertent ingestion of contaminated house dust or soil, may also contribute to daily oral intakes. Non-dietary ingestion of OP pesticides is not likely to be future concern since many have been phased out of residential use in the United States (USEPA 2006b). However, this may be an important pyrethroid exposure pathway for people who regularly use pyrethroids in their homes or yards (Lu*, et al.* 2009).

Percent Contribution of Composite Type to Daily Pesticide Intake. Figure 3.2 presents box plots of the percent contribution of each composite type to total daily intake (mg/d) of the target pesticides. On average, below-ground vegetables did not contribute

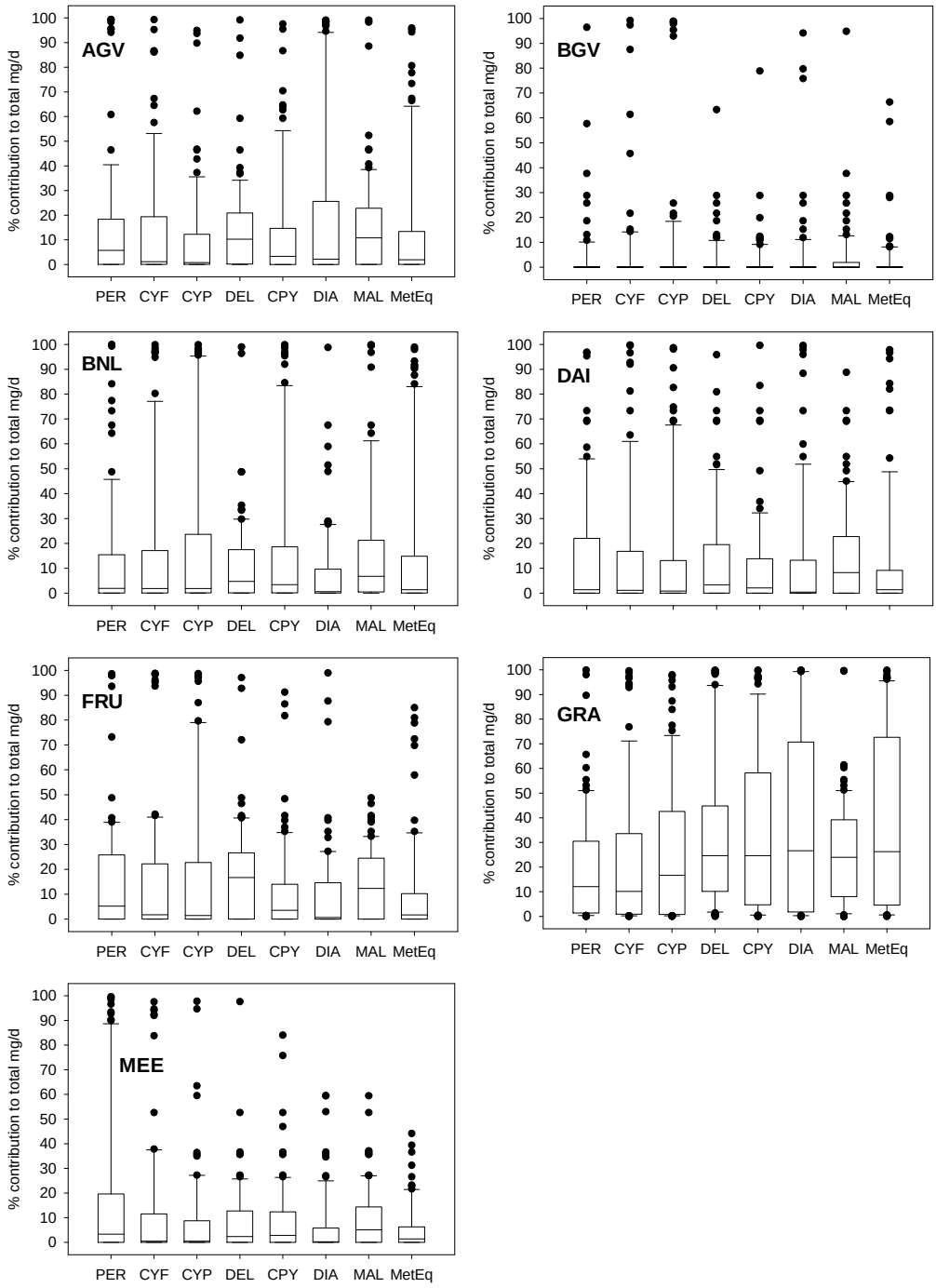


Figure 3.2 Box plots of the distributions of the percent contribution of each composite type to total daily intake (n = 84) of the target pesticides. AGV = above-ground vegetables, BGV = below-ground vegetables, BNL = beans/nuts/legumes/miscellaneous, DAI = dairy, FRU = fruit/fruit juice, GRA = grains, MEE = meat/fish/eggs; PER = permethrin, CYF = cyfluthrin, CYP = cypermethrin, DEL = deltamethrin, CPY = chlorpyrifos, DIA = diazinon, MAL = malathion, MetEq = methamidophos equivalent chlorpyrifos + diazinon + malathion. Lower box boundary = 25th percentile, line within box = median, upper box boundary = 75th percentile, whiskers = 10th and 90th percentiles, • = observation outside 10th or 90th percentile.

appreciably to the daily intakes of our study participants, while grains contributed the most (10-30%). Median percent contributions for the remaining composite types were generally 0-10%. The plots illustrate the episodic nature of dietary pesticide exposures; on some days, a particular composite type could account for 0% or 100% of the total daily intake. The plots also illustrate the fact that a variety of food types, not just fruits and vegetables, can contribute to daily intakes.

Study limitations. In any study involving compositing foods there is a possibility that the wrong items were put into certain composite jars. We evaluated this potential source of error by examining the food logs. In the below-ground vegetables, dairy, fruit/fruit juices, grains, and meat/fish/eggs samples, < 10% of the individual foods in those jars were placed there by mistake. Eleven percent of foods in the above-ground vegetable jars were mistakenly put there while 20% of foods in the beans/nuts/legumes/miscellaneous jars were mistakenly put there. Thus, we believe that our classification scheme accurately describes the types of foods analyzed in most categories except perhaps beans/nuts/legumes/miscellaneous. The most commonly misclassified foods in this category were cookies, corn twists, granola bars, cereal, and raspberry jelly.

Similarly, although we instructed participants to separate foods the best they could (*e.g.* place dry cereal in the grains jar and milk in the dairy jar), this was difficult for complex recipe foods, such as pizza, burritos and others. Our compositing key instructed participants to categorize these foods by main ingredient. For example, it told them to

put pizza (including sauce, cheese and toppings) into the grains jar, since the greatest portion of the mass of a typical slice of pizza is likely to be bread. As a result, certain composite samples contained traces of foods belonging in different categories. This does not apply to composite samples containing a single type of food (23% of above-ground vegetables, 65% of below-ground vegetables, 41% of beans/nuts/legumes/miscellaneous, 54% of dairy, 36% of fruit/fruit juices, 15% of grains, and 67% of meat/fish/eggs samples). Complex recipe foods accounted for < 5% of the total foods in the above-ground vegetables, below-ground vegetable, fruit/fruit juices, and grains categories, and < 10% of foods in the beans/nuts/legumes/miscellaneous, dairy, and meat/fish/eggs categories. Consequently, for the majority of samples analyzed, we believe the composite group is an accurate descriptor of the types of foods comprising them.

A third limitation of our study is the fact that our analytical method did not meet our quality control standards for the fats/oils samples. As a result, we were not able to capture 100% of the total daily pesticide intake for participants who ate fats/oils. Five participants supplied us with a total of 17 fats/oils samples in both sampling cycles. The most common items were salad dressing, mayonnaise, margarine, and olive oil. On the days we collected fats/oils samples, their mean percent contribution to total daily grams eaten by participant was 2.1% (± 2.5%). Because they comprised such a small portion of our participants' diets, fats/oils were not likely to contribute significantly to their pesticide intakes.

Fourth, we report pesticide concentrations in food samples, but cannot say whether or not the pesticides were in/on the foods as purchased or added as a contaminant during food handling in participants' homes. This type of information would be useful for mitigating exposures. We did collect household pesticide use data from certain participants, and a number of samples with detectable residues came from participants who said they did not use pesticides. A more quantitative investigation of the actual source of pesticides in/on foods might involve sampling kitchen surfaces and/or foods before they are brought into participants' homes.

Implications for Dietary Pesticide Exposure Assessment. Although we collected and analyzed over 400 composite samples, our study only involved 12 adults. It is difficult to generalize findings from such a small sample to a larger population. Nonetheless, our findings have several important implications for dietary pesticide exposure and risk assessment. First, we found evidence of frequent dietary exposure to the target pesticides among our participants. Total daily intakes generally did not exceed health-based guidelines except in a small number of cases when cumulative OP intakes were considered. Second, we found certain pesticides at or above their tolerance limits in individual foods or mixtures of foods, thus tolerance limits might not constitute a valid upper bound for risk assessment. Third, we found pesticides in approximately half the samples we analyzed that contained only items labeled "organic," thus it may not be reasonable to assume that "organic" items contain no pesticides.

We also showed that dietary exposures can come from a range of food types, including grains, and beans/nuts/legumes, and not just the fruits and vegetables that might traditionally be considered the major contributors to dietary pesticide intakes. Lastly, our results illustrate the episodic nature of dietary pesticide exposures. The food types contributing most to intakes on one day might differ from those driving intakes on another. The exceptions were grains, which consistently accounted for the majority of chlorpyrifos and diazinon intakes among our study participants.

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CHAPTER 4: METHOD DEVELOPMENT FOR MULTI-RESIDUE PESTICIDE EXTRACTION FROM RESIDENTIAL DUST

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Introduction

Although many researchers have focused on studies of outdoor environmental exposure to hazardous chemicals, researchers have recently begun to investigate chemical exposures attributed to indoor environments, such as homes and offices, where people spend the majority of their time (Regueiro, *et al.* 2007). In fact, Americans spend up to 90% of their time indoors (Brown, *et al.* 1994; Gurunathan, *et al.* 1998; Molhave, *et al.* 1997). Non-dietary exposure to pesticides occurs primarily in the home (Lewis, *et al.* 1994; Simcox, *et al.* 1995). Approximately three-quarters of American households use pesticides to control or eliminate indoor and outdoor pests (Kiely, *et al.* 2002). Indoor environments may be concentrating pesticide levels in mediums, such as air and house dust, due to inadequate ventilation (Garcia-Jares, *et al.* 2009). Protection from sunlight and severe environmental conditions, such as extreme heat or humidity, may amplify this by slowing indoor degradation processes in air or house dust (Garcia-Jares, *et al.* 2009; Hong, *et al.* 2001; Kiely, *et al.* 2002; Rudel, *et al.* 2003). Most studies developed to assess children's exposure to pesticides show that the greatest quantity of pesticides and highest concentrations are found in household dust in comparison to air, soil, and food (Lewis, *et al.* 1994; Whitmore, *et al.* 1993). Ingestion, inhalation, and dermal absorption of household dust is a significant exposure route to pesticides for small children primarily because they spend much of their time on the floor and easily come into contact with dust via hand-to-mouth or object-to-mouth contact (Lewis, *et al.* 1994; Maertens, *et al.* 2004; Simcox, *et al.* 1995). Consequently, analyzing pesticide levels in house dust is an important step in evaluating children's pesticide exposure.

OP and pyrethroid pesticides are two classes of pesticides that have been used extensively in residential settings in the United States (Kiely, *et al.* 2004; Landrigan, *et al.* 1999). OP and pyrethroid pesticides are semi-volatile and non-volatile, respectively. In addition, they have been shown to preferentially bind to the particle phase (*e.g.* particulate matter or house dust). In 2001 and 2002, the U.S. EPA banned the residential use of chlorpyrifos and diazinon, OP pesticides, due to the potential harmful effects to inhabitants, especially children (He 1994; USEPA 2000a; b). Since OP pesticides have been withdrawn from residential use, the use of pyrethroid pesticides for residential use has increased (Adgate, *et al.* 2000; Barro, *et al.* 2006). Synthetic derivatives of chrysanthemic and pyrethric acid, pyrethroid pesticides are produced based upon the structure of natural pyrethrins – esters of chrysanthemic and pyrethric acid bonded to one of three alcohols via an ester linkage (Regueiro, *et al.* 2007; Starr, *et al.* 2008). Pyrethroid pesticides are also used in other household products, such as pet flea and tick treatments and treatment for head lice (He 1994; Regueiro, *et al.* 2007).

House dust is a complex matrix – characterized by a high organic carbon content from the presence of skin tissues, hair fibers, and mites – from which it is difficult to extract and evaluate pesticides and other organic contaminants (Maertens, *et al.* 2004; Regueiro, *et al.* 2006). Dust particle equilibrium concentrations are higher in the gas phase of indoor air; as a result, dust acts as a reservoir for semi-volatile organic compounds, such as OP and pyrethroid pesticides (Butte and Heinzow 2002). There is no gold standard or consistent methodology available for the study or comparison among residential dust pesticide exposure studies (Julien, *et al.* 2008). Approximately 40 methods exist for the

analysis of organic contaminants in indoor dust samples (Garcia-Jares, *et al.* 2009). Only a quarter of available methods has been used to analyze pesticides, including OP and pyrethroid pesticides, and fewer methods have been developed to analyze these pesticides in house dust samples (Garcia-Jares, *et al.* 2009; Regueiro, *et al.* 2007). The majority of methods that analyze pesticides in indoor dust collect samples via conventional vacuum cleaners and paper dust bags, conventional vacuum cleaners modified with Soxhlet filter tubes or cellulose extraction thimbles, or High Volume Small Surface Samplers (HVS3) equipped with Teflon catch bottles (Berger-Preiss, *et al.* 2002; Berger-Preiss, *et al.* 1997; Leng, *et al.* 2005; Lu, *et al.* 2004; Rudel, *et al.* 2003). Researchers typically sieve the collected samples to remove larger pieces and achieve more homogeneity within the sample. Then dust samples are weighed and then extracted with solvent via pressurized liquid extraction (PLE), microwave-assisted solvent extraction (MASE), ultrasound-assisted extraction, MSPD, or Soxhlet extraction and determined via GC (Garcia, *et al.* 2007; Regueiro, *et al.* 2006; Saito, *et al.* 2003; Sjodin, *et al.* 2001). In most cases, researchers remove analytical interferences via multi-step procedures, which introduce several inherent problems, such as risk of analyte and time loss and sample contamination (Regueiro, *et al.* 2007). Soxhlet extraction with determination via GC is the most commonly used method (Garcia-Jares, *et al.* 2009).

Excluding Soxhlet extraction, previous methods for OP and pyrethroid pesticide extraction from house dust (*e.g.* PLE) are less solvent-intensive (Garcia-Jares, *et al.* 2009; Ingerowski, *et al.* 2001; Shoeib, *et al.* 2005; Wilford, *et al.* 2005). Soxhlet extraction is lengthy taking 15-18 h and uses large volumes of organic solvent (≥ 250 mL) (Garcia-

Jares, *et al.* 2009). Using GC equipped with MS, ECD, or FID to determine pesticides of interest, researchers obtained limits of detection LOD in these studies that ranged from 50-1000 ng/g (Berger-Preiss, *et al.* 2002; Berger-Preiss, *et al.* 1997; Leng, *et al.* 2005; Lu, *et al.* 2004; Rudel, *et al.* 2003). Of the few studies that describe methods for analyzing OP or pyrethroid pesticides in house dust, two utilize HVS3s with Teflon catch bottles to collect dust samples (Lu, *et al.* 2004; Simcox, *et al.* 1995). In both studies, researchers extracted OP pesticides from 150 μm dust samples with 50 mL acetone in one minute via ultrasound-assistance followed by determination with GC-MS (Lu, *et al.* 2004; Simcox, *et al.* 1995). The original method by Simcox, *et al*. (Simcox, *et al.* 1995) is void of the additional cleanup steps, and researchers report a percent recovery range of 72-106%, relative standard deviation (RSD) ≤ 20, and a limit of quantitation between 11-40 ng/g. Lu, *et al*. (Lu, *et al.* 2004) were able to obtain a LOD range of 180-560 ng/g. Although Lu, *et al*. added additional cleanup steps to the established method by Simcox, *et al*. to remove additional organics and particulates that produce GC-MS interferences, they did not examine fortified samples or report values for percent recoveries or RSDs of associated samples (Lu, *et al.* 2004; Simcox, *et al.* 1995). Another study by Starr, *et al*. used a similar method to obtain pyrethroid but not OP pesticides (Starr, *et al.* 2008). Starr and coworkers (Starr, *et al.* 2008) presented a pesticide recovery range of 23 ± 1% to 101 ± 2% and the LOD ranged from 1-60 ng/g.

Although Lu, *et al*., and Simcox, *et al*. present accurate techniques to analyze house dust (Lu, *et al.* 2004; Simcox, *et al.* 1995), our aim was to develop a method to analyze both OP and pyrethroid pesticides at the same time. Both studies used 2.5 g dust and 50 mL of

acetone as the extraction solvent per sample. In addition to the 50 mL of acetone used, another 278-282 mL of cyclohexane was used to clean up the sample (Lu, *et al.* 2004; Simcox, *et al.* 1995). Simcox and coworkers used sand for their fortified samples in the absence of standard "clean" dust samples, stated that "there was doubt that sand was a representative matrix," and did not adjust their field sample results based on field spike recovery data (Simcox, *et al.* 1995). Finally, none of the methods – Lu, *et al*., Simcox, *et al*., or Starr, *et al*. – have been shown to be multi-residue methods for use with both OP and pyrethroid pesticides (Lu, *et al.* 2004; Simcox, *et al.* 1995; Starr, *et al.* 2008). Here, we present a method for the determination of three OP and four pyrethroid pesticides in house dust samples. We show the application of several types of QuEChERS-like extractions followed by a SPE cleanup to house dust samples (Anastassiades, *et al.* 2003; Fillion, *et al.* 2000; Supelco 2005). Quantitation is carried out by GC-ECD and accuracy, linearity, and repeatability are evaluated.

Materials and Methods

Reagents and Materials. Hexane (HPLC grade), methanol (HPLC grade), acetonitrile (HPLC grade), toluene (HPLC grade), and Na_2SO_4 (ACS grade) were purchased from Sigma-Aldrich, Inc. (St. Louis, MO). NaCl (ACS grade) was purchased from J. T. Baker (Phillipsburg, NJ). Supelclean™ ENVI-CARB-II/PSA SPE cartridges (Bed A: 500 mg ENVI-CARB-II; Bed B: 300 mg PSA) were purchased from Sigma-Aldrich, Inc. (Bellefonte, PA). Helium (zero grade) and nitrogen (zero grade) gas were 99.999% ultra high purity, obtained from Specialty Gases Southeast, Inc. (Suwanee, GA). The water used was obtained from an ultrapure 18.2 MΩ·cm Milli-Q® water (Millipore, Billerica, MA) system.

The HVS3 was purchased from CS3, Inc. (Sandpoint, ID). The TurboVap LV was obtained from Zymark (Hopkinton, MA). The 15-mL glass centrifuge tubes and snap caps were purchased from VWR (Suwanee, GA). Adjustable single-channel pipetters were obtained from Eppendorf North America (Westbury, NY; Calibrated Nov 2007). The Vortex-Genie® 2 was purchased from Scientific Industries, Inc. (Bohemia, NY). The centrifuge used was obtained from International Equipment Co. (Needham Heights, MA). Sieves used were W.S. Tyler U.S.A. Standard Testing Sieves (stainless steel), 2-mm (U.S. No. 10) for initial screening and 250-µm (U.S. No. 60) with American Society for Testing and Materials specification E11 (Salisbury, NC). A Model 2510 Bransonic® Tabletop Ultrasonic Cleaner (Danbury, CT) was used to sonicate samples.

Standards. Pesticide reference standards were obtained from the National Center for Environmental Health, Centers for Disease Control and Prevention (CDC, Atlanta, GA) or ChemService, Inc. (West Chester, PA). Stock solutions and working standard solutions were prepared in acetonitrile. Mixed fortification standards, each containing three OP (diazinon, malathion, and chlorpyrifos) and four pyrethroid (permethrin, cyfluthrin, cypermethrin, and deltamethrin) pesticides at 10 μg/mL were prepared in acetonitrile from stock standard solutions.

Trace-cleaning Procedure. Samples were handled with trace-cleaned glass, metal or Teflon equipment. Trace-cleaning consisted of washing with warm tap water and a 1% Alconox solution (Alconox, Inc., White Plains, NY) followed by thorough rinsing with tap water, then three times with de-ionized water and a final time with ultrapure Milli-Q® water. Equipment was left to air-dry or dried in an oven at 150°C, then rinsed once with HPLC-grade hexane (Sigma-Aldrich, St. Louis, MO).

Dust Collection and Sieving. Dust samples were collected by trace-cleaned HVS3 from carpets in the living room of one home and in bedrooms of two homes selected for convenience because of their proximity to Emory University (Pang*, et al.* 2002). The HVS3 is designed to capture particles > 5 μm in diameter using an aluminum cyclone separator and Teflon sampling train (Pang*, et al.* 2002). A 1 m x 1 m template of floor or carpet was marked by tape. Sampling began by using the HVS3 and moving the nozzle forward and back along the left edge of the area marked by the template, covering an area 7.5 cm (3") wide (HVS3 nozzle width) and 1 m long. This area was vacuumed eight

times (four back-and-forth passes) moving the sampler in a straight line at about 0.5 m/s so that it took ca. two seconds to travel 1 m. After four back-and-forth passes on the first strip, the technician gradually angled over to the next 7.5 cm wide strip and repeated four back-and-forth passes. The technician repeated this process until he/she had sampled the 1-m^2 area of the template. Sampling was completed when either the entire rug had been vacuumed or 10 g were collected, whichever came first. If 6 mm (1/4 inch in height from bottom of Teflon collection cup) of material was obtained, no further vacuuming was needed. If less than 6 mm was collected, another 1-m^2 was marked off and vacuumed. When enough dust was collected, the Teflon catch bottle was capped, secured with lab tape, wrapped in foil to protect it from light, placed in a Ziploc bag and cooler with cold packs, and transported to the laboratory.

At the laboratory, dust samples were sieved to ≤ 250 μm the same day they were collected. If this was not possible, they were temporarily stored in a trace-cleaned, tightly sealed amber glass jar at -20°C for up to three days before processing. We manually sieved dust samples to 2 mm for coarse particle removal and also to 250 μm, the relevant size for ingestion exposures (Calabrese*, et al.* 1996). All samples were analyzed for background pesticide concentrations as well as in fortified form. No detectable background levels were noted.

Extraction Procedures. Briefly, ~ 0.10 g of ≤ 250 μm dust was placed in a 15-mL disposable glass centrifuge tube and 5 mL acetonitrile or methanol was added. We

performed a series of different extraction processes to find the most sensitive extraction procedure for the dust samples (Table 4.1 details different extraction processes tested).

Table 4.1. Study Extraction Processes Used.

Extraction	Spike Level	Solvent	Vortexed	Sonicated	Centrifuged
A	200 ng g-1	MeCN	3 min	n/a	5 min
B	500 ng g-1	MeCN	3 min	n/a	5 min
C	500 ng g-1	MeCN	3 min	30 min	5 min
D*	500 ng g-1	MeOH	3 min	n/a	5 min

*solvent exchange 3 mL MeOH to MeCN for SPE cleanup

SPE Cleanup. ENVI-CARB-II/PSA cartridges were conditioned with 5 mL of 25% v/v toluene in acetonitrile. Na_2SO_4 was added on top of each SPE cartridge to a depth of ~ 2 mm. A 2 mL aliquot of the organic extract was loaded onto the cartridge, which was then eluted with 10 mL of 25% v/v toluene in acetonitrile. The eluant was collected in a 15-mL disposable glass centrifuge tube and placed in a TurboVap LV and evaporated under a stream of air at 10 psi and 35°C for 15 minutes and again at 25 psi and 35°C for 30 minutes to an approximate volume of 800 μL. We eluted the cartridge once more with 10 mL of 25% v/v toluene in acetonitrile, adding it to the reduced volume of the first eluant. The combined eluants were then evaporated to dryness using the TurboVap LV first at 10 psi and 35°C for 15 minutes and then at 25 psi and 35°C. Samples were reconstituted in 1 mL of acetonitrile and stored at -20°C.

During processing Extract A, the second elution with 10 mL was performed 24 hours after the initial elution of 10 mL, which was allowed to evaporate overnight in the 15-mL disposable centrifuge tube in a fume hood at ambient temperature. Extracts B-D were cleaned up without this 24-hour time gap.

Instrumental Analysis. A Hewlett-Packard Model 5890A Series II GC equipped with an Agilent Technologies (Santa Clara, CA) model electron-capture detector (ECD) and a 7683B Series Injector autosampler (Agilent Technologies, Inc., Santa Clara, CA) was used. The DB-5 (Agilent Technologies, Inc., Santa Clara, CA) GC column used was 30 m, 0.25 mm i.d., 0.25 μm film thickness [5% phenyl, 95% dimethylpolysiloxane] (Agilent Technologies, Inc., Santa Clara, CA). The temperature programming began at 80°C, held 2 minutes, 80-280°C at 10°C/min to 280°C then held for 13 minutes. The helium carrier gas was at a constant flow of 2 mL/min and nitrogen makeup gas flow was 60 mL/min. The injection was 1.0 μL (splitless). Other relevant analytical parameters included: 2 mm i.d. single taper injection liner, injection port temperature 240°C, detector temperature 280°C.

Fortification Studies. Twelve ~ 0.10 g dust samples were weighed into 15-mL disposable glass centrifuge tubes (n = 3 for each Extraction A-D). Three were fortified with 10 μg/mL fortification standard solution to achieve a final concentration of 200 ng/g (Extraction A). Nine were fortified with 10 μg/mL fortification standard solution to achieve a final concentration of 500 ng/g (Extractions B-D). The 12 samples were extracted and 300 μL of the cleaned extracts transferred to GC sample vials. We prepared blank (unfortified) dust samples by adding 0.10 g samples to 15-mL disposable glass centrifuge tubes and performing Extraction B followed by SPE cleanup.

Identification, Quantification, and Confirmation of Pesticides in Dust Samples. Solvent standards were prepared from stock standards at various concentrations (1, 5, 10,

25, 50, 100, 150, 200, 250, 500, and 1000 ng/g) and used to create an 11-point calibration curve for quantification. Method detection limits were calculated for each analyte using a power regression model. We defined the lowest concentration used in the calibration curve, 1 ng/g, to be the LOD for each pesticide. Detection limits were verified by injection of the samples prepared at 1 ng/g to ensure that discernible peaks had a signal-to-noise ratio > 3. Sample extracts and standards were injected on the GC-ECD. Peaks were identified by comparing their retention times to the retention times of standards.

Results

All samples were analyzed for background pesticide concentrations as well as in fortified form. No detectable background levels were noted.

Table 4.2. Mean percent recoveries of OP and pyrethroid pesticides extracted from dust samples fortified at 500 ng/g.[a] Values in parentheses are coefficients of variation.

	Extractions			
	A	B	C	D
	200 ng/g	500 ng/g	500 ng/g	500 ng/g
diazinon	99.4 (2)	99.8 (7)	84.7 (26)	69.5 (18)
malathion	105.2 (8)	93.9 (6)	89.6 (12)	71.6 (5)
chlorpyrifos	95.6 (12)	125.7 (18)	108.7 (33)	89.4 (8)
permethrin	116.6 (6)	117.8 (8)	102.0 (18)	158.6 (36)
cyfluthrin	100.7 (7)	100.2 (18)	118.5 (12)	126.6 (13)
cypermethrin	134.9 (17)	141.0 (7)	116.8 (28)	118.3 (2)
deltamethrin	149.1 (17)	171.1 (9)	162.4 (16)	165.0 (4)

[a]n=3; three samples were fortified at each of the fortification levels

Recoveries. Table 4.2 summarizes mean percent recoveries by analytes, extraction types and fortification levels. Mean percent recoveries ranged from 69.5%-171.1% across all dust samples and replicates, with two-thirds of mean percent recoveries between 80-120%. The values for the coefficients of variance ranged from 2 to 36% with the majority of coefficients of variance below 15%. Extractions A and B gave mean percent recoveries between 95.6-125.7% for OP pesticides while mean percent recoveries ranged from 100.2-171.1% for pyrethroid pesticides. In the cases of Extractions A and B, cypermethrin and deltamethrin had mean percent recoveries greater than 120%. In Extractions A and B, all coefficients of variance were ≤ 18%. Extraction C gave mean percent recoveries between 80-120% for all pesticides except deltamethrin (162.4%). Coefficients of variance ranged from 12-33% in Extraction C data. Extraction D

presented lower mean percent recoveries (69.5-89.4%) for OP pesticides compared to Extractions A-C (84.7-125.7%). Also, Extraction D gave an elevated permethrin mean percent recovery of 158.6%, versus the 102.0-117.8% observed with Extractions A-C With Extractions A-D, deltamethrin showed a consistently higher mean percent recovery ranging from 149.1-171.1%.

Chromatography. Most of the GC chromatograms showed little interference from the sample matrix for extracts B-D. GC chromatograms of Extract A showed more interference from the sample matrix than those of Extracts B-D. Figure 4.1 shows the chromatograms of the seven pesticides in Extract B. This chromatogram is indicative of

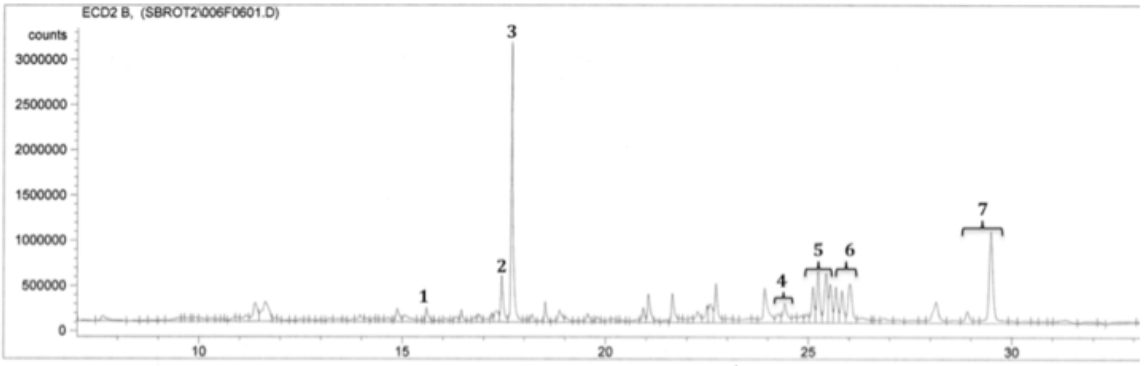

Figure 4.1. GC-ECD chromatogram of a dust sample extract B fortified with 500 ng g^{-1} OP and pyrethroid pesticides. X-axis = time in min. Y-axis = area counts. **1**: diazinon; **2**: malathion; **3**: chlorpyrifos; **4**: permethrin; **5**: cyfluthrin; **6**: cypermethrin; **7**: deltamethrin.

the primary interferences observed during the study. None of the multiple peaks co-eluted or interfered with peaks of target analytes. Stable chromatographic retention times allowed for reliable identification of unknown peaks. For example, the retention time of diazinon (~ 15.616 min) did not vary by more than ± 0.004 min during the course of a 9-hour analytical run. Minimal peak broadening, tailing, and peak matrix interference were observed. Baseline resolution was achieved the majority of the time to include peaks

differing in retention times by < 0.3 min. Consequently, retention times were used to estimate accurately the identity of unknown peaks.

Discussion

Recoveries. Among Extractions A-D, Extractions A and B methods gave the most optimal (70-120%) mean percent recoveries. Similar to Lu, *et al*., Simcox, *et al*., and Starr, *et al*., we sonicated samples in Extraction C to extract analytes (Lu, *et al.* 2004; Simcox, *et al.* 1995; Starr, *et al.* 2008). Lu, *et al*., Simcox, *et al*., and Starr and coworkers sonicated dust samples for ≤ 10 min versus the 30 min sonication we used in Extraction C (Lu, *et al.* 2004; Simcox, *et al.* 1995; Starr, *et al.* 2008). The mean percent recovery ranged between 84.7-116.8% for the investigated pesticides, except for deltamethrin (162.4%). Mean percent recoveries for malathion and diazinon using Extraction C were 7% and 15%, respectively, lower than the comparable mean percent recoveries using Extractions A and B. In Extractions A and B, sonication was not used to extract pesticides. One explanation for this drop in mean percent recovery with Extraction C may be that during prolonged sonication reversible binding between the diazinon or malathion and dust sample is occurring. Pesticide adsorption is a reversible process that binds pesticides to the surface of dust particles. The adsorption and desorption of pesticides between dust particles and the dust extraction solution is possible (Harrison 1998) – steady-state adsorption is unlikely (Johnson 2008). Grove and Chough showed that reversible binding between soil samples and dicloran, a fungicide may be occurring via thorough extraction experiments (Groves and Chough 1970). This decreased mean percent recovery may also be supported by the fact that we sieved dust sample to 250 μm and not 150 μm (Lu, *et al.* 2004; Simcox, *et al.* 1995; Starr, *et al.* 2008). Although dust samples are not necessarily less homogeneous, higher organic carbon fractions, which readily adsorb analyte, have been found in 250 μm samples. This fact coupled with

prolonged analyte-matrix interactions offers the possibility of reduced mean percent recovery of analytes due to many interactions that could occur, such as interactions with highly charged cations (Eskilsson and Mathiasson 2000).

Extraction D presented lower mean percent recovery (69.5-89.4%) for the three OP pesticides investigated in the study. In Extraction D, methanol was used as the extraction solvent. OP pesticides are more polar than pyrethroid pesticides, and acetonitrile is a more polar extraction solvent than methanol. Accordingly, the more polar OP pesticides are not as readily extracted from the dust samples by methanol. This may account for the lower mean percent recovery for the OP pesticides using Extraction D. Thus, we found Extractions A and B to be the most accurate methods for OP and pyrethroid pesticide extraction from household dust in this work.

Although no target analytes were detected in blank samples, some analyte mean percent recoveries exceeded 120%, especially those of deltamethrin. Deltamethrin mean percent recoveries ranged from 149.1-171.1% across all extraction procedures used. This increased mean percent recovery did not increase with concentration and the eluting peak did not change in size across the two fortification levels. As a result, the high bias for deltamethrin can be attributed to an interference resulting from ECD response enhancement and/or co-elution of another compound resulting from interactions within the fortified dust samples during the extraction processes.

Extraction Procedure. Lu, *et al*. and Simcox, *et al*. used a 2.5 g sample in 50 mL of

acetone that was sonicated for 1 min (Lu, *et al.* 2004; Simcox, *et al.* 1995). We use a 0.1 g sample and one-tenth of the amount of solvent to extract both OP and pyrethroid pesticides from dust samples. The cleanup method in both the Lu, *et al*. and Simcox, *et al*. studies employed gel permeation chromatography columns in which 1.5 mL of extract was eluted with 278-282 mL cyclohexane (Lu, *et al.* 2004; Simcox, *et al.* 1995). Our cleanup procedure uses 20 mL solvent, significantly less solvent. Starr and colleagues used 0.5 g dust in 12 mL of solvent to extract analytes using vortexing to initially wet the dust sample (Starr, *et al.* 2008). Extractions A-D use less solvent (5 mL). Similar to the Lu, *et al*. and Simcox, *et al*. methods, Starr and colleagues sonicated samples for 10 min to extract pesticides (Lu, *et al.* 2004; Simcox, *et al.* 1995; Starr, *et al.* 2008). Starr, *et al*. cleaned extracts by passing them through two cartridges, a C_{18} cartridge on top of an aminopropyl cartridge (Starr, *et al.* 2008). Although no additional solvent was used to clean up dust samples in the Starr, *et al*. study, additional waste was created by using two SPE cartridges (Starr, *et al.* 2008). The method we present reduces waste by using one dual-layer SPE cartridge.

We present a multi-residue method for the extraction and cleanup of OP and pyrethroid pesticides in house dust. The use of a GC-ECD allows for the unambiguous determination of the compounds at levels as low as 1 ng/g. Our quantitative, multi-residue method has advantages over previously published methods of reduced solvent use, simple extraction and cleanup steps, and rapid determination via GC-ECD. Future work may involve extending the method for use with additional pesticides.

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CHAPTER 5: CONCLUSIONS AND FUTURE DEVELOPMENT

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Conclusions

In this research, we investigated many multi-residue methods until ultimately discovering an extraction and cleanup method for use for trace analysis of OP and pyrethroid pesticides in food and residential dust and other potential matrices. In the first analytical method described, we fully developed and validated an improved method for the determination of OP and pyrethroid pesticides in food. Effective extraction of pesticide residues from food matrices was demonstrated using this multi-residue extraction method. The method was a rapid, high-throughput, accurate, multi-residue method for the analysis of pesticide residues in a variety of food samples using modifications of existing methods. The procedure involved pesticide extraction from food samples with acetonitrile followed by a salting-out with NaCl and cleanup of the extract with a multilayer solid-phase extraction cartridge composed of a Supelclean^{TM} ENVI-CARB-II top layer and a primary-secondary amine bottom layer separated by a polyethylene frit. To evaluate the method, we performed fortification studies at 50, 100, and 200 ng/g for three OP and four pyrethroid pesticides in 16 different foods. Instrumental analysis was carried out by capillary GC-ECD. Confirmatory analysis was performed by GC coupled with mass spectrometry (MS) in the selected-ion monitoring (SIM) mode. Mean percent recoveries for each fortification level ranged from 49-146% with 80% of mean percent recoveries between 80-120%.

Using the developed food method, we collected and analyzed over 400 food composite samples in the Children's Environmental Exposure to Pesticides Pilot study involving 12 adults. Data from that study presented many significant conclusions about dietary

pesticide exposure of a small sample population. The pilot study showed that participants were frequently being exposed to OP and pyrethroid pesticides via food ingestion of a range of food types, but generally their total daily pesticide intakes did not surpass health-based guidelines. Another notable finding was that some pesticides were found at or above tolerance limits in individual foods or combinations of foods. This implies that tolerance limits may be antiquated and need to be re-evaluated. Additionally, pesticide residues were detected in approximately half the samples labeled "organic" invalidating the fact that items denoted "organic" are completely void of pesticides. Finally, we found dietary pesticide exposures to be sporadic in nature as food types demonstrating the highest pesticide intake on one day may differ from those causing the most pesticide intake on a different day. This is supported by the fact that the adult diet may be episodic in nature as well. Still, grain sample composites consistently proved to cause chlorpyrifos and diazinon intakes among study participants.

As pesticide residue exposure occurs many times in residential settings, we also developed an analytical method to analyze OP and pyrethroid pesticides in household dust in order to determine the potential exposure of persons to pesticides residues via this exposure matrix. Four different extraction procedures were coupled with identical SPE cleanup techniques followed by GC-ECD analyses. We evaluated the accuracy of each procedure via fortification and recovery studies at 200 and 500 ng/g for three OP and four pyrethroid pesticides in household dust. The most efficient extraction protocol evaluated included pesticide extraction from dust samples again with acetonitrile and cleanup of the resulting extract with a Supelclean^{TM} ENVI-CARB-II/PSA dual-layer SPE cartridge.

Using the optimal extraction procedure, the spiked mean percent recoveries of 0.1 g dust samples fortified at 200 ng/g and 500 ng/g were between 99.4-149.1% with a coefficient of variation between 2-17% and a method detection limit of 1.0 ng/g. Mean percent recovery across all pesticides, extractions, and fortification levels ranged from 69.5-171.1% with approximately two-thirds of mean percent recoveries between 70-120%.

Like many in the pesticide field forging new methods, we have attempted to reduce the overall solvent usage by greatly reducing the sample size to one gram. Many reasons for this include the ability to run forensic samples with limited sample, use less toxic chemicals per sample, and manipulate more samples at less cost in the laboratory setting. Nevertheless, as sample size decreases, heterogeneity becomes more problematic. For example, the discussion of the black bean sample ultimately led to the recommendation to homogenize the matrix prior to future sampling and analysis. Another important issue is that as sample compositing is a part of any method, there should be mention of how to handle heterogeneous matrices prior to sampling. In short, sample matrix interferences and low mean percent recoveries of target pesticides, OP and pyrethroid pesticides, due to sample preparation procedures are well-known in the area of conventional multi-residue analysis. Still, we present a simple, cost-effective and high-throughput multi-residue method to determine pesticide residues in various food products and dust samples at trace levels using modifications of existing methods.

In an environment where many researchers are endeavoring to run 100+ pesticide residues per sample, we selected a realistic population of seven compounds to study.

More importantly, we examined a variety of sample types at various fortification levels in hopes of conducting a study with an expanded scope. We hope that the issues we encountered and discussed with the food and house dust methods will help shed light on the complexity of pesticide residue analysis and the many challenges faced when approaching method development.

Future Development

One of the primary incentives of method development in this study was to create a method that we would use to measure a person's total environmental exposure to parent pesticide compounds via food and dust and compare the total parent pesticide intake to measurements of pesticide metabolites found in urine samples that were also collected from participants in the Atlanta adult study. As collaborators at the CDC are currently testing the urine samples collected, we are unable to examine the information and make any correlations between the total pesticide intake and the total pesticide metabolite output. A future development would be to study the dose-effect response that may be present in individuals based upon these data.

In future studies, the further development of sample compositing and homogenization methods needs to be evaluated to increase accuracy, precision, recovery and other quality control criteria of the developed analytical method to guarantee the generation of dependable data. Samples may need to be lyophilized or homogenized with a tissue homogenizer prior to extraction and cleanup to ensure a uniform sample aliquot is being used during these methodological steps.

The analytical methods presented have been minimally evaluated for use with fats and oil, and they have not been shown effective for extracting OP and pyrethroid pesticides from this type of matrix. Additional development of the analytical method for the analysis of fats and oils may also be needed in future studies. This is particularly

important since many insecticides are lipophilic, and fatty matrices may be reservoirs for these compounds.

Another limitation of the current research may have been the instrumentation used. Many researchers have been using GC-MS or LC-MS/MS for pesticide residue analysis. For future studies other detection methods should be evaluated to detect multi-residue pesticides and to achieve better sensitivity and selectivity with a faster chromatographic analysis time although GC-ECD proved effective in our study. Optimization of the current GC-ECD temperature programming may be considered. Moreover, confirmatory analysis data may prove to be more consistent by analyzing the same samples on the same GC-ECD utilizing two different types of capillary columns.

As a final point, researchers need to investigate the impact of matrix effects in pesticide residue analysis of food, house dust, and other matrices. Few studies in the literature expound on matrix effects and the ramifications it has on pesticide residue analysis. In the current study, we attempted to reduce matrix effects via method of standard addition. However, we still attribute some increased mean percent recoveries to matrix effects unaware of its cause or origin. A study of the cause and origin of matrix effects as relative to this study would ultimately further reduce or eliminate its impact and strengthen the method.