

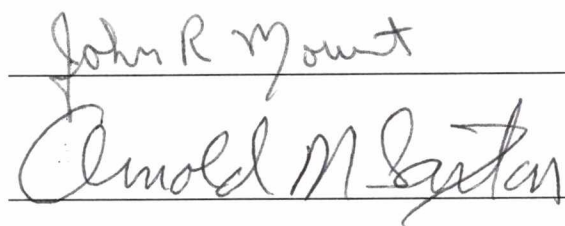
To the Graduate Council:

I am submitting herewith a thesis written by Chen-Chun Shao entitled "RELIABILITY OF METHODS FOR EXTRACTION OF VEGETABLES AND CLEAN-UP FOR SELECTIVE ION MONITORING OF SELECTED ORGANOPHOSPHATE AND N-METHYLCARBAMATE INSECTICIDES." I have examined the final copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Food Science and Technology.



Sharon L. Melton, Major Professor

We have read this thesis
and recommended its acceptance:



Accepted for the Council:



Associate Vice Chancellor
and Dean of The Graduate School

**RELIABILITY OF METHODS FOR EXTRACTION OF
VEGETABLES AND CLEAN-UP FOR SELECTIVE ION
MONITORING OF SELECTED ORGANOPHOSPHATE AND
N-METHYLCARBAMATE INSECTICIDES**

A Thesis

Presented for the

Master of Science

Degree

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Chen-Chun Shao

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DEDICATION

This thesis is dedicated to my parents

Mr. Kuang-Tsu Shao

and

Mrs. Shui-Chiung Tseng

who always have been giving me endless love and encouragement.

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Special thanks go to my family for their support and love throughout my graduate studies. Finally, sincere appreciation is extended to Antonia Tsao for her patience and understanding during the pursuit of my goals.

ABSTRACT

The reliabilities of two multiresidue methods (MRMs) for 11 organophosphates and N-methylcarbamate insecticides (INs) in 3 vegetables or VEGs (corn, navy beans and spinach) were determined and compared. MRM 1 involved acetonitrile extraction followed by florisil clean-up and MRM 2, ethyl acetate-acetone extraction and reverse phase C₁₈ clean-up. INs were measured using an internal standard method and GC-MS selective ion monitoring (SIM). Reliability was determined by percent recovery (PR), coefficient of variation (CV) and sensitivity. The PR of each MRM was dependent upon IN, VEG and method. Each MRM was precise ($< \pm 15\%$ CV) for each IN in each VEG. Naled could not be analyzed by either MRM in any VEG (PRs < 8); carbofuran, carbaryl, fenamiphos and azinphos-methyl could not be measured by MRM 1 (PRs < 21). Six INs: phorate, dyfonate, disulfoton, methyl parathion, malathion and chlorpyrifos in all VEGs could be analyzed accurately because the PRs were between 80 or 110 or by multiplying levels by a correction factor or CF (PR/100). MRM 2 analyzed accurately the same 6 INs as did MRM 1 plus the 4 INs: carbofuran, carbaryl, fenamiphos and azinphos-methyl not measurable by MRM 1. CFs are necessary for some of these latter INs in MRM 2 for accurate results. All of the INs measured accurately by MRMs 1 and 2, except for azinphos-methyl, could be

measured by SIM in spinach extracts near or below the allowable levels. Thus, MRM 2 was the best method for the most INs.

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CHAPTER I

INTRODUCTION

The introduction of a substantial amount of insecticides into the environment over the last few decades has led to great public concern. The toxic insecticides can cause acute or chronic poisoning in humans resulting in allergic reactions, oncogenic effects and even death. A survey showed that one-half of U.S. consumers are concerned about pesticide residue on fresh products and two-thirds of consumers were willing to pay up to 10% higher prices to acquire certified pesticide residue-free fresh products (Ott, 1990). However, current U.S. Department of Agriculture (USDA) grades and standards are not the most appropriate criteria for providing consumers enough information regarding pesticide residues in food (Figueroa, 1995). Therefore, rapid and accurate methods for monitoring pesticide residues in food are necessary in order to provide consumers a risk-free consuming space.

Current multiresidue methods (MRMs) used by the Food and Drug Administration (FDA) for analysis of insecticide residues in food are insufficient to meet the demand for analyzing all insecticides rapidly, efficiently and with less pollution. The reason for this is that the majority of insecticide residue analyses are still carried out by a classical approach involving a large amount of solvent for extraction and clean-up procedures. Several MRMs have been proposed in recent

years for determination of insecticide residues in crops by simplifying and miniaturizing the extraction and clean-up procedures combined with different chromatographic and other detective techniques (Fodor-Csorba, 1992; Niessner et al., 1996; Torres et al., 1996; Wan and Wong, 1996).

The Food Science and Technology Department at The University of Tennessee (FST-UT) successfully analyzes vegetable samples from Bush Brothers & Company (Dandridge, TN) for selected organochlorine and organophosphate residues using a modified AOAC (1990) method proposed by Yang (1993). This MRM uses acetonitrile to extract vegetable samples followed by an ethyl ether (EE) in petroleum ether (PE) partition and with or without florisil solid phase extraction clean-up with the resultant extract being determined by gas chromatography-mass spectrometry (GC-MS).

Through 3 years of multiresidue analysis, we have found that the clean-up procedure is necessary in order to maintain the high performance of GC-MS analysis. The lack of clean-up procedure normally results in a GC-MS that requires frequently cleaning and service; this maintenance service provided by a technician is expensive. Kadenczki et al. (1992) also reported that omitting the clean-up can have negative effects on the quality of the analysis.

In addition to the pesticides that are analyzed currently in vegetables (spinach, cabbage, kale, collards, mustard greens, turnip greens, corn and dry beans) from Bush Brothers & Company, other pesticides used in the vegetables produced by

Bush Brothers & Company are also of interest. Eleven insecticides, including 9 organophosphates and 2 N-methylcarbamate listed in Table 1, are allowed in the production of Bush Brothers & Company vegetables (CPCR, 1996). In order to extend the number of pesticides that can be monitored by the MRM used by FST-UT, the reliability of this MRM for measuring the 11 insecticides listed in Table 1 needs to be determined.

Although the current MRM used by FST-UT is reliable and accurate, it still has the common drawbacks of MRMs, such as time consumption, labor intensity and use of large amounts of toxic solvents. In order to join the trend for more rapid, efficient, and less polluting methods, a new MRM that can be used in the routine analysis of pesticide residues in FST-UT is needed. An improved method reported by Saul et al. (1995) used solid phase extraction (SPE) with a reverse phase C₁₈ bonded cartridge to clean up extracts containing organophosphates and N-methylcarbamates, including those listed in Table 1. This method provides the benefits of speed, convenience, and reduced solvent consumption. Other reports also showed that reverse solid phase extraction can be used successfully in the analysis of organic compounds in pharmaceutical fluids and environmental water and can replace traditional liquid-liquid partition (Loconto and Gaiand, 1989; Well and Michael, 1987). Saul et al. (1995), using this reverse phase C₁₈ SPE clean-up, confirmed positive results on the detection of organophosphate and N-methylcarbamate insecticides by GC-MS. However, they did not provide any

Table 1--Organophosphate and N-methylcarbamate insecticides allowed for production of Bush Brothers & Company vegetables

Insecticide	Type
Naled	Organophosphate
Phorate	Organophosphate
Carbofuran	N-methylcarbamate
Dyfonate	Organophosphate
Disulfoton	Organophosphate
Methyl parathion	Organophosphate
Carbaryl	N-methylcarbamate
Malathion	Organophosphate
Chlorpyrifos	Organophosphate
Fenamiphos	Organophosphate
Azinphos-methyl	Organophosphate

information about the accuracy or precision of the analysis method for these insecticide residues.

In order to apply this new clean-up technique in the pesticide residue analysis of vegetable samples, the reliability of this method reported by Saul et al. (1995) needs to be determined for the insecticides listed in Table 1. The reliability of this reverse phase SPE method must be as good as the current MRM used by FST-UT, and the analysis time and amount of solvent used in this reverse phase SPE method also must be less than that of the current MRM used by the FST-UT for the 11 insecticides (Table 1) in order for the new method to be used in routine multiresidue analysis by FST-UT.

Another index that helps to determine the reliability of any method is the sensitivity of the overall method for any given analyte. To be truly representative of the method, an extract of a sample, which is considered to be "dirty" in that many co-extractives in combination with the pesticides are removed by solvent extraction, needs to be used to determine the limit of detection of each pesticide analyzed in a MRM. Such a sample in Bush Brothers & Company vegetables is spinach, which is dark green and contains chlorophyll. Determination of sensitivity for each insecticide in sample matrices rather than of a simple insecticide solution is the more realistic approach for assessing the reliability of any method.

Therefore, the objectives of this research were as follows:

- (1). To determine the reliability (accuracy and precision) of the current FST-UT extraction and clean-up method for the eleven insecticides listed in Table 1 in selected Bush Brothers & Company vegetables (corn, navy beans and spinach).
- (2). To determine the reliability (accuracy and precision) of the reverse SPE method, which was modified in order to be more cost-effective and time efficient for the same insecticides (Table 1) and vegetables (corn, navy beans and spinach).
- (3). To compare the reliability (accuracy and precision) of the two multiresidue methods for each insecticide listed in Table 1.
- (4). To determine the sensitivity of the GC-EIMF measurement of each of the eleven insecticides in spinach extracts prepared by the two methods.

CHAPTER II

LITERATURE REVIEW

I. INSECTICIDES AND HUMANS

The insecticide is a chemical or mixture of chemicals used for controlling insects and their relatives through lethal effect (Cutkomp and Eesa, 1994).

Insecticides are not only applied in agriculture to increase crop production but also are used in homes to prevent pests. According to Smith (1993), twenty-three percent of pesticide sales in the United States were insecticides in 1992. The value of insecticides sold in United States was over two trillion dollars in 1991, and half of them were used in agriculture.

The use of insecticides can be traced back to the burning of brimstone (sulfur) for fumigation around 1,000 B.C. (Ware, 1989). The first synthetic organic insecticides for public use were dinitro compounds and thiocyanates (Matsumura, 1985). The introduction of DDT discovered in Switzerland in 1939 brought another revolution of synthetic insecticides in pest control. Following World War II the demand for safer insecticides made organophosphates the most used insecticides.

Insecticides can be divided into systemic and non-systemic insecticides. Systemic insecticides are those that are capable of being absorbed by a plant into

the plant vascular system. They are less affected by weather since they are inside the plant (Cutkomp and Eesa, 1994). Normally they are toxic to any sucking insect feeding on the plant tissue (Ware, 1989). Non-systemic insecticides are those that cannot be translocated to a plant vascular system. They are applied to the foliage and subjected to weather.

About one-third of world crops are destroyed by pests during growing, harvesting and storage (Ware, 1989). Estimated average losses per year from insect damage in crops are 11-19% (Metcalf, 1980). Therefore, insecticides are necessary to reduce crop loss before their economic value is affected dramatically. Currently, insecticides are still the most efficient and economic methods to control pests. However, overuse of insecticides causes problems. One problem is the development of resistance in insects, leading to more toxic and higher levels of insecticides needed to maintain the same result. Another issue is environmental pollution and safety. Each year approximately 45,000 cases of human poisoning due to pesticides occur worldwide and about 50 of these cases result in death (Pimentel and Levitan, 1986).

Public concern about the effects of pest control chemicals on human health and environmental quality has increased greatly. Consumer worries about the pesticide residues in food are higher than those for cholesterol, fat, salt and other compounds (Moye, 1991). Another survey also showed that pesticide residue in food was the most important factor affecting consumers' confidence in food safety

(Scroggins, 1991). In the United States, the Environmental Protection Agency (EPA) has the authority to register pesticides used under the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) (Duke et al., 1993). Toxicity tests are required for any insecticide before approval (Duke et al., 1993). Toxicity of insecticides is evaluated based on acute and chronic effects. Acute effect is expressed in terms of the LD₅₀ (for "lethal dose"). This value represents the amount of poison per unit weight required to kill 50% of a particular population of the animal tested, and is expressed as milligrams per kilogram or sometimes as milligrams per body. (Matsumura, 1985). The test for chronic effects requires that registered insecticides present no oncogenic risk to humans (Duke et al., 1993).

The U.S. EPA also has the responsibility for setting the tolerance limits for pesticide residues that can be present in foods. The tolerance is the maximum amount of pesticide residue that can legally remain on the food or feed (Ware, 1989). The Food and Drug Administration (FDA) is charged with enforcing these tolerances set by the EPA for all foods except meat and poultry, which are under the administration of the U.S. Department of Agriculture (USDA). Tolerances for all legal pesticides have not been established in all foods. Table 2 lists the tolerances of 11 insecticides allowed in corn, navy beans and spinach (Anon, 1996).

Table 2--Tolerances^a allowed in corn, navy beans and spinach for 11 selected insecticides (Anon., 1996)

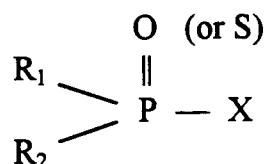
Insecticide	Vegetable		
	Corn	Navy beans	Spinach
Naled	0.5	0.5	3.0
Phorate	0.5	0.1	N/A ^b
Carbofuran	25.0	N/A	N/A
Dyfonate	0.1	0.1	0.1
Disulfoton	5.0	0.75	0.75
Methyl parathion	1.0	1.0	1.0
Carbaryl	100	10.0	12.0
Malathion	8.0	8.0	8.0
Chlorpyrifos	8.0	0.7	N/A
Fenamiphos	N/A	N/A	N/A
Azinphos-methyl	N/A	0.3	2.0

^aTolerance is expressed as parts per million (ppm).

^bTolerance is not available.

II. ORGANOPHOSPHATE AND N-METHYLCARBAMATE INSECTICIDES

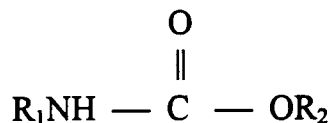
Organophosphate insecticides are normally esters, amides, or thiol derivatives of phosphoric or phosphonic acid (WHO, 1986b). The general formula is as follows:



where R₁ and R₂ may be any group (alcohol, phenol, mercaptan, amide, alkyl or aryl) either bonded to the phosphate directly or via O, N, or S indirectly and where the X can be any substituted and branched aliphatic, aromatic, or heterocyclic linked to phosphate directly or via O or S.

The insecticidal action for organophosphates was found by Gerhard Schrader in Germany around 1934 (Matsumura, 1985). In 1946, tetraethyl pyrophosphate (TEPP) was the first organophosphate introduced to agriculture (Ware, 1989). The organophosphates account for 30% of the registered synthetic insecticides and acaricides in the United States and the number is still increasing (Matsumura, 1985). Organophosphates have replaced organochlorines as the most used insecticides due to the concerns of persistence and pollution effects for the latter (Hassall, 1990).

N-methylcarbamate insecticides are N-substituted esters of carbamic acid (WHO, 1986a). The general formula is as follows:



where R₁ is a methyl group and R₂ is an aromatic or aliphatic moiety.

Carbamates are the latest anticholinesterase insecticides. The first carbamate insecticides were introduced by Geigy Chemical Company in Switzerland in 1952 (Ware, 1989). Low mammalian oral and dermal toxicity and a broad spectrum of insecticidal activity have made carbamate insecticides the most popular pesticides (Ware, 1989). Another advantage of carbamates is their short persistence on plants, and most of them degrade into their metabolites shortly after application (WHO, 1986a).

The biochemical mode of action for organophosphate and N-methylcarbamate insecticides is the interference with the passage of impulses in the insect nervous system. The nervous transmission in the axons is accomplished by the exchange of Na⁺ and K⁺ between axonal membranes. In synapse the transmission is by the acetylcholine (ACh). The ACh is then hydrolyzed to acetic acid and choline by cholinesterases (ChE) so the synapse will be cleared for another transmission. The organophosphate and N-methylcarbamate insecticides exert their toxic action by attaching to the ChE and inhibit the ACh from being hydrolyzed by ChE. As a

result, no more ChE is available for the ACh (Fig. 1). The electric transmission circuit becomes jammed due to the accumulation of ACh in the insect, resulting in restlessness, hyperexcitability, paralysis, and death (Ware, 1989).

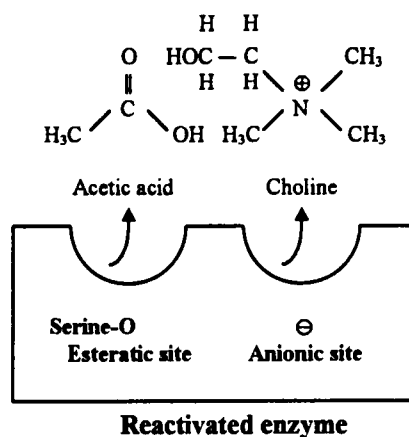
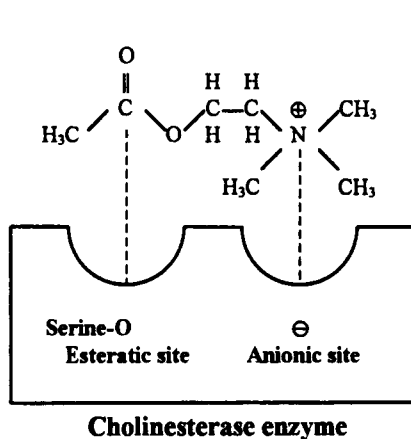
III. OFFICIAL INSECTICIDE ANALYSES

Insecticide analysis normally is done by multiresidue methods (MRMs) or single residue methods (SRMs). MRMs can analyze a number of insecticides simultaneously; SRMs, however, only analyze a single insecticide. Therefore, MRMs more nearly meet the practical needs for insecticide screening. SRMs can be used to analyze insecticides that are not covered by MRMs.

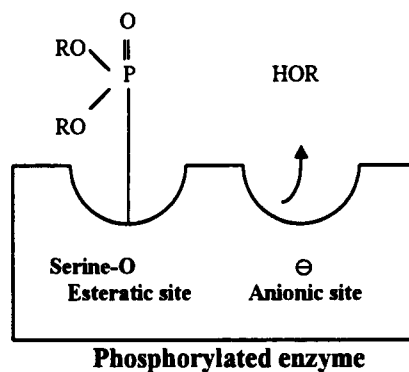
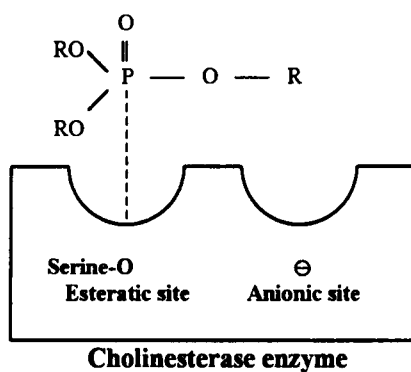
Currently, the five most common MRMs in volume I of the *Pesticide Analytical Manual* (PAM) are used by the FDA for pesticide monitoring (PAM, 1994) (Table 3). Each of them is also included in the AOAC (1990) *Official Methods of Analysis*. The Luke method (Luke et al., 1975; Luke et al., 1981), Mills method (Mills, 1959), MOG method (Mills et al., 1963) and Storherr method (Storherr et al., 1971) can be used to analyze organophosphates. The Krause method (Krause, 1980) can be used to analyze N-methylcarbamates. These methods can detect over 300 pesticides and related compounds.

The basic steps of MRMs include sampling, extraction, clean-up, separation and detection (Moye, 1991). These steps include using acetone or acetonitrile as

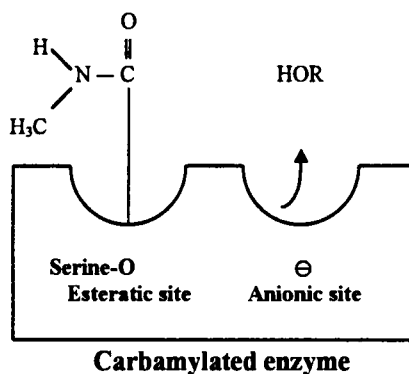
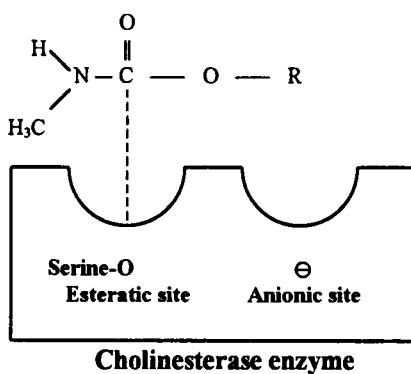
(A). Acetylcholine



(B). Organophosphate



(C). Carbamate



(A). Acetylcholine is hydrolyzed to acetic acid and choline resulting in reactivated enzyme. (B). Organophosphate is hydrolyzed and phosphorylates the enzyme by attaching to serine at the esteratic site. (C). Carbamate is hydrolyzed and carbamylates the enzyme by also attaching to serine at the esteratic site.

Fig.1--The mode of inhibition of organophosphate and N-methylcarbamate insecticides (Ware, 1989)

Table 3--Multiresidue methods commonly used by Food and Drug Administration (FDA)

Method	Pesticides ^a	Food	Instrument ^b
Mill method ^c	OPs, OCs	Fatty	GC
Mill, Onley and Gaither method ^d	OPs, OCs	Nonfatty	GC
Luke method ^e	OPs, OCs, ONs	Nonfatty	GC
Storherr method ^f	OPs	Nonfatty	GC
Krause method ^g	MCs	Nonfatty	HPLC

^aOPs: organophosphates ; OCs: organochlorines; ONs : organonitrogens; MCs: N-methylcarbamates.

^bGC: gas chromatography; HPLC: high-performance liquid chromatography.

^cMills (1959).

^dMills et al. (1963).

^eLuke et al. (1975) and Luke et al. (1981).

^fStorherr et al. (1971).

^gKrause (1980).

the extraction solvents, using liquid-liquid partition (LLP) and chromatographic techniques to remove interfering compounds, and finally using gas chromatography (GC) or high-performance liquid chromatography (HPLC) with selected detectors to identify pesticides. The primary drawback of these existing MRMs is that they can not detect all current pesticides. First, approximately 400 pesticides have established tolerances (Anon., 1996), only about half of them can be detected with these five MRMs (Anon., 1995). Second, these five MRMs are too time-consuming and labor intensive. They cannot efficiently prevent contaminated product from reaching the market. Third, they require large amounts of hazardous solvents, resulting in disposal problems and possible pollution. The testing procedures result in $10^8 - 10^9$ times more pollution than do the pesticides being determined (Steinwandter, 1992).

IV. MODIFIED INSECTICIDE ANALYSES

To ameliorate the disadvantages of the official MRMs, numerous methods have been developed (Fodor-Csorba, 1992; Motohashi et al., 1996; Torres et al., 1996; Wan and Wong, 1996; Yang et al., 1996). Basically, these new methods simplify, miniaturize, and improve the conventional extraction and clean-up procedures. Meanwhile, existing instruments or new techniques to analyze different pesticides in different matrices are used.

Improved Extraction and Clean-up

In older methods, the sample size is usually around 100 grams, so the volume of solvent required may be several hundred milliliters. Bushway et al. (1995) used 5 grams of potato, fruit or juice samples for pesticide analysis and only 40 ml of solvent for extracting. Holstege et al. (1994) used minicolumns to screen organophosphates, organochlorines, and N-methylcarbamates in plant and animal tissues, with recovery from 77 to 113%. Both miniaturization schemes reduced solvent use and time consumption.

Extraction of organophosphates with ethyl acetate and sodium sulfate without further clean-up was also described by Steinwandter (1990). This method can reduce solvent use to 1/10 – 1/100 of the original use level. Miyahara et al. (1994) analyzed organophosphates in vegetable and fruit samples and validated this method by GC-MS and flame photometric detector (FPD).

Solid phase extraction (SPE) has been used to replace classical LLP to reduce time and solvent consumption. Lee et al. (1991) used acetonitrile extraction and C₁₈ minicolumn clean-up on the analysis of organophosphates, organochlorines and N-methylcarbamate in fruits and vegetables and was able to detect pesticides at the 0.01 ppm level. Odanaka et al. (1991) reported that using C₁₈ minicolumn for clean-up of extracts of some crops showed acceptable recovery for organophosphates and N-methylcarbamates. Consalter and Guzzo (1991) showed applicable result in analyzing organophosphates with Sep-Pack C₁₈. Using a

modified clean up with a florisil minicolumn instead of the classical florisil column in some organophosphates also showed improved results in some vegetable extracts (Yang, 1993).

Some approaches omitted the clean-up procedure to diminish pollution due to the solvent. Liao et al. (1991) used acetonitrile for extraction to determine pesticide residues in different crop samples without a clean-up procedure. The average recovery of 143 compounds in 13 crops was 92%.

Determination by Gas Chromatography / Mass Spectrometry

Gas Chromatography (GC) is a separation technique in which the sample is subjected to a competitive distribution between mobile and stationary phases. GC is normally used for samples that are volatile and thermally stable. With the development of capillary columns, capillary GC (cGC) has become an important tool for insecticide analysis (Loconto and Gaind, 1989). Various methods have been developed for insecticide analysis. Hernández et al. (1990) extracted organophosphates from fruit samples and determined them by cGC/MS. The percent of recovery was between 81 and 129%. Carbamates and organophosphates extracted from four fruit and vegetable samples were analyzed by cGC-CI/MS and had recoveries from 73 to 120% (Mattern et al., 1990). The advantages of capillary columns over packed column are higher resolving power, lower retention time, lower bleed, longer life and greater reproducibility; they have replaced the classical packed columns (Willard et al., 1988).

The primary detectors employed for pesticide analysis are the electron capture detector (ECD), the nitrogen-phosphorous detector (NPD), the alkali flame ionization detector (AFID), and the Hall electrolytic conductivity detector (HECD) (OTA, 1988). The major problems with these detectors are that separate detection methods are required for better identification of various pesticides. Second, compounds with the same retention time will cause false positive results. Fortunately, mass spectrometric detection (MSD) can overcome the defects mentioned above and is often used by regulatory agencies to confirm the presence or absence of pesticide residues in food (Luke et al., 1988).

The analysis of samples using MSD is dependent on successful ionization. Electron impact (EI) and chemical ionization (CI) are the most common ionization method in MS (Mattern and Rosen, 1992). In EI, sample molecules are bombarded with a beam of electrons emitted from a filament composed of rhenium or tungsten metal. These collisions extract electrons from sample molecules, forming ionized molecules. Finally, positive ions are propelled into the analyzer and give a spectrum characteristic for the sample (Nielsen, 1994). In CI, ionization is achieved by the reaction of the sample with ionized gas such as methane or isobutane (Mattern and Rosen, 1992). The combination of GC and MSD, so-called GC-MS, was introduced by Holmes and Morrell (1957) and Gohlke (1959). Today GC-MS has become the most powerful technique for identifying compounds.

Although GC-MS with electron impact ionization (GC-EIMS) is the most widely used technique in pesticide residue analysis (Torres et al., 1996), it may be limited by its sensitivity. Selective ion monitoring (SIM), which measures only a few selected ions, can greatly improve the sensitivity problem (Stan, 1989). The ion selected is usually the most abundant in the mass spectrum of the compound to be analyzed.

For over 30 years, SIM has had widespread application in medical, biological and environmental analyses. Liao et al. (1991) used GC-EIMS with SIM mode to analyze 143 pesticides in crop samples and had good recovery. Miyahara et al. (1994) used GC-EIMS with the SIM mode to analyze organophosphates in 9 crop samples. Benfenati et al. (1990) used GC-EIMS with the SIM mode to analyze 50 pesticides for checking the contamination of water. In addition to identification and confirmation, the SIM mode also can be applied to quantification.

N-methylcarbamates also can be analyzed by GC-EIMS with the SIM mode although it is usually investigated using the HPLC method. The Food Science and Technology Department at the University of Tennessee also used the GC-EIMS with the SIM mode to screen organochlorines and organophosphates in products from Bush Brothers & Company (Melton, 1996).

V. CHARACTERISTICS OF ELEVEN INSECTICIDES TO BE ANALYZED

The eleven pesticides to be analyzed were all insecticides. Nine of them were organophosphates (naled, phorate, dyfonate, disulfoton, methyl parathion, malathion, chlorpyrifos, fenamiphos, and azinphos-methyl) and two of them were N-methylcarbamate (carbofuran and carbaryl).

Three of the eleven insecticides are leading pesticides ranked by volume sales in U.S. in 1991-1992. They are chlorpyrifos, carbaryl and malathion (Smith, 1993). All of these 11 insecticides were included in the 1994 FDA pesticide residue monitoring program (Anon., 1995). Only naled and fenamiphos were not detected in the fiscal year 1994 under regulatory and incidence / level monitoring. Following are the properties of 11 insecticides analyzed in this research project (Milne, 1995; U.S. EPA, 1988; Worthing and Walker, 1995).

Naled ($C_4H_7Br_2Cl_2O_4P$; MW = 381) is a non-systemic insecticide. Technical grade naled is a yellow liquid with a slightly pungent odor. It is insoluble in water and readily soluble in aromatic and chlorinated solvents. It is rapidly hydrolyzed in water. Acute oral LD_{50} (rat) is 430 mg/kg. It can be used on numerous crops but use in grain bins is not approved.

Phorate ($C_7H_{17}O_2PS_3$; MW = 260) is a systemic insecticide. Technical grade phorate is a clear mobile liquid. Solubility in water is 50 mg/L and readily

miscible with alcohol, ketone, ether, ester and aromatic solvents. It is stable at 25°C for 2 years. Acute oral LD₅₀ (rat) is 1.0 mg/kg. It can be used on a variety of crops.

Carbofuran (C₁₂H₁₅NO₃; MW = 221) is a systemic insecticide. Technical grade carbofuran is a colorless crystal solid. Solubility in water is 700 mg/L; other solubilities are 150 g/kg acetone and 140 g/kg acetonitrile. It is unstable in alkaline solutions. Acute oral LD₅₀ (rat) is 8 - 14 mg/kg. It can be used to control most soil and foliage pests.

Dyfonate (C₁₀H₁₅OPS₂; MW = 246) is a soil insecticide. Technical grade dyfonate is a clear colorless liquid with an aromatic odor. Solubility in water is 13 mg/L and it is miscible with acetone and ethanol. It is stable at temperatures below 100°C. Acute oral LD₅₀ (rat) is 24.5 mg/kg. It can be used on soil.

Disulfoton (C₈H₁₉O₂PS₃; MW = 274) is a systemic insecticide. Technical grade disulfoton is a colorless oil with a characteristic odor. Solubility in water is 25 mg/L and it is soluble in most organic solvents. Fifty percent can be lost by hydrolysis at 20°C. Acute oral LD₅₀ (rat) is 2.6-12 mg/kg. It can be used on seeds.

Methyl parathion (C₈H₁₀NO₅PS; MW = 263) is a non-systemic insecticide. Technical grade methyl-parathion is a colorless crystal or a tan liquid. Solubility in water is 55 – 60 mg/L and it is soluble in light petroleum. It can be hydrolyzed by alkali. Acute oral LD₅₀ (rat) is 14 mg/kg. It is effective against many insects.

Carbaryl ($C_{12}H_{11}NO_2$; MW = 201) is a mild systemic insecticide. Technical grade carbaryl is a colorless crystal. Solubility in water is 850 mg/L and is 400 - 450 g/kg in dimethylformamide. It is stable at temperatures below 70°C. Acute oral LD₅₀ (rat) is 850 mg/kg. It can be used on wide variety of fruit and vegetable crops.

Malathion ($C_{10}H_{19}O_6PS_2$; MW = 330) is a non-systemic insecticide. Technical grade malathion is a clear amber liquid. Solubility in water is 145 mg/L and it is miscible with most organic solvents. Acute oral LD₅₀ (rat) is 2800 mg/kg. It can be used in a wide range of agricultural and horticultural crops.

Chlorpyrifos ($C_9H_{11}Cl_3NO_3PS$; MW = 351) is a non-systemic insecticide. Technical grade chlorpyrifos is a colorless crystal with a mild mercaptan odor. Solubilities include 2 mg/L water, 6.5 kg/kg acetone and 450g/kg methanol. The hydrolysis rate increases with the pH and temperature. Acute oral LD₅₀ (rat) is 135 - 163 mg/kg. It can be used to control pests in soil and on foliage.

Fenamiphos ($C_{13}H_{22}NO_3PS$; MW = 303) is a systemic insecticide. Technical grade fenamiphos is a colorless waxy solid. Solubility in water is 700 mg/L and it is readily soluble in isopropanol and toluene. Acute oral LD₅₀ (rat) is 15.3 – 19.4 mg/kg. It is recommended for overall application.

Azinphos-methyl ($C_{10}H_{12}N_3O_3PS_2$; MW = 317) is a non-systemic insecticide. Technical grade azinphos-methyl is a colorless crystal. Solubility in water is 33

mg/L and in toluene is greater than 1 kg/L. Acute oral LD₅₀ (rat) is 16.4 mg/kg. It can be used on wide variety of fruits, vegetables, nuts, and crops.

CHAPTER III

MATERIALS AND METHODS

I. MULTIRESIDUE METHODS APPLIED

Experimental Design

Prior to the quantitative analysis of any insecticide, the repeatability of the instrumental measurement was tested over a period of time by determining the relative response factor, RRx , of each of 11 insecticides (Table 1) to an internal standard, anthracene-d10, in 3 different standard solutions, 2 different times, 5 days apart. The instrumental measurement of RRx for each insecticide was run in triplicate during the first time period and in duplicate for the second time period. The model for RRx was as follows:

$$RRx = \mu + S + T + \varepsilon$$

where μ = mean RRx , S = standard solution, T = period or time of analysis on the GC-MS, and ε = error. The coefficient of variation of RRx was determined as a measurement of the instrumental precision within a time period.

The experimental design for the recovery of each of 11 insecticides in Table 1 was a completely randomized design, which included vegetables, methods and replications. A replication consisted of 3 vegetables and 2 methods, and 3

replications were run. The order in which each vegetable-method-replication combination was run, was randomized across vegetable, method and replication. All vegetable-method-replication combinations (n=18) of vegetables spiked with a known concentration of each of the 11 insecticides and all vegetable-method combinations (n=6) of unspiked vegetables were extracted, cleaned-up and prepared for instrumental analysis during a single day. These spiked and unspiked samples were analyzed instrumentally in the same order as they were extracted for the concentrations of the 11 insecticides using a selected set of ions and during another time for 8 insecticides using an alternate set of ions. The percent recovery (PR) of each insecticide on each time of analysis for each vegetable-method-replication combination was calculated. The model for PR was as follows:

$$PR = \mu + V + M + R + V \times M + \varepsilon$$

where μ = mean PR, V = vegetable, M = method, R = replication, and ε = error. R was included in the model to check the consistency of the PR.

The coefficient of variation (CV) for the PR of each of the 11 insecticides in each vegetable-method combination was calculated for the first time of instrumental analysis and for the PR of each of 8 insecticides for the second time of instrumental analysis. The calculation included determination of the standard deviation in PR across replications; therefore, the model for CV was as follows:

$$CV = \mu + V + M + I + V \times M + V \times I + M \times I + \varepsilon$$

where μ = mean CV, V = vegetable, M = method, I = insecticide, and ϵ = error.

Materials and Samples

All insecticide standards — naled, phorate, carbofuran, dyfonate, disulfoton, methyl parathion, carbaryl, malathion, chlorpyrifos, fenamiphos, and azinphos-methyl — were purchased from Chem Service, Inc. (West Chester, PA). The purity of each of these 11 insecticides was more than 98%. The internal standard, anthracene-d10, was bought from Sigma Chemical Co. (St. Louis, MO).

Florisil Sep-Paks (1-g) were bought from Millipore Corp. (Bedford, MA). C₁₈ Sep-Paks were obtained from Waters Associates (Milford, MA). Ashless filter paper was obtained from Schleicher & Schuell (Keene, NH). Celite, anhydrous sodium sulfate and sodium chloride bought from Fisher Scientific (Springfield, NJ), were of a special grade for pesticide residue analysis. Pesticide grade solvents — acetonitrile, petroleum ether, ethyl ether, methanol, acetone, and ethyl acetate — were all purchased from Fisher Scientific (Springfield, NJ).

Dry navy beans and dry corn were obtained from Bush Brothers & Company (Dandridge, TN). Spinach samples were purchased from a local supermarket in Knoxville, TN. All samples were stored at -18°C before use. Each sample was mixed thoroughly prior to sampling. The vegetable samples were analyzed for insecticides at the target ions monitored in this study.

Instrumentation

A Shimadzu Model 9AM gas chromatograph (GC) (Shimadzu Scientific Instruments Inc., Columbia, MD) interfaced with a Shimadzu Model QP-1000 mass spectrometer (MS) was employed to analyze samples. The gas chromatography (GC) was fitted with a splitless injector and a 30-m $\times$ 0.25-mm i.d. PTE-5 fused-silica capillary column (Supelco Co., Inc., Bellefonte, PA). A high purity of helium with a flow rate at 1.1 mL/min was used as the carrier gas. The GC temperature program was set to hold the oven at 50°C for 2.5 min, increase the temperature from 50 to 240°C at a rate of 20°C/min, hold the temperature at 240°C for 7 min, and finally increase the temperature at the same rate to 280°C and hold for 20 min. The injector temperature was maintained at 250°C.

The mass spectrometer (MS) was equipped with a quadrupole mass analyzer and was operated in the electron impact (EI) mode over a mass range selected between 40 – 400 M/z at 70 eV. The GC transfer line and the ion source temperature were kept at 250°C. The Shimadzu Model QP-1000 MS used in this study can be set in two desired modes: either in the cyclic scan mode, EIMS, or the selected ion monitoring (SIM) mode, EIMF. In order to maintain the performance, the mass spectrometer (MS) was calibrated by perfluorotri-butylamine (PFTBA) once during the 10 days of analysis. The PFTBA is one of

the most frequently compounds that is recommended by the EPA for calibration and tuning the quadrupole analyzer.

Analysis of Retention Time and the Most Abundant Ion

One μl of each insecticide or internal standard containing the concentration listed in Table 4, which gave less than maximum detection threshold, was analyzed on GC-EIMS. The retention time (RT) and the M/z of most abundant ion for each of the 11 insecticides and the internal standard were determined from their respective mass spectrum using the temperature program mentioned above by GC-EIMS analysis.

Programming the Mass Spectrometer

After determining the retention time and the most abundant ion, the program used for Shimadzu QP 1000 mass spectrometer was selected for GC-EIMF analysis. The program could detect specific ion of interest at the time selected without any interference of other insecticides.

Acetonitrile Extract with Florisil Clean-up Method

The following method (Yang, 1993), which is modified from the AOAC (1990) method, is currently used by the Department of Food Science and Technology at The University of Tennessee for pesticide analysis. Prior to extraction, vegetable samples (100-g) was spiked with 1.00-mL of standard solution containing 11 insecticides and internal standard at concentrations listed in Table 4.

Table 4--Concentration of 11 insecticides and internal standard used to spike vegetables in the analysis of GC-EIMS

Compound	Concentration ($\mu\text{g}/\mu\text{L}$)
Naled	0.07
Phorate	0.07
Carbofuran	0.15
Dyfonate	0.15
Disulfoton	0.15
Methyl parathion	0.15
Carbaryl	0.15
Malathion	0.20
Chlorpyrifos	0.30
Fenamiphos	0.30
Azinphos-methyl	0.80
Anthracene-d10 ^a	0.10

^aInternal standard.

Sample preparation: Corn and navy bean samples were soaked with pesticide-free water overnight. Spinach samples were chopped. One hundred grams of soaked or chopped sample, 200-mL acetonitrile, and 10-g Celite were placed in a 0.45-L Mason jar and blended on an Ostracizer blender at high speed for 2 min. The mixture was filtered with suction into a 250-mL graduated cylinder through a 12-cm Buchner funnel containing 11-cm diam. sharkskin filter paper.

Extraction and clean-up: One half portion of the filtrate was transferred to a 500-mL separatory funnel; 100-mL of 6% ethyl ether (EE) in petroleum ether (PE) (v/v) was added, followed by 300-mL water and 5-mL saturated sodium chloride solution. The separatory funnel was shaken vigorously for 45 sec and then the mixture was allowed to separate. After discarding the aqueous layer, the EE/PE layer was washed twice with 50-mL distilled water each time. The bottom aqueous layer was discarded. Water was removed by passing the EE/PE layer into a 100-mL graduated cylinder through a filter funnel containing 15-g anhydrous sodium sulfate over glass wool. Twenty-five mL of extract was dried to near dryness under nitrogen flow on a steam bath. Dried extract was dissolved in 1-mL of 6% EE/PE and then loaded on a 1-g florisil Sep-Pak cartridge attached to a vacuum manifold (Baxter Scientific Products, Stone Mount, GA). Samples were eluted with 10-mL 6% EE/PE followed by 10-mL 15% EE/PE and 10-mL 35% EE/PE. Eluates were dried to near dryness under nitrogen flow on a steam bath.

Dried eluates were dissolved in 1-mL acetone and stored at -18°C under N_2 prior to GC-EIMF analysis.

Ethyl Acetate-Acetone Extract with Reverse Phase Clean-up Method

This following method was modified from the method proposed by Saul et al. (1995) by eliminating the HPLC clean-up and heating on a steam bath under nitrogen flow to concentrate samples. One milliliter of the standard solution containing 11 insecticides and the internal standard (Table 4) was added to samples prior to extraction.

Sample preparation: Corn and navy bean samples were soaked with pesticide-free water overnight. Spinach samples were chopped. Thirty-five grams of each of soaked or chopped sample was mixed with 35-mL saturated sodium sulfate solution in a 0.45-L Mason jar and blended on an Ostracizer blender at high speed for 1 min.

Extraction and clean-up: The mixture was extracted with 70-mL of 5% acetone in ethyl acetate (v/v). Centrifugation was carried out at 2000 rpm for 10 min using a Sorval RC-2B centrifuge (Ivan Sorval Inc., Norwalk, CT). Then 50-mL of the solvent was dried to near dryness on a steam bath under nitrogen flow. The dried sample was dissolved in 2-mL of methanol and then diluted to 10% methanol with water. After activating the C_{18} bonded phase cartridge with 5-mL methanol followed by 5-mL water, the sample was loaded on C_{18} bonded phase

cartridge. After washing the cartridge with 10-mL water, the insecticides were eluted with 2-mL methanol and stored at -18°C under N_2 prior to GC-EIMF analysis.

II. METHOD RELIABILITY EVALUATION

Analysis of Relative Response Factors

Three sets of standard solutions, each containing 11 insecticides and an internal standard, were prepared (Table 5). The retention time of each insecticide or internal standard and the M/z of the most abundant ion determined by GC-EIMS analysis for each insecticide/internal standard were used to set up the program for GC-EIMF. Each of the three standard solutions was analyzed by GC-EIMF on 2 separate periods (days) during this study. The average relative response factors (RR_x s) were determined for each insecticide. The RR_x was determined by the following equation:

$$RR_x = \frac{A_x \times C_s}{A_s \times C_x}$$

where A_x = peak area of insecticide, C_s = concentration of internal standard, A_s = peak area of internal standard, C_x = concentration of insecticide.

Table 5--Concentrations ($\mu\text{g}/\mu\text{L}$) of insecticides and internal standard in three standard solutions

Compound	Standard solution		
	1	2	3
Naled	0.10	0.04	0.07
Phorate	0.10	0.04	0.07
Carbofuran	0.15	0.10	0.15
Dyfonate	0.15	0.10	0.15
Disulfoton	0.15	0.10	0.15
Methyl parathion	0.15	0.10	0.20
Carbaryl	0.15	0.10	0.20
Malathion	0.15	0.10	0.20
Chlorpyrifos	0.15	0.10	0.20
Fenamiphos	0.15	0.20	0.25
Azinphos-methyl	0.50	1.00	1.80
Anthracene-d10 ^a	0.10	0.10	0.10

^aInternal standard.

Percentage Recoveries and Coefficients of Variation

The concentration of each insecticide for each vegetable under the two different multiresidue methods was determined by the following equation:

$$Cx = \frac{Ax \times Cs}{As \times RRx}$$

where Ax = peak area of insecticide, Cs = concentration of internal standard, As = peak area of internal standard, and RRx = average RRx determined from three standard solutions for each insecticide during the period (single day) in which samples were analyzed by GC-EIMF.

The percentage recovery (PR) of each insecticide for each vegetable under the two different MRMs was determined by the following equation:

$$PR (\%) = \frac{(Cx - Y)}{Z} \times 100$$

where Cx = concentration of insecticide in spiked sample, Y = concentration of insecticide in unspiked sample, and Z = concentration of insecticide added to spiked sample. The percentage recovery is a measurement of accuracy of a method with the method having a percentage recovery closest to 100 being the most accurate for any one pesticide (Pomeranz and Meloan, 1987). The precision of each method for each insecticide was determined by the coefficient of variation

(CV) across replication (Pomeranz and Meloan, 1987).

Statistical Analysis

Initially, all dependent variables were analyzed as of a function of independent variables, their interactions and replication by general linear method (GLM) in SAS (SAS institute Inc., 1990). When replication was not significant ($P > 0.05$), it was pooled with the error term.

The relative response factors for each insecticide were analyzed statistically as a function of times (periods) of measurement and standard solutions using GLM. Percentage recovery for each insecticide was analyzed statistically as a function of vegetable analyzed, method of analysis and their interaction using GLM. The coefficient of variation was analyzed as a function of vegetable, method, insecticide, vegetable $\times$ method, vegetable $\times$ insecticide, and method $\times$ insecticide. The three way interaction (vegetable $\times$ method $\times$ insecticide) was used as the error term.

The significant probability of differences (PDIFF) among least-squares means of the relative response factor for each insecticide among standard solutions ($n=3$) and periods of analysis ($n=2$), of percentage recovery for each insecticide among all vegetable-method combinations ($n=6$), and in CVs among vegetable-method combinations ($n=22$) were calculated. A probability level of 0.05 ($P < 0.05$) was taken as the level for a significant difference between two means.

III. RELIABILITY OF ALTERNATE IONS (M/Z) FOR MEASURING INSECTICIDES

Besides selection of the most abundant ion (base peak) produced during GC-EIMS analysis of a specific insecticide for quantitative measurement in the vegetable extracts, an alternate ion, which was at a greater M/z or close to the M/z of the base peak of a single insecticides, was selected for each of 8 insecticides. The 8 insecticides included carbofuran, dyfonate, methyl parathion, carbaryl, malathion, chlorpyrifos, fenamiphos and azinphos-methyl. The alternate ion was used to measure the respective insecticide in the unspiked and spiked vegetable extracts prepared by the two previously described methods. For each insecticide in each vegetable extract, the percent recovery as a measure of accuracy and the coefficient of variation (precision) were determined as described previously. The percent recovery and CV of each insecticide were analyzed using the GLM procedure of SAS Institute Inc. (1990) as a function of type of vegetable, analysis method and their interaction. The method giving the percent recovery closest to 100% and the smallest CV was the most accurate, precise method for any given insecticide. The mean CV was analyzed statistically also as a function of vegetable, method, insecticide and all respective two way interactions in an effort to determine the most precise method for a MRM of the 8 insecticides. Significantly different Least-square means were identified by the PDIFF option of

SAS Institute Inc. (1990). A probability level of 0.05 ($P < 0.05$) was taken as a level defining a significant difference between two means.

IV. DETERMINATION OF SENSITIVITY

GC-EIMF was set to maximum sensitivity (Gain = 5.0) for each set of target ions. Concentrated extracts of unspiked spinach prepared by two methods (acetonitrile extract with florisil clean-up or ethyl acetate-acetone extract with reverse phase clean-up) were obtained. Samples (2 μ L) of the spinach extract from each method were analyzed by GC-EIMF to determine the noise level (maximum vertical height) for the M/z of each insecticide within ± 0.2 min of its retention time; this height was measured by a ruler in cm. Each spinach extract was spiked with a concentration of each insecticide that gave a peak height less than the maximum possible for its M/z on the GC-EIMF chromatogram at a gain of 5.0. The amount of insecticide that gave two times the peak height determined as the noise level for its respective M/z peak in the GC-EIMF analysis of the spinach extract was determined as the estimated detection limit for that insecticide (Pomeranz and Meloan, 1987).

CHAPTER IV

RESULTS AND DISCUSSION

I. MULTIRESIDUE METHODS APPLIED

Analysis of Retention Time and the Most Abundant Ion

The retention time and the most abundant ion for each of 11 insecticides and the internal standard analyzed by GC-EIMS are listed in Table 6. The mass spectrum for each compound is shown in Appendix A. These spectra are comparable to those of Hites (1992). A concentration of each compound was analyzed that gave less than the maximum detection threshold (total ion count 409,500) for the most abundant ion in order to determine the most abundant ion of each insecticide correctly.

Programming the Mass Spectrometer

Using the retention time and the most abundant ion determined by GC-EIMS for each insecticide, the MS program shown in Table 7 was used for GC-EIMF. Two target ions (109 and 75) in ion set one (11.8 – 12.5 min), four target ions (164, 109, 88, and 188) in ion set two (12.6 – 13.4 min), four target ions (109, 144, 125, and 97) in ion set three (13.5 – 14.5 min), and two target ions (303 and 77) in ion set four (14.6 – 20.0 min) were analyzed.

Table 6--Retention times and mass/charge (M/z) of the most abundant ion monitored for 11 insecticides and internal standard in GC-EIMS analysis

Compound	Retention time (min)	Ion monitored (M/z) ^a
Naled	12.2	109
Phorate	12.4	75
Carbofuran	12.7	164
Dyfonate	13.0	109
Disulfoton	13.1	88
Methyl parathion	13.7	109
Carbaryl	13.9	144
Malathion	14.0	125
Chlorpyrifos	14.2	97
Fenamiphos	15.2	303
Azinphos-methyl	18.7	77
Anthracene-d10 ^b	13.2	188

^aM/z = mass/charge where charge = +1.

^bInternal standard.

Table 7--GC-EIMF program for analysis of 11 insecticides and internal standard

Compound	Ion set	Channel	Time period (min)	Ion monitored (M/z)
Naled	1	1	11.8 – 12.5	109
Phorate		2	11.8 – 12.5	75
Carbofuran	2	1	12.6 – 13.4	164
Dyfonate		2	12.6 – 13.4	109
Disulfoton		3	12.6 – 13.4	88
Anthracene-d10 ^a		4	12.6 – 13.4	188
Methyl parathion	3	1	13.5 – 14.5	109
Carbaryl		2	13.5 – 14.5	144
Malathion		3	13.5 – 14.5	125
Chlorpyrifos		4	13.5 – 14.5	97
Fenamiphos	4	1	14.6 – 20.0	303
Azinphos-methyl		2	14.6 – 20.0	77

^a Internal standard.

II. METHOD RELIABILITY EVALUATION

Analysis of Relative Response Factors

The average relative response factor (RRx) of each insecticide in different standard solutions is listed in Table 8. The RRx was averaged across two times of analysis, 5 days apart. The RRxs among standard solutions were not significantly different. Table 9 shows that the RRxs and coefficients of variation (CVs) for 11 insecticides averaged across standard solutions for two different analysis times. The RRxs for naled, carbofuran, methyl parathion, carbaryl, and azinphos-methyl were not significantly different between the 2 times of analysis. The RRxs for phorate, dyfonate, disulfoton, malathion, chlorpyrifos, and fenamiphos increased significantly with the increased time period between analyses. The increase in RRx results from the contamination of the mass spectrometer ion source and analyzer and reflects decreased sensitivity of the mass spectrometer (Yang, 1993). Liao et al. (1991) presented that the GC-MS could not endure multiple analyses without loss of sensitivity. The increase of RRx is due to the decrease in the abundance of the ion used to detect the internal standard at a given concentration or an increase in the abundance of the ion used to measure a given insecticide. The change of ion abundance is dependent on a given M/z rather than on a M/z range in GC-EIMS range (Yang, 1993). Our study confirmed these results. The analysis of variance of the relative response factors for each insecticide is

Table 8--Mean relative response factors^a of each insecticide to internal standard (anthracene-d10) in different standard solutions analyzed by selected ion monitoring on GC-EIMF

Insecticide	Standard solution ^b		
	1	2	3
Naled	0.7745	0.6599	0.6970
Phorate	4.6576	3.9606	4.1391
Carbofuran	0.5671	0.5130	0.5341
Dyfonate	0.9120	0.9644	0.9072
Disulfoton	1.1807	1.1986	1.0944
Methyl parathion	0.2717	0.2417	0.2992
Carbaryl	0.4294	0.4468	0.4661
Malathion	0.3437	0.3718	0.3613
Chlorpyrifos	0.2189	0.2068	0.1892
Fenamiphos	0.6095	0.6482	0.6480
Azinphos-methyl	0.1569	0.1542	0.1665

^aAveraged across two times of analysis 5 days apart.

^bEach standard solution contained different concentrations of each insecticide.

Table 9--Mean relative response factors^a (RRx) averaged across standard solutions and coefficient of variation (CV) of insecticides to internal standard (anthracene-d10) analyzed two different times

Insecticide	Analysis time ^b			
	1 ^c		2 ^d	
	RRx	CV (%)	RRx	CV (%)
Naled	0.7764a	8.70	0.6444a	9.61
Phorate	2.7326b	4.08	5.5831a	13.66
Carbofuran	0.5184a	4.72	0.5411a	8.80
Dyfonate	0.6380b	4.93	1.2104a	5.55
Disulfoton	0.7999b	3.53	1.5159a	5.67
Methyl parathion	0.2726a	11.39	0.2690a	10.81
Carbaryl	0.4963a	6.62	0.4047a	7.79
Malathion	0.3193b	0.91	0.3985a	6.71
Chlorpyrifos	0.1647b	2.03	0.2451a	11.46
Fenamiphos	0.4971b	9.66	0.7733a	1.51
Azinphos-methyl	0.1696a	13.53	0.1488a	14.27

^aMeans in the same row followed by unlike letters are different ($P < 0.05$).

^bAnalysis time 1 and 2 were separated by a 5 day period.

^cn = 3.

^dn = 2.

presented in Appendix B (B-1 to B-11).

Percentage Recoveries

The concentrations of insecticides based on selected ions in unspiked samples are shown in Table 10. The concentrations were used to calculate percent recovery. Two insecticides, dyfonate and chlorpyrifos, were found in measurable levels only in unspiked navy beans using the ethyl acetate-acetone extract and reverse clean-up method. The presence of a monitored ion did not positively indicate the presence of the insecticide; it could have been due to the interference with GC-EIMF analysis at the selected ion. The presence of the suspected insecticide was not confirmed by GC-EIMS analysis. In this study, interference existed in some target ions of interest in unspiked samples and this affected the ion to be monitored. Thus, the influence needed to be corrected.

The least-squares means of percentage recoveries for each insecticide from different vegetables and multiresidue methods are listed in Table 11. The analysis of variance of the percent recoveries for each insecticide as a function of independent variable vegetable, method, replication, and interaction is shown in Appendix C (C-1 to C-11). No significant differences were found among replications for any insecticide.

Naled. The percent recovery of naled was significantly different among vegetables and methods but no significant vegetable $\times$ method interaction was

Table 10--Concentration of insecticides in unspiked samples for different multiresidue methods

Insecticide	Method ^a	Concentration (µg/g)		
		Corn	Navy beans	Spinach
Naled	1	0	0	0
	2	0	0	0
Phorate	1	0	0	0
	2	0	0	0
Carbofuran	1	0	0	0
	2	0	0	0
Dyfonate	1	0	0	0
	2	0	1.04	0
Disulfoton	1	0	0	0
	2	0	0	0
Methyl parathion	1	0	0	0
	2	0	0	0
Carbaryl	1	0	0	0
	2	0	0	0
Malathion	1	0	0	0
	2	0	0	0
Chlorpyrifos	1	0	0	0
	2	0	2.75	0
Fenamiphos	1	0	0	0
	2	0	0	0
Azinphos-methyl	1	0	0	0
	2	0	0	0

^a1 = acetonitrile extract with florisil clean-up; 2 = ethyl acetate-acetone extract with reverse phase clean-up.

Table 11--Least-squares mean^{ab} percent recoveries for 11 insecticides from different vegetables extracted and cleaned up by different methods

Insecticide	Method ^c	Vegetable		
		Corn	Navy beans	Spinach
Naled	1	7a	3b	5ab
	2	5ab	2b	4b
Phorate	1	65d	85c	69d
	2	61d	143a	103b
Carbofuran	1	3d	8d	3d
	2	145c	338a	265b
Dyfonate	1	84c	102c	107c
	2	98c	181b	238a
Disulfoton	1	23d	46cd	34d
	2	67b	109a	54c
Methyl parathion	1	37d	87c	33d
	2	147b	306a	318a
Carbaryl	1	12d	21d	5d
	2	256c	463a	351b
Malathion	1	55e	79d	41e
	2	114c	253a	228b
Chlorpyrifos	1	164b	139c	137c
	2	106d	203a	201a
Fenamiphos	1	1c	8c	9c
	2	128b	245a	226a
Azinphos-methyl	1	3c	16c	2c
	2	130b	345a	152b

^an = 3.

^bFor each insecticide, means in a row or column followed by an unlike letter are different ($P < 0.05$).

^c1 = acetonitrile extract with florisil clean-up; 2 = ethyl acetate-acetone extract with reverse phase clean-up.

found (Appendix C) (C-1). An average of 2 to 7% recovery was found for naled across vegetable and method (Table 11) with coefficients of variation, which ranged from 8.1 to 13.4% (Table 12). The recovery of naled was higher from corn using acetonitrile extraction with the florisil clean-up method than was recovered from navy beans by either method or from spinach by the method using reverse phase SPE clean-up. The low recovery of naled can be explained by its rapid hydrolysis in water. More than 90% of naled in water is hydrolyzed within 48 hr at room temperature (U.S. EPA, 1988). Both methods involved contact with water in the extraction and clean-up steps. The naled most likely was especially susceptible to hydrolysis during steam-bath drying, when moisture would condense on the nitrogen tube and drip into the sample. That hydrolysis rate may have been increased greatly by the high temperature of the steam bath. Agüera and Contreras (1993) used anhydrous sodium sulfate and ethyl acetate to extract green pepper fortified with naled, which was analyzed by GC-MS. They reported a 70% recovery, undoubtedly, addition of sodium sulfate scavenged the water reducing naled hydrolysis.

Phorate. The percent recovery of phorate was significantly affected by vegetable, method, and vegetable $\times$ method interaction (Appendix C) (C-2). Average recovery of phorate across vegetable and method ranged from 61 to 143% (Table 11), and the coefficient of variation ranged from 3.2 to 8.9% (Table 12).

Table 12--Mean coefficients of variation^a (CVs) for 11 insecticides from different vegetables extracted and cleaned up by different methods

Insecticide	Method ^b	Vegetable		
		Corn (%)	Navy beans (%)	Spinach (%)
Naled	1	9.7	13.1	9.2
	2	13.4	8.1	10.4
Phorate	1	8.4	3.2	8.9
	2	7.2	10.1	7.1
Carbofuran	1	14.9	6.7	8.3
	2	9.4	5.3	1.1
Dyfonate	1	14.2	8.2	7.4
	2	12.7	1.3	1.4
Disulfoton	1	6.7	5.1	7.9
	2	7.4	7.3	7.6
Methyl parathion	1	11.2	10.1	9.3
	2	12.4	13.0	2.9
Carbaryl	1	3.9	7.4	9.3
	2	12.0	12.2	10.2
Malathion	1	9.3	13.0	4.8
	2	4.0	4.9	4.1
Chlorpyrifos	1	7.9	3.8	7.0
	2	2.2	6.3	4.4
Fenamiphos	1	2.5	4.6	9.0
	2	8.8	9.8	5.6
Azinphos-methyl	1	8.1	4.8	7.7
	2	9.4	9.3	8.7

^an = 3.

^b1 = acetonitrile extract with florisil clean-up; 2 = ethyl acetate-acetone extract with reverse phase clean-up.

Highest levels of phorate were recovered from navy beans receiving the reverse phase clean-up method. This recovery of 143 % may indicate the presence of interfering compounds at the M/z 75 used to analyze phorate for this vegetable-method combination. However, the lack of a measurable amount of phorate in the unspiked sample at M/z of 75 makes this explanation questionable. The recovery of 103% indicated that the reverse phase clean-up was suitable for phorate in spinach samples and is similar to 112% recovery reported by Benfenati et al. (1990) of phorate from water using reverse phase clean-up followed by the GC-MS analysis.

Liao et al. (1991) reported a 96% recovery of phorate from vegetable extracts receiving no clean-up prior to GC-EIMF analysis. The lower recovery of phorate from the florisil clean-up method may be due to the use of the florisil cartridge. Florisil is suitable for non-polar pesticides, such as organochlorines, and presents problems with moderately polar to polar pesticides (OTA, 1988). Therefore, the moderately polar phorate apparently did not completely elute from the florisil cartridge. However, with the possible exception of phorate analysis by the reverse phase clean-up of navy beans, the precision of either method for the vegetables is such ($\leq 10\%$) that a correction factor may be applied to make methods accurate (Pomeranz and Meloan, 1987).

Carbofuran. The percent recovery of carbofuran was significantly affected by

vegetable, method, and vegetable $\times$ method interaction (Appendix C) (C-3).

Average recovery of carbofuran across vegetable and method ranged from 3 to 338% (Table 11), with a coefficient of variation ranged from 1.1 to 14.9% (Table 12). The higher recovery especially from navy beans was found with reverse phase clean-up method. Liao et al. (1991) used acetonitrile to extract vegetables and found an average recovery of 110% for carbofuran. Nash (1990) extracted carbofuran from water on a C₁₈ reverse phase cartridge and found an average 77% recovery.

The extremely low rate of recovery resulting from the florisil clean-up method most likely was due to the relatively high polarity of carbofuran, which was not eluted from the florisil cartridge. Another reason for its low recovery might be that the thermal instability of carbofuran led to its degradation during the GC analysis (Müller and Stan, 1990). In addition, liquid-liquid partition (LLP), which was used in our florisil clean-up method, might give lower recoveries due to the formation of an emulsion (Odanaka et al., 1991). The extremely high recoveries of carbofuran from the reverse phase clean-up method most likely are due to co-extractives eluted from the C₁₈ cartridge, resulting in the interference on the GC-EIMF analysis of carbofuran detected using the ion at M/z of 164.

Dyfonate. The percent of recovery for this insecticide was significantly affected by vegetable, method and vegetable $\times$ method interaction (Appendix C) (C-4). Average recovery of dyfonate across vegetable and method ranged from 84

to 238% (Table 11), with a coefficient of variation ranging from 1.4 to 14.2% (Table 12). The recovery of dyfonate in navy beans (102%) and spinach (107%) treated with the florisil clean-up method indicated this method was accurate. The recovery in corn (98%) receiving reverse phase clean-up was also very accurate. However, as a MRM, the acetonitrile extract with florisil clean-up provides the most accurate method for dyfonate with detection at M/z 109 in the vegetables analyzed. Acetonitrile extraction has shown fairly accurate results for dyfonate previously. Fillion et al. (1995) reported an 88% recovery of dyfonate from vegetable and fruit samples with an acetonitrile extraction and confirmation by GC-EIMF analysis.

A relatively large amount of dyfonate was recovered from spinach and navy beans with ethyl acetate-acetone extraction and reverse phase clean-up. Its higher recovery from spinach might be due to plant pigments or lipids in spinach that interfered with the abundance of ion monitored ($M/z = 109$).

Disulfoton. The percent recovery of disulfoton was significantly affected by vegetable, method, and vegetable $\times$ method interaction (Appendix C) (C-5). Average recovery of disulfoton across vegetable and method ranged from 23 to 109% (Table 11), with a coefficient of variation that ranged from 5.1 to 7.9% (Table 12). The lower recovery was found in all vegetables extracted by acetonitrile followed by florisil clean-up method. The more accurate recovery was

found in navy beans with ethyl acetate-acetone with the reverse phase clean-up method.

Since Liao et al. (1991) found 84% recovery of disulfoton in vegetable samples with acetonitrile extraction and GC-MF analysis, the disulfoton most likely was not eluted from the florisil cartridge in the present study. Ambrus and Thier (1986) investigated the elution behavior of organophosphates on florisil and found that the disulfoton only partially eluted from the florisil when using ethyl ether in petroleum ether; however, disulfoton could be quantitatively eluted from florisil with a solvent mixture of benzene, ethyl ether, and acetone. Thus, changing the solvent polarity can elute the more polar organophosphates from florisil. Lower percent recoveries of disulfoton from corn and spinach compared with that from navy beans can not really be explained at the time; however, it is possible that disulfoton was bound by chemical components present in these two vegetables but not in the navy beans.

Methyl parathion. The percent recovery of methyl parathion was significantly affected by vegetable, method, and vegetable $\times$ method interaction (Appendix C) (C-6). Average recovery of methyl parathion across vegetable and method ranged from 33 to 318% (Table 11), with a coefficient of variation ranging from 2.9 to 13.0% (Table 12). An acceptable recovery from navy beans (87%) showed that methyl parathion could be recovered with a florisil clean-up. Ambrus and Thier (1986) also proved that the methyl parathion could be quantitatively eluted from

florisil using 6% EE in PE. However, the lower recovery of methyl parathion in corn and spinach with the florisil clean-up method showed that the recovery of the same insecticide depended on the vegetable. While the polarity of the florisil cartridge has been shown to differ from batch to batch (OTA, 1988), the precision of the analysis of methyl parathion in each of the vegetables by method 1 (Table 12) is such that it is unlikely that these differences contributed to the significantly lower percent recovery in corn and spinach. Although Liao et al. (1991) reported 105% recovery of methyl parathion using acetonitrile for extraction and no clean-up, these latter researchers extracted only fresh vegetables and did not determine the percent recovery from corn, navy beans or spinach. However, the fact that they did obtain full recovery of methyl parathion from fresh vegetables, indicated that perhaps methyl parathion was bound by some component in the corn and spinach which was not present in the navy beans.

Higher recovery, far in excess of 100%, was found in vegetable samples receiving the reverse phase clean-up method rather than the florisil clean-up method. Holland et al. (1994) reported 150% recovery of methyl parathion from white wine when methyl parathion was extracted by C₁₈ reverse phase clean-up. The co-extractives, which cannot be removed from vegetables, may have given an enhanced interference of the target ion (M/z 109) monitored, but the effect was not found in the unspiked sample (Table 10).

Carbaryl. The percent recovery of carbaryl was significantly affected by vegetable, method, and vegetable $\times$ method interaction (Appendix C) (C-7). Average recovery of carbaryl across vegetable and method ranged from 5 to 463% (Table 11), with a coefficient of variation ranging from 3.9 to 12.2% (Table 12). Extremely high levels in excess of 250% of carbaryl were recovered when using the reverse phase clean-up method; navy beans had the highest level of recovery. These high levels of recovery may indicate interference with measurement of the carbaryl at M/z 144. These interfering components might be due to plant wax or other components; Ambrus and Their (1986) reported that the C₁₈ reverse phase cartridge was not effective in removing plant wax in the analysis of organophosphates. A high recovery of carbaryl (230%) also was reported in wine samples using C₁₈ reverse phase extraction with GC-ECD and GC-NPD analysis (Holland et al., 1994). The latter report showed that the co-extractives not removed by the reverse phase clean-up enhanced the recovery of carbaryl even using these detectors. However, once again the effect of interfering compounds with carbaryl analysis in unspiked samples at M/z 144 did not result in a measurable amount in those samples.

Much lower recoveries of carbaryl were found using a florisil clean-up for all vegetables than in the reverse phase clean-up method (Table 11). Liao et al. (1991) reported 128% recovery of carbaryl extracted from fresh vegetables using acetonitrile extraction and no clean-up of the extract before determined by GC-

MF. The lower recoveries using the florisil clean-up can be explained by polarity of carbaryl, which could not be completely recovered from the florisil cartridge using EE/PE solvent. Increasing the polarity of the elution solvent might help elute the polar insecticides. Another reason that might have resulted in low recoveries is that the N-methylcarbamates such as carbaryl, are thermally labile and may be decomposed during GC analysis (Müller and Stan, 1990).

Conventional methods use high performance liquid chromatography for routine analysis for thermally labile N-methylcarbamates, although some reports indicate that direct GC analysis can be used (Kendrick et al., 1991; Wigfield et al., 1993). The splitless injection used in the present experiment at the initial low temperature in the GC column should cause little degradation of the N-methylcarbamates during GC analysis.

Malathion. The percent recovery of malathion was affected significantly by vegetable, method, and vegetable $\times$ method interaction (Appendix C) (C-8). Average recovery of malathion across vegetable and method was between 41 and 228% (Table 11), with a coefficient of variation ranging from 4.0 to 13.0% (Table 12). Highest recoveries of malathion were found in navy beans and spinach receiving the reverse phase clean-up method. This indicated that there was interference with detection of malathion at M/z of 125. The interference may be from co-extractives not removed by the reverse phase clean-up method; however, more research is needed to determine the type of interference.

Lower recoveries of malathion were obtained from vegetable samples treated with the florisil clean-up method than when treated with reverse phase clean-up method; however, the recoveries also depended on the vegetables receiving the same clean-up method. Liao et al. (1991) showed a 120% recovery of malathion using an acetonitrile extraction and no further clean-up. The recovery of the moderately polar malathion could be improved, perhaps, if the florisil clean-up were eliminated.

Chlorpyrifos. The percent recovery of chlorpyrifos was affected significantly by vegetable, method, but not vegetable $\times$ method interaction (Appendix C) (C-9). Average recovery of chlorpyrifos across vegetable and method ranged from 106 to 201% (Table 11), with a coefficient of variation ranging from 2.2 to 7.9% (Table 12). An acceptable recovery was obtained from the corn samples (106%) receiving the reverse phase clean-up method. Agüera and Contreras (1993) reported a 98% recovery of chlorpyrifos from vegetable and fruit samples using sodium sulfate and ethyl acetate followed by no further clean-up before GC-MS analysis. Benfenati et al. (1990) extracted chlorpyrifos from water with a reverse phase clean-up and GC-MS analysis and found a recovery of 85%. Relatively high levels of chlorpyrifos were recovered from navy beans and spinach receiving reverse phase clean-up. Perhaps, co-extractives eluted with the chlorpyrifos by methanol somehow enhanced the response of the GC-EIMF analysis of chlorpyrifos at M/z 97.

Fenamiphos. The percent recovery of fenamiphos was significantly affected by vegetable, method, and vegetable $\times$ method interaction (Appendix C) (C-10). Average recovery of fenamiphos across vegetable and method ranged from 1 to 245% (Table 11), with a coefficient of variation ranging from 2.5 to 9.8% (Table 12). Recoveries greater than 100% were found in vegetable samples treated with a reverse phase clean-up, indicating that interference existed in GC-EIMF analysis at the selected ion of M/z 303 was enhanced in some manner.

The extremely low recovery of fenamiphos from all vegetables treated with the florisil clean-up method indicated that the polar fenamiphos could not be recovered very well from florisil with EE/PE solvents used. Yang (1993) reported that some organophosphates with high polarity were completely lost during the florisil clean-up with the same solvent system.

Azinphos-methyl. The percent recovery of azinphos-methyl was significantly affected by vegetable, method, and vegetable $\times$ method interaction (Appendix C) (C-11). Average recovery of azinphos-methyl across vegetable and method ranged from 2 to 345% (Table 11), with a coefficient of variation ranging from 4.8 to 9.4% (Table 12). High recovery was found from vegetables with reverse phase clean-up; the navy beans had the highest recovery. Loconto and Gaind (1989) reported a 105% recovery of azinphos-methyl using reverse phase clean-up and a GC-NPD fitted with a DB-5 column for analysis. Holland et al. (1994) presented a 180% recovery from wine samples using C₁₈ reverse phase extraction with GC-

ECD and GC-NPD analysis. The high recovery obtained from the reverse phase clean-up method showed that components, which enhanced ECD and NPD detection, existed in the wine extract.

The low recovery from the florisil clean-up method indicated that the azinphos-methyl either did not elute from the florisil cartridge or did not partition into EE/PE solution. Mattern et al. (1991) found that azinphos-methyl could not be recovered from corn using the florisil clean-up and GC-CIMS analysis. The result was similar to ours, which had only 3% recovery. The lack of recovery of organophosphates receiving a florisil clean-up could be improved by either using more polar eluting solvents or eliminating the clean-up step. Pylypiw (1993) also indicated that using acetonitrile will extract more undesirable polar co-extractives, which require the clean-up step that usually lowers pesticide recovery.

Coefficients of Variation

When the mean CVs were analyzed as a function of vegetable, method, insecticide and all possible two-way interactions among these three independent variables (Appendix D), there were no significant effects at the $P < 0.05$ level. However, when the Least-squares means for CV were separated across insecticide-method combinations by PDIFF (Table 13), differences were found among the CVs of the 22 insecticide-method combinations. Method 2 for carbofuran, dyfonate, malathion and chlorpyrifos and method 1 for fenamiphos by method 1 were more precise ($P < 0.05$) than measurements of naled by either method;

Table 13--Least-squares mean (n=3) coefficients of variation^a (CVs) for 11 insecticides analyzed in different vegetables by two different methods

Insecticide	Method ^b	CV (%)
Naled	1	10.6ab
	2	10.6ab
Phorate	1	6.8bcde
	2	8.1abcde
Carbofuran	1	10.0ab
	2	5.3cde
Dyfonate	1	9.9ab
	2	5.1cde
Disulfoton	1	6.6bcde
	2	7.4abcde
Methyl parathion	1	10.2ab
	2	9.4abc
Carbaryl	1	6.9bcde
	2	11.5a
Malathion	1	9.0abcd
	2	4.4e
Chlorpyrifos	1	6.3bcde
	2	4.3e
Fenamiphos	1	5.4cde
	2	8.1abcde
Azinphos-methyl	1	6.9bcde
	2	9.2abcd

^aMeans in a column followed by unlike letters are different ($P < 0.05$).

^b1 = acetonitrile extract with florisil clean-up; 2 = ethyl acetate-acetone extract with reverse phase clean-up.

carbofuran, dyfonate or methyl parathion by method 1 or carbaryl by method 2 ($CVs \leq 5.4\%$ versus $CVs \geq 9.9\%$). In addition, for carbofuran, dyfonate, carbaryl and malathion, method 2 (extraction with ethyl acetate-acetone followed by reverse phase clean-up) was more precise (lower CVs; $P < 0.05$) than method 1 (extraction with acetonitrile followed by florisil clean-up). In general, then method 2 gave more precise measurements of more insecticides than did method 1. However, all CVs (Table 12) were below 15%, a level of precision reported to be acceptable in the analysis of pesticides in vegetables by a multiple residue analysis method (Gretch and Rosen, 1987).

Conclusions

Thus, for method 1 in which the vegetables were extracted by acetonitrile followed by partitioning into 6% EE in PE and clean-up by elution from a florisil column with solvents containing up to 35% EE in PE (v/v), only dyfonate could be accurately determined in navy beans and spinach. However, the precision of the method was satisfactory for all insecticides, and the percent recoveries for dyfonate in corn and phorate, disulfoton, methyl parathion, malathion and chlorpyrifos in all three vegetables were large enough (> 22) for correction factors to be applied to the quantities of these insecticides determined by this method to obtain accurate results (Pomeranz and Meloan, 1987). A correction factor of a precise method is determined by dividing 100% by the actual percent recovery for the analyte measured by the method. The level of the analyte determined by the

method is multiplied by the factor to get an accurate estimate. However, five of the insecticides, naled, carbofuran, carbaryl, fenamiphos, and azinphos-methyl were too polar either to be partitioned into 6% EE in PE (v/v) or to be eluted from a florisil cartridge by eluting solvents containing up to 35% EE in PE (v/v). The recoveries for each of these latter insecticides from each vegetable were, for the most part, less than 10%. More polar solvents need to be used for partitioning and elution from the florisil column if these insecticides are to be measured using method 1.

Contrary to the results with method 1, results with method 2 showed promise for the detection of all insecticides except naled. However, the reverse phase clean-up method did not remove as many co-extractives as did method 1. These co-extractives enhanced GC-EIMF measurement of several insecticides resulting in recoveries far in excess of 100% were obtained for several of the insecticides. For any insecticide, though, this was dependent upon the vegetable. For example, phorate could be determined in spinach accurately using method 2 with detection at M/z 75 ion, but not accurately in corn or navy beans. Method 2 also could determine accurately dyfonate, chlorpyrifos, and possibly malathion in corn, and disulfoton in navy beans without the application of a correction factor. Otherwise, with the exception of these latter examples in specific vegetables and naled in all three vegetables, correction factors would have to be applied to obtain accurate results for the quantity of insecticide present.

By method 2 the recoveries of eight of the insecticides: dyfonate in navy beans and spinach and carbofuran, methyl parathion, carbaryl, malathion, chlorpyrifos, fenamiphos, and azinphos-methyl in all three vegetables were in excess of 100% due to enhancement of co-extractives for responses at the M/z ions chosen for insecticide measurement by GC-EIMF. This enhancement possibly could be reduced or eliminated by selection of another ion of a known M/z present in the mass spectrum of each insecticide for GC-EIMF analysis. Alferness and Iwata (1994) indicated that selecting high-mass ions results in a reduced amount or lack of interference in GC-EIMF analysis. Mattern and Rosen (1992) showed that the relative probability of interference with M/z in the range of 50 to 99, 100 to 159, 160 to 219, 220 to 279, 280 to 339, were 187:83:49:13:1, respectively. Therefore, for each of these latter insecticides, a new target ion was selected, which had a higher M/z than that used previously or was close to the M/z of the previous target ion if that ion had the highest M/z and was also the most abundant ion for that insecticide. All unspiked and spiked vegetable samples extracted and cleaned up by the two methods were reanalyzed by GC-EIMF using the new target ions for these eight insecticides.

III. RELIABILITY OF ALTERNATE IONS (M/Z) FOR MEASURING INSECTICIDES

Percentage Recoveries

The new target ions used to detect and measure 8 insecticides in three vegetables: corn, navy beans and spinach, which were extracted and cleaned-up by two methods, are given in Table 14. The Least-squares means for the percent recoveries for 8 insecticides from different vegetables and extraction methods using the new target ions are shown in Table 15. The percent recovery of each insecticide was significantly affected by vegetable, method and vegetable $\times$ method interaction except for no differences ($P > 0.05$) between methods for chlorpyrifos and among vegetables for dyfonate (Appendix E) (E-1 to E-8).

Selection of the higher/alternate ions of a given M/z improved the interference problem vegetable extracts receiving the reverse phase clean-up method. The recoveries of the 8 insecticides extracted from all vegetables by method 2 (reverse phase clean-up) and measured by the new target ions were all reduced from those using the first set of target ions (Tables 11 and 15). Accurate results (recoveries between 95 and 105%) were obtained using method 2 and the new target ions for dyfonate in spinach, methyl parathion in corn and spinach, and chlorpyrifos in navy beans. Correction factors could be applied to make method 2 and detection by the new target ions accurate for dyfonate in corn and navy beans, methyl

Table 14--New target ion selected for 8 insecticides from different vegetables extracted and cleaned up by different methods

Insecticide	New ion monitored (M/z)
Carbofuran	122
Dyfonate	137
Methyl parathion	263
Carbaryl	145
Malathion	173
Chlorpyrifos	199
Fenamiphos	288
Azinphos-methyl	132

Table 15--Least-squares mean^{ab} percent recoveries for 8 insecticides from different vegetables extracted and cleaned up by different methods at new selected target ions

Insecticide	Method ^c	Vegetable		
		Corn	Navy beans	Spinach
Carbofuran	1	2c	5c	2c
	2	61b	71a	75a
Dyfonate	1	84b	58c	61c
	2	57c	86b	96a
Methyl parathion	1	23d	30c	21d
	2	101b	118a	103b
Carbaryl	1	9e	15d	4f
	2	44b	89a	40c
Malathion	1	32e	43d	49d
	2	114c	222a	136b
Chlorpyrifos	1	77b	78b	81b
	2	57c	99a	83b
Fenamiphos	1	1d	5c	3cd
	2	33a	34a	23b
Azinphos-methyl	1	1d	8b	1d
	2	3c	26a	3c

^an = 3.

^bFor each insecticide means in a row or column followed by an unlike letter are different ($P < 0.05$).

^c1 = acetonitrile extract with florisil clean-up; 2 = ethyl acetate-acetone extract with reverse phase clean-up.

parathion in navy beans, chlorpyrifos in corn and spinach, azinphos-methyl in navy beans, and carbofuran, carbaryl, malathion, and fenamiphos in all three vegetables (Table 15).

For measurement of azinphos-methyl in method 2 extracts, the new target ion with a M/z 132 did not really show enough recovery to be used for quantitation in corn or spinach and the 26% recovery in navy beans really should be higher (Table 15) for accurate results, even with applied correction factor. The recoveries of azinphos-methyl using the ion with a M/z of 77 to measure the insecticide in method 2 extracts of corn and spinach were 130 and 152%, respectively (Table 11); application of a correction factor to the measurements using M/z 77 ion detection could give fairly accurate results for azinphos-methyl in corn and spinach. However, the 345% recovery of this insecticide from method 2 extracts of navy beans using the M/z 77 ion for detection may be too high to obtain accurate results even when applying a correction factor.

Selection of new target ions generally reduced the percent recoveries in method 1 extracts of all vegetables when compared with that from the first set of target ions (Tables 11 and 15). For several insecticides, method 1 using florisil clean-up and the first set of target ions, resulted in percentage recoveries close to 100% or of high enough recoveries to be corrected for accurate results (Table 11). However, the selection of the new target ion of M/z 199 instead of 97 for chlorpyrifos reduced the percent recoveries in the method 1 extracts of the three

vegetables from 137 - 164 (Table 11) to 77 - 81 (Table 15). Perhaps, the ion with a M/z of 199 is better for measurement and detection of chlorpyrifos than the ion with a M/z of 97.

Coefficients of Variation

Means for the CVs of the percent recoveries of each of the 8 insecticides for each vegetable-method combination are shown in Table 16. The CVs for all insecticide-method-vegetable combinations were less than 15%, a level of precision acceptable in the analysis of pesticides (Gretch and Rosen, 1987).

When the mean CVs were analyzed as a function of vegetable, method, insecticide and all possible two-way interactions among these three independent variables (Appendix F), only method was significantly different. However, when the Least-squares means for CV were separated across insecticide-method combinations (Table 17), differences were found among the CVs of the 16 insecticide-method combinations. For 2 insecticides, carbofuran and methyl parathion, CVs for method 1 were higher than for method 2. For 4 other insecticides, dyfonate, carbaryl, chlorpyrifos and azinphos-methyl, the CVs for method 1 appeared to be greater than the CVs for method 2, and for 2 other insecticides, malathion and fenamiphos, the CVs for method 2 appeared to be greater than those for method 1. Overall, using the new target ions (Table 14) to measure the 8 insecticides resulted in a more precise analysis by method 2 ($P < 0.05$) than by method 1 (mean CV of 5.1 versus 8.0%, respectively).

Table 16--Mean coefficients of variation^a (CVs) for 8 insecticides from different vegetables extracted and cleaned up by different methods using new selected target ions

Insecticide	Method ^b	Vegetable		
		Corn (%)	Navy beans (%)	Spinach (%)
Carbofuran	1	13.28	9.36	10.49
	2	2.91	4.07	5.81
Dyfonate	1	9.62	4.56	5.53
	2	2.95	6.92	4.93
Methyl parathion	1	6.04	5.89	11.45
	2	3.36	2.13	2.66
Carbaryl	1	6.24	11.81	7.71
	2	8.27	1.66	6.51
Malathion	1	3.29	6.20	6.21
	2	5.27	2.37	8.57
Chlorpyrifos	1	12.76	6.00	4.88
	2	7.10	3.06	1.07
Fenamiphos	1	6.52	8.58	8.49
	2	5.39	4.16	12.76
Azinphos-methyl	1	12.27	7.48	8.34
	2	5.89	7.90	2.57

^an = 3.

^b1 = acetonitrile extract with florisil clean-up; 2 = ethyl acetate-acetone extract with reverse phase clean-up.

Table 17--Least-squares mean (n=3) coefficients of variation^a (CVs) for 8 insecticides analyzed by new target ions (M/z) in different vegetables by two different methods

Insecticide	Method ^b	CV (%)
Carbofuran	1	11.0a
	2	4.3cd
Dyfonate	1	6.6abcd
	2	4.9bcd
Methyl parathion	1	7.8abc
	2	2.7d
Carbaryl	1	8.6abc
	2	5.5bcd
Malathion	1	5.2bcd
	2	5.4bcd
Chlorpyrifos	1	7.9abc
	2	3.7cd
Fenamiphos	1	7.9abc
	2	8.4abc
Azinphos-methyl	1	9.4ab
	2	5.5bcd

^aMeans in a column followed by unlike letters are different ($P < 0.05$).

^b1 = acetonitrile extract with florisil clean-up; 2 = ethyl acetate-acetone extract with reverse phase clean-up.

While an analytical chemical definition of an accurate, precise method is one resulting in 95 to 105% recovery and a CV of $\pm 5\%$, the recovery range increases and the magnitude of the CV increases with each magnitude decrease in analyte concentration measured (Pomeranz and Meloan, 1987). As already stated, Gretch and Rosen (1987) reported that a CV of $\pm 15\%$ was acceptable for the precision of an analytical method for measurement of pesticides. Burke (1968) considered a 80 to 110% recovery acceptable for the accuracy of a pesticide analysis method.

Conclusions

Either method tested using alternate ions in this study is precise enough (CV < 15%) to be used for the analysis of any of the 8 insecticides in either corn, navy beans or spinach using the second set of target ions (Table 14). With this latter set of target ions, method 1 could be used for an accurate measurement of dyfonate in corn and chlorpyrifos in spinach. Compared with these results using the set of target ions in Table 14, method 2 could accurately measure the level of methyl parathion in corn; dyfonate, carbaryl, and chlorpyrifos in navy beans; and dyfonate, methyl parathion, and chlorpyrifos in spinach.

An accurate estimate of the level of any insecticide with greater than 22% recovery using the second set of target ions (Table 14) probably could be made with the application of a correction factor. However, as percent recovery decreases, difficulty in measuring analyte concentration increases. Thus, accurate results probably could be obtained with method 1 for carbofuran, carbaryl,

fenamiphos or azinphos-methyl in any of the vegetables using the target ions listed in Table 14. Also, it would be nearly impossible to determine accurate amounts of azinphos-methyl in corn or spinach using method 2 with these same target ions.

On the Shimadzu QP-1000, the GC-EIMF or selective ion monitoring mode, only 16 target ions can be monitored in any one analysis; 4 ions at a time may be measured during any one time period for 4 different time periods. On new Shimadzu GC-mass spectrometers, up to 32 ions can be monitored during any one time period for 32 different time periods or over 1,000 different ions during any one analysis (Dodgen, 1997). A combination of ions with different mass to charge (M/z) ratios can be used to detect and measure any one insecticide. Thus, with method 2 using the reverse phase clean-up, on a newer mass spectrometer, two or more target ions could be used to measure 10 of the insecticides in vegetable extracts, and the ion(s) giving the most accurate estimate for any one insecticide-vegetable combination could be used for quantitative analysis of that insecticide in that vegetable.

IV. DETERMINATION OF SENSITIVITY

The GC-EMIF minimum detectable quantities (MDQs) for 11 insecticides detected by the target ions listed in Table 6 in spinach extracts prepared by the two different methods are shown in Table 18. For method 1, MDQs ranged from

Table 18--GC-EIMF minimum detectable quantities^a (μg) for 11 insecticides in spinach extract with different methods

Insecticide	Ion monitored (M/z)	Method ^b	
		1	2
Naled	109	0.0220	0.0130
Phorate	75	0.0070	0.0021
Carbofuran	164	0.0002	0.0002
Dyfonate	109	0.0013	0.0021
Disulfoton	88	0.0005	0.0001
Methyl parathion	109	0.0720	0.0280
Carbaryl	144	0.0023	0.0010
Malathion	125	0.0230	0.0125
Chlorpyrifos	97	0.0880	0.0420
Fenamiphos	303	0.0005	0.0003
Azinphos-methyl	77	0.4460	0.8860

^aEstimated as amount to give two times the maximum GC-EIMF baseline noise on the most sensitive GC-EIMF setting for each spinach extract within ± 0.2 min of insecticide GC-EIMF retention time.

^b1 = acetonitrile extract with florisil clean-up; 2 = ethyl acetate-acetone extract with reverse phase clean-up.

0.0005 to 0.4460 μg for the 11 insecticides, and for method 2, MDQs ranged from 0.0001 to 0.8860 μg . The highest MDQs in spinach extracts from both methods were for azinphos-methyl; this insecticide essentially was not recovered in method 1 extracts and had large interference in method 2 extracts (Table 11) when measured using the ion of M/z 77. With the exception of azinphos-methyl, methyl parathion and chlorpyrifos in spinach extracts from method 1, the MDQs for all of the other insecticides were less than 0.5 μg .

The MDQs for 8 insecticides using a second set of different target ions (Table 14) than used previously are shown in Table 19. Again, the insecticide with the greatest detection in spinach extract from both methods was azinphos-methyl. In general, for any one insecticide, when measured at the alternate target ion (Table 14), the MDQ remained nearly at the same level or increased slightly with compared with the MDQ determined using the primary target ion (Table 6). One possible reason for this observation, is the fact that the primary target ion was at the M/z of the most abundant ion formed from the insecticide during mass spectrometric analysis. Another consideration is that for several of the insecticides, which were analyzed using alternate target ions, there was interference from co-extractives with detection at the primary target ions resulting in excess of 100% recovery or enhanced GC-EIMF response (Table 11).

MDQs of eight of the insecticides studied have been established by Kadenczki et al. (1992) using a nitrogen-phosphorus-detector (NPD) in analysis of pesticides.

Table 19--GC-EIMF minimum detectable quantities^a (μg) for 8 insecticides in spinach extract with different methods using new target ions

Insecticide	Ion monitored (M/z)	Method ^b	
		1	2
Carbofuran	122	0.0006	0.0003
Dyfonate	137	0.0053	0.0024
Methyl parathion	263	0.0605	0.0350
Carbaryl	145	0.0064	0.0016
Malathion	173	0.0210	0.0150
Chlorpyrifos	199	0.1650	0.1400
Fenamiphos	288	0.0004	0.0003
Azinphos-methyl	132	0.6130	0.9220

^aEstimated as amount to give two times the maximum GC-EIMF baseline noise on most sensitive GC-EIMF setting for each spinach extract within ± 0.2 min of insecticide GC-EIMF retention time.

^b1 = acetonitrile extract with florisil clean-up; 2 = ethyl acetate-acetone extract with reverse phase clean-up.

These MDQs are listed in Table 20. The MDQs determined by NPD are much lower than the MDQs determined by GC-EIMF in the spinach extracts. Two possible reasons for these disparities are (1) the inherent sensitivity of the detectors with the NPD being two to three orders of magnitude more sensitive than the GC-EIMF (Nielsen et al., 1994; Pomeranz and Meloan, 1987), and (2) the probability of interference from spinach co-extractives with GC-EIMF analyses. Although Kadenczki et al. (1992) did not report how they determined the MDQs on the NPD, sensitivity measurements for pesticides commonly are made in pure solvents and not food extracts (Liao et al., 1991; Saul et al., 1995), thus eliminating possibilities of interference with pesticide measurements.

To convert MDQs of the insecticides in the spinach extracts to limits of detection (ppm) in the present study, the MDQ of each insecticide should be multiplied by 40. Thus, if the MDQs from method 2 in Table 18 for naled, dyfonate, disulfoton, methyl parathion, carbaryl, malathion and azinphos-methyl are multiplied by 40, the detection limits for these insecticides in spinach extracts are 0.52, 0.084, 0.004, 1.12, 0.04, 0.5 and 35 $\mu\text{g/g}$ spinach, respectively. With the exception of methyl parathion and azinphos-methyl, these detection limits from GC-EIMF are below the tolerances for the insecticides in spinach (Table 2), and for methyl parathion, the detection limit (1.12 ppm) is only marginally greater than the allowed tolerance (1 ppm) for its presence in spinach. However, it is obvious,

Table 20--Minimum detectable quantities (MDQs) for 8 insecticides reported from other literature (Kadenczki et al., 1992)^a

Insecticide	MDQs (μ g)
Phorate	0.00003
Carbofuran	0.00050
Dyfonate	0.00002
Methyl parathion	0.00002
Carbaryl	0.00050
Malathion	0.00005
Chlorpyrifos	0.00001
Azinphos-methyl	0.00010

^aBy GC-NPD.

that a method of detection other than GC-EIMF is necessary to measure azinphos-methyl in spinach at or below its tolerance (2.0 ppm).

CHAPTER V

SUMMARY

The reliabilities of two multiresidue methods (MRMs): method 1, which used an acetonitrile extract with florisil clean-up reported by Yang (1993), and method 2, which used ethyl acetate-acetone extract with reverse phase clean-up as reported by Saul et al. (1995), for three vegetables (corn, navy beans and spinach) for 11 organophosphate and N-methylcarbamate insecticides were determined and compared. The insecticides included naled, phorate, carbofuran, dyfonate, disulfoton, methyl parathion, carbaryl, malathion, chlorpyrifos, fenamiphos and azinphos-methyl. The reliability of each method was assessed by determining the accuracy (percent recovery) and precision (coefficient of variation) for each insecticide in each vegetable and sensitivity (minimum detectable quantity) for each insecticide in spinach. An accurate method had 80 - 110% recovery with a recovery closest to 100% being the most accurate; a precise method had a coefficient of variation (CV) of $\pm 15\%$ with the most precise method having the smallest CV; and the most sensitive method had the smallest minimum detectable quantity (MDQ) in a spinach extract prepared by each method.

A replication consisted of spiking each vegetable with a known amount of each of the 11 insecticides, extracting an unspiked vegetable sample and the spiked vegetable sample by each of the two methods, and analyzing the amount of each

insecticide in the unspiked and spiked sample by gas chromatography-mass spectrometry (GC-MS). Three replications were run. The insecticides were analyzed on a Shimadzu Model 9-AM gas chromatograph equipped with a 30-m x 0.25-mm i.d. fused silica PTE-5 capillary column interfaced with a Shimadzu Model QP1000 mass spectrometer using electron ionization (EI). Splitless injection was used, and an internal standard method using anthracene-d10 was used for quantification of each insecticide. A temperature program was used which allowed resolution of each of the 11 insecticides and the internal standard. The mass spectrum of each insecticide was obtained, and the mass/charge (M/z) of the most abundant ion (base peak) where $z = 1$ was determined and used in selective ion monitoring (SIM). For each of 8 insecticides, an alternate ion with a M/z close to or greater than that of the base peak was selected also and used in SIM. The percent recovery (PR) was analyzed statistically as a function of vegetable extracted ($n=3$), method ($n=2$) and their interaction for each insecticide. The CV was analyzed as a function of vegetable, method, pesticide ($n=11$) and all two way interactions for the SIM using base peak ions and for the alternate ions for 8 insecticides.

The accuracy of any one MRM for a given insecticide was dependent largely upon the insecticide, the vegetable and the method. Each method was precise enough ($CVs < \pm 15\%$) to analyze any of the 11 insecticides investigated. Using the alternate ions in SIM across 8 insecticides, method 2 was ($P < 0.05$) more

precise ($CV = 5.1\%$) than method 1 ($CV = 8.0\%$).

Using the recovery of 80 to 110% as a measure of accuracy showed that naled was not recovered well enough ($PRs < 8$) by either MRM to be analyzed accurately. Also, percent recoveries for carbofuran, carbaryl, fenamiphos, and azinphos-methyl for method 1 using either of their target ions in SIM analysis were really not great enough ($PRs < 21$) for these insecticides to be analyzed using that method.

Using the base peak in SIM to measure the levels of phorate, dyfonate and possibly, disulfoton, methyl parathion and malathion, gave high enough recoveries in extracts of corn, navy beans and spinach for method 1 to be used for their analysis. However, a correction factor ($100/PR$) would have to be applied to amounts of phorate and methyl parathion in corn and spinach extracts and of disulfoton and malathion in extracts from all three vegetables by method 1 for acceptable accuracy. Using an alternate ion at a higher M/z than its base peak ion (199 versus 97) for chlorpyrifos on method 1 extracts from all three vegetables decreased the PRs from a range of 129 - 164 to 77 - 81, allowing fairly accurate analysis of chlorpyrifos by method 1. Thus, without consideration of sensitivity, method 1 could be used to determine precisely and accurately (with the application of a correction factor to insecticides with PRs between 23 and 80 or over 110), 6 of the 11 insecticides studied. These 6 insecticides include phorate, dyfonate, disulfoton, methyl parathion, malathion, and chlorpyrifos. The MDQs of each of

these 6 insecticides (Table 18) can be converted to the limits of detection in spinach by being multiplied by 40. With the exception of methyl parathion, method 1 using SIM can measure the levels of the other insecticides near or below their allowable limits in spinach and other vegetables (Table 2).

For four of the insecticides, carbofuran, carbaryl, fenamiphos and azinphos-methyl, which could not be analyzed using method 1, method 2 could be used. Phorate, dyfonate, disulfoton, methyl parathion, malathion, and chlorpyrifos also could be analyzed successfully in extracts of all three vegetables, but depending upon the vegetable and insecticide, correction factors would have to be applied to obtain accurate results. Thus, without consideration of sensitivity, method 2 is the best MRM for the analysis of the most insecticides in the vegetables, corn, navy beans and spinach, investigated in this study.

Although mass spectrometry is not the most sensitive method for detection of organophosphate and N-methylcarbamate insecticides, the sensitivity of the method 2 using SIM was low enough to measure in spinach, 6 of 7 insecticides at or below levels allowed (Table 2). Those 6 insecticides included naled, dyfonate, disulfoton, methyl parathion, carbaryl and malathion. Method 2 using SIM was not very sensitive for azinphos-methyl and could not measure that insecticide anywhere near its tolerance in spinach. For the other insecticides, phorate, carbofuran, and chlorpyrifos, for which tolerances were found in the literature for corn and/or navy beans (Table 2), method 2 also could measure these insecticides

at or below their allowable levels if the sensitivity were the same as in spinach. It is also of interest to note that if enough naled could have been recovered, then method 2 also could have measured that insecticide near or below its allowable level in spinach. Therefore, method 2 could provide precise, accurate (if a correction factor is applied where PR is between 23 and 80 or greater than 110), measurement of 9 of the 11 insecticides investigated in this study in corn, navy beans and spinach, at or below levels allowed in these products. These 9 insecticides include phorate, carbofuran, dyfonate, disulfoton, methyl parathion, carbaryl, malathion, chlorpyrifos, and fenamiphos.

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APPENDICES

APPENDIX A.

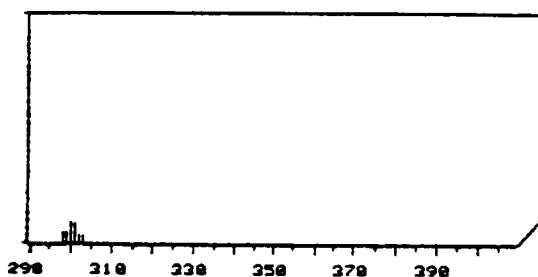
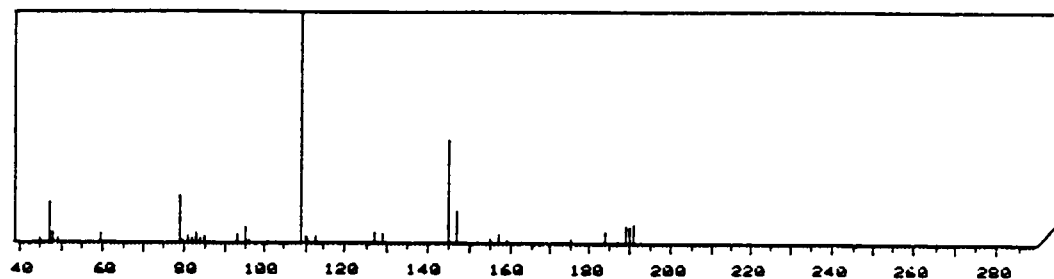
Mass spectra of eleven insecticides

(a).

FILENAME:STD .01
COMMENT:MASS SPECTRA

MODE: EI
EI: 70 GAIN: 2.5
IS TEMP: 250 SCAN SPEED: 10
COL TEMP: 234

DATA	R.T.	PEAK	MASS	RANGE	BASE	PEAK	TOTAL	RAW-	E.G.
22	12.2	142	43-	305	165700(	109)	695440	0-	0
		62	44-	305	165700(	109)	629228	0-	0



(b).

FILENAME:STD .01
COMMENT:MASS SPECTRA

MODE: EI
EI: 70 GAIN: 2.0
IS TEMP: 250 SCAN SPEED: 10
COL TEMP: 239

DATA	R.T.	PEAK	MASS	RANGE	BASE	PEAK	TOTAL	RAW-	E.G.
2	12.4	105	41-	264	330100(	75)	1208715	0-	0
		45	41-	262	330100(	75)	1143720	0-	0

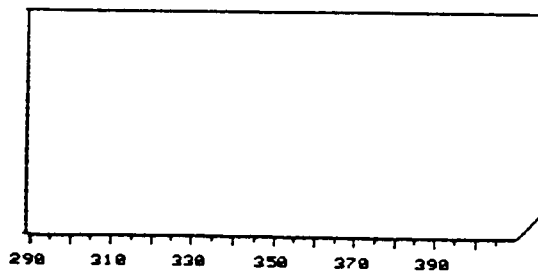
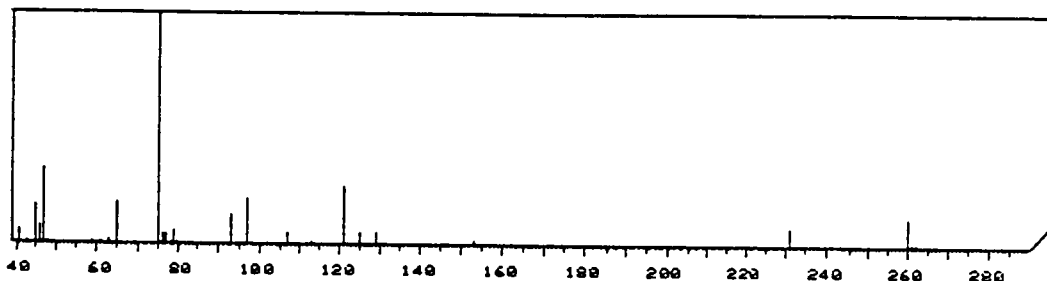


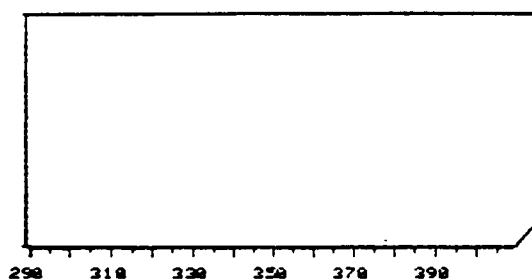
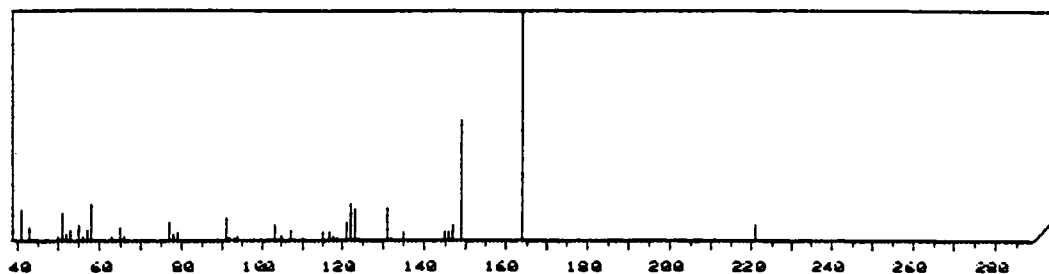
Fig. A-1--Mass spectra of the (a) naled and (b) phorate

(a).

FILENAME: STD .02
COMMENT: MASS SPECTRA

MODE: EI
EUI: 70 GAIN: 2.0
IS TEMP: 250 SCAN SPEED: 10
COL TEMP: 242

DATA	R.T.	PEAK	MASS	RANGE	BASE	PEAK	TOTAL	RAW	E.G.
9	12.7	25	41-	221	208000	(164)	346977	0-	0
		53	41-	221	208000	(164)	331649	0-	0



(b).

FILENAME: STD .01
COMMENT: MASS SPECTRA

MODE: EI
EUI: 70 GAIN: 2.0
IS TEMP: 250 SCAN SPEED: 10
COL TEMP: 245

DATA	R.T.	PEAK	MASS	RANGE	BASE	PEAK	TOTAL	RAW	E.G.
11	13.0	122	41-	249	246900	(109)	953614	0-	0
		49	43-	248	246900	(109)	877270	0-	0

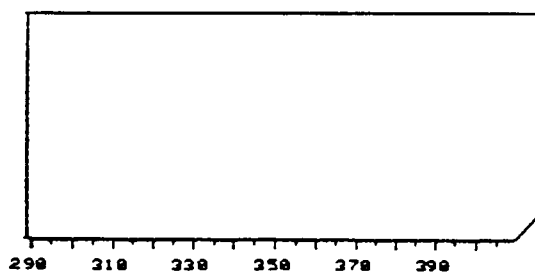
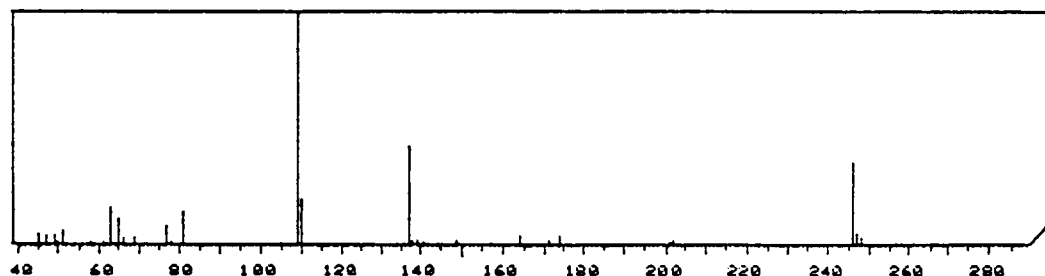


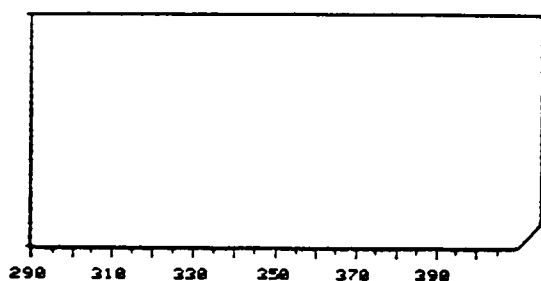
Fig. A-2--Mass spectra of the (a) carbofuran and (b) dyfonate

(a).

FILENAME: STD .01
COMMENT: MASS SPECTRA

MODE: EI
EI: 70 GAIN: 2.0
IS TEMP: 250 SCAN SPEED: 10
COL TEMP: 248

DATA	R.T.	PEAK	MASS RANGE	BASE PEAK	TOTAL	RAW- B.G.
6	13.1	135	41- 381	227000(82)	1001566	0- 0
		56	41- 276	227000(82)	1013077	0- 0



(b).

FILENAME: STD .01
COMMENT: MASS SPECTRA

MODE: EI
EI: 70 GAIN: 2.5
IS TEMP: 250 SCAN SPEED: 10
COL TEMP: 260

DATA	R.T.	PEAK	MASS RANGE	BASE PEAK	TOTAL	RAW- B.G.
39	13.7	151	42- 381	116100(109)	740513	0- 0
		82	43- 265	116100(109)	695462	0- 0

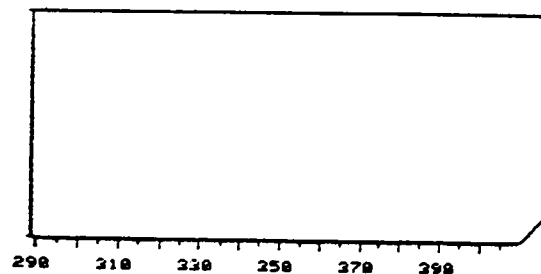
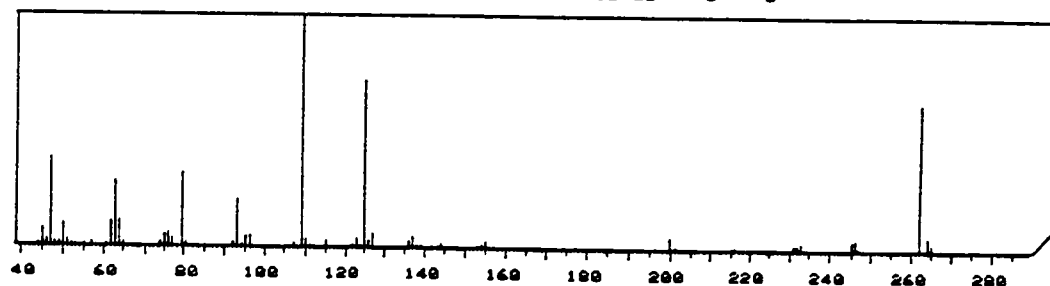


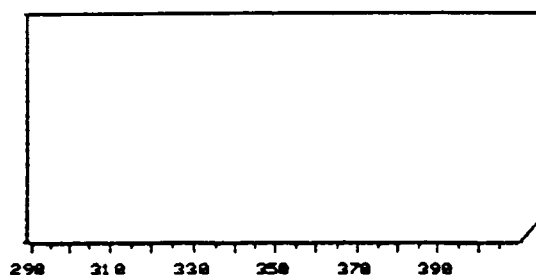
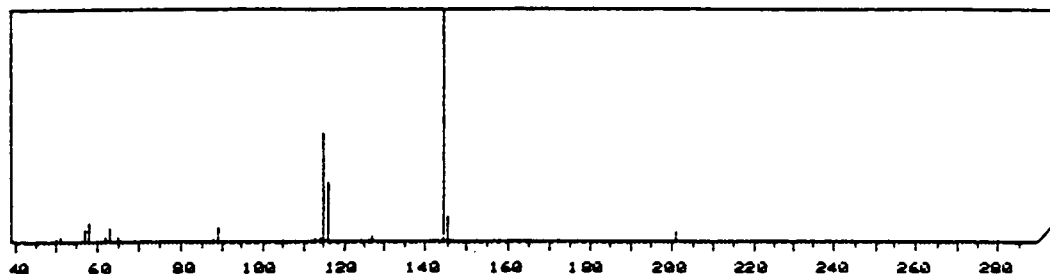
Fig. A-3--Mass spectra of the (a) disulfoton and (b) methyl parathion

(a).

FILENAME:STD .01
COMMENT:MASS SPECTRA

MODE: EI
EU: 70 GAIN: 2.0
IS TEMP: 250 SCAN SPEED: 10
COL TEMP: 265

DATA	R.T.	PEAK MASS RANGE	BASE PEAK	TOTAL	RAW- E.G.
61	13.9	58 43- 201	172300(144)	447955	0- 0
		58 43- 201	172300(144)	447955	0- 0



(b).

FILENAME:STD .01
COMMENT:MASS SPECTRA

MODE: EI
EU: 70 GAIN: 2.0
IS TEMP: 250 SCAN SPEED: 10
COL TEMP: 259

DATA	R.T.	PEAK MASS RANGE	BASE PEAK	TOTAL	RAW- E.G.
10	14.0	142 41- 331	92800(125)	842134	0- 0
		142 41- 331	92800(125)	842134	0- 0

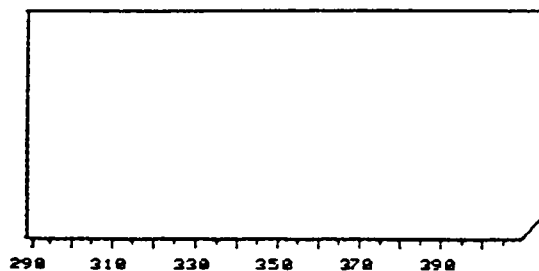
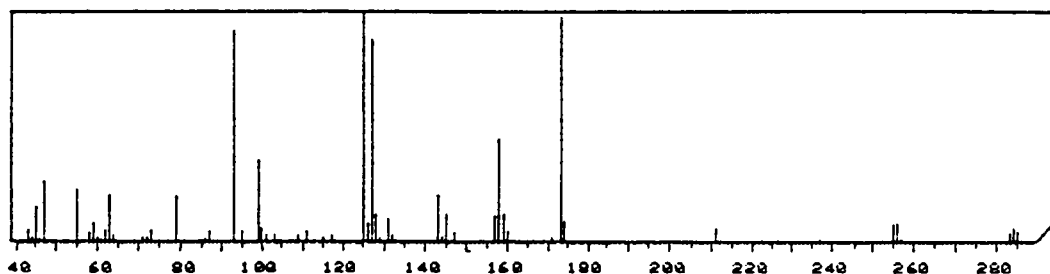


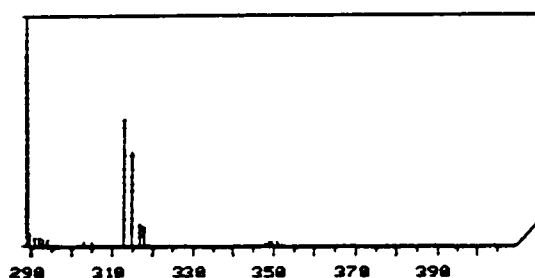
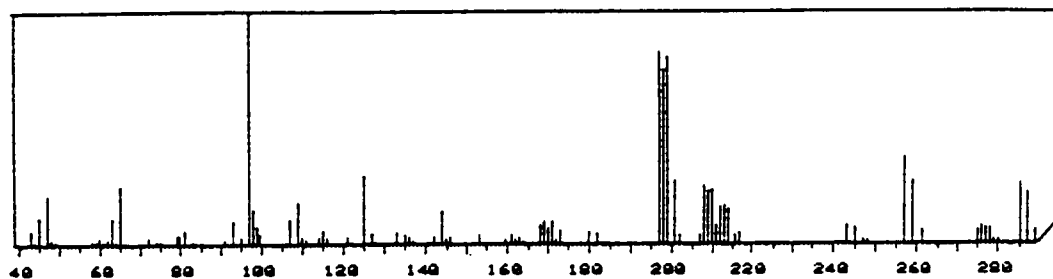
Fig A-4.--Mass spectra of the (a) carbaryl and (b) malathion

(a).

FILENAME:STD .01
COMMENT:MASS SPECTRA

MODE: EI
EI: 70 GAIN: 2.0
IS TEMP: 250 SCAN SPEED: 10
COL TEMP: 264

DATA	R.T.	PEAK	MASS RANGE	BASE PEAK	TOTAL	RAW	E.G.
15	14.2	202	41- 253	68200(97)	917529	0-	0
		156	43- 352	68200(97)	895908	0-	0



(a).

FILENAME: MIX .03
COMMENT: ORI+D-10

MODE: EI
EU: 70 GAIN: 4.5
IS TEMP: 250 SCAN SPEED: 10
COL TEMP: 270

DATA	R.T.	PEAK	MASS	RANGE	BASE	PEAK	TOTAL	RAW	B.G.
312	18.7	195	37-	400	43700	77	592379	0-	0
		186	40-	400	43700	77	592909	0-	0

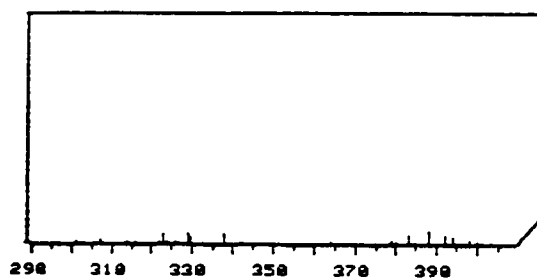
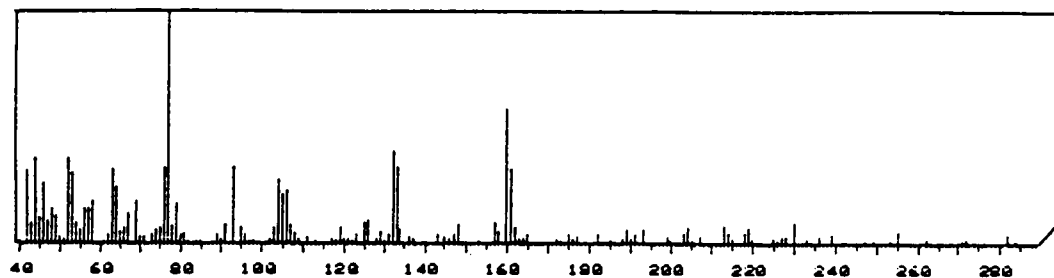


Fig. A-6--Mass spectra of the (a) azinphos-methyl

APPENDIX B.

**Tabulated analyses of variance for the relative response factors of eleven
insecticides**

B-1--Analysis of variance for the relative response factor (RRx) of naled averaged across standard solutions and two different times

General Linear Models Procedure

Dependent Variable: RRx

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	0.10543887	0.02108777	3.67	0.0434
Error	9	0.05165143	0.00573905		
Corrected Total	14	0.15709031			
		R-Square	C.V.	Root MSE	RRx Mean
		0.671199	10.46873	0.07576	0.72365

Source	DF	Type III SS	Mean Square	F Value	Pr > F
STD	2	0.03280369	0.01640185	2.86	0.1094
TIME	1	0.06272915	0.06272915	10.93	0.0091
STD*TIME	2	0.00750258	0.00375129	0.65	0.5432

Tests of Hypotheses using the Type III MS for STD*TIME as an error term

Source	DF	Type III SS	Mean Square	F Value	Pr > F
STD	2	0.03280369	0.01640185	4.37	0.1861
TIME	1	0.06272915	0.06272915	16.72	0.0549

B-2--Analysis of variance for the relative response factor (RRx) of phorate averaged across standard solutions and two different times

General Linear Models Procedure

Dependent Variable: RRx

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	31.6537935	6.3307587	42.31	0.0001
Error	9	1.3466467	0.1496274		
Corrected Total	14	33.0004402			
	R-Square	C.V.	Root MSE		RRx Mean
	0.959193	9.988129	0.38682		3.87277

Source	DF	Type III SS	Mean Square	F Value	Pr > F
STD	2	1.8023280	0.9011640	6.02	0.0219
TIME	1	29.2519865	29.2519865	195.50	0.0001
STD*TIME	2	1.0500595	0.5250297	3.51	0.0747

Tests of Hypotheses using the Type III MS for STD*TIME as an error term

Source	DF	Type III SS	Mean Square	F Value	Pr > F
STD	2	1.8023280	0.9011640	1.72	0.3681
TIME	1	29.2519865	29.2519865	55.71	0.0175

B-3--Analysis of variance for the relative response factor (RRx) of carbofuran averaged across standard solutions and two different times

General Linear Models Procedure

Dependent Variable: RRx

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	0.01453327	0.00290665	1.33	0.3328
Error	9	0.01962076	0.00218008		
Corrected Total	14	0.03415403			
	R-Square	C.V.	Root MSE		RRx Mean
	0.425521	8.851464	0.04669		0.52750

Source	DF	Type III SS	Mean Square	F Value	Pr > F
STD	2	0.00879585	0.00439792	2.02	0.1889
TIME	1	0.00185941	0.00185941	0.85	0.3798
STD*TIME	2	0.00497542	0.00248771	1.14	0.3617

Tests of Hypotheses using the Type III MS for STD*TIME as an error term

Source	DF	Type III SS	Mean Square	F Value	Pr > F
STD	2	0.00879585	0.00439792	1.77	0.3613
TIME	1	0.00185941	0.00185941	0.75	0.4784

B-4--Analysis of variance for the relative response factor (RRx) of dyfonate averaged across standard solutions and two different times

General Linear Models Procedure

Dependent Variable: RRx

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	1.20354838	0.24070968	17.08	0.0002
Error	9	0.12683391	0.01409266		
Corrected Total	14	1.33038229			
		R-Square	C.V.	Root MSE	RRx Mean
		0.904664	13.69344	0.11871	0.86693

Source	DF	Type III SS	Mean Square	F Value	Pr > F
STD	2	0.01224385	0.00612192	0.43	0.6605
TIME	1	1.17955522	1.17955522	83.70	0.0001
STD*TIME	2	0.01417163	0.00708582	0.50	0.6209

Tests of Hypotheses using the Type III MS for STD*TIME as an error term

Source	DF	Type III SS	Mean Square	F Value	Pr > F
STD	2	0.01224385	0.00612192	0.86	0.5365
TIME	1	1.17955522	1.17955522	166.47	0.0060

B-5--Analysis of variance for the relative response factor (RRx) of disulfoton averaged across standard solutions and two different times

General Linear Models Procedure

Dependent Variable: RRx

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	1.88009897	0.37601979	15.19	0.0004
Error	9	0.22273558	0.02474840		
Corrected Total	14	2.10283455			
		R-Square	C.V.	Root MSE	RRx Mean
		0.894078	14.48205	0.15732	1.08628

Source	DF	Type III SS	Mean Square	F Value	Pr > F
STD	2	0.02979871	0.01489936	0.60	0.5683
TIME	1	1.84579784	1.84579784	74.58	0.0001
STD*TIME	2	0.00945350	0.00472675	0.19	0.8294

Tests of Hypotheses using the Type III MS for STD*TIME as an error term

Source	DF	Type III SS	Mean Square	F Value	Pr > F
STD	2	0.02979871	0.01489936	3.15	0.2408
TIME	1	1.84579784	1.84579784	390.50	0.0026

B-6--Analysis of variance for the relative response factor (RRx) of methyl parathion averaged across standard solutions and two different times

General Linear Models Procedure

Dependent Variable: RRx

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	0.00920920	0.00184184	6.57	0.0077
Error	9	0.00252120	0.00028013		
Corrected Total	14	0.01173040			
	R-Square	C.V.	Root MSE		RRx Mean
	0.785071	6.171699	0.01674		0.27119

Source	DF	Type III SS	Mean Square	F Value	Pr > F
STD	2	0.00793810	0.00396905	14.17	0.0017
TIME	1	0.00004688	0.00004688	0.17	0.6921
STD*TIME	2	0.00074395	0.00037198	1.33	0.3124

Tests of Hypotheses using the Type III MS for STD*TIME as an error term

Source	DF	Type III SS	Mean Square	F Value	Pr > F
STD	2	0.00793810	0.00396905	10.67	0.0857
TIME	1	0.00004688	0.00004688	0.13	0.7565

B-7--Analysis of variance for the relative response factor (RRx) of Carbaryl averaged across standard solutions and two different times

General Linear Models Procedure

Dependent Variable: RRx

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	0.04063060	0.00812612	4.73	0.0213
Error	9	0.01544564	0.00171618		
Corrected Total	14	0.05607624			
	R-Square	C.V.	Root MSE		RRx Mean
	0.724560	9.012952	0.04143		0.45964

Source	DF	Type III SS	Mean Square	F Value	Pr > F
STD	2	0.00516867	0.00258434	1.51	0.2728
TIME	1	0.03018091	0.03018091	17.59	0.0023
STD*TIME	2	0.00478062	0.00239031	1.39	0.2972

Tests of Hypotheses using the Type III MS for STD*TIME as an error term

Source	DF	Type III SS	Mean Square	F Value	Pr > F
STD	2	0.00516867	0.00258434	1.08	0.4805
TIME	1	0.03018091	0.03018091	12.63	0.0709

B-8--Analysis of variance for the relative response factor (RRx) of malathion averaged across standard solutions and two different times

General Linear Models Procedure

Dependent Variable: RRx

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	0.02550255	0.00510051	9.26	0.0024
Error	9	0.00495873	0.00055097		
Corrected Total	14	0.03046128			
	R-Square	C.V.	Root MSE		RRx Mean
	0.837212	6.687035	0.02347		0.35102

Source	DF	Type III SS	Mean Square	F Value	Pr > F
STD	2	0.00193627	0.00096814	1.76	0.2269
TIME	1	0.02259143	0.02259143	41.00	0.0001
STD*TIME	2	0.00153699	0.00076849	1.39	0.2967

Tests of Hypotheses using the Type III MS for STD*TIME as an error term

Source	DF	Type III SS	Mean Square	F Value	Pr > F
STD	2	0.00193627	0.00096814	1.26	0.4425
TIME	1	0.02259143	0.02259143	29.40	0.0324

B-9--Analysis of variance for the relative response factor (RRx) of chlorpyrifos averaged across standard solutions and two different times

General Linear Models Procedure

Dependent Variable: RRx

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	0.02649605	0.00529921	14.83	0.0004
Error	9	0.00321558	0.00035729		
Corrected Total	14	0.02971163			
		R-Square	C.V.	Root MSE	RRx Mean
		0.891774	9.601607	0.01890	0.19686

Source	DF	Type III SS	Mean Square	F Value	Pr > F
STD	2	0.00212031	0.00106016	2.97	0.1024
TIME	1	0.02327524	0.02327524	65.14	0.0001
STD*TIME	2	0.00171772	0.00085886	2.40	0.1457

Tests of Hypotheses using the Type III MS for STD*TIME as an error term

Source	DF	Type III SS	Mean Square	F Value	Pr > F
STD	2	0.00212031	0.00106016	1.23	0.4476
TIME	1	0.02327524	0.02327524	27.10	0.0350

B-10--Analysis of variance for the relative response factor (RRx) of fenamiphos averaged across standard solutions and two different times

General Linear Models Procedure

Dependent Variable: RRx

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	0.28890277	0.05778055	33.25	0.0001
Error	9	0.01563900	0.00173767		
Corrected Total	14	0.30454178			
	R-Square	C.V.	Root MSE		RRx Mean
	0.948647	6.860630	0.04169		0.60760

Source	DF	Type III SS	Mean Square	F Value	Pr > F
STD	2	0.00477388	0.00238694	1.37	0.3016
TIME	1	0.27451962	0.27451962	157.98	0.0001
STD*TIME	2	0.00695132	0.00347566	2.00	0.1911

Tests of Hypotheses using the Type III MS for STD*TIME as an error term

Source	DF	Type III SS	Mean Square	F Value	Pr > F
STD	2	0.00477388	0.00238694	0.69	0.5929
TIME	1	0.27451962	0.27451962	78.98	0.0124

B-11--Analysis of variance for the relative response factor (RRx) of azinphos-methyl averaged across standard solutions and two different times

General Linear Models Procedure

Dependent Variable: RRx

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	0.00652279	0.00130456	1.00	0.4708
Error	9	0.01177145	0.00130794		
Corrected Total	14	0.01829424			
	R-Square	C.V.	Root MSE		RRx Mean
	0.356549	22.42775	0.03617		0.16125

Source	DF	Type III SS	Mean Square	F Value	Pr > F
STD	2	0.00040329	0.00020165	0.15	0.8593
TIME	1	0.00156171	0.00156171	1.19	0.3029
STD*TIME	2	0.00428630	0.00214315	1.64	0.2473

Tests of Hypotheses using the Type III MS for STD*TIME as an error term

Source	DF	Type III SS	Mean Square	F Value	Pr > F
STD	2	0.00040329	0.00020165	0.09	0.9140
TIME	1	0.00156171	0.00156171	0.73	0.4832

APPENDIX C.

Tabulated analyses of variance for percent recoveries of eleven insecticides

C-1--Analysis of variation for percent recovery of naled from different vegetables extracted and cleaned up by different methods

General Linear Models Procedure

Dependent Variable: PR

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	7	39.8344499	5.6906357	26.64	0.0001
Error	10	2.1359862	0.2135986		
Corrected Total	17	41.9704361			
		R-Square	C.V.	Root MSE	PR Mean
		0.949107	10.13919	0.46217	4.55823

Source	DF	Type III SS	Mean Square	F Value	Pr > F
VEG	2	30.6479273	15.3239637	71.74	0.0001
METHOD	1	7.9088529	7.9088529	37.03	0.0001
REP	2	0.7318484	0.3659242	1.71	0.2292
VEG*METHOD	2	0.5458213	0.2729106	1.28	0.3205

C-2--Analysis of variation for percent recovery of phorate from different vegetables extracted and cleaned up by different methods

General Linear Models Procedure

Dependent Variable: PR

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	7	14764.3872	2109.1982	31.31	0.0001
Error	10	673.5791	67.3579		
Corrected Total	17	15437.9663			
		R-Square	C.V.	Root MSE	PR Mean
		0.956369	9.353236	8.20719	87.7470

Source	DF	Type III SS	Mean Square	F Value	Pr > F
VEG	2	7948.85266	3974.42633	59.00	0.0001
METHOD	1	3825.50138	3825.50138	56.79	0.0001
REP	2	43.49559	21.74780	0.32	0.7313
VEG*METHOD	2	2946.53754	1473.26877	21.87	0.0002

C-3--Analysis of variation for percent recovery of carbofuran from different vegetables extracted and cleaned up by different methods

General Linear Models Procedure

Dependent Variable: PR

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	7	326108.845	46586.978	599.31	0.0001
Error	10	777.342	77.734		
Corrected Total	17	326886.187			
	R-Square	C.V.	Root MSE		PR Mean
	0.997622	6.942283	8.81670		127.000

Source	DF	Type III SS	Mean Square	F Value	Pr > F
VEG	2	29690.935	14845.468	190.98	0.0001
METHOD	1	269094.760	269094.760	3461.73	0.0001
REP	2	246.203	123.102	1.58	0.2527
VEG*METHOD	2	27076.947	13538.473	174.16	0.0001

C-4--Analysis of variation for percent recovery of dyfonate from different vegetables extracted and cleaned up by different methods

General Linear Models Procedure

Dependent Variable: PR

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	7	56015.7971	8002.2567	120.89	0.0001
Error	10	661.9428	66.1943		
Corrected Total	17	56677.7399			
	R-Square	C.V.	Root MSE		PR Mean
	0.988321	6.014310	8.13599		135.277

Source	DF	Type III SS	Mean Square	F Value	Pr > F
VEG	2	20292.2025	10146.1012	153.28	0.0001
METHOD	1	25206.7450	25206.7450	380.80	0.0001
REP	2	235.3537	117.6769	1.78	0.2185
VEG*METHOD	2	10281.4959	5140.7479	77.66	0.0001

C-5--Analysis of variation for percent recovery of disulfoton from different vegetables extracted and cleaned up by different methods

General Linear Models Procedure

Dependent Variable: PR

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	7	13883.7804	1983.3972	118.79	0.0001
Error	10	166.9635	16.6963		
Corrected Total	17	14050.7438			
	R-Square	C.V.	Root MSE		PR Mean
	0.988117	7.345248	4.08612		55.6294

Source	DF	Type III SS	Mean Square	F Value	Pr > F
VEG	2	4261.55068	2130.77534	127.62	0.0001
METHOD	1	8089.41002	8089.41002	484.50	0.0001
REP	2	74.78117	37.39059	2.24	0.1572
VEG*METHOD	2	1458.03849	729.01924	43.66	0.0001

C-6--Analysis of variation for percent recovery of methyl parathion from different vegetables extracted and cleaned up by different methods

General Linear Models Procedure

Dependent Variable: PR

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	7	249029.405	35575.629	86.39	0.0001
Error	10	4117.848	411.785		
Corrected Total	17	253147.253			
	R-Square	C.V.	Root MSE		PR Mean
	0.983733	13.12123	20.2925		154.654

Source	DF	Type III SS	Mean Square	F Value	Pr > F
VEG	2	37033.966	18516.983	44.97	0.0001
METHOD	1	188429.066	188429.066	457.59	0.0001
REP	2	98.755	49.378	0.12	0.8883
VEG*METHOD	2	23467.618	11733.809	28.50	0.0001

C-7--Analysis of variation for percent recovery of carbaryl from different vegetables extracted and cleaned up by different methods

General Linear Models Procedure

Dependent Variable: PR

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	7	598315.579	85473.654	80.36	0.0001
Error	10	10636.978	1063.698		
Corrected Total	17	608952.557			
	R-Square	C.V.	Root MSE		PR Mean
	0.982532	17.67611	32.6144		184.511

Source	DF	Type III SS	Mean Square	F Value	Pr > F
VEG	2	35779.630	17889.815	16.82	0.0006
METHOD	1	532708.291	532708.291	500.81	0.0001
REP	2	236.698	118.349	0.11	0.8958
VEG*METHOD	2	29590.960	14795.480	13.91	0.0013

C-8--Analysis of variation for percent recovery of malathion from different vegetables extracted and cleaned up by different methods

General Linear Models Procedure

Dependent Variable: PR

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	7	123691.499	17670.214	245.39	0.0001
Error	10	720.088	72.009		
Corrected Total	17	124411.587			
	R-Square	C.V.	Root MSE		PR Mean
	0.994212	6.627064	8.48580		128.048

Source	DF	Type III SS	Mean Square	F Value	Pr > F
VEG	2	20243.0275	10121.5137	140.56	0.0001
METHOD	1	88344.3263	88344.3263	1226.85	0.0001
REP	2	74.8586	37.4293	0.52	0.6099
VEG*METHOD	2	15029.2863	7514.6432	104.36	0.0001

C-9--Analysis of variation for percent recovery of chlorpyrifos from different vegetables extracted and cleaned up by different methods

General Linear Models Procedure

Dependent Variable: PR

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	7	22184.3842	3169.1977	36.58	0.0001
Error	10	866.4509	86.6451		
Corrected Total	17	23050.8351			
	R-Square	C.V.	Root MSE		PR Mean
	0.962411	5.877200	9.30833		158.380

Source	DF	Type III SS	Mean Square	F Value	Pr > F
VEG	2	4740.2790	2370.1395	27.35	0.0001
METHOD	1	17184.4960	17184.4960	198.33	0.0001
REP	2	212.9788	106.4894	1.23	0.3332
VEG*METHOD	2	46.6304	23.3152	0.27	0.7694

C-10--Analysis of variation for percent recovery of fenamiphos from different vegetables extracted and cleaned up by different methods

General Linear Models Procedure

Dependent Variable: PR

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	7	192806.676	27543.811	174.62	0.0001
Error	10	1577.336	157.734		
Corrected Total	17	194384.012			
	R-Square	C.V.	Root MSE		PR Mean
	0.991885	12.21402	12.5592		102.826

Source	DF	Type III SS	Mean Square	F Value	Pr > F
VEG	2	13325.415	6662.708	42.24	0.0001
METHOD	1	169078.808	169078.808	1071.93	0.0001
REP	2	150.031	75.016	0.48	0.6349
VEG*METHOD	2	10252.421	5126.211	32.50	0.0001

C-11--Analysis of variation for percent recovery of azinphos-methyl from different vegetables extracted and cleaned up by different methods

General Linear Models Procedure

Dependent Variable: PR

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	7	268739.257	38391.322	146.41	0.0001
Error	10	2622.165	262.217		
Corrected Total	17	271361.423			

R-Square	C.V.	Root MSE	PR Mean
0.990337	15.00325	16.1931	107.931

Source	DF	Type III SS	Mean Square	F Value	Pr > F
VEG	2	47900.391	23950.195	91.34	0.0001
METHOD	1	183971.251	183971.251	701.60	0.0001
REP	2	103.649	51.824	0.20	0.8238
VEG*METHOD	2	36763.966	18381.983	70.10	0.0001

APPENDIX D.

**Tabulated analysis of variance for coefficient of variance of percent recovery for
eleven insecticides**

D--Analysis of variance for coefficient of variation (CV) of percent recovery for 11 insecticides from different vegetables extracted and cleaned up by different methods

General Linear Models Procedure

Dependent Variable: CV

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	45	581.244202	12.916538	1.91	0.0598
Error	20	135.595776	6.779789		
Corrected Total	65	716.839977			
	R-Square	C.V.	Root MSE		CV Mean
	0.810842	33.33352	2.60380		7.81136

Source	DF	Type III SS	Mean Square	F Value	Pr > F
VEG	2	43.712518	21.856259	3.22	0.0612
METHOD	1	3.607347	3.607347	0.53	0.4742
INSECT	10	141.546627	14.154663	2.09	0.0774
VEG*METHOD	2	28.232058	14.116029	2.08	0.1509
VEG*INSECT	20	206.605182	10.330259	1.52	0.1771
METHOD*INSECT	10	157.540470	15.754047	2.32	0.0521

APPENDIX E.

Tabulated analyses of variance for percent recoveries for eight insecticides at new selected target ions

E-1--Analysis of variation for percent recovery of carbofuran from different vegetables extracted and cleaned up by different methods at new selected target ions

General Linear Models Procedure

Dependent Variable: PR

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	7	20032.6446	2861.8064	860.75	0.0001
Error	10	33.2478	3.3248		
Corrected Total	17	20065.8924			
	R-Square	C.V.	Root MSE		PR Mean
	0.998343	5.084374	1.82340		35.8628

Source	DF	Type III SS	Mean Square	F Value	Pr > F
VEG	2	195.3215	97.6608	29.37	0.0001
METHOD	1	19672.6836	19672.6836	5916.99	0.0001
REP	2	28.0927	14.0463	4.22	0.0468
VEG*METHOD	2	136.5467	68.2734	20.53	0.0003

E-2--Analysis of variation for percent recovery of dyfonate from different vegetables extracted and cleaned up by different methods at new selected target ions

General Linear Models Procedure

Dependent Variable: PR

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	7	4235.66562	605.09509	22.21	0.0001
Error	10	272.49003	27.24900		
Corrected Total	17	4508.15565			
	R-Square	C.V.	Root MSE		PR Mean
	0.939556	7.071812	5.22006		73.8150

Source	DF	Type III SS	Mean Square	F Value	Pr > F
VEG	2	198.95430	99.47715	3.65	0.0645
METHOD	1	630.48005	630.48005	23.14	0.0007
REP	2	16.98223	8.49112	0.31	0.7391
VEG*METHOD	2	3389.24903	1694.62452	62.19	0.0001

E-3--Analysis of variation for percent recovery of methyl parathion from different vegetables extracted and cleaned up by different methods at new selected target ions

General Linear Models Procedure

Dependent Variable: PR

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	7	31630.5713	4518.6530	649.47	0.0001
Error	10	69.5742	6.9574		
Corrected Total	17	31700.1455			
		R-Square	C.V.	Root MSE	PR Mean
		0.997805	3.993444	2.63769	66.0506

Source	DF	Type III SS	Mean Square	F Value	Pr > F
VEG	2	587.0688	293.5344	42.19	0.0001
METHOD	1	30979.7535	30979.7535	4452.77	0.0001
REP	2	2.9527	1.4763	0.21	0.8124
VEG*METHOD	2	60.7963	30.3982	4.37	0.0433

E-4--Analysis of variation for percent recovery of carbaryl from different vegetables extracted and cleaned up by different methods at new selected target ions

General Linear Models Procedure

Dependent Variable: PR

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	7	15102.7633	2157.5376	540.33	0.0001
Error	10	39.9302	3.9930		
Corrected Total	17	15142.6934			
		R-Square	C.V.	Root MSE	PR Mean
		0.997363	5.942861	1.99825	33.6244

Source	DF	Type III SS	Mean Square	F Value	Pr > F
VEG	2	3121.9107	1560.9554	390.92	0.0001
METHOD	1	10569.6107	10569.6107	2647.02	0.0001
REP	2	12.6471	6.3236	1.58	0.2526
VEG*METHOD	2	1398.5947	699.2974	175.13	0.0001

E-5--Analysis of variation for percent recovery of malathion from different vegetables extracted and cleaned up by different methods at new selected target ions

General Linear Models Procedure

Dependent Variable: PR

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	7	80908.7620	11558.3946	270.51	0.0001
Error	10	427.2788	42.7279		
Corrected Total	17	81336.0408			
		R-Square	C.V.	Root MSE	PR Mean
		0.994747	6.585167	6.53666	99.2633

Source	DF	Type III SS	Mean Square	F Value	Pr > F
VEG	2	11195.0492	5597.5246	131.00	0.0001
METHOD	1	60833.0454	60833.0454	1423.73	0.0001
REP	2	7.3326	3.6663	0.09	0.9184
VEG*METHOD	2	8873.3348	4436.6674	103.84	0.0001

E-6--Analysis of variation for percent recovery of chlorpyrifos from different vegetables extracted and cleaned up by different methods at new selected target ions

General Linear Models Procedure

Dependent Variable: PR

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	7	2657.04906	379.57844	14.10	0.0002
Error	10	269.20472	26.92047		
Corrected Total	17	2926.25378			
		R-Square	C.V.	Root MSE	PR Mean
		0.908004	6.544610	5.18849	79.2789

Source	DF	Type III SS	Mean Square	F Value	Pr > F
VEG	2	1400.04391	700.02196	26.00	0.0001
METHOD	1	5.46702	5.46702	0.20	0.6619
REP	2	27.75088	13.87544	0.52	0.6123
VEG*METHOD	2	1223.78724	611.89362	22.73	0.0002

E-7--Analysis of variation for percent recovery of fenamiphos from different vegetables extracted and cleaned up by different methods at new selected target ions

General Linear Models Procedure

Dependent Variable: PR

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	7	3470.52504	495.78929	146.96	0.0001
Error	10	33.73666	3.37367		
Corrected Total	17	3504.26169			
		R-Square	C.V.	Root MSE	PR Mean
		0.990373	11.09114	1.83675	16.5606

Source	DF	Type III SS	Mean Square	F Value	Pr > F
VEG	2	113.61014	56.80507	16.84	0.0006
METHOD	1	3217.35681	3217.35681	953.67	0.0001
REP	2	1.78874	0.89437	0.27	0.7724
VEG*METHOD	2	137.76934	68.88467	20.42	0.0003

E-8--Analysis of variation for percent recovery of azinphos-methyl from different vegetables extracted and cleaned up by different methods at new selected target ions

General Linear Models Procedure

Dependent Variable: PR

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	7	1444.33391	206.33342	180.92	0.0001
Error	10	11.40452	1.14045		
Corrected Total	17	1455.73843			
		R-Square	C.V.	Root MSE	PR Mean
		0.992166	14.66139	1.06792	7.28389

Source	DF	Type III SS	Mean Square	F Value	Pr > F
VEG	2	970.234078	485.117039	425.37	0.0001
METHOD	1	249.314450	249.314450	218.61	0.0001
REP	2	3.216944	1.608472	1.41	0.2887
VEG*METHOD	2	221.568433	110.784217	97.14	0.0001

APPENDIX F.

**Tabulated analysis of variance for coefficient of variance of percent recovery for
eight insecticides at new selected target ions**

F--Analysis of variance for coefficient of variation (CV) of percent recovery for 8 insecticides from different vegetables extracted and cleaned up by different methods at new selected target ions

General Linear Models Procedure

Dependent Variable: CV

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	33	349.414812	10.588328	1.28	0.3216
Error	14	116.201579	8.300113		
Corrected Total	47	465.616392			
	R-Square	C.V.	Root MSE		CV Mean
	0.750435	43.99860	2.88099		6.54792

Source	DF	Type III SS	Mean Square	F Value	Pr > F
VEG	2	8.988029	4.494015	0.54	0.5936
METHOD	1	107.161633	107.161633	12.91	0.0029
INSECT	7	54.776958	7.825280	0.94	0.5055
VEG*METHOD	2	3.604154	1.802077	0.22	0.8075
VEG*INSECT	14	106.808304	7.629165	0.92	0.5615
METHOD*INSECT					

VITA

Chen-Chun Shao was born in Changhua, Taiwan on February 25, 1968. He graduated from Changhua Senior High School in 1986. In 1991, he received a Bachelor of Science Degree from the Food Science Department of National Chung Hsing University, Taichung, Taiwan. After graduating, he served in the army for 2 years. From 1993 to 1994, he served as a research assistant at the Institute of Molecular Biology of National Chung Hsing University.

In June 1995, he entered the Food Science and Technology Department at The University of Tennessee, Knoxville. He is a member of Gamma Sigma Delta, the Honor Society of Agriculture, and the Institute of Food Technologists. Upon completion of the Master of Science degree he plans to go on for his Ph.D. in the Food Science and Technology Department at The University of Tennessee, Knoxville, for another graduate career.