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PART ONE

DEVELOPMENT OF ANALYTICAL METHODS FOR THE DETERMINATION OF SELECTED "ORGANIC PESTICIDES"

PART TWO

DDT AND IT'S METABOLITES IN SOIL AND AIR AT AN AGRICULTURAL SITE NEAR SOUTH HAVEN, MICHIGAN: DETERMINATION AND IMPLICATIONS

By

CHRISTINE VANDERVOORT

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ABSTRACT

PART ONE

DEVELOPMENT OF ANALYTICAL METHODS FOR THE DETERMINATION OF SELECTED "ORGANIC PESTICIDES"

By

CHRISTINE VANDERVOORT

Part one of this research was conducted to develop a multiple pesticide analytical residue method for the determination of the "Organic Pesticides", Nicotine, Pyrethrum, Rotenone, and Warfarin. This research determined the optimum detection and chromatographic conditions for the analytical determination of these four pesticides in a multiresidue scheme. The desire to achieve a multiresidue method that was efficient (accurate and precise) and economical yielded a method that did not meet desired lower limit of detection. The four pesticides had physical properties quite different from each other which required the method to change pH from neutral to basic and then to an acidic pH. The second part of the method development work involved pesticides that did not have individual residue analytical methods that were previously published. The pesticides analyzed were α-terthienyl, azadiractin, ryanodine, and veratridine. The chemical and physical properties of these four pesticides had many similarities and thus facilitated their simultaneous extraction, separation, and detection.

PART TWO

DDT AND IT'S METABOLITES IN SOIL AND AIR AT AN AGRICULTURAL SITE NEAR SOUTH HAVEN, MICHIGAN: DETERMINATION AND IMPLICATIONS

By

CHRISTINE VANDERVOORT

The second part of this research involved analysis of air and soil samples to determine the spatial distribution of DDT and it's metabolites (sum of DDT) in Southwestern Michigan. Historically, levels of DDT have been elevated in this geographical region. The levels were found to be easily quantified with gas chromatography (GC) during the sampling period from April 1998 to August 1998. Historically this site has been under extensive fruit and vegetable farming and received high inputs of DDT in the past. The research supports the postulate that the elevated levels of DDT in the area are due to volatilization from the soil. The soil samples from the site had DDT levels elevated above outlying areas. The ratio of DDT to its metabolites also supports the view that the DDT was from past spraying before the 1973 ban in the United State of DDT. Calculations were determined from the residue data to estimate the time for the soil to dissipate the residues and it was found to range from a few years to thousands of years.

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PART ONE

DEVELOPMENT OF ANALYTICAL METHODS FOR THE DETERMINATION OF SELECTED "ORGANIC PESTICIDES"

INTRODUCTION

Natural compounds with pesticidal activity are being considered and used as replacements and enhancements to the present synthetic pesticide arsenal. Thousands of secondary plant compounds are being isolated and tested for biological activity in "Natural Product" Laboratories. Secondary plant compounds, very likely, developed their biological activities in response to plant pests. The toxicological and environmental properties of these novel compounds are many times a mystery due to limited testing. Natural pesticides may be a mixture of several biologically active constituents as in Sabadilla which contains several alkaloids. The mixtures may have synergistic and additive effects that cause major complication to determining absolute responsibility of a biological action to one chemical. The regulatory hurdles to bring a product of this nature to market may be prohibitive if handled as a synthetic pesticide. Natural pesticides play an important role in nature and to humans. Through their manipulation and exploitation humans may

find important and effective uses of natural products. Continued research will provide a valuable group of chemicals for known and as of yet unknown uses.

The use of cultural pest control techniques known collectively as "organic farming" produces food that has a demand by a certain segment of consumers. Organic farming, projected to be responsible for 1.5% of domestic food and fiber production with annual growth rates of about 20% since 1990 (Mahoney, 1998). Many farmers are adopting organic production methods because of problems with pesticides such as resistance and environmental concerns. Although producers and consumers of organic foods have a variety of motives for their beliefs in this alternative type of farming technology, both are concerned about the possible health hazards of pesticide residues and believe "organic farming" eliminates this risk. A popular national magazine "Self" wrote an article on organic produce with comments to readers' questions such as this: "Organic produce is sold locally and in season, as a result, it tends to be fresher, retaining more vitamins and minerals than conventionally grown produce shipped from far away. All else being equal, organic tomatoes, for example, are as nourishing as regular ones-minus those pesky pesticides." (Sullivan, 1998).

Conventionally grown produce offers the same nutritional value if brought to market immediately. Organic produce is under more stress than conventional produce due to more disease control and fewer alleochemicals produced as protectants against the pathogens. This article offers scientifically backed information rather than emotional hype.

Federal laws concerning "organic farming" have been introduced but no action has been taken, so presently there are no national regulations that control this group. December 16, 1997 a draft proposal for organic farming regulations was put out for comment. Agriculture Secretary Dan Glickman received 200,000 comments to the draft proposal. The initial organic regulation proposal for organic farming approved foods from engineered crops, crops that had biosolids applied (municipal sludge), and irradiated foods to fall under the organic label. The majority of comments opposed foods from engineered crops, biosolids application, and irradiated food under the organic food production label. Glickman concluded that those practices would not be included in the organic rule (Glickman, 1998). The public comment to proposed regulations was to tighten restrictions on livestock feed, limit antibiotics, reduce acceptable levels of pesticide

residues and give small farmers more authority. A revised national regulation was supposed to come out for comment later in 1998. Regulation for "organic farming" have been defined in a few states along with organic grower groups that regulate what may be used to produce "organic" food. Most organic growers use the 10% rule, which says a food can be regarded as organic if it has 10% or less residues of the EPA tolerance. This also qualifies most conventional foods as "organic". Organic farming follows a rule that soil must not have had pesticides applied in the previous 36 months. With the ubiquitous nature of DDT, dieldrin, and other organochlorine chemicals, can the soil ever be free of pesticides? Residues of DDT and its metabolites occur in detectable quantities in California soils (Odermatt, 1993).

"Organic" labels without certification is merely an unverified manufacturer's packaging claim (Smillie, 1999). "Certified organic," does not mean pesticide free, chemical free, minimally processed or more nutritious. A certified organic facility must present inspectors with documentation to track the production of the raw agricultural product.

The perceptions of organic farming versus high input farming bring concerns for health and economics. Claims made about "Organic food" include:

"Organic food" has been grown, without toxic pesticides or artificial fertilizers, grown in soil whose humus content was increased by the additions of organic matter, grown in soil whose mineral content was increased with application of natural mineral fertilizers, has not been treated with preservatives, hormones, antibiotics, etc (Steffan, 1971).

The conventional farmer relies on machinery and chemicals to increase productivity and maintain profitability or off farm inputs. The organic farmer and the conventional farmer both must maintain profitability to continue in their businesses. The organic farmer usually receives a premium price for organically grown food, where as the high input farmer produces disease and insect free produce and generally of higher quantity and quality.

The approach to crop protection for the farmer has been a systematic one, were the organic farmer will try to rely on biological rather than chemical control when possible. An example being, in response to a fungal attack on a crop on a conventional farm the control

measure will be pesticides. An organic farmer will look at the nutritional status and stress on the crop and its ability to resist the disease to an extent that the yields are not greatly diminished. The organic agriculturist has a philosophy with many commonalties to other organic agriculturists but to confound that they also have many alternative philosophies. The important holistic nature of the organic farm implies interactions between crops, soil, animals, and the social structure of the family. Many of the theories in organic farming have not been stated in clear scientific terms but rather in social and emotional terms. Some of the common elements are balanced crop rotation, green cover, animal byproducts, shallow plowing, no synthetic pesticides, and pest control through biological and avoidance techniques. The conventional farmer also uses similar techniques to the organic farmer with the exception of synthetic pesticide use. The sustainable agriculture movement has many sound practices such as cover crops to supply essential nutrients like nitrogen and provide a natural habitat for beneficial predators. The cover crops such as vetch, peas, and clover also reduce soil erosion and hinders emergence of weeds. When soil under conventional farming has synthetic fertilizers substituted with organic fertilizer the soil

maintains better tilth, i.e. texture, and their ability to retain moisture is improved (Steffan, 1971), although organic fertilizers do not insure nominal levels of nutrients. Sustainable agriculture has been in response to environmental and social costs that have come from enormous yields from conventional farming such as large petroleum, pesticide, and nutrient inputs from off the farm and this causes reliance on government subsidies and bank loans. Generally organic farmers have smaller farms which rely on farm resources rather than off farm, even though organic farmers also must rely on bank loans to remain profitable.

Many of the beliefs of organic farmers have not been scientifically tested. Claims of zero pesticide residues in fresh or processed foods must be viewed with incredulity since no extensive residue studies on active ingredients have been conducted. The assumption that organic chemicals degrade rapidly has a major problem associated with it, in that the applications have no regulated preharvest interval, number of applications, application rates, or formulations or active ingredients. "Natural" pesticides may be applied at harvest and at high rates but they are not required to have residue analysis for the active ingredients or metabolites of toxicological

concerns. The need for a precise determination of the residues applied to organic products will allow assurance to the consumer that they are truly consuming safe food.

This proposed research will investigate the development of analytical methods for the determination of selected "organic" pesticides on and in foods. The mere unorthodoxy of organic farming should not be used to automatically reject this technology; but research programs should be conducted to evaluate the magnitude and fate of the active ingredients found in "natural" pesticides in fresh and processed agricultural products to better understand the safety of "organic" foods.

OBJECTIVES

Objective-I

The research focus was on the development of methodology for detecting residues of natural organic chemicals applied by organic farming techniques. The initial stage involved analysis of natural pesticides, which have published standard residue methods and their integration into a possible multi residue scheme. The target chemicals for this stage were nicotine, rotenone, pyrethrums, and warfarin. The hypothesis is that all of the chemicals can be analyzed using one multi residue

method to achieve accurate, precise, and µg/g residue values. The null hypothesis is that the chemicals can not be analyzed at sufficiently low levels to determine typical quantities of pesticide on or in food through a multi residue method.

Objective-II

The second stage of the research involved the development of residue methods for the "natural" pesticides for which there are not available sensitive (µg/g or ng/g) and selective published analytical methods. Analytical methods were developed for the following active ingredients: α-terthienyl (marigold), azadirachtin (neem), ryanodine & dehydroryanodine (ryania), cevine, sabadine, cevadine, and veratridine (sabadilla). The hypothesis was that all of the chemicals can be analyzed using one multi residue method to achieve accurate, precise, and µg/g residue values. The null hypothesis is that the chemicals can not be analyzed at sufficiently low levels to determine typical quantities of pesticide on or in food through a multi residue method.

LITERATURE REVIEW

Pesticide analysis involves large quantities of organic solvent use and waste. The focus in recent years has been to reduce laboratory-generated waste and lower the detection limits. Several techniques have been examined to reduce waste and generate lower levels of detection of the target analyte. Supercritical fluid extraction and solid phase extraction have had some success. The supercritical equipment has been expensive and thus inaccessible to some laboratories. Solid phase extraction (SPE) has been used in cleanup steps and this provides some reduction in solvent extraction over solvent partitioning. SPE may be used in isolation of analytes from each other and then elution of only a select chemical.

ORGANIC FARMING & FOOD INDUSTRY

Organic foods have annual growth rates around 20 % (Mahoney, 1998). The market appears to be headed for continued growth. Organic foods are great for processing because they don't need to be cosmetically perfect as in the fresh fruit market. William Breene, professor emeritus, University of Minnesota says, "It should be remembered that

neither can it be proven that they are healthier nor can they said to be pesticide free", (Mahoney, 1998). The organic foods account for about 1.5% of domestic food sales and they have had a growth rate of about 20% since 1990 for a total revenue of $4 billion (Mahoney, 1998). The natural foods market has many claims that don't have traditional efficacy testing. A "Natural" product KOLESTOP® has phytosterols (plant sterols) that are found in the fat-soluble fractions of plants and are effective in improving circulating lipid to reduce risk of coronary heart disease. Chemically similar to cholesterol, phytosterols inhibit the absorption of cholesterol. Phytosterol consumption in humans under a wide range of study conditions has been shown to reduce plasma total and low density lipoprotein (LDL) cholesterol. Most studies report no effect of phytosterol administration in high density lipoprotein (HDL) cholesterol or triglyceride levels (Jones, et al.., 1997). This product has many claims without the traditional FDA review.

CHARACTERISTICS AND USES OF THE PESTICIDES

α-Terthienyl

Chemistry and Source: Terthienyls are released from the roots of growing *Asteraceaes* (marigolds) and have shown a strong nematicidal activity. α-Terthienyl's molecular formula is shown in Figure 1.0.

Pharmacology: Marigolds secrete toxic compounds of a α-terthienyl type into the soil, which kills nematodes. To be effective marigolds must be planted as a solid crop and grown for 90 days to begin secreting α-terthienyl to reduce the nematode population. Marigolds also act as a trap crop. Nematodes enter their roots but are unable to complete their life cycle and die without reproducing.

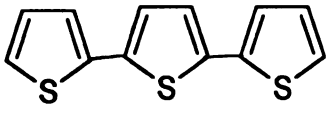

Figure 1.0 α-terthienyl Molecular Structure

Formulation: Plant-derived products are not sold commercially for control of nematodes. The actual plant can be planted as the controlling agent.

Azadirachtin (Neem)

Chemistry and Source: Azadirachtin has been derived from the seeds of the neem tree, *Azadirachta indica*, which has a wide distribution throughout Asia and Africa (Merck Index, Eleven Edition, 1989). Azadirachtin's molecular formula is shown in Figure 1.1

Pharmacology: The observation that the desert locust did not eat the leaves of the neem tree and another closely related tree species, led to the isolation and identification of azadirachtin in 1967. Since then, azadirachtin has been shown to have repellent, antifeedent, and/or growth regulating insecticidal activity against a large number of insect species and some mites. It has also been reported to act as a repellent to nematodes. Neem extracts have also been used in medicines, soap, toothpaste

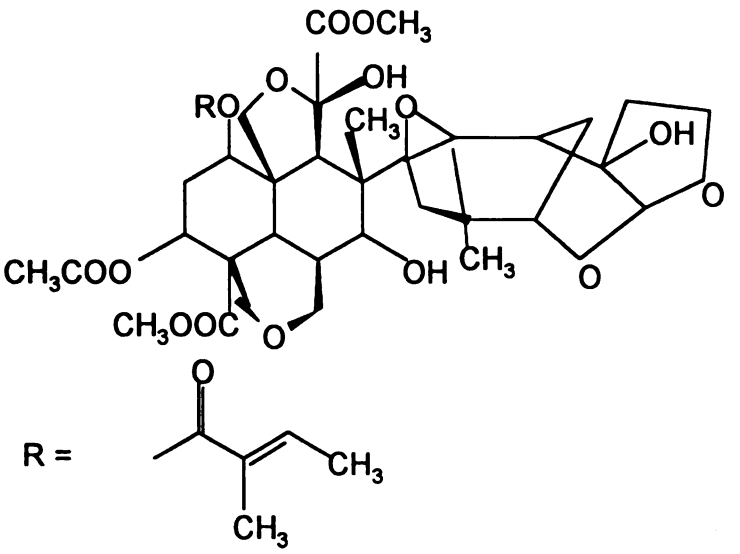

Figure 1.1 Azadirachtin Molecular Structure

and cosmetics (Author unknown http://www.calchemico.com/neem.html). Toothpaste with neem extracts has claims that they prevent tooth decay and periodontal disease.

Formulation: The most common commercial formulations of neem is Neemix, which is available for fruit tree, and lists leafminers, mealybugs, aphids, fruit flies, caterpillars and

psylla as insects that will not feed on treated trees. Azadirachtin has shown good activity against spotted tentiform leafminer in tests in past years, but the formulation that was available at that time was somewhat phytotoxic. In insecticide trials in 1992 with another azadirachtin product called Margosan-O, the product showed good activity against leafhopper. Margosan-O does not include a label for fruit crops, however. Azadirachtin has a relatively short environmental life and a low mammalian toxicity (rat oral LD_{50} >10,000 mg/kg). It can be used up to and including the day of harvest, with reentry permitted without protective clothing after the spray has dried. It has toxicity to fish and aquatic invertebrates.

Nicotine

Chemistry and Source: Nicotine has a tertiary amine composed of pyrrolidine and pyridine rings found in dried leaves *of Nicotiana tabacum* and *N. rustica*. The extract is a colorless to pale yellow, oily liquid that is very hygroscopic and turns brown on exposure to air or light. Nicotine has two pKs, pK_1 at 6.16 (15 ° C) and pK_2 at 10.96 (Merck Index, Eleven Edition, 1989). Nicotine forms salts in acids and double salts with many metals and acids. Nicotine's molecular formula has been given in Figure 1.2.

Pharmacology: Nicotine functions mainly as an excitatory

stimulus in the central and peripheral nervous system. Transmission at the neuromuscular junction is associated with increased cation conductance. K^+ is allowed to leave the post-junction area as Na^+ enters. The peripheral nervous system nicotine reacts like acetylcholine at ganglion and neuromuscular sites. Nicotine's toxic action is due to both stimulation and blocking of autonomic ganglia and skeletal muscles at the neuromuscular junction.

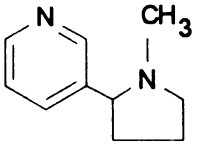

Figure 1.2 Nicotine Molecular Structure

Formulation: Black Leaf 40 is a 40% nicotine sulfate formulation that may be sprayed or added to hydrated lime and spread as a dust (Cook, 1998).

Pyrethrum

Chemistry and Source: This compound will be produced in the flowers of *Chrysanthemum cinerariaefolium* and was the forerunner of the synthetic pyrethroid insecticides. The active insecticidal ingredients are obtained from the flowers. There are four active ingredients, Cinerin I,

Cinerin II, Jasmolin I, Jasmolin II (Casida, 1995).

Pharmacology: Pyrethrum has a relatively non-toxic relationship to humans and other mammals, although the dust produces allergy attacks in people who are allergic to ragweed pollen. The acute oral LD_{50} ranged from 750 to 1000 mg/kg (Extoxnet, 1994). Pyrethrum has been shown to be toxic to fish, but "relatively" non-toxic to honey bees.

Natural pyrethrums are contact poisons which act on the nervous system to cause a "knockdown" that causes the insect not to be able to move or fly away. To assure a lethal dose, pyrethrum was often sprayed with other synergists and insecticides. Pyrethrum's molecular formula is shown in Figure 1.3

Formulation: There are not nearly as many commercially available formulations of this chemical as there are for rotenone, but it has availability as an emulsifiable concentrate, in combination with rotenone, or alone as a wettable powder. Pyrethrum cost the least expensive of these four materials. Depending on the rate

R = CH_3 for Pyrethrin I

R = $COOCH_3$ for Pyrethrin II

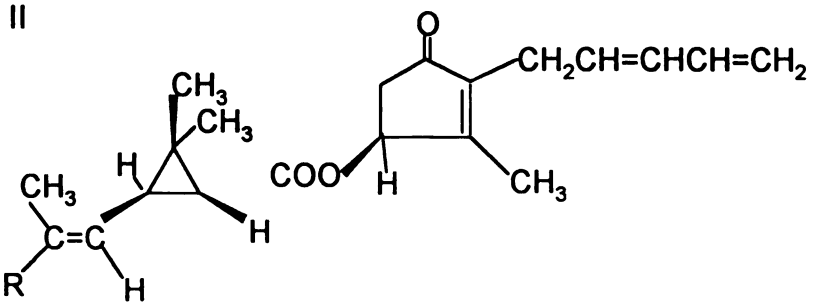

Figure 1.3 Pyrethrum Molecular Structure

used, it may be less expensive than many synthetic insecticides. It also may be synergized by piperonyl butoxide (PBO). Pyrethrum is labeled for use against a large number of pests. An addendum to the label for one formulation of pyrethrum showed it to be moderately to highly effective (61-100% control) against the following pests of fruit: grape leafhopper, potato leafhopper, leaf curl plum aphid, blueberry flea beetle, blueberry thrips and blueberry sawfly. It may be used efficaciously against cranberry fruitworm and also will be quickly broken down in the environment and may be used up to and including the day of harvest.

Rotenone

Chemistry and Source: Rotenone has been extracted from the root of various plants of the *Derris* or *Lonchocarpus* species from Southeast Asia, Central and South America. The molecular formula for Rotenone, $C_{23}H_{22}O_6$ ({2R-(2α,6aα,12aα)]-

1,212,12a-tetrahydro-8,9-dimethoxy-2-(1-=methylethenyl)[1] benzopyranol[3,4-b]furo[2,3-h][1]benzopyran-6(6aH)-one) has been determined. Derris root has long been used as a fish poison and its insecticidal properties were known to the Chinese long before it was first isolated in 1895. Formulated product may be available as at least 118 formulated products from a large number of manufacturers. Rotenone's molecular formula may be found in Figure 1.4

Pharmacology: Rotenone's selectivity has been found to be a non-systemic contact and stomach poison. Site of action may be in electron transport chain (The Pesticide Manual, Tenth Edition, 1994). It may be synergized by the addition of PBO, which also comes from botanical material. Rotenone is less expensive than synthetic insecticides, but is moderately priced for a botanical. It was the most commonly mentioned of the botanicals in pre-synthetic literature and has shown that it was at least somewhat effective against a large number of insect pests. These include: pear psylla, strawberry leafroller, European corn borer, European apple sawfly, cherry fruit fly, apple maggot, cranberry fruitworm, raspberry fruitworm, pea aphid (with similarity to rosy apple aphid), European red mite and two-spotted spider mite, codling moth, plum curculio, Japanese beetle and tarnished plant bug. Unfortunately, Rotenone has shown toxicity to ladybird beetles and predatory mites. But, it has been shown

to be non-toxic to syrphid flies that feed on aphids, and to honeybees. Rotenone is rapidly degraded in sunlight, lasting a week or less. Of the botanicals mentioned here, rotenone has the most toxicity to humans and other mammals. The acute oral LD_{50} ranges from 12-2000 mg/kg in various animals (Extoxnet, 1993). In small doses it may be irritating or numbing to mucous membranes. Use as a potent piscicide has been known because of the high toxicity to fish, having been commonly used as a fish poison. Toxicity has also been shown to occur in birds and pigs.

Formulation: A recent regulatory development

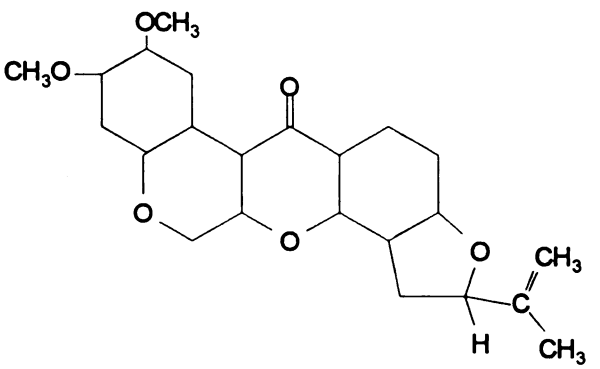

Figure 1.4 Rotenone Molecular Structure

illustrates the tenuous situation of many minor-use materials and may end up rendering rotenone unavailable for use on many crops. According to a USDA news release and as quoted in the Federal Register (July 20, 1995), the Rotenone Task Force has announced that it plans to delete all the agricultural uses from rotenone labels because of the cost of reregistration; these uses include all tree

fruits and small fruits. The registrants plan to maintain rotenone uses for fish control and flea/tick/mite control on dogs and cats. They will reconsider their plans for deletion if someone shows a willingness to develop the necessary data for reregistration.

Ryania

Chemistry and Source: A product of the roots and stems of *Ryania speciosa* of Trinidad, ryania acts as both a stomach and contact poison on target insects. It was found that Ryanodine was the most expensive of the materials covered in this research, and also was not as readily available as rotenone or pyrethrum. Ryanodine, the active ingredient, was formulated as a wettable powder and labeled for use against the codling moth in apples. It has also shown to be toxic to the European corn borer and may control cranberry fruitworm. In tests it provided excellent control of a pest complex comprising codling moth, oriental fruit moth and lesser appleworm. It also controlled aphids, white apple leafhopper and spotted tentiform leafminer. It has been shown to be more persistent than rotenone or pyrethrum and also more selective. Generally it has not been found to be very harmful to pest predators and parasites, but has been shown to be somewhat toxic to the predators *Atractotomus maliand*

Diaphnocoris spp. It may also be used up to 24 hours before harvest. Ryania's molecular formula is shown in Figure 1.5.

Pharmacology: Ryania's insecticidal properties act as a stomach poison and ryania often depresses the insects feeding initially, so that it undergoes a long period of inactivity before death. It has residual properties longer than the other botanicals. Relative to rotenone, ryania has a moderate toxicity in acute or chronic oral toxicity

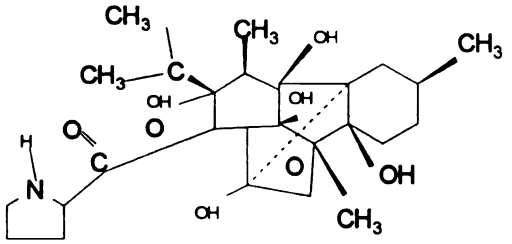

Figure 1.5 Ryania Molecular Structure

testing done in mammals; this was partly why much attention has been given to this insecticide in recent years. The acute oral LD_{50} of ryania ranges from 750 to 1200 mg/kg, less toxic than rotenone and slightly more toxic than pyrethrum. It is toxic to fish.

Formulation: Ryan 50 a product of the roots and stems of *Ryania speciosa* of Trinidad. It has also been shown to be toxic to the European corn borer and may control cranberry fruitworm. In recent tests it provided excellent control of a pest complex comprising codling moth, oriental fruit moth

and lesser appleworm. It also controlled aphids, white apple leafhopper and spotted tentiform leafminer. Rotenone has been found to be more persistent than rotenone or pyrethrum and also more selective. It generally has not been harmful to pest predators and parasites, but it has been found to be somewhat toxic to some minor predatory mites. It may be used up to 24 hours before harvest.

Sabadilla

Chemistry and Source: The source of sabadilla was found to be the seed of a tropical lily, *Veratrum Sabadilla and V. Officinale* that contains several toxic alkaloids (Grieve, 1995). The alkaloids of toxicological importance are cevadine, veratridine, cevine, and sabadine. Sabadilla's molecular formula may is shown in Figure 1.6.

Pharmacology: In previous articles about botanical insecticides printed in Scaffolds (Kain, 1995), it was stated that sabadilla was not toxic to honeybees. However, the information provided by different sources since then has been ambiguous. Some say, that it is relatively non-toxic to honeybees and others (including the manufacturer) say it has been found to be toxic. The confusion may lie in the fact that sabadilla has shown to be toxic to honeybees on contact, but without any residual activity. In the interest of playing it safe (especially given the current state of

bee health), it would probably be best to consider sabadilla a hazard to honeybees and follow all necessary precautions to prevent their exposure to the material. Sabadilla has been shown to be less toxic to mammals than rotenone or pyrethrum; the acute oral LD_{50} was determined to be greater than 4000 mg/kg.

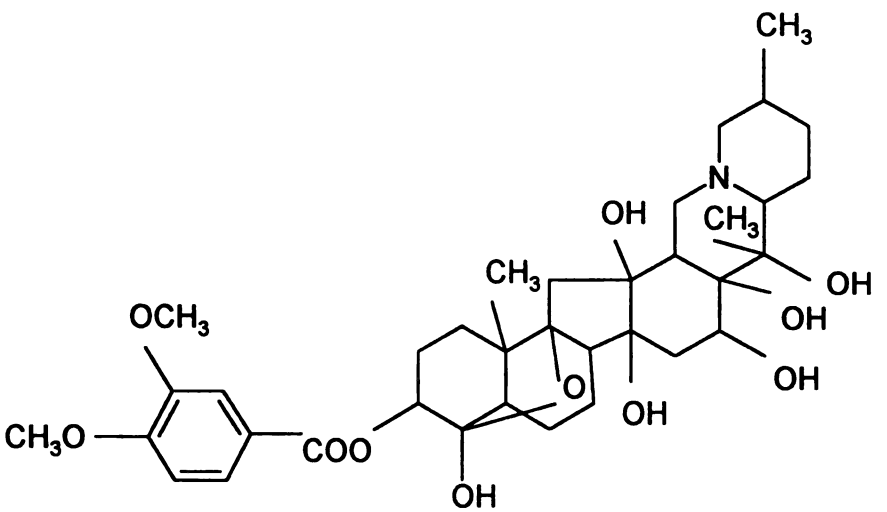

Figure 1.6 Sabadilla Molecular Structure

Formulation: There are very few commercial formulations of this material. It may be found as a dust that may also be added to water and sprayed, but clogging of the nozzles may occur. It will control potato leafhopper and is somewhat effective. Sabadilla mixed with lime or sulfur or dissolved in kerosene to provide a base to facilitate application (Douglas, 1996). Sabadilla was found to be moderately priced for a botanical (similar to rotenone). It has little

effect on predators/parasitoids, except for the predatory mite *Typholdromus pyri*, to which it was extremely toxic in recent tests by Joe Kovach (Kain, 1995). Sabadilla may be used up to 24 hours before harvest. Apple is the only deciduous tree fruit crop specifically mentioned on the label of the one product found registered for use in New York State.

Warfarin

Chemistry and Source: Warfarin has a colorless, crystalline structure and a formulation of $C_{19}H_{16}O_4$ (4-hydroxy-3-(3-oxo-1-phenylbutyl)-2H-1-benzopyran-2-one). The anticoagulant ability of warfarin was discovered and reported in 1944 and by 1952 was registered for use in the United States. Warfarin's molecular formula is shown in Figure 1.7.

Pharmacology: Warfarin inhibits normal function of Vitamin K in blood coagulation. With continuous exposure severe bleeding and death occur (Warfarin, 1995). The LD_{50} of various animals ranged from 1 to 1200 mg/kg (Extoxnet, 1995). Warfarin poisoning symptoms include mucus membrane bleeding, hematomas in joints, cerebral hemorrhage leading to paralysis and death.

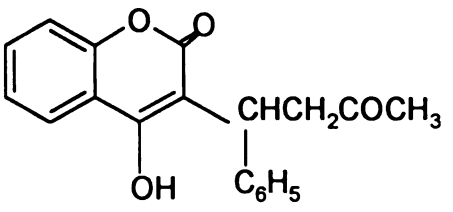

Figure 1.7 Warfarin Molecular Structure

Formulation: The compound comes as ready-to-use bait, concentrate, powder, liquid concentration, and various powders and dust formulations.

ANALYTICAL METHOD DEVELOPMENT FOR LIQUID CHROMATOGRAPHY

Reverse Phase Liquid Chromatography (RPLC) has been modified by many different sorptive materials and mobile phases since the initial chromatography columns. The separation of analytes by differential migration from a narrow application zone in a porous sorptive medium. Column chromatography, thin layer chromatography (TLC), and paper chromatography are subdivisions of solution chromatography based on the sorptive material. The analytes move due to solution-solid adsorption and partition distributions.

Analytes move by zone migration which has a fraction of the of the mobile phase velocity, R. At the molecular level each molecule adsorbs to the stationary phase and its migration will be stopped while other molecules move on. Each molecule goes through a stop and go path but statistically all the molecules that are the same are moving at the same theoretical velocity. Since each molecule has a specific velocity which includes t_a (time adsorbed) and t_d (time desorbed) then R is shown by:

$$R = (t_a)/(t_a + t_d)$$

The effect of separation depends on the solute migration to regions of lower concentration, eddy diffusion or differential path tortuosity, and the propensity of a gaussian concentration profile to precede. The result of

the above phenomena is an initial separation of analytes and also a band broadening of individual analytes. Temperature can be used to effect the resolution of compounds because diffusion has temperature dependency.

Adsorption depends on the phenomenon of molecule being held on the surface of a solid support. A molecule adsorbed to the surface of an absorbent will have a potential energy, P_e, due to intermolecular forces holding it there, and a kinetic energy, K_e, due to vibrational movement. When K_e exceeds P_e the molecule will leave the surface and the molecules move on. K_e depends on mass, shape, and temperature which contribute to the band broadening effect seen in chromatography. Adsorption is a reversible process and characterized by weak forces. Examples of adsorption chromatography are columns which utilize adsorbents such as calcium carbonate, silica gel, aluminum oxide, charcoal, etc. and ether, carbon tetrachloride, alcohols, acetone, and water as solvents.

Partitioning will determine the equilibrium distribution of an analyte between two immiscible solvents. Partition chromatography has been seen with column, paper, and thin layer chromatography. The reverse phase notation refers to the solid phase part of a non-polar stationary compound such as silicone oil, C_{18}, or paraffin. The mobile phase will be relatively polar, as compared to the

stationary phase, solvent such as methanol, acetonitrile, and water mixtures. Reverse phase separations are useful for nonpolar hydrocarbons because the nonpolar compounds will differentially be retarded as they move with the mobile phase. In a typical C_{18} column the anayltes partition with the column's octadecyl molecules through Van der Waals forces. The more polar compounds travel through the column faster due to weaker interaction with the octadecyl column packing material. A typical order of elution from the column would be strong Lewis acids (carboxylic acids), weak Lewis acids (alcohols, phenols), strong Lewis bases (amines), weak Lewis bases (ethers, aldehydes, ketones), permanent dipoles ($CHCL_3$), induced dipoles (CCL_4), then aliphatic hydrocarbons. The mobile phase for RP-C_{18} column should typically be a mixture of water with methanol or acetonitrile. Varying the water content of the mobile phase can optimize the separation. As the organic content of the mobile phase increases the retention time of the analytes is decreased. The organic molecules spend more time in the organic phase as polarity decreases.

Chromatography deals with the separation of chemicals, which then need to be detected by another technique. Many suitable detectors are available such as spectrometers, mass detectors, and refractive index detectors. The molecule's chemical and physical characteristics provide modes of

detection and from them determine the appropriate means of detection.

The molecules absorb in the UV region of the electromagnetic (EM) spectrum makes them suitable for UV detection. Electromagnetic waves, as its name implies, are composed of two components: a oscillating electric field and a oscillating magnetic field mutually perpendicular to each other. The two waves are in phase. The EM spectrum has wavelengths from 10^{-14} to 10^{7} m and the UV spectrum from 10^{-8} (vacuum UV-10 nm) to 3.5×10^{-7} m (Near UV-350 nm). EM radiation composition will be of discrete photons of energy. The photon energy can be quantized by the equation:

$$E = h\nu = hc/\lambda$$

E represents energy in joule (J), ν for frequency (s^{-1}), h for Planck's constant (6.63×10^{-34} Js), λ for wavelength (m), and c for the speed of light (3×10^{8} m/s).

Spectroscopy deals with the interaction of EM radiation with sample material. As the radiation enters into the sample the beam may reflect, refract within the sample, scatter, absorb the radiation or be transmitted through the sample. A sample that was stimulated by the input of energy in the form of EM radiation will have absorbed energy and then be in a higher energy state or cause expulsion of an electron and be ionized. The results of photon absorption

by the sample leads to a reduction in the intensity of the EM radiation being transmitted through the sample. Relaxation of an excited species can occur by emission of a photon (photoluminescence) or release of kinetic energy.

UV radiation absorption causes electronic excitation. The actual amount of energy absorbed for a change in electrons Energy State may be related by:

$$(E_1 - E_o) = h\nu$$

In organic molecules there are three general types of electrons; sigma bonded electrons, pi electron bonds, and n electrons. Sigma electrons form high-energy bonds and UV radiation will not have sufficient energy to excite sigma bonded electron. Sigma bonds are found in saturated bonds and this feature makes these compounds ideal solvents for UV absorption spectroscopy. Pi bonded electrons are found in aromatic and conjugated compounds. N electrons are nonbonding electron pairs found in N, O, S, or halogen compounds and these electrons can be excited by UV absorption. The absorbed energy necessary to cause an electronic excitation varies slightly due the different vibrational and rotational molecular energy levels which leads to an absorption band where an atomic spectra is a sharp line.

For an electronic transition to occur the electron must not change its spin orientation as it goes from an unexcited state to an excited one. A compound that does not change spin orientation is called a singlet and one that changes is a triplet. The triplet conversion is a forbidden transition and occurs very rarely. When a molecule emits energy from the singlet state to ground it is referred to as fluorescence. Singlet-triplet transitions occur rarely and they are called inter system crossing. When an electron return from a triplet state to ground a phosphorescence emission occurs. A singlet to ground occurs in about 10^{-8} seconds and triplet to ground 10^{-2} to 100 seconds.

To determine if a molecule will absorb in the UV range a transition from a bonding or lone-pair orbital to an unfilled non-bonding or anti-bonding orbital must be available. The chromophore (electrons responsible for the absorption) must be identified. Some examples of chromophores are ketones that have a n to π^* transition which are examples of a lone-pair of electrons on oxygen goes to the an anti-bonding orbital.

UV spectroscopy can be used for both qualitative and quantitative analysis. For qualitative identification a scan is done and compared to knowns for shape and specific spectral absorbance areas. UV absorption spectra overlap considerably for different compounds so this is not a

decisive technique for identifying a compound. Once the compound has been identified UV may be a powerful tool for quantitative analysis.

Absorption follows definite physical laws. Transmittance has been defined as the ratio of I_1 (intensity of radiation leaving the sample) to I_o (intensity of radiation entering the sample):

$$T = I_1 / I_o$$

and may be related to absorbance by:

$$A = -\log T = abc$$

Absorptivity will be represented by a, b which will be path length, and c the concentration. These relationships show the logarithmic relationship between transmittance and concentration and the linear relationship between absorbance and concentration. These relationships hold true for dilute solutions. UV absorption is sensitive 100 ppb to 1 ppm.

GENERAL CONSIDERATIONS FOR ANALYTICAL METHOD DEVELOPMENT

Method development will involve finding common solvents, columns, and detection methods. The method development process shall be approached according to the following sections:

- Establish Criteria for the Method
- Method Development

The establishment of criteria will pertain to the proposed MDL of about 1.0 ppm being sought. The recoveries will be done with a variation in precision of less than 30% of the mean and accuracy between 60% and 130%. The validation will determine the working concentration range, to include the level of quantitation (LOQ) and approximately 10 X this value to encompass anticipated residues found. The standard curve range will be determined for general shape, ie. linear, exponential, or polynomial fit and the upper range of the curve. The acceptance criteria for goodness of fit of the curve, r^2, will be greater than 0.95.

A minimum validation data set will include:

- Two samples at the MDL run concurrently with control samples.
- Two samples fortified at the maximum concentration of the validation range run concurrently with control samples.

- One reagent blank.

The method will include lists of equipment, materials and reagents, the stepwise procedure used to execute the method, a summary of the validation results, the appropriate validation data, representative chromatograms and a discussion of the results. When considering extraction of the sample the technique will consider the nature of the sample.

To determine the correct extraction method the sample matrix and analytes are considered. When using a SPE column the analytes interact with the packing material and are preferentially retained or eluted. The analyte will absorb to the packing material and the contaminants will pass through or alternately the analyte will pass through and the contaminates will be retained on the column. Several packing phases are available for specific purposes. Normal Phase, Reversed Phase, Ion-Pairing, and Ion-Exchange packings are available. Selection of the proper SPE tube involves knowing:

- Degree of contamination
- Sample complexity
- Analyte concentration range
- Analyte solubility in solvents
- Strength of analyte/sorbent interaction

- Sample volume

SPE tubes come in several sizes, from 1 ml to 60 ml, to assure optimal sample extraction and cleanup capabilities. SPE tubes are conditioned prior to sample introduction to activate the packing material according to the packing material and compounds of interest. Sample volumes from microliters to liters may be added to the SPE tube. Reverse phase packings lose their extraction efficiency and sample recoveries go down as the volume of sample increases because the packing material loses its activation brought on by preconditioning the column. The column was washed after sample introduction with a solvent in which the analyte has a low solubility. The wash volume typically should be about the same volume as the tube. The analyte was then eluted off the column with a solvent that has a strong affinity for it. The sample was then reduced or brought to volume for injection onto the analytical instrument.

RESEARCH

METHOD I - NICOTINE, WARFARIN, ROTENONE, AND PYRETHRUM

The initial research was initiated by looking at the individual pesticides, their chemical, physical properties, and referenced methods found in Appendices A-D. These four chemicals were chosen because of their frequency of use in organic farming systems. Solubility was the first parameter looked at. The analyte of interest was put into individual test tubes and different solvents were added to evaluate the solubility. The results are given in Table 1.0:

Each test tube was shaken and then evaluated for precipitate or phase separation in the tube. All four chemicals showed solubility in methanol, acetonitrile, and acetone.

Reference data agreed with the experimental data. The choice of the solvent was made also in conjunction with mobile phase (HPLC) and gas chromatography (GC) compatibility. Methanol was chosen as the solvent because of solubility of the analyte, ultraviolet (UV) and visible absorption properties, and volatility concerns.

Table 1.0 Solubility of Solvents

Chemical	Methanol	Acetone	Acetonitrile	Water
Nicotine	Soluble	Soluble	Soluble	Soluble
Pyrethrum I & II	Soluble	Soluble	Slightly Soluble	Insoluble
Rotenone	Soluble	Soluble	Slightly Soluble	Slightly Soluble
Warfarin	Soluble	Soluble	Soluble	Soluble (Alkaline)

Obviously the material must be soluble in the solvent, invisible to the detector and nonreactive with the method. Additionally, volatility of the solvent must be considered because it could have detrimental effects on the concentration of the standards due to evaporation over time.

The maximum absorbances of solvents under consideration are given in Table 1.1.

Table 1.1 Maximum Absorbance at Specific Wavelengths (nm)

Solvent	nm / A	nm / A	nm / A	nm / A	nm / A
Methanol	205/1.0	225/.16	250/.02	300/.005	400/.005
Acetontrl	190/1.0	205/.1	225/.01	250/.005	350/.005
Water	254/.001	-	-	-	-
Acetone	330/1.0	340/.06	350/.01	375/.005	400/.005

Methanol and acetonitrile had the most favorable UV absorbance maxima (ie. below 205 nm).

Volatility was looked at in terms of solvent storage of standards. Table 1.2 shows the vapor pressure of

solvents under consideration. Acetonitrile and methanol had favorable vapor pressures for decreasing solvent loss in standards over time in storage. Hexane and other nonpolar solvents were not used because the analytes were insoluble in them.

Table 1.2 Vapor Pressure

Solvent	Vapor Pressure (Torr)
Methanol	**125**
Acetonitrile	**88.8 @ 25° C**
Acetone	**184.5**
Hexane	**120**

solvents under consideration. Acetonitrile and methanol had favorable vapor pressures for decreasing solvent loss in standards over time in storage. Hexane and other nonpolar solvents were not used because the analytes were insoluble in them.

The HPLC, GC, and capillary electrophoresis (EC) chromatographic aspects of the method were investigated, with particular attention to compatibility with the instruments. The flame ionization detector (FID) was considered for GC applications since it detects most carbon based molecules. Each analyte showed minimal response at greater than 100 ppm levels. This level was too high for residue work, which should be 10^{-2} to 10^{-4} times the 100 ppm

level. Nicotine was easily detected using the nitrogen/phosphorus detector (NPD) on the 5890 Hewlett Packard GC with a Carbowax column.

EC was abandoned as a chromatographic technique due to overlapping peaks and drifting during runs. The analytes being pH dependent were influenced by the temperature, which was not held constant. EC could be promising if only the slightly polar chemicals, Warfarin and Nicotine were considered.

HPLC was investigated as to mobile phase compatibility with the analyte and analyte separation from the mobile phase and other analytes, initially along with the wavelength for combined best detection. Ultraviolet (UV) and visible (Vis) absorbance maximas were determined for each analyte using a Gilford UV/Vis Spectrometer. The absorbance maximas are given in Table 1.3. The wavelength was given relative to the strongest absorbing wavelength as 1.00.

The spectra for each individual analyte were taken to determine optimum wavelength. The choice of a common wavelength for all four chemicals was determined as a summation of absorbances over all the analytes at a particular wavelength. The most favorable wavelength for the simultaneous detection of all the analytes was 280 nm.

Table 1.3 Absorbance Maximas

Chemical	λ (nm) / A	λ (nm) / A	λ (nm) / A
Nicotine	**215 / 0.95**	**260 / 0.93**	**265 / 1.00**
Pyrethrum I	**209 / 0.89**	**222 / 1.00**	**240 / 0.89**
Pyrethrum II	**209 / 0.89**	**222 / 1.00**	**240 / 0.89**
Rotenone	**226 / 0.95**	**241 / 0.94**	**290 / 1.00**
Warfarin	**215 / 1.00**	**285 / 0.40**	**309 / 0.41**

They are given in Table 1.4. The 280 nm was selected because the sum of the absorbances was 1.72, which was the greatest sum.

Various solvents were tried to optimize the resolution between the analytes and achieve the best peak shapes. The problem with optimizing was achieving satisfactory results for all four analytes. Nicotine has a basic character with pK_1 at 6.16 (15 ° C) and pK_2 at 10.96 while warfarin has a slightly acidic molecule. As long as the mobile phase was organic with a neutral pH the molecules

Table 1.4 Analyte Absorbance Sums at Given Wavelengths

Chemical	Retention Time	227 nm	254 nm	280 nm	295 nm
Nicotine	1.75	0.17	0.67	0.67	0.17
Pyrethrum I	3.00	0.12	0.04	0.02	-
Pyrethrum II	3.30	0.04	0.06	0.06	-
Rotenone	2.90	0.40	0.40	0.17	0.20
Warfarin	1.85	0.30	0.16	0.80	0.17
Response Sum		1.03	1.33	1.72	0.54

stayed non-ionic. Ionic molecules would not be retained by the C_{18} column and be eluted with or before the solvent peak. Methanol was found to be the best solvent for the standards and as the mobile phase for the HPLC due to all the chemicals were soluble in it and the solvent front on the HPLC did not interfere with the analytes as they came off the detector. When the solvents and mobile phases were mixed the column would have conditioning problems and not recover to a stable baseline in time for the analyte to elute. Buffered mobile phases such as a phosphate buffer at ~pH 10 worked well with nicotine but not warfarin. The desire to use one mobile phase to alleviate the need for reconditioning the column between analyte injections was chosen, except for Nicotine had to be chromatographed

separately with a Na_2HPO_4 buffered acetonitrile mobile phase.

The three analytes, Warfarin, Rotenone, and Pyrethrum had retention times of 1.72, 2.92, and 3.00 minutes respectively. Nicotine was determined with Na_2HPO_4 buffered acetonitrile mobile phase to maximize recoveries and will be considered at later. The resolution between peaks may be found in Table 1.5 and calculated by the following formula:

$$R_s = (v_2 - v_1)/\{(w_2 + w_1) * 0.5\}$$

Peak resolution has the ratio of the difference between two peaks retention times, v_n, of analyte n divided by the average, w_n peak width for peak n. The resolution between Warfarin and Rotenone represent totally resolved peaks in that the tangents of the peaks to the baseline of the chromatogram do not intersect, i.e. non overlapping lines. This is not the case with Rotenone and Pyrethrum. The use of different wavelengths provides for sufficient separation via wavelength and chromatographic separation.

Rotenone detection was at 254 nm and Pyrethrum at 227 nm for increased sensitivity after initial method development work at 280 nm. Part of the problem with separation of Rotenone and Pyrethrum has to do with the fact that Pyrethrum has several different fractions. The slightly

different molecular formulas had subtle differences on the partitioning while traveling through the column.

The slight differences brought about broadening of the analytical peak which in turn cause peak overlap. Values of Rs ≥ 1 represent "totally separated" peaks.

Table 1.5 Resolution between Peaks

Chemical	Retention Time (min)	Wavelength (nm)	Width (min)	Resolution R_s
Warfarin	1.72	280	0.32	-
Rotenone	2.92	254	0.64	2.50
Pyrethrum	3.00	227	0.79	0.11

Column efficiency can be calculated by determining height equivalent to one theoretical plate (HETP). HETP was determined by dividing the length of the column by the number of theoretical plates. Theoretical plates represent a concept of the number of partitioning steps an analyte would go through as it traverses the length of the column. The larger N would represent the better efficiency because more partitioning occurred. Theoretical plates were determined for the analytes by the following formula:

$$N = 16(t_r/W_b)^2$$

In the above formula t_r represents retention time and W_b equals the peak width at the baseline. The values of HETP and N is shown in Table 1.6.

Warfarin and Pyrethrum had the best HETP, which imply that, the choice of mobile phase and column provide good chromatographic conditions for these analytes. The column was a Brownlee Laboratory reverse phase C_{18} (RP-C_{18})

Table 1.6 Theoretical plate and HETP values

Chemical	Retention Time (min)	Peak Width (min)	Column length (mm)	Theoretical Plates	HETP
Warfarin	1.72	0.48	250	364.17	0.69
Rotenone	2.92	1.94	250	55.10	4.54
Pyrethrum	3.00	0.81	250	344.77	0.73

Spheri-10 column, 250 mm X 4.6 mm. A Waters 501 HPLC pump with a Rheodyne 7125 injector was used for the analysis. Detection was with a Milton Roy variable wavelength Spector Monitor® 3100 model connected to a Spectra-Physics SP4270 integrator. The RP-C_{18} column was chosen because of its versatility with many organic compounds and ease in functioning over a pH range of pH 2 to pH 7. A concern with this column was degradation by hydrolysis of the silica matrix. It also degrades under basic conditions that are

preferred for the Nicotine analysis. Once the analytes are separated from each other the concentration was determined from the UV absorbance measurements.

QUANTITATION

The analyte was isolated (by HPLC) and put into a solution with a non UV absorbing solvent. Calibration curves are prepared by plotting absorbance vs concentration or transmittance vs concentration and a linear regression line determined and the unknown concentration calculated from the regression line. UV detection can be coupled with chromatography using a flow through cell and isolation of different fractions of the sample. If background interferences exist extracting the sample without an analyte would allow for subtraction of unwanted absorbance.

Nicotine was run with a basic Na_2HPO_4 buffered acetonitrile (60:40) mobile phase on a Develosil™ ODS-UG Speri-5, 150 mm X 4.6 mm column. The Develosil™ ODS-UG provides stability at high pH values. The mobile phase was pH adjusted to about pH 10. The pump, detector and integrator are the same as used for the previous three analytes.

Standard curves were run for all of the analytes, they are shown in Figures 1.16 - 1.19. The results of the linear regression are given in Table 1.7. The regression line does

not use (0,0) as a point in the equation the line. Each line was determined with three to four standards within a typical concentration range to be used for validation.

Nicotine was done with a Na_2HPO_4 buffered acetonitrile mobile phase at ~ pH 10. At pH 10 Nicotine will have some ionization due to the pK_as. The single ionization will be to the extent that of A/HA^+ will be 0.1096 or about 90% ionized. The double ionization will be to 0.014% double ionized. The extraction was started with individual chemicals and then when consistent recoveries were obtained the method was combined with another chemical.

Table 1.7 Linear Regression for Standards

Chemical	y-intercept	x-coefficient	r^2
Nicotine	**1000000**	**971232**	**0.975**
Pyrethrum	**-95663**	**65896**	**1.000**
Rotenone	**430717**	**129359**	**0.997**
Warfarin	**4046**	**147895**	**1.000**

Calibration curves were prepared by plotting absorbance vs concentration and a linear regression line determined. The unknown concentration was then calculated from the regression line. UV detection can be coupled with the chromatography using a flow through cell. If

background interferences exist extracting of unwanted absorbance.

Standard curves were run for all of the analytes, they are shown in Figures 1.8 - 1.11. The results of the linear regression are given in Table 1.7. The regression line does not use (0,0) as a point in the equation line. Each line was determined with three to four standards within a typical concentration range to be used for validation.

The extraction was started with individual chemicals and when consistent recoveries were obtained another analyte would be added to the method to coextract.

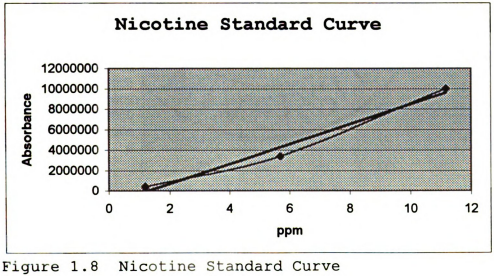

Figure 1.8 Nicotine Standard Curve

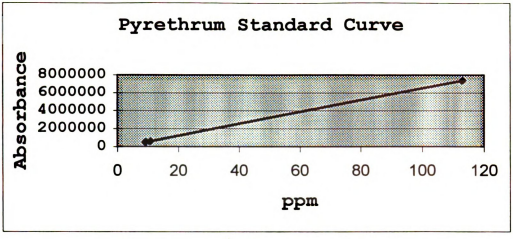

Figure 1.9 Pyrethrum Standard Curve

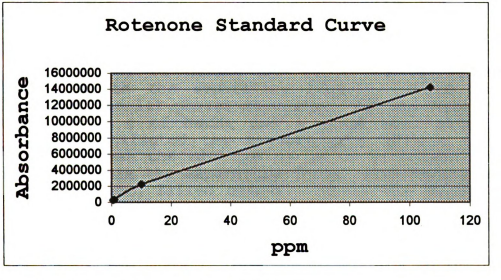

Figure 1.10 Rotenone Standard Curve

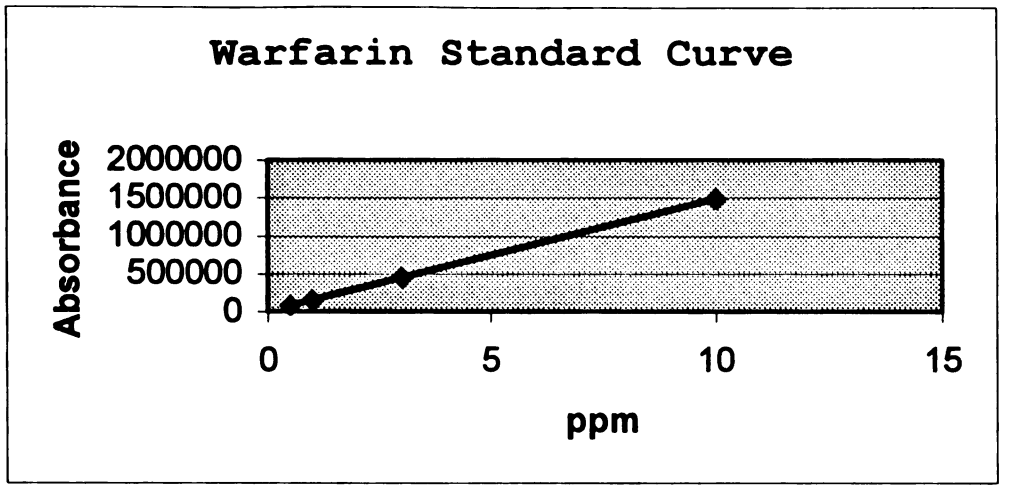

Figure 1.11 Warfarin Standard Curve

Warfarin and Rotenone were the first two chemicals to be extracted together. The method followed may be found below:

1. Weighed 10 g of sample into a separatory funnel.
2. Added 50 ml of hexane and 30 ml of saturated NaCl solution to the separatory funnel. Shook for two minutes and let phases separate. Put the hexane layer through 5 g of Na_2SO_4 and a glass wool plug filled funnel. Collected the hexane layer into a turbo-vap tube. Repeat the addition of 50 ml of hexane two more times. Rinse the separatory funnel with hexane and add to the Na_2SO_4 filled funnel. Rinse the Na_2SO_4 filled funnel with hexane and add to turbo vap tube. Reduce the volume of hexane to ~ 0.5 ml and take it up with methanol to 2ml for HPLC analysis.
3. HPLC analysis will be done at 1 ml/min with methanol as the mobile phase on a C-18 column.
   A wavelength of 254 nm and 280 nm for detection of Rotenone and Warfarin respectively was used.

The method provided modest recoveries of 34%-54% for Warfarin and good recoveries for Rotenone of 86%-115%. Since Warfarin has a slight acidic nature there was a loss

of warfarin to the aqueous phase. Adjusting the pH to less than 5.5 with an aqueous solution of dilute HCl was added to the method after the first hexane extraction and then repeat the extraction with 50 ml of fresh hexane. This brought the recoveries for Warfarin up to 63%-68%. The recoveries were low for typical residue work but showed promise with the added concern of introducing two more chemicals through the same method. Work to improve the recoveries will be continued later with the addition of other solvents for increased extraction recoveries.

Pyrethrum was the next chemical added to the extraction method. The results again showed good promise with recoveries between 80% - 117% with the combined method. Nicotine was added to the method next with recoveries of 17% 33%. The original method for Nicotine is given in Appendix A. The method was reviewed to review the pH concerns that Nicotine has due to its two basic nitrogen atoms. Initially the sample was cleaned with an acidic water wash and then brought to a basic pH with 10 N NaOH until pH was between 8 and 9 and then 50 ml of dichloromethane was added. The dichloromethane portion was saved and the aqueous portion was again extracted with dichloromethane and the extracts were combined. This would combine nicely with the method for the first three chemicals. The first method with all four chemicals included a basic extraction by adjusting the pH

with concentrated NaOH until the pH was greater than 12. The extraction was repeated with 80 ml of fresh hexane. The recoveries were up to 102% at 6 µg/ml spike level. At lower levels the baseline noise interfered to a greater extent relative to the peak of interest.

The final method may be found in Appendix F. In this method the sample is first extracted at a neutral pH to obtain the neutral species with dichloromethane. NaCl is added to the aqueous phase to push nonionic molecules out of the aqueous phase into the dichloromethane. At a neutral pH Nicotine's speciation has a ratio of 6:94:0.01 of the double ionized, single ionized, to the nonionized species respectively. This accounts for the poor recoveries at the lower pHs for Nicotine. The aqueous phase was then made basic to assure that Nicotine would be nonionic and extractable by the hexane. The speciation at pH 12 of Nicotine was 91.6:8.4 Nicotine to single ionized Nicotine. The aqueous phase was then acidified to assure Warfarin would be protonated to allow for preferred residence concentration to be in the organic solvent.

The overall recoveries for the combined method are given in Table 1.8. The four chemicals showed wide ranges of recoveries within each chemical. Warfarin though it had

Table 1.8 Percent Recoveries for Fortified Samples

Chemical	Percent Recovery Mean (n=2)	Spike Concentration (µg/ml)	Range %
Nicotine	83.8	8.0	66-102
Pyrethrum	98.5	5.0	80-117
Rotenone	100.5	4.9	86-115
Warfarin	65.5	2.0	63-68

a smaller range from 63%-68% of recoveries had the lowest mean recovery of 65.5%, attributable to the pH changes during extraction. At each step the analyte was lost to some degree.

The chemicals were evaluated for Limit of Detection (LOD) and Limit of quantitation (LOQ) and the results is shown in Table 1.9. The LOD represents the lowest

Table 1.9 LOD and LOQ Values

Chemical	LOD µg/ml	LOQ µg/g
Nicotine	1.19	8.0
Pyrethrum	0.81	5.0
Rotenone	0.5	4.9
Warfarin	0.5	2.0

concentration of a standard detected with the UV detector of a standard injected into the HPLC. LOQ represents the lowest concentration of a spiked sample put through the extraction method, then injected into the HPLC and detected and quantified with acceptable recoveries. In the multimethod the level of detection of 1 µg/g was not achieved but could be lowered if done as individual methods (non published data from Dr. Matthew Zabik's laboratory).

FUTURE RESEARCH

The partitioning between the aqueous phase was favored for each chemical by the different pHs and NaCl additions. In choosing chemicals to analyze together for future research the pK_a would preferably be closer and the chemicals would all be neutrals, bases, or acids. The combination of the three has been quite troublesome and the recoveries were not especially good for all chemicals. Another point of consideration was the mode of detection in that the wavelengths of maximum detection varied for the four chemicals. This could be easily evaluated by the use of a diode array detector for multiple simultaneous wavelength detection.

Further work could also be done in trying solid phase extraction (SPE) to eliminate the large volumes of solvent required to achieve modest recoveries. The solvent savings both in purchases and in disposal cost are a driving force in laboratories. The reduced exposure to hazardous solvents would be another advantage of SPE.

METHOD II - SABADILLA, α-TERTHIENYL, RYANIA, AND AZADIRACHTIN

The research started with evaluation of each chemical's solubility in organic solvents and Absorptivity to UV-Vis radiation for detection. Table 2.0 shows the solvents of choice for each chemical.

Table 2.0 Solubility of Solvents

Chemical	Hexane	Methanol	Acetone
α-Terthienyl	Soluble	Soluble	Soluble
Azadirachtin	Insoluble	Soluble	Soluble
Ryanodine	Insoluble	Soluble	Soluble
Veratridine	Insoluble	Soluble	Soluble

Methanol was chosen as the solvent of choice because of both solubility of the analyte and lower volatility compared to acetone.

The UV-Vis maximas are given in Table 2.1 for the chemicals. Sabadilla has a composition of over 30 alkaloids with two of primary toxicological concern. Strong UV chromophores exist only for Veratridine so that will be the component analyzed for with the HPLC-UV detection system available (Zang, 1997). The other alkaloids can be detected using HPLC-MS for detection.

Ryania's active ingredients of toxicological concern are Ryanodine and Dehydroryanodine and the standard used was composed of both constituents. Azadirachtin has a very poor UV maximum in that it occurs very close to the wavelength were solvents also absorb electromagnetic radiation.

Table 2.1 Maximum Absorbance at Specific Wavelengths (nm)

Chemical	λ (nm) / A	λ (nm) / A	λ (nm) / A
α-Terthienyl	224 / 0.81	252 / 0.91	350 / 1.00
Azadirachtin	217 / 0.72	232 / 1.00	NA
Ryanodine	210 / 0.30	269 / 1.00	NA
Veratridine	239 / 1.00	271 / 0.93	298 / 0.74

The initial HPLC work was done at 254 nm with methanol at 1.0 ml/min. Resolution was calculated using the equation on page 47 in the text. Values are given in Table 2.2. Ryanodine, Azadirachtin and Veratridine overlap at concentrations greater than about 2 µg/ml, which can be determined by the low resolution between the three chemicals. Column efficiency was evaluated by looking at HETP and theoretical plates. The results are given in Table 2.3. Azadirachtin and α-Terthienyl did have the best theoretical plate counts, which allows for better separation efficiency. Slowing down the mobile phase to 0.5 ml/min

provided better resolution but poorer recoveries due to the peaks flatten out at the slower flow rate.

Table 2.2 Resolution of Between Peaks

Chemical	Retention Time (min)	Wavelength (nm)	Width (min)	Resolution R_s
Ryanodine	3.24	260	0.70	-
Azadirachtin	3.46	260	0.50	0.37
Veratridine	3.60	260	0.80	0.22
α-Terthienyl	4.77	260	0.75	1.51

The individual UV-Vis spectra for each analyte were done and ultraviolet (UV) and visible (Vis) absorbance maximas were determined for each analyte using a Gilford UV/Vis Spectrometer. HPLC retention time

Table 2.3 Theoretical plate and HETP values

Chemical	Retention Time (min)	Peak Width (min)	Column length (mm)	Theoretical Plates	HETP
α-Terthienyl	4.77	0.75	250	647	0.39
Azadirachtin	3.46	0.50	250	766	0.33
Ryanodine	3.24	0.70	250	343	0.73
Veratridine	3.60	0.80	250	324	0.77

data for calculating resolution, theoretical plates, and HETP was determined from the chromatograms run on the. This

data was run at 254 nm on a HPLC system, this will be found described later in the text in detail. The standard curves are in Figures 2.0-2.3.

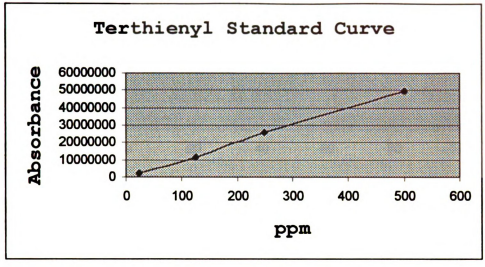

Figure 2.0 α-Terthienyl Standard Curve

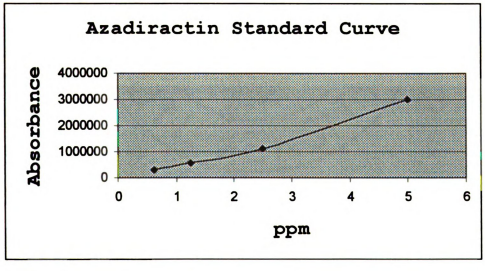

Figure 2.1 Azadirachtin Standard Curve

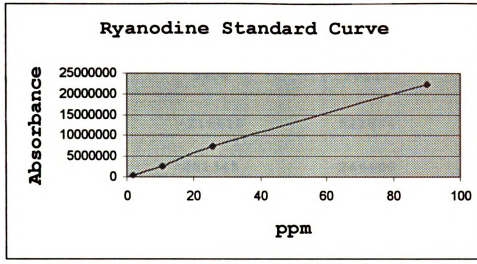

Figure 2.2 Ryanodine Standard Curve

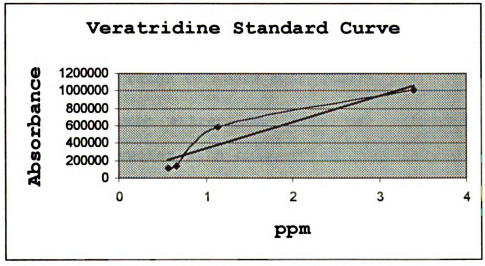

Figure 2.3 Veratridine Standard Curve

Table 2.4 Linear Regression for Standards

Chemical	y-intercept	x-coefficient	r^2
α-Terthienyl	5904	99516	0.99
Azadirachtin	-216618	621894	0.98
Ryanodine	281345	246666	0.99
Veratridine	30288	301965	0.89

All of the standard curves had good linearity with r^2 greater than 0.98 with the exception of Veratridine. The standard curves for each analyte are in Figures 2.8 - 2.11. The Veratridine standard curve had a showed log relationship with concentration, i.e. Response = ln concentration. This also shows that Veratridine should be evaluated with a linear standard curve only over a small concentration range to avoid non-linearity.

Solid phase extraction (SPE) was considered at for extraction purposes with this set of chemicals due to their hydrophobic and nonionic characteristics. These characteristics make them good for SPE in that a common solvent could be used to elute them. SPE has generally attractive characteristics as an analytical extraction method due to the ease of handling, time saving alternative to liquid/liquid extraction, significantly reduced solvent

usage, and also may be used to remove interference compounds.

The method given in Appendix F was followed. The sample extract was put on the 6 ml Bakerbond SPE Octadecyl (C_{18}) Reversed Phase column as a mixture of all four analytes in methanol with 6 replications. The SPE columns were attached to a vacuum manifold to provide a uniform flow rate of the solvents. The spike amounts ranged from 0.36-1.08 µg for Ryanodine, 0.28-0.84 µg for Veratridine, and 0.02-0.06 µg for both α-Terthienyl and Azadirachtin. After elution of the analyte the sample was analyzed using HPLC. The HPLC column was a Brownlee Laboratory reverse phase C_{18} (RP-C_{18}) Spheri-10 column, 250 mm X 4.6 mm. A Waters 501 HPLC pump with a Rheodyne 7125 injector was used for the analysis. Detection was with a Milton Roy variable wavelength Spector Monitor® 3100 model connected to a Spectra-Physics SP4270 integrator. Recoveries of the analysis are given in Table 2.5. The recoveries using the integrator calculated area produced recoveries greater than 130% for Ryanodine, Azadirachtin, and α-Terthienyl. This was due to high background relative to the analyte. Using the height of the peak for the previously mentioned three analytes the recoveries were less than 130%.

Table 2.5 Percent Recoveries for Fortified Samples

Chemical	Integrator Area	Hand Measured Height	Best Measurement
α-Terthienyl	161	78	78
Azadirachtin	232	111	111
Ryanodine	194	93	93
Veratridine	119	152	119
Overall Mean ± Std Dev	177 ± 48	108 ± 32	100 ± 18

Veratridine showed the opposite effect to recoveries. To optimize the recoveries of Veratridine was be measured with height and the other three the integrator value was used.

The LOD and LOQ values for the chemicals is shown in Table 2.6. Similar the previous method when the analytes are analyzed by themselves the LOQ can be reduced.

Table 2.6 LOD and LOQ Values

Chemical	LOD µg/ml	LOQ µg/g
α-Terthienyl	5.0	0.02
Azadirachtin	0.625	0.02
Ryanodine	1.8	0.36
Veratridine	3.4	0.28

RESULTS AND CONCLUSIONS

Further work could be done with HPLC/MS that would differentiate the mass and structural form present, so analytes that elute very close on a HPLC column could be known. Biological samples are complex and require more separation than water samples using capillary electrophoresis (CE) could also be employed. The essential part of a complex systems has to do with finding more than one way of separating compounds. The different forms of separation may act differently, ie. ionic compounds could be isolated from nonpolar compounds with CE even if they absorbed at the same wavelength.

Organic pesticides are essentially the same as conventional synthetic pesticides and should be treated accordingly. Oral LD_{50} of Nicotine and Rotenone have values of 60 mg/kg in rats and Malathion has a value of 5500 mg/kg. Malathion is under Environmental Protection Agency (EPA) review under FQPA (Food Quality Protection Act) at this time because of its human toxicity. The very reason that some plants develop chemical arsenals against pest should assure you that organically derived chemicals have similar and if not more potent toxicological actions both to humans and other living organisms.

The government regulates food safety by two major federal laws which are administered by EPA for Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) and Health and Human Services/Food and Drug Administration (HHS/FDA) the Federal Food, Drug, and Cosmetic Act (FDCA). FDCA establishes tolerances for pesticide residues in food and tolerances are enforced by HHS/FDA for most foods and US Department of Agriculture/Food Safety and Inspection Service (USDA/FSIS) for meat, poultry, and some egg products. Food safety should follow consistent regulations for pesticides and additives to assure both a safe exposure level through eating and environmental sources have a minimal impact for non target organisms. To foster the naïve attitude that organic foods are safer than conventional foods will ironically be a disservice to the very people you intend to protect from pesticide and additive exposure. Increasing assurance of a safe food will be through recognizing and educating the public of chemical toxicity without differentiation of chemical source.

APPENDIX A

SOP FOR DETERMINATION OF NICOTINE IN CROPS

TITLE: **Determination of Nicotine in Crops**

DATE: September 12, 1995

RESPONSIBLE PERSON: Chris Vandervoort

SCOPE: This SOP will provide for quantitation of residues of nicotine in crops matrices

REFERENCES: Sheen, Shuh, Detection of Nicotine in Foods and Plant Material, Journal of Food Science, 53:5:1988:p1572-1573.

TERMINOLOGY: GC = Gas Chromatography

HAZARDS & PRECAUTIONS:

1. Weigh 10 g of sample and mix with 30 ml water, 1 ml of 3 N HCl, and 30 g $(NH_4)_2SO_4$. Heat in steam bath for 30 minutes with stirring. Cool slightly and filter through Whatman # 1 filter with vacuum. Transfer to a 250 ml separatory funnel with approximately 2, 3-4 ml portions of saturated $(NH_4)_2SO_4$.

2. Add 50 ml of dichloromethane and shake for at least 30 seconds. Discard dichloromethane portion.

3. Add 10 N NaOH til pH is between 8 and 9. Add 50 ml of dichloromethane and shake for at least 1 minute. Save dichloromethane into a 125 ml separatory funnel. Repeat with fresh 50 ml of dichloromethane and combine extracts.
4. Add 5 ml 1 N H_2SO_4 and shake for at least 1 minute. Discard the dichloromethane.

5. Transfer the aqueous phase to a Turbo-Vap tube with 2-3 ml of water and add to tube. Add 1 g Na_2SO_4 to the tube. and place in a 90 ° C water bath with N_2 to remove dichloromethane. Cool and add 10 N NaOH til pink color persists in phenolphthalein. Cool and add

sufficient Na_2SO_4 to saturate. Add 1 ml of benzene or (toluene).

6. GLC analysis of nicotine analytical parameters.
   20 m Carbowax .25 mm column NP detector.

APPENDIX B

SOP FOR DETERMINATION OF PYRETHRUM IN CROPS

TITLE: **Determination of Pyrethrum in Crops**

DATE: October 25, 1995

RESPONSIBLE PERSON: Chris Vandervoort

SCOPE: This SOP will provide for quantitation of residues of pyrethrum in crop matrices

REFERENCES: Fujie, G.H. and O.H. Fullmer. (1978) Determination of Cis- and trans-pyrethrum residues in plant, animal, and soil matrices by gas chromatography. J. Agric. Food Chem., 26, p 395-398.

TERMINOLOGY: GC = Gas Chromatography

1. Extraction

a. Place 10 g sample into a 250 Erlenmeyer flask and add 50 ml of a 2:1 mixture of hexane:isopropanol.
b. Blend for ~ 3 minutes and pour off the solvent through a Buchner funnel with Whatman # 4 filter into a 250 ml separatory funnel.
c. Add an additional 50 ml of 2:1 mixture and blend for ~ 1 minute.
d. Add ~ 2 g of Celite and filter through the Buchner funnel and rinse the filter cake with 25 ml of hexane.
e. Add 125 ml of 10 % NaCl to separatory funnel and shake for ~ 1 minute.
f. Transfer the aqueous layer to another separatory funnel.
g. Add 100 ml of 10 % NaCl to the first separatory funnel and shake for ~ 1 minute. Transfer lower aqueous phase to the second separatory funnel and drip the solvent through anhydrous sodium sulfate into a round bottom flask. Rinse separatory funnel with an additional 5 ml of hexane and drip through anhydrous sodium sulfate.
h. Add an additional 50 ml of hexane to second separatory funnel and shake for ~ 1 minute. Discard aqueous layer and drip hexane through anhydrous sodium sulfate.

i. Reduce volume < 1 ml, add hexane to 10 ml.

2. Florisil Cleanup

a. Prepare a 1 cm i.d. glass column with 2 g of Florisil activated at 135 ° C and topped with 1 cm of anhydrous sodium sulfate.
b. Prewash column with 20 ml of 9:1 hexane:ethyl ether, two 5 ml of hexane and discard.
c. Add sample to column using two 2 ml hexane rinses of sample container. Elute 40-55 ml 9:1 hexane:ethyl ether.

3. Gas Chromatography

a. Use FID detector for detection.

APPENDIX C

SOP FOR DETERMINATION OF ROTENONE IN CROPS

TITLE: **Determination of Rotenone in Crops**

DATE: November 1, 1995

RESPONSIBLE PERSON: Chris Vandervoort

SCOPE: This SOP will provide for quantitation of residues of rotenone in crops matrices

REFERENCES: Ho, J.S. and W.L. Budde. (1994) Investigation of the Natural Pesticide Rotenone in Water Using Liquid-Solid Disk Extraction, Supercritical Fluid Elution, and Liquid Chromatography/Particle Beam Mass Spectrometry. Analytical Chemistry, Vol.66, No.21p 3716-3722.

Dawson, V.K. and J.L. Allen. (1988) Liquid Chromatographic Determination of Rotenone in Fish, Crayfish, Mussels, and Sediments. J. Assoc. Off. Anal. Chem., Vol.71, No. 6. p. 1094-1096.

Bushway, R.J. (1983) Reverse Phase Radial Compression High Performance Liquid Chromatography Determination of Rotenone in Formulations. J. Assoc. Off. Anal. Chem., Vol. 66, No. 3. p. 793-796.

TERMINOLOGY: **GC = Gas Chromatography**

1. Extraction
   a. Place 10 g sample into a Sorval mixing cup with 25 ml of methanol and thoroughly mix for 5 minutes. Pour supernatant into a Gelman type A.E glass fiber filter. Repeat three time and combine supernatant.
   b. Add supernatant to separatory funnel with 500 ml of 0.1 N HCl and extract with 20 ml hexane three times. Evaporate hexane to dryness.

2. Silica gel column
   a. Transfer extract with 5 ml of toluene to 500 x 22 mm silica gel column with glass wool followed by 5 cm of Na_2SO_4, silica gel. Rinse flask and column with five 5 ml toluene portions and discard. Do not let the column go dry. Elute column with 70 ml of toluene:acetone (97 +3). Take to dryness and bring to volume for LC with methanol.

3. LC
   a. Reverse phase C-18 column with UV detection at 295 nm. Mobile phase is methanol:water (70:30). Flow rate 1 ml/min.

APPENDIX D

SOP FOR DETERMINATION OF WARFARIN IN CROPS

TITLE: **Determination of Warfarin in Crops**

DATE: November 1, 1995

RESPONSIBLE PERSON: Chris Vandervoort

SCOPE: This SOP will provide for quantitation of residues of warfarin in crops matrices

REFERENCES: Akhtar, S. & L.C. Bailey, 1985. Simultaneous Liquid Chromatography Determination of Warfarin and Sulfaquinoxaline in Cornmeal-Based Rodenticide. , J. Assoc. Off. Anal. Chem. Vol. 68, No. 6. p. 1139-1142.

Jones, A. 1996. HPLC Determination o Anticoagulant Rodenticide Residues in Animal Livers, Bull. Environ. Contam. Toxicol. 56:8-15.

1

TERMINOLOGY: GC = Gas Chromatography

1. Extraction-Plant material

a. Weigh 1 g of sample into a screw top vial and shake with 8 ml of acetonitrile. Let settle and filter supernatant through 0.45 mm membrane and collect in a centrifuge tube. Repeat three times with fresh acetonitrile and add supernatants together. Wash residue with 5 ml acetonitrile twice and add to filter.

2. Liquid Chromatography

a. Use reverse-phase C-18 column with UV detector set at 280 nm. Use mobile phase of 1.113 g of heptanesulfonic acid sodium salt in 500 ml of LC grade H_2O added to 50 ml of acetonitrile and adjust the pH to 3.5 with HCl. Set the flow rate a 1 ml/min and ca 2500 psig.

APPENDIX E

SOP FOR DETERMINATION OF NICOTINE, ROTENONE, PYRETHRUM, AND WARFARIN IN CROPS

TITLE: Determination of Nicotine, Rotenone, Pyrethrum, and Warfarin in Crops

DATE: October 6, 1998

RESPONSIBLE PERSON: Chris Vandervoort

SCOPE: This SOP will provide for quantitation of residues of nicotine in crops matrices

1. Weigh 10 g of sample and place in a separatory funnel with 20 ml of 0.5 N NaCl. Extract with 80 ml of dichloromethane. Allow phases to separate and put dichloromethane layer through glass wool stoppered funnel with ~ 5 g of Na_2SO_4 into a round bottom flask. Repeat with 80 ml of fresh dichloromethane.

2. Adjust the pH with concentrated NaOH until the pH is greater than 12. Add 80 ml of hexane and shake for 2 minutes. Allow phases to separate and put hexane layer through Na_2SO_4 into a round bottom flask. Repeat with 80 ml of fresh hexane.

3. Adjust the pH to less than 5.5 with of the aqueous solution with HCl. Extract with 50 ml of hexane and put hexane layer through the Na_2SO_4 funnel. Combine all hexane filtrates together. Repeat with 50 ml of fresh hexane.

4. Reduce the volume of hexane to dryness and take it up with methanol for HPLC analysis.
   a. HPLC analysis will be done at 1.0 ml/min with methanol as the liquid phase on a C-18 column for pyrethrum, rotenone, and warfarin. Pyrethrum detection is at 227 nm, rotenone is at 254 nm, and warfarin is at 280 nm.
   b. Nicotine was done on a Develosil™ ODS-UG Speri-5, 150 mm X 4.6 mm column with a Na_2HPO_4 buffered acetonitrile mobile at ~ pH 10. Detection was at 254 nm.

APPENDIX F

SOP FOR DETERMINATION OF α-Terthienyl, Azadirachtin, Ryanodine, and Veratridine (αARV) IN CROPS

TITLE: **Determination of αARV in Crops**

DATE: September 12, 1998

RESPONSIBLE PERSON: Chris Vandervoort

SCOPE: This SOP will provide for quantitation of residues of αARV in crops matrices

REFERENCES: Zang,X., Fukuda, E.K., and Rosen, J. D. Multiresidue Analytical Procedure for Insecticides Used by Organic Farmers, Journal Agric. Food Chemistry, 46:1988:p2206-2210.

TERMINOLOGY: HPLC = High Pressure Liquid Chromatography

HAZARDS & PRECAUTIONS:

1. 10 g sample added to 100 ml of 1:9 water:acetonitrile. The mixture was homogenized and let settle for 30 minutes. The extract was filtered through a 1.5 µm pore size glass filter and rinsed with acetonitrile. The acetonitrile was removed with a Turbo-Vap.

2. A 6 ml Bakerbond SPE Octadecyl (C_{18}) Reversed Phase column was conditioned with 6 ml methanol followed by 6 ml of H_2O.

3. The sample was placed on the column and passed through the column at rate of 1-2 ml per minute. The column was vacuumed dried for 5 minutes. The column was washed with 3 ml of H_2O and eluted with 3-4 ml of methanol.

4. Liquid Chromatography
   Use reverse-phase C-18 column with UV detector set at 254 nm. Use mobile phase of HPLC grade methanol. Set the flow rate between 0.5-1 ml/min and ca 2500 psig.

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INTRODUCTION

DDT or p,p'-dichlorodiphenyltrichloroethane was first synthesized in 1874 in Germany. Paul Muller discovered the insecticidal efficacy of DDT in 1939 and was awarded the Nobel Prize in medicine and physiology in 1948 for it (Mischke, et al.., 1985). DDT was synthesized by condensing chloral hydrate with chlorobenzene. DDT is a broad spectrum and relatively cheap pesticide and this made it a good candidate for extensive use throughout the world.

DDT and its metabolites are extremely persistent in the environment. Due to the persistence of DDT and global forces and physical forces acting on DDT, it has been distributed throughout the globe. DDT and its metabolites are extremely lipophilic and hence, bioaccumulate throughout the food chain. DDT was banned in 1973 due to scientific concerns about environmental persistence and bioaccumulation in wild animals.

OBJECTIVES

Objective-III

This area of investigation was to determine the sources of the elevated DDT and its metabolites that have been measured in South Haven, Michigan since 1990 by several

researchers. Table 3.0 has some of the previous research values from other studies.

Table 3.0 Projects that have Quantified DDT and its Isomers in South Haven, MI

Study Dates	p,p'DDT Maximum Level[a]	p,p'DDT Maximum Date	p,p'DDT Avg Level[a]	p,p'DDE Maximum Level[a]	p,p'DDE Maximum Date	p,p' DDE Avg Level[a]
7/8/91 to 8/9/91	574	7/20/91	339 ± 167	1944	8/2/91	1331 ± 351
5/18/92 to 2/25/94	1907 1807	6/5/92 6/18/93	321 262	3614 5416	6/5/92 6/18/93	648 935

[a] measured in pg/m^3

The research was done in cooperation with Michigan Department of Environmental Quality - Air Division and US Environmental Protection Agency. There exist two probable hypotheses for the elevated levels. The area historically had high inputs of DDT due to the large fruit farming industry. One hypothesis is that DDT is already present in the soil and becomes volatilized with the increasing temperature and tillage practices in the spring of each year. Kelthane is an organochlorine miticide used on a wide variety of fruit, vegetable, ornamental and field crops. Kelthane is manufactured from DDT. In 1986, use of Kelthane was temporarily canceled by the EPA because of concerns raised by high levels of DDT contamination. However, it was reinstated when it was shown that modern manufacturing processes can

produce technical grade Kelthane which contains less than 0.1% DDT.

The other source of DDT could be long range transport from other locations in North America. Mexico in 1997 agreed to stop using chlordane and DDT over the next 10 years Government representatives said {(2)Author unknown}. DDT is used to kill mosquitoes, which carry malaria in Mexico. Pesticide use in Mexico still impacts the US from Mexican fruits and vegetables or pesticides that are blown across the border and into the water supply. The research looked at both air samples and soil samples from the same area to assess the impact of the soil on the air samples through volatility and soil surface disturbance from farming activites. p,p'-DDT, o,p'-DDT, p,p'-DDD, o,p'-DDE, p,p'-DDE, o,p'-DDD,and Kelthane are the analytes that were analyzed for this study.

LITERATURE REVIEW

Persistence in the Environment

A controversy over persistence of organic pollutants and natural degradation by microbial action has been brewing by various researchers. It has been reported that naturally occurring organisms in sediments play an important role in breaking down the chlorinated compounds. A massive DDT-contaminated Superfund site off the California coast

(contaminated by Montrose Chemicals) has at stake a remediation decision that will affect millions of people and could cost hundreds of millions of dollars to clean up. The proposed method of cleanup was to cover the site with a thick sand cap. The finding that DDE (1,1-dichloro-2,2-bis(chlorophenyl)-ethylene), a byproduct of the pesticide DDT, can naturally degrade comes from laboratory experiments performed at Michigan State University's (MSU's) Center for Microbial Ecology in East Lansing and many other places (Quensen, 1998). It was shown 40 years ago that the breakdown products of DDT accumulate in the environment by many researchers. Sediments collected from the Superfund site on the Palos Verdes Shelf, had important findings because they showed that degradation products from DDT use may not be as persistent as previously thought. The MSU group suggest that natural processes might be significantly reducing the risk posed by these contaminated sediments (Renner, 1998). The research showed that the dechlorination does go beyond the DDE metabolite, which was previously shown and confirmed by them.

The pathway of DDT breakdown to DDE, DDD, and DDMU is shown in Figure 3.0. DDT loses a HCl to go to DDE or loses a Cl and gains a H to become DDD (1,1'-(2,2-dichloroethylidene)-bis[4-chlorobenzene]). DDD goes to DDMU (1,1'-(2-chloroethenyldene)-bis[4-chlorobenzene]) by loss of

HCl. The DDMU structure has one less Cl- than DDE as seen in Figure 3.0.

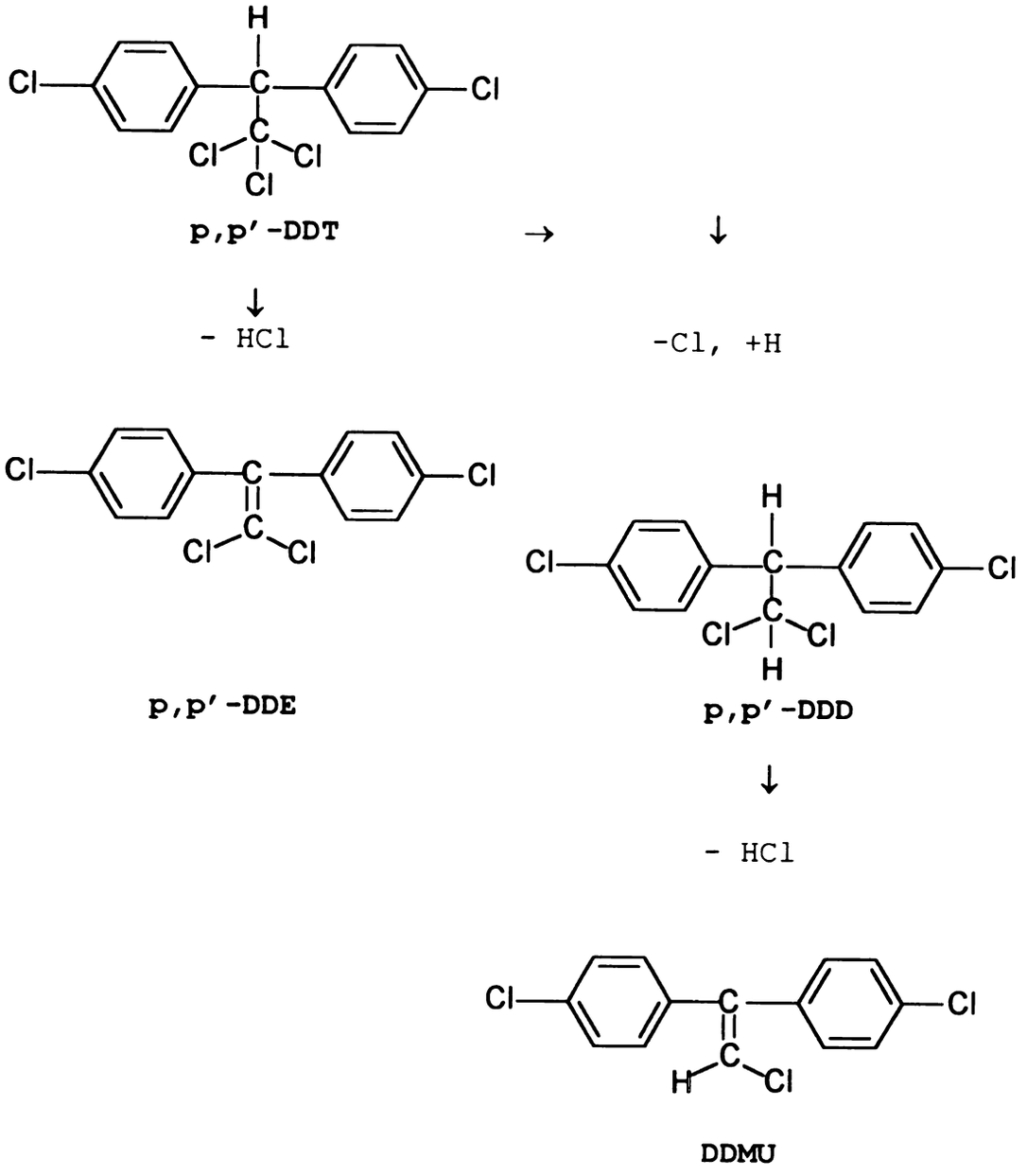

Figure 3.0 DDT Degradation Pathway

The research conducted by Renner at MSU involved microbial degradation of sediments of DDE to DDMU, which has one less chlorine than DDE. The DDMU is also found not to bioaccumulate as readily as the parent compounds. The research has some real life uncertainty for bacterial "dechlorinators". They are often less viable in the environment and with competition from other organisms and therefore die out. Properties of the target chemicals is shown in Table 3.1.

Table 3.1 Properties of DDT, DDE, DDD, and Kelthane

Chemical	Henry's Law Constant $Pa \cdot m^3/mol$	Vapor Pressure (Pa)	Log K_{oc}
o,p - DDE	245[a]	Negligible	6.00[b]
p,p - DDE	245[a]	Negligible	6.00[b]
o,p - DDD	Not Known	Negligible	4.64[b]
p,p - DDD	Not Known	Negligible	4.64[b]
o,p - DDT	0.86[a]	2.5×10^{-5} [c]	5.14-6.26[b]
p,p - DDT	0.86[a]	2.5×10^{-5} [c]	5.14-6.26[b]
Kelthane	Not Known	Not Known	Not Known

a (Iwata, 1993), b (Odermatt, 1993), c (WHO, 1979)

Toxicity

DDT and its metabolites are readily absorbed and stored in fatty tissue of many organisms. DDT is readily absorbed through the gastrointestinal tract with increased absorption in the presence of fatty acids. Aquatic organisms reach an equilibrium concentration with age and with water concentration. These organisms are then ingested and further biomagnification occurs on up the food chain. DDT has been shown to be moderately to slightly toxic to mammalian species via the oral route with oral LD_{50}s ranging from 113 to 800 mg/kg in rats up to greater than 100 mg/kg in sheep and goats (Extoxnet, 1993).

Acute toxicity due to one-time administration of 100 mg/kg of DDT to rats showed increased blood levels of liver enzymes and cellular changes in the central nervous system of monkeys (WHO, 1979). Humans, exposed to acute concentrations show symptoms of nausea, diarrhea, increased liver enzyme activity, irritation of eyes, nose, and throat, and convulsions at higher doses.

Chronic exposure to DDT and its metabolites to several bird species have shown a mechanism of eggshell thinning and other reproductive implications.

Atmospheric Transport

Atmospheric transport occurs as a result of soil/air partitioning. Initially the contaminants are deposited after spraying or plant decomposition into the soil matrix. The soil acts as an environmental sink for the aromatic hydrocarbons (ArH) (Hippelein, 1998). In warm climates the ArH show increased volatilization due to the temperature dependency on vapor pressure. The ArH are then partitioned into the air and transported to cooler regions. In the cooler regions the ArH then partition back into the soil as they condense. The particle bound ArH are also deposited into the soil through wet or dry deposition. The soil concentration and temperature drive the magnitude of the ArH air concentration. The annual cycling of sum of all DDT and its metabolites (ΣDDT) concentrations have been shown to follow a temperature dependent path as seen in data collected in Egbert, ON, Canada (Hoff, 1992). The data has low concentrations in the winter months of about 50 pg/m^3 and a high in July of 220 pg/m^3. The distribution of ΣDDT has a sinusoidal shape with a maximum in summer and minimum in winter. The best fit for distribution of ΣDDT was found to be Lorentzian, which is measured by:

$$X(\tau) = X_{min} \{1 + (A_M\Gamma^2)/(\tau - \tau_{max})^2 + \Gamma^2)\}$$

where A_M amplitude of cycle, τ month, τ_{max} month of highest concentration, and Γ half-width in months of the distribution.

The magnitude and direction of the air/soil partitioning can be determined by the soil/air equilibrium partition coefficient K_{SA}. K_{SA} can be calculated by:

$$K_{SA} = K_{SW} / K_{AW}$$

K_{SW} is the soil/water partition coefficient and K_{AW} is the air/water partition coefficient (Hippelein, 1998).

Vaporization and movement of a chemical has an inherent relationship to vapor pressure, which depends on environmental conditions. The surface moisture also determines the rate of volatilization, a dry surface may retard vaporization by as much as 25 times (Spencer, 1990).

The movement away from the soil surface is diffusion controlled, close to the surface very little vertical movement occurs. Once the chemical has made it into the overlying air space where wind and other turbulent factors can move the chemical away from the soil surface. Wind contributes to the local movement of chemicals and then planetary forces move the chemicals onto a much larger scale away from the local area for long range transport (Spencer , 1990).

Volatilization will be short term in the northern latitudes and occur generally in summer when air and water temperatures are high. The volatilization will create fluxes of contaminants out of the water and soil during the summer months with corresponding fluxes back into soil and water in the winter months (McConnell, 1993).

To determine a mass balance of ΣDDT the assumption of a steady state will be assumed. The total mass will reside in the soil, water, or air. The weighted average for each partition compartment will be determined by:

$$\text{Total Mass} = K_S M_S + K_A M_A + K_W M_W$$

K_S, K_A, and K_W are the partition coefficient for soil, air, and water respectively and M_S, M_A, and M_W are for the masses for the corresponding compartment. DDT inputs into the environment have been identified to come from atmospheric sources (Swackhammer, 1988). A concentration gradient has been observed for ΣDDT to be higher in south-central Ontario and the lower Great Lakes than northwestern Ontario as a result of past agriculture and industrial uses in the Midwest. Long-range transport from regions to the south west were DDT may still be used illegally (Muir, 1993) may contribute to the DDT in the north. Helton conducted a study before DDT was banned in 1967 and 1968 and found DDT to be present in all atmospheric samples taken from nine

areas throughout the United States (Stanley, 1971). Air samples collect 1989 to 1990 showed 160 fold increase in levels of ΣDDT in samples collected from tropical Asia, where DDT is still used for agriculture and vector control, than from the Bering Straits (Iwata, 1993). The corresponding samples taken from adjacent ocean water had a 6.4 fold concentration factor. This data suggests that extensive usage is still occurring in tropical Asia. Another confirmation of the transport theory relies on the ratio of p,p'-DDT to p,p'-DDE (T/E) in that commercial products contain only a small portion of DDE and the T/E ratios differ due to sample location in the globe. Low ratios of T/E are seen in the North Pacific and North Atlantic basins compared to samples from the tropical Asian areas being sprayed. p,p'-DDT will be converted to p,p'-DDE due to UV absorption and metabolism by organisms during and before transport.

Soil and Sediment Concentrations

Trends in ΣDDT deposition and use can be traced in sediments from reservoirs due to their increased sediment rates over natural lakes (Van Metre, 1997). Van Metre's study showed high ranges of ΣDDT of 27 to 74 ug/kg in the sediment samples for 1965 to reductions of up to 93% in samples from 1990. The temporal concentration trends show a correlation to the use and nationwide ban in 1972 of DDT.

The two of the reservoirs had overall 58 to 78% of the ΣDDT due to DDE.

A soil study conducted in California in 1985 looked at speciation of the DDT resides in soils as a function of the ΣDDT (Odermatt,et al.., 1993). The results showed a range for DDT residues of 0 to 80%, DDD of 0 to 35%, and DDE of 15 to 100%. The mean ratios were 39% DDT, 8% DDD, and 58% DDE. The ratios support that the residues are from historical applications. These ratios were not looked at for the isomeric ratios, which would have been of interest when, back calculating to the formulated product. DDT as a group of contaminants would preferentially stay in the organic phase of soil and when bound unavailable for UV and microbial degradation.

Degradation of DDT

The formulated technical mixture nominally contains 14.9% o,p'-DDT, 77.1% p,p'-DDT, 0.1% o,p'-DDE, 4.0% p,p'-DDE, 0.1% o,p'-DDD, and 0.3% p,p'-DDD. A mixture analyzed 40 years later in our laboratory contains 22% o,p'-DDT, 70% p,p'-DDT, 4% o,p'-DDE, 3% p,p'-DDE, 0.6% o,p'-DDD, and 0% p,p'-DDD. A paper written by Müller-Herold, 1996, looked at the dominant contributions to decay for DDT and found a rapid decay in the atmosphere and a high solubility in soil.

The global limiting lifetime of DDT was calculated to

be 83 days, using the weighted average of 16 years in the soil, 1 year in water, and 7.4 days in the atmosphere.

RESEARCH

Sample Collection

The research samples were obtained from Coloma, Michigan and South Haven, Michigan. Coloma was considered to be a control (uncontaminated site) and South Haven had the historically high ΣDDT residues. The sample schedule is given in Table 3.2. A map of the general geographical area is shown in Figure 3.1 and a localized map for the sampling sites is shown in Figure 3.2. At South Haven there were 3 PUF samplers and Coloma had one. Sample A was on the south end of a notill corn field and B and C were collocated on the east side of the same corn field. Sample D was from Coloma which was a grass covered vacant parcel.

A field blank was an air canister and quartz fiber filter sample that was opened at the sample site, put into the sampler, immediately removed and closed, then brought back to the laboratory for analysis. A trip blank is similar to the field blank but it was not opened in the field. The purpose of these samples was to assess any possible contamination from unexpected sources. Continuous wind speed, wind direction, and temperature were collected at Coloma and the South Haven sites.

Table 3.2 Sampling schedule for 1998 Air and Soil Samples

Run Date	Air Sample	Soil	Field	Trip
4/14/98	X	X	X	
4/20/98	X			X
4/26/98	X			
5/02/98	X			
5/04/98	X		X	
5/06/98	X			
5/08/98	X			
5/10/98	X			
5/12/98	X		X	
5/14/98	X			
5/16/98	X			
5/19/98	X			
5/25/98	X		X	
5/31/98	X			
6/06/98	X			X
6/12/98	X			
6/18/98	X			
6/24/98	X		X	
6/30/98	X			
7/06/98	X			
7/12/98	X			
7/18/98	X		X	
7/24/98	X			
7/30/98	X			
8/05/98	X			
8/11/98	X		X	
8/17/98	X			
8/19/98	X			
8/23/98	X	X	x	

Figure 3.1 General Geographical Area of the Study

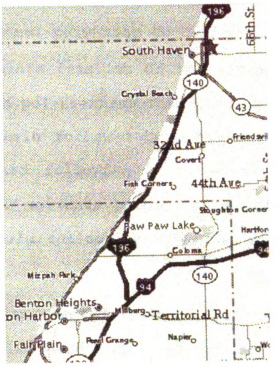

Figure 3.2 Localized Sampling Map

Air samples were collected with an Andersen PS-1 PUF sampler that was operated for approximately 24 hours at a nominal level of one meter above the soil surface. A nominal air volume of 350 m^3 was collected. The sampler head contained a 10.2 cm Whatman GF/A glass fiber filter (QF) with a nominal pore size to collect particles > 0.1 μm for the particulate fraction of the air sample. Following the filter was a polyurethane foam plug (PUF) then nominally 10 g of XAD_2 resin followed by another PUF. The PUF and XAD_2 were cleaned following a 7 day cleaning cycle with multiple organic solvents before being put into the sample thimbles for field collection (see the actual procedure in Appendix G).

The soil was sampled at the beginning and the end of the study. Samples were taken adjacent to the air sampling equipment and at three depths within the soil. Each soil core was nominally 10 inches and after being brought to the laboratory it was divided into three equal portions. Each portion was about 3.3 inches, and after dividing, the sample sections were mixed to obtain a homogenous sample. At each site a duplicate was taken for determining precision. Along the field's diagonals a sample was taken at SH. Each transect was a random composite of about 15 individual soil samples collected from SE-NW and SW-NE corners of the corn field to obtain a sample that represented the whole field.

After collection the samples were placed in a cooler at < 0°C and transported to the laboratory within 12 hours for continued storage at nominal -20°C until extraction to reduce any further degradation or chemical changes before extraction. The samples were analyzed within seven days from sampling to decrease any changes that may occur during storage.

Analytical Preparation and Cleaning

Initially all the material to be used in the analytical portion of the research had to be cleaned to remove interfering material to give a sufficiently clean background for nanogram to picogram detection of the analytes. The complete procedure may be found in Appendices G and H. All organic reagents used were pesticide-grade.

Extraction

Extraction took two days for each sample set. Each sample set contained at least one concurrent spiked sample. The air samples were extracted as the vapor phase and the particulate phase separately. The procedures were similar except for the size of glassware and proportion of solvent needed for extraction. The samples were placed in soxhlet extractor with 50:50 acetone/hexane and extracted for 18 to 24 hours. The detailed extraction procedure is shown in Appendix I.

The soil samples were extracted following a similar procedure as the air and the detailed procedure is shown in Appendix J.

Silica Gel Column Chromatography

Following extraction, the extracts were cleaned up on a 4% deactivated silica gel column to remove interfering contaminants and the volume reduced for gas chromatography. The gas chromatography analyte peaks had consistent retention times and good baseline separation so that not all of the samples were put through a silica column. If the resulting chromatograph had co-eluting peaks that could not be separated with the Hewlett-Packard (HP) software by manual integration then the extract was put through a silica gel column.

Gas Chromatography

The samples were reduced to about 2ml volume for GC analysis with a Turbo-Vap evaporator and further diluted after the first injection if needed to reduce concentrations at the GC. The GC was a 5890 Series II Hewlett-Packard (HP) equipped with a Ni^{63} electron capture detector using HP 3365 ChemStation for data acquisition and reporting. The IBM compatible computer had a dual channel interface to connect the ChemStation software to the GC. The injector was set at 250°C and the detector at 350 °C. The oven was initially

holds at 100°C for one minute and then began ramped at 1 °C/minute to 240°C, at this time all of the analytes had eluted off the column. The column was cleaned by ramping at 10 °C/minute to 280 °C. The total run time was 144 minutes. The GC column was a J & W Scientific DB-5 column with an internal diameter of 0.25 mm with a 0.1 μm film, 30 m long and the analytes were off the column in 108 minutes.

RESULTS AND CONCLUSIONS

The vapor and particulate fractions were analyzed separately to differentiate metabolic ratios in the air samples that may have occured. The soil samples were then analyzed in relationship to the air samples.

Air Samples

The air monitoring data provided detectable residue of all of the analytes during the study. It was determined that the Coloma (CLM) site was not a true control due to the presence of analyte residues. The residue levels found at CLM were significant but generally not elevated above the South Haven (SH) site. The magnitude of values measured at SH were consistent from year to year and independent of the research team conducting the analysis determined from data provided by Michigan Department of Environmental Quality. The sample concentrations may be found in Appendix K. Each site can be viewed for the total analytes per sample in vapor phase, particulate phase, and total air sample (Vapor + Particulate) in Figures 3.3 to 3.70. Also note that the y axis for sites A, B, and C are greater than D and are all different. Generally the CLM site can be considered less impacted than the SH site with about a two fold difference in residue levels.

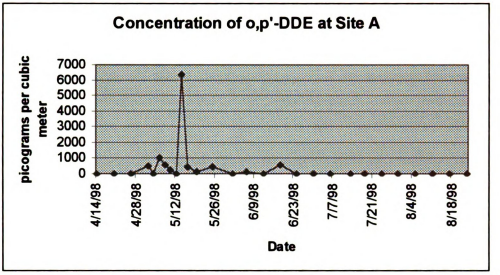

Figure 3.3 Concentration of Vapor Phase o,p'-DDE at SH Site A

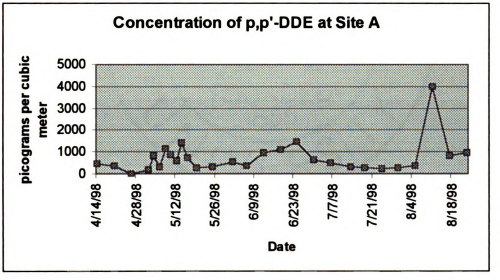

Figure 3.4 Concentration of Vapor Phase p,p'-DDE at SH Site A

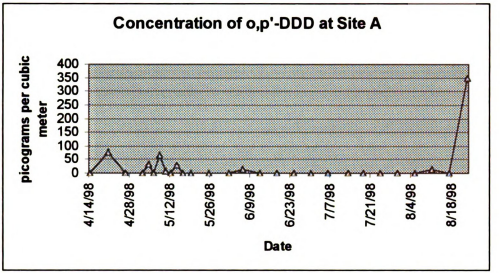

Figure 3.5 Concentration of Vapor Phase o,p'-DDD at SH at Site A

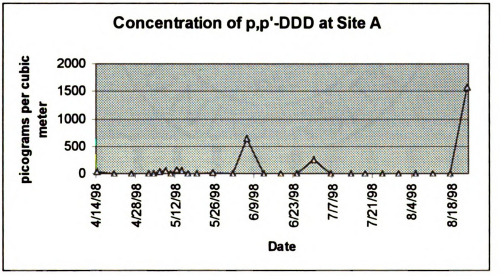

Figure 3.6 Concentration of Vapor Phase p,p'-DDD at SH at Site A

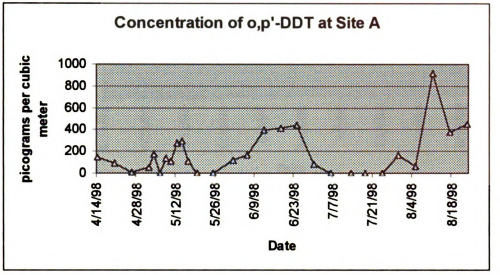

Figure 3.7 Concentration of Vapor Phase o,p'-DDT at SH at Site A

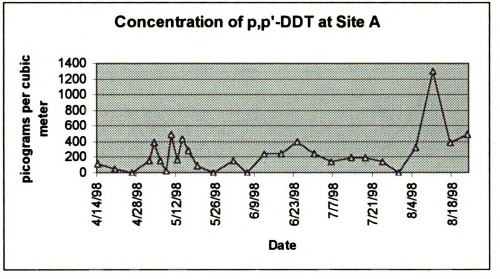

Figure 3.8 Concentration of Vapor Phase p,p'-DDT at SH at Site A

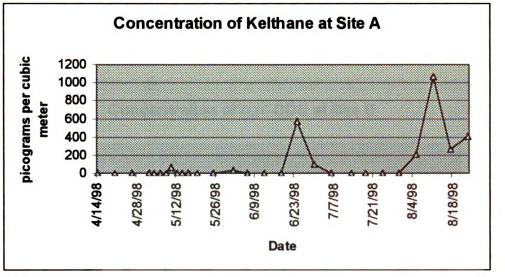

Figure 3.9 Concentration of Vapor Phase Kelthane at SH at Site A

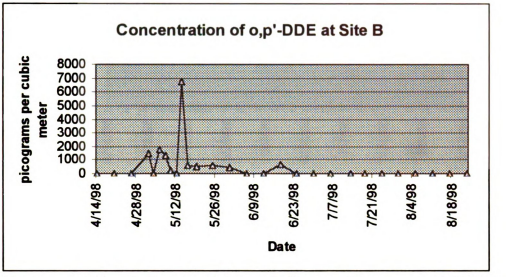

Figure 3.10 Concentration of Vapor Phase o,p'-DDE at SH at Site B

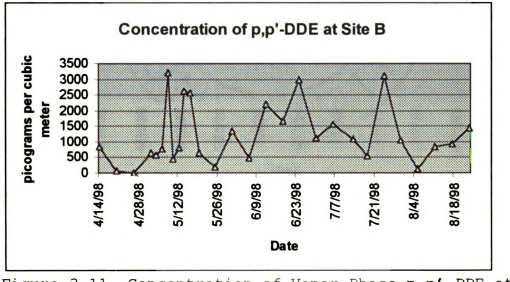

Figure 3.11 Concentration of Vapor Phase p,p'-DDE at SH at Site B

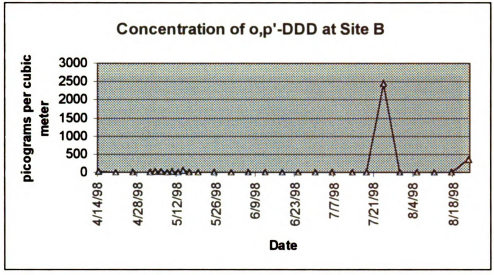

Figure 3.12 Concentration of Vapor Phase o,p'-DDD at SH at Site B

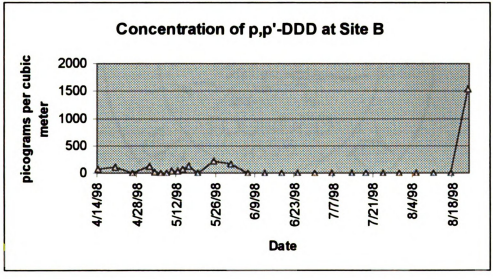

Figure 3.13 Concentration of Vapor Phase p,p'-DDD at SH at Site B

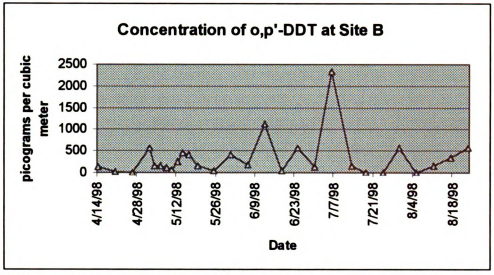

Figure 3.14 Concentration of Vapor Phase o,p'-DDT at SH at Site B

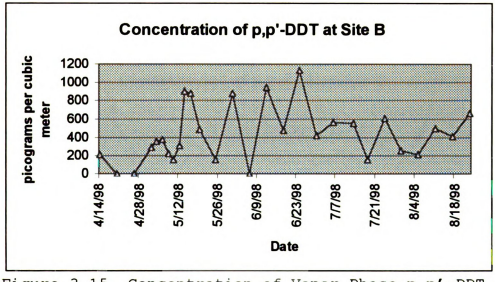

Figure 3.15 Concentration of Vapor Phase p,p'-DDT at SH at Site B

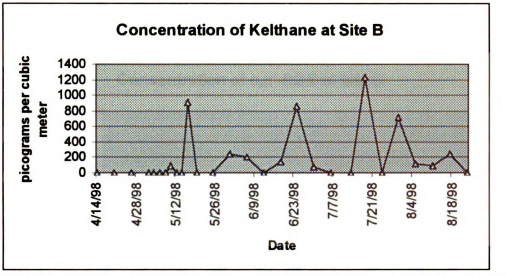

Figure 3.16 Concentration of Vapor Phase Kelthane at SH at Site B

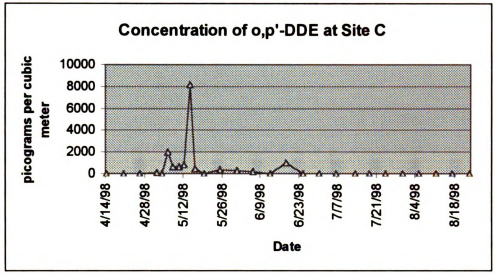

Figure 3.17 Concentration of Vapor Phase o,p'-DDE at SH at Site C

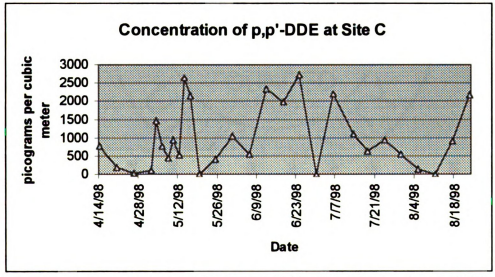

Figure 3.18 Concentration of Vapor Phase p,p'-DDE at SH at Site C

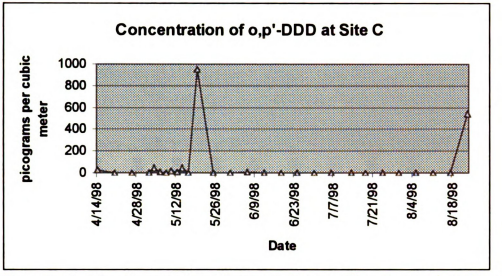

Figure 3.19 Concentration of Vapor Phase o,p'-DDD at SH at Site C

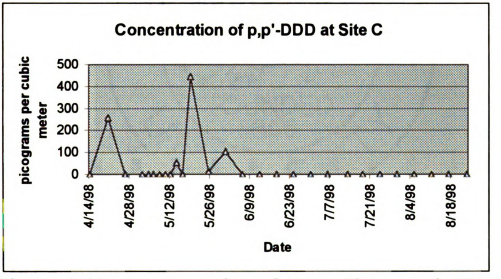

Figure 3.20 Concentration of Vapor Phase p,p'-DDD at SH at Site C

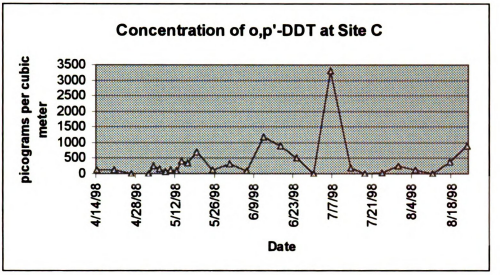

Figure 3.21 Concentration of Vapor Phase o,p'-DDT at SH at Site C

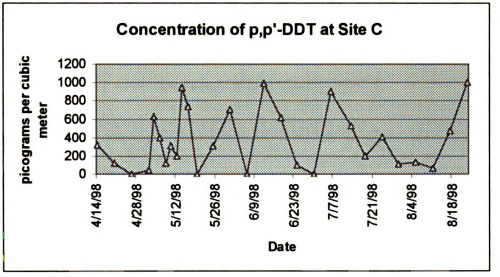

Figure 3.22 Concentration of Vapor Phase p,p'-DDT at SH at Site C

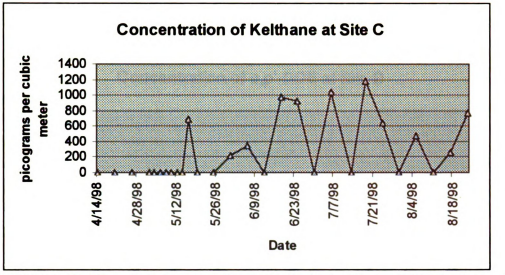

Figure 3.23 Concentration of Vapor Phase Kelthane at SH at Site C

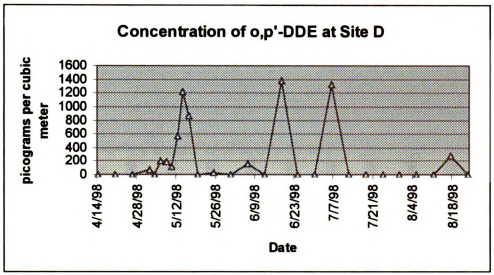

Figure 3.24 Concentration of Vapor Phase o,p'-DDE at CLM Site D

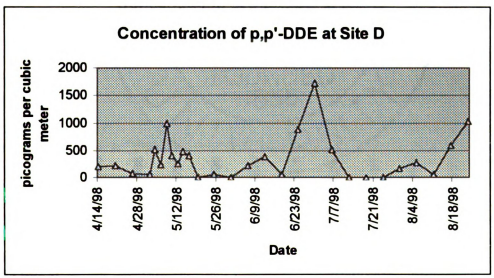

Figure 3.25 Concentration of Vapor Phase p,p'-DDE at CLM Site D

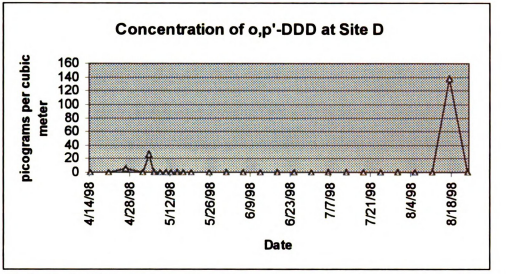

Figure 3.26 Concentration of Vapor Phase o,p'-DDD at CLM Site D

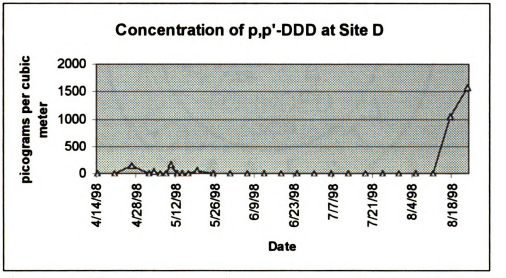

Figure 3.27 Concentration of Vapor Phase p,p'-DDD at CLM Site D

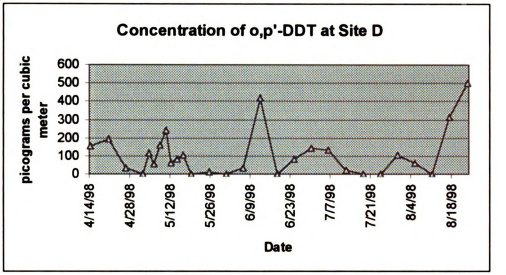

Figure 3.28 Concentration of Vapor Phase o,p'-DDT at CLM Site D

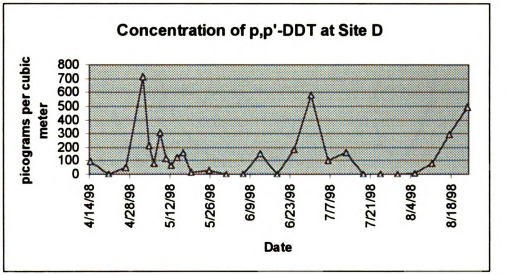

Figure 3.29 Concentration of Vapor Phase p,p'-DDT at CLM Site D

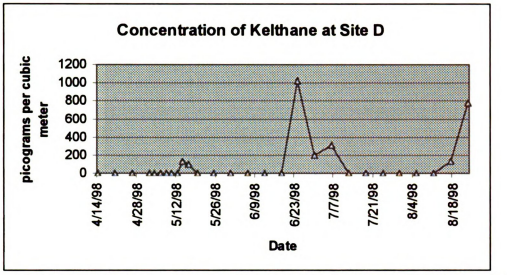

Figure 3.30 Concentration of Vapor Phase Kelthane at CLM Site D

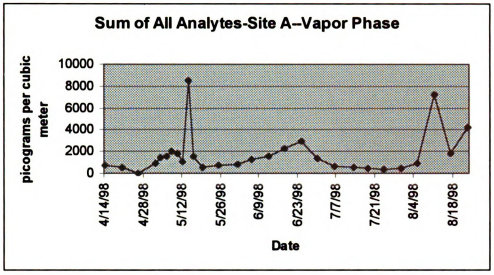

Figure 3.31 All Analytes for SH Site A Vapor Phase

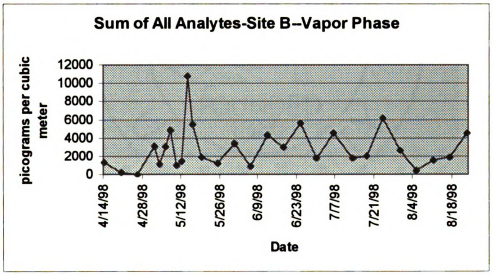

Figure 3.32 All Analytes for SH Site B Vapor Phase

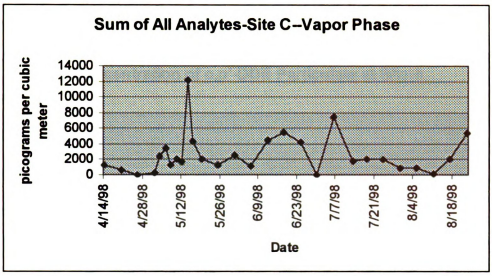

Figure 3.33 All Analytes for SH Site C Vapor Phase

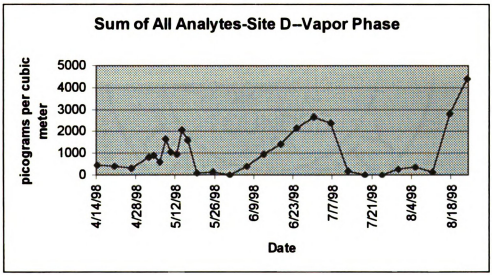

Figure 3.34 All Analytes for CLM Site D Vapor Phase

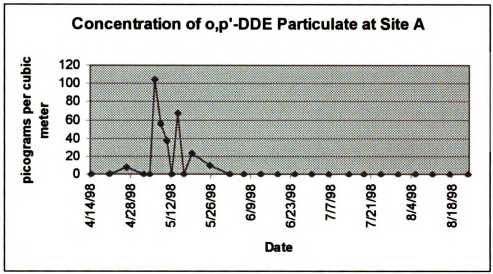

Figure 3.35 Concentration of o,p'-DDE Particulate at SH at Site A

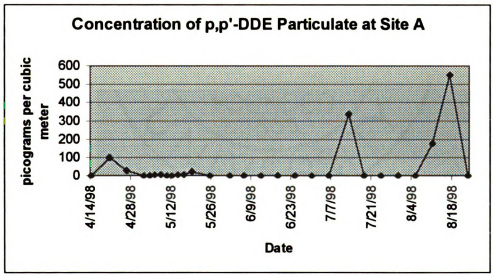

Figure 3.36 Concentration of p,p'-DDE Particulate at SH at Site A

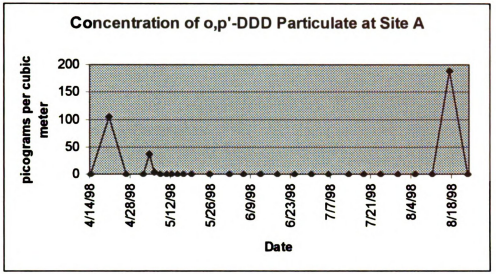

Figure 3.37 Concentration of o,p'-DDD Particulate at SH at Site A

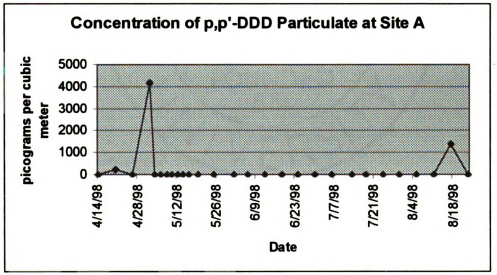

Figure 3.38 Concentration of p,p'-DDD Particulate at SH at Site A

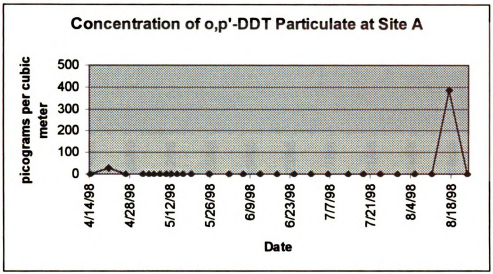

Figure 3.39 Concentration of o,p'-DDT Particulate at SH at Site A

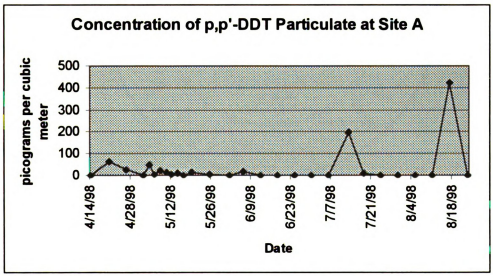

Figure 3.40 Concentration of p,p'-DDT Particulate at SH at Site A

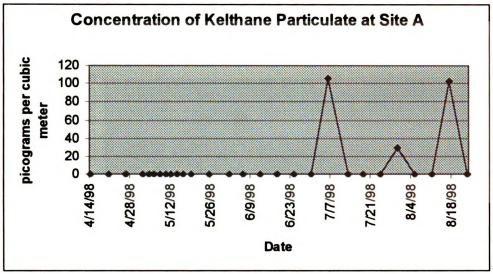

Figure 3.41 Concentration of Kelthane Particulate at SH at Site A

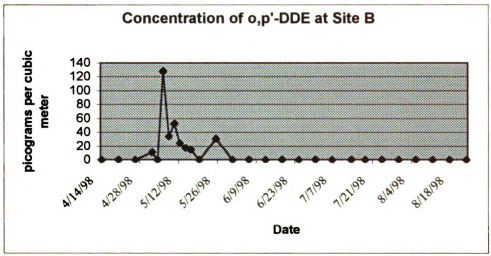

Figure 3.42 Concentration of o,p'-DDE Particulate at SH at Site B

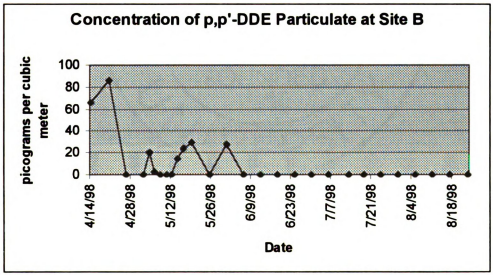

Figure 3.43 Concentration of p,p'-DDE Particulate at SH at Site B

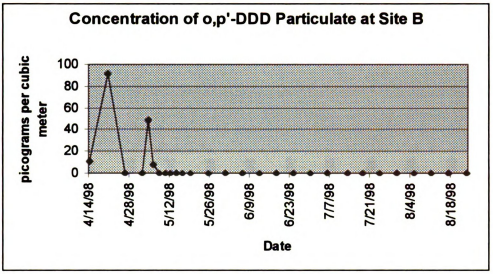

Figure 3.44 Concentration of o,p'-DDD Particulate at SH at Site B

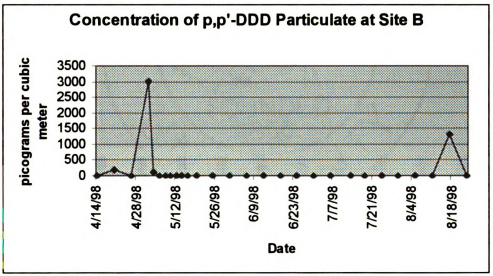

Figure 3.45 Concentration of p,p'-DDD Particulate at SH at Site B

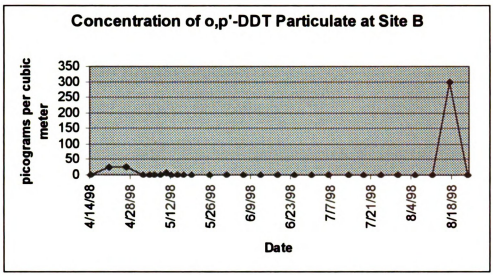

Figure 3.46 Concentration of o,p'-DDT Particulate at SH at Site B

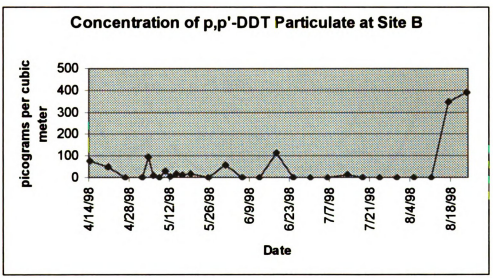

Figure 3.47 Concentration of p,p'-DDT Particulate at SH at Site B

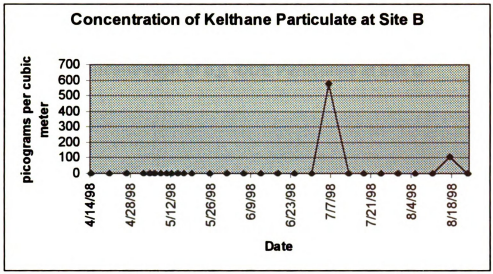

Figure 3.48 Concentration of Kelthane Particulate at SH at Site B

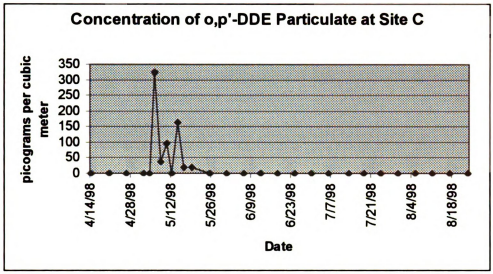

Figure 3.49 Concentration of o,p'-DDE Particulate at Site C

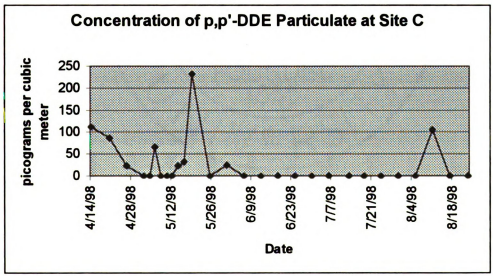

Figure 3.50 Concentration of p,p'-DDE Particulate at SH at Site C

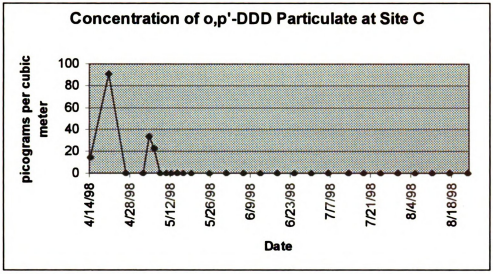

Figure 3.51 Concentration of o,p'-DDD Particulate at SH at Site C

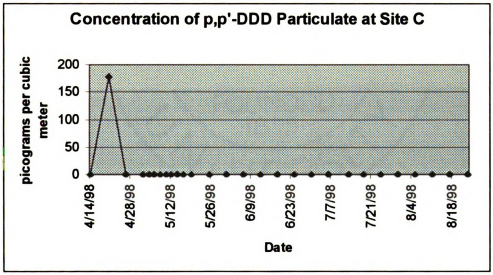

Figure 3.52 Concentration of p,p'-DDD Particulate at SH at Site C

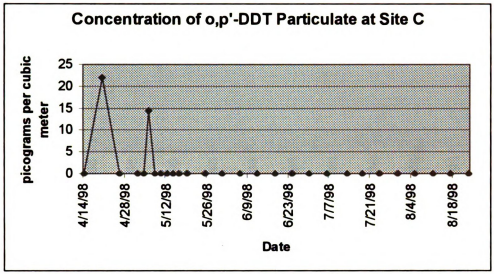

Figure 3.53 Concentration of o,p'-DDT Particulate at SH at Site C

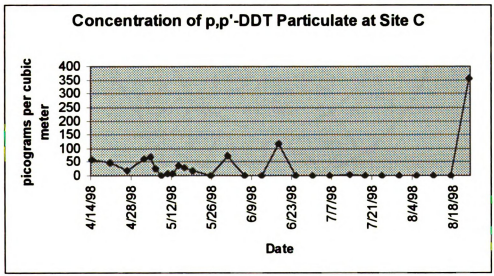

Figure 3.54 Concentration of p,p'-DDT Particulate at SH at Site C

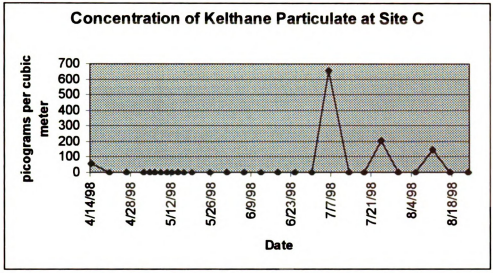

Figure 3.55 Concentration of Kelthane Particluate at SH at Site C

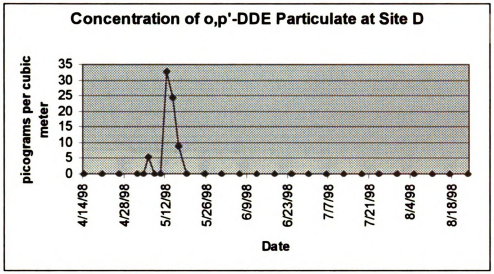

Figure 3.56 Concentration of o,p'-DDE Particulate at CLM Site D

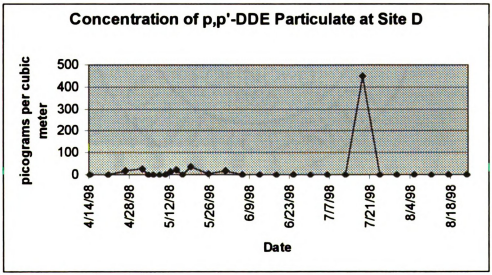

Figure 3.57 Concentration of p,p'-DDE Particulate at CLM Site D

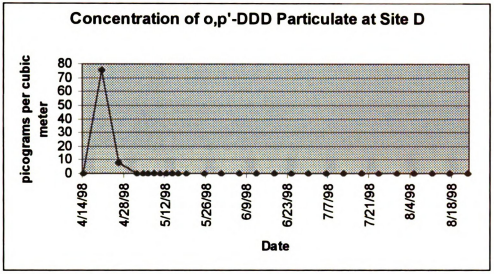

Figure 3.58 Concentration of o,p'-DDD Particulate at CLM Site D

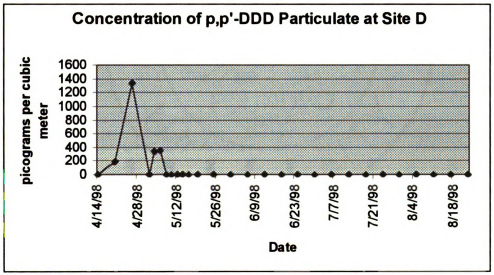

Figure 3.59 Concentration of p,p'-DDD Particulate at CLM Site D

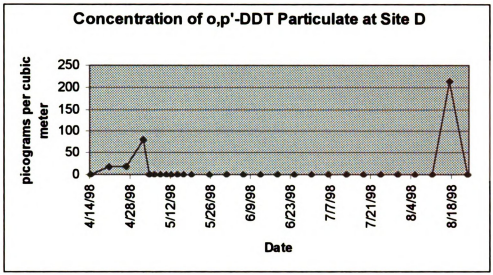

Figure 3.60 Concentration of o,p'-DDT Particulate at CLM Site D

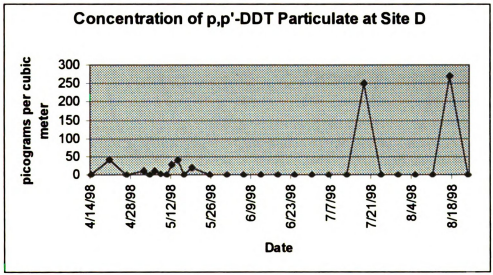

Figure 3.61 Concentration of p,p'-DDT Particulate at CLM Site D

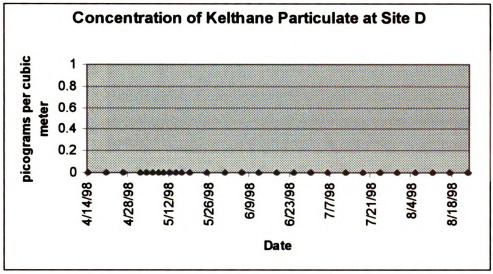

Figure 3.62 Concentration of Kelthane Particulate at CLM Site D

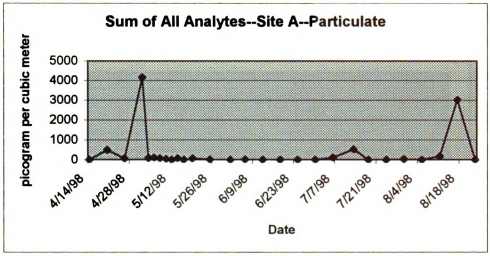

Figure 3.63 All Analytes for SH Site A Particulate

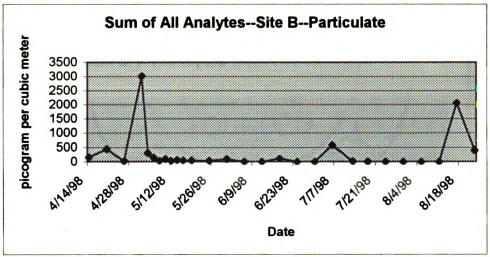

Figure 3.64 All Analytes for SH Site B Particulate

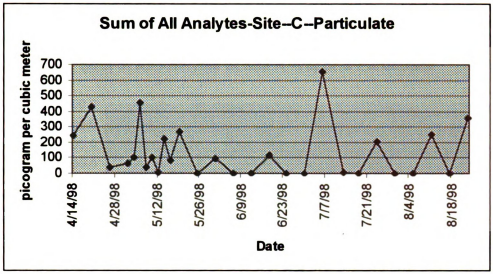

Figure 3.65 All Analytes for SH Site C Particulate

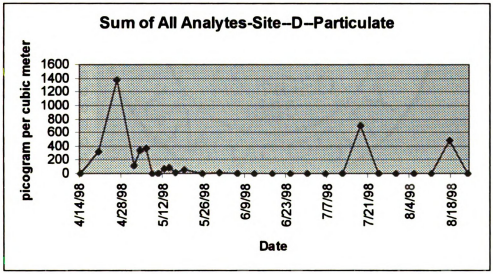

Figure 3.66 All Analytes for CLM Site D Particulate

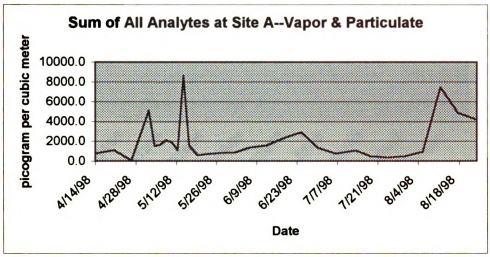

Figure 3.67 Sum of All Analytes for South Haven Site A Particulate & Vapor Phase

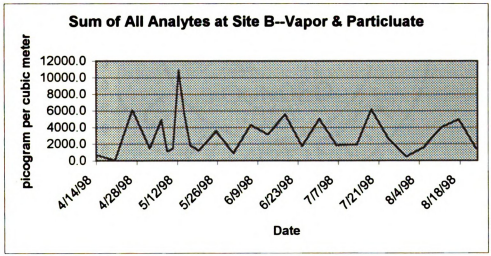

Figure 3.68 Sum of All Analytes for South Haven Site B Particulate & Vapor Phase

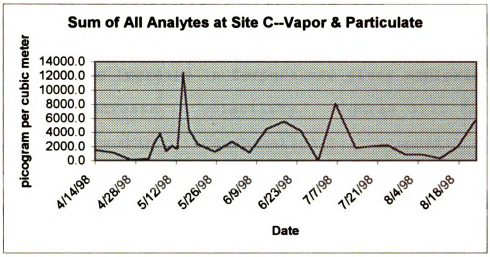

Figure 3.69 Sum of All Analytes for South Haven Site C Particulate & Vapor Phase

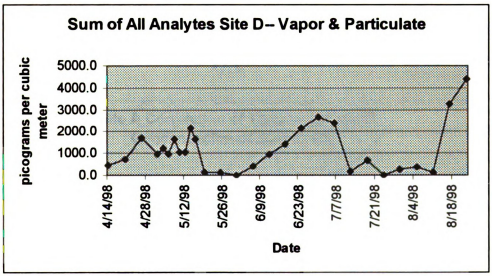

Figure 3.70 Sum of All Analytes for South Haven Site D Particulate & Vapor Phase

The concentration in the air of ΣDDT should show a direct relationship to the temperature of the environment. A 10 degree increase in temperature gives approximately a 3-4 times increase in volatility. Figures 3.71 to 3.72 show the relationship of concentration to atmospheric temperature for Site A and Site B. The temperature data for all sites is shown in Appendix L. Figure 3.73 shows the relationship of temperature to concentration for all of the data with the calculated exponential correlation equation. The equation found was:

$$\text{Concentration} = 896e^{0.1287\text{Temperature}}$$

A r^2 of 0.8128 was found, indicating a good correlation of the data. This information helps in the validation of the entire process from sampling to analytical detection.

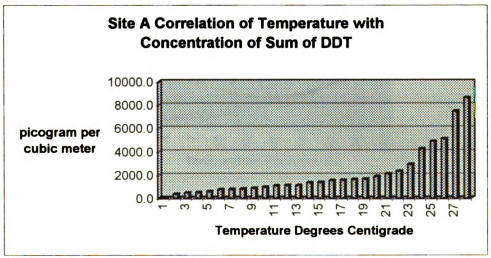

Figure 3.71 Site A Correlation of Temperature with Concentration of ΣDDT

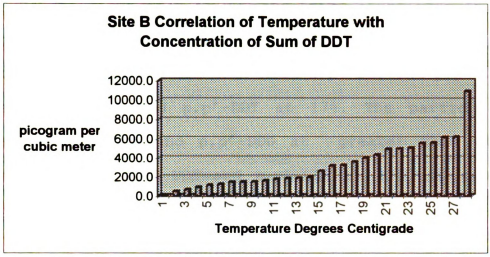

Figure 3.72 Site B Correlation of Temperature with Concentration of ΣDDT

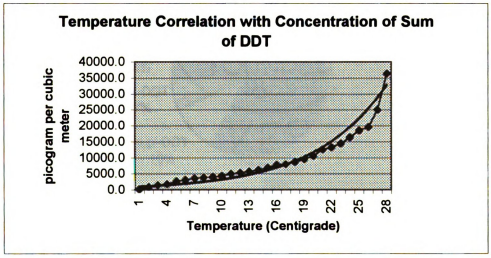

Figure 3.73 Temperature Correlation with Concentration of ΣDDT for all air samples

The dispersion of DDT and it's metabolites in the vapor phase (Figure 3.74) shows o,p'-DDE and p,p'-DDE at greater than 50% of the total ΣDDT with o,p'-DDD and p,p'-DDD at 17% and o,p'-DDT and p,p'-DDT at 17%. The particulate phase shows o,p'-DDD and p,p'-DDD at greater than 66% of the total ΣDDT, o,p'-DDE and p,p'-DDE at 12% and o,p'-DDT and p,p'-DDT at 16%.

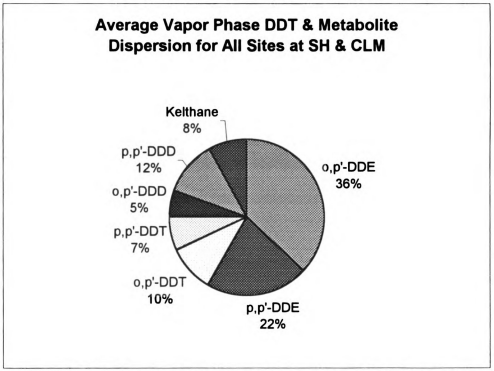

Figure 3.74 Average Vapor Phase DDT & Metabolite Dispersion for All Sites at SH & CLM

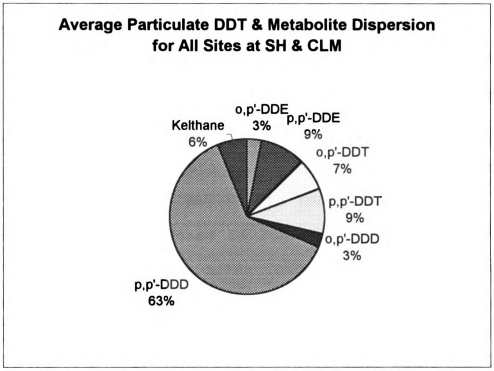

Figure 3.75 Average Particulate DDT & Metabolites Dispersion for All Sites at SH & CLM

The particulate has the major portion of the residue, 66, in DDD and the vapor phase has themajor portion in DDE at 58%. This indicates that the residues are aged because the parent DDT is a smaller percent of the ΣDDT.

One aspect for determining the age of the DDT residues is through the ratio of it's metabolites to the parent compound and that data can be seen in Figures 3.74 to 3.80. The site specific pie charts for percent the DDT and it's metabolites are found in Figures 3.76 to 3.79 and Figure 3.80 represents all sites combined. DDE was found at 2.4 times the concentration of DDT and 4.8 times the concentration of DDD in the vapor phase. These ratios indicate that the DDT has changed from the initial concentration were DDT composes 95% (in the formulated product) of the ΣDDT to 26% (of the environmental sample over all sites and locations). Using the half-life equation to calculate expected concentrations of DDE after legal application ceased in 1973, it was found that DDT degraded according to the soil half-life of 16 years. From our laboratory data 100% DDT goes to 27% DDT (normalizing the 95% formulated product to 100% for clarity of calculation) and follows the equation:

$$\log C = \log C_o - kt/2.303$$

The results show a half-life of 13.2 years (actual calculation is shown in Appendix Q) which indicates both

soil, air, and water were contributing to the degradation of DDT to obtain a hybrid half-life between 1 year in water, 16 years in soil, and 7.4 days in the atmosphere. The half-life supports the idea that the controlling factor in degradation of DDT was soil and thus the atmospheric concentration are a result of the soil burden volatilizating during the warm weather and when there is no snow cover.

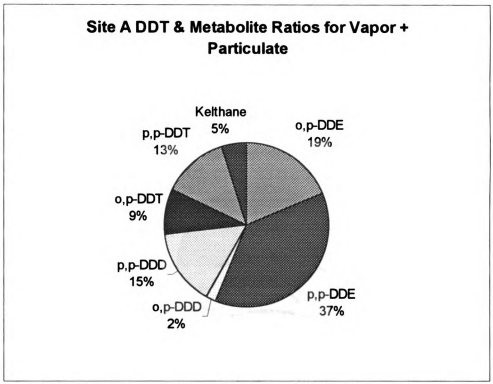

Figure 3.76 Site A DDT & Metabolite Ratios for Vapor + Particulate

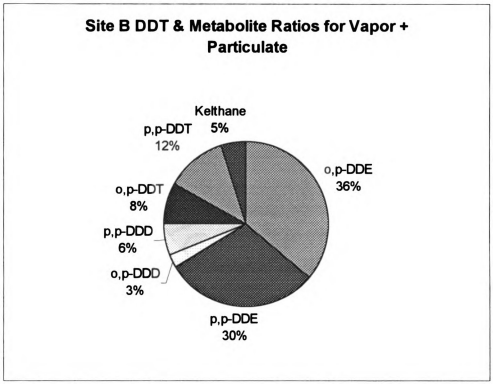

Figure 3.77 Site B DDT & Metabolite Ratios for Vapor + Particulate

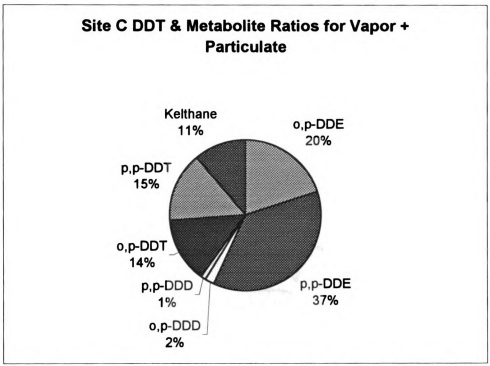

Figure 3.78 Site C DDT & Metabolite Ratios for Vapor + Particulate

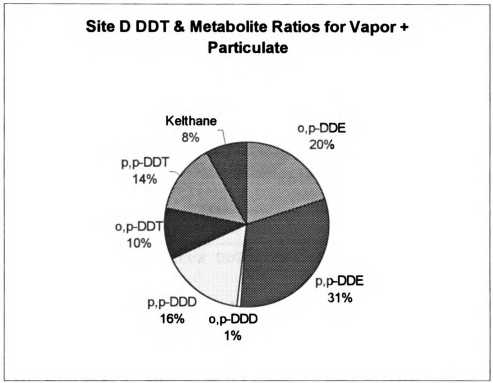

Figure 3.79 Site D DDT & Metabolite Ratios for Vapor + Particulate

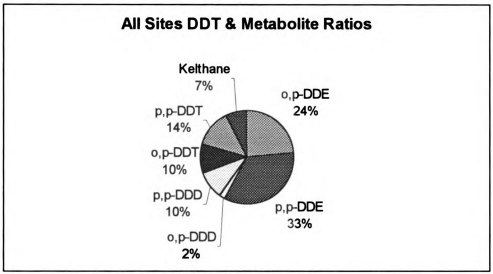

Figure 3.80 All Sites DDT & Metabolite Ratios

The data show a 2.5 fold greater concentration of ΣDDT in the vapor phase over the particulate phase. The ΣDDT concentration shows a positive correlaion with increasing temperature and this could account for the increased levels of ΣDDT in the vapor phase compared to the particulate phase. The amount of DDT and metabolites brought into the air column was used to determine the amount of ΣDDT that moved off the sites during the sampling period to the atmosphere for long range transport. The air sample was taken from an effective height of 1 m above the soil surface. Over 24 hours the sample collected an average volume of 328 m^3. The basic assumption for these calculations was that the air was sampled from 1 m above to 1 m below the sampler inlet. To determine the average amount of chemical moved away from one hectare of the field the following equations were used:

$$A = C \times 20000$$

A is amount of ΣDDT in a hectare volume 2m high
C is concentration of the ΣDDT in pg/m^3
20000 is volume above a hectare that is 2m high by 100m long by 100m wide

$$\#Sweeps = windvelocity(meter/day)(1/100m)$$

Sweeps of a hectare per day
Wind velocity converted to meters per day
100m is the length of one side of a square hectare

$$Amount/Day = A \times \#Sweeps$$

Amount per Day represents the amount of the ΣDDT moving off a hectare per day. The resultant average wind speed for the sampling date is shown in Appendix N for determination of direction of movement of the ΣDDT. The amount of ΣDDT moving off of one hectare was calculated from the data and is shown in Figure 3.81 and Figure 3.84 for all sites. The average value of ΣDDT moving off of Site A was 98 mg/day, Site B was 194 mg/day, Site C was 136 mg/day and Site D was 53 mg/day. The contribution from the soil ΣDDT burden to the total air concentration can be seen from this data. It shows a greater than 2 fold increase in ΣDDT at site SH to CLM.

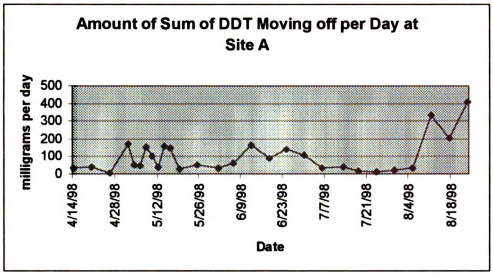

Figure 3.81 Amount of ΣDDT Moving Off Site A per Day

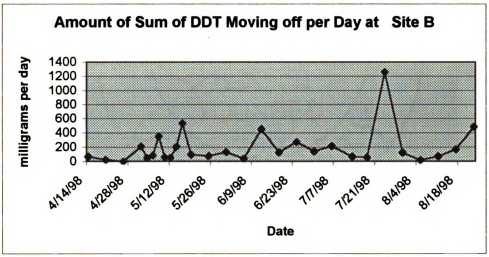

Figure 3.82 Amount of ΣDDT Moving Off Site B per Day

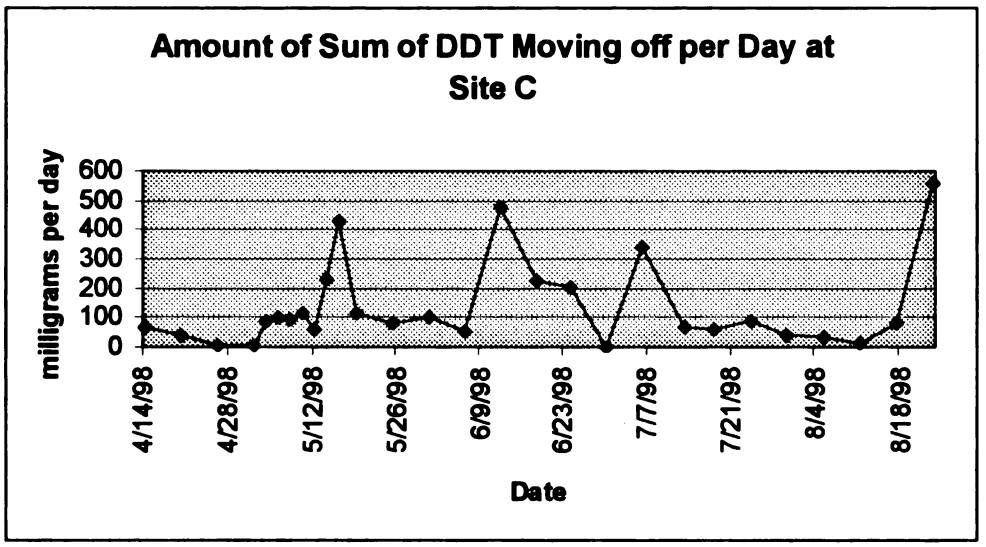

Figure 3.83 Amount of ΣDDT Moving Off Site C per Day

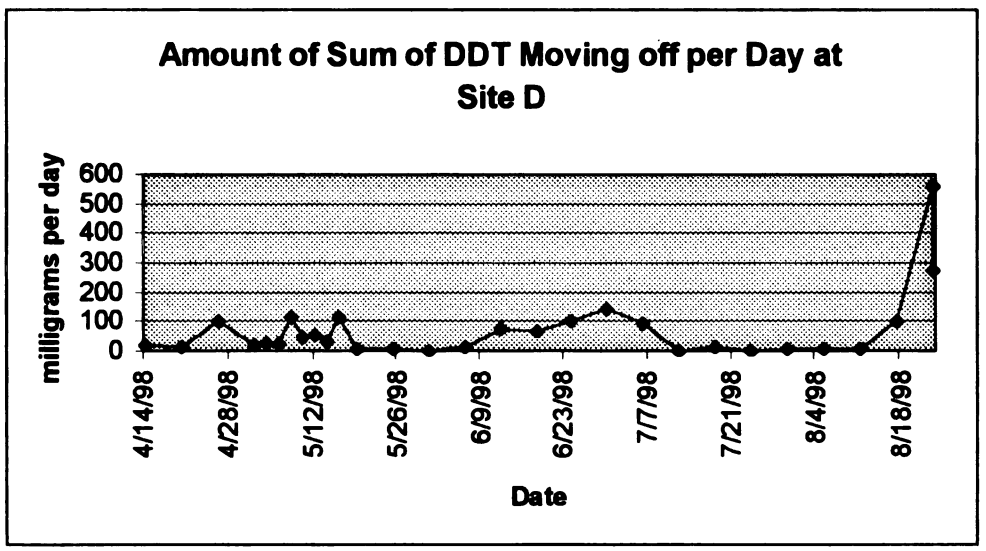

Figure 3.84 Amount of ΣDDT Moving Off Site D per Day

Soil Data

The soil data is shown in Appendix M for p,p'-DDT, o,p'-DDT, p,p'-DDE, o,p'-DDE, p,p'-DDD, o,p'-DDD, and Kelthane. The data was summed for all DDT for each site and at each section of the sample (top, middle, and bottom), the result is shown in Table 3.3. The middle third showed the highest residues of 57% followed by the top at 36%, and the bottom third had 8% of the total concentration. The middle section was 1.6 times more concentrated then the top section and the middle was 7.1 times more concentrated than the bottom. Site BC showed the greatest residues at 4.3 µg/g average in the mid sampling level, with Site A at 4.1 µg/g average in the mid sampling level, and Site D at 0.4 µg/g average in the mid samplling level. The data shows that Site BC had 1.8 times as much ΣDDT as Site A and 7.4 times the amount found at Site D. This would imply with the greater burden of ΣDDT being in the soil at SH then the increased air concentrations would also be at SH. This was found to be the case, as can be seen in Figures 3.3 to 3.70 and verified in the data found in Appendix K.

Table 3.3 Soil Data of Summed DDT Concentrations

Sample Site	Top Subsample (ng/g)	Middle Subsample (ng/g)	Bottom Subsample (ng/g)	% Site Contamination
A	1126	4067	0.22	33%
BC	4081	4360	793	59%
D	377	442	400	8%
% Position Contamination	36%	57%	8%	

The total amount of ΣDDT in the soil that was sampled was used to determine the net loss of ΣDDT from the total soil burden. The amount of ΣDDT in the soil was determined by taking the average soil concentration times the volume of soil in one hectare 10 inches deep times the average bulk density of sandy loam soil ($1.5 g/cm^3$). The weight of one hectare of soil 25.4 cm deep was found to be 3.81×10^6 kg. Table 3.4 shows the maximum, average and minimum amount of the ΣDDT in the soil at the two sites. The SH soil averaged 5.5 times more ΣDDT, on a calculated basis, as the CLM site.

Table 3.4 Amount of ΣDDT found in one hectare of soil 25.4 cm deep

Site	High (kg/ha)	Average (kg/ha)	Low (kg/ha)
SH	111.6	79.4	47.2
CLM	NA	14.5	NA

NA - Not Available

The percent loss due to volatilization from the soil was calculated to determine and estimate the time needed to move all of the DDT out of the soil profile. The following equation was used for these calculations:

$$\%MovedOff/Year = \frac{AmountMovedOff/Year}{TotalSoilBurden} \times 100\%$$

Table 3.5 shows the results using average and high (worst case scenarios) for levels of ΣDDT to disappear per year from the soil profile. The range for SH goes from 0.04% to 0.95% per year ΣDDT loss from the soil. CLM has a range from 0.13% to 1.4% per year loss from the soil. If these numbers are consistent for the years to come a theoretical calculation for time before soil burden of the ΣDDT goes to zero would be 100% divided by the percent loss per year. The calculation predicts 2500 years at the worst case (slowest degradation) to 105 years for the average case at SH. For CLM the numbers range from 769 years to 71 years for the soil to be free of ΣDDT.

Table 3.5 Percent Loss of ΣDDT per year from one hectare for the two Locations

Site	Concentration Level	Soil	Burden	Per	Location
		SH	SH	SH	CLM
		High	Average	Low	Average
A	High	0.13	0.18	0.31	NA
	Average	0.03	0.04	0.07	NA
B	High	0.40	0.57	0.95	NA
	Average	0.06	0.09	0.15	NA
C	High	0.18	0.25	0.43	NA
	Average	0.04	0.06	0.10	NA
D	High	NA	NA	NA	1.4
	Average	NA	NA	NA	0.13

The metabolites were then looked at separately for percent loss per year and the amount of time to reduce the entire soil burden to zero under consistent conditions as found during the 1998 sampling period. These calculation is shown in Table 3.6. o,p'-DDE had the shortest time for complete dissipation at 4 years, and p,p'-DDT had the longest at 6958 years. Although the numbers are derived from assumptions (consistent atmospheric and land use conditions) to facilitate the calculation the trends show that the ΣDDT will continue to be a long term contaminant and available for exposure to the environment and animal life.

Table 3.6 The percent loss per year and Amount of Years to Reduce the Soil Burden to Zero concentration per Metabolite

Metabolite	Percent Loss per Year	Years to Soil Burden to Zero Concentration
o,p'-DDE	24.4	4
p,p'-DDE	0.09	1081
o,p'-DDD	0.34	298
p,p'-DDD	1.16	86
o,p'-DDT	0.19	526
p,p'-DDT	0.01	6958
Kelthane	0.16	636

Conclusions of Soil Impact on Air Concentrations

SH shows a 2 fold and greater air concentration level over CLM which reflect the fact that the soil burden at SH was almost 6 times more concentrated than CLM. Another factor that shows the soil contributes heavily to the air concentration can be seen with the correlation (r^2 of 0.81) of concentration with temperature. If the atmospheric concentration was coming from the air alone then heating should cause lower concentrations due to volume expansion and dilution of the contaminant. This was not seen in the data, evident by the temperature to concentration correlation of r^2 of 0.81.

The soil data supports the theory long term residence in the soil because the highest concentration was in the mid section of the soil sample at both sites. This would indicate downward movement of the contaminants rather then fresh deposition. This site has been under notil farming for several years.

The overall ratios of DDT to the metabolites indicate the DDT has degraded following a $t_{1/2}$ of 13.2 years. The vapor phase concentration will generally be 5 to 10 fold more concentrated than the particulate phase.

There may be many implications with long term availability of contaminants that can be seen with the

calculations to zero concentration levels being in several years to thousands of years. We have seen with many previous studies of ΣDDT, concentration levels in wildlife will continue to bioaccumulate and compounds of toxicological concern such as DDT will continue to create symptoms in organisms that are exposed. Since DDT has been so persistent, the organisms that die will release the contaminants for future uptake by others and there is a recycling of old contaminants.

Levels of DDT continue to persist in the Great Lakes and other bodies of waters and with present trends of long-term degradation there will continue to be hundreds of years before levels are diminished. The ambient levels that are seen in the US are now from applications many years ago and/or from long distance transport.

There is evidence of feminization of snapping turtles, alligators, and panther occurring at sites contaminated with p,p'-DDE (de Solla,et al.., 1998). The continued evidence of endocrine disruption from organochlorine contaminants suggests the impact due to DDT will continue.

Great Lake (GL) fish consumers were compared to non-GL fish consumers and p,p'-DDE, p,p'-DDT, and o,p'-DDT were detected in all subjects and only DDE was detected in the control group (Anderson, et al.., 1998) in blood samples. o,p´-DDT has induced growth of breast tumors by estrogenic

inhibitory action in human breast cancer cells (Verma, 1998).

FUTURE WORK

The data supports the persistence of residues many years after legal use in the US has been discontinued due to the ratios of DDT to the metabolites both in the soil and air samples. In future studies the ratio of p,p'-DDT to the metabolites should be looked at to determine the movement and age of the residues.

A study under consideration now will look at the same site and include three additional sites in areas considered to not be highly impacted with ΣDDT, determined by soil sampling results conducted during the fall of 1998 and winter/spring of 1999. The samplers will also be set collocated with one of the samplers set to collect air from the prevailing wind direction. The two will be compared to find if there does exist a directional component that should be considered to determine the source of the ΣDDT. This data should help discriminate between the amount of long range transport that has contributed to the elevated levels found in South Haven, MI and old ΣDDT from past inputs.

APPENDIX G

METHOD III - DDT, DDD, DDE, AND KELTHANE - AIR

A. Glassware cleaning:

1. MeOH or CH_2Cl_2 rinse if dirty before soap wash
2. Soap wash
3. If still dirty 50:50 H_2SO_4:HNO_3 soak overnight
4. Tap water rinse
5. DI water rinse
6. Dry
7. Muffle 450 ° C for four hours with foil (always use dull side of foil towards glass) on open ends
8. Cool & Store

B. Stainless Steel tools:

1. Soap wash
2. Tap water rinse
3. DI water rinse
4. Dry
5. Wrap in foil & Store

C. Pasteur pipettes & vials:

1. Wrap glass in foil or place in a beaker and cover with foil
2. Muffle at 450 ° C for four hours

D. Teflon:

1. Sonicate for 15 minutes in CH_2Cl_2
2. Place in 70 ° C oven for two hours
3. Store in sealed jar

E. Glass Wool:

1. Put in beaker and cover with foil
2. Muffle at 450 ° C for four hours
3. Store

APPENDIX H

METHOD III - DDT, DDD, DDE, AND KELTHANE - AIR

Reagent Preparation

A. Sodium Sulfate (Na_2SO_4):

1. Put Na_2SO_4 in beaker and muffle at 450 ° C for four hours
2. Store in 100 ° C oven

B. XAD_2: XAD_2 is and absorptive polyaromatic resin used for sample preparation and fractionation. In this study it was used as an absorptive sampling device. It has a high affinity for absorption of hydrophobic compounds up to 20,000 molecular weight.

Day 1

1. Place XAD_2 in extractor plugged with glass wool
2. Rinse with tap water many times, stirring to remove foam and small particles.
3. Rinse with small amount of MeOH three times to remove the water
4. Add 500 ml of MeOH to 1 l flask
5. Add 20 boiling chips
6. Assemble soxhlet
7. Turn on heater (60-65 setting)
8. Turn on chilled water
9. Cover soxhlet with foil
10. Extract for 24 hours

Day 2

1. Turn off and cool 15-30 minutes
2. Flush MeOH from soxhlet as possible.
3. Add 500 ml of acetone to 1 l flask
4. Add 20 boiling chips
5. Turn on heater (45 setting)
6. Cover soxhlet with foil
7. Extract for 24 hours

Day 3

1. Turn off and cool 15-30 minutes
2. Flush Acetone from soxhlet as possible.
3. Add 500 ml of hexane to 1 l flask
4. Add 20 boiling chips
5. Turn on heater (40-45 setting)
6. Cover soxhlet with foil
7. Extract for 24 hours

Day 4

1. Turn off and cool 15-30 minutes
2. Flush Hexane from soxhlet as possible.
3. Add 500 ml of CH_2Cl_2 to 1 l flask
4. Add 20 boiling chips
5. Turn on heater (40-50 setting)
6. Cover soxhlet with foil
7. Extract for 24 hours

Day 5

1. Turn off and cool 15-30 minutes
2. Flush CH_2Cl_2 from soxhlet as possible. Wait 15 minutes
3. Add 100 ml of hexane to 1 l flask. Wait 15 minutes and flush. Repeat at least 3 times, until the level in the siphon tube is the same as in the soxhlet.
4. Add 500 ml of hexane to a 1 l flask
5. Add 20 boiling chips
6. Turn on heater (40-45 setting)
7. Cover soxhlet with foil
8. Extract for 24 hours. Flushing may need to be induced before it flushes on its own.

Day 6

1. Turn off and cool 15-30 minutes
2. Flush hexane from soxhlet as possible.
3. Add 500 ml of 1:1 acetone:hexane to a 3 l flask
4. Add 20 boiling chips
5. Turn on heater (40-45 setting)
6. Cover soxhlet with foil
7. Extract for 24 hours

Day 7

1. Turn off and cool 15-30 minutes
2. Flush Acetone/Hexane from soxhlet as possible.
3. Pour XAD_2 in a beaker and dry overnight at 65 ° C
4. Store in amber bottle in freezer at - 20 ° C for up to three months
5. Keep subsample in separate jar for checking lab blank and matrix spike

C. Quartz Fiber Filters (QF): QF are quartz fiber filters placed on the front of the air sampler to collect the particulate fraction of the sample. The nominal particle size collected was > 0.1 µm (Monosmith, 1996).

1. Each QF is wrapped in aluminum foil and muffled at 450 ° C for four hours
2. Stored in freezer inside a plastic bag

APPENDIX I

Extraction of XAD_2 resins and Quartz fiber filters (QF)

1. Supplies:
Soxhlet extractor (55/50 & 24/40)
Condenser (55/50)
500 ml Round Bottom (RB)
Pipettes
Boiling chips
Acetone
Hexane
Standards
CH_2Cl_2 squirt bottle
MeOH squirt bottle
Glass wool
Foil
Heating mantle

2. XAD_2 and QF Extraction:

Day 1 Sample set will have samples & one blank, duplicate, & recovery QA sample

Label date of collection, sample type, and extraction start day

Thoroughly rinse inside of each piece of glassware in contact with the sample first with MeOH then followed by CH_2Cl_2

Add 5-6 clean Teflon chips to RB
Pour 175 ml of acetone & 175 ml of hexane into RB
Put Glass wool into siphon tube
Transfer sample to soxhlet extractor: Either XAD_2 or QF

Rinse container to remove all XAD_2
Spike samples at this time onto the XAD_2 or QF
Turn on the heating mantles to ~45
Turn on water
Cover soxhlet with foil
Extract for 18 to 24 hours

3. Remove solvent:
Day 2 Cool for 15 -20 minutes

Pour off solvent and store dark cool place
Remove XAD_2 & boiling chips
Rotary evaporate or Turbo-Vap to 2-5 ml
Add 75 ml of hexane and take to 2-5 ml
Add 75 ml of hexane and take to 2 ml

4. Silica Column Chromatography:

Supplies:

Hexane
MeOH
CH_2Cl_2
Glass wool
Na_2SO_4
4 % deactivated silica

Activation/Deactivation of silica

Put silica in beaker with foil at 100 ° C
Put thermostat to 300 ° C keep in oven over night

Turn oven to 100 ° C, don't remove silica until the oven has cooled

Put silica on counter for ~5-10 minutes
Put silica into a desiccator for 2 hours
Deactivate with 4 % w/v with DI
Shake for about 15 minutes
Silica may be stored for 3 days in a stoppered flask

Item	QF	XAD_2
Silica	4-6 g	4-6 g
Column size	3.5 inches	3.5 inches
Na_2SO_4	0.5 inches	0.5 inches
Elution volume (1^{st} & 2^{nd} fraction)	25 ml 1^{st} hexane 2^{nd} 1:1 hexane:CH_2Cl_2	25 ml 1^{st} hexane 2^{nd} 1:1 hexane:CH_2Cl_2
Switching volume	4 ml	4 ml
Elution volume (3^{rd} fraction)	30 ml 3^{rd} MeOH	30 ml 3^{rd} MeOH

Place ~ 1 cm Glass wool in column
Fill column with hexane and add silica in a hexane slurry, tamp column to pack silica

Add Na_2SO_4

Wash column with 25 ml of hexane **!!NEVER LET COLUMN GO DRY!!**

Add sample

Elute 1st fraction with 25 ml of hexane at ~ 1 drip/second

Add 4 ml of 1:1 hexane:CH_2Cl_2 switching solvent

Elute the 2nd fraction with 25 ml of 1:1 hexane:CH_2Cl_2

Add 4 ml of MeOH switching solvent

Elute the 3rd fraction with 30 ml of MeOH

Compound	Fraction
p,p'-DDE	1st
o,p-DDE	1st
p,p'-DDD	2nd
o,p-DDD	2nd
p,p'-DDT	2nd
o,p-DDT	2nd
Kelthane	2nd or 3rd

Reduce volume to ~ 2 ml with N_2

Put in 2 ml GC autosampler vials and now ready for the GC.

APPENDIX J

METHOD III - DDT, DDD, DDE, AND KELTHANE - SOIL

Reagent Preparation

Sodium Sulfate (Na_2SO_4):

1. Put Na_2SO_4 in beaker wash with CH_2Cl_2 and muffle at 400 ° C for four hours
2. Store in 100 ° C oven

Extraction of soil

1. Supplies:
   Soxhlet extractor (55/50 & 24/40)
   Condenser (55/50)
   500 ml Round Bottom (RB)
   Thimbles
   Pipettes
   Boiling chips
   Acetone
   Hexane
   Standards
   CH_2Cl_2 squirt bottle
   MeOH squirt bottle
   Glass wool
   Foil
   Heating mantle

2. Extraction:
   Determine percent water by putting weigh about 10 g of soil and place in a oven 100 ° C over night.
   Reweigh the soil and determine the weight loss and calculate the percent weight loss.
   Mix 10 g of soil with 10 g anhydrous Na_2SO_4 and place in a extraction thimble
   Place 300 ml of 1:1 Acetone/Hexane in 500 ml RB with 1-2 clean boiling chips
   Extract for 16-24 hours at 4-6 cycles/hour
   Drain the extract through ~ 10 g of anhydrous Na_2SO_4 into a Turbo-Vap tube
   Add ~ 10 ml of hexane to Turbo-Vap tube to remove acetone and reduce volume to ~ 10 ml

4. Silica Column Chromatography:
   Supplies:

   Hexane
   MeOH

CH_2Cl_2
Glass wool
anhydrous Na_2SO_4 dried at 400 ° C for 4 hours
4 % deactivated silica 100-200 mesh

Activation/Deactivation of silica

Put silica in beaker with foil at 160 ° C, in oven over night

Put silica on counter for ~5-10 minutes
Put silica into a desiccator for 2 hours
Deactivate with 3.3 % w/v with DI
Shake for about 15 minutes
Silica may be stored for 3 days in a stoppered flask
Store in a desiccator
Place ~ 1 cm Glass wool in 1 cm diameter column
Fill column 3 g of silica
Top with 2-3 cm of anhydrous Na_2SO_4
Wash column with 10 ml of hexane **!!NEVER LET COLUMN GO DRY!!**
Don't collect first hexane, stop when almost to top of anhydrous Na_2SO_4
Add sample, rinse with 1-2 ml hexane twice
Elute 1^{st} fraction with 80 ml of hexane at ~ 1 drip/second
Elute the 2^{nd} fraction with 50 ml of 1 hexane
Add 4 ml of CH_2Cl_2 switching solvent
Elute the 3^{rd} fraction with 15 ml of CH_2Cl_2
The CH_2Cl_2 (3^{rd} fraction) **must be** changed to hexane for GC
Reduce volume to ~ 0.5 ml with Turbo-Vap
Add 10 ml of hexane
Reduce volume to ~ 1ml with stream of N_2
Put in 2 ml GC autosampler vials and now ready for the GC.

APPENDIX K

Concentration Data Combined for Vapor & Particulate for All Sites

Concentration Total							
Date	**o,p-DDE**	**p,p-DDE**	**o,p-DDD**	**p,p-DDD**	**o,p-DDT**	**p,p-DDT**	**Kelthane**
Site A	**pg/m3**	**pg/m3**	**pg/m3**	**pg/m3**	**pg/m3**	**pg/m3**	**pg/m3**
4/14/98	0	453	1.7	38.7	143.6	121.5	0
4/20/98	0	461.3	182.3	209.9	121.5	113.3	0
4/26/98	8.2	27.2	0	0	10.9	27.2	0
5/2/98	517.2	204	0	4160.9	54.6	158	0
5/4/98	0	827.7	70.6	0	175.1	440.7	0
5/6/98	1104.8	331.4	2.8	36.8	0	155.8	0
5/8/98	644.1	1149.7	65	62.1	135.6	53.7	0
5/10/98	312.5	869.3	8.5	0	110.8	508.5	65.3
5/12/98	0	579.4	0	70.6	273.5	167.6	0
5/14/98	6409.2	1418.5	30.8	52.3	292.3	443.1	0
5/16/98	421.8	716.8	0	0	112.1	286.1	0
5/19/98	162.8	308.1	0	0	0	101.7	0
5/25/98	450.7	305.9	0	26.3	0	6.6	0
6/1/98	0	552	0	0	118.5	156.1	37.6
6/6/98	110.7	384.4	13	645	166.1	19.5	0
6/12/98	0	947.7	0	0	393.8	249.2	0
6/18/98	561.3	1107.4	0	0	417.2	239.3	0
6/24/98	0	1489.9	0	0	439.6	399.3	573.8
6/30/98	0	656.5	0	255.3	82.1	240.1	97.3
7/6/98	0	506.2	0	0	0	139.8	105.6
7/13/98	0	666.7	0	0	0	397.3	0
7/18/98	0	273.7	0	0	0	200	0
7/24/98	0	212	0	0	0	144.9	0
7/30/98	0	284.8	0	0	162.3	0	29.8
8/5/98	0	352.2	0	0	59.7	323.9	210.7
8/11/98	0	4187.2	13.7	0	913.2	1292.2	1064
8/17/98	0	1383.6	188.4	1373.3	763.7	804.8	366.4
8/23/98	0	959.1	348	1576	450.3	482.5	403.5

Concentration Data Combined for Vapor & Particulate for All Sites

Concentration Total Date Site B	o,p-DDE pg/m3	p,p-DDE pg/m3	o,p-DDD pg/m3	p,p-DDD pg/m3	o,p-DDT pg/m3	p,p-DDT pg/m3	Kelthane pg/m3
4/14/98	0	899.7	25.8	74.5	140.4	289.4	0
4/20/98	0	157.9	91.4	285.3	52.6	52.6	0
4/26/98	0	0	0	0	26.9	0	0
5/2/98	1470.4	647.9	0	3143.7	583.1	281.7	0
5/4/98	0	610.3	83.1	157.6	151.9	452.7	0
5/6/98	1868.9	774.9	31.3	0	151	378.9	0
5/8/98	1360	3217.1	0	0	111.4	220	0
5/10/98	258.7	456.4	23.3	29.1	72.7	186	90.1
5/12/98	24.6	815.4	9.2	43.1	261.5	310.8	0
5/14/98	6741.2	2644.1	47.1	76.5	450	923.5	0
5/16/98	582.4	2600	0	120.6	411.8	897.1	908.8
5/19/98	545.2	670.6	0	0	151.6	498.5	0
5/25/98	609.2	190.8	0	221.5	40	153.8	0
5/31/98	420.1	1381.9	0	163.2	402.8	937.5	246.5
6/6/98	0	484.9	0	0	177.3	0	210.7
6/12/98							
6/18/98	686.1	1664.2	0	0	54.7	594.9	138.7
6/24/98	0	2985.3	0	0	584.6	1136	860.3
6/30/98	0	1123.3	0	0	133.3	420	73.3
7/6/98	0	1566.3	0	0	2336.6	563.1	576.1
7/13/98	0	1094.3	0	0	154.7	562.3	0
7/18/98	0	560.6	0	0	0	155.3	1227
7/24/98	26467	3106.6	2459.6	0	0	606.6	0
7/30/98	0	1044.6	0	0	583.6	252.8	713.8
8/5/98	0	143.9	0	0	0	214	110.7
8/11/98	0	840.9	0	0	155.8	490.3	87.7
8/17/98	0	923.3	0	1303.1	641.1	756.1	355.4
8/23/98	0	1441.6	353.9	1535.7	571.4	1048.7	0

Concentration Data Combined for Vapor & Particulate for All Sites

Concentration Total

Date Site C	o,p-DDE pg/m3	p,p-DDE pg/m3	o,p-DDD pg/m3	p,p-DDD pg/m3	o,p-DDT pg/m3	p,p-DDT pg/m3	Kelthane pg/m3
4/14/98	0	881.8	43.2	0	118.2	377.5	60.5
4/20/98	0	279.5	90.4	432.9	158.9	169.9	0
4/26/98	0	47	0	0	5.5	19.3	0
5/2/98	61.3	119.8	0	0	0	103.1	0
5/4/98	0	1463.1	79.5	0	267	698.9	0
5/6/98	2389.5	848.8	32	0	162.8	427.3	0
5/8/98	724.9	426.9	0	0	60.2	126.1	0
5/10/98	709.5	930.2	16.8	0	139.7	318.4	0
5/12/98	823.4	524.2	11.4	0	94	208	0
5/14/98	8303.8	2660.8	50.1	56	413	985.3	0
5/16/98	452.8	2175.9	0	0	342	772	697.1
5/19/98	20.5	231	950.3	444.4	701.8	17.5	0
5/25/98	408.6	425.7	0	14.3	125.7	308.6	0
5/31/98	234.1	1075.1	0	106.9	306.4	774.6	216.8
6/6/98	183.9	554.8	6.5	0	90.3	0	345.2
6/12/98	0	2346.7	0	0	1186.7	986.7	0
6/18/98	1015.3	1993.9	0	0	896	737	978.6
6/24/98	0	2724.8	0	0	516.8	97.3	926.2
6/30/98	0	0	0	0	0	0	0
7/6/98	0	2211.5	0	0	3305.1	897.3	1695
7/13/98	0	1107.6	0	0	191	527.8	0
7/18/98	0	626.7	0	0	0	200	1183
7/24/98	0	931.3	0	0	20.6	408.9	852.2
7/30/98	0	558.6	0	0	248.3	110.3	0
8/5/98	0	134.6	0	0	134.6	128.4	474
8/11/98	0	104.6	0	0	0	70.8	147.7
8/17/98	0	897.5	0	0	378.1	473.5	254.4
8/23/98	0	2180.1	540.4	0	897.5	1360.2	767.1

Concentration Data Combined for Vapor & Particulate for All Sites

Concentration	Total						
Date	o,p-DDE	p,p-DDE	o,p-DDD	p,p-DDD	o,p-DDT	p,p-DDT	Kelthane
Site D	pg/m3	pg/m3	pg/m3	pg/m3	pg/m3	pg/m3	pg/m3
4/14/98	0	201.1	0	0	152.2	95.1	0
4/20/98	0	221.6	75.7	186.5	210.8	40.5	0
4/26/98	0	85.5	13	1497.4	51.8	54.4	0
5/2/98	72	85.3	0	0	80	722.7	0
5/4/98	0	516.2	27	370.3	113.5	210.8	0
5/6/98	217.4	236.4	0	356	54.3	92.4	0
5/8/98	186.8	986.3	0	0	159.3	313.2	0
5/10/98	116.5	411.9	0	173.4	243.9	113.8	0
5/12/98	611.4	274.5	0	0	59.8	95.1	0
5/14/98	1250	505.4	0	0	81.5	163	135.9
5/16/98	873.1	398.8	0	0	102.7	160.1	102.7
5/19/98	0	37.9	0	54.2	0	35.2	0
5/25/98	23.8	58.2	0	0	10.6	31.7	0
5/31/98	0	19	0	0	0	0	0
6/6/98	156.3	223.7	0	0	32.3	0	0
6/12/98	0	390.9	0	0	420.2	153.1	0
6/18/98	1381.8	48.5	0	0	0	0	0
6/24/98	0	876.9	0	0	83.1	181.5	1019
6/30/98	0	1724.6	0	0	142	576.8	202.9
7/6/98	1325.8	522.5	0	0	132	101.1	306.2
7/12/98	0	0	0	0	23.4	163.9	0
7/18/98	0	450.2	0	0	0	250.8	0
7/24/98	0	0	0	0	0	0	0
7/30/98	0	161	0	0	102.7	0	0
8/5/98	0	281.4	0	0	59.9	6	0
8/11/98	0	48	0	0	0	78.1	0
8/17/98	275.4	592.8	137.7	1038.9	524	559.9	134.7
8/23/98	0	1031.7	0	1584.5	500	489.4	778.2

APPENDIX L

Concentration Data Combined for Vapor Phase for All Sites

Data	o,p-DDE ng/Samp	p,p-DDE ng/Samp	o,p-DDD ng/Samp	p,p-DDD ng/Samp	o,p-DDT ng/Samp	p,p-DDT ng/Samp	Kelthan ng/Samp
Site A							
4/14/98	0	164	0.6	14	52	44	0
4/20/98	0	131	28	0	34	18	0
4/26/98	0	0	0	0	4	0	0
5/2/98	180	71	0	0	19	55	0
5/4/98	0	293	12	0	62	138	0
5/6/98	353	116	0	13	0	53	0
5/8/98	208	405	23	22	48	11	0
5/10/98	97	306	3	0	39	174	23
5/12/98	0	197	0	24	93	55	0
5/14/98	2061	460	10	17	95	141	0
5/16/98	143	242	0	0	38	97	0
5/19/98	48	98	0	0	0	31	0
5/25/98	134	93	0	8	0	0	0
6/1/98	0	191	0	0	41	54	13
6/6/98	34	118	4	198	51	0	0
6/12/98	0	308	0	0	128	81	0
6/18/98	183	361	0	0	136	78	0
6/24/98	0	444	0	0	131	119	171
6/30/98	0	216	0	84	27	79	32
7/6/98	0	163	0	0	0	45	0
7/13/98	0	99	0	0	0	59	0
7/18/98	0	78	0	0	0	55	0
7/24/98	0	60	0	0	0	41	0
7/30/98	0	86	0	0	49	0	0
8/5/98	0	112	0	0	19	103	67
8/11/98	0	878	3	0	200	283	233
8/17/98	0	243	0	0	110	112	77
8/23/98	0	328	119	539	154	165	138

Concentration Data Combined for Vapor Phase for All Sites

Data	o,p-DDE ng/Samp	p,p-DDE ng/Samp	o,p-DDD ng/Samp	p,p-DDD ng/Samp	o,p-DDT ng/Samp	p,p-DDT ng/Samp	Kelthan ng/Samp
Site B							
4/14/98	0	291	5	26	49	74	0
4/20/98	0	26	0	38	10	0	0
4/26/98	0	0	0	0	0	0	0
5/2/98	518	230	0	48	207	100	0
5/4/98	0	206	12	6	53	124	0
5/6/98	611	271	8	0	53	130	0
5/8/98	464	1126	0	0	39	77	0
5/10/98	71	157	8	10	23	53	31
5/12/98	0	265	3	14	85	99	0
5/14/98	2286	894	16	26	153	307	0
5/16/98	193	876	0	41	140	301	309
5/19/98	187	220	0	0	52	165	0
5/25/98	188	62	0	72	13	50	0
5/31/98	121	390	0	47	116	253	71
6/6/98	0	145	0	0	53	0	63
6/12/98	0	637	0	0	324	272	0
6/18/98	188	456	0	0	15	131	38
6/24/98	0	812	0	0	159	309	234
6/30/98	0	337	0	0	40	126	22
7/6/98	0	484	0	0	722	174	0
7/13/98	0	290	0	0	41	145	0
7/18/98	0	148	0	0	0	41	324
7/24/98	7199	845	669	0	0	165	0
7/30/98	0	281	0	0	157	68	192
8/5/98	0	39	0	0	0	58	30
8/11/98	0	259	0	0	48	151	27
8/17/98	0	265	0	0	98	117	71
8/23/98	0	444	109	473	176	203	0

Concentration Data Combined for Vapor Phase for All Sites

Data	o,p-DDE ng/Samp	p,p-DDE ng/Samp	o,p-DDD ng/Samp	p,p-DDD ng/Samp	o,p-DDT ng/Samp	p,p-DDT ng/Samp	Kelthan ng/Samp
Site C							
4/14/98	0	267	10	0	41	111	0
4/20/98	0	70	0	93	50	44	0
4/26/98	0	9	0	0	2	0	0
5/2/98	22	43	0	0	0	15	0
5/4/98	0	515	16	0	94	222	0
5/6/98	710	269	3	0	51	138	0
5/8/98	239	149	0	0	21	44	0
5/10/98	220	333	6	0	50	112	0
5/12/98	289	184	4	0	33	71	0
5/14/98	2759	894	17	19	140	321	0
5/16/98	133	658	0	0	105	228	214
5/19/98	0	0	325	152	240	0	0
5/25/98	143	149	0	5	44	108	0
5/31/98	81	363	0	37	106	243	75
6/6/98	57	172	2	0	28	0	107
6/12/98	0	704	0	0	356	296	0
6/18/98	332	652	0	0	293	203	320
6/24/98	0	812	0	0	154	29	276
6/30/98	0	0	0	0	0	0	0
7/6/98	0	732	0	0	1094	297	344
7/13/98	0	319	0	0	55	151	0
7/18/98	0	188	0	0	0	60	355
7/24/98	0	271	0	0	6	119	188
7/30/98	0	162	0	0	72	32	0
8/5/98	0	44	0	0	44	42	155
8/11/98	0	0	0	0	0	23	0
8/17/98	0	254	0	0	107	134	72
8/23/98	0	702	174	0	289	323	247

Concentration Data Combined for Vapor Phase for All Sites

Data	o,p-DDE ng/Samp	p,p-DDE ng/Samp	o,p-DDD ng/Samp	p,p-DDD ng/Samp	o,p-DDT ng/Samp	p,p-DDT ng/Samp	Kelthan ng/Samp
Site D							
4/14/98	0	74	0	0	56	35	0
4/20/98	0	82	0	0	71	0	0
4/26/98	0	26	2	60	13	21	0
5/2/98	27	21	0	0	0	267	0
5/4/98	0	191	10	11	42	78	0
5/6/98	78	87	0	0	20	30	0
5/8/98	68	359	0	0	58	113	0
5/10/98	43	152	0	64	90	42	0
5/12/98	213	96	0	0	22	25	0
5/14/98	451	177	0	0	30	45	50
5/16/98	286	132	0	0	34	53	34
5/19/98	0	0	0	20	0	6	0
5/25/98	9	21	0	0	4	12	0
5/31/98	0	0	0	0	0	0	0
6/6/98	58	83	0	0	12	0	0
6/12/98	0	120	0	0	129	47	0
6/18/98	456	16	0	0	0	0	0
6/24/98	0	285	0	0	27	59	331
6/30/98	0	595	0	0	49	199	70
7/6/98	472	186	0	0	47	36	109
7/12/98	0	0	0	0	7	49	0
7/18/98	0	0	0	0	0	0	0
7/24/98	0	0	0	0	0	0	0
7/30/98	0	47	0	0	30	0	0
8/5/98	0	94	0	0	20	2	0
8/11/98	0	16	0	0	0	26	0
8/17/98	92	198	46	347	104	97	45
8/23/98	0	293	0	450	142	139	221

APPENDIX M

Concentration Data Combined for Particulate Phase for All Sites

Site A Date	o,p-DDE ng/Sam	p,p-DDE ng/Sam	o,p-DDD ng/Sam	p,p-DDD Ng/Sam	o,p-DDT ng/Sam	p,p-DDT ng/Sam	Kelthan ng/Sam
4/14/98	0	0	0	0	0	0	0
4/20/98	0	36	38	76	10	23	0
4/26/98	3	10	0	0	0	10	0
5/2/98	0	0	0	1448	0	0	0
5/4/98	0	0	13	0	0	18	0
5/6/98	37	1	1	0	0	2	0
5/8/98	20	2	0	0	0	8	0
5/10/98	13	0	0	0	0	5	0
5/12/98	0	0	0	0	0	2	0
5/14/98	22	1	0	0	0	3	0
5/16/98	0	1	0	0	0	0	0
5/19/98	8	8	0	0	0	4	0
5/25/98	3	0	0	0	0	2	0
6/1/98	0	0	0	0	0	0	0
6/6/98	0	0	0	0	0	6	0
6/12/98	0	0	0	0	0	0	0
6/18/98	0	0	0	0	0	0	0
6/24/98	0	0	0	0	0	0	0
6/30/98	0	0	0	0	0	0	0
7/6/98	0	0	0	0	0	0	34
7/13/98	0	99	0	0	0	59	0
7/18/98	0	0	0	0	0	2	0
7/24/98	0	0	0	0	0	0	0
7/30/98	0	0	0	0	0	0	9
8/5/98	0	0	0	0	0	0	0
8/11/98	0	39	0	0	0	0	0
8/17/98	0	161	55	401	113	123	30
8/23/98	0	0	0	0	0	0	0

Concentration Data Combined for Particulate Phase for All Sites

Site B Date	o,p-DDE ng/Sam	p,p-DDE ng/Sam	o,p-DDD ng/Sam	p,p-DDD Ng/Sam	o,p-DDT ng/Sam	p,p-DDT ng/Sam	Kelthan ng/Sam
4/14/98	0	23	4	0	0	27	0
4/20/98	0	31	33	65	9	19	0
4/26/98	0	0	0	0	10	0	0
5/2/98	4	0	0	1068	0	0	0
5/4/98	0	7	17	49	0	34	0
5/6/98	45	1	3	0	0	3	0
5/8/98	12	0	0	0	0	0	0
5/10/98	18	0	0	0	2	11	0
5/12/98	8	0	0	0	0	2	0
5/14/98	6	5	0	0	0	7	0
5/16/98	5	8	0	0	0	4	0
5/19/98	0	10	0	0	0	6	0
5/25/98	10	0	0	0	0	0	0
5/31/98	0	8	0	0	0	17	0
6/6/98	0	0	0	0	0	0	0
6/12/98	0	0	0	0	0	0	0
6/18/98	0	0	0	0	0	32	0
6/24/98	0	0	0	0	0	0	0
6/30/98	0	0	0	0	0	0	0
7/6/98	0	0	0	0	0	0	178
7/13/98	0	0	0	0	0	4	0
7/18/98	0	0	0	0	0	0	0
7/24/98	0	0	0	0	0	0	0
7/30/98	0	0	0	0	0	0	0
8/5/98	0	0	0	0	0	0	0
8/11/98	0	0	0	0	0	0	0
8/17/98	0	0	0	374	86	100	31
8/23/98	0	0	0	0	0	120	0

Concentration Data Combined for Particulate Phase for All Sites

Site C Date	o,p-DDE ng/Sam	p,p-DDE ng/Sam	o,p-DDD ng/Sam	p,p-DDD Ng/Sam	o,p-DDT ng/Sam	p,p-DDT ng/Sam	Kelthan ng/Sam
4/14/98	0	39	5	0	0	20	21
4/20/98	0	32	33	65	8	18	0
4/26/98	0	8	0	0	0	7	0
5/2/98	0	0	0	0	0	22	0
5/4/98	0	0	12	0	0	24	0
5/6/98	112	23	8	0	5	9	0
5/8/98	14	0	0	0	0	0	0
5/10/98	34	0	0	0	0	2	0
5/12/98	0	0	0	0	0	2	0
5/14/98	56	8	0	0	0	13	0
5/16/98	6	10	0	0	0	9	0
5/19/98	7	79	0	0	0	6	0
5/25/98	0	0	0	0	0	0	0
5/31/98	0	9	0	0	0	25	0
6/6/98	0	0	0	0	0	0	0
6/12/98	0	0	0	0	0	0	0
6/18/98	0	0	0	0	0	38	0
6/24/98	0	0	0	0	0	0	0
6/30/98	0	0	0	0	0	0	0
7/6/98	0	0	0	0	0	0	217
7/13/98	0	0	0	0	0	1	0
7/18/98	0	0	0	0	0	0	0
7/24/98	0	0	0	0	0	0	60
7/30/98	0	0	0	0	0	0	0
8/5/98	0	0	0	0	0	0	0
8/11/98	0	34	0	0	0	0	48
8/17/98	0	0	0	0	0	0	0
8/23/98	0	0	0	0	0	115	0

Concentration Data Combined for Particulate Phase for All Sites

Site D Date	o,p-DDE ng/Sam	p,p-DDE ng/Sam	o,p-DDD ng/Sam	p,p-DDD Ng/Sam	o,p-DDT ng/Sam	p,p-DDT ng/Sam	Kelthan ng/Sam
4/14/98	0	0	0	0	0	0	0
4/20/98	0	0	28	69	7	15	0
4/26/98	0	7	3	518	7	0	0
5/2/98	0	11	0	0	30	4	0
5/4/98	0	0	0	126	0	0	0
5/6/98	2	0	0	131	0	4	0
5/8/98	0	0	0	0	0	1	0
5/10/98	0	0	0	0	0	0	0
5/12/98	12	5	0	0	0	10	0
5/14/98	9	9	0	0	0	15	0
5/16/98	3	0	0	0	0	0	0
5/19/98	0	14	0	0	0	7	0
5/25/98	0	1	0	0	0	0	0
5/31/98	0	7	0	0	0	0	0
6/6/98	0	0	0	0	0	0	0
6/12/98	0	0	0	0	0	0	0
6/18/98	0	0	0	0	0	0	0
6/24/98	0	0	0	0	0	0	0
6/30/98	0	0	0	0	0	0	0
7/6/98	0	0	0	0	0	0	0
7/12/98	0	0	0	0	0	0	0
7/18/98	0	149	0	0	0	83	0
7/24/98	0	0	0	0	0	0	0
7/30/98	0	0	0	0	0	0	0
8/5/98	0	0	0	0	0	0	0
8/11/98	0	0	0	0	0	0	0
8/17/98	0	0	0	0	71	90	0
8/23/98	0	0	0	0	0	0	0

Appendix N

Sample Volume, Pressure, and Temperature Data for All Sites

Pressure data collected at Holland
Temp. data collected at each site: Coloma (260210014) and S. Haven (260050002)

Date	Sampler No.	Sampled Volume, m^3	avg P, mm Hg	avg T, deg C
4/14/98	c	347	737	11
4/14/98	b	349	737	11
4/14/98	a	362	737	11
4/14/98	d	368	737	11
4/20/98	d	370	747	10
4/20/98	c	365	747	11
4/20/98	b	361	747	11
4/20/98	a	362	747	11
4/26/98	d	386	744	8
4/26/98	c	362	744	9
4/26/98	B	372	744	9
4/26/98	A	368	744	9
5/2/98	D	375	736	9
5/2/98	c	359	736	9
5/2/98	d	355	736	9
5/2/98	a	348	736	9
5/4/98	d	370	739	14
5/4/98	c	352	739	14
5/4/98	b	349	739	14
5/4/98	a	354	739	14
5/6/98	d	368	740	18
5/6/98	c	344	740	19
5/6/98	b	351	740	19
5/6/98	a	353	740	19
5/8/98	d	364	737	19
5/8/98	c	349	737	19
5/8/98	b	350	737	19
5/8/98	a	354	737	19
5/10/98	d	369	743	15
5/10/98	c	358	743	15
5/10/98	b	344	743	15
5/10/98	a	352	743	15
5/12/98	d	368	741	18
5/12/98	c	351	741	18
5/12/98	b	325	741	18
5/12/98	a	340	741	18
5/14/98	d	368	746	18
5/14/98	c	339	746	19

Date	Sampler No.	Sampled Volume, m^3	avg P, mm Hg	avg T, deg C
5/14/98	a	325	746	19
5/16/98	d	331	743	22
5/16/98	c	307	743	22
5/16/98	b	340	743	22
5/16/98	a	339	743	22
5/19/98	d	369	744	22
5/19/98	c	342	744	22
5/19/98	b	343	744	22
5/19/98	a	344	744	22
5/25/98	d	378	743	13
5/25/98	c	350	743	13
5/25/98	b	325	743	13
5/25/98	a	304	743	13
5/31/98	d	368	736	20
5/31/98	c	346	736	20
5/31/98	b	288	736	20
6/1/98	a	346	740	15
6/6/98	d	371	747	10
6/6/98	c	310	747	10
6/6/98	b	299	747	10
6/6/98	a	307	747	10
6/12/98	d	307	735	22
6/12/98	c	300	735	21
6/12/98	b	286	735	21
6/12/98	a	325	735	21
6/18/98	d	330	744	24
6/18/98	c	327	744	24
6/18/98	b	274	744	24
6/18/98	a	326	744	24
6/24/98	d	325	744	27
6/24/98	c	298	744	27
6/24/98	b	272	744	27
6/24/98	a	298	744	27
6/30/98	d	345	737	21
6/30/98	c	316	737	21
6/30/98	b	300	737	21
6/30/98	a	329	737	21
7/6/98	d	356	745	23
7/6/98	c	331	745	24
7/6/98	b	309	745	24
7/6/98	a	322	745	24
7/12/98	d	299	746	20
7/13/98	c	288	744	23
7/13/98	b	265	744	23
7/13/98	a	297	744	23
7/18/98	d	331	744	23
7/18/98	c	300	744	23

Date	Sampler No.	Sampled Volume, m^3	avg P, mm Hg	avg T, deg C
7/18/98	a	285	744	23
7/24/98	d	334	747	21
7/24/98	c	291	747	21
7/24/98	b	272	747	21
7/24/98	a	283	747	21
7/30/98	d	292	745	21
7/30/98	c	290	745	22
7/30/98	b	269	745	22
7/30/98	a	302	745	22
8/5/98	d	334	747	22
8/5/98	c	327	747	22
8/5/98	b	271	747	22
8/5/98	a	318	747	22
8/11/98	d	333	746	21
8/11/98	c	325	746	21
8/11/98	b	308	746	21
8/11/98	a	219	746	21
8/17/98	d	334	745	23
8/17/98	c	283	745	23
8/17/98	b	287	745	23
8/17/98	a	292	745	23
8/23/98	d	284	741	27
8/23/98	c	322	741	27
8/23/98	b	308	741	27
8/23/98	a	342	741	27

APPENDIX O

Soil Concentration Data Site A

Soil Data in ug/g Site/Date	Site A/East			Site A/West		
April	**Top**	**Middle**	**Bottom**	**Top**	**Middle**	**Bottom**
o,p-DDE	0	34	0	13	13	0
p,p-DDE	1	3044	0	1341	1434	0
o,p-DDD	0	194	0	66	71	0
p,p-DDD	0	395	0	125	68	0
o,p-DDT	0	781	0	317	361	0
p,p-DDT	3	6804	0	2575	2816	0
Kelthane	0	3369	0	1609	1873	0
Sept						
O,p-DDE	40	80	0	40	0	0
P,p-DDE	11700	1411	260	7750	0	100
O,p-DDD	480	760	0	320	0	0
P,p-DDD	210	150	0	120	0	0
O,p-DDT	3860	6170	20	2730	10	30
P,p-DDT	22180	232290	280	14920	50	180
Kelthane	1750	2400	20	920	0	20

Soil Concentration Data Site BC

Site/Date	Site BC/North			Site BC/South		
April	**Top/dup**	**Middle**	**Bottom**	**Top**	**Middle**	**Bot/dup**
o,p-DDE	19.5	18	4	0	26	1
p,p-DDE	2563.5	2055	570	2101	3184	472.5
o,p-DDD	140	84	20	95	149	14.5
p,p-DDD	308	59	28	204	521	24.5
o,p-DDT	673	463	129	528	709	101.5
p,p-DDT	5582.5	4012	1009	4040	6121	796
Kelthane	2878.5	0	427	0	0	198
Sept						
o,p-DDE	10	0.01	0	10	0	0
p,p-DDE	23350	22270	310	25700	4700	0
o,p-DDD	0	0	10	0	30	0
p,p-DDD	40	100	0	70	10	0
o,p-DDT	410	310	0	530	120	0
p,p-DDT	8410	7230	30	10500	2820	0
Kelthane	400	350	10	410	110	0

Soil Concentration Data Site D

Site/Date	Site D/East			Site D/West		
April	**Top**	**Mid/dup**	**Bottom**	**Top**	**Middle**	**Bottom**
o,p-DDE	0	0	2	0	6	3
p,p-DDE	0	125.5	241	271	535	439
o,p-DDD	0	2	2	4	5	4
p,p-DDD	0	8.5	9	16	20	35
o,p-DDT	0	9	22	110	91	58
p,p-DDT	0	113.5	242	1096	840	528
Kelthane	0	41	0	187	0	0
Sept						
o,p-DDE	10	20	30	0	10	20
p,p-DDE	2650	2350	1580	2320	2270	2770
o,p-DDD	10	10	10	10	20	30
p,p-DDD	20	10	10	20	20	40
o,p-DDT	500	390	40	270	550	970
p,p-DDT	3810	2570	1950	3110	4110	7550
Kelthane	70	50	20	60	130	260

APPENDIX P

Wind Speed and Direction for Sample Days at SH

Date	Wind Speed (mph)	Resultant Wind Direction
4/14/98	6.1	SSE
4/20/98	4.9	W
4/26/98	9.9	ENE
5/2/98	4.4	WNW
5/4/98	4.5	WNW
5/6/98	3.4	SSE
5/8/98	9.3	NE
5/10/98	7	NE
5/12/98	4.6	SSE
5/14/98	2.4	W
5/16/98	12.5	WSW
5/19/98	6.5	SSW OR WSW
5/25/98	8.3	WNW
6/1/98	4.8	WSW OR ESE
6/6/98	6	WNW
6/12/98	13.6	SW
6/18/98	5.2	ESE
6/24/98	6.3	SW
6/30/98	10.3	NW
7/6/98	5.4	SW
7/13/98	5	W
7/18/98	4	W
7/24/98	5	NW
7/30/98	6	NNW OR NW
8/5/98	4.6	E
8/11/98	5.8	NNE
8/17/98	5.5	SW
8/23/98	12.6	SW

Wind Speed and Direction for Sample Days at CLM

Date	Wind Speed (mph)	Resultant Wind Direction
4/14/98	5	NW
4/20/98	3	N
4/26/98	8	ENE
5/2/98	3	W
5/4/98	3	NE
5/6/98	4	SSE
5/8/98	9	NE
5/10/98	6	NNW
5/12/98	7	NE
5/14/98	2	SSE
5/16/98	9	WSW
5/19/98	5	SW
5/25/98	5	WNW
6/1/98	4	W
6/6/98	10	NW
6/12/98	6	SW
6/18/98	6	SE
6/24/98	7	S
6/30/98	5	NNW
7/6/98	2	SSW
7/13/98	2	WNW
7/18/98	2	SE
7/24/98	4	NNW
7/30/98	4	NNW
8/5/98	3	ENE
8/11/98	5	N OR NNW
8/17/98	4	SW
8/23/98	8	SSW

APPENDIX Q

Calculation of experimental half-life of DDT

Known Data:

log 27 = log 100 - (k x 25 years)/2.303

So k = 0.052

So log 50 = log 100 - (0.052 x years)/2.303

Therefore years = 13.2 years = $t_{1/2}$

Appendix R

Soil Texture and Moisture Content

Sample name	Soil Depth*	Carbon %	Sand %	Silt %	Clay %	Moisture %	Type
A 110 SE 15 N	2	1.43	68.9	18.4	12.7	16.0	sandy loam
A 110 SE 15 N	M4	1.14	66.6	22.7	10.7	12.1	sandy loam
A 110 SE 15 N	B4	0.41	60.9	18.4	20.7	10.8	sandy clay loam
A 15 N 105 of SE	2	1.73	64.9	22.4	12.7	16.0	sandy loam
A 15 N 105 of SE	M4	1.59	62.9	22.4	14.7	16.2	sandy loam
A 15 N 105 of SE	B4	0.42	80.9	24.4	14.7	12.9	sandy loam
Coloma W	2	0.52	84.9	4.7	10.4	6.1	loamy sand
Coloma W	M4	0.64	90.3	5.4	4.4	7.6	sand
Coloma W	B4	1.57	84.9	4.4	10.7	10.5	loamy sand
Coloma 50 E	2	1.55	81.2	7.9	10.9	13.4	loamy sand
Coloma 50 E	M4	0.85	84.5	9.2	6.4	11.2	loamy sand
Coloma 50 E	B4	0.64	86.5	8.2	5.4	8.0	loamy sand
B/C 15 W 270 SE	2	1.72	72.9	14.4	12.7	16.3	sandy loam
B/C 15 W 270 SE	M4	1.22	78.9	8.4	12.7	10.5	sandy loam
B/C 15 W 270 SE	B4	0.77	74.9	10.4	14.7	9.2	sandy loam
B/C 15 W 268 N of SE	2	1.82	72.9	16.4	10.7	13.6	sandy loam
B/C 15 W 268 N of SE	M4	1.80	78.6	10.7	10.7	14.2	sandy loam
B/C 15 W 268 N of SE	B4	1.01	76.9	14.4	8.7	13.2	sandy loam
SW2NE	Comp.	1.22	66.9	18.4	14.7	14.1	sandy loam
SE2NW	Comp.	1.25	70.9	14.4	14.7	12.7	sandy loam

*2=top two inches
M4=middle 4 inches
B4=bottom 4 inches
comp.=composite

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