# EXTRACTION OF PESTICIDE RESIDUES IN SOILS USING MULTIVARIATE OPTIMIZATION AND SUPERCRITICAL FLUID EXTRACTION

by

Min Michael Zhou

A dissertation submitted to the Faculty of the University of Delaware in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Plant and Soil Sciences

Fall 1996

**EXTRACTION OF PESTICIDE RESIDUES IN SOILS USING MULTIVARIATE OPTIMIZATION AND SUPERCRITICAL FLUID EXTRACTION**

by

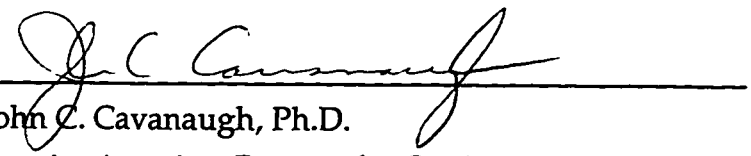
Min Michael Zhou

Approved: 

Donald L. Sparks, Ph.D.
Chairman of the Department of Plant and Soil Sciences

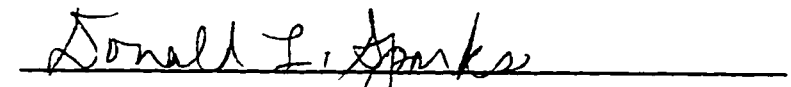
Approved: 

John C. Nye, Ph.D.
Dean of the College of Agricultural Sciences

Approved: 

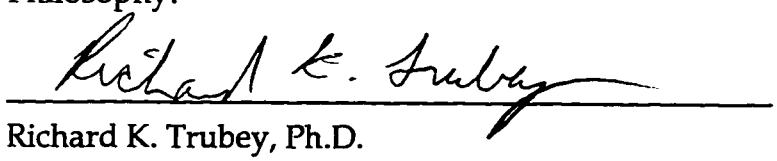
John C. Cavanaugh, Ph.D.
Interim Associate Provost for Graduate Studies

I certify that I have read this dissertation and that in my opinion it meets the academic and professional standard required by the University as a dissertation for the degree of Doctor of Philosophy.

Signed 

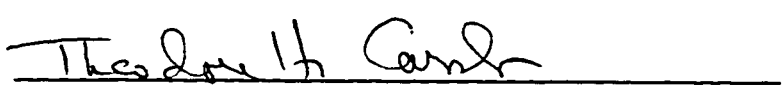
Donald L. Sparks, Ph.D.
Distinguished Professor in charge of dissertation

I certify that I have read this dissertation and that in my opinion it meets the academic and professional standard required by the University as a dissertation for the degree of Doctor of Philosophy.

Signed 

Richard K. Trubey, Ph.D.
Member of dissertation committee

I certify that I have read this dissertation and that in my opinion it meets the academic and professional standard required by the University as a dissertation for the degree of Doctor of Philosophy.

Signed 

Theodore H. Carski, Ph.D.
Member of dissertation committee

I certify that I have read this dissertation and that in my opinion it meets the academic and professional standard required by the University as a dissertation for the degree of Doctor of Philosophy.

Signed _____

Mark Radosevich, Ph.D.
Member of dissertation committee

I certify that I have read this dissertation and that in my opinion it meets the academic and professional standard required by the University as a dissertation for the degree of Doctor of Philosophy.

Signed _____

Zenaida O. Keil, Ph.D.
Member of dissertation committee

## TABLE OF CONTENTS

## LIST OF TABLES

**Appendix II**

## LIST OF FIGURES

## ACKNOWLEDGEMENTS

There are many people to whom I am indebted for their guidance and support throughout my graduate sojourn. My failure to mention any of these people herein is not reflective of the extent of my indebtedness to them.

Several people deserve particular recognition. I wish to convey my sincerest appreciation to the chairperson of my supervisory committee, Dr. Donald L. Sparks, for his helpful advice and guidance throughout these research projects. I would like to thank Dr. Richard K. Trubey and Dr. Theodore H. Carski for their continued supervision, technical expertise, and constructive comments during the projects, and also to thank Dr. Mark Radosevich and Dr. Zenaida O. Keil for their constructive comments and helpful support during the course of my projects.

In addition, I would like to thank Dr. Julia R. Wheeler and Dr. Sidney S. Goldberg for their supervision, encouragement and support for the research (within DuPont Agricultural Products Department), and to thank Thomas M. Priester and Fred Q. Bramble and other colleagues for their collaborative research and efforts throughout the projects.

I am grateful to DuPont Agricultural Products Department, Global Technology Division for the financial and other related material supports, and for their expressed interest in my investigations.

The deepest gratitude is extended to my family, particularly to my father, who has persistently endured me as a budding analytical/ environmental chemist and encouraged me to pursue further education for a Ph.D. degree.

Finally, to my wife, Sheree, mere words can not express the depth of my appreciation for her loving and caring attitude. I owe it all to you in so many ways. I can only hope to repay you in the remainder of my earthly days. If not, there is always eternity.

## RATIONALE AND SCOPE OF RESEARCH

For many years, pesticides have been extensively used in agriculture, forestry, public health, and domestic gardening/households. Modern pesticides greatly benefit our society, but they may pose environmental concerns, particularly as potential soil and groundwater contaminants. Knowledge of the environmental fate in soil is important to ensure the environmental safety of pesticide use. Most laboratory data regarding sorption/desorption kinetics do not exactly reflect real environmental situations. The occurrence and significance of bound pesticide residues in soil have become important issues in dealing with persistence, degradation, and biological availability of pesticide residues.

The awareness of the presence of bound residues in soil and plants has increased dramatically. The organic fraction of a soil appears to have the potential for forming bound residues with pesticides or products arising from their degradation. Currently the United States Environmental Protection Agency (USEPA) espouses two primary methods for the extraction of semivolatile organic contaminants and pesticides from soils. These classical methods, the sonication and the Soxhlet sequential extraction, are widely used in environmental laboratories. Unfortunately, these methods are time consuming and involve the use of large volumes of solvents that are often

toxic or even carcinogenic. Not only is the purchase of these solvents expensive, but their safe disposal is equally costly. Also, during most soil extractions, the solvent extracts must be concentrated. In this process, the excess solvent is usually evaporated in a hood and vented to the atmosphere. These evaporated solvents contribute to our present air pollution problems.

Analysis of bound residues of pesticides in soil has always been a challenging problem. The most frequently used method for analysis and quantification of bound residues of pesticides has been by total combustion of the solvent-extracted sample to convert bound $^{14}C$ residues to $^{14}CO_2$. The $^{14}CO_2$ is trapped in basic solvents and radioassayed by liquid scintillation counting, leaving little change of characterizing the bound residues.

Supercritical fluid extraction (SFE) has already been shown to be a viable alternative to more conventional sample preparation techniques. One of the biggest advantages of SFE over the use of standard liquid extractions is the fact that many sample preparations can be done with nonpolluting, nontoxic fluids such as $CO_2$. The use of SFE on an industrial scale has occurred for many years, but it was not until recently that SFE has been applied to analytical scale sample preparation. The potential advantages of this technique come from the unique properties of supercritical fluids. However, analytical SFE is currently an evolving technique in which many experimental parameters and problems have yet to be properly defined. The traditional approach to experimentation is exploring one variable at a time. This involves holding all variables constant except one, which is varied across a range. This approach can be very costly, time consuming, and not suitable

for understanding interactions among variables, i.e., how variables work together in synergistic or antagonistic ways. Nevertheless, a multivariate optimization scheme (MOS) has not yet been employed to investigate the complex soil systems as well as SFE parameter interactions and their effects on the extractability of pesticide residues in soils. To reveal these potential effects, the objectives of this research are:

- To study sorption/desorption equilibria and kinetics of three major herbicides in selected soils and the effect of pesticide residence time (aging) on their desorption and the formation of "bound" residues.

- To develop a multivariable optimization scheme (MOS) for supercritical fluid extraction (SFE). To explore the applicability of the SFE technique for extracting 'bound' pesticide residues in soils, compared to some conventional methods.

- To identify the extracted residues (i.e., parents and metabolites formed under laboratory and field conditions) using different techniques.

Sorption/desorption equilibria and kinetics of three herbicides were conducted with several selected soils. Preliminary results discussed in Chapter 1 help us in understanding and interpreting the behavior of these pesticides and potential formation of bound residues in the soil environment. Using a multivariate optimization scheme (MOS), SFE methods were systematically optimized for both freshly fortified and aged samples, which

enable us to better understand the differences between laboratory spiked and field aged samples. Significant findings are detailed in Chapter 2. Then a comparison study was performed with other extraction methodologies such as Soxhlet, sonication, surfactant addition, and accelerated solvent extraction (ASE). Analytical data are evaluated and summarized in Chapter 3. To understand the characteristics of the three herbicides, and the reliability of the data from the SFE, identification and semiquantification of parent compounds and their respective degradation products were investigated and are discussed in respective Chapters 1, 2, and 3.

In addition to the above results, future research is also suggested/recommended in the "Findings and Future Research" section, to encourage continued efforts in these important areas.

## ABSTRACT

Modern pesticides are generally recognized as significantly benefiting our ability to satisfy the world's need for abundant, safe, affordable food and fiber. Pesticides reach the soil environment by direct or indirect applications from aerial and ground sprays. The main processes affecting the ultimate fate of pesticides in soil are retention by soil materials (adsorption/desorption processes), transformation processes (biological and chemical degradation), and transport into soil, to the atmosphere, and to surface or groundwater.

Sorption/desorption equilibria and kinetics of atrazine, diuron, and bensulfuron methyl were conducted using a batch technique on several selected soils. The sorption/desorption distribution coefficients ($K_a/K_d$) of diuron and bensulfuron methyl were calculated using the Freundlich equation. Results indicated that the content of organic matter was the major variable contributing to diuron and bensulfuron methyl sorption. The low $1/n_d$ (isotherm slope for desorption) values showed that both pesticides were not readily desorbed from the soils tested. The rates of sorption were more rapid than those of desorption for atrazine and bensulfuron methyl, especially in the case of bensulfuron methyl on the soil containing a high level of organic matter (57.5%). In this soil sorption was extremely fast, compared to the other soils that contained high clay (56.4%) or high sand (91.6%). The slow rates of

desorption were presumably associated with the heterogeneous nature of the soil, and potential hysteresis phenomena. Hysteresis was observed, at various degrees, depending on the pesticides and soils tested. The energy of activation values for both sorption ($E_a$ = 11-25 kJ/mol) and desorption ($E_d$ = 18-38 kJ/mol) suggested that transport or diffusion control is rate-limiting for both processes. This study also showed that the desorption of bensulfuron methyl was almost irreversible, particularly with high contents of soil organic matter. This was correlated to the $K_d$ values obtained from the isotherm equilibrium experiments. The stronger sorption of bensulfuron methyl than diuron suggested a potential different sorption mechanism.

A multivariate optimization scheme (MOS) was used to investigate the effects of environmental variables [i.e., soil organic matter %, clay minerals %, various pesticides, residence time of pesticide (aging), etc.] and supercritical fluid extraction (SFE) parameters (i.e., pressure, temperature, extraction duration, extraction mode, etc.) on the extractability of pesticide residues from soil samples. MOS offered the opportunity to systematically and simultaneously examine the interaction and effects among important soil variables and extraction parameters. MOS is a highly efficient technique for studying a large number of variables and identifying optimal extraction conditions.

Pesticide residence time had a major influence on binding processes for all tested pesticides. Extractability as a function of soil composition was greatly dependent on the particular pesticide examined. Bensulfuron methyl was extracted with the greatest difficulty from soils containing both high

levels of organic matter and clay. Atrazine was extracted more easily than diuron. High organic matter and high clay content led to strong binding for diuron and atrazine, respectively.

Effects of SFE parameters on extractability were apparently related to the nature of residues (e.g., freshly fortified versus aged residues). For freshly fortified samples, analyte solubility in supercritical fluid and/or modified supercritical fluid was the critical factor as indicated by the strong influence of pressure on extraction efficiency. For aged samples, temperature was an important determinant of extraction efficiency, indicating that mass transfer or diffusion processes were rate-limiting. The presence of modifier and extraction duration also significantly impacted extractability of aged pesticides.

Several aged soil samples (with atrazine, diuron, bensulfuron methyl) were extracted by Soxhlet, sonication, surfactant extraction, accelerated solvent extraction (ASE), and supercritical fluid extraction (SFE) for comparison. Among solvent extraction, ASE provided better extraction efficiency than the conventional methods. This is probably due to the elevation of temperature and pressure that create a subcritical phase for the extraction. This results in an increase of solvating power and solubility of analyte into the extraction liquid. Surfactant extraction yielded better extractability, particularly in the case of diuron and atrazine, than other solvent extractions (i.e., Soxhlet).

SFE was the best approach to recover aged residues from the soils. With the aid of a surfactant as a modifier, additional bound residues can be extracted using the optimal SFE method, especially for aged atrazine and diuron. Prewetting of aged samples was found to be effective in accelerating the extraction rate.

For aged atrazine and diuron extraction, elevated temperature appeared to be a significant element in effectively recovering or extracting the analytes from the soils. However, it seemed to be detrimental in recovering bensulfuron methyl, which was presumably associated with thermal degradation that occurred in the extraction. To optimize the SFE procedures, characteristics of the analyte(s) should be also carefully taken into account. Analytical results suggested that interaction time was important to the extraction, but fresh supercritical fluid using a dynamic mode may cause better extraction rate and efficiency of the aged residues. The combination of an initial static followed by a dynamic mode extraction apparently enhanced extraction rate and efficiency.

Pesticide degradation occurred mostly in the environment (i.e, soil system), but in some cases, it was also observed under laboratory conditions (i.e., during sorption/desorption experiments, sample aging, analytical procedure, etc.). The rate and extent of the degradation basically depended on the characteristics of the pesticide and the nature of the soil conditions/ composition. Identification and semiquantification were conducted using TLC-Bioassay, LC-RadioChem, LC-UV-DAD, and LC or GC/MS techniques. In general, bensulfuron methyl degraded very rapidly even in the soil-

aqueous system (during sorption/desorption isotherms and sample aging). Major transformation compounds were identified as sulfonamide, O-desmethyl-bensulfuron methyl (ODM-DPX-F5384), and homosaccharin. There was also thermal degradation during the SFE process at an elevated temperature (> 80°C). Diuron degradation was observed in the field studies (degradation and lysimeter studies). 3,4-Dichlorophenylurea (DCPU) and N-(3,4-dichlorophenyl)-N'-methylurea (DCPMU) were present to a various extent, depending on the length in the field. The percent of DCPU and DCPMU detected increased with the increase in sampling intervals. It was surprising that diuron was decomposed to dichloroaniline (DCA) with the presence of either Celite® 545 or silica homogeneous material during the SFE extraction at above 120°C. Atrazine was considered to be the most stable compound, compared to bensulfuron methyl and diuron. No degradation took place under any laboratory conditions (sorption/desorption experiments, sample aging, and SFE procedures at elevated temperature, i.e., 150°C). Atrazine showed some degree of transformation after an extended period of time (aging) in the field, resulting in dealkylation and/or hydroxylation.

# Chapter 1

# PESTICIDES IN THE SOIL ENVIRONMENT

## 1.1 Introduction

### 1.1.1 Pesticides in the Environment

The increasing use of pesticides in agriculture remains a controversial issue. One of the main concerns is the hazard of soil pollution by pesticides and their impact on soil fertility. Pesticides reach the soil by direct application, to control soil pests and for uptake by plants, and also indirectly, from aerial and ground sprays (Graham-Bryce, 1981). The main processes affecting the efficiency and ultimate fate of pesticides in soil are retention by soil materials (sorption-desorption processes), transformation processes (biological and chemical degradation), and transport into soil, to the atmosphere, and to surface and ground water.

After reaching the soil, most pesticide formulations are distributed primarily into the soil solution and then onto the surfaces of the solid phase and/or into the soil atmosphere, gravitating toward a dynamic equilibrium. The uptake of pesticides by soils (usually termed sorption or adsorption) and their release (desorption) have been considered from the very beginning of pesticide use as key processes (Alexander, 1965). The availability of pesticides for uptake by the target organisms and for movement in solution or in the

gaseous phase, as well as their chemical and biological transformation processes, are all affected by sorption-desorption.

As discussed, pesticides reach the soil either by direct application or indirectly, following their application on plant canopies. Upon reaching the land pesticides are subjected to complex physico-chemical and biological transformations. There are several reasons for attempting to understand the fate and behavior of pesticides in soil: the need to improve the efficiency of soil-applied compounds, the need to minimize their potential adverse effects on soil fertility, and the need to minimize the risk of environmental pollution due to their transfer to the groundwater and atmosphere.

### 1.1.2 Environmental Fate of Pesticides in Soils

The first reports on soil adsorption of synthetic organic pesticides appeared shortly after World War II as a result of the increasing use of these compounds. It had been observed that the extremely efficient and persistent insecticide DDT and other persistent chlorinated hydrocarbon insecticides used to control malaria vectors and other insects rapidly lost their toxicity when sprayed on the internal surfaces of houses in several African countries (Hadaway and Barlow, 1952). Since these first observations, and the introduction of new classes of pesticides, adsorption has become a central issue in pesticide studies. The techniques used were developed from visual observation to bioassays, to direct adsorption measurements both in the field and in the laboratory, and later to the use of modern sophisticated spectroscopic and microscopic methods and particle-scattering techniques.

Although the subject has been studied intensively, adsorption of pesticides by soils is not yet fully understood. However, the available information provides some understanding of the factors affecting adsorption and the mechanisms and lays the basis for assessing the behavior of pesticides in soil and in the environment.

Seven factors are known to influence the fate and behavior of pesticides in soil systems: (1) chemical decomposition, (2) direct/indirect photochemical decomposition, (3) microbial decomposition, (4) volatilization, (5) movement, (6) plant or organism uptake, and (7) adsorption. The phenomenon of adsorption-desorption directly or indirectly influences the magnitude of the effect of the other six factors. Adsorption, therefore, appears to be one of the major factors affecting the interactions occurring between pesticides and soil colloids.

### 1.1.3 Nature of Soil Colloids

The chemical and physical properties of soils are influenced strongly by soil constituents which have high specific surface areas or highly reactive surfaces. Since high specific surface area is associated with small particle size, the colloidal fraction of the soil will be the dominant factor in influencing interactions between pesticide molecules and the soil. The colloidal constituents of soils may be divided into the organic fraction and the mineral fraction. The humic colloid fraction has not been completely characterized, but it appears that much of the reactivity of this fraction is embodied in the fraction designated "humic acid." The mineral fraction is composed of

crystalline clay minerals and crystalline and amorphous oxides and hydroxides. Humic acid has been described by van Dijk (1966) as being globular, polydisperse, and irregular polycondensate. Humic acids are polybasic acids with at least two kinds of acid groups, i.e., carboxyl and phenolic hydroxyl groups. The cation exchange capacity (CEC) of humic acid is higher than that of clay minerals, being of the order of 200 to 400 meq/100 g.

Functional groups such as carboxyl, amino, phenolic hydroxyl, and alcoholic hydroxyl, in addition to directly affecting the adsorption of cationic and anionic pesticide by humic acid, may also provide sites for hydrogen bonding interactions with the pesticide molecules. Because of the complexity of humic acid and the experimental difficulties in applying spectroscopic techniques to the study of interactions between two groups of organic compounds of considerable complexity, relatively little information on the mechanism of adsorption of pesticides by organic matter is available.

The uptake of nonionic organic compounds and pesticides by soil in aqueous systems is affected by chemical partition into soil organic matter (Chiou et al., 1979; Chiou, 1981; Chiou and Shoup, 1985; Sawhney and Brown, 1989). In contrast to adsorption, the term partition or partitioning is used to denote an uptake in which the sorbed organic chemicals permeate into the network of an organic medium by forces common to solution (i.e., by van der Waals forces). The partition uptake is analogous to the extraction of an organic compound from water into an organic phase. When the organic phase is a solid (i.e., soil organic matter), partition is distinguished from

adsorption by the homogeneous distribution of the sorbed materials through the entire volume of the solid phase (Sawhney and Brown, 1989).

Most agricultural soils are mixtures of different materials including organic matter, microbial biomass, and inorganic crystalline and noncrystalline compounds. The particle size of mineral grains varies from colloidal dimensions to pebbles. Soil-pesticide interactions may be better understood if the interactions of the soil components of organic matter, clay, and other minerals with the pesticide are better understood. Clay minerals, having small particle size and hence large surface area per unit weight, are important in the overall sorption behavior of clay soils and sediments. Large surface areas are important for sorption of pesticides (Karickhoff, 1984), but it is likely that sorption occurs most strongly at chemically or crystallographically specific sites (Karickhoff and Brown, 1978; Terce, 1983; Glass, 1987; Borggaard and Streibig, 1988; Breen, 1991; Morillo et al., 1991; Laird et al., 1992). Sites with high charge density demonstrate electrostatic attraction for ionic pesticides (Karickhoff and Brown, 1978; Glass, 1987; Morillo et al., 1991), and acidic sites can protonate nonionic pesticides at a pH near the $pK_a$ of the pesticide (Terce, 1983; Borggaard and Streibig, 1988; Breen, 1991).

In contrast to adsorption and transport, which are transfer processes, degradation is the most widespread phenomenon contributing to the disappearance of pesticides from soils. Soil is an ideal medium inducing transformation reactions of pesticides (Graham-Bryce, 1981). The usually moist and aerated upper layer of agricultural soils provides proper conditions

for chemical changes (mainly hydrolysis and oxidation reactions) occurring in the soil solution. At the same time, adsorption strongly affects the availability of pesticides for transformation reactions.

However, the most important soil characteristic related to pesticide degradation is probably the rich microbial population, capable of attacking a wide variety of chemical compounds. The first studies of soil persistence of pesticides were carried out with phenoxyacetic acid herbicides in the first decade after their introduction (1945-1955) and indicated microbial degradation. A few years later this disappearance pathway was demonstrated for other groups of pesticides, such as some cyclodiene and organophosphorus insecticides, and the s-triazine herbicides. These accumulating data, and the "principle of microbial infallibility" pervading scientific thought at that time (Alexander, 1965), led to the opinion that microbial degradation is responsible for the detoxification of all the toxic compounds reaching the soil. However, studies of pesticide degradation, carried out in the late 1960's, mainly with organophosphates and s-triazines, showed that, in addition to microbiological processes, nonbiological degradation could play an important role in the transformation of pesticides in soils.

### 1.1.4 Kinetic Studies of Pesticides in Soils

The primary soil components responsible for pesticide sorption are clay minerals, and especially, humic materials. Pesticides can be divided into cationic, basic, acidic, and nonionic classes (Saltzman and Yaron, 1986). The

fate of pesticides and organic pollutants in the environment is strongly dependent on their sorptive behavior. Sorption affects not only physical transport of these materials but also their degradation.

The sorptive behavior of pesticides can be studied from either equilibrium or kinetic viewpoints. While both are important, perhaps the time-dependent processes are least understood. As environmental concerns intensify about groundwater pollution, waste disposal, and soil detoxification, it will become increasingly important to better understand the kinetics and mechanisms of pesticide interactions with soils (Sparks, 1989; Sparks, 1995).

The rate of sorption and desorption of pesticides on soils and soil constituents has been investigated by a number of researchers (Hance, 1967) and is dependent on the type of sorbent, pesticide, and rate of mixing. For example, sorption seems much slower on humic substances (Khan, 1973). Other factors that may affect the kinetics are swelling of the sorbent and temperature (Hance, 1967).

Hance (1967) investigated the rate of sorption and desorption of four pesticides (monuron, linuron, atrazine, and chlorpropahm) on two soils, a soil organic matter fraction, and bentonite, a 2:1 smectitic clay mineral. An equilibrium in sorption was reached in 24 hours for almost every system. Desorption was slower than sorption.

The sorption and desorption of pesticides by soils and soil constituents has generally been characterized by an initial rapid rate followed by a much slower approach to an apparent equilibrium (Haque et al., 1968; Leenheer and

Ahlrichs, 1971); Khan, 1973; McCall and Agin, 1985). The initial reaction(s) have been associated with diffusion of the pesticides to and from the surface of the sorbent, while the slower reactions have been related to particle diffusion of the pesticides into and out of micropores of the sorbent.

An example of this kind of relationship is shown in the work of Leenheer and Ahlrichs (1971) for sorption of carbaryl and parathion on soil humic materials. An apparent equilibrium was attained in 2 hours or less, and the amount of pesticides sorbed at equilibrium increased as temperature increased.

Steinberg et al. (1987) studied the persistence of 1,2-dibromoethane (EDB) in soils and found that low amounts of the organic were released with time, particularly if EDB had not been freshly added to the soil. They suggested that the slow release rate was due to EDB being trapped in soil micropores where release is influenced by extreme tortuosity and/or steric restrictions. It was estimated, based on a radial diffusion model, that 23 and 31 years would be required for a 50% equilibrium in EDB release to occur from two Connecticut soils.

A novel technique using on-line microfitration and HPLC analysis has been used to study sorption kinetics and sorption equilibrium of the herbicide atrazine with the clay minerals montmorillonite, kaolinite, and illite and the clay fraction of a soil (Gilchrist et al., 1993). Fast and slow labile sorption have been observed for atrazine along with a reversible but kinetically slow sorption/desorption process that is consistent with diffusion of pesticide into

the interior of the clay particles. In the study, labile sorption capacity, mole fraction site coverage, labile sorption equilibrium function, and distribution coefficients were determined for the clay minerals in aqueous slurries with atrazine. The identification and quantitative description of key chemical species avoid some of the commonly reported hysteresis.

The pesticide sorption behavior of specific clay minerals has been studied by several researchers (Karickhoff and Brown, 1978; Terce and Calvet, 1978; Terce, 1983; Glass, 1987; Borggaard and Streibig, 1988; Singh et al., 1989; Breen, 1991; Morillo et al., 1991; Laird et al., 1992). It has been found that some sorption phenomena have not yet been fully explained. Two outstanding problems are the characterization of the sorption capacity of clay particles for labile and reversible but slow species (Gamble and Khan, 1990; Gamble and Ismaily, 1992) and the characterization of the formation of bound residues of pesticides in clays and soils (Di Toro and Horzempa, 1982; Macalady and Wolfe, 1984; Karickhoff and Morris, 1985).

Equilibrium partitioning of organic compounds between water and soil has been the subject of extensive study over the last 2 decades (Hamaher and Thompson, 1972; Karickhoff (Ed), 1980; Means et al., 1980; Karickhoff, 1984). More recently, interest has grown in considering kinetic effects which govern the movement of contaminants in these media under non-equilibrium conditions (Di Toro and Horzempa, 1982; Karickhoff and Morris, 1985; Pignatello, 1990; Ball, 1991; Miller and Pedit, 1992; Pavlostathis and Mathavan, 1992; Pignatello et al., 1993). Kinetics are important in many transport and biodegradation processes which operate at shorter time scales than are

required for equilibrium to be established. Sorbed nonionic organic compounds often display biphasic desorption kinetics from soils and sediments where a labile component of the compound desorbs readily and reversibly, while a resistant component desorbs orders of magnitude more slowly (Di Toro and Horzempa, 1982; Karickhoff and Morris, 1985).

Several processes have been proposed as being responsible for non-equilibrium sorption. Rate-limiting processes have been grouped into two general classes: transport related and sorption related (Brusseau and Rao 1989; Brusseau et al., 1991). Three different diffusive mass-transfer related mechanisms can cause sorption-related nonequilibrium: film diffusion, retarded intraparticle diffusion (RIPD), and intrasorbent diffusion. Film diffusion will not be considered further, as many researchers have shown that this mechanism is generally insignificant in comparison to other mechanisms for the uptake/release of organic chemicals (Brusseau and Rao, 1989).

### 1.1.5 Pesticide Transformation Products

Pesticides applied in the environment are transformed by biological or nonbiological processes into one or more transformation/degradation products. For most pesticides, transformation results in detoxification to innocuous products. Major degradation products of some currently used pesticides, however, play an important role in pest control and environmental contamination. Pesticide transformation is any process in which a change takes place in the molecular structure of a pesticide. The transformation of a pesticide can occur immediately after, or even before, application, during

storage. The chemicals formed by the different transformation processes are referred to by several names (Somasundaram and Coats, 1991).

### 1.1.6 Properties of Degradation Products

Characteristics influencing the environmental significance of pesticide degradation products include water solubility, vapor pressure, and carcinogenic and mutagenic potential. Although most degradation products of pesticides are converted into less toxic or nontoxic materials, some degradation products may be biologically and/or environmentally active. In general, many degradation products are more soluble in water than their parent compounds (Somasundaram et al., 1991). This increased solubility favors their mobility to groundwater.

However, pesticide transformation is mainly a beneficial process resulting in detoxification of the parent compound. For some pesticides, in contrast, the products formed can be of significance in both crop protection and environmental contamination. Yet, for many of the currently used pesticides, the fate and significance of their degradation products are not clearly understood.

### 1.1.7 Current Techniques in Identifying Degradation Products of Pesticides

The derivation of structural information from spectroscopic data is now an integral part of identification and confirmation of unknown compounds (i.e., degradation products of organic contaminants). Analytical techniques in

these areas have been primarily Fourier transform infrared spectrometry (FTIR), nuclear magnetic resonance (NMR), gas chromatography/mass spectrometry (GC/MS), gas chromatography/mass spectrometry/mass spectrometry (GC/MS/MS), liquid chromatography/mass spectrometry (LC/MS), or liquid chromatography/mass spectrometry/mass spectrometry (LC/MS/MS).

Among the above mentioned techniques, FTIR and NMR usually generate characteristic data regarding functional groups and structural information of the molecules with relatively less sensitivity than MS. It is also required to have some extent of purity and quantity for identification. However, GC/MS or LC/MS techniques enable researchers to identify and quantify trace levels of target analyte(s) in a complex matrix (Karasek and Clement, 1988).

### 1.1.8 Applications of GC/MS and LC/MS

Although GC/MS is in an advanced stage of development, new techniques, applications, and instrumentation are continually being introduced. Many of these are minor modifications of existing methods, or are for specialized uses such as extended mass range and the analysis of substances of low volatility. New developments in such diverse fields as gas chromatography, ionization method, mass analysis, computer techniques, and mathematical procedures such as pattern recognition are all of potential values to GC/MS. Among the most significant developments are the techniques of tandem mass spectrometry (MS-MS) and Fourier transform

mass spectrometry (FTMS). These developments show how unique applications of existing technology can lead to new analytical capabilities. Computers are essential to both MS/MS and FTMS because of the vast amounts of data that have to be processed-even more than for conventional GC/MS analysis.

The application of a mass spectrometer as a universal, yet extremely selective and sensitive detector in gas chromatography has revolutionized the identification and measurement of unknown organic compounds. The efficient use of GC/MS has made possible the capability to analyze numerous components in a complex environmental sample.

The simultaneous analysis of 25 pesticides in soybean and rice was performed by a gas chromatograph with dual electron-capture detection (ECD) and nitrogen-phosphorus detection (NPD) (Hong et al., 1993). The gas chromatography properties of the 25 pesticides were also investigated. Confirmatory analysis was achieved based on the retention time and characteristic fragment using the technique of GC/MS.

Jackson (1993) reported that the combination of a gas chromatograph, matrix-isolation FTIR spectrometer and mass spectrometer in a single instrument showed necessary selectivity and sensitivity in analyzing caffeine at picogram levels. It was almost three orders of magnitude more sensitive than the conventional lightpipe GC-FTIR instruments. The presence of multiple detectors (MS, FID) is seen as a significant advantage. The complementary nature of MS and FTIR data can be exploited with parallel

detectors to give complete and confident structural and isomeric identification of unknowns, as demonstrated with pesticide mixtures.

Analysis of pesticides and PCB in water, soil and plant materials using GC/MS was reviewed by Cochrane et al. (1994). A variety of adsorbents (i.e., silica, modified aluminas, etc.) have been used in a variety of ways to eliminate interfering coextractives. Successful identification and quantitation of pesticides and PCB residues has relied heavily on gas chromatography with element-specific detectors including mass spectrometry. In this review, several specific examples were provided from current laboratory operations.

More recently GC/MS methodology was investigated by Barinova et al. (1995) to determine residues of chlorinated pesticides in potatoes. Aldrin, dieldrin, dilor, heptachlor, alpha- and gamma- hexachlorocyclohexanes, DDT and its metabolites were studied. Gas chromatography with packed columns may result in errors in the identification of chlorinated pesticides. A combination of gas chromatography and mass spectrometry provided reliable identification of the pesticides, even with interfering compounds present.

The research efforts were mainly inspired by the great success of combined capillary gas chromatography/mass spectrometry (GC/MS) in solving analytical problems. However, the development of LC/MS turned out to be a demanding and challenging task, because many of the new pesticides and their degradation products are thermally labile. LC/MS has grown to become a mature and routinely used technique in many areas of application.

GC/MS is widely used as the major MS method in environmental analysis (Barcelo, 1988; Cairns et al., 1989). However, many of the target compounds (i.e., many pesticides and their metabolites) are not amenable to GC analysis. Therefore, the role of LC in environmental analysis is of growing importance. It is almost impossible to review all applications of LC/MS, which have received extensive coverage. Selected applications in the field of environmental analysis for pesticides will be discussed.

Carbamate pesticides, since their thermal lability prohibits GC/MS, are compatible with a variety of LC/MS interfaces (Browm et al., 1990). Quantitative data on the LC/MS analysis of carbamates are given by various authors (Bellar and Budde, 1988; Behymer et al., 1990; Voyksner and Bursey, 1984).

The thermospray (TSP) interface is used for the quantitative carbamate analysis (Bell and Budde, 1988; Voyksner et al., 1984; Chiu et al., 1989), and the identification of metabolites (Saar and Salomon, 1990). Generally, better detection limits are achieved with TSP compared to direct liquid introduction (DLI). However, the improvement in the detection limit in TSP relative to DLI is less than would be expected considering the difference in effective mass flow to the MS ion source with these two techniques (split ratio).

Quantitative analysis of carbamates using the particle beam interface (PBI) is reported by Behymer et al. (1990) and Bellar et al. (1990). In general, the reported detection limits are between 100 and 300 ng, which appears to be insufficient for practical quantitative pesticide residue analysis.

Sulfonylureas form a group of relatively new and selective herbicides. The compounds are highly thermally labile and cannot readily be derivatized and are therefore not amenable to GC/MS. The LC/MS analysis of this group of herbicides has been described with various interfaces. The Moving-belt interface (MBI) analysis of sulfonylureas is described by Barefoot and Reiser (Barefoot and Reiser, 1987; Barefoot and Reiser 1989), and for two of its metabolites, but no molecular weight information is obtained. The compounds show extensive fragmentation due to bond cleavage on either side of the carbonyl group, allowing the calculation of molecular weight.

In comparison with the probe electron impact (EI), given by Shalaby (1985), the MBI EI spectrum gives more information, e.g., the peaks at m/z = 300 and 257 are not observed in the probe EI spectrum. In ammonia chemical ionization (CI) spectra obtained with the MBI, weak protonated molecules are observed for sulfometuron methyl and its hydroxylated metabolite, but not for the glucoside of the hydroxylated metabolites (Barefoot and Reiser, 1989).

Sulfonylureas and their metabolites have also been studied using packed microcapillary columns coupled to a continuous-flow fast atom bombardment (CF-FAB) interface (Barefoot and Reiser, 1989). The on-line FAB spectra of sulfometuron methyl and its metabolites show strong protonated molecules and more low intensity, useful fragment ions. No quantitative data are given. It was apparent that the highly labile sulfonylurea herbicides are difficult to analyze with LC/MS. Extreme care must be taken to obtain direct molecular-weight information from the intact

molecules. From the interfaces tested, only CF-FAB gave strong intact protonated molecules for the parent compounds and their metabolites.

Supercritical fluid extraction of atrazine and polar metabolites from sediments followed by confirmation with LC/MS was reported recently (Papilloud et al., 1996). An optimized nondegrading supercritical fluid extraction enabled the reproducible extraction of atrazine and its metabolites from the sediments, showing also that atrazine was metabolized in its hydroxylated analogue, involving chemical abiotic degradation. It was the first time use of SFE followed by LC/MS of the polar atrazine metabolites from an environmental matrix.

### 4.1.5 Significance in Identifying Degradation Products of Pesticides

Pesticide transformation may occur during sample preparation, sorption/desorption equilibria, extraction, sample storage, shipment, and especially after field application biologically by microorganisms and nonbiologically through chemical reactions in the soil system.

Identification and semiquantification of degradation products are vitally important in order to clearly understand the real situation of laboratory or field samples before and after any of the available extraction approaches. Degradation processes may occur in the environment (i.e., soil system) as well as in analytical procedures, especially with elevated temperature and/or caustic conditions. TLC-Bioassay, LC-RadioChem, LC/UV-DAD, LC/MS, and/or GC/MS can be used to identify or confirm the presence of potential degradation compounds (metabolites) during different processes.

Experimental findings will enable environmental scientists to understand the characteristics of parent compounds and their respective metabolites in predicting the fate of pesticides and potential degradates in the surface and subsurface environments.

Identification and semiquantification of potential degradation products were performed and are discussed in each of the following chapters, respectively.

## 1.2 Methods and Materials

### 1.2.1 Sorption/Desorption Equilibrium Studies of Diuron and Bensulfuron Methyl on Selected Soils

Four different soils (Cecil sandy loam, Woodstown sandy loam, Keyport silt loam and Flanagan sandy loam) were used for preliminary studies (collaborative studies within DuPont). Characterization data (pH, % sand, % silt, % clay, % organic matter, etc.) are given in Appendix II.

#### 1.2.1.1. Sorption Isotherm Study

Aqueous (0.01 N $CaSO_4$) solutions of the $^{14}C$-labeled test compound were prepared to have nominal initial concentrations ($C_1$) of 0.2, 0.5, 1.0 2.5, 5.0 mg/L. One-half milliliter (0.5 mL) aliquots of each solution, sampled in triplicate, were mixed with 20 mL of a Formula-947™ Scintillation Cocktail (New England Nuclear, DuPont Medical Products, Boston, Mass) and analyzed for total $^{14}C$ by liquid scintillation counting (LSC, Tracor® Analytic

Mark III Liquid Scintillation System; Model 6881, TM Analytic Inc., Elk Grove Village, IL).

Twenty-mL aliquots of each standard solution were mixed with 20 g of soil (oven-dried equivalents, Mettler Model PE600 2-place top-loading balance, Mettler Instrument Corp., Hightstown, NJ) in screw-capped, Poly-seal, 4-oz polyethylene bottles. The capped bottles and their contents were shaken for 24 hours in a constant temperature water bath maintained at 25°C (Gyrotory Shaker Model G-76, New Brunswick Scientific, New Brunswick, NJ), and then centrifuged at 1000 x G (2000 rpm) for 10 minutes (IEC HN-SII Tabletop Centrifuge, Damon/IEC Division, Needham Hts., Mass). Three 0.5-mL aliquots of the supernatant were removed from each bottle, mixed with 15 mL of Formula-947 Scintillation Cocktail, and the concentration of radioactivity in the aqueous phase ($C_2$) was determined by LSC. All concentration values were expressed as μg/mL or μg/g (ppm).

#### 1.2.1.2 Desorption Isotherm Study

Soil and water from the highest concentration level of each sorption study were used for the desorption study. Each test system was identified with an alpha-numeric code. After discarding a known volume of the supernatant, fresh 0.01 N calcium sulfate solution was added until the original total weight and 1:1 (w/w) soil to water ratio was obtained. The volume of supernatant discarded varied from soil to soil depending on its moisture capacity and filtration characteristics. The bottles were shaken in the constant temperature shaker bath maintained at 25°C for another 24 hours and the new

concentration of radioactivity in the supernatant ($C_2$) was determined again. This procedure was repeated a total of five times (5 consecutive days) using fresh 0.01 N calcium sulfate solution for each equilibration.

### 1.2.2 Sorption/Desorption Kinetic Studies of Bensulfuron Methyl and Atrazine on Selected Soils

#### 1.2.2.1 Sorption Kinetic Study

Three soils (a, Belhaven with high organic matter content: 56.7%; b, Cullera with high clay content: 57.5%; c, Miaka with high sand content: 91.7%) and Alliston sandy loam (from Canada) were used to study sorption/desorption kinetics of bensulfuron methyl and atrazine, respectively. Soil characterization data are given in Appendix II. A batch technique was chosen for the study, because generally pesticide sorption/desorption rates are considered to be relatively slow.

Aqueous (0.01 N $CaCl_2$) solutions of the test compound were prepared to have nominal initial concentrations of 0.5 and 5.0 μg/mL or ppm. (20 mL for 10 g soil). One-quarter milliliter (0.25 mL) aliquots of each solution taken in duplicate, were mixed with 15 mL of Formula-947™ Scintillation Cocktail (New England Nuclear) and analyzed for total $^{14}C$ by liquid scintillation counting (LSC).

Twenty-mL aliquots of each standard solution were mixed with 10 g of soil (oven-dried equivalents) in screw-capped, Poly-seal, 4-oz polyethylene bottles. The capped bottles and their contents were shaken (moderate speed

setting) for up to 60 hours in a constant temperature water bath maintained at 25°, 40°, and 60°C, respectively. At a predetermined interval (0, 5, 15, 30, 60 minutes, 2, 4, 8, 16, 32 and 60 hours), samples were centrifuged at 1000 x G (2000 rpm) for 3 minutes when necessary (if too turbid). Then two 0.25-mL aliquots of the supernatant were removed from each bottle, mixed with 15 mL of Formula-947 Scintillation Cocktail, and the concentration of radioactivity in the aqueous phase was determined by LSC. All concentration values were expressed as fraction sorbed (0-1 or 0-100% ).

#### 1.2.2.2 Desorption Kinetic Study

After the sorption kinetic studies, 20 mL of fresh 0.01N $CaCl_2$ was added to the soils. The capped bottles and their contents were shaken for up to 32 hours in a constant temperature water bath maintained at 25°, 40°, and 60°C, respectively. At a predetermined interval (0, 15, 30, 60, 120, 240 minutes, 8, 16, 20 and 32 hours), samples were centrifuged at 1000 x G (2000 rpm) for 3 minutes when necessary (if too turbid). Then two 0.25-mL aliquots of the supernatant were removed from each bottle, mixed with 15 mL of Formula-947 Scintillation Cocktail, and the concentration of radioactivity in the aqueous phase was determined by LSC. All concentration values were expressed as fraction desorbed (0-1 or 0-100%).

### 1.2.3 Identification and Semiquantification of Potential Degradation Compounds from the Sorption/Desorption Studies

Aqueous samples were taken from both sorption and desorption studies over a period of time to identify any potential degradation during the

studies. Aqueous samples were acidified to pH 3.2 and extracted three times with methylene chloride at 1:1 (v/v) ratio. Aliquots of the concentrate and nonradiolabeled reference standards were spotted on EM 500μ silica and 200 μ $SiC_{18}$ TLC plates and developed in $CH_2Cl_2/CH_3OH/23\%NH_4OH$ (144/50/4, v/v/v) and $CH_2Cl_2$/acetonitrile/acetic acid/water (150/27/2.5/0.3, v/v/v/v), respectively. The bensulfuron methyl and metabolites/degradates were detected and quantified with a Berthold LB2832 automatic-TLC analyzer (Berthold Scientific, Wiessbaden, Germany) and identified by coelution of authentic reference standards. These analyses were performed on the aqueous extracts both before sorption and after the last desorption phase of the experiments. An additional extraction was done on water samples which had been incubated for the same period of time as the desorption phase aqueous extracts but without soil. Aqueous samples were also analyzed by reversed phase LC with a gradient mobile phase separation. LC column: Zorbax® Rx-$C_{18}$ 4.6 mm ID, 250 mm, 5 μm (Mac-Mod Analytical); Mobile phase: 20% acetonitrile held for initial 5 minutes, gradient to 30% in 10 minutes, then to 50% in 5 minutes, and held for 5 minutes, with $NaH_2PO_4$ buffered at pH 3.2; Flowrate: 1.0 mL/min; Injection volume: 25-50 μL; UV detection: 254 nm.

### 1.2.4. Chemicals and Reagents

The radiolabeled reference standards used in the study were: [phenyl-$^{14}$C(U)]diuron with a radiochemical purity of 98.3%, [$^{14}$C] atrazine with a radiochemical purity of 99%, [phenyl-$^{14}$C(U)]bensulfuron methyl, and [pyrimidine-2-$^{14}$C]bensulfuron methyl with a radiochemical purity of 99%,

and related standards of degradation compounds were synthesized by DuPont New England Nuclear (NEN) Products (Billerica, Mass.).

Analytical standards of diuron (DPX-14740-149, 99.7% purity), bensulfuron methyl (DPX-F5384, 99.3% purity), atrazine (INY-0150, 100.0% purity), and related standards of degradation compounds were synthesized by DuPont Agricultural Products, E. I. du Pont de Nemours and Company (Wilmington, Del.). The structures of the compounds are given in Appendix I.

All water used in the studies was deionized using a Milli-Q® water purification system (Millipore Corp. Milford, Mass). Chemical reagents were Fisher Scientific reagent grade, ACS grade, and HPLC grade where applicable.

## 1.3 Results and Discussion

To understand the sorptive nature of pesticides in different soil matrices, the sorption/desorption equilibrium and kinetic studies were performed on selected soils for bensulfuron methyl, diuron, and atrazine.

### 1.3.1 Sorption/Desorption Equilibrium Studies of Diuron And Bensulfuron Methyl on Selected Soils

In the sorption studies, the concentration of radioactivity sorbed onto the soil after equilibration ($C_s$) was determined by subtracting the concentration of radioactive diuron in the aqueous solution at equilibrium ($C_2$) from the initial concentration in the standard solution ($C_1$). The $C_2$ and $C_s$ values were plotted on a log-log scale to generate Freundlich isotherms. The sorption distribution coefficient ($K_a$) of diuron on each soil was calculated as

the $C_s$ value corresponding to a $C_2$ value of the concentration at 1 μg/mL samples. Coefficients of sorption per unit of organic matter values ($K_{a/OM}$) were calculated from each $K_a$ value using the equation

$$K_{a/OM} = K_a(100\%)/\%OM$$

where % OM is the percent organic matter in the corresponding soil sample. The slope of each line ($1/n_a$) was calculated using a least square regression curve fitting program.

In the desorption studies, the initial concentrations of radioactive diuron in the aqueous phases ($C_1$ and $C_2$) and on the soil ($C_s$), prior to the first desorption equilibration, were determined from the sorption study. The concentration of radioactivity removed from the soil ($C_{2a}$) was determined at each subsequent equilibrium period (Days 1-5) by subtracting the initial concentration of radioactivity in the aqueous phase at the beginning of the day ($C_1$) from the concentration at the end of the day ($C_2$). The concentration remaining on the soil was determined by subtracting the $C_{2a}$ value from the previous day's $C_s$ value. The $C_2$ and $C_s$ desorption data were similarly plotted on a log-log scale. Desorption distribution coefficients ($K_d$), coefficients of desorption per unit of organic matter ($K_{d/OM}$), and the slope of the isotherms ($1/n_d$) were determined in a manner analogous to the sorption study.

Theoretical desorption distribution coefficients ($K_d'$) were calculated from the experimental $K_a$ values using the Hornsby equation (Wu et al., 1975):

$$K_{d'} = K_a\,(n_a/n_d) \times S_m\,(1-n_a/n_d)$$

where $S_m$ is maximum concentration of test substance on the soil.

For diuron studies, the concentration data from the sorption studies were plotted in Figures 1.1-1.4. Calculated sorption characteristics (i.e., $K_a$, $K_{a,OM}$ and $1/n_a$) for diuron on each soil are summarized in Table 1.2. Diuron was moderately sorbed to the two sandy loam soils ($K_a$ = 4.0 and 6.7), but was fairly strongly sorbed to the Flanagan silt loam ($K_a$ =18) and the Keyport silt loam ($K_a$ = 12), which have 4.3 and 4.7% organic matter, respectively. The fact that the slopes ($1/n_a$) were not significantly different from 1.0 indicated that sorption was not significantly affected by the concentration of diuron. The correlation coefficients (r) for the soils' percent organic matter versus $K_a$ was 0.98 for diuron. This indicated a very strong relationship between soil organic matter content and $K_a$. The good agreement in diuron $K_{a,OM}$ values on each soil indicated that the organic matter content was the major variable contributing to diuron sorption.

The concentration data from the desorption studies are plotted (Figures 1.1-1.4). Calculated desorption characteristics (i.e., $K_d$, $K_d'$, $K_{d,OM}$ and $1/n_d$) are summarized in Table 1.2. $K_d'$ values (i.e., the numbers in parentheses), calculated from the experimental $K_a$ values, were in excellent agreement with the experimental $K_d$ values, suggesting a strong relationship between $K_d$ and % organic matter.

The primary soil components responsible for pesticide sorption are clay minerals and especially, humic materials of organic matter. As with most pesticides (nonionic and neither acidic nor basic), organic matter appeared to

be the soil constituent most important for pesticide sorption in the studies. The higher the content of organic matter in the soil, the less desorption would occur. The molecular structure of the pesticide and the pH of the soil strongly affected the degree of sorption (Saltzman and Yaron, 1986).

The effect of atrazine treatments on adsorption properties of a brown soil from the Lorraine plain, eastern France, was studied (Berhard-Bitaud et al., 1994). A strong correlation between the soil organic matter (SOM) content with cropping time was emphasized in the absence of weeds, as a result of the specific effect of the herbicide. Not only the amount of atrazine adsorbed, but the adsorption kinetics, were affected by the treatment. Adsorption assays were also carried out on samples from plots with treated and untreated soils, and with controlled SOM content. No effect of the herbicide could be demonstrated after one year, whereas the influence on the SOM quality was questioned.

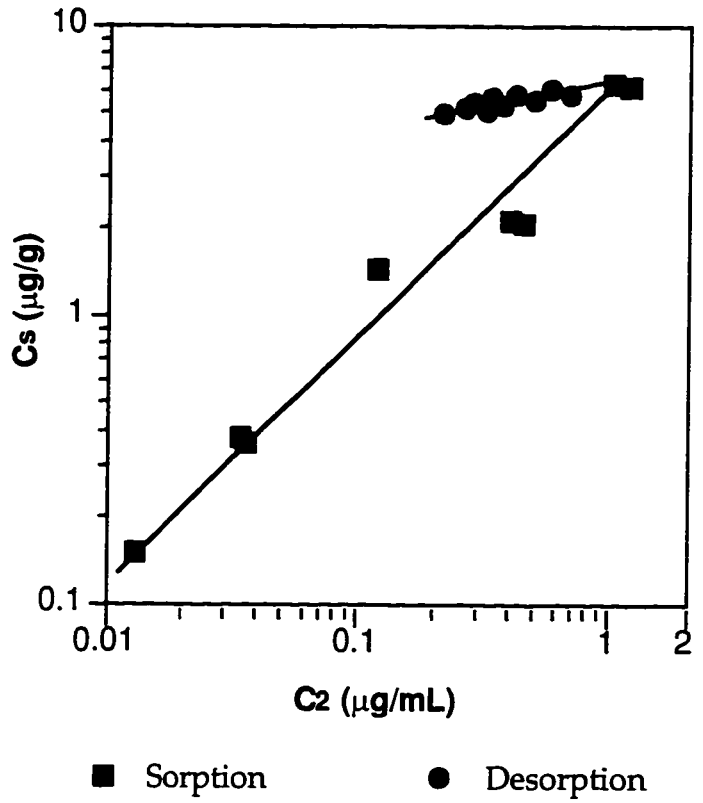



**Figure 1.1 Sorption/desorption isotherm for diuron on Cecil sandy loam soil**

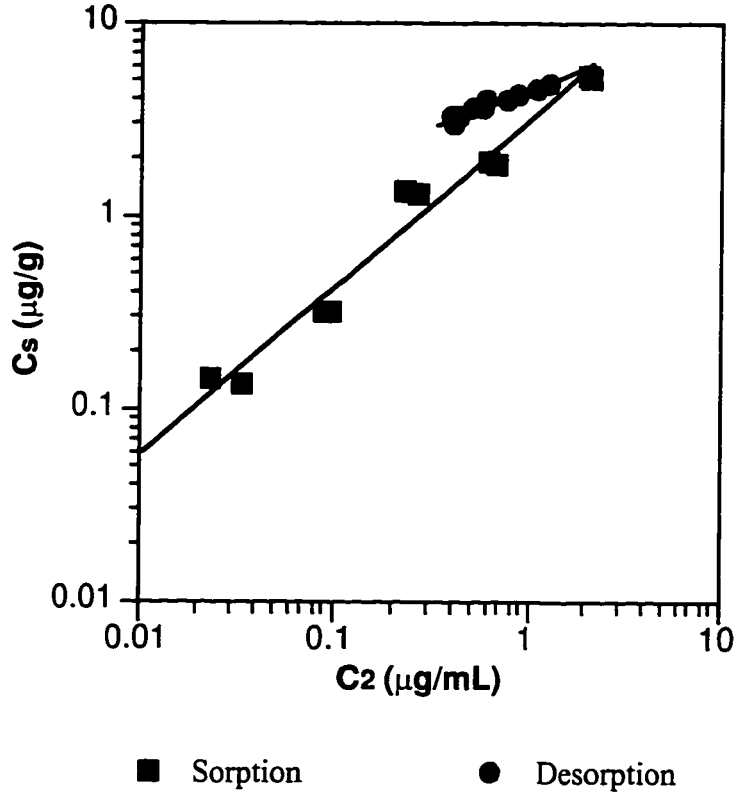



**Figure 1.2 Sorption/desorption isotherm for diuron on Woodstown sandy loam soil**

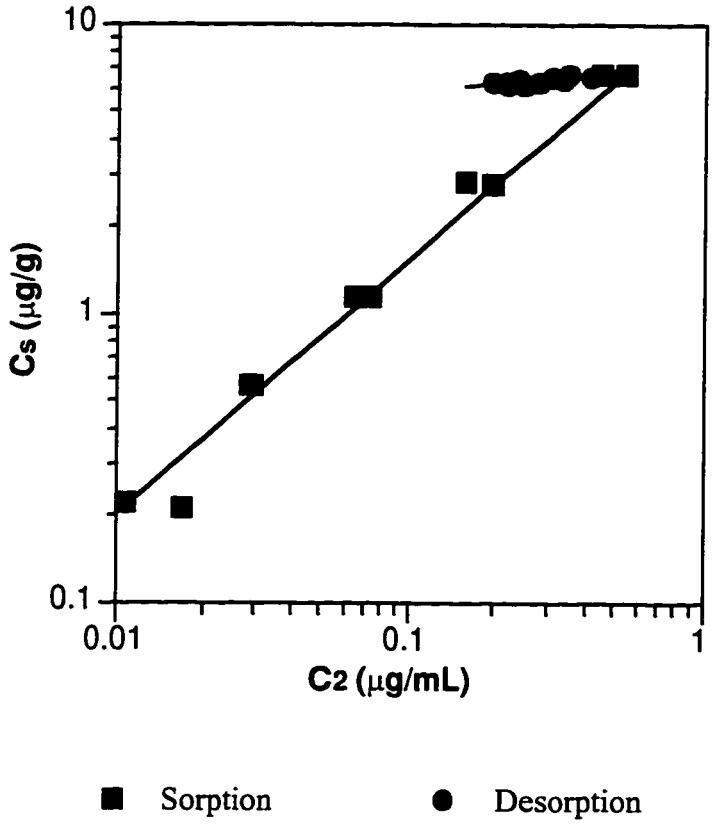



**Figure 1.3 Sorption/desorption isotherm for diuron on Keyport silt loam soil**

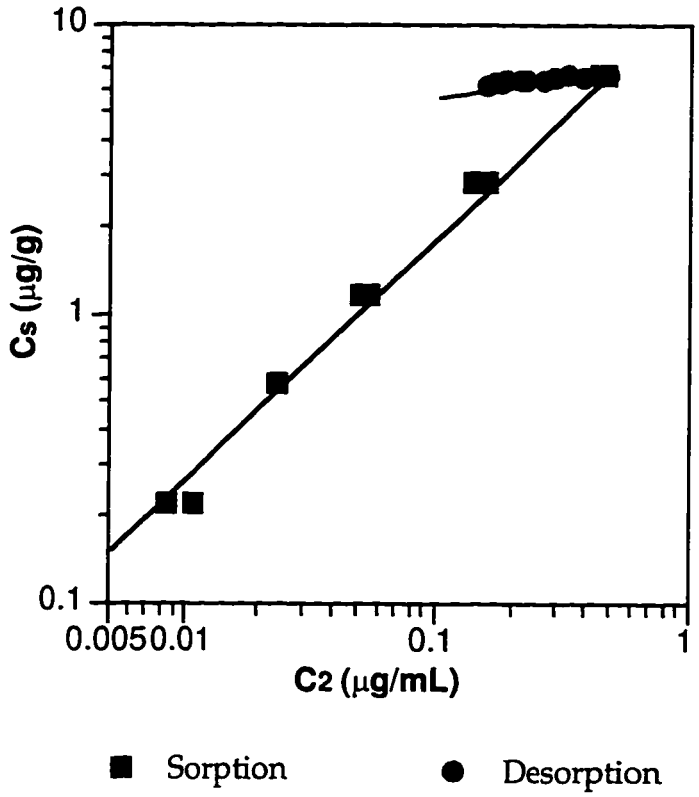



**Figure 1.4 Sorption/desorption isotherm for diuron on Flanagan silt loam soil**

Similarly, the experimentally measured and calculated concentration data from the bensulfuron methyl sorption studies are plotted in Figures 1.5-1.8. Calculated sorption characteristics (i.e., $K_a$, $K_{a,OM}$, and $1/n_a$) for bensulfuron methyl on each soil are summarized in Table 1.2. The correlation coefficient (r) of $K_a$ versus percent organic matter was 0.82. This indicated that only a fair correlation of $K_a$ with percent organic matter for bensulfuron methyl. Bensulfuron methyl was weakly sorbed to the two sandy loam soils ($K_a$ = 1.4 and 2.5), but was strongly sorbed to the Flanagan silt loam ($K_a$ = 14), and the Keyport silt loam ($K_a$ = 12) which have 4.3 and 7.5% organic matter, respectively. The $K_{a,OM}$ values for the Cecil sandy loam (i.e., 120), Woodstown sandy loam (i.e., 130) and Keyport silt loam (i.e., 160) were in reasonably good agreement. The high value (i.e. 230) on Flanagan silt loam and the moderate correlation coefficient (r = 0.8) suggested that other variables (i.e. cation exchange capacity) may also be important in the sorption of bensulfuron methyl.

The fact that the slopes ($1/n_a$) were not significantly different from 1.0 indicated that sorption was not significantly affected by the concentration of bensulfuron methyl over the concentration range studied. The correlation coefficients (r) for the soils' percent organic matter versus $K_a$ was 0.92 for bensulfuron methyl. This indicated a very strong relationship between soil organic matter content and $K_a$. The good agreement in bensulfuron methyl $K_{a,OM}$ values on each soil indicated that the organic matter content also was the major variable contributing to bensulfuron methyl's sorption.

The concentration data from the bensulfuron methyl desorption studies are plotted in Figures 1.5-1.8. Calculated desorption characteristics (i.e., $K_d$, $K_d'$, $K_{d,OM}$ and $1/n_d$) are summarized in Table 1.2. $K_d'$ values (i.e., the numbers in parentheses), calculated from the experimental $K_d$ values, were in excellent agreement with the experimental $K_d$ values. The consistency of the $K_{d,OM}$ values and inverse relationship of $1/n_d$ values with % organic matter ($r = 0.80$) (Figure 1.9, where a line can be established) indicated that the desorption properties of bensulfuron methyl were directly, but not strongly or only, related to the soil organic matter content (possible ion exchange mechanism). The low $1/n_d$ values (i.e., $1/n_d = <0.3$) indicated that bensulfuron methyl was only gradually desorbed from the test soils.

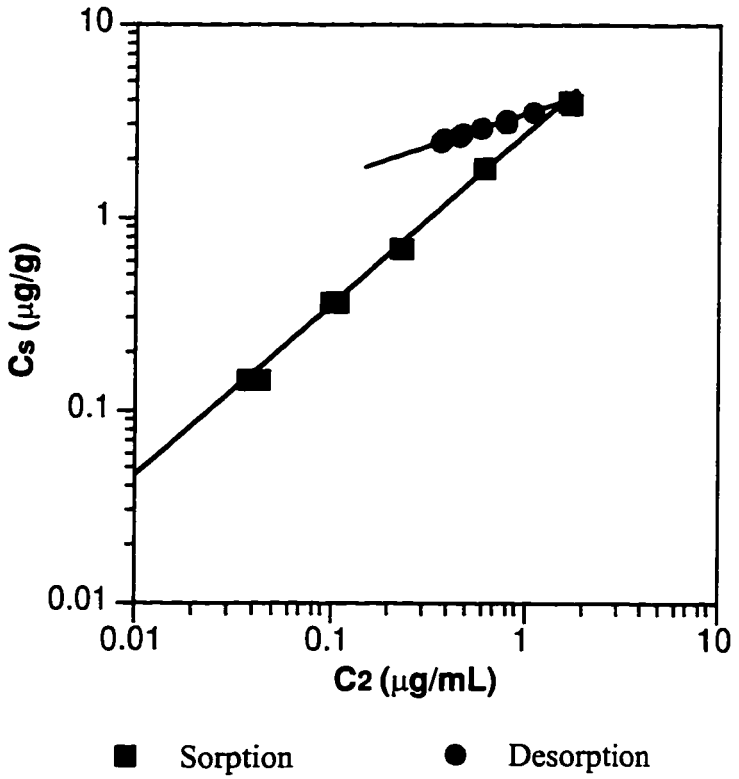



**Figure 1.5 Sorption/desorption isotherm for bensulfuron methyl on Cecil sandy loam soil**

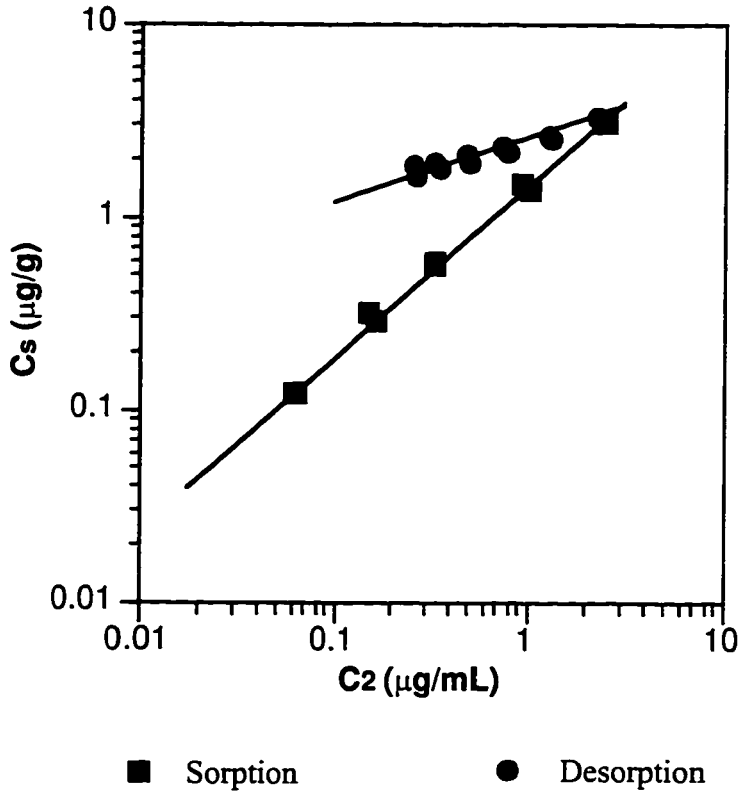



**Figure 1.6 Sorption/desorption isotherm for bensulfuron methyl on Woodstown sandy loam soil**

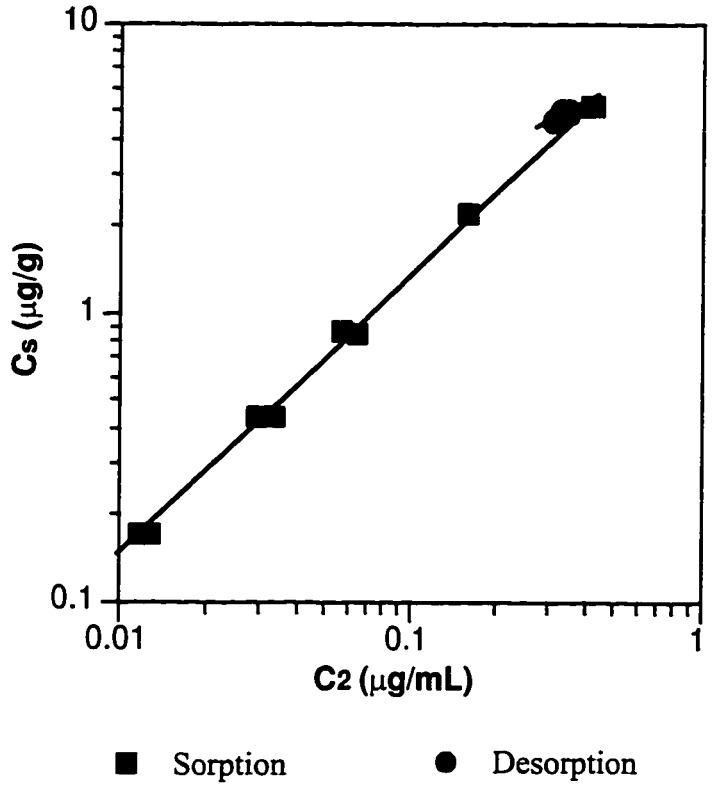



**Figure 1.7 Sorption/desorption isotherm for bensulfuron methyl on Keyport silt loam soil**

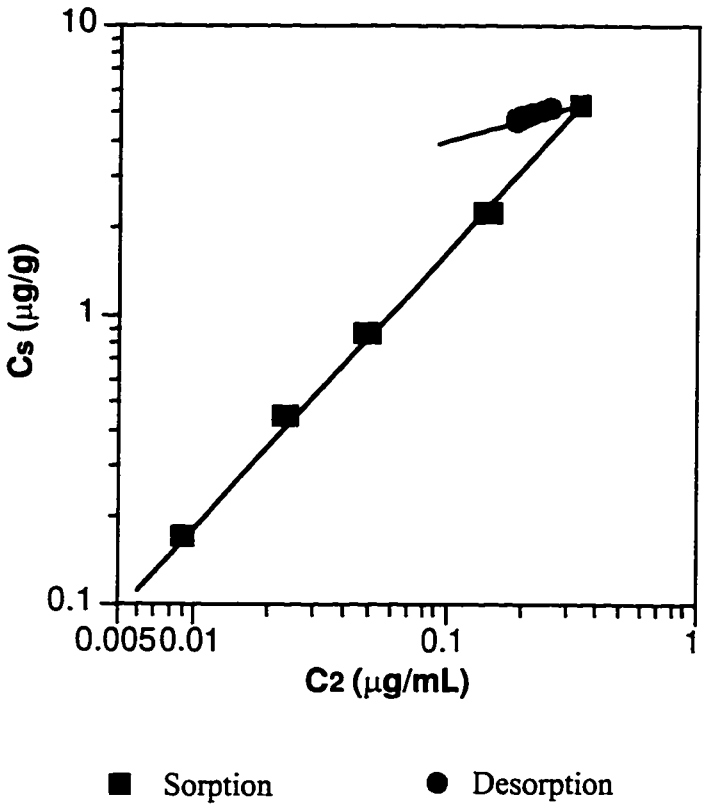
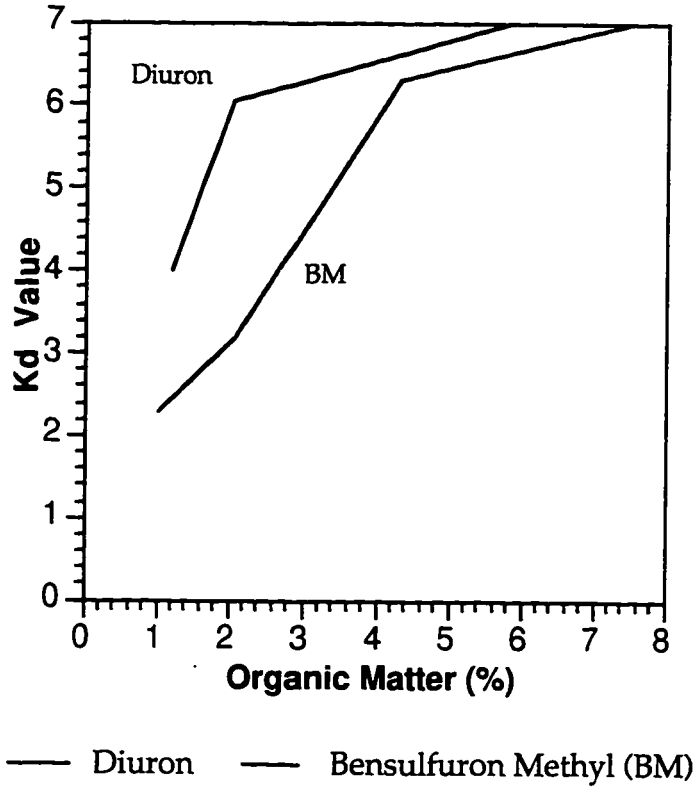



**Figure 1.8 Sorption/desorption isotherm for bensulfuron methyl on Flanagan silt loam soil**




Diuron — Bensulfuron Methyl (BM)

**Figure 1.9 Relationship between $K_d$ values and OM%**

**Table 1.1 Comparison of $K_a$ values (sorption partition coefficients)**

**Cecil sandy loam soil**

| Compound | K observed | K LL predicted* | K predicted | K UL predicted* |
|---|---|---|---|---|
| **Bensulfuron Methyl** | 2.50 | 1.00 | 1.76 | 3.09 |
| **Diuron** | 6.70 | 2.62 | 6.09 | 14.16 |

**Woodstown sandy loam soil**

| Compound | K observed | K LL predicted* | K predicted | K UL predicted* |
|---|---|---|---|---|
| **Bensulfuron Methyl** | 1.40 | 0.45 | 0.83 | 1.54 |
| **Diuron** | 4.00 | 1.38 | 3.36 | 8.16 |

* LL Lower limit predicted; UL Upper limit predicted

The partition coefficients (K values) for both diuron and bensulfuron methyl were evaluated by comparing the experimental results (observed) to predicted values using $R_f$ values (mobility index) obtained from a thin-layer chromatography (TLC) method with a linear regression least-square fit

approach (unpublished research), as given in Table 1.1 above. Both upper and lower limit values were obtained within a 95% confidence interval (CI). These fairly comparable results indicated that these isotherm equilibrium studies were valid and reliable in understanding/interpreting the sorption/desorption behavior in these soils. The pH-dependent sorption and the relationship between pH and the dissociation constant with pH suggested a potential ion exchange mechanism, as observed in the bensulfuron methyl studies.

Sorption/desorption characteristics of diuron and bensulfuron methyl are summarized in Table 1.2. Unfortunately, no data were obtained for bensulfuron methyl desorption from Keyport silt loam soil, because almost no desorption occurred. This was probably due to the high organic matter, high CEC and low pH of this soil.

Sorption of diuron and bensulfuron methyl at extended periods of time were conducted. No significant increase in sorption was observed during 24-72 hours, when an apparent equilibrium was reached. However, more sorption occurred after 20 days. It is likely that if the sorbent particles have a small pore structure, or if the sorbent processes are characterized by a very slow step, a steady state, which could be reached quite quickly, may be mistaken for the true equilibrium. Unless a study is continued for a long enough time the slow sorption may not be observed. Several possible sorption mechanisms have been proposed to ascribe this apparent equilibrium. Hamaker and Thompson (1972) reported that the amount of atrazine absorbed by some soils could be doubled over a period of 60 days.

Multiple site sorption was assumed, which was attributed to a combination of low interaction or affinity sites, and high interaction or affinity sites (Sparks, 1995).

Yuyama et al. (1987) studied soil and water relationship in the behavior of bensulfuron methyl, and concluded that the degree of soil binding was increased by high clay and organic matter contents and low pH. The Freundlich constant of the soil samples, ranging from 2.1 to 112, was highly correlated with their total CEC.

Steinberg et al. (1987) studied the persistence of 1,2-dibromoethane (EDB) in soils and found that low amounts of the organic were released with time, particularly if EDB had not been freshly added to the soil. They suggested that the slow release rate was due to EDB being trapped in the soil micropores where the release rate was influenced by extreme tortuosity and/or steric restrictions. It was estimated, based on a radial diffusion model, that 23 and 31 years would be required for a 50% equilibrium in EDB release to occur from two Connecticut soils. The results implied that desorption for some pesticides after a period of aging time would be very difficult or even impossible to evaluate using conventional approaches for desorption such as batch techniques and other similar methodologies.

Ma and Selim (1994) reported that sorption/desorption isotherms of atrazine on a Sharkey clay soil exhibited a strong hysteric behavior. The extent of observed hysteresis increased with retention/residence time. Attempts were made to describe atrazine retention based on a modified 2nd-

order approach where heterogeneity of adsorption sites was assumed. Two retention sites were considered: type 1 (Se) represented that retained on noncatalytic sites with low binding energy, and type 2 (Sk) was that retained on catalytic sites that result in the formation of strong interactions with matrix surfaces. A 3rd type (Si) represented irreversible sites occupied by hydroxyatrazine following hydrolysis or other physico-chemical transformations. The rates of the reactions were assumed to be a function of vacant or available sites which were equally accessible to either Se or Sk.

**Table 1.2 Sorption/desorption characteristics of diuron and bensulfuron methyl (BM) on selected soils**

**Sorption characteristics**

| Soil type | $K_a$ | | $K_{a,OM}$ | | $1/n_a$ | |
|---|---|---|---|---|---|---|
| | Diuron | BM | Diuron | BM | Diuron | BM |
| Woodstown Sandy Loam | 4.0 | 1.4 | 260 | 130 | 0.85 | 0.88 |
| Cecil Sandy Loam | 6.7 | 2.5 | 240 | 120 | 0.81 | 0.89 |
| Flanagan Silt Loam | 18 | 14 | 330 | 230 | 0.87 | 0.93 |
| Keyport Silt Loam | 12 | 12 | 280 | 160 | 0.92 | 0.97 |

**Desorption characteristics**

| Soil type | $K_d$ ($K_{d'}$) | | $K_{d,OM}$ | | $1/n_d$ | |
|---|---|---|---|---|---|---|
| | Diuron | BM | Diuron | BM | Diuron | BM |
| Woodstown Sandy Loam | 4.2 (4.2) | 2.4 (2.5) | 380 | 220 | 0.51 | 0.26 |
| Cecil Sandy Loam | 6.1 (5.9) | 3.3 (3.2) | 290 | 160 | 0.54 | 0.30 |
| Flanagan Silt Loam | 6.8 (7.0) | 6.4 (6.3) | 160 | 150 | 0.68 | 0.18 |
| Keyport Silt Loam | 7.0 (7.0) | - | 150 | - | 0.63 | - |

### 1.3.2 Kinetic Studies of Bensulfuron Methyl and Atrazine on Selected Soils

Three soils (a, Belhaven with high organic matter content: 56.7%; b, Cullera with high clay content: 57.5%; c, Miaka with high sand content: 91.7%) were used to study sorption/desorption kinetics of bensulfuron methyl. One soil (Alliston soil from Canada) was selected for a similar study of sorption/desorption kinetics for atrazine. Characterization data for these soils are given in Appendix II.

Sorption rates (the slopes of ln A vs. *t* plot, where A is the concentration of a sorbate at a time *t*) of atrazine on the Alliston soil and bensulfuron methyl on the above three soils were not apparently affected by varying the concentration of sorbate, suggesting a possible first order kinetics. The sorption rate of atrazine on the Alliston soil was fairly fast. An increase in temperature resulted in an increase in the sorption rate. The desorption rate was generally lower than the sorption rate at a specific temperature. During atrazine sorption studies, apparent equilibria were reached within 3-6 hours depending upon the temperature used (Figure 1.10). It was much easier to desorb atrazine from the soil than bensulfuron methyl (Figures 1.11-1.12). For instance, approximately 78% (0.78 fraction) of the sorbed atrazine could be desorbed after the sorption process.

Surprisingly, the sorption of bensulfuron methyl to the Belhaven soil (high organic: 57%) was extremely rapid. Equilibrium was reached within 10-25 minutes depending upon the temperature used (Figure 1.13). It was

reported (Khan, 1973) that sorption seemed much slower on humic substances than other components (i.e., clay minerals). Fast sorption on the high organic soil was probably associated with the low pH (pH 4.0), which would protonate the sorbate. Thus it would become more hydrophobic favoring sorption, and rapid partitioning onto the soil particles. Because of the high specific surface areas, almost 98% of bensulfuron methyl was sorbed at an apparent equilibrium. This was also attributed partially to the high cation exchange capacity of this high organic soil, as observed in the equilibrium studies.

Sorption to the Cullera clay soil was rather slow (Figure 1.15), probably due to the high pH of the soil (pH 8.1). At this pH, bensulfuron methyl could be readily deprotonated or ionized resulting in a negatively charged species. In addition, the soil colloid would be negatively charged at pH 8.1. Both negatively charged sorbate and sorbent would repel each other causing much slower sorption than usual. The sorption rate for the Miaka sandy soil was higher than that for the Cullera soil, presumably with a relatively neutral pH (pH 6.2). The $pK_a$ value for bensulfuron methyl is 5.9, which would not cause obvious ionization and protonation at this pH. In addition, the Miaka sandy soil did not contain either high clay (4.4%) or high organic matter (1.0%), or high CEC (3.8 meq/100g), which appeared to be the major determinants for bensulfuron methyl sorption.

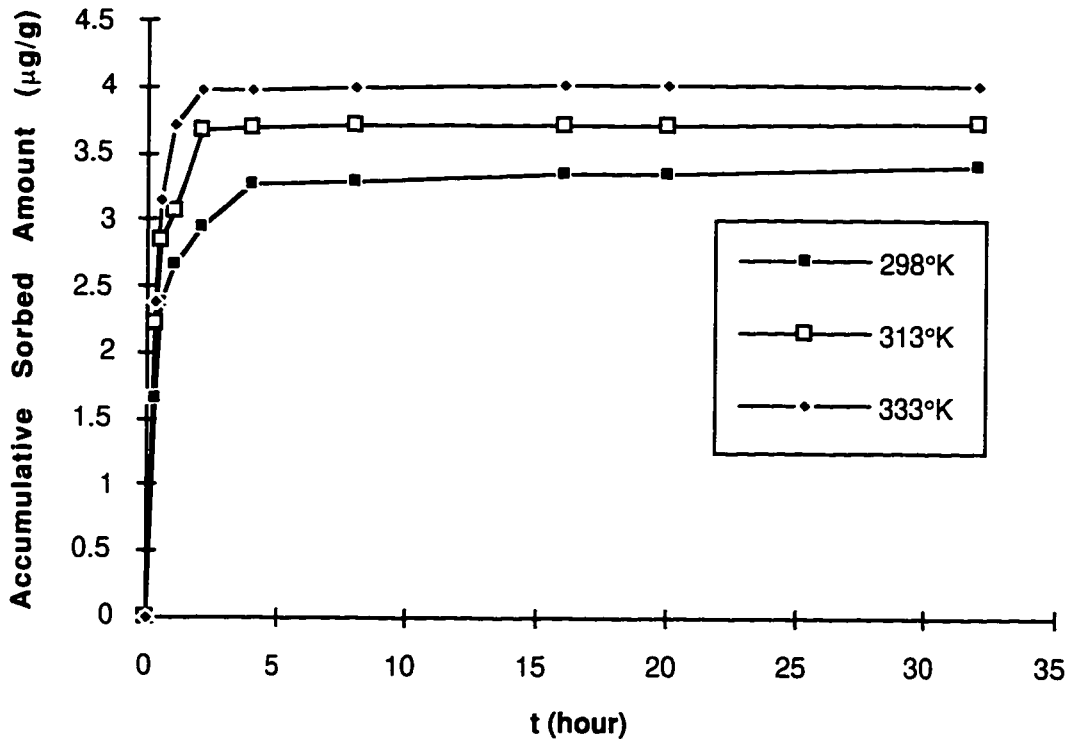



**Figure 1.10 Plot of accumulative sorbed amount vs. time for atrazine sorption on the Alliston soil**

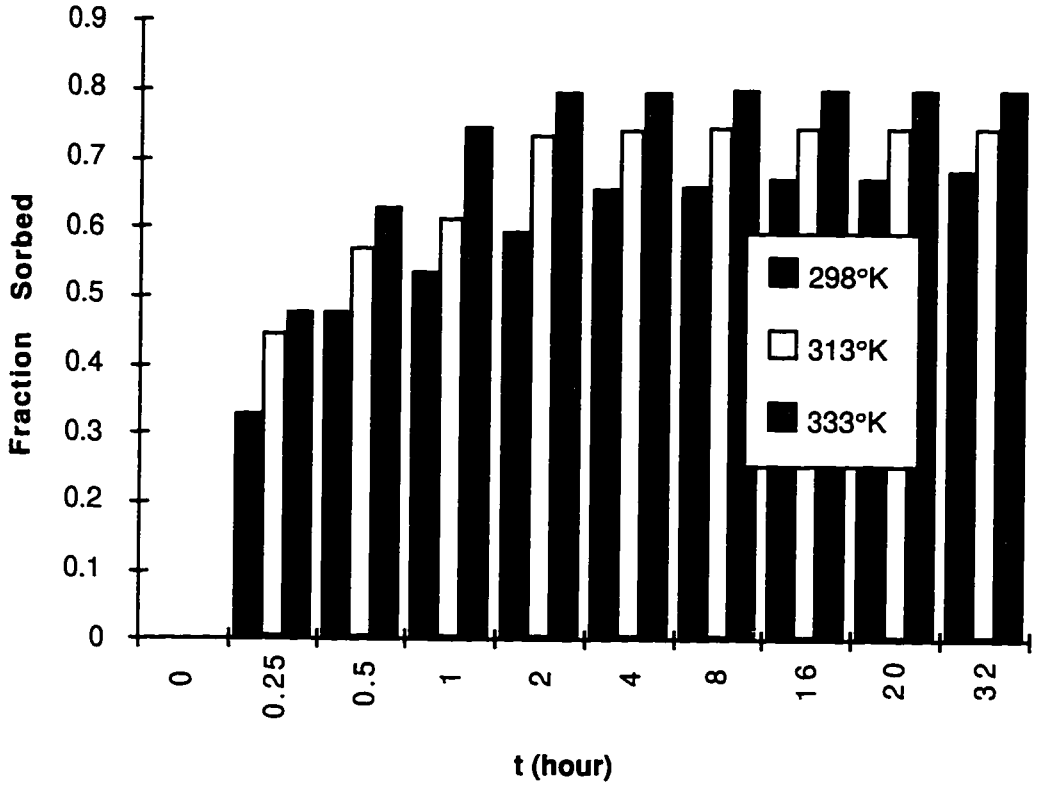



**Figure 1.11 Sorption kinetics of atrazine on Alliston soil at different temperatures**

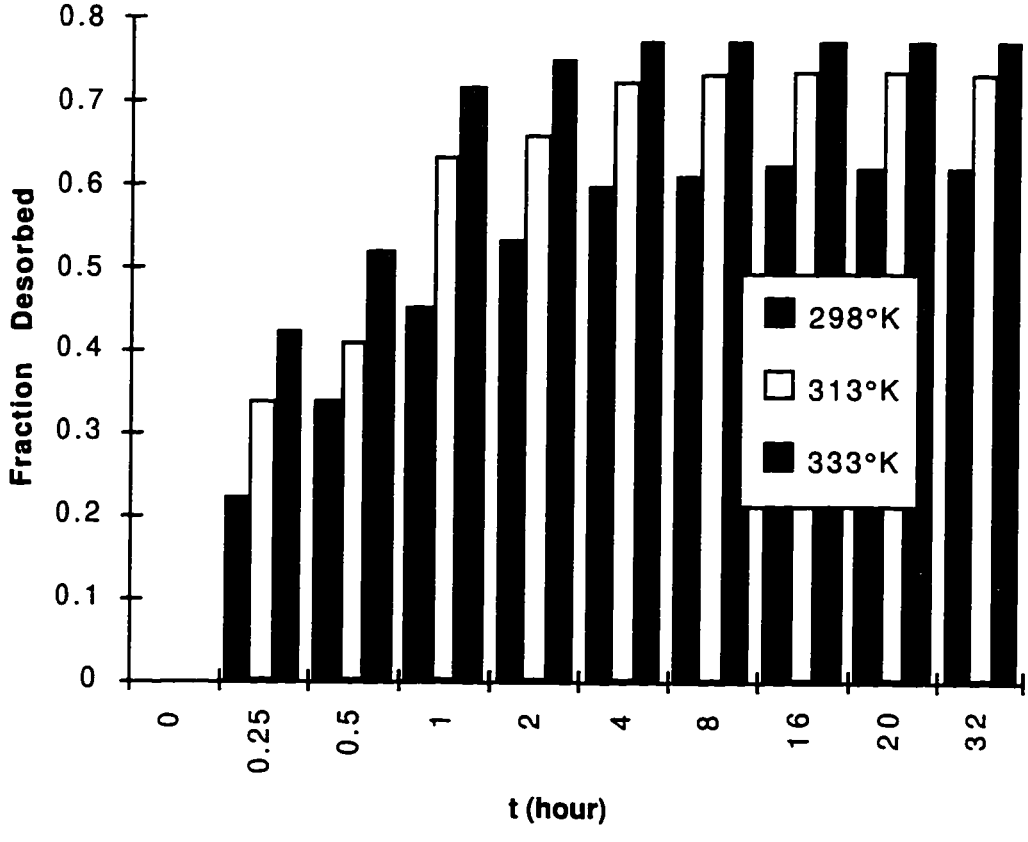



**Figure 1.12 Desorption kinetics of atrazine from Alliston soil at different temperatures**

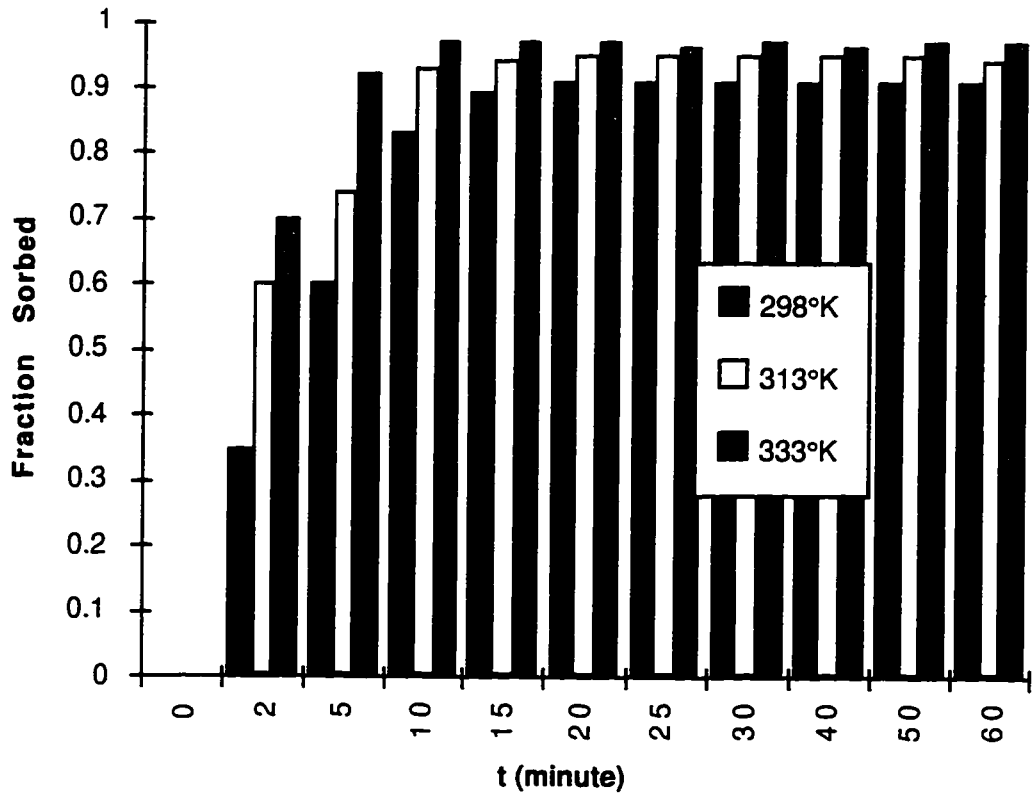



**Figure 1.13 Sorption kinetics of bensulfuron methyl on Belhaven soil at different temperatures**

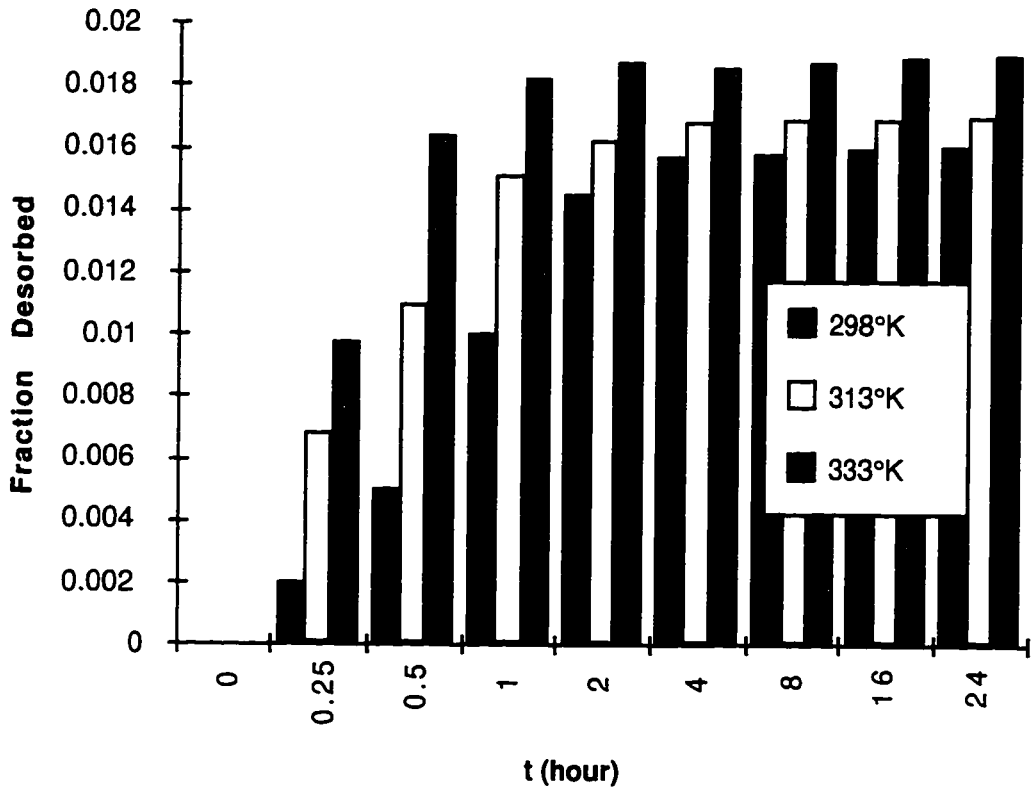



**Figure 1.14 Desorption kinetics of bensulfuron methyl from Belhaven soil at different temperatures**

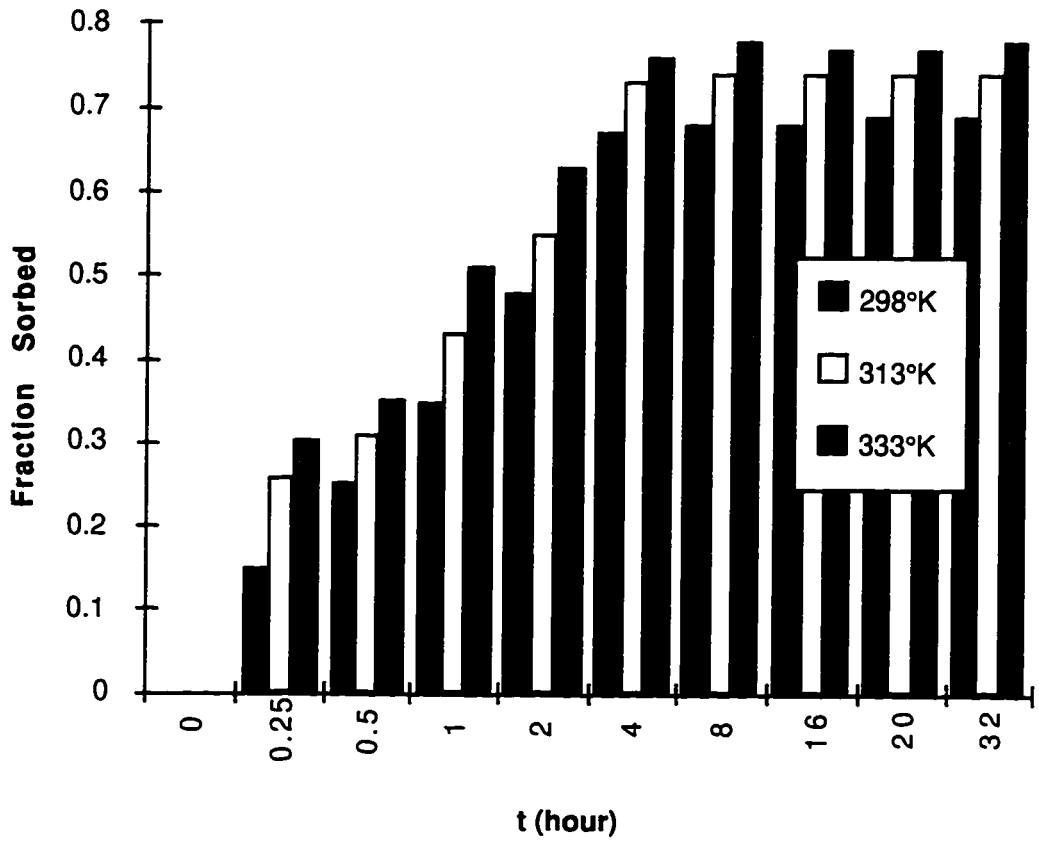



**Figure 1.15 Sorption kinetics of bensulfuron methyl on Cullera soil at different temperatures**

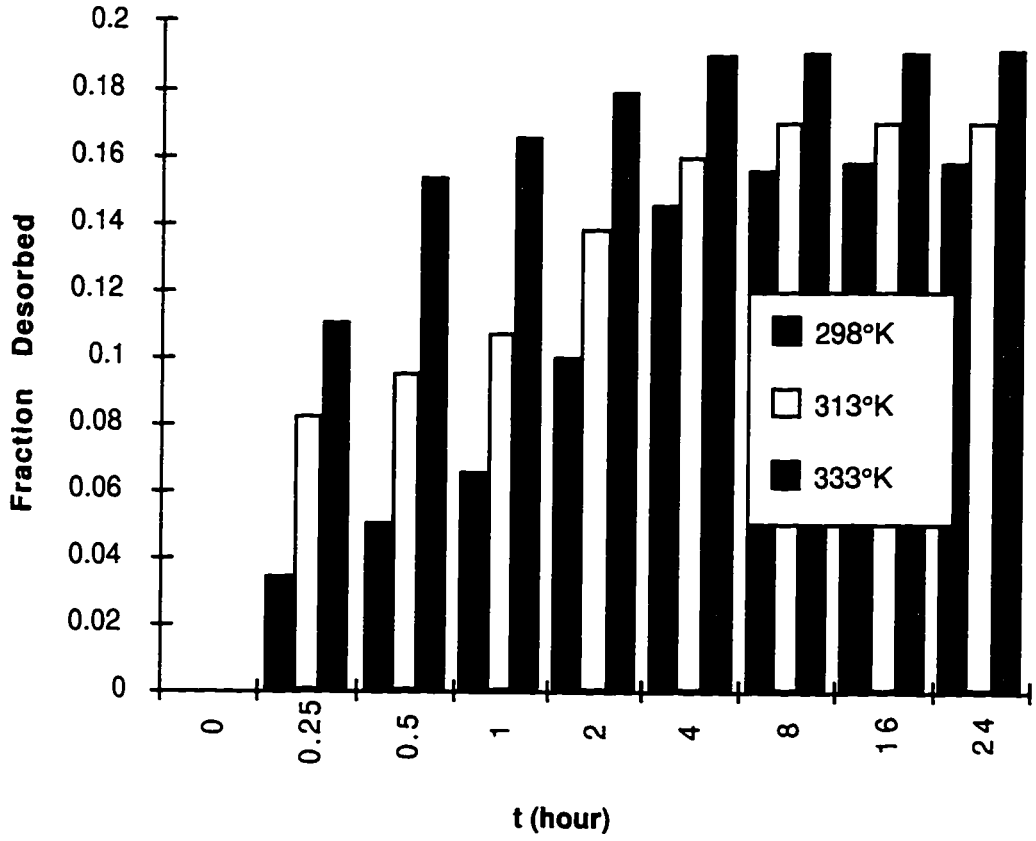



**Figure 1.16 Desorption kinetics of bensulfuron methyl from Cullera soil at different temperatures**

The energy of activation values for sorption were derived from the Arrhenius equation with a plot of ln k vs. 1/T (Figure 1.17). Energy of activation values for sorption were 24.4 kJ/mol, 19.3 kJ/mol, and 13.7 kJ/mol for the Belhaven high organic soil, the Cullera clay soil, and the Miaka sandy soil, respectively. It was expected that the clay soil would have a higher sorption rate than the sandy soil. In this case, the pH of the Spain clay soil was very high resulting in a highly negatively charged surface. In addition, bensulfuron methyl can be protonated or deprotonated by changing pH. At pH above 6, the analyte may be negatively charged, which might cause slower sorption kinetics. Using a similar approach, the energy of activation values for desorption were 38.0 kJ/mol, 28.2 kJ/mol, and 20.8 kJ/mol for the Belhaven high organic soil, the Cullera clay soil, and the Miaka sandy soil, respectively.

The choice of kinetic methodology would affect the determination of magnitude of energy of activation (Ogwada and Sparks, 1986). To minimize differences, a batch technique with a similar shaking speed was used throughout the studies, to measure the rates of sorption and desorption at various temperatures. The energies of activation values for both sorption and desorption were derived from the Arrhenius and van't Hoff equations by plotting ln k vs. 1/T (slope = - E/R) as indicated above. Results are given in Table 1.3. In general, energies of activation values for desorption were higher than those for sorption. The higher values for the energies of activation for the desorption vs. those for the sorption suggested that extra energy was required to overcome the barrier of energy for desorption processes in the

heterogeneous soils. These values also indicated that transport or diffusion control was rate-limiting for both sorption and desorption processes.

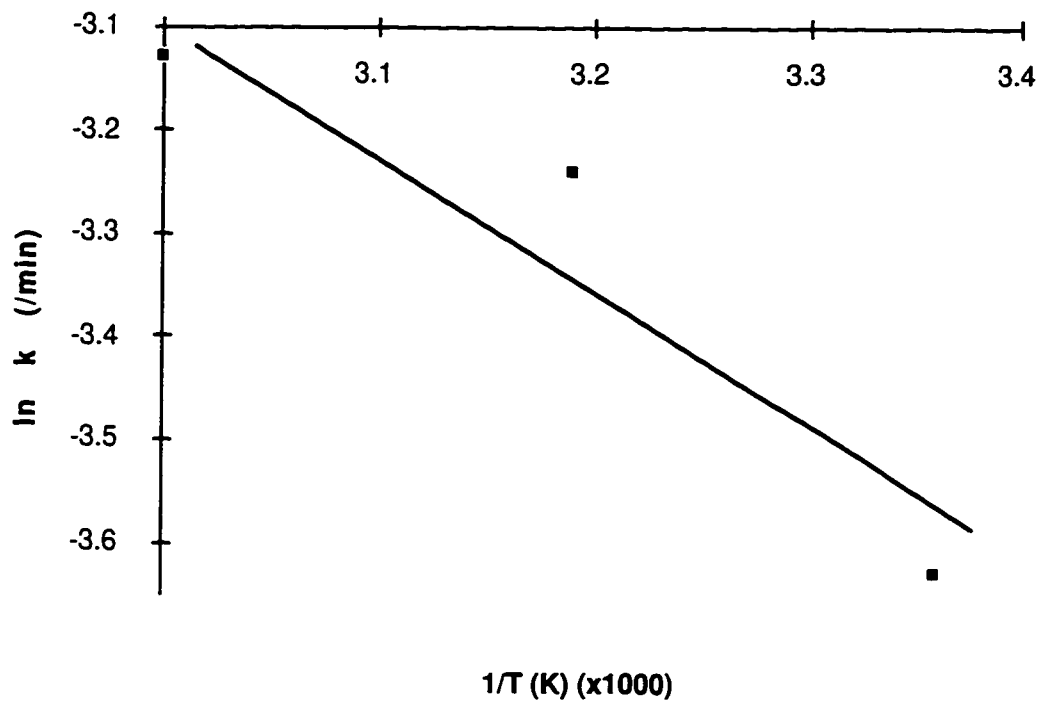



**Figure 1.17** **Plot of ln k vs 1/T for atrazine sorption on the Alliston soil** (slope = - 1.4 and $R^2$ = 0.923)

Hysteresis was observed to various extents depending on the pesticides and soils tested, particularly with the Belhaven high organic soil from North Carolina. The amount sorbed reached 98% within 15 minutes, but only 2% of the sorbed fraction was desorbed after extended desorption times at the same temperature. The energy of activation values for both sorption ($E_a$ = 11-24 kJ/mol) and desorption ($E_d$ = 18-38 kJ/mol) suggested that transport or diffusion control was rate-limiting for both processes. The study also showed that the sorption of bensulfuron methyl was almost irreversible, particularly as organic matter content increased. Organic matter was correlated to the $K_d$ values obtained from the isotherm equilibrium experiments. It was surprising that the sorption kinetics for the Belhaven organic soil was one order of magnitude higher than that for the Cullera clay soil. This may be associated with relatively lower pH of the Belhaven soil. Sorption rate increased up to six fold as the temperature increased from 25°C to 60°C. This was consistent with Bronsted's reaction rate theory, in which reaction rates become higher with increasing temperature.

The rates of desorption were generally lower than those for sorption for bensulfuron methyl, especially in high OM soil. Desorption from this soil was extremely slow compared to the incredibly high sorption rate (0.00013 $min^{-1}$ vs. 0.215 $min^{-1}$ at 298K). This could partially be ascribed to the high surface area of organic matter, which acted as a sponge to sorb or soak up organics rapidly, and the structure of interlayer sites of the clay minerals [montmorillonite and vermiculite (2:1 clay with interlayer spaces that are partially collapsed)] (Sparks and Jardine, 1981). As indicated earlier, the data

showed that desorption of bensulfuron methyl from the Belhaven soil was almost nonexistent. The poorly accessible exchange sites of the organic matter might also play a role in the slower desorption kinetics (Figure 1.13-1.14).

Hysteresis was also observed in the desorption study of bensulfuron methyl from the Cullera clay soil (Figure 1.15-1.16). As indicated in the soil characterization data (Appendix II), this soil primarily contains vermiculite and montmorillonite, which would make desorption more difficult. The high CEC of this soil might also attribute to the obvious hysteresis in this case.

Sorption at initial times may have been diffusion of the herbicide molecules to the surface of the soil particles. However, at longer times the sorption rate became slow which could be due to the intraparticle diffusion of the molecules into the interior of the soil particulates, as observed with atrazine sorption onto the Alliston soil (Figure 1.10). The relatively fast rate of sorption, low activation energy values, and heat of activation suggested a physisorption, possibly involving van der Waals forces and hydrophobic bonding between the herbicide molecules and soil surface in an aqueous system.

Fast and slow labile sorptions have also been observed for atrazine along with a reversible but kinetically slow sorption/desorption process (Gilchrist et al., 1993), which is consistent with diffusion of pesticide into the interior of the clay particles. Labile sorption capacity, mole fraction site coverage, labile sorption equilibrium function, and distribution coefficient were determined for the clay minerals in aqueous slurries with atrazine.

The rate of sorption and desorption of pesticides on soils and soil constituents is dependent on the type of sorbent, pesticide, and rate of mixing. Other factors that may affect the kinetics are swelling of the sorbent and temperature (Hance, 1967). The sorption of pesticides by soils and soil constituents such as clay minerals and humic substances has generally been characterized by an initial rapid rate followed by a much slower approach to an apparent equilibrium (Haque et al., 1968; Khan, 1973; McCall and Agin, 1985).

**Table 1.3 Sorption/desorption rate and energy of activation of bensulfuron methyl and atrazine on selected soils[a]**

| Rate Coefficient $min^{-1}$ (K) | Bensulfuron Methyl | | | Atrazine |
|---|---|---|---|---|
| | Miaka | Cullera | Belhaven | Alliston |
| $k_a/k_d$ (298K)[b] | 0.0197/0.0040 | 0.0108/0.0023 | 0.215/0.00013 | 0.0266/0.0168 |
| $k_a/k_d$ (313K) | 0.0306/0.0057 | 0.020/0.0057 | 0.458/0.00045 | 0.0391/0.0277 |
| $k_a/k_d$ (333K) | 0.0357/0.010 | 0.0241/0.0078 | 0.602/0.00066 | 0.0433/0.0365 |
| $E_a/E_d$ kJ/mol[c] | 13.7/20.8 | 19.3/28.2 | 24.4/38.0 | 11.6/18.1 |
| $\Delta H°$ kJ/mol[d] | - 7.1 | - 8.9 | - 13.6 | - 6.5 |

a Soil physiochemical data are in Appendix II.

b $k_a$ sorption rate; $k_d$ desorption rate for nonaged samples (calculation was based on first-order kinetic equation: $\ln A/A_0 = kt$, where A is the concentration at $t$ time, and $A_0$ is the original concentration)

c $E_a$ energy of activation for sorption; $E_d$ energy of activation for desorption for nonaged samples (derived from Arrhenius equation with a plot of ln k vs. 1/T °K: slope = - E/R, where R is Gas constant)

d $\Delta H°$ standard energy of heat or enthalpy ($\Delta H = E_a - E_d$)

The initial reaction(s) have been associated with diffusion of the pesticides to and from the surface of the sorbent, while the slower reaction(s) have been related to particle diffusion of the pesticides into and out of micropores of the sorbent. A similar example of this kind of relationship was shown in the work of Leenheer and Ahlrichs (1971) for sorption of carbaryl and parathion on soil humic materials. An apparent equilibrium was reached in two hours or less, and the amount of pesticide sorbed at equilibrium increased as temperature increased.

Desorption became more difficult when the residence time increased, as observed in the desorption of atrazine and bensulfuron methyl from Alliston sandy loam and Cullera clay soils, respectively, after having been aged for four weeks. Desorption rates were much slower for both aged atrazine and bensulfuron methyl in the soil up to four weeks, than those without aging. In both cases, desorption rates decreased and hysteresis became more apparent (Figure 1.18-1.19), compared to Figures 1.12 and 1.16. More hysteresis could occur with an increase in residence time of sorbed pesticides in soils. For example, approximately 78% of atrazine could be desorbed without aging, compared to only 26% of atrazine desorbed from the aged samples. In addition, energy of activation values for the desorption from aged samples were higher (Table 1.4) than those without aging (Table 1.3).

It was apparent that desorption rates decreased as the residence time increased (Table 1.4). However, the energy of activation values for the desorption increased from 28.2 to 45.4 kJ/mol for bensulfuron methyl, and 18.1 to 30.3 kJ/mol for atrazine, respectively. This suggested that more energy

was necessary to overcome the energy barrier for the desorption process than that for desorption in the nonaged samples.

Attempts to desorb compounds such as bensulfuron methyl from the Cullera clay soil and atrazine from the Canadian Alliston sandy loam soil were almost unsuccessful (showing a much slower rate) after six months of aging. This could be due to the sorbed analytes being relocated and trapped in the soil micropores where the release rate would be influenced by extreme tortuosity and/or steric restrictions.

A study was reported by Pignatello et al. (1993). A field soil long-contaminated with atrazine and metolachlor was eluted under saturated flow and compared to the elution of herbicides freshly injected onto an identical column. The mobility of the injected herbicides was far greater that the native. A two-compartment diffusion sorption model-having a fast compartment S1 in rapid exchange with water and a slow compartment S2 with exchange by radial diffusion kinetics gave good simultaneous fits to native and injected elution curves and predicted flow rate effects and postleaching soil herbicide profiles. The absence of particle-size effects on desorption rates suggests that the diffusive medium of S2 is microparticles distributed among all particle-size fractions.

Sorption/desorption equilibria and kinetics of selected pesticides (atrazine, diruon, bensulfuron methyl) demonstrated an initial rapid rate followed by a much slower approach to an apparent equilibrium. The rate of sorption/desorption was dependent on the type of sorbent (different soils),

pesticide, and temperature. As the residence time of sorbed pesticide in the soils increased, less desorption occurred. The preliminary sorption/ desorption data can assist in macroscopically understanding and interpreting potential sorption/desorption mechanisms and/or binding strengths. This could be especially useful in learning about "bound residues".

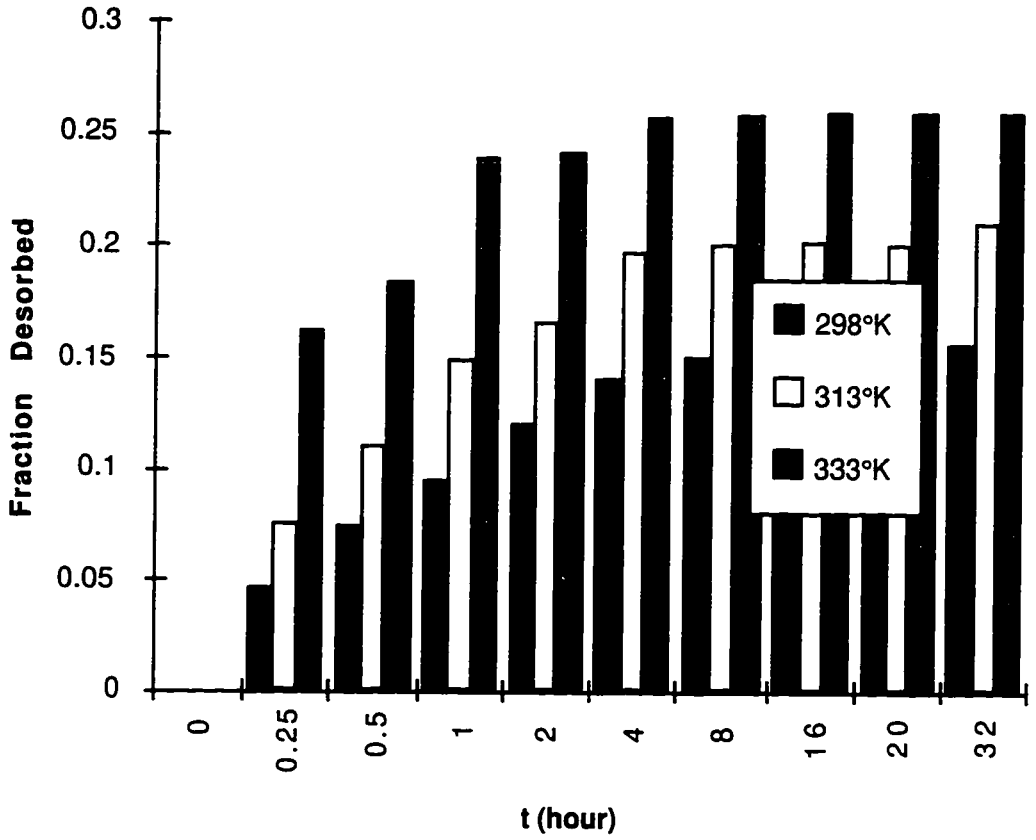



**Figure 1.18 Desorption kinetics of atrazine from Alliston Soil at different temperatures after aging for four weeks**

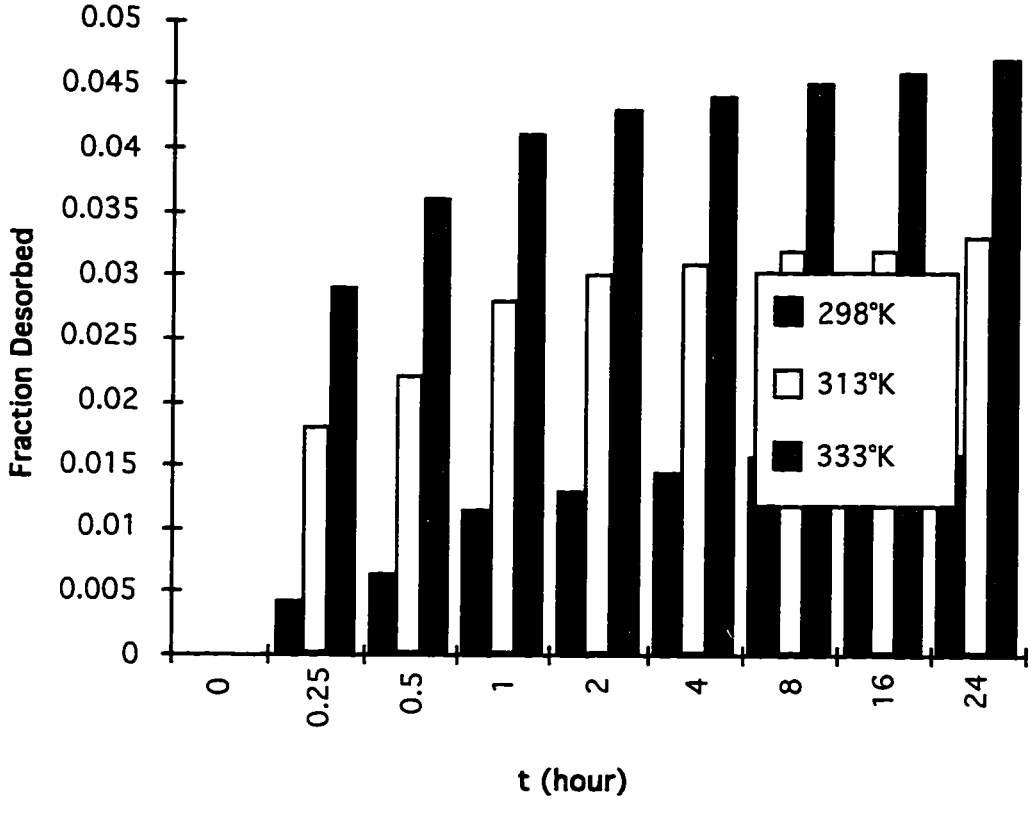



**Figure 1.19 Desorption kinetics of bensulfuron methyl from Cullera soil at different temperatures after aging for four weeks**

**Table 1.4 Desorption rate and energy of activation of bensulfuron methyl and atrazine on selected soils after having been aged for four weeks**

| (°K) | Bensulfuron Methyl | | Atrazine | |
|---|---|---|---|---|
| | Cullera $k_d$ ($min^{-1}$) | Aged Cullera $k_{d'}$ ($min^{-1}$) | Alliston $k_d$ ($min^{-1}$) | Aged Alliston $k_{d'}$ ($min^{-1}$) |
| 298 °K | 0.0023 | 0.00028 | 0.0168 | 0.0032 |
| 313 °K | 0.0057 | 0.0012 | 0.0277 | 0.0052 |
| 333 °K | 0.0078 | 0.00196 | 0.0365 | 0.012 |
| | $E_d$ (kJ/mol) | $E_{d'}$ (kJ/mol) | $E_d$ (kJ/mol) | $E_{d'}$ (kJ/mol) |
| | 28.2 | 45.4 | 18.1 | 30.3 |

$k_{d'}$ and $E_{d'}$ values for aged samples.

### 1.3.3 Identification and Semiquantification of Potential Degradation Compounds from the Sorption/Desorption Studies

Bensulfuron methyl and metabolites/degradates were detected and quantified with a Berthold LB2832 automatic-TLC analyzer. Bensulfuron methyl (DPX-F5384), methyl 2-(aminosulfonylmethyl)benzoate (sulfonamide), homosaccharin, ODM-DPX-F5384 and FA-DPX-F5384 were identified by coelution of authentic reference standards. These analyses were performed on the aqueous extracts both before sorption and after the last desorption phase of the experiments. An additional extraction was done on water samples which had been incubated for the same period of time as the desorption phase aqueous extracts but without soil.

Identification and semiquantification of the aqueous extracts from the final desorption equilibration were summarized in Table 1.5. Bensulfuron methyl degraded to sulfonamide, and unidentified polar compounds (probably including pyrimidine-amine) on the sandy loams. On the Keyport silt-loam, bensulfuron methyl degraded to the aforementioned compounds and 2-[[[[[(4,6-dimethoxy-2-pyrimidinyl)amino]carbonyl]amino]sulfonyl] methyl] benzoic acid (FA-DPX-F5384), ODM-DPX-F5384 and homosaccharin. While DPX-F5384 degraded to a large extent over Keyport silt loam (73 to 92%, respectively), it did not appear to degrade any more rapidly over Cecil and Woodstown sandy loams than in water that was incubated without soil (23, 21 and 28%, respectively). The percent of bensulfuron methyl extracted from the aqueous phase of the last desorption day was inversely proportional to the organics found in the soil from which it was desorbed, and was likely to

reflect the relative number of active microorganisms in that soil sample. The effect of this degradation on $K_a$ is that the apparent $K_a$ would be lower on soils where bensulfuron methyl decomposed more rapidly providing that the radiolabeled degradates were less readily sorbed onto the soil.

**Table 1.5 Degradation of bensulfuron methyl on selected soils**

| | % of Recovered Radioactivity in Aqueous Phase[a] | | | | | |
|---|---|---|---|---|---|---|
| **Compound** | **Day 0** | **Day 5** | **Cecil** | **Woodstown** | **Flanagan** | **Keyport** |
| DPX-F5384 | 87 | 72 | 77 | 79 | 27 | 8 |
| Sulfonamide | 6 | 18 | 13 | 10 | 58 | 44 |
| Homosaccharin | ND[c] | ND | ND | ND | ND | 2 |
| FA-DPX-F5384 | ND | ND | ND | ND | ND | 11 |
| ODM-DPX-F5384 | ND | ND | ND | ND | ND | 6 |
| Polar[b] | 5 | 7 | 7 | 6 | 12 | 26 |

[a] After the final desorption from respective soils: Day 0 is the initial values from aqueous phase, and Day 5 is after 5 days incubation without soil.

[b] Radioactivity not extracted from the acidified aqueous phase with methylene chloride.

[c] Not detected under the conditions described.

There were no degradation from both diuron and atrazine sorption/desorption studies. Representative LC chromatograms and MS spectra are shown in Figures A.1-A.6 (Appendix IV) for bensulfuron methyl, diuron, atrazine and their potential degradation compounds, respectively. Baseline separation for these compounds was achieved by a gradient LC mobile phase buffered with 5 mM $NaH_2PO_4$ at pH 3.25. The degradation compounds were identified initially by their retention times, compared to the standards under identical LC conditions, and then confirmed by LC/MS where applicable.

Bensulfuron methyl was degraded fairly rapidly during sorption and desorption studies. Major degradation compounds (sulfonamide, homosaccharin, FA-DPX-F5384, and polar degradates such as pyrimidine-amine) were identified by the LC-UV-DAD and confirmed by LC/MS where applicable. Both diuron and atrazine were very stable under laboratory conditions such as sorption/desorption and aging studies (Figures A.2 and A.6).

As known from earlier studies, bensulfuron methyl degraded fairly rapidly during the sorption/desorption studies. These observations explained why these degradation products were found in the aged samples (Chapter 3, **section 3.3.5**).

# Chapter 2

# EXPERIMENTAL DESIGN FOR MULTIVARIATE OPTIMIZATION FOR SUPERCRITICAL FLUID EXTRACTION

## 2.1 Introduction

### 2.1.1 Experimental Design

An experimental design approach is a set of tools that systematically applies statistical methods to the experimental process. It enables researchers, scientists or engineers to vary a number of variables according to a plan or design in order to evaluate their effects. It was invented in the early 1920s in Britain by Sir R. A. Fisher, a geneticist and member of the Royal Society, as an alternative to handling massive amounts of data, which were largely inadequate to answer posed questions. Since then, new designs have continued to expand the application and power of the discipline. The process of developing new designs continues today.

It is often wise for an experimenter to use a strategic approach to experimentation that involves designing second (or even a third) experiment based on results from the first designed study. Three types of experimental situations, coupled with three types of experimental designs are: 1, Examine a large number of control variables (perhaps 30 or so) operating in a process to determine which one or ones have an effect on a particular response.

Screening designs are used for this purpose; 2, Understand how variables interact with each other to jointly influence responses. Experimental design is based on the assumption that variables, e.g., temperature, pressure, and time, often interact (i.e., operate in synergistic or antagonistic ways). Interaction designs identify such relationships; 3, Understand how the collection of interconnected variables influences responses over a wide range of values even though not all of those values have been directly tested. Response surface designs are used on this level to model how variables behave. In other words, the screening designs are to identify important variables for future study. The interaction designs are to understand effects of important variables and their interactions. The response surface designs are to develop a prediction model for a process and optimization. Armed with this understanding, the experimenter can develop a process which will meet objectives (i.e., optimization) for performance and/or quality.

### 2.1.2 Design Model

It often happens that when one begins a project, there are more variables than can be easily investigated. A successful strategy is to concentrate on the first-order effects and argue that a variable without a substantial first-order effect is unlikely to have large second order or interaction effects. Given that one desires to estimate the first-order effects, and to ignore higher order effects, the questions are how many trials to take and what design to use. In general, there are two collections of designs for screening: the Plackett-Burman (1946) and the Morris-Mitchell (1983) linear designs. Several design options are available for choosing a response surface:

interaction, quadratic, central composite in cube, partial cubic, central composite in sphere, etc. (Wheeler, 1994).

### 2.1.3 Principle of Multivariate Optimization Scheme (MOS)

The multivariate optimization scheme (MOS) mainly involves experimental design with testing variables, models, etc. Basically, there are two experimental directions: screening trials and response surface trials. Screening can be thought of as sifting dirt through a mesh in search of lost jewels. Only items bigger than the mesh spacing are of interest to the analyst. It will shorten a large list of variables at the early stages of experimentation. At this time one usually wants to just identify the important variables for further study or to get the combination of variables that works reasonably well. Response surfaces or contour plots are the goal if one wants to characterize, optimize, and/or predict one's results.

For the same number of variables one needs to run more trials in order to obtain a response surface than to just screen out which variables are important. There are many types of variables. The two major distinctions are between the *response variables* and the *control variables*. The response variables or responses are the ones that one observes and in which one measures changes in output from a process. The control variables or controls are the varied inputs or parameters which affect the responses.

In general, the traditional approach to experimentation is exploring one variable at a time. This involves holding all variables constant except one, which is varied across a range. This approach can be very costly, time

consuming, and inadequate to uncover information about interactions among variables, i.e., how variables work together in synergistic or antagonistic ways.

Three experimental strategies are widely used in experimental designs. First, screening designs are employed to look at a large number of control variables to determine which one or ones have an effect on a particular response, or to identify important variables for future studies. Second, interaction designs are usually conducted to understand how variables interact with each other to jointly influence responses. Third, response surface designs are used to develop a prediction model for understanding variable interactions and optimization for a process and performance.

### 2.1.4 Applications of MOS for SFE

Selection of optimum conditions for SFE is extremely complex. The most common approaches for SFE optimization conditions are usually based on simple univariate sequential optimization procedures. However, such one-parameter optimization schemes would not be effective in locating the true optimum parameter zone in some cases, and therefore, a multivariate, simultaneous optimization approach is preferable.

Systematic optimization schemes for SFE have rarely been investigated. Optimization of supercritical fluid chromatography (SFC) has been studied (Crow and Foley, 1990) using a sequential simplex method and (Li et al., 1990; Ong et al., 1991) using the overlapping resolution mapping scheme. Recently, a two-level, two-factor ($2^2$) factorial design approach was used to optimize the

temperature and pressure for the SFE of amine hydrochloride (Bicking, 1992). With this approach, however, only two variables were optimized simultaneously.

Optimization of the parameters in SFE was studied by Reindl and Hofler (1994). SFE was investigated as a sample cleanup and concentration method for the determination of polynuclear aromatic hydrocarbons (PAHs) in soil samples by HPLC. It was found that methanol contents of 5% or 10% toluene as cosolvents were necessary to achieve equivalent extraction yields for SFE compared to Soxhlet extraction. A single variable approach was used for optimization in the study. For real world soil samples, drying agents were used to trap the contained water of the soil sample and elemental copper granules were used to trap organosulfur compounds. Similar extraction yields were achieved for samples when the methanol content in carbon dioxide was increased to 8% (mol).

A systematic evaluation of the parameters affecting the supercritical fluid extraction (SFE) of the following primary aromatic amines was performed: 1,4-phenylenediamine, 2,4-diaminotoluene, benzidine, 4,4'-methylenebiis(2-chloroaniline), 3,3'-dimethylbenzidine, and 3,3'-dichlorobenzidine. Nitrous oxide was utilized as the supercritical fluid, along with six different chemical modifiers. The effect of modifier concentration was examined, as were the effects of pressure, extraction temperature, time, and volume. 1,6-hexanediamine in methanol was demonstrated to be an effective SFE modifier. The extraction was optimized with a modifier concentration of 5% in methanol, a pressure of 350 atm, three extraction

volumes, and two modifier addition cycles. Again, a single variable approach was used for optimization in the study.

Hitchen et al. (1993) used an experimental design with mutilinear regression to examine the relative contribution of the main experimental variables during SFE. Six steroidal compounds of various solubilities in supercritical $CO_2$ were considered. The results indicated that the density of the supercritical fluids had the greatest effect on the solubilization and transfer of steroid from extraction cell to collection device. The minimum number of cell volumes of supercritical $CO_2$ required for effective extraction was experimentally determined.

Most useful two-level multivariable factorial designs can be generated from Hadamard matrices, which were discovered by Jacques Hadamard (Diamond, 1981), a French mathematician. Use of these matrices for experiment design was first described by Plackett and Burman, who limited their use to saturated designs. This author, however, has shown the applicability of these matrices to most two-level experiment designs.

Bicking (1992) studied a two-level, two-factor ($2^2$) factorial design approach, which was used to optimize SFE conditions for the determination of an amine hydrochloride in avian feed. The recovery was maximized by optimizing the temperature and pressure. Optimum conditions also provided a lower level of nonvolatile extractables. The optimized method demonstrated excellent recovery at levels as low as 5 μg/g. In another study, a central composite experimental design strategy was used to optimize

temperature and pressure conditions for SFE of hydrocarbons from solid samples (Bicking et al., 1993). Linear regression of the resulting data allowed for the construction of a percent recovery plot that could be applied over a wide range of extraction conditions. The data also provided some insights into the effect of operating parameters on the SFE experiment. Soil samples were then analyzed for both oil and grease and total petroleum hydrocarbon (TPH) content by SFE. The results were compared with standard procedures using chlorofluorocarbon (Freon®, DuPont) solvent.

An experimental design optimization method was demonstrated for evaluation of the resolution of the performance mixture used in the proposed ASTM test to determine the aromatic content of aviation fuels (Fraile and Sanchez, 1993). The method used a Doehlert experimental matrix test to optimize the resolution and analysis time by varying the pressure and temperature of the supercritical carbon dioxide mobile phase. The separation between saturated and aromatic compounds was optimized using only seven experiments. With this procedure, the analysis time required for determination of the total aromatic content of more complex samples was reduced to less than 10 minutes. This systematic experimental design approach enables the reliable establishment of separation conditions suitable for the ASTM test in minimum time and with minimum effort.

An interpretive method for predicting optimum conditions for SFE was developed by Li et al. (1994). The proposed scheme was used for the optimization of temperature, initial pressure, and pressure gradient for the extraction of vitamin E. In the study, eleven experiments were conducted to

obtain global optimum conditions. The validity of the scheme was confirmed by comparing the predicted extraction efficiencies with the experimental values. The application of the predicted optimal SFE conditions for the extraction of vitamin E in corn oil was demonstrated.

### 2.1.5 Supercritical Fluid Extraction (SFE) as an Emerging Technique

A material becomes a supercritical fluid (SF) when it is maintained at temperatures and pressures above the critical point. It is defined by a critical temperature and a critical pressure above which the substance is neither a gas nor a liquid, but possesses properties of both. At temperatures and pressures above the critical point the substance cannot be liquified regardless of the pressure exerted on it, and it is called a supercritical fluid. Supercritical fluids exhibit several properties that make them desirable as extraction solvents. The solvent strength of pure supercritical fluids is generally directly related to the density of the liquid. Since the density of the fluid is a function of its pressure and temperature, precise control of the pressure and temperature can be used to obtain a solvent with a more narrow window of solvating strength than is the case with liquid solvents. It is possible, therefore, to perform selective extractions using supercritical fluids, something that is often not achievable with organic solvents.

In comparison to liquids, supercritical fluids would be expected to give rise to more rapid mass transfer, and therefore faster extractions, based on the following reasons: 1) the high diffusivities observed in supercritical fluids promote rapid molecular diffusion. This means that the solubilized solutes

will diffuse into the bulk fluid faster to be transported away from the matrix; 2) the low kinematic viscosities (viscosity divided by density) enhance free-convection mass transfer; 3) Low kinematic viscosities also allow increased turbulence in an extraction system, which contributes to more rapid forced-convection mass transfer; 4) The lower viscosities of fluids allow them to penetrate the pores and interstices of the matrix better, which promotes rapid mass transfer and solute extraction. These physical parameters are summarized as follows:

| | Gas | SF | Liquid |
|---|---|---|---|
| Density ($g/cm^3$) | $10^{-3}$ | 0.1-1 | 1 |
| Diff. Coeff. ($cm^2/s$) | $10^{-1}$ | $10^{-3}$ - $10^{-4}$ | $< 10^{-5}$ |
| Viscosity ($g/cm \cdot s$) | $10^{-4}$ | $10^{-3}$ - $10^{-4}$ | $10^{-2}$ |

In addition, the advantage of using supercritical fluids as extraction solvents can also be realized if fluids that are gases at atmospheric pressure are used. While using supercritical fluids for extraction, they can be allowed to escape into the air after decompression, and a clean extract in little or no organic solvent remains. Since extraction can be done with SFE within minutes instead of hours, there are considerable savings in time and cost from

the reduced use of organic solvents, as well as reduction in hazardous waste disposal. A schematic diagram of an SFE system is shown in Figure 2.1.

Another common practice in SFE that should be mentioned in connection with the physicochemical properties of supercritical fluids is the use of cosolvents, entrainers, or modifiers. These are compounds that are added to the primary fluid to enhance the extraction efficiency of the compounds of interest. For example, methanol is a common cosolvent added to $CO_2$ to improve the recovery of many analytes. There are two basic reasons for this. The first is to overcome "matrix effects" caused by the solutes being strongly bound to the matrix via chemisorption or physisorption mechanisms. An example of this is the extraction of pesticides from soil. To recover pesticides such as aldrin, dieldrin, or DDT from an incurred or natural soil that has been weathered, the use of methanol or acetone in the $CO_2$ is necessary to get quantitative recovery; pure $CO_2$ cannot extract the bound pesticides by itself (Khan, 1995). The second reason is to increase the polarity of the primary fluid and, hence, its solvent strength. An example of this would be the extraction of ionic surfactants from sewage sludge. Pure $CO_2$ does not solubilize these compounds, but $CO_2$ with 30 to 40% methanol can extract these polar compounds (Bright and McNally (Eds), 1992). Many organic cosolvents in concentrations from 0.5 to 40% (wt/wt) have been used in SFE with good success (McNally and Wheeler, 1988a).

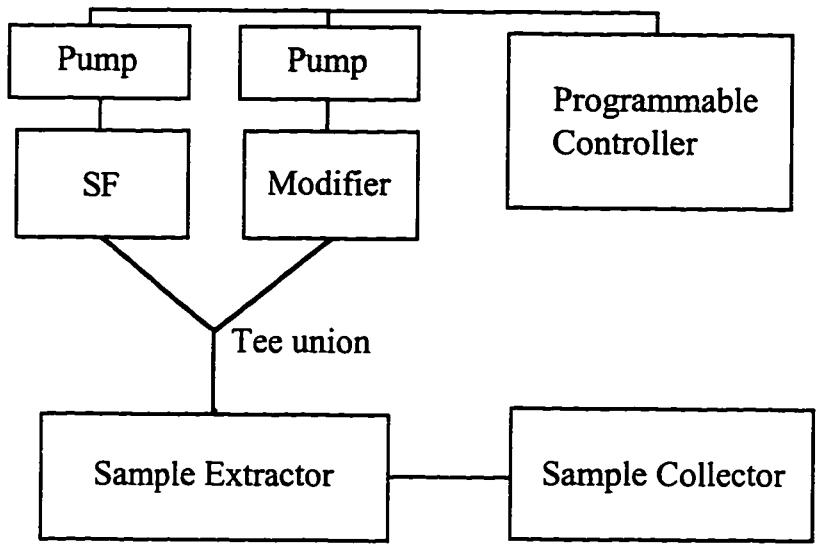



**Figure 2.1 A schematic diagram for an SFE system**

### 2.1.5.1 Choice of Operating Conditions

Two basic parameters in SFE are the extraction recovery (the proportion of the amount of solute extracted with respect to its initial amount, usually expressed as a mass percentage) and the extraction rate (extraction recovery per unit time at a given velocity of the supercritical fluid through the cell), the latter decreasing exponentially with time. The realization of the extraction of a specific solute from a matrix necessitates the optimization of several parameters, mainly the pressure, temperature, the possible addition of an organic modifier to the fluid and flow rate. The optimization of the extraction system will relate to the configuration of the trapping region, which is often determined by the mode of operation.

### 2.1.5.2 Operation Parameters

*Influence of pressure-* Four parameters are extremely helpful in the understanding of solute behavior in supercritical media, and thus in executing successful analytical supercritical fluid extraction (King, 1989; Andersen et al., 1990): (i) the miscibility or threshold pressure (Giddings et al., 1968; Giddings et al., 1969), which corresponds to the pressure at which the solute partitions into the supercritical fluid; (ii) the pressure at which the solute reaches its maximum solubility; (iii) the fractionation pressure range, which is the pressure region between the miscibility and solubility maximum pressures; and (iv) a knowledge of the physical properties of the solute, particularly its melting point (in fact most solutes dissolve better when they are in their liquid state, i.e., above their melting point). Fluid pressure is the main parameter

that influences extraction recovery. An elevation of this pressure at a given temperature results in an increase in the fluid density, which means a better solubility of the solutes. Consequently, the higher the extraction pressure, the smaller is the volume of fluid necessary for a given extraction (McNally and Wheeler, 1988b). However, high pressure is not always recommended for complex matrices owing to the higher solubility of most solutes when the pressure is elevated; thus the extract can become very complex and, consequently, its analysis becomes very difficult.

*Influence of temperature-* At a constant pressure the density of $CO_2$ decreased when the temperature rises. This effect becomes more pronounced as the compressibility increases. The temperature also affects the volatility of the solute. Hence the effect of a temperature elevation is difficult to predict because of its dependence on the nature of the sample. For a non-volatile solute, a higher temperature would result in lower extraction recovery owing to a decrease in solubility. On the other hand, for a volatile solute, there is a competition between its solubility in $CO_2$ (which decreases as the temperature increases) and its volatility (which rises with increasing temperatures). For example, when the temperature increases from 80 to 120°C, the extraction recovery of diuron from soil with methanol-modified $CO_2$ is enhanced from 75% to 99% (Wheeler and McNally, 1989).

*Addition of a modifier-* The low polarity of $CO_2$ limits its use to the extraction of relatively apolar or moderately polar solutes. Thus, a small amount of a polar organic solvent (methanol, acetonitrile, water, etc.), called a "modifier" or entrainer", is usually added to the supercritical fluid for the

extraction of more polar solutes. The nature of the modifier depends on the nature of the solute to be extracted (Walsh et al., 1987); for example, the extraction of diuron is greatly enhanced with methanol instead of acetonitrile as a modifier, probably because of hydrogen bonding which could exist between diuron and methanol (McNally and Wheeler, 1988a). A reasonable starting point consists of selecting a modifier that is a good solvent in its liquid state for the target analyte.

It should be noted that the addition of large amounts of modifier will considerably change the critical parameters of the mixture (Crowther and Henion, 1985; Gurdial et al., 1991). As a result, binary mixtures of carbon dioxide and an organic solvent are often used in a subcritical state, where the diffusion coefficients are smaller than in a supercritical state.

Modifiers can be introduced as mixed fluids in the pumping system with a second pump and a mixing chamber (Taguchi et al., 1991), or by simply injecting the modifier as a liquid into the sample before extraction (Janda et al., 1989) (the latter way being less successful because it leads to concentration gradients within the matrix). Alternatively, one may use directly a cylinder tank of modified $CO_2$, but this is much more expensive; besides, as the tank becomes empty, the content of modifier tends to increase.

*Influence of fluid velocity and flow rate-* The speed of the supercritical fluid flowing through the cell has a strong influence on the extraction efficiencies. The slower the fluid speed, the deeper it penetrates the matrix. The fluid speed can be expressed by the linear velocity, which is strongly

dependent on the flow rate and the cell geometry. For a given extraction cell, the flow rate can be easily changed by using a new restrictor with a different inside diameter. Decreasing the flow rate results in a lower linear velocity and usually in increased extraction recoveries (as a result of an extended contact between the supercritical fluid and the sample) (Hawthorne et al., 1991). However, this entails longer extraction times. On the other hand, high flow rates can result in a decrease in the recovery either by inducing an elevated pressure drop through the extraction cell, or by increasing analyte loss during decompression of the fluid. Thus an optimum flow rate has to be found. Typical values are approximately 1 mL/min of compressed fluid (with extraction cells of I.D. ca. 1 cm), which corresponds to ca. 500 mL/min of gas after decompression.

*Influence of the nature of the matrix*- Factors such as the particle size, shape, surface area, porosity, moisture, concentration of extractable solutes and the nature of the matrix will affect the analytical results. In the same way the interactions between solutes and active sites of the matrix can necessitate strict extraction conditions.

*Efficiency of the solute trapping system*- Once the compounds of interest are in the supercritical extraction fluid, the next step is to isolate them for further analyses. Generally, this is accomplished by decompression of the fluid through a restrictor (often heated to prevent the formation of pieces of ice that could plug the restrictor). Trapping becomes more difficult when either the solute is more volatile or the flow-rate is higher. The collection

technique therefore need to be efficient. Most often, SFE is coupled to chromatographic techniques, either "off-line" or "on-line" (Hawthorne, 1990).

### 2.1.5.3 Mode of Operation System

As equally important as the decompression region, the analyte trapping region must be functioning properly to ensure good recovery of the extracted compounds. The trapping region may be as simple as a vial containing an organic solvent through which the extractor effluent passes, or it may be much more complex. Cryogenically cooled packed-bed traps and gas chromatograph ovens have been used. The configuration of the trapping region is often determined by the mode of extraction, either on-line or off-line.

*On-Line Supercritical Fluid Extraction-* With on-line SFE, the extraction process replaces the normal sample injection process into the chromatograph, and the extraction apparatus is directly coupled with the chromatographic instrument used for the analysis subsequent to the extraction. On-line SFE has the advantage of being inherently sensitive because all of the extracted analytes can be loaded directly onto the chromatographic column. There is also less sampling with on-line extraction, and the entire system is easily automated. The trapping of analytes is usually better controlled because it is an instrument-controlled parameter. There are some drawbacks with on-line SFE that must be considered. When performing on-line or coupled SFE, the SFE parameters, the analyte trapping conditions, and the chromatographic separation all must be understood and optimized for the analysis to be successfully completed. A sample extracted on-line is dedicated to the

coupled chromatographic system. Once the on-line SFE analysis is completed, the extract is no longer available for evaluation using different techniques or chromatographic parameters (Vannoort et al., 1990).

*Off-Line Supercritical Fluid Extraction-* In contrast to on-line SFE, off-line SFE is performed with the analysis and extraction systems completely separated and decoupled. This is inherently simpler because only the extraction and analyte trapping need to be considered. Once collected, the analytes can be subjected to several analytical techniques depending on the information desired from the sample. This technique allows the extraction and the chromatographic systems to do what they do best, and that is sample preparation and separations, respectively. This gives rise to higher throughput and better utilization than with on-line techniques in which either the chromatograph or extractor sit idle (Mulcahey et al., 1991).

With the advantages of off-line SFE, one must not forget that disadvantages do exist. Off-line techniques may not be as sensitive as on-line procedures. For example, on-line SFE/GC using a 1-mg sample in the extraction cell yields the same sensitivity as the off-line SFE of a 1-g sample when the analytes are collected in 1 mL of solvent followed by analysis using a 1-μL injection on-column for GC (Richter, 1992).

*Dynamic and Static Supercritical Fluid-* Analytical SFE has been conducted using three different modes: 1) dynamic (in which the supercritical fluid is continuously flowing through the cell), 2) static (in which the cell is pressurized with supercritical fluid and the extraction is allowed to proceed

without any outflow of the supercritical fluid until extraction is completed, and 3) a combination of static followed by dynamic extraction. In practice for static extraction, the cells are pressurized with the supercritical fluid, the inlet and outlet valves are closed for a given period, both valves are opened, and the fluid flows dynamically through the cell carrying the extracted analytes away.

Both dynamic and static SFE have been used to achieve quantitative results, but dynamic extraction might be expected to yield more rapid recoveries by continuously providing pure extraction fluid to the sample. Dynamic extractions can also be performed without any valves between the extraction cell and the collection medium. The elimination of the valve between the extraction and collection media is attractive, particularly for the extraction of trace compounds, since the chances for analyte loss or contamination are reduced. Static extractions, however, have the advantage that modifiers can be added directly to the extraction cell prior to the pressurization step. This static step can cause the swelling of the matrix in some cases, which can make the solutes more readily accessible for extraction (Wheeler and McNally, 1989). Another potential advantage of static extraction is that it is possible to do extractions with less fluid than with dynamic extraction, with no restrictor needed (thus fewer clogging problem).

### 2.1.6 Applications of SFE in Environmental Analysis

Supercritical fluids are becoming an acceptable alternative to conventional liquid solvents for the rapid analytical-scale extraction of

environmental samples. The most common fluid to date has been supercritical $CO_2$ because of its reasonable critical properties, low toxicity, and chemical inertness. In addition, supercritical $CO_2$ has Lewis base characteristics, induced dipole interactions, and quadruple interactions that allow it to solvate numerous compounds ranging in polarity from nonpolar to moderately polar. However, poor recoveries associated with certain analyte/matrix combinations (even at low analyte concentrations) in analytical-scale supercritical fluid extraction of real environmental samples indicate that a suitable supercritical fluid must not only be able to solvate analytes of interest, but must possess properties that allow it to interact with the analyte and the matrix to efficiently partition the analyte into the bulk supercritical fluid. While $CO_2$ is a relatively good solvent, these solvating interactions are weak and it has been shown that pure $CO_2$ often cannot extract environmentally persistent as well as long time aged (bound) pollutants.

SFE has been applied to a broad range of environmental samples. The efficiency of this technique depends both on the nature of the solute to be extracted and on the characteristics of the matrix (Chester et. al., 1992). Some of the first data on the use of SFE to remove pesticide residues were reported by Stahl and Rau (1984). In this work, the authors showed the successful extraction of HCH, aldrin, DDT, and -endosulfan from senna leaves. These results were preliminary, but they demonstrated the potential of SFE for the removal of pesticide contaminants while leaving much of the matrix interference behind. In recent years, the SFE has drawn increasing attention

among analytical and environmental chemists (Richter, 1992). A review of application of SFE in pesticide analyses in the soil matrix is discussed below.

It was not until 1986 that SFE was applied to the determination of pesticides in soils. Capriel et al. (1986) investigated the extraction of bound residues in soil. They used supercritical methanol at 250°C and 150 bar as the extraction fluid. The extraction efficiency of SFE was compared to high temperature distillation (HTD) for the recovery of $^{14}C$ labeled pesticides and metabolites including atrazine, prometryn, deltamethrin, diuron, 2,4-D, methylparathion, dieldrin, carbofuran, and some of their associated metabolites. Identification of the compounds was done by GC/MS, and quantification was performed using combustion followed by liquid scintillation or GC with either an electron capture detector (ECD) or nitrogen phosphorus detector (NPD). Several different soils, all with varying composition, were investigated. Under the conditions used, the soil type seemed to have no influence on recovery of the analytes. In all cases, the recovery of the $^{14}C$-labeled compounds was better with SFE than with HTD.

The extraction of DDT was studied by Brady et al. (1987) using much milder conditions. In the study, $CO_2$ at 40°C and 100 atm pressure and a flow rate of 0.7 g/s (65 mL/min) was used as the extraction fluid. Quantification was performed using GC with an ECD. The moisture content and pore size of the soils were measured to determine their effect on extraction efficiency. It was found that DDT was not completely removed from soils with high organic matter. This was presumably due to the interaction of the DDT with humic acid components in the soil. The addition of water to the soils did not

reduce the amount of DDT extracted, but it did slow down the extraction rate, so the extraction had to proceed for a period of time. The highest recovery achieved with these mild conditions was 70%. In the same study, the recovery of a less polar material, Arochlor 1254, was investigated. The polychlorinated biphenyls (PCBs) were easily extracted (90% under the identical conditions). In order to study the effects of long-term exposure of soil to these compounds, the extraction rate of a laboratory-fortified soil and a soil from a spill site were compared. The laboratory-fortified soil has a more rapid decrease of concentration vs. time than the incurred sample, but in both cases, the maximum recovery was 70% as compared to standard Soxhlet extraction procedures.

Wright et al. (1987) reported that SFE recoveries higher than 90% from sediment can be achieved by pure $CO_2$ for propazine, terbutylazine, atrazine and cyanazine. Simazine, as a very poorly soluble compound in pure $CO_2$, requires addition of methanol to the supercritical fluid. The entrainer can be added directly to the sediment in the extraction chamber. Compounds leaving the restrictor from the SFE apparatus were trapped into a few $cm^3$ of methanol. Methanolic solution was concentrated, and then analyzed by capillary GC/FID (flame ionization detection) and HPLC/DAD (diode-array detection) at 225 nm.

Engelhardt and Groß (1988) showed the feasibility of extracting lindane, aldrin, and DDT from soil. They extracted fortified soil samples with $CO_2$ at 138 bar for 15 min, and the analysis was done by packed column

supercritical fluid chromatography (SFC) coupled on-line with the SFE system. No recovery data were reported in this work.

McNally and Wheeler (1988a and 1988b) and Wheeler and McNally (1989) had reported studies on the extraction of urea and sulfonylurea herbicides from soil and other matrices. In these studies, the extraction temperature, flow rate, pressure, and extraction fluid modifier type and concentration were examined for their effect on the extraction efficiency of diuron andlinuron from soil. Analyses of the extracts were performed using liquid scintillation counting of the $^{14}C$-labeled compounds. In some cases, further analyses were performed by HPLC or GC.

Increases in temperature yielded increased extraction efficiencies of diuron and linuron. Higher extraction efficiencies over shorter time periods resulted from increases in extraction pressures. Increases in flow rates yielded higher extraction efficiencies until the pressure drop across the extraction cell caused by the high flow rate became significant. Higher concentrations of modifiers gave better recovery of the compounds, but the identity of the entrainers also influenced the results. Methanol gave the best results with diuron, and ethanol was best with linuron. In the second study, static and dynamic extraction were investigated.

For this system, static extraction seemed to give quantitative recovery in a shorter period of time than did dynamic extraction. This may be because swelling of the sample matrix may occur during static extraction and not during dynamic extraction.

Studies of the sulfonylurea herbicides yielded many of the same conclusions as the work with the urea herbicides. Few recovery data were reported in this work, but the ability to extract and analyze sulfonylurea herbicides from soil in less than 45 min was shown with on-line SFE/SFC instrumentation.

The extraction efficiency of s-triazine herbicides from sediment was reported by Janda et al. (1989). Simazine, atrazine, propazine, terbutylazine, and cyanazine were fortified onto dried sediment samples. SFE was performed using $CO_2$ at 48°C and 230 bar for 30 min. In some cases, methanol was added to the extraction cell prior to extraction with the $CO_2$. The extracted analytes were trapped in methanol. Analyses of the extracts were done using GC or HPLC. At the 150- to 400-ppb level, essentially 100% recovery of the five compounds was achieved. However, aged residues were not tested.

Lopez-Avila et al. (1990) had reported on a preliminary study investigating the use of SFE for the extraction of pesticides and other pollutant materials as part of a proposed U. S. Environmental Protection Agency protocol. The effects of extraction pressure, temperature, moisture content, time, static or dynamic mode of extraction, cell volume, and sample size were studied. There were 41 organochlorine and 47 organophosphorus pesticides fortified on clean sand or soil. Conditions used were $CO_2$ with 10% methanol, 70°C, and 250 atm for 30 min. Analyses were conducted by GC-ECD. Excellent recoveries were achieved for 38 of the 41 organochlorine pesticides. Only the recoveries for hexachorocyclopentadine, chlorbenzilate, and DBCP

were low. All but five of the organophosphorus pesticides were recovered almost quantitatively. Diazinon, phorate, demeton-S, demeton-O, and TEPP gave low recoveries, possibly due to hydrolysis caused by the methanol or decomposition during the GC analyses.

Supercritical fluid extraction of organophosphate and organochlorine pesticides from soils was investigated and compared to the classical sonication and Soxhlet extractions (Snyder et al., 1992). Four soils, sand, clay, top soil, and river sediment, were fortified with 12 organochlorine and organophosphate pesticides. These soils were extracted with supercritical $CO_2$ modified with 3% MeOH at a pressure of 350 atm and a temperature of 50°C. Overall average recoveries of the 12 pesticides were greater than 85% for each of the soil matrices, and the overall average relative standard deviation (RSD) for all the pesticides and soil was 5.1%. Secondly, a large batch of topsoil was specially prepared and fortified with the same pesticides and repetitively extracted using SFE (n=9) and the classical sonication (n=9) and Soxhlet (n=8) extractions. The recovery data and precision of each extraction was evaluated statistically. It was found that overall average recoveries of the 12 pesticide compounds for the sonication, Soxhlet, and supercritical fluid extractions were 94.7%, 93.1%, and 91.6%, respectively. SFE demonstrated the best precision of the three extraction methods with the overall average relative standard deviation being 2.94%. Lastly, native soils contaminated with organochlorine pesticides were repetitively extracted using SFE and the sonication extraction. Comparable precisions between SFE and the sonication method were obtained. Also SFE performed equally as

well as the sonication extraction in recovering a number of organochlorine pesticides from the native soil samples.

The effect of polar modifiers on matrix swelling in clay, soil and plant materials has been examined under supercritical conditions (Fahmy et al., 1993). In the study, the extent of swelling of modifier-saturated plant and clay matrices was observed via a high-pressure sapphire view cell. Extractions of $^{14}C$-labeled solutes from these matrices were conducted separately. It was found that greater swelling occurred in the saturated fluid system; pure supercritical $CO_2$ did not cause matrix swelling in any of the systems examined. Modifier polarity seems to predict high extraction rate and efficiency and swelling in organic matrices such as pea leaves. Kinetic effects of swelling suggest that longer contact times should produce greater swelling and higher recoveries, but the majority of swelling takes place very rapidly. The swelling process of supercritical carbon dioxide is unique when compared to supercritical nitrous oxide. In general, matrix swelling was found to be an important factor in modifier-enhanced supercritical fluid extraction; maximum swelling was not found at maximum pressure. Greater recoveries from clay and pea leaves saturated with different types of modifiers were enhanced by matrix swelling.

Hawthorne et al. (1993) investigated the factors controlling quantitative supercritical fluid extraction of environmental samples. They found that because of the heterogeneous nature of environmental samples, the partitioning step may be controlled by analyte solubility in the extraction

fluid, kinetic limitations, and/or the ability of the extraction fluid to interrupt matrix-analyte interactions.

Snyder et al. (1993) studied the effect of instrumental parameters and soil matrix on the recovery of organochlorine and organophosphorus pesticides from soil using SFE. They concluded that $CO_2$ modified with 3% methanol yielded much higher extraction efficiencies of the organochlorine and organophosphorus pesticides, which were fortified into a variety of soils, than did $CO_2$ alone.

In addition, when a small amount of moisture was present in the tested topsoil, the water actually acted like a modifier. Increased recoveries of the polar pesticides were obtained by SFE when the $CO_2$ was entrained with water. They also found that density has an effect on recoveries for $CO_2$ and methanol-modified $CO_2$. Generally, as density increased the recovery of the pesticides increased. However, a threshold density was reached above which maximum recoveries of the pesticides were obtained. This was attributed to the increased solvating power of the supercritical fluid at the higher density.

Temperature was found to have little effect on recoveries of most of the pesticides over a range of 40-120°C. The efficiency of the supercritical fluid extraction was not greatly increased by a static extraction conducted prior to the dynamic removal (Synder et al., 1993).

Locke (1993) reported that use of SFE to recover fluometuron herbicide from soil was successful and comparable to that obtained using conventional methods. Soil was treated with $^{14}C$-ring-labeled fluometuron, air-dried, and

stored for 6 months at 5°C. The $^{14}C$ was then extracted from soil with methanol by conventional extraction (twice with MeOH/$H_2O$ 80:20 v/v; 2:1 extract/soil v/w) and with $CO_2$ using a supercritical fluid extractor. The SFE method development included adding modifiers and varying $CO_2$ fluid density, extraction temperature, sample mass, and extraction time. Adding $H_2O$ to modify the sample was the single most effective variable which improved recovery. Extraction temperatures above 50°C lowered recovery, presumably because of thermal instability of fluometuron. The optimum $CO_2$ density at 50°C was 0.80 g /mL. Static extraction times greater than 6 min and dynamic extraction times greater than 18 min did not significantly improve recovery. Recovery using optimum SFE conditions was comparable to that obtained using conventional extraction methods. Extraction of aged field samples indicated that this technology can also be used to extract some common fluometuron metabolites.

A kinetic study of supercritical fluid extraction of organic contaminants from heterogeneous environmental samples with carbon dioxide and elevated temperature was reported (Langenfeld et al., 1995). It was found in the study that native analytes were extracted more slowly than fortified analytes, suggesting that additional processes affect SFE rates of native analytes. A kinetic model was used that could help distinguish between these processes and included terms for matrix-fluid mass transport, as well as partitioning and bulk mass transport in the supercritical fluid. The results of this study show that increasing the extraction temperature is a simple and effective method to increase SFE rates while still exploiting the advantages of

supercritical $CO_2$, and can be used regardless of whether slow SFE rates are due to poor partitioning into the fluid or limited by slow desorption due to strong analyte-matrix interactions.

More recently, the use of supercritical carbon dioxide with and without methanol as a modifier to extract bound $^{14}C$ pesticide residues from soil, plants, and wheat samples was described (Khan, 1995). The $^{14}C$ material extracted was trapped in methanol, radioassayed, and then analyzed by various chromatographic techniques. Optimal supercritical fluid extraction conditions for extraction were obtained for each pesticide by varying the temperature, pressure, and amount of modifier. Supercritical carbon dioxide modified with methanol improved the recovery of bound $^{14}C$ residues from soil and plant samples. Supercritical methanol was found to be less efficient than supercritical carbon dioxide or methanol-modified supercritical carbon dioxide for the extraction of bound pesticide residues. Analysis of the extracts indicated that the $^{14}C$ bound residues in soil, plants, and wheat samples were present in the form of parent compounds and/or metabolites.

## 2.2 Methods and Materials

There are two experimental designs in the multivariable optimization scheme: a screening design and a response surface design. The screening experimental design was used to define the significance of independent variables for both soil environmental variables and SFE parameters. The experimental design model was developed by ECHIP® Inc. (Hockessin, Del.), and primarily used to narrow down the number of variables to several

important ones for response surface experimental design. The response surface design was then employed to elucidate the interactions and relationships among the independent variables, and lead to an optimization scheme. Two groups of independent variables (i.e., "soil environmental variables" and "SFE parameters") were studied to determine the effects of environmental variables and SFE parameters on the extractability of pesticides in soils.

Threshold values of SFE parameters (pressure, temperature, modifier) were determined by fortifying each of the analytes (radiolabeled $^{14}C$ analyte at 2,000 dpm/g) onto Celite® 545 individually and extracting by SFE. The ranges of SFE parameters were varied to locate the threshold ranges. For instance, pressure was varied from 140-352 atm, temperature ranged from 30-70 °C (limited to bensulfuron methyl, which is thermally labile), the modifier percentage ranged from 5-30% (surfactant Triton® X-100 0.5% with acetonitrile and acidic water with 0.1N HCl, 10/80/10 v/v/v). Extraction was initiated by 3 minutes static followed by 7 minutes dynamic mode at a flow rate of 0.5 mL/min. The extractant was collected in Atomlight® and measured by LSC. An ISCO SFE system 2300 (ISCO, Lincon, Neb.), consisting of two Model 260D pumps; one system controller (electronic); one Model SFX 2-10 dual-chamber extractor module, with associated valves, fittings, mixing tee, and connecting tubing; 10-mL S.S. sample cartridges; and two extractant collection vials (20 x 150 mm) contained in plastic Erlenmeyer flasks; was used in the study.

General procedures for sample preparation using SFE may be obtained from Chapter 3 (**3.2.4 Supercritical Fluid Extraction**).

### 2.2.1 Soil Environmental Variables

Three soils (Belhaven high organic matter, 57.5% from North Carolina, Cullera high clay, 56.7% from Spain, and Miaka high sand, 91.6% from Florida; detailed information on the soil characterization are given in Appendix II) were chosen for soil environmental variable studies. Nine soils were generated by mixing the three soils with different ratios yielding desirable pooled samples for the studies. The four variables were the contents of organic matter, and clay, residence time of pesticide in the soil, and type of pesticide (Table 2.2). The effect of various independent variables on the binding strength/unextractability were investigated using a multivariate optimization scheme, which uses a quadratic model from ECHIP® software.

To reveal the difference in soil matrix-pesticide interaction, SFE parameters were optimized, but extended extractions were not performed in this phase of the study. To minimize the extraction efficiency due to poor solubility of the analytes in SFE process, threshold values were measured and identical SFE conditions were used for each extraction.

### 2.2.2 Supercritical Fluid Extraction Parameters

In a preliminary test, seven variables were screened for a further response surface test: SFE temperature and pressure, extraction time (length), extraction modifier, sample size, analyte concentration level, and soaking time

(if applicable) involving preaddition of modifier to extraction vessel. This screening design was used to determine the top four important variables for further studies. Using the multivariate optimization scheme (MOS), four major variables were selected from the screening experiments. The variables were pressure, temperature, modifier (%), and length of extraction.

The response surface design was used to optimize the extraction procedure. In initial experiments it was found that the relative importance of the SFE parameters varied depending upon whether freshly fortified samples or aged samples were extracted. To accurately reveal the significance of the variables, fortified and aged samples were extracted and evaluated. Soil samples (either freshly fortified or aged) were weighed directly into the extraction vessel (0.25-2.0 g). Modifier was added to the sample vessel prior to SFE for static extraction for freshly fortified samples, and continuously delivered by pump for dynamic extraction for aged/native samples. Experimental design and trials are given in Table 2.3-2.4 (i.e., bensulfuron methyl for fresh fortification, and atrazine for aged Alliston soil).

### 2.2.3 Experimental Design for Multivariate Optimization Scheme

Statistical experimental design and analysis provide an optimal method to discover the relationship between a response and one or more control variables. If $y$ is a response, i.e., recovery or extraction efficiency, and $X_1$, $X_2$, $X_3$...., are a set of control variables (i.e., temperature, pressure, time, etc., then one can suppose a functional relationship between the two, like $y = f(X_1, X_2, X_3,...)$.

Continuous variables are the most common design variables. For calculation, one can write polynomial models as an approximation to a hypothetical response surface. The most common models are linear, interaction, quadratic and partial cubic. The partial cubic model contains interaction terms of the third degree but does not contain cubic terms in a single variable. In this study, a quadratic model was selected as follows if three variables are studied:

$$y = a_0 + a_1X_1 + a_2X_2 + a_3X_3 + a_{12}X_1X_2 + a_{23}X_2X_3 + a_{13}X_1X_3$$

$$+ a_{11}X_1^2 + a_{22}X_2^2 + a_{33}X^2 + a_{123}X_1X_2X_3$$

or if four variables are studied:

$$y = a_0 + a_1X_1 + a_2X_2 + a_3X_3 + a_4X_4 + a_{12}X_1X_2 + a_{14}X_1X_4 + a_{23}X_2X_3 + a_{13}X_1X_3$$

$$+ a_{24}X_2X_4 + a_{34}X_3X_4 + a_{11}X_1^2 + a_{22}X_2^2 + a_{33}X_3^2 + a_{44}X_4^2 + a_{1234}X_1X_2X_3X_4$$

in which the $a$ values are coefficients and the X values represent experimental conditions (temperature, pressure, modifier, and length of extraction) expressed as percentages of the experimental ranges on the respective axes. The coefficients were obtained using a modified version of the ECHIP® Basic program. The sequential trials are listed in Table 2.3-2.4.

In this study, a MOS based on a quadratic or central composite model was used for surface response design, to systematically elucidate the influence of soil environmental variables and SFE parameters on the extractability of freshly fortified and aged pesticide residues from soil samples. The

combination of statistically designed experiments with the versatility of SFE efficiently leads to an elucidation of soil matrix effects, and greatly increases our ability to probe important aspects of pesticide binding, and efficiently recover as much bound residue as possible.

Desktop Apple® Macintosh IIvx (Apple Computer, Inc., 20525 Mariani Avenue, Cupertino, CA), ECHIP® software program (ECHIP, Inc., Hockessin, Del.), Microsoft® EXCEL, version 4.0 , WORD version 5.0 (Microsoft Corporation, Redmond, Wash.), DeltaGraph® Pro 3.5.1 (MindVision® Software Corp.,), and PowerPoint®, version 3.0 (Microsoft Corporation, Redmond, Wash.) were employed for data analysis and graphing.

### 2.2.4 Sample Preparation for Soil Environmental Variables

All of the control and aged soil samples were air dried, and sieved (250 μm). For the soil environmental variable experiments, mixed soils were air dried, weighed (2 grams) in a scintillation vial, fortified with atrazine, diuron and bensulfuron methyl at 20,000 dpm/g ($^{14}C$ radiolabeled compound), respectively, and dried under a stream of nitrogen to minimize microbe activity. Samples were stored at approximately 4-7°C in a refrigerator to ensure as little degradation as possible. Fortifying solutions were prepared by reconstituting them into an aqueous solution after evaporating off organic solvent in the standard solutions. For example, 1.0 mL of standard solution (10 μg/mL or 100,000 dpm/mL in acetonitrile) was transferred into a 15 mL graduated tube and evaporated down to approximately <0.2 mL and brought

up to 10 mL with deionized water to yield 1.0 μg/mL or 10,000 dpm/mL standard.

Threshold values of extraction were determined from preliminary experiments. Universal SFE parameters [300 atm, 60°C, with a modifier of 20% (acetonitrile/acidic water with 0.1 N HCl/0.5% Triton ®X-100 surfactant 80/10/10 v/v/v] were selected for the study to minimize differences in extractability due to poor solubility of the analyte in modified supercritical fluid carbon dioxide (SC-$CO_2$). The extraction was initiated by 3 minutes static mode followed by 7 minutes dynamic mode at a flowrate of 0.5 mL/min. 0.25-1 gram of prepared soil samples were weighed directly in the extraction cell. Modifier(s) (Triton® X-100/acetonitrile/water 0.5% 10/80/10 v/v/v) were delivered by pump during the extraction (20% modifier to $CO_2$, dynamic). Length of extraction (10 minutes), temperature (70°C), pressure (246 atm), and dynamic extraction were used for the study. Extracts were collected in a 25-mL test tube with 10-mL Atomlight® cocktail for LSC, or 50/50 v/v acetonitrile/water for LC analysis.

### 2.2.5 Sample Preparation for SFE Optimization

For freshly fortified experiments, soil samples were air dried, weighed (2 grams) in a scintillation vial, fortified with atrazine, diuron and bensulfuron methyl at 20,000 dpm/g ($^{14}C$ radiolabeled compound), respectively, and extracted using SFE procedures for the fortified samples. Aged soil samples that were used included Danish and Swedish soils from diuron field degradation studies, Canadian Alliston soil from Canadian Agricultural

Research Centre in Ottawa for atrazine, and a local Keyport soil from bensulfuron methyl studies. These samples were air dried, passed through 250 μm sieve, weighed (2 g) directly in an extraction cell, and extracted using the SFE with both static and dynamic modes to determine which mode was better. Then the multivariate optimization scheme was used to optimize SFE parameters for the extraction rate and efficiency studies (refer to Chapter 3 for details).

For SFE parameters optimization experiments, preliminary tests suggested that the static mode with a relatively short extraction period (5-10 min) was sufficient for the freshly fortified samples, but the dynamic mode with an extended extraction period (30 min) was necessary to obtain high extraction efficiency for the field aged samples. For individual analytes, SFE parameters were (140-352 atm, 40-70°C, 5-30% modifier) for bensulfuron methyl, (140-352 atm, 40-100°C, 5-30% modifier) for diuron, and (140-352 atm, 40-150°C, 5-30% modifier) for atrazine. Note that bensulfuron methyl is thermally labile, therefore the upper limit of the temperature was set at 70°C; diuron is relatively stable at temperatures up to 100°C. Atrazine is very stable at an elevated temperature, up to the maximum temperature for the SFE system (150°C).

Because of the inherent limitation of two- or three- dimensional (2-D or 3-D) contour plots, only two variables vs. response (extractability) could be plotted/viewed at a time. However, different variables could be shown in either 2-D or 3-D contour plots to demonstrate the influence of each variable on the extractability. In a summary result sheet provided by the ECHIP®

software, the degree of significance among variables was summarized over the test range.

0.25-1 gram of aged soil samples were weighed directly in the extraction cell. Modifier(s) (methanol/acidic water with 0.1N HCl, 80/20 v/v; acetonitrile/acidic water with 0.1N HCl, 80/20 v/v; 0.5% Triton® X-100/acetonitrile/acidic water with 0.1N HCl, 10/80/10 v/v/v) were added to samples prior to SFE extraction (static), or by pump delivery during the extraction (5-30% modifier to $CO_2$, dynamic). Both dynamic and static modes were evaluated for further studies. Extended extraction (from 5 minutes to 30 or 60 minutes in some cases) was used to recover as much bound residues as possible. A dynamic SFE was employed for the optimization scheme. For extraction rate/efficiency studies, extracts were collected at predetermined intervals (every 5 minutes for dynamic and static extractions). Extracts were collected in acetonitrile, and evaporated to almost dryness using N-EVAP® in a water bath at approximately 50°C and reconstituted into either Atomlight® cocktail (LSC) or 1:1 (v/v) acetonitrile/water (LC) and analyzed by either LSC or LC-UV (where applicable).

### 2.2.6 Identification and Semiquantification of Potential Degradation Compounds from the SFE Studies

To determine potential degradation products, which might present in the original samples prior to the SFE procedures, fortified samples were studied under identical conditions, which these samples were extracted by SFE at different temperatures (40, 70, and 80°C for bensulfuron methyl; 50,

100, and 120°C for diuron; 100, and 150°C for atrazine) with or without modifier (10/80/10 v/v/v, 0.5% Triton® X-100/methanol/0.1N HCl) at a constant pressure of 241 atm.

Extracts or aliquots were concentrated (TurboVap®, if needed), filtered through 0.2 μm filter (Nylon Acrodisc®, Gelman Sciences, VWR Scientific Distributor), and analyzed by reversed phase LC-UV-DAD (HP 1090M Liquid Chromatograph with UV-DAD Detector, Hewlett-Packard) and confirmed by LC/MS and/or LC/MS/MS (LC/MS SSQ 7000 or LC/MS/MS TSQ 7000, Finnigan Mat, with thermospray or electrospray where applicable. LC column: Zorbax® Rx-$C_{18}$ 4.6 mm ID, 250 mm, 5 μm (Mac-Mod Analytical); Mobile phase: 20% acetonitrile held for initial 5 minutes, gradient to 30% in 10 minutes, then to 50% in 5 minutes, and held for 5 minutes, with $NaH_2PO_4$ buffered at pH 3.2; Flowrate: 1.0 mL/min; Injection volume: 25-50 μL; UV detection: 254 nm.

### 2.2.7 Chemicals and Reagents

Analytical standards of diuron (DPX-14740-149, 99.7% purity), bensulfuron methyl (DPX-F5384, 99.3% purity), atrazine (INY-0150, 100.0% purity), and related degradation compounds were synthesized by DuPont Agricultural Products, E. I. du Pont de Nemours and Company(Wilmington, Del.). The structures of the compounds are given in Appendix I.

Deionized water was obtained from a Milli-Q®- water purification system (Millipore Corp. Milford, Mass). HCl, methanol, acetonitrile, Triton® X-100, and other chemicals were Fisher Scientific/ACS reagent grade.

## 2.3 Results and Discussion

MOS is a very powerful tool for development of an analytical method, especially for the case of SFEwhich involves many response variables. In traditional sample extraction methods, such as Soxhlet extraction, the extraction process often produces a fairly large volume of diluted solution of analyte(s) plus other coextracted components in the liquid extraction solvent. The extraction step is usually followed by a concentration step in which most of the solvent is driven off, leaving a small volume of concentrated solution. Often the concentrated solution is subjected to a fractionating process (such as column chromatography or liquid-liquid back extraction) where large volume fractions are obtained-necessitating more concentration before the solution can be ready for introduction into an analytical instrument. Compared to the conventional approaches, in contrast to typical liquid solvents, the solvent power of supercritical fluid is variable and selectable. Extraction conditions determine which, and to what extent, various components within the sample are soluble in the fluid. Fractionation can be achieved by adjusting extraction conditions in a sequential, stepwise manner on the same sample. During any one extraction step, classes of compounds are dissolved and removed from the sample by the supercritical fluid (this solution can be concentrated-often up to the point of being saturated when bulk extractables are encountered). The solution is expanded to lower pressure to remove the solvent, and the extracted components precipitate out of the expanding gas. The key point here is that the extraction fluid is removed automatically (saving time,

eliminating steps, the user can achieve "extraction-of-a-fraction" and concentration simultaneously).

In the preliminary experiments, the threshold values of SFE parameters (pressure, temperature, modifier concentration, etc.) were determined initially for each of the analytes on Celite® 545 (atrazine, diuron, and bensulfuron methyl). The threshold values were 241 atm, 30°C and without modifier for atrazine, 176 atm, 30°C and with 5% modifier for diuron, and 241 atm, 40°C and with 20% modifier for bensulfuron methyl, respectively. The universal SFE parameters of 211 atm, 60°C and 20% modifier were selected for multivariate optimization scheme experiments.

### 2.3.1 Screening Design

Studies of SFE optimization conducted in laboratories have traditionally dealt with freshly fortified samples, which would frequently behave very differently from environmental soil samples. In other words, these data sometimes do not reveal the real situation for many environmental soil samples. Many pesticides will bind to soils very tightly after aging for a period of time (Khan, 1982). Experimental conditions of SFE may be best suited for non-aging samples, but may not be optimal for aging samples. Furthermore, the soil samples were always immediately extracted after spiking to avoid the risk of decomposition or the loss of analytes. Obviously the disadvantages of using fortified samples is that they show higher recoveries than "real" samples. This is probably due to stronger matrix-analyte interactions (e.g., chemical and physisorption) in field aged samples.

For the initial screening design, a linear model was chosen for the evaluation.

$$y = a_0 + a_1X_1 + a_2X_2 + a_3X_3 + a_4X_4 + a_5X_5 + a_6X_6 + a_7X_7$$

Where $a$ is the coefficient for each individual variable, and $X$ denotes independent variables for the design experiments.

Samples were fortified with diuron at different concentrations, and proceeded SFE extraction under the following conditions: extraction length was about 10 minutes with a static mode at a temperature of 60°C, and a pressure of 242 atm, with 20% modifier of 0.5% Triton® X-100/acetonitrile/acidic water with 0.1N HCl (10/80/10 v/v/v) (Table 2.1). SFE extraction efficiencies indicated that SFE temperature, pressure, modifier, and extraction length were more significant than other variables for better extractability.

### 2.3.2 Surface Response Design

Among seven variables in the SFE procedure, four of the variables such as temperature, pressure, modifier, and length of extraction seemed to be more important than the other variables.

A quadratic or central composite model was used for the surface response design with three or four independent variables. Effects of soil environmental variables [i.e., organic matter content %, clay minerals content %, residence time of pesticide residues (months), different species of pesticides

**Table 2.1 Screening design for SFE parameter evaluation**

| Trial # | T °C | P (atm) | Length (min) | Modifier (mL) | Weight (g) | Level (dpm) | Soaking (min) | Rec (%) |
|---|---|---|---|---|---|---|---|---|
| 1 | 40 | 352 | 8 | 3 | 1 | 5000 | 1 | 86 |
| 2 | 100 | 141 | 2 | 1 | 8 | 5000 | 10 | 75 |
| 2 | 100 | 141 | 2 | 1 | 8 | 5000 | 10 | 70 |
| 3 | 40 | 141 | 8 | 3 | 8 | 5000 | 1 | 86 |
| 3 | 40 | 141 | 8 | 3 | 8 | 5000 | 1 | 93 |
| 4 | 100 | 352 | 2 | 1 | 1 | 50000 | 1 | 89 |
| 4 | 100 | 352 | 2 | 1 | 1 | 50000 | 1 | 80 |
| 5 | 100 | 141 | 2 | 3 | 8 | 50000 | 1 | 84 |
| 5 | 100 | 141 | 2 | 3 | 8 | 50000 | 1 | 78 |
| 6 | 40 | 352 | 8 | 1 | 1 | 5000 | 10 | 72 |
| 7 | 40 | 352 | 2 | 1 | 8 | 50000 | 10 | 68 |
| 8 | 100 | 141 | 8 | 3 | 1 | 5000 | 1 | 86 |
| 9 | 100 | 141 | 8 | 1 | 1 | 50000 | 10 | 75 |
| 10 | 40 | 352 | 2 | 3 | 8 | 5000 | 1 | 97 |
| 11 | 100 | 352 | 2 | 3 | 1 | 5000 | 10 | 101 |
| 12 | 40 | 141 | 8 | 1 | 8 | 50000 | 1 | 46 |
| 13 | 100 | 352 | 8 | 1 | 8 | 5000 | 1 | 93 |
| 14 | 40 | 141 | 2 | 3 | 1 | 50000 | 10 | 77 |

(atrazine = 0, diuron = 2, and bensulfuron methyl = 3)] and SFE parameters [temperature, pressure, modifier %, and length of extraction (min)] on the extractability of residues from soils were examined and evaluated.

### 2.3.3 Effect of Soil Environmental Variables on Extractability

The multivariate optimization scheme is a very powerful tool for the evaluation of the impact of both environmental variables and SFE parameters on the extractability of freshly fortified and aged pesticide residues from soils. The response surface for the quadratic design experiment, together with the full design, are shown in Table 2.2 for the evaluation of soil environmental variables on the extractability.

In preliminary studies, the following independent variables were determined to be significant: organic matter content, clay mineral content, length of analyte residence time (aging period) in soil, and type of pesticide. Systematic evaluation of the independent variables was conducted subsequently using a multivariate approach. The experimental design table is given below (Table 2.2).

Analyte residence time was a significant factor for all pesticides studied, especially for bensulfuron methyl during a 12 months (Figure 2.2). The extractability decreased from approximately 93 to 54% for bensulfuron methyl, from 98 to 69% for diuron, and from 100 to 73% for atrazine, respectively, with increase in residence time from 0 to 12 months. Other work in our laboratory indicated that the sorption mechanism for bensulfuron

methyl, which may be associated with pH and the cation exchange capacity (CEC) of the soil, might be different from that for diuron and atrazine.

Both soil organic and clay contents (%) had a significant impact on the extraction of the aged bensulfuron methyl in soil aged for 12 months. It appeared that bensulfuron methyl binding was more dependent on the organic content than clay content (Figures 2.3-2.5). With a 12 month residence time, extractability of bensulfuron methyl decreased to 32% when organic matter content increased to 28%. The strong effect of organic matter content on binding of bensulfuron methyl was clearly shown in Figure 2.5. For the 6 months aging period, the effect of a clay mineral content as high as 15% was not significant; A large decrease in bensulfuron methyl extractability was observed when clay content increased to 28% (Figure 2.4). Atrazine was the easiest analyte extracted; clay content had greater impact on extractability than did organic matter content (Figures 2.4-2.5). In the case of diuron, both organic and clay contents affected the extractability, but not as significantly as for bensulfuron methyl. It is reasonable that the high CEC of the Belhaven soil might also contribute to the stronger binding of bensulfuron methyl than that for diruon, as observed in the sorption/desorption studies. This was not tested as an independent environmental variable.

Mixtures of soil samples would be expected to differ from the original soils due to different characteristics as such their cation exchange capacity (CEC), pH, and specific surface areas, all of which are important in pesticide sorption. It is likely that such soil samples, prepared under laboratory conditions, may not truly reflect the natural soil environment. Therefore, the

degree of significance among the soil environmental variables potentially indicated interactions and relationships among the test variables, but should not be considered as a quantitative evaluation of binding strength and mechanisms. However, these results should be useful in qualitatively understanding the nature of the interaction of field aged residues with soil constituents and understanding bound residues in the real soil environment.

### 2.3.4 Effect of SFE Parameters on Extractability

Many studies of SFE optimization conducted in the laboratories have mostly dealt with freshly fortified samples, which frequently behave very differently from environmental soil samples. Many pesticides bind to soils very tightly after aging for a period of time (Khan, 1982). Fortified samples were convenient, but often showed higher recoveries than field aged samples. This can be explained by stronger matrix-analyte interactions (e.g., chemisorption and physisorption) in field aged samples.

For freshly spiked samples, supercritical fluid density was critical to the extractability of pesticide residues as indicated by the strong influence of pressure increase (Figures 2.6-2.8). This suggested that analyte solubility in the modifier supercritical fluid was the crucial factor in the extraction. In some cases, the polarity of the modified supercritical fluid was also important to recover relatively polar analyte such as bensulfuron methyl (Figure 2.9). More than 15% modiifier should be used to achieve higher recovery for bensulfuron methyl.

The degree of significance among the test SFE parameters was apparently related to the nature of the analyte(s) and residence time in soils (aged and nonaged). Temperature and modifier (%) became more important than pressure in the SFE extraction for aged diuron samples (Figures 2.10-2.12). For aged atrazine sample (approximately 3.5 years in a field), the length of extraction became important for the higher extractability (Figure 2.13). This clearly indicated that mass transfer or diffusion control is rate limiting in the SFE process. In addition, pressure seemed to be also effective for aged atrazine samples (Figure 2.14). This could be associated with the high temperature employed in the extraction (150°). To maintain certain threshold values of SC-$CO_2$ density, a relatively high pressure should be applied. This was not the case for bensulfuron methyl and diuron, since a fairly low temperature was used in the extraction.

Threshold values of temperature and pressure for extracting freshly fortified diuron, bensulfuron methyl, and atrazine from inert (Celite 545®) material were derived from varying the pressure while maintaining a reasonable value for temperature (60°C), and vice versa (i.e. pressure of 210 atm). The values for either temperature or pressure, which yield recovery values above 90% were considered to be threshold values for each compound.

In general, recovery increased as both pressure and temperature increased, but it reached a plateau when both parameters exceeded threshold values for recovering a particular analyte. Beyond this threshold, an increase in either parameter was no longer important for the extraction process. The density of supercritical carbon dioxide is directly proportional to the increase

in pressure, but inversely proportional to the increase in temperature. It was understood that heterogeneous soil samples would behave differently compared to the homogeneous matrix Celite 545®. However, these threshold values would give qualitative measurements in terms of desorption threshold for freshly fortified samples.

Surfactant as a modifier to supercritical fluid has not been reported to date. Interestingly, a modifier containing surfactant Triton® X-100 yielded better extraction rates and efficiencies for most aged soils in these studies except for bensulfuron methyl in the aged samples. This is presumably related to the particular sorption mechanism associated with bensulfuron methyl (unpublished research).

Multivariate optimization schemes were performed for both freshly fortified bensulfuron methyl, and diuron samples (Table 2.3), and field aged atrazine and diuron samples (Table 2.4). Four independent variables were evaluated for the optimization of extraction. Experimental conditions are listed below:

**Table 2.2 Effect of environmental variables on the extractability of three pesticides from soils using SFE**

| Trail # | Pesticide[a] | Length (Mon) | Clay (%) | OM (%) | Recovery (%) |
|---|---|---|---|---|---|
| 18 | 2 | 0 | 15 | 2 | 94 |
| 12 | 0 | 0 | 28 | 15 | 94 |
| 11 | 0 | 12 | 2 | 15 | 68 |
| 1 | 0 | 0 | 2 | 2 | 99 |
| 17 | 1 | 12 | 2 | 2 | 80 |
| 15 | 1 | 12 | 15 | 28 | 50 |
| 20 | 0 | 6 | 0 | 28 | 72 |
| 9 | 2 | 12 | 28 | 15 | 34 |
| 6 | 2 | 12 | 2 | 28 | 36 |
| 5 | 0 | 0 | 2 | 28 | 96 |
| 2 | 2 | 12 | 2 | 2 | 79 |
| 16 | 1 | 0 | 2 | 28 | 93 |
| 4 | 0 | 12 | 28 | 2 | 47 |
| 19 | 0 | 6 | 28 | 2 | 65 |
| 3 | 2 | 0 | 28 | 2 | 90 |
| 3 | 2 | 0 | 28 | 2 | 90 |
| 1 | 0 | 0 | 2 | 2 | 100 |
| 13 | 1 | 6 | 28 | 28 | 60 |
| 4 | 0 | 12 | 28 | 2 | 47 |
| 7 | 2 | 0 | 28 | 28 | 83 |
| 8 | 0 | 12 | 28 | 28 | 38 |
| 5 | 0 | 0 | 2 | 28 | 96 |
| 2 | 2 | 12 | 2 | 2 | 78 |
| 14 | 2 | 6 | 14 | 28 | 58 |
| 10 | 2 | 0 | 2 | 15 | 92 |

[a] Numbers represented for individual pesticide (atrazine = 0, diuron = 1, and bensulfuron methyl = 2), respectively.

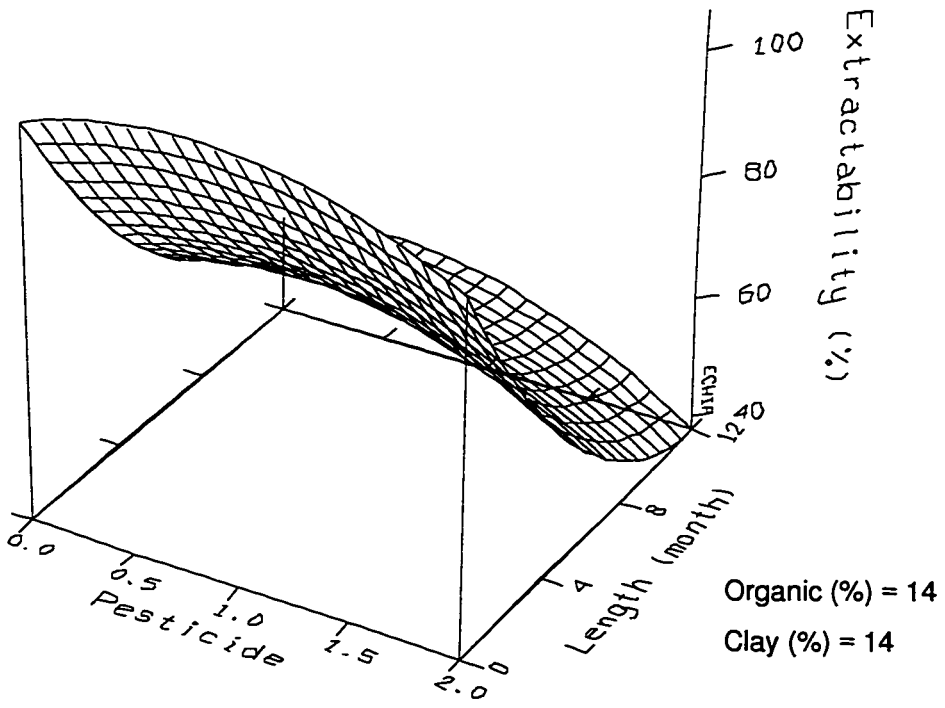



**Figure 2.2 Effect of pesticide residence time on extractability for three pesticides using SFE**

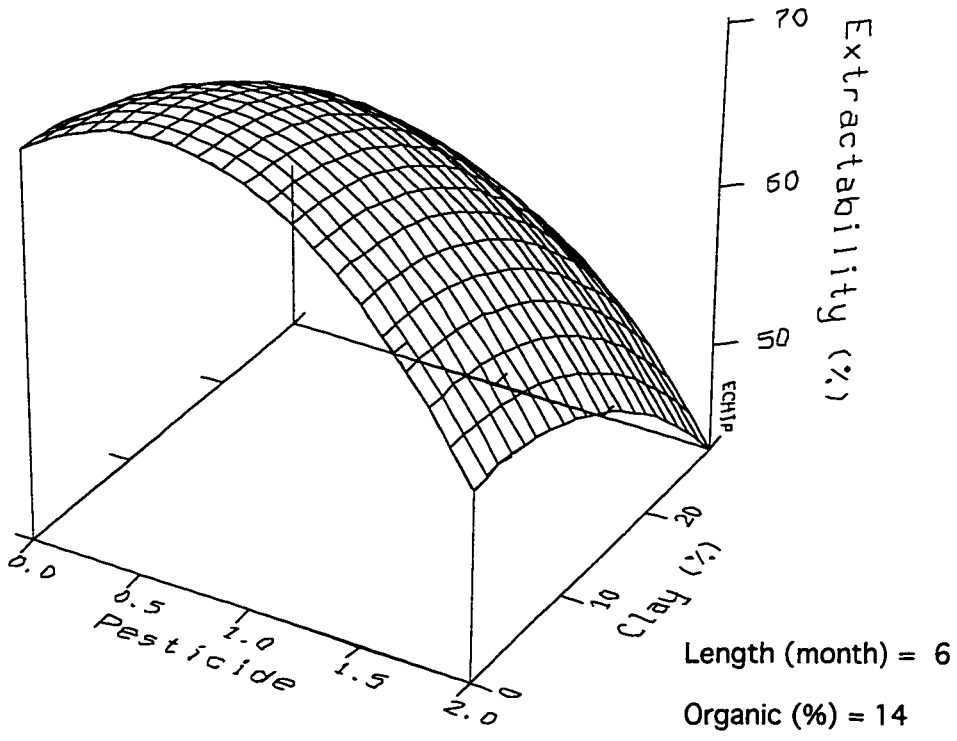



**Figure 2.3 Effect of clay content on extractability for three pesticides using SFE (6 months)**

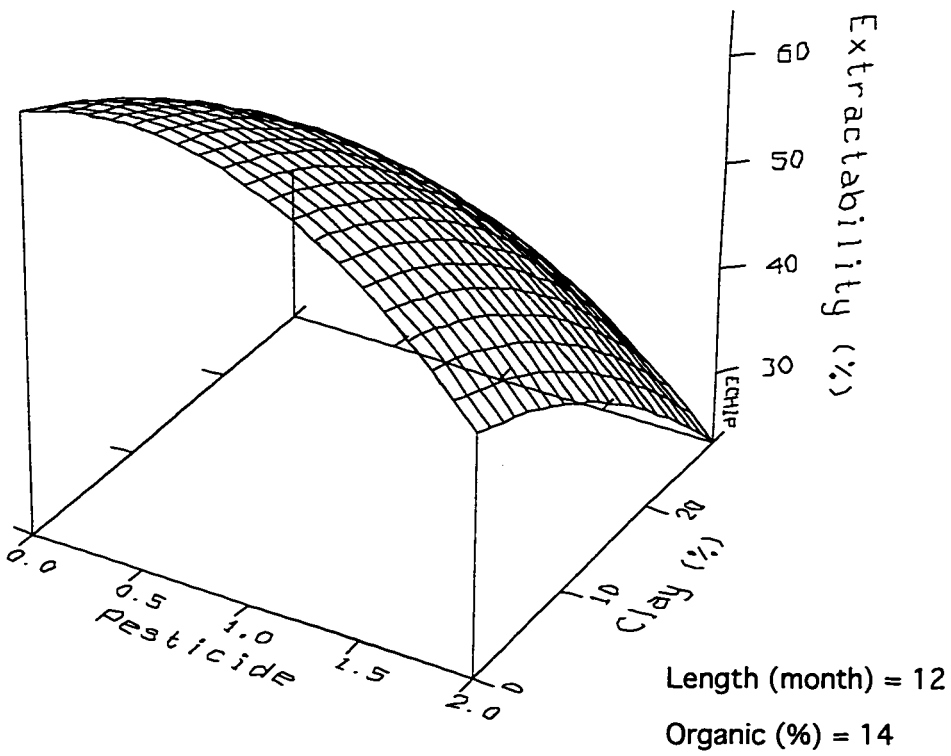



**Figure 2.4 Effect of clay content on extractability for three pesticides using SFE (12 months)**

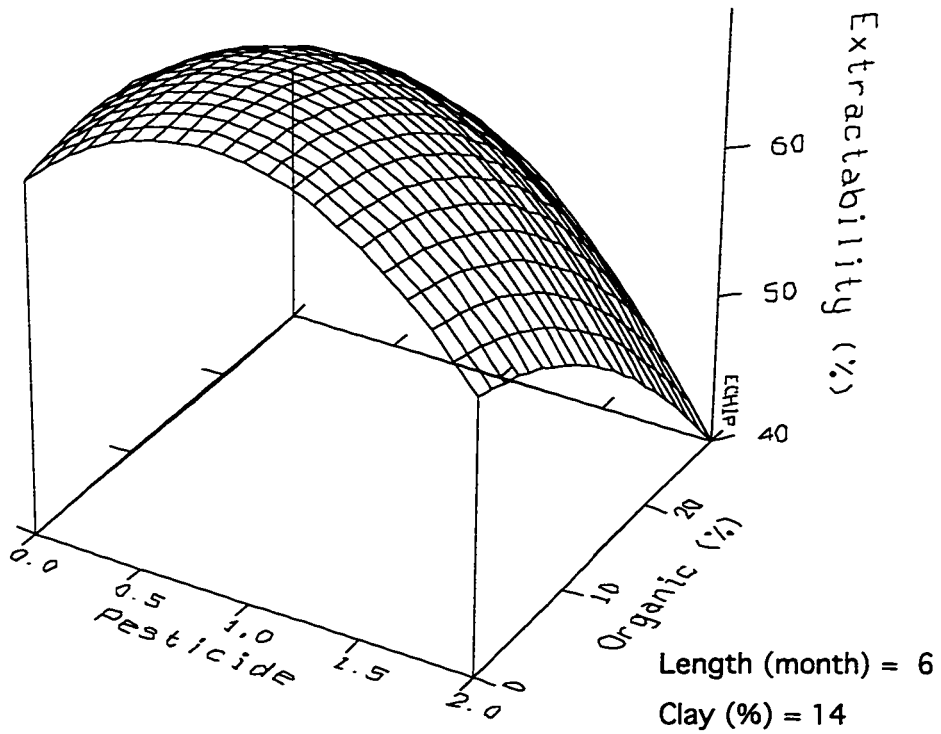



**Figure 2.5 Effect of organic matter content on extractability for three pesticides using SFE (6 months)**

**Table 2.3 Effect of SFE parameters on the extractability of fortified bensulfuron methyl (BM) from Alliston soil using the MOS**

| Trail # | T (°C) | P (atm) | Length (min) | Modifier (%) | Recovery (%) |
|---|---|---|---|---|---|
| 13 | 55 | 246 | 10 | 30 | 92 |
| 2 | 70 | 352 | 2 | 5 | 72 |
| 4 | 40 | 352 | 10 | 5 | 69 |
| 5 | 40 | 140 | 2 | 30 | 88 |
| 17 | 55 | 352 | 2 | 5 | 71 |
| 6 | 70 | 352 | 2 | 30 | 100 |
| 12 | 40 | 140 | 10 | 17.5 | 88 |
| 3 | 70 | 140 | 10 | 5 | 57 |
| 20 | 40 | 246 | 2 | 30 | 88 |
| 4 | 40 | 352 | 10 | 5 | 68 |
| 3 | 70 | 140 | 10 | 5 | 59 |
| 2 | 70 | 352 | 2 | 5 | 69 |
| 5 | 40 | 140 | 2 | 30 | 87 |
| 14 | 70 | 246 | 6 | 30 | 94 |
| 1 | 40 | 140 | 2 | 5 | 48 |
| 18 | 70 | 140 | 6 | 5 | 65 |
| 1 | 40 | 140 | 2 | 5 | 46 |
| 16 | 55 | 140 | 2 | 30 | 86 |
| 11 | 40 | 352 | 2 | 17.5 | 96 |
| 7 | 70 | 140 | 10 | 30 | 87 |
| 8 | 40 | 352 | 10 | 30 | 93 |
| 15 | 55 | 352 | 6 | 30 | 97 |
| 19 | 40 | 246 | 10 | 5 | 68 |
| 9 | 70 | 352 | 10 | 17.5 | 96 |
| 10 | 70 | 140 | 2 | 17.5 | 87 |

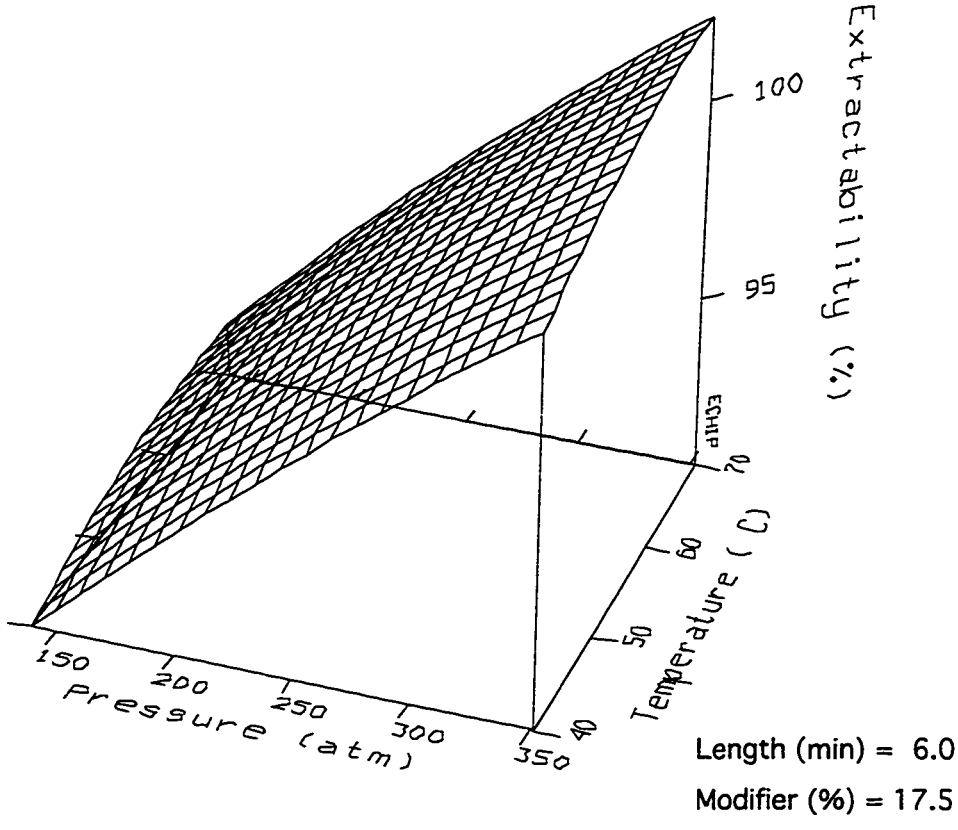
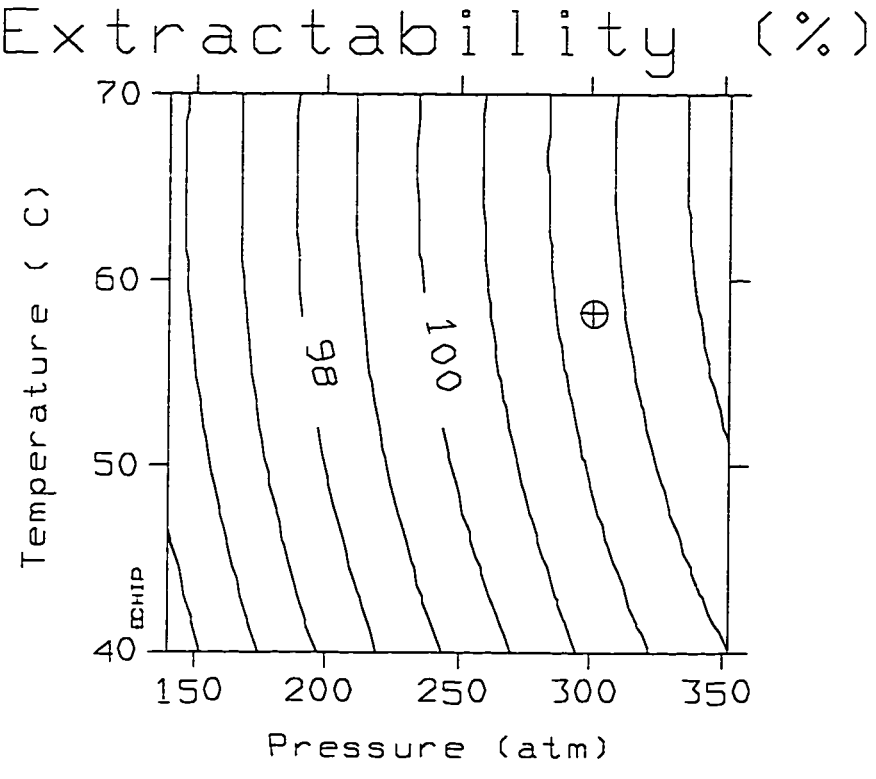



**Figure 2.6 SFE extractability of bensulfuron methyl from fortified Alliston soil (effect of pressure and temperature)**

Unfortunately, it was impossible to recover aged residues from heterogeneous soils within a relatively short time for the extraction. According to preliminary studies, the initial extraction rate would reflect representative extraction efficiency for the entire process. Therefore, optimization experiments were conducted based on the initial three extractions with an assumption that accelerated SFE would yield better recovery.

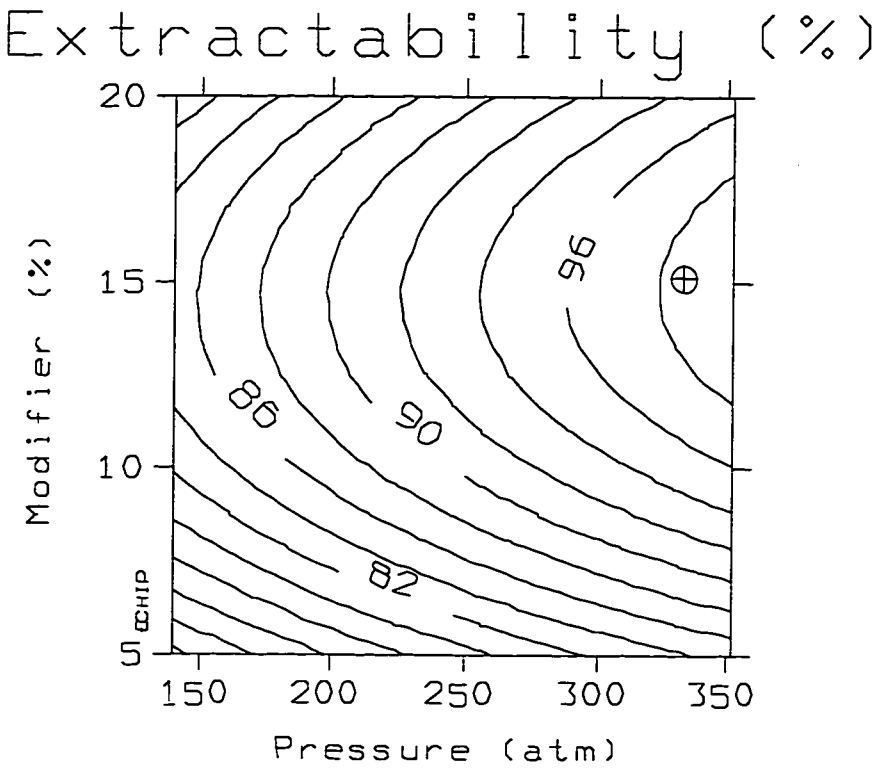
Temperature seemed to have significant effects on the recovery of field aged residues. Recovery increased significantly from 76 to 86% (diuron, Figure 2.11) when elevating temperature from 40 to 100°C for 20 minutes, and from 69 to 88%, (atrazine, Figure 2.14) with an increase in temperature from 40 to 150°C for 20 minutes. However, elevated temperature would not be applicable for thermally labile compounds such as bensulfuron methyl.

For the field aged residues, SFE extraction kinetics seemed to heavily relate to mass transfer or diffusion controlled process, therefore solubilization of analyte in the supercritical fluid was no longer an important parameter for better extraction rate and efficiency.




| Pressure=301 atm | | Temperature=58°C |
|---|---|---|
| Value | Low Limit | High Limit |
| 102 | 94 | 111 |

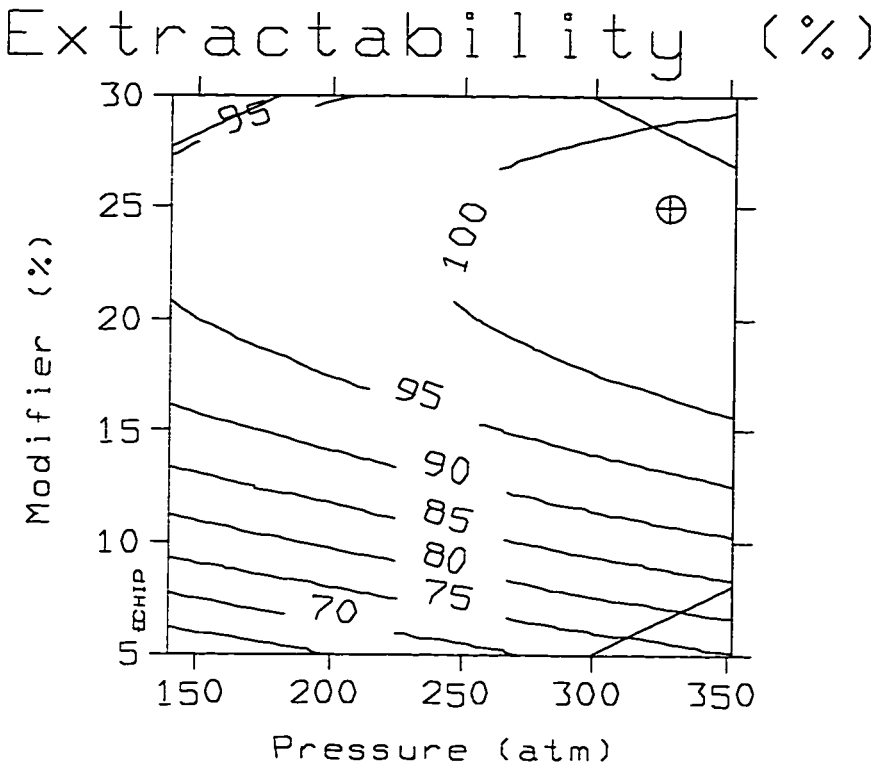
**Figure 2.7 2-D optimization for bensulfuron methyl from fortified Alliston soil (effect of pressure and temperature)**




Temperature (°C) = 70 Length (min) = 6

| Pressure=333 atm | | Modifier=15 % |
|---|---|---|
| Value | Low Limit | High Limit |
| 98 | 86 | 111 |

**Figure 2.8 2-D optimization for fortified atrazine from Alliston soil (effect of pressure and modifier)**




Length (min) = 5.5 Temperature (°C) = 65

| Pressure=328 atm | | Modifier=25 % |
|---|---|---|
| Value | Low Limit | High Limit |
| 103 | 96 | 111 |

**Figure 2.9 2-D optimization for bensulfuron methyl from fortified Alliston soil (effect of pressure and modifier)**

**Table 2.4 Effect of SFE parameters on the extractability of aged atrazine from Alliston soil using the MOS**

| Trail # | T (°C) | P (atm) | Length (min) | Modifier (%) | Recovery (%) |
|---|---|---|---|---|---|
| 10 | 150 | 140 | 10 | 17.5 | 75 |
| 12 | 40 | 140 | 30 | 17.5 | 42 |
| 3 | 150 | 140 | 30 | 5.0 | 82 |
| 4 | 40 | 352 | 30 | 5.0 | 54 |
| 16 | 95 | 140 | 10 | 30 | 75 |
| 14 | 150 | 246 | 20 | 30 | 83 |
| 17 | 95 | 352 | 10 | 5.0 | 74 |
| 13 | 95 | 246 | 30 | 30 | 86 |
| 1 | 40 | 140 | 10 | 5.0 | 35 |
| 8 | 40 | 352 | 30 | 30 | 53 |
| 5 | 40 | 140 | 10 | 30 | 43 |
| 2 | 150 | 352 | 10 | 5.0 | 80 |
| 2 | 150 | 352 | 10 | 5.0 | 80 |
| 20 | 40 | 246 | 10 | 30 | 39 |
| 4 | 40 | 352 | 30 | 5.0 | 54 |
| 11 | 40 | 352 | 10 | 17.5 | 46 |
| 1 | 40 | 140 | 10 | 5.0 | 35 |
| 19 | 40 | 246 | 30 | 5.0 | 41 |
| 15 | 95 | 352 | 20 | 30 | 80 |
| 6 | 150 | 352 | 10 | 30 | 79 |
| 3 | 150 | 140 | 30 | 5.0 | 82 |
| 18 | 150 | 140 | 20 | 5.0 | 79 |
| 9 | 150 | 352 | 30 | 17.5 | 90 |
| 5 | 40 | 140 | 10 | 30 | 43 |
| 7 | 150 | 140 | 30 | 30 | 88 |

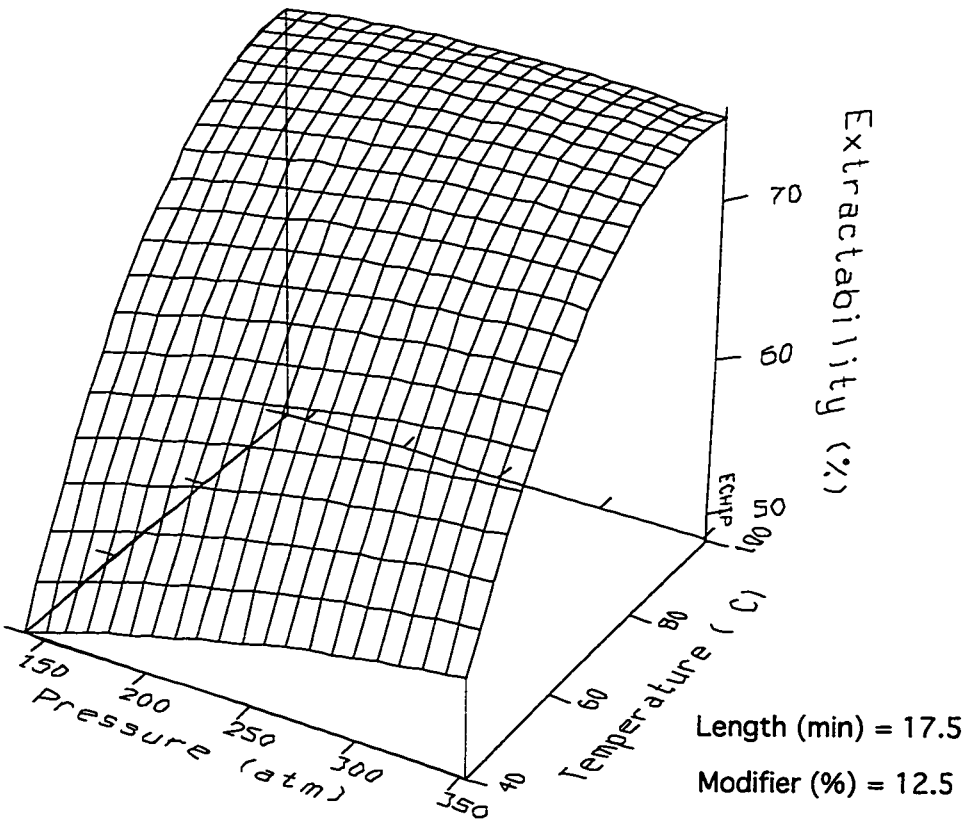



**Figure 2.10 SFE extractability of diuron from aged Danish soil (day 100) (effect of pressure and temperature)**

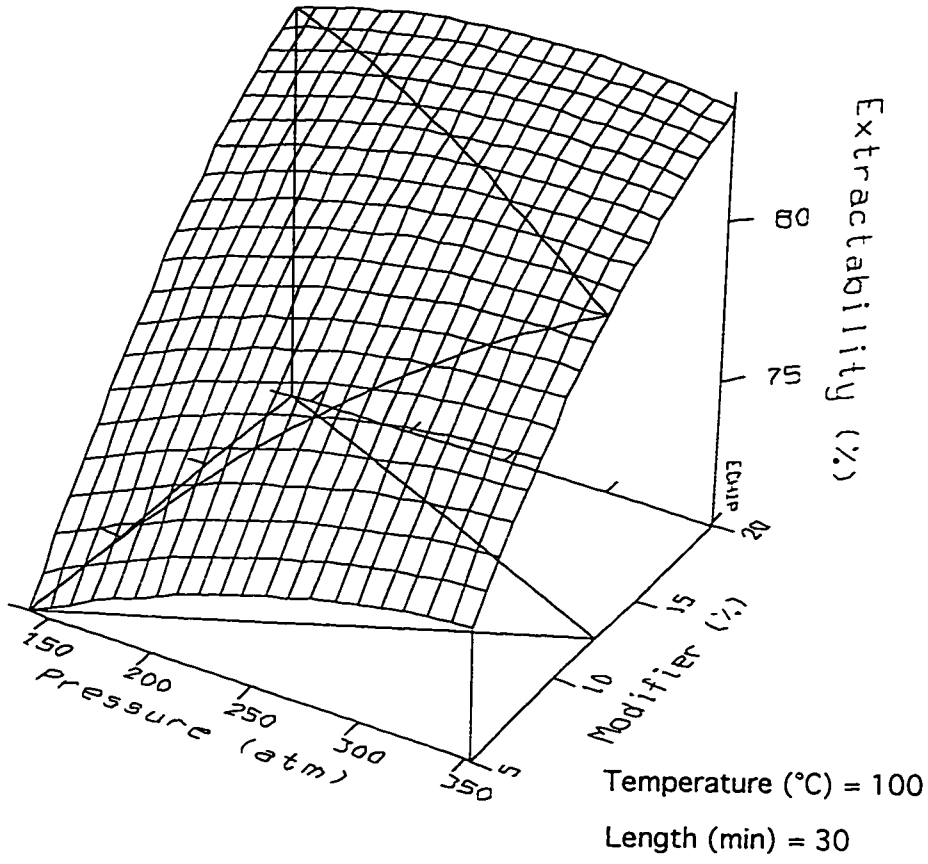



**Figure 2.11 SFE extractability of diuron from aged Danish soil (day 100) (effect of pressure and modifier)**

Using initial static and followed by dynamic extraction, more than 80% of aged atrazine residues were extracted from Alliston soil within 20-30 minutes (Figure 2.13). Elevation of temperature was crucial to recovering the "bound atrazine". Addition of modifier was also significant for the extraction. Among the tested modifiers, use of surfactant yielded the highest recovery (especially when temperature was increased to 150°C), while other modifiers (such as acetonitrile and methanol) were not as effective as surfactant addition.

Some proponents of the static method believe that the long exposure to solvent allows the matrix to swell, thus improving the penetration of carbon dioxide into its interstices and increasing analyte recovery. Those who prefer the dynamic method claim that continual exposure of analyte to fresh solvent enhances partitioning of the analyte into the mobile phase (Hawthorn et al., 1993). As with other SFE operating criteria, selection should be based on actual performance. It was observed that extended length of extraction for the static mode did not increase extraction rate and efficiency, but elevated temperature and repeated extraction with fresh modified SC-$CO_2$ would significantly enhance the recovery of diuron and atrazine.

Prewetting the matrix sample with modifier and proceeding with initial static extraction followed by dynamic extraction yielded a faster extraction rate than either static or dynamic extraction only. This simply allows the matrix to swell, resulting in better exposure to supercritical fluid and modifier, especially in the case of extracting native or bound residues. This demonstrated that the mode of SFE (either dynamic or static) and length

of extraction are crucial in recovering bound residues, while pressure is no longer an important parameter for the extraction. This also suggested that mass transfer or the slow diffusion process is determining factor for the extraction.

As discussed earlier, the importance of operational parameters for SFE would vary from case to case depending upon the analyte to be extracted as well as the nature of residues in the soils (i.e., residence time and type of soils). As indicated in Figure 2.8, an increase in $CO_2$ density (increase in pressure) was very effective in extracting freshly fortified atrazine. However, it was not as significantly effective as for the aged atrazine (Figures 2.13-14)

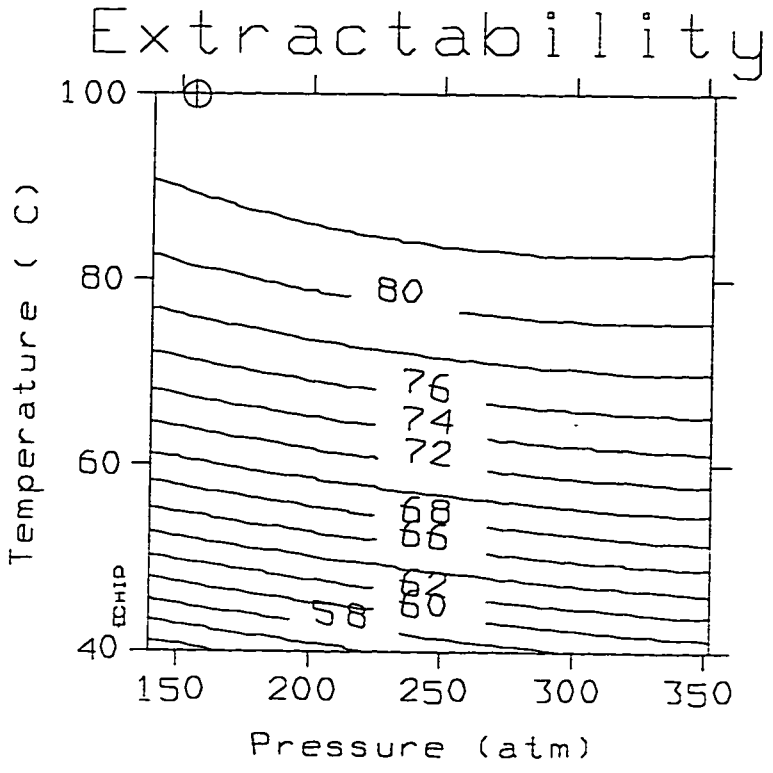
Addition of modifier seemed to enhance recovery in all cases, but excessive amounts of modifier resulted in detrimental effect. This could be due to the oversaturated liquid which would affect supercritical density and interaction with the matrix and analyte.

Interestingly, the significance of the variables was different in recovering aged residues from soil samples, compared to what were found in recovering freshly fortified samples. It was assumed that recovering aged residues would be predominated by mass transport or diffusion control processes. An increase in supercritical fluid density did not enhance the extraction efficiency. However, an increase in temperature considerably improved the extraction rate and recovery.

MOS offered the opportunity to systematically and simultaneously examine the interaction and effect plots among important soil variables and

extraction parameters. MOS is a highly efficient technique for studying a large number of variables and identifying optimal extraction conditions. Pesticide residence time had a major influence on binding processes for all tested pesticides. Extractability as a function of soil composition was greatly dependent on the particular pesticide examined. Effects of SFE parameters on extractability appeared to depend on the nature of residues (e.g., freshly fortified versus aged residues). For freshly fortified samples, analyte solubility in supercritical fluid and/or modified supercritical fluid was the critical factor as indicated by the strong influence of pressure on extraction efficiency. For aged samples, temperature was an important determinant of extraction efficiency, indicating that mass transfer or diffusion processes were rate-limiting. The presence of modifier and extraction duration also significantly impacted extractability of aged pesticides.

More evaluation and discussion on the analytical results will be elaborated in Chapter 3.




Length (min) = 28 Modifier (%) = 30

| Pressure=155 atm | | Temperature=100 °C |
|---|---|---|
| Value | Low Limit | High Limit |
| 83 | 77 | 89 |

**Figure 2.12 2-D optimization for aged diuron (day 100) (effect of pressure and temperature)**

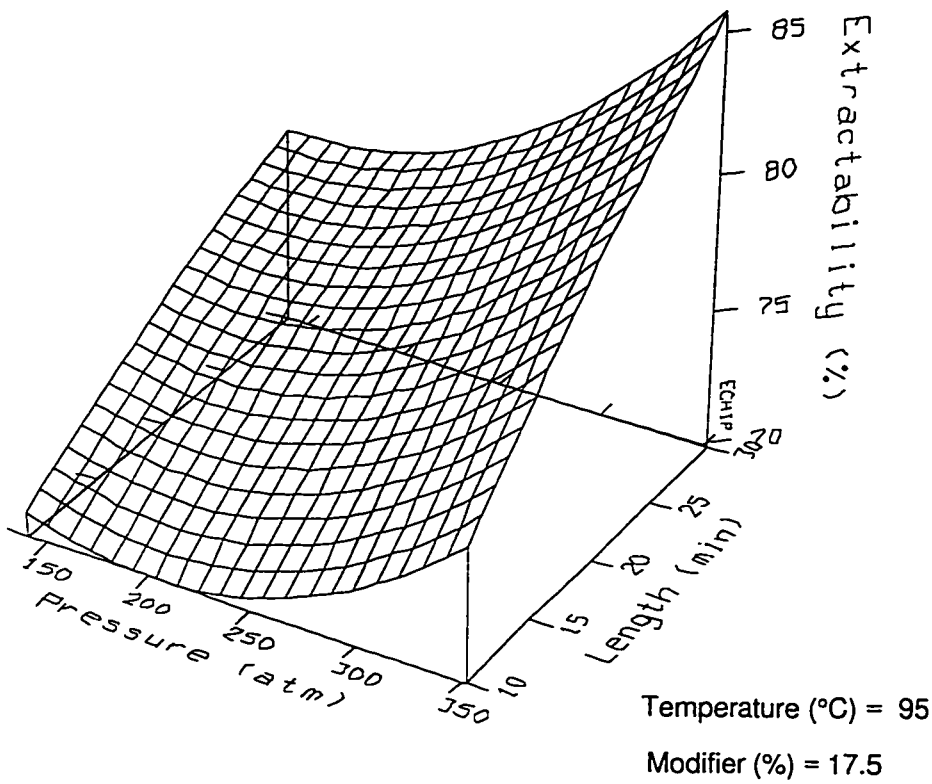



**Figure 2.13 SFE extractability of atrazine from aged Alliston aoil (3.5 years) (effect of pressure and length)**

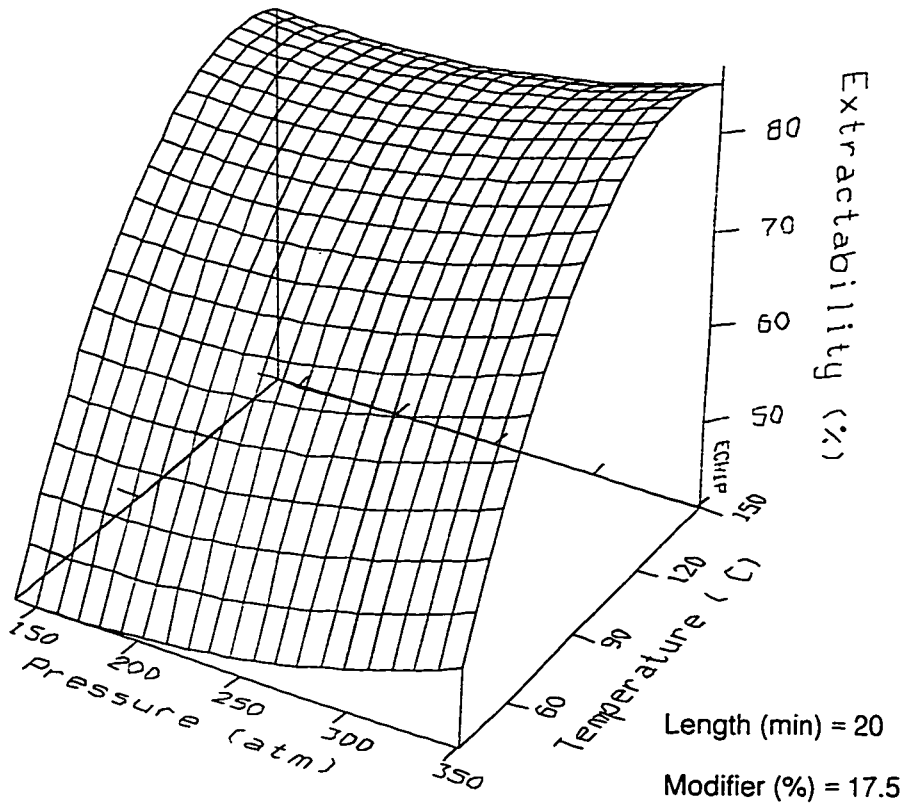



**Figure 2.14 SFE extractability of atrazine from aged Alliston soil (3.5 years) (effect of pressure and temperature)**

### 2.3.5 Identification and Semiquantification of Potential Degradation Compounds from the SFE Studies

To identify potential degradation products due to SFE procedures, fortified samples were conducted under identical conditions, followed by SFE. It was observed that no degradation products were found under the conditions specified in the SFE procedures. However, the thermal degradation products were observed as homosaccharin, pyrimidine amine and FA-DPX-F5384 for bensulfuron methyl at an elevated temperature of 80°C with both the soil and silica matrices (Appendix IV, Figure A.1).

With the homogeneous materials of silica and Celite®545 as sample matrices, diuron was decomposed to 3,4-dichloroaniline (DCA) and an unknown peak, which appeared to be a potential dimer of DCA, tetrachloroazobenzene (TCAB or TCAOB) at a temperature above 120°C during the SFE extraction (Appendix IV, Figure A.2). Dimer formation was not confirmed by MS. There was no thermal degradation in the presence of soil under identical SFE conditions. It seemed that both silica and Celite® 545 could facilitate the degradation and polymerization reactions.

No thermal degradation was observed for atrazine, while temperature was elevated up to 150°C, which is the upper limit for the SFE system (Appendix IV, Figure A.6).

These results indicated that the conditions used in the SFE were appropriate and analytical results regarding degradation profiles for field

aged samples were reliable, accurate, and valid. However, experimental results clearly indicated that the characteristics of analytes must be considered in the process of optimizing SFE parameters for better extractability of aged residues.

# Chapter 3

# PESTICIDE RESIDUES IN SOILS AND EXTRACTION METHODOLOGIES

## 3.1 Introduction

### 3.1.1 Bound Pesticide Residues

A definition of what was considered to constitute residue in soil was published in the US Federal Register (1975) and discussed in more detail by Kaufman (1976). This referred to binding of the pesticide residue only with the humic fractions of soil and since we now believe that residues may also bind to clay and clay-humin fractions this definition has since been superceded. Alternative definitions have been proposed from time to time by Khan (1982), Klein and Scheunert (1982), Kearney (1982), and by Fuhr (1987). There are all similar and based on the extraction rate and efficiency of the bound residue using either extraction methods commonly used in residue analysis or methods that do not significantly change the nature of the residues. In all these definitions unextractable residues, which result from the incorporation of $^{14}CO_2$ and small fragments recycled through metabolic pathways leading to natural products, are excluded. The definition reported by Kearney (1982) represented the view of the Pesticide Commission of the International Union of Pure and Applied Chemistry (IUPAC).

Any definition of a "bound" residue based on defined extraction systems must be arbitrary and not necessarily well correlated with lack of biological availability. Since there is no single "bound fraction" or "complex", such as the lignin complex reported for the chloroanilines or the adsorbed residues of parents or metabolites of other pesticides, it has been suggested by Pillmoor et al., (1984) that bound residues in plants should be defined on a case by case basis. It may be that bound residues in soil could be defined as "residues of the intact pesticide or degradation products derived from it that are no longer able to exert their original biological activity to any significant extent and/or can not be extracted from the soil by extraction methods which do not degrade the compound unless such methods are able to destroy the soil structure without affecting the compound.

It has been known for more than 30 years that when some pesticides, or their degradation products, enter soil they become bound to the organic matter or clay mineral fraction of the soil (Bailey and White, 1964). In the bound state they are very difficult to remove/extract and characterize, and tend to lose their biological activity. Because of the difficulty in extraction and identification it was generally only possible to demonstrate the presence of these soil bound residues with the use of radiolabeled pesticides. Many of those pesticides formerly believed to be readily degraded and "lost" from soil, were later shown to form these bound residues, formerly undetected, and it became apparent that the concept of persistent and non-persistent residues needed reconsideration (Katan et al., 1976).

Bound pesticide residues were also shown to occur in plant and other biological material, as well as in soil, and the whole subject received its first public review in 1976 (Kaufman et al., 1976). Since that time many publications have appeared and it has become clear that bound pesticide residues in soil are formed to a greater or lesser extent with all classes of pesticides so far investigated, and with many compounds constitute the major part of the soil residue. The formation of bound residues of pesticides in soil and in plants has been generally reviewed by Klein and Scheunert (1982), Khan (1982), Roberts (1984), and Fuhr (1987). The regulatory aspects of bound residues in plants have also been considered by Kovcas (1986).

### 3.1.2 Sorption Mechanisms

The processes by which pesticides become bound to organic matter or to clay colloids have been discussed by Stevenson (1976) and White (1976), respectively. In general there are two broad mechanisms by which organic chemicals, and pesticides in particular, interact with the soil colloids and become bound, via sorption or by chemical reaction.

Sorption mechanisms have been discussed in detail by Bailey and White (1964, 1970) and by Hamaker and Thompson (1972). Sorption may be by purely physical means as with Van der Waals forces, or the attraction may be chemical in character. A common type of chemical adsorption is electrostatic bonding (coulombic forces). This is the attraction of an organic anion to metal ions in the soil colloids. Hydrogen bonding and co-ordination through an attached metal ion (ligand exchange) are also commonly involved

in the adsorption process. Humic substances also contain well demonstrated concentrations of free radicals, probably of the semiquinone type, and the existence of charge transfer complexes with pesticides capable of being ionized has been demonstrated in some instances (Senesi , 1981; Bartha and Hsu, 1976).

It is to be expected that more than one type of adsorption mechanism may be responsible for the binding, thus Van der Waals forces may be combined with electrostatic attraction or with hydrogen bonding, and in some instances the type and strength of binding changes with time (Stevenson, 1976; White, 1976). There are also indications that the same pesticide may be adsorbed both to organic matter and to the clay mineral fractions of soil, and that changes can occur with the gradual movement of pesticide residues from one matrix to another as time progresses. In the case of the s-triazines and substituted ureas the form of electrostatic binding is almost always associated, or aided by hydrogen bonding and in the case of ureas by physical forces (Hance, 1969).

In order to fully appreciate the wide variety of sorption phenomena for organic compounds on soil, it is important that one recognizes the heterogeneous nature of the soil (i.e., the makeup of organic matter and mineral matter in various proportions) in response to variable system conditions. It is known from previous studies that sorption of nonionic organic compounds by soil in aqueous systems is controlled mainly by the organic matter content of the soil (Goring, 1967; Hamaker and Thompson, 1972; Chiou et al., 1979; Karickhoff et al., 1979). By contrast, sorption by dry

and subsaturated soils from nonpolar organic solvents (Hance, 1965; Yaron and Saltzman, 1972; Chiou et al., 1985) is determined mainly by the mineral type and content.

Triazines can also adsorb to clay minerals presumably by similar mechanisms. In the case of the expanding lattice clay minerals, there is evidence from laboratory experiments that triazines and paraquat can be adsorbed within interlayer spacings (Weber, 1970; Knight and Denny, 1970). The s-triazine herbicides are also more strongly adsorbed regardless of soil pH. Furthermore, as divalent cations, they have the potential for reacting with more than one negatively charged site on soil colloids or with free radical sites (Ledwith and Woods, 1970; Khan, 1973).

Once pesticides enter soil it is difficult to determine the respective involvement of organic matter and clay in the binding process. Knight and Tomlinson (1967) oxidized the organic matter in a soil and showed that the adsorptive capacity of the soil for paraquat was not significantly altered suggesting clay minerals were the major adsorbents. However, no valid interpretation can be made of these results because of the probable release, or exposure of fresh clay adsorption sites brought about by the vigorous treatment of the soils. Furthermore paraquat and diquat form strong adsorption bonds to organic matter and indeed to plant foliage (Damanakis et al., 1970; Brian, 1967).

Acidic pesticides have a tendency to be adsorbed to cations on the organic exchange sites and also are aided by hydrogen bonding and Van der

Waals forces. Thus the phenoxyalkanoic acids, and other pesticides that contain an ionizable acid group, are adsorbed by these processes (Stevenson, 1976). The rapid deactivation of glyphosate by organic and mineral soils has also been attributed primarily to adsorption through metal coordination (Spankle et al., 1975a), although hydrogen bonding almost certainly plays a part. It has further been shown that phosphate in the soil competes with glyphosate for adsorption sites, suggesting that the phosphonic acid group is primarily involved (Spankle et al., 1975). Adsorption of acidic pesticides is also likely to be significant in allophane soils and soils rich in amorphous oxides which are believed to carry a net positive charge.

### 3.1.3 Chemical Interaction

The second main type of bound residue is that which is formed by chemical reaction of the pesticide, or more usually its degradation product, with natural organic substances in the soil (Stevenson, 1976; Bartha and Hsu, 1976). A stable chemical linkage is formed, and such binding would be expected to increase the persistence of the residue in soil, while causing it to lose its chemical identity. The substances most prone to condensation reactions with organic substance in soil are aromatic amines and phenols. Thus most of the chloroanillines liberated by partial degradation of the urea herbicides, acylanilides and phenylcarbamates form bound residues by chemical bonding to organic matter (Hsu and Bartha, 1976). Nitroanilline herbicides (Helling and Krivonak, 1978), parathion (Katan et al., 1976), trifluralin (Golab et al., 1979), diflubenzuron (Nimmo et al., 1986), and many other pesticides which are readily converted to aromatic amines are probably

chemically incorporated into organic matter by a similar mechanism, which may involve condensation with polyphenols or quinones with the synthesis of humic-like substances.

Similarly, phenolic or quinone residues formed by the partial degradation of many phenoxy herbicides, insecticides, and fungicides may react chemically with amino substituents in the organic matter or polymerize to become part of the soil humus (Stott et al., 1983). Unextractable radioactivity in soil, following the application of some compounds e.g., 2,4-D, has been attributed to the natural incorporation of $^{14}CO_2$ (Smith and Muir, 1980).

### 3.1.4 Extent and Nature of Bound Residues

Pesticide residues which become bound in soil may be the parent chemical or its degradation products. In many cases persistent soil bound residues result from both the parent and one or more degradation products. Klein and Scheunert (1982) and Khan (1982) have listed those pesticides which have been shown to form bound residues, ranging from a few percent to 90% of the applied chemical; typically they are between 20 and 70%. The data have been summarized by Khan and Dupont (1987). Information quantifying these bound pesticides soil residues is now quite extensive, with typical examples of various classes, summarized by Calderbank (1989).

The processes which restrict the amount of chemical available for binding are obviously degradation processes, such as chemical, photochemical, and metabolism on plants or weeds before the chemical

reaches the soil and mainly chemical and microbial decomposition within the soil. Some compounds form bound residues on plant materials which may subsequently reach the soil. In some instances, discussed by Weber (1970), there is evidence that adsorption to clay surfaces can accelerate chemical decomposition, but generally, adsorption and other binding processes markedly reduce degradation rates (Hamaker and Goring, 1976).

Raghu and Drego (1986) reported the formation of bound residues of lindane in five Indian soils under aerobic and flooded conditions with and without organic amendments. The bound residues varied from 5 to 35% after one year of application. Flooding of the soil reduced the concentration of bound residues from 28 to 10%. The amendment of flooded soil with green manure further decreased the amount of bound residues. Under both aerobic and flooded conditions more bound residues were associated with the fulvic acid fraction of the soil organic matter.

Synthetic pyrethroid insecticides have also been found to leave a considerable amount of bound residues (up to 63%) in soil (Zhang et al., 1984). In a recent study, rapid formation of bound residues of cypermethrin was observed in four different soils (Barooah, 1991). The bound residues in all the soils increased with time, irrespectively of their physicochemical properties. After 120 days of incubation, the highest level of bound residues was 54% in the Pantnagar soil with the highest organic matter treated with $^{14}$C-cis-cypermethrin.

Smith and Milward (1983) detected traces of bound residues of simazine in soils one year after application. Adopting non-destructive methods these authors showed that the herbicides, picloram and triallate, formed bound residues even in field-weathered soils. Similar occurrence and long persistence of bound residues of dinitroaniline herbicides in soil have also been reported (Golab, 1979). They found that after three years of application considerable amounts of trifluralin and oryzalin (35-38% of the applied $^{14}C$) remained as bound residues in soil.

It has been observed that persistent organochlorine pesticides form comparatively less bound residues in soil than the non-persistent organophosphate and other classes of pesticides. Accumulation of bound residues in soil increases with time. The bound residues may consist of the parent compounds and/or their degradation products (Agnihotri and Barooah, 1994).

### 3.1.5 Aging of Bound Residues

Although the adsorption process should, strictly speaking, be reversible in practice, few studies have been carried out on desorption, and those that have, indicate that desorption is almost invariably much slower (Hamaker and Thompson, 1972). The difficulty of desorption or extraction increases with time and also with soil processes such as drying and rewetting (Graham-Bryce, 1967). It is apparent that a portion of the chemical becomes more firmly held than the average. This is probably what has been referred to as the bound residue. However, bound residues appear to become even more firmly

bound with time and the whole process appears to be progressive, and is dependent upon the soil's dynamic condition.

There is abundant evidence that, with longer residence time in soil, bound pesticide residues tend to lose all biological activity and become even more resistant to degradation and extraction. This phenomenon has been referred to as "aging" of residues, and although it is of vital importance regarding the environmental significance of bound residues, is very poorly understood.

It seems likely that two main mechanisms are involved in the aging process, viz. a redistribution of chemical from weaker to stronger adsorption sites and/or slow chemical incorporation into the humin fraction. It is probable that chemisorption is involved in the continually strengthening adsorption process. Chemisorption is characterized by an initial rapid adsorption followed by a gradual increase in adsorption over several weeks or more. This may be interpreted as a rapid physical adsorption followed by the slow establishment of the stronger chemisorption bond. The nature of this bond is not well defined except that its strength approaches that of a true chemical bond (Hamaker and Thompson, 1972). For those chemicals which are strongly adsorbed initially, such as the bipyridylium herbicides, one would expect a much slower rate of transfer. Furthermore, if the occupation of strong bonding sites requires a steric or energy barrier to overcome, there would be even slower rates of redistribution of the adsorbed chemical.

It is generally accepted that polar molecules can substitute for water in the interlayer spacing of expanding layer minerals (Greenland, 1965). It has been demonstrated in model experiments with clay minerals that paraquat, diquat, and the triazine herbicide prometon were adsorbed by montmorillonite, and located within the clay lattice (Weber and Weed, 1968). Other triazine herbicides (Weber, 1970) and polar compounds such as picloram and parathion have also shown to be strongly adsorbed on montmorillonite, including the interlayer spacing (Biggar et al., 1978). In the complex heterogeneous environment of the soil all these molecules are predominantly adsorbed to organic matter and it is largely a matter of conjecture whether they could migrate to the interlayer spacing of expanding type clays. Nevertheless, this type of migration is theoretically feasible. Even with a strongly adsorbed molecule such as paraquat there is free exchange between the adsorbed molecules and the low concentration in the soil solution (Knight and Denny, 1970), and Burns and Audus (1970) showed, in laboratory experiments, that paraquat could slowly transfer through a dialysis membrane from adsorption sites on organic matter in the inside to inorganic components of soil on the outside. In another experiment (Damanakis et al., 1970), herbicidally active paraquat residues in a sphagnum peat soil were deactivated by the addition of a clay mineral. A very high level (1,700 mg/kg or above) of paraquat was used in both experiments which leaves the possibility of transfer of normal low residues in the real soil situation still open to question.

Despite the lack of convincing evidence it is clear that adsorption in soil is not necessarily dominated by one adsorption site or one mode of adsorption and it is clearly feasible and probable that changes in the type of adsorption could be responsible for the increasing strength of binding of pesticide residues with time.

The alternative main mechanism by which bound residues age is most likely a result of covalent bond formation, i.e., by chemical incorporation of the pesticide residue into the humin fraction of the soil. This type of interaction has been most commonly observed with degradation products of the urea and anilide herbicides and also phenolic products from the phenoxy herbicides. It may also occur with the parent polychlorophenol soil sterilants, disinfectants, and herbicides. Such incorporation will occur slowly as the molecules are incorporated by natural processes into the complex polymeric humin fractions of soil and may well be preceded by adsorption processes.

### 3.1.6 Capacity of Soils for Binding

As indicated earlier the clay and organic colloids of the soil have an extremely large surface area and can provide millions of square meters of active surface in the top centimeters of a single hectare (Ahlrichs, 1972). Most soils are capable of processing and adsorbing several thousand kilograms of natural organic material which enters many agricultural soils each year (Jenkinson and Rayner, 1977). Although some pesticides will reach soil already bound to plant material to some degree, most will enter soil, at least in part, in the solution phase. The environment of the soil provides large

expanses of solid-solution interfaces and the few kilograms of organic pesticides which are applied, or reach the soil should not present any additional problem for the adsorptive capacities of normal soils.

The cation exchange capacity of soils will clearly be of some relevance in estimating the quantity of basic or easily protonated pesticides capable of being adsorbed. Many organic chemicals of this nature can be adsorbed by clay minerals in quantities up to and beyond their cation exchange capacities (Bailey and White, 1970). Weber and Weed (1968) showed that the cationic herbicides, diquat and paraquat, could be adsorbed by montmorillonite and kaolinite to approximately the cation exchange capacity (CEC) values of the clays. However, the maximum paraquat adsorbed by seven soils, with highly variable clay contents, was found to range from 27% to 63% of their CEC values.

At this level of adsorption, however, there are considerable amounts of free pesticides in the soil solution in equilibrium with the adsorbed materials, and it would appear there is a gradation in binding strengths. The more weakly bound materials are phytotoxic to plants because of this higher concentration of chemical in the soil solution (Tucker et al, 1969). These authors characterized the binding as "loosely" bound if the chemical could be displaced from the soil with saturated ammonium chloride solution. The portion of the chemical which could not be removed by this procedure was referred to as tightly bound (Calderbank, 1989).

Furthermore, there is evidence that many pesticides are sorbed slowly. For example, Hamaker and Thompson (1972) quote data showing that the amount of atrazine adsorbed by some soils could be doubled over a period of 60 days. They pointed out that if the adsorbent particle has a small pore structure, or if the adsorbent process contains a very slow step, a steady state, which would be reached quite quickly, may be mistaken for the true equilibrium. Unless a study is continued long enough it may miss a slow drift in the apparent "equilibrium" with time as the rate limiting step is overcome. Paraquat, and probably other herbicides which are rapidly deactivated in soil, presumably reach some of the firm adsorption sites very quickly. Even with these chemicals, which are more easily studied than others, there is a lack of data on long term adsorption (Hamaker and Thompson, 1972).

It must be concluded that it is extremely difficult to predict the amounts of pesticide residues which soils are capable of binding and deactivating, but in any event, the level of bound residues is likely to be extremely low compared with the large amounts of natural organic substances entering soil each year.

### 3.1.7 Degradation of Bound Residues

Soil organic matter and natural organic chemicals, such as carbohydrates, protein, fats, and simpler substances released from dead microorganisms, plants, roots, and detritus, as well as the host of naturally occurring chemicals of unknown toxicity entering soil are subject to the same adsorption and binding processes as pesticides. It is well known that organic

matter becomes associated with clay (Greenland, 1965) and its association into microaggregates is difficult to separate (Edwards and Bremner, 1967; Jenkinson and Rayner, 1977). The latter authors classified organic matter fractions according to their half-lives which ranged from less than 1 year to almost 2,000 years. They considered that the fraction with a half-life of almost 50 years was physically adsorbed to the clay, while the fraction with the longest half-life was probably chemically bound to components of the soil. The authors point out, however, that none of the fractions were completely resistant to degradation.

It is well proven that some of the better defined naturally occurring chemicals also become adsorbed in soil and this process slows down their degradation. Thus mononucleotides, nucleic acids and nucleoproteins are adsorbed to clays and their decomposition is retarded (Goring and Bartholomew, 1952). Similarly the presence or addition of an allophanic material reduced the losses of glucose carbon by about 25% and of the Carbon of polysaccharides by 36 to 65% in normal agricultural soils (Zunino et al., 1982). Sorensen (1972) showed that $^{14}C$-labeled glucose and cellulose formed fractions in three soils which became resistant to degradation with half-lives of 5 to 9 years. Pinck and Allison (1951) also showed that the adsorption of organic compounds by clay minerals influences their availability to soil microbes.

It is not surprising then to find that the rates of degradation of organic pesticides in soil are also considerably reduced when they become bound to soil colloids. With radiolabeled atrazine 83% of the $^{14}C$ was still present in soil

9 years after its application and 50% of this residue, which included parent compound, represented bound material (Capriel and Haisch, 1983; Capriel et al., 1985).

A related experiment carried out over 20 years with unlabeled atrazine was unfortunately unable to account for the bound residue (Khan and Saidak, 1981). Khan (1982a) showed that another triazine, prometryn, formed a high proportion of bound residues as the parent compound one year after application. Glyphosate, which is degraded fairly rapidly in many soils (Ruepel et al., 1977), becomes very resistant to degradation when strongly bound in some volcanic ash soils and has a half-life of 22 years (Nomura and Hilton, 1977). Paraquat is readily degraded by many soil organisms in culture solution (Baldwin et al., 1966; Smith et al., 1976) or when weakly adsorbed (Burns and Audus, 1970) but is much more resistant to degradation when in the field soil environment (Calderbank, 1968; Riley et al., 1976). Paraquat bound residues in a sandy loam soil were calculated to have a half-life of about 6.6 years (Hance et al., 1980, 1985). Bound residues of many other pesticides have been detected in soil from 1 to 6 years after their application (Constenla et al., 1984; Smith and Muir, 1984; Hsu and Bartha, 1976; Stott et al., 1983), but in most cases it is impossible to assign half-life values to the bound residue because of the difficulty in quantifying it without the use of radiolabeled material. Bartha (1971) estimated that 3,4-dichloroaniline bound residues, derived from propanil, would have a residual life in soil of as much as 10 years.

Despite the high capacity of soils to bind the relatively small amounts of pesticide residues entering them, the question arises that if bound residues persist for many years could there be an indefinite and continuing build up of pesticide bound residues in soils, the so called "burdening" of soils? This seems improbable when considering the substantial evidence for the slow microbial decomposition of bound residues and the analogy with the turnover of natural soil organic matter, present in vastly greater quantities in soil (Sauerbeck, 1980).

Hamaker and Goring (1976) have discussed the kinetics of pesticide residue decomposition in soil when part of the residue becomes bound and relatively unavailable. They suggest that the formation of bound residues may be responsible for degradation reactions of pesticides in soil deviating from first-order kinetics and have proposed a model in which the labile (degradable) pesticide is in equilibrium with bound residues which are assumed to be unavailable for degradation. The model was used to estimate the residue which would accumulate from repeated additions. It was clear that there would be an accumulation, but only up to a steady state and the level of bound residue reached rapidly declined when the annual addition ceased. When the amount remaining was 10% of each annual application the accumulation was calculated to be 2 x first year residue.

It is reasonable to assume, based on current evidence that the adsorbed parent, or metabolite, is in equilibrium in trace amounts with the soil solution and microbially or chemically degraded and/or that the bound residue is available for slow degradation. There is certainly ample evidence for bound

residue degradation (Nomrua and Hilton, 1977; Khan and Ivarson, 1981; 1982; Hance et al., 1980, Rache and Lichtenstein, 1985).

Bearing in mind all these assumptions, it is apparent that bound residues will not accumulate indefinitely but will plateau when the amount being degraded each year will be equal to the quantity of new pesticide added to the soil each year (Fuhr, 1987).

### 3.1.8 Biological Consequences of Binding

When pesticides become bound in soil they tend to lose their biological activity (Kaufman et al., 1976) especially with longer residence time in soil; indeed many lose their chemical identity before the binding process (e.g., Bartha, 1971). Since bound residues undoubtedly persist in soil for long periods, concern has been expressed that these residues may be available for uptake by plants and have other consequences on soil life or fertility. Alternatively, that microbiological processes, changes in soil chemistry due to environmental changes, or changes in agricultural practices could release these materials into the soil solution. This might then allow their uptake into crop plants or their leaching into groundwater (Kearney, 1976).

The release and bioavailability of bound residues has been reviewed by Lichtenstein (1980), Khan (1982), Klein and Scheunert (1982), and more recently by Fuhr (1987) and Khan and Dupont (1987).

Weber (1976) classified bound residues according to whether they were fixed or biologically available, and by their ease of extraction rate and

efficiency from soil. When pesticides become bound by adsorption processes, rather than covalently bound to soil a proportion will always be available in the soil solution, depending on the amount of chemical applied and the strength of binding. Even strongly bound herbicides, such as paraquat and diquat which are normally not taken up from plant roots (Calderbank, 1968; Riley and Gratton, 1974), are in equilibrium with traces of free herbicide in the soil solution (Knight and Denny, 1970). Normally such low concentrations are either not adsorbed by the plant or are below the level of harmful physiological effect. Indeed weakly adsorbed paraquat is available in certain artificial situations for uptake by plants (Damanakis et al., 1970; Tucker et al., 1969). Several other pesticides have been examined for the uptake of nonextractable soil-bound residues by roots and the data have been summarized by Klein and Scheunert (1982). Small amounts of radioactivity were detected in the plants, but the amounts were generally below 1% of the total residue bound in the soil.

In carrying out uptake studies with plants it is sometimes difficult to ensure that all the extractable residue had been removed from the soil and only residues of bound material remain. Consequently it is feasible that these traces of radioactivity could have been derived from unbound material not completely removed. Alternatively, it could have originated from slow mineralization of the bound residue by microorganisms or the actual uptake of radioactive products bound in the soluble fulvic acid fraction.

The uptake of soil bound residues of methyl parathion by earthworms has also been demonstrated, but the residue remains bound in the worms (Fuhreman and Lichtenstein, 1978).

The relevance of these uptake studies to real life in field soils has been seriously questioned (Fuhr, 1987), and furthermore, there is no evidence that any of the absorbed radioactivity possesses biological activity. On the contrary, such direct experimental work that has been carried out, e.g., Lichtenstein et al. (1977) suggests that bound residues lose their original (parent) biological activity. There is also ample evidence that bound residues of pesticides on plant material are not absorbed to any significant extent by the digestive system of animals (Calderbank et al., 1968; Khan and Dupont, 1987; Akhatar, 1987).

Even if the original pesticide, or some noxious metabolite, could be released, all evidence suggests that microbial degradation of bound residues is a slow process. Consequently quantities of any such toxic material would be extremely small and subject to the normal, relatively rapid, degradation processes affecting "free" pesticides in the soil. In other words, the second degradative process would be expected to be faster than the first so that the toxic material would not be expected to accumulate to exert a significant effect. It is entirely predictable that the slow release of bound residues into soil solution will be of little toxicological or ecological concern in practice. The uptake of toxic trace elements from soil, such as cadmium. lead, mercury, and arsenic, into food crops should be of much greater concern and occupy relatively more attention (Wiersma et al., 1986).

### 3.1.9 Regulatory Perspective of Bound Residues

Clearly the important matter is not so much how the residue is defined but the question of its biological availability. Thus from a regulatory point of view, even if the parent chemical is non-detectable and regardless of whether the residue is extractable or not, it will still be necessary to establish the nature and extent of any persistent biological effects in the soil. Conversely, it should not be a matter of concern if the parent chemical is detectable but is in a firmly bound state and showing no significant biological effects, as discussed earlier.

A survey of EPA's current regulatory requirements related to bound pesticide residues cited in the Pesticide Assessment Guidelines, Subdivision O, Residue Chemistry, including evaluation of representative historical examples taken from this Agency's registration files, indicated the need to develop specific regulatory criteria for determining the nature and significance of bound pesticide residues in soil and other environmental samples. Accordingly, a suggested sequential testing approach addressing this need was developed to (a) differentiate bound residues and natural constituents and to (b) determine the regulatory significance of bound pesticide residues via a 5-step sequential tier approach. The latter tier approach evaluated the (1) location, (2) amount, (3) chemical nature, (4) toxicological significance, and (5) analytical methods of analysis of bound pesticide residues (Kaufman et al., 1976).

In summary, a significant proportion, which typically ranges from 20 to 70% of pesticides applied in agriculture, remains in the soil as a persistent

residue bound to the soil colloids. The bound residue may be the parent chemical or degradation product(s) or combinations of both. In only a few instances can the soil bound residue be fully characterized and monitored.

Two main mechanisms, viz surface adsorption processes and covalent bond formation, appear to be involved, with the latter process predominating in the binding of degradation products. Organic matter is largely responsible, at least in the initial binding processes, but certain expanding clays, which bind some pesticides more firmly than organic matter, may be involved.

With longer residence time in the soil, bound pesticide residues "age" i.e., as time progresses residues tend to become more firmly bound, more resistant to degradation and show little evidence of any biological activity. This may be the result of the migration of residues to less accessible binding sites which requires an energy barrier to be overcome and/or the slow chemical incorporation of residues into the humic fraction of soil.

### 3.1.10 Current Approaches for Bound Residue Analysis

Analysis of bound residues of pesticides in soil has always been a challenging problem. Using radiolabeled pesticides, it has been possible to show the presence of bound residues in soil as combustion of the extracted samples produced $^{14}CO_2$. The most frequently used method for analysis and quantification of bound residues of pesticides has been by total combustion of the solvent-extracted sample to convert bound $^{14}C$ residues to $^{14}CO_2$ which is trapped in basic solvents and radioassayed by liquid scintillation counting, leaving little scope for characterization of the bound residues. Alternatively,

strong acid or alkaline hydrolysis has been used for solubilizing bound residues. However, these drastic methods often result in destruction of the identity of the bound residues. Fortunately analytical methods are being improved upon and a few novel techniques have been developed like pyrolysis in nitrogen (Balba et al., 1979), high temperature distillation, HTD (Khan and Hamilton, 1980), or new solvent extraction methods involving boron trifluoride-methanol pretreatment of the sample and supercritical fluid methanol (Capriel et al., 1986) and supercritical fluid carbon dioxide (SC-$CO_2$). More recently it was reported that accelerated solvent extraction (ASE) became one of the alternatives for the extraction (Richter and Felix, 1994).

### 3.1.11 SFE as an Emerging Technique for Extracting Bound Residues

Using SFE, sample preparation can be done faster, cheaper, and more completely than can be achieved by conventional extraction procedures. SFE is not necessarily a panacea for all sample preparation problems, but it seems to be ideally suited for the extraction of pesticides from many matrices.

In general, SFE has been successfully applied to a wide range of environmental samples and numerous interesting results have been obtained. This new technique, still in its developmental stage, will probably become a routine sample preparation technique. Nevertheless, one needs to always bear in mind that "SFE must not be considered as a magic technique but as an important weapon in the analyst's armory" (Bartle and Clifford, 1991). A detailed review of its application can be found in Chapter 2.

## 3.2 Methods and Materials

### 3.2.1 Soxhlet and/or Sonication Extraction

Aged soil samples with atrazine, diuron, and bensulfuron methyl were extracted using Soxhlet procedures and sonication procedures to remove as much extractable analytes as possible prior to the supercritical fluid extraction (SFE). Soxhlet extraction and sonication extraction were similar to the methods cited in the EPA methods (Methods 3540 and 3550).

***Soxhlet extraction***- Duplicate soil samples (0.5-2 g) were extracted initially in methanol/water (9/1 v/v) for 2 hours, followed by two extractions in methanol, and finally a 24 hour methanol Soxhlet extraction. All extracts were combined and evaporated to almost dryness using TurboVap® at 50°C, brought up to an appropriate volume with either Atomlight® cocktail (LSC) or 1:1 (v/v) acetonitrile/water (LC) and analyzed by either LSC or LC-UV (where applicable).

***Sonication extraction***- Duplicate soil samples (0.5-2 g) were then extracted by sonicating in acetonitrile/acidic water with 0.1N HCl/surfactant (80/10/10 of 0.5% Triton® X-100) for 10 hours twice (2x10 hours). All extracts were combined and evaporated to almost dryness using TurboVap® at 50°C, brought up to an appropriate volume with either Atomlight® cocktail (LSC) or 1:1 (v/v) acetonitrile/water (LC) and analyzed by either LSC or LC-UV (where applicable).

### 3.2.2 Surfactant Extraction

Duplicate 10-g test soil aliquots were weighed into tared 250-mL centrifuge bottles. A stir bar and 80 mL of extraction solution (0.5% Triton® X-100, acidic water with 0.1N HCl/acetonitrile 10/10/80, v/v/v) was added to each test sample. The test samples were stirred with magnetic stirrers for approximately 2 hours at the greatest speed sustainable. The extracts were combined in a common TurboVap® flask by either filtering extract plus soil (0.45 μm), or by centrifugation separation of the extract and soil and filtering the decanted extract (0.45 μm). The combined extract was reduced to <5 mL in a TurboVap® at fan setting B (5000 rpm), transferred in acetone and water washes to a 25-mL volumetric flask, and diluted to volume in water. Extracts were stored frozen prior to analysis.

Equivalent aliquots (1-3 mL, depending on radioactive concentration) were transferred from each of the 2 extracts to a 50-mL graduated cylinder. 0.5-1 mL of calibration standard was added to the sample solution. The sample solution was diluted to 30 mL with water. The sample solution was filtered (0.2 μm) into a 50-mL polypropylene centrifuge tube prior to LC analysis. An HPLC system with a supplemental pump (Kratos) and switching valve (VICI) were used to determine the analytes of interest by UV-VIS (9065), radiochemical (Ramona 92) detectors, and on-line fraction collector (Foxy) (see a diagram in Appendix III). This was a collaborative study within DuPont.

### 3.2.3 Accelerated Solvent Extraction (ASE)

Duplicate soil samples were weighed (0.5-2 g) in an extraction cell (11 mL), and extracted in acetonitrile/acidic water with 0.1N HCl/0.5% Triton® X-100 surfactant (80/10/10, v/v/v) for 5 minutes of initial dynamic followed by 15 minutes of static extraction twice (with total volume of 2 x15 mL, 30 mL) at temperatures of 70°C, 100°C, and 150°C, and a pressure of 141 atm for bensulfuron methyl, diuron, and atrazine, respectively. The extract was then evaporated down to almost dryness by N-EVAP® at approximately 50°C using a water bath, and reconstituted into either Atomlight® cocktail (LSC) or 1:1 (v/v) acetonitrile/water (LC) and analyzed by either LSC or LC-UV (where applicable).

### 3.2.4 Supercritical Fluid Extraction (SFE)

Freshly fortified and/or aged soil samples were extracted using supercritical fluid extraction with both static and dynamic modes to determine which mode is better than the other. Then a multivariate optimization scheme was used to optimize SFE parameters for the extraction rate and efficiency studies (refer to Chapter 2 for details).

0.25-1 gram of aged soil samples were weighed directly in the extraction cell. Modifier(s) (methanol/acidic water with 0.1N HCl 80/20 v/v; acetonitrile/acidic water with 0.1N HCl 80/20 v/v; 0.5% Triton® X-100/acetonitrile/acidic water with 0.1N HCl 10/80/10 v/v/v) were added to samples prior to SFE extraction, or by pump delivery during the extraction (5-30% modifier to $CO_2$). SFE temperatures were 70°C, 100°C, and 150°C with

pressure at 352 atm for bensulfuron methyl, diuron, and atrazine, respectively. Both dynamic and static modes of extraction were evaluated for further studies. Extended extractions (from 5 minutes to 30, or 60 minutes in some cases) were conducted to recover as much bound residues as possible. For extraction rate studies, extracts were collected at predetermined intervals (every 5 minutes for dynamic and static extraction). Extracts were collected in acetonitrile, and evaporated to almost dryness using N-EVAP® at approximately 50°C, using a water bath and reconstituted into either Atomlight® cocktail (LSC) or 1:1 (v/v) acetonitrile/water (LC) and analyzed by either LSC or LC-UV (where applicable).

### 3.2.5 Extraction Rates/Efficiency by SFE

To understand desorption/extraction kinetics, both homogeneous material (Celite® 545) and heterogeneous soil samples were studied. In the experiments, freshly fortified and aged samples were examined and evaluated using dynamic and static SFE methods. In dynamic extraction, extracts were collected and analyzed at a predetermined interval (every 5 minutes) and up to 60 minutes. In a similar manner, static extractions were proceeded for every 5 minutes and repeated extractions up to 60 minutes. Note that additional interaction time in the process of extract collection was not counted for 5 minutes, usually ranging from an additional 5-10 minutes for the repeated static extraction. As a result, extraction/ interaction time would be longer for the repeated static extraction than the dynamic extraction.

### 3.2.6 Fortified and Aged Pesticide Residues in Different Soils

Samples with atrazine (aged) and without atrazine (control) were obtained from the Canadian Agricultural Research Center (Ottawa). Samples with diuron and bensulfuron methyl (aged) and without diuron and bensulfuron methyl (control) were obtained from some DuPont studies. Samples with freshly fortified analyte(s) were prepared during the course of this study.

Celite 545® as a homogeneous material was chosen for freshly fortified and aged experiments under laboratory conditions (stored in a refrigerator at approximately 4°C) for investigation, which compared to freshly fortified and aged soil heterogeneous samples under identical conditions.

### 3.2.7 Identification and Semiquantification of Potential Degradation Compounds from the Field Studies

The test soil samples were extracted using surfactant extraction (see Chapter 3 for details), filtered with 47 mm Nylaflo® 0.45 μm Nylon membrane filters, concentrated on TurboVap® (Zymark Corp.), centrifuged at 6000 rpm (Damon/IEC Division and Sorvall Instruments® Model RC5C centrifuge), and filtered through 0.2 μm syringe filter (Acrodisc®) prior to HPLC analysis.

The HPLC system consists of a supplemental pump (Kratos) and switching valve (VICI) which were used to inject 20 mL of the prepared 30 mL extract solutions on an analytical column at 2 mL/min during the initial 10 minutes of the HPLC analysis. At 10 minutes, the switching valve was rotated

to direct the mobile phase from the analytical pump (9010) to the analytical column for chromatographic separation. UV-VIS (9065) and Radiochemical (Ramona 92) detectors were on-line, and fractions were collected (Foxy) for LSC analysis during the entire HPLC run. Approximate delays of 0.4 minutes and 1.5 minutes from the UV-VIS were observed for the Ramona and Foxy, respectively. The analytical conditions follow: Injection volume: 10 minutes @ 2 mL/min column loading with Kratos pump; Mobile phase: gradient from 2% to 20% methanol in 10 minutes, to 55% in 4 minutes, hold for 7 minutes, and then to 100% in 11 minutes, aqueous with 0.2% formic acid at a flow rate of 1.0 mL/min. Other parameters for Ramona 92 detector, Foxy fraction collector, and LSC are given in Appendix III.

The calculations used to determine extract recovery (dpm/g), residue recovery (dpm/g), extraction efficiency (% of the bound residue), the % AR in surfactant extracts, and LC recoveries (% of total dpm, and % AR) were mentioned in Chapter 3. Results are rounded at the end of the calculation.

Representative MS source conditions were: temperature: 250°C; thermospray probe temperature: 100°C SIM acquisition: PDMU: m/z 136.9, 177.9; CPDMU: m/z 170.9, 212; CPMU: m/z 184.9/186.9; DCPMU: m/z 218.9, 221; Diuron: m/z 205, 207, 233; ODM-DPX-F5384: m/z 91, 141, 149; Pyrimidine amine: m/z 124, 155; Sulfonamide: m/z 149, 230; Homosaccharin: m/z 104, 198; DPX-F5384: m/z 91, 141, 149; Hydroxyatrazine (HAT): m/z 198, 239; desethylatrazine (DEA): m/z 188, 229; Atrazine: m/z 216, 257.

### 3.2.8 Chemicals and Reagents

Analytical standard of diuron, N'-(3,4-dichlorophenyl)-N'-methylurea (IN-15654-12, 99.9% pure), (3,4-dichlorophenyl)-urea(IN-R915-7, 98.4% purity), N'-(3-chlorophenyl)-N,N-dimethylurea (IN-12894-4, 99.1% purity), N-(3-chlorophenyl)-N'-methylurea (IN-15454-1, 99.9% purity), and 3,4-dichlorobenzenamine (IN-17239-10, 99.3% purity) plus a qualitative standard (3-chlorophenyl)urea (IN-74955-0), bensulfuron methyl (IN-F5384-83, 98.7% purity), sulfonamide (IN-N5297-2, 96.3 % purity), homosaccharin (IN-B6895-1, 97.8% purity), pyrimidine amine (IN-J290-3, 95.7% purity), O-desmethyl bensulfuron methyl (IN-F7880-1, 89.5% purity), hydroxypyrimidinal bensulfuron methyl (IN-N8989-1, 92.3%), atrazine (IN-Y0150, 100.0% purity), de-ethyl-atrazine (92.7% purity), 2,6-diamino-atrazine (IN-W0625, 98.7% purity) and 4-hydroxy-2,6-diamino-atrazine (IN-C4974, 98.8% purity) were synthesized by DuPont Agricultural Products, E. I. du Pont de Nemours and Company. The structures of the compounds are shown in Appendix I.

All water (distilled and deionized) were obtained from a Milli-Q®-water purification system (Millipore Corp. Milford, Mass). Chemical reagents were Fisher reagent grade, ACS grade, and HPLC grade where applicable.

## 3.3 Results and Discussion

### 3.3.1 Soxhlet, Sonication, and Accelerated Solvent Extraction

Several aged soil samples were extracted by Soxhlet, sonication, surfactant extraction, and accelerated solvent extraction (ASE) methods.

These results, given in Table 3.2, were compared to the optimal SFE data. Among the conventional methods, the sonication method yielded better recovery than the Soxhlet method, especially for the aged diuron and atrazine. Surfactant extraction gave additional extraction efficiency for the aged samples with diuron and atrazine. It was observed that surfactant extraction did not increase the extraction rate and efficiency significantly for bensulfuron methyl, probably due to a possible different sorption mechanism and pH dependent characteristics for this particular analyte, compared to atrazine and diuron.

Accelerated solvent extraction (ASE) has become commercially available recently, and was experimentally tested in the study for comparison. The ASE method is based on the adjustment of known extraction conditions to higher temperatures and pressures. It accelerates the desorption of analytes from the sample surface and their dissolution into the solvent. The ASE method yielded better extraction efficiency than those conventional methods, with a considerably short time and less solvent involved.

With the aid of surfactant-addition in the extraction solvent, ASE provided better recovery than the conventional solvent extractions mentioned above (methanol/water or acetonitrile/water), for all aged samples tested. In the ASE extraction, elevation of temperature creates subcritical phases for the extraction solvent, resulting in an increase of solvating power and solubility of analyte into the extraction liquid. This novel technique is promising to replace the conventional solvent extractions (Table 3.2).

### 3.3.2 Surfactant Extraction

For the extraction of aged diuron from Danish soils, either acidic or surfactant modifiers such as hydrochloric acid and Triton® X-100 were shown to be most successful, and their use resulted in better yield in recovery than conventional Soxhlet extractions. Soxhlet and sonication approaches have been widely used for extracting organic compounds from solids such as soil and sediment samples. Both of these methods are time-consuming and require the use of large volumes of solvent, which create disposal problems. The need for more-efficient and more-economical methods of sample preparation has generated considerable interest in supercritical fluid extraction as an alternative for extracting organic pollutants from solid matrices.

The radioactive concentrations (dpm/g) determined for the extracted soil residues were used to calculate the extraction efficiencies (refer to Table 3.1). The calculations used to determine extract recovery (dpm/g), residue recovery (dpm/g), extraction efficiency (% of the bound residue), the % AR in surfactant extracts, and LC recoveries (% of total dpm, and % AR) were as follows:

**(% of Total dpm)** = The % of DPM of TOTAL sum for peak fractions

$$\textbf{(\% AR)} = \frac{\text{DPM\% of TOTAL sum for peak fraction x Extract \%AR}}{100}$$

**Extraction efficiency (%)**

$$= \frac{\text{Extracted Residues (dpm/g) x 100}}{\text{Extracted Residues (dpm/g) + Unextracted Residues (dpm/g)}}$$

**Extraction efficiency (% AR)**

$$= \frac{\text{Bound Residues (\%AR) x Extraction Efficiency (\%)}}{100}$$

Significant non-extractable residues, 27-44% of the applied radioactivity (% AR), were detected in the loamy sand and sandy loam soil samples from a diuron study. This supplemental study was conducted to provide additional experimental data for the characterization of soil bound residues using surfactant extraction techniques on previously extracted test soil samples generated in the original study.

Test soil samples were supplied by Inveresk Research International, Ltd. (Scotland). The test soil samples came from 4 of 5 incubation groups in the original study and are representative of all soil types used. The samples received for analysis in this supplemental study are listed in Table 3.1.

These Danish soil samples were initially extracted in methanol:water (9:1, v:v), followed by 1-2 extractions in methanol, and finally a 24 hour methanol Soxhlet extraction if residue recoveries were below 90% of the applied radioactivity (% AR). Bound residues, up to 44% AR, remained in the test soil samples by the end of the 100-day testing period. These soil samples were reextracted using the surfactant extraction procedure described above. Extraction efficiencies ranged from 41.9%-98.0% of the bound residues or 1%-22% AR. The highest recovery (98%, 1% AR) was from the 0-day Danish

sandy loam test soil sample. Diminishing recoveries were observed with increased sampling interval. The recoveries from the 100-day test soil samples (the longest sampling interval) ranged from 49.4%-58.2% of the bound residues or 8%-22% AR.

**Table 3.1 Recovery of bound residues for diuron from different soils using surfactant extraction**

| Soil Description | Sampling Interval (days) | Bound Residue (% AR[a]) | Extraction Efficiency (%[b]) |
|---|---|---|---|
| Swedish loamy sand | 100 | 35.5 | 49.8 |
| Danish sandy loam | 100 | 25.7 | 58.2 |
| Danish sandy loam | 0 | 1.4 | 98.0 |
| Danish sandy loam | 32 | 15.0 | 51.9 |
| Danish sandy loam | 64 | 22.5 | 41.9 |
| Danish sandy loam | 100 | 44.7 | 49.4 |

[a] % of the Applied Radioactivity

[b] % extraction efficiency

The extraction recovery for the 100-day Swedish loamy sand test soil was 49.8% of the bound residue or 17.7% AR. The surfactant extraction increased the total extract recovery from 51% to 69% AR. Diuron was determined to be 11% of the total dpm in collected fractions or 1.9% AR. DCPMU was determined to be 13% of the total dpm in collected fractions or 2.3% AR. DCPU was determined to be 17% of the total dpm in collected fractions or 3.0% AR.

The extraction recovery for the 100-day Danish sandy loam test soil incubated at 20°C was 58.2% of the bound residue or 15.0% AR. The surfactant extraction increased the total extract recovery from 65% to 80% AR. Diuron was determined to be 36% of the total dpm in collected fractions or 5.4% AR. DCPMU was determined to be 30% of the total dpm in collected fractions or 4.5% AR. DCPU was determined to be 6% of the total dpm in collected fractions or 0.9% AR. The extraction recoveries for the Danish sandy loam test group ranged from 98.0% to 41.9% of the bound residue or 1.3% to 22.1% AR. The surfactant extraction recovery increased the total extract recovery for the 100-day sample from 45% to 65% AR. Diuron was determined to be from 85% (0 day) to 17% (100 day) of the total dpm in collected fractions or 1.1% to 3.7% AR, respectively. DCPMU was determined to be from 0% (0 day) to 18% (100 day) of the total dpm in collected fractions or 0% to 4.0% AR, respectively. DCPU was determined to be from 0% (0 day) to 14% (100 day) of the total dpm in collected fractions or 0.2% to 3.1% AR, respectively. More discussion is given in **Section 3.3.5**.

The surfactant extraction method used in these experiments extracted significant amounts of the bound residues from the test soil samples. Recoveries ranged from 98.0% to 41.9% of the bound residues for sampling intervals ranging from 0 to 100 days, respectively. These recoveries represent increases of up to 22 % AR and improved the range of total extract recoveries from 43%-95% AR in the original study to 65%-98% AR. The percent of bound residues extracted was inversely related to the sampling interval (longer sampling intervals yielded lower recoveries).

### 3.3.3 Supercritical Fluid Extraction

Preliminary experiments were conducted to determine whether the static or dynamic mode of SFE extraction would be employed for multivariate optimization scheme, which is elaborated in the Chapter 2. Both freshly fortified and aged analyte(s) were evaluated.

Releasing an analyte (pesticide) from a matrix (soil) requires a knowledge and understanding about the solubility of the solute, the rate of transfer of the solute from the sorbent to the solvent phase, and interactions of the solvent phase with the sorbent. These processes have frequently been illustrated with the extraction (or "desorption") triangle shown in Figure 3.1. In this case, pesticide(s) refer to the analyte, soil samples refer to the matrix, and SC-$CO_2$ or modified SC-$CO_2$ refer to the solvent. There are kinetics and physical/chemical interactions involved between matrix and analyte. Similarly, there are kinetics, physical/chemical interactions, and solvent/solute solubility involved between analyte and solvent. There are kinetics, swelling, and physical/chemical interactions involved between the matrix and solvent. Collectively, these factors control the effectiveness of the SFE process, if not extraction process in general. However, to obtain a better comprehension of their contributions, hypotheses for solubility, sorbent, and kinetic interactions must be considered individually.

Extraction rate and efficiency were performed on both homogeneous material (Celite® 545) and a heterogeneous sample (Alliston soil). Atrazine was fortified onto both matrices, and extracted after fortification and aging for

100 days. In general, no apparent difference was observed for the freshly fortified samples, but it became more difficult to extract the analyte from heterogeneous sample than from the homogeneous material (Figure 3.2). This suggested that additional energy of activation be required to overcome the barrier for desorption/release after aging. It is very likely to require even more energy to recover the analyte of interest after field aging through a weathering process.

Some researchers have reported that solubility does not completely explain recoveries of some analytes from complex matrices (Sunol and Chen, 1985; Peter, 1984; Kruckonis and McHugh, 1986). An understanding of the interaction of the modifier and supercritical fluid with the matrix is important to comprehend potential phenomena affecting extractions.

Among three modifiers (Methanol/acidic $H_2O$ 80/20 v/v; Acetonitrile/acidic $H_2O$ 80/20 v/v; 0.5% Triton® X-100 surfactant/acidic $H_2O$/Acetonitrile 10/10/80 v/v/v), modifier (Triton® X-100) yielded the highest recovery compared to the other two modifiers (Figure 3.3). The modifier of acetonitrile resulted in better recovery than methanol (Figure 3.4-3.5). Recovery was enhanced with an increase in the percentage of the modifiers (from 5 to 30%) for all cases. Recently more studies have been reported using a relatively higher percentage of modifier to improve extraction efficiencies, particularly for recovering polar contaminants (Khan, 1995). Many early studies had dealt with modifiers up to only 5-10%.

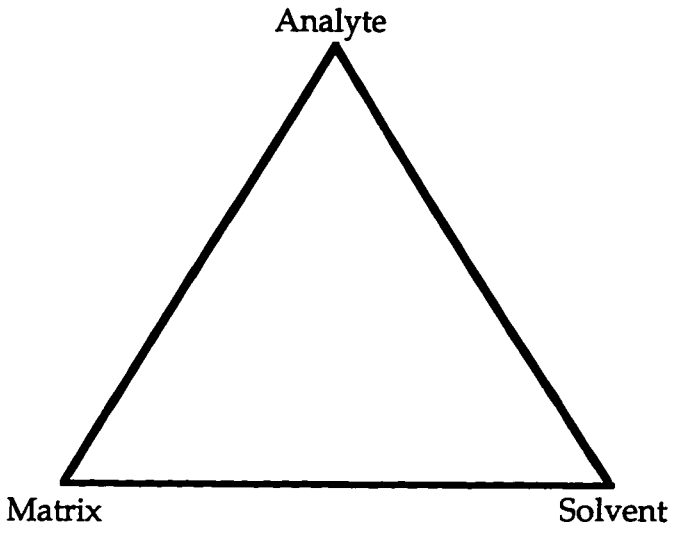



**Figure 3.1 Triangle relationships among analyte, matrix, and solvent in the SFE process**

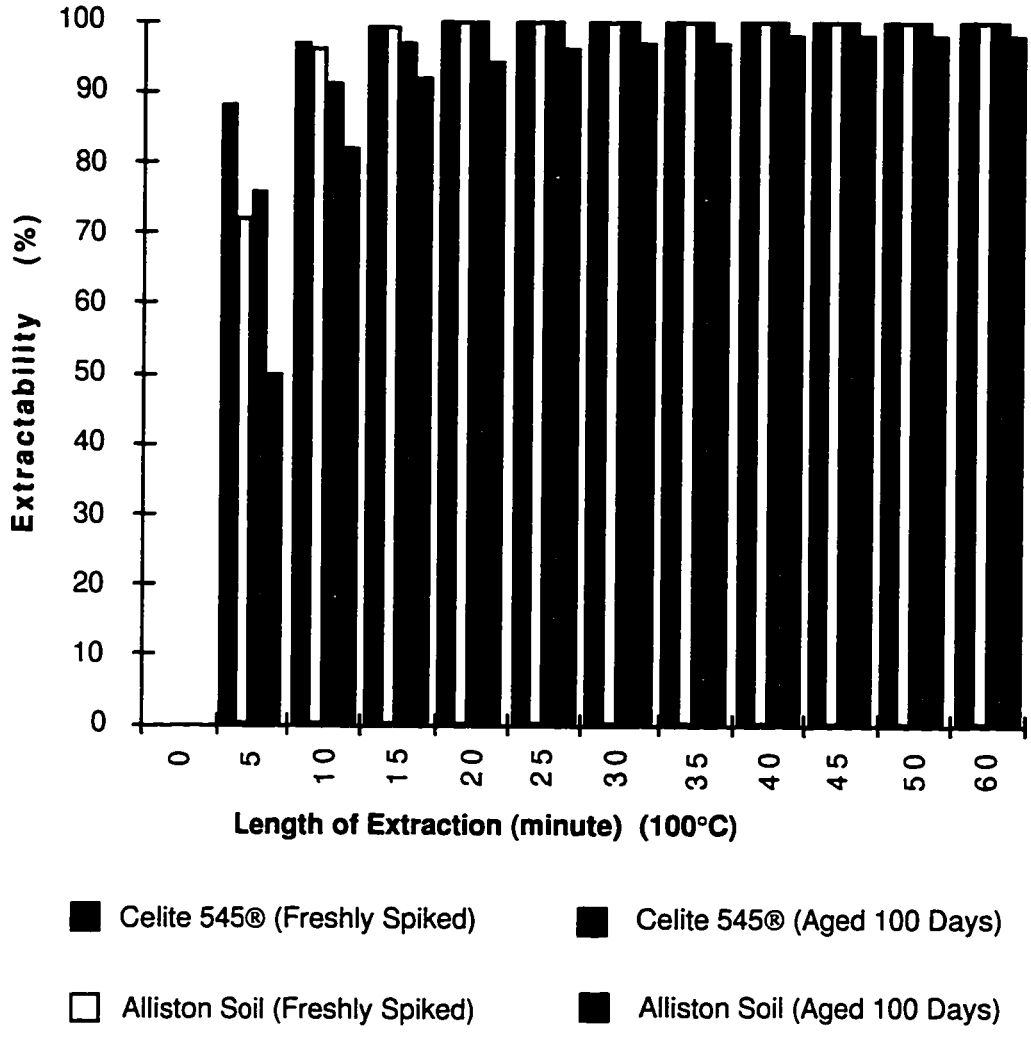



**Figure 3.2 Extraction rate/efficiency of atrazine from fortified and aged Celite® 545 and Alliston soil**

Unfortunately, since the role that the modifier plays in analytical-scale SFE of heterogeneous environmental samples is not well understood, choosing a modifier for an application has been highly empirical. Furthermore, other factors such as the effects of modifier concentration, the sample matrix, and the analyte type can complicate the choice. To date, very few investigations have focused on these parameters in modified SFE applications. In addition, modifier(s) commonly used are polar organic solvent(s) which may assist nonpolar supercritical fluid $CO_2$ to recover some polar analytes.

Langenfeld et al. (1994) reported that several modifiers were investigated to understand their role in analytical-scale supercritical fluid extraction of environmental samples. It was found that the modifier identity was more important than modifier concentration for increasing extraction efficiencies. Acidic/basic modifiers including methanol, acetic acid, and aniline greatly enhanced the extraction of PCBs. In general, increasing the modifier concentration from 1 to 10% (v/v) had little effect on PCB and low molecular weight PAHs recoveries, although the recoveries of high molecular weight PAHs from urban air particulate matter were enhanced significantly at higher modifier concentrations. Although there is no definite theory that explains modifier selection for SFE, it appears that modifiers should be selected on the basis of matrix characteristics and the target analytes.

Surfactant extractions have been reported for conventional extractions (Ishii et al., 1977; Hinze, 1992). At low surfactant concentrations above the critical micelle concentration (CMC), typically less than 15 wt%, micellar

solutions of nonionic surfactants can be induced in this range by varying the temperature. In some cases, surfactant micellar-rich phase will be formed and concentrated by hydrophobic organic components originally present in the sample subjected to the phase separation step. The factors that influence both the phase separation behavior and extraction efficiency were critically assessed (Hinze, 1992). In theory, the surfactant would compete with the organic (hydrophobic) in terms of binding sites in the matrix, and ultimately release organic contaminants in the matrix system.

Surfactants as a modifier to supercritical fluids have not been reported to date. Interestingly, a modifier of surfactant Triton® X-100 yielded much better extraction rates and efficiencies for most aged soils in the studies with one exception for bensulfuron methyl in the aged samples, which did not apparently enhance extraction rates and efficiencies. It was presumably associated with a different sorption mechanism (possible ion exchange and partitioning) for the analyte, which might be different from that for diuron and atrazine.

Fortified analytes are not exposed to the same active sites as the native analytes. Because of the heterogeneous nature of environmental samples, the partitioning step may be controlled by analyte solubility in the extraction fluid, kinetic limitations, and/or the ability of the extraction fluid to interrupt matrix analyte interactions. It is becoming increasingly clear that the high solubility of a particular species in the supercritical fluid is not a sufficient condition to yield high extraction efficiencies, and that the ability of the supercritical fluid to overcome matrix-analyte interactions is often more

important than the high solubility for achieving quantitative recoveries. The quantitative extraction of a particular analyte from an environmental sample can be viewed as a three step process: first, the analyte must be efficiently (and rapidly) partitioned from the sample matrix into the bulk supercritical fluid. Second, the analyte must be swept from the sample extraction cell. Finally, the analyte must be efficiently or quantitatively collected in a form that is compatible with the method used for analysis of the extract. Extraction rate/efficiency of homogeneous (Celite® 545) and heterogeneous (Alliston soil) materials resulted in differences in aged samples as indicated in Figure 3.2.

Supercritical fluid extraction (SFE) appeared to be a useful/alternative for pesticide desorption studies, particularly for those aged or weathered pesticide desorption processes. SFE has superior solvating power over other organic solvents, to release or "desorb" native or aged pesticide from soils. As a result, supercritical fluid $CO_2$ with or without modifier(s) may be used as a desorbing agent in desorption studies.

Two primary methods are commonly employed to measure sorption and desorption kinetics on soil/soil constituents-batch and flow. In comparison, two similar techniques can be performed to determine those kinetics using SFE-static and dynamic extractions. In this case, the static SFE is similar to the batch technique, while the dynamic SFE is comparable to the flow technique with one exception in that the SFE technique does not use stirred mixing, as is used in conventional kinetic methods. Either one has its own advantages and disadvantages. The types of reactions that are studied

and their relative time scales are important considerations in choosing a kinetic method. As known, some weathering and pesticide reactions in soils and on soil constituents, are slow, diffusion-controlled processes. In these systems, certain batch and flow methods would be quite satisfactory. Regardless of the kinetic method one chooses, controlling the temperature is imperative, simply because most reaction rates are strictly temperature-dependent. It is obvious that neither of them is a panacea for kinetic studies of heterogeneous systems such as soils.

**Table 3.2 Comparison of recovery of residues from soils using different extraction methods[a]**

| | Recovery (%) (n) | | | | |
|---|---|---|---|---|---|
| Sample[b] | Soxhlet | Sonication | Surfactant | ASE | SFE |
| Swedish Loamy Sand (Day 100) | 65 (2) | 70 (2) | 78 (2) | 83 (2) | 88 (2) |
| Swedish Sand (Day 100) | 86 (2) | 89 (2) | 92 (2) | 94 (2) | 96 (2) |
| Danish Sandy Loam (Day 0) | 74 (2) | 80 (2) | 87 (2) | 89 (2) | 96 (4) |
| Danish Sandy Loam (Day 100) | 55 (2) | 62 (2) | 75 (2) | 77 (2) | 88 (4) |
| Keyport Silt Loam (BM) | 70 (2) | 71 (2) | 73 (2) | 74 (2) | 80 (4) |
| Belhaven OM (BM) | 67 (2) | 68 (2) | 70 (2) | 69 (2) | 76 (4) |
| Alliston Sandy Loam (Yr 3.5) | 75 (2) | 77 (2) | 82 (2) | 84 (2) | 91 (4) |

[a] Methods were Soxhlet, Sonication, Surfactant Extraction, Accelerated Solvent Extraction (ASE), and Supercritical Fluid Extraction (SFE).

[b] Sample: Swedish and Danish soils were analyzed for diuron; Keyport and Belhaven soil were analyzed for bensulfuron methyl; Alliston soil was analyzed for atrazine.

### 3.3.4 Extraction Rates/Efficiencies by SFE

As mentioned earlier, extraction rate and efficiency were performed on both a homogeneous material (Celite® 545) and a heterogeneous sample (Alliston soil). Atrazine was fortified onto both matrices, and extracted after fortification and aging for 100 days. In general, no apparent difference was observed for the freshly fortified samples, but it became more difficult to extract the analyte from the heterogeneous sample than that from the homogeneous material (Figure 3.2).

Determining the recovery of added or fortified analytes from environmental samples is a routine procedure in developing and validating new extraction methods, particularly in the absence of standard reference materials. While the use of fortification to determine extraction efficiencies from relatively homogeneous samples such as sorbent resins may be valid (since the fortified analytes are likely to be exposed to the same active sites on the resin as the analytes collected from a sample), spiking analytes onto heterogeneous environmental matrices such as soil and sediments may not be a reliable means of representing the extraction behavior of native analytes. Fortified analytes on real-world samples may not be situated on (or exposed to) the same binding sites as those of the native analytes because of the kinetic and diffusional limitations of the sorption process, and several possible interactions may exist simultaneously between a particular analyte and a complex matrix. Furthermore, spiking techniques usually require an organic solvent to deposit the fortified analytes onto the sample matrix, and the solvent may affect the chemical integrity of the sample and is generally not

comparable with the deposition conditions experienced by the native analytes. Since fortified analytes may often be retained less on or in the environmental matrices than the native analytes, the use of fortified recovery studies may overestimate the efficiencies of extraction methods.

The results of this study demonstrate that commonly used spiking methods are frequently not valid for determining quantitative extraction conditions for heterogeneous matrices using either SFE or conventional liquid solvent extractions. None of the spiking procedures investigated were able to accurately represent the native analytes, which were normally bound much more strongly in or on the environmental matrices than the fortified analytes. However, the relative extraction rates of fortified and native analytes can be used to investigate the location and binding of pollutants in or on the different sorption sites available with heterogeneous environmental matrices. The results suggest that using different extraction approaches (i.e., sequential extractions with supercritical $CO_2$ and organically modified $CO_2$, and finally by conventional liquid solvent extraction) is more valid than the fortified recovery studies for determining quantitative extraction conditions for native pollutants from complex samples.

Unfortunately, real-world environmental samples often contain pollutants that have undergone several processes (weathering, aging, chemisorption, trapping in interstitial pores, etc.), making them more resistant to extraction. Although studying SFE solely with frontal elution methods or fortifiers may describe some of the processes involved in SFE, Alexandrou and Pawliszyn (1989) and Burford et al. (1993) demonstrated that great

discrepancies exist between the extraction rates of artificially introduced analytes (i.e., fortified analytes) and analytes that were generated or contaminated onto the soil matrix (i.e, native analytes). These results suggest that in order to understand the kinetic process involved in analytical-scale SFE of real-world environmental samples, the extraction rates of the native analytes must be studied.

The major challenge in developing a model is that it is difficult to distinguish between various mechanisms based on experimental SFE results. For example, Bartle et al. (1990) developed a diffusional-based model to explain the SFE process by adopting the "hot-ball" model that describes heat transfer within a spherical particle, and more recently extended this model to include terms for partitioning at the sample interface and desorption from the sample surface (Bartle et al., 1992). The model predicted an exponential extraction profile with an initial rapid extraction phase that was associated with analytes located near the surface, followed by a slow diffusion-limited phase from analytes located in the interior of the matrix, and fit the experimental data reasonably well. However, other mathematical relationships based on completely different theories and mechanisms can show the same behavior, making these theories experimentally indistinguishable. Fortunately, the abundance of adjustable parameters of supercritical fluids allows one to easily change extraction conditions and observe their effect on SFE rates. For example, a change in extraction pressure, temperature, or fluid composition can be used to distinguish between mechanisms (Langenfeld et al., 1995).

Although good experimental fits with the three-rate-constant desorption model were obtained, in reality, heterogeneous environmental samples may have hundreds of different sites with different desorption energies. Furthermore, apparent rate constants that were dependent on SFE conditions were determined, as opposed to theoretical site-specific rate constants where the mole fraction distribution between sites should be constant regardless of the SFE temperature. For example, SFE by 40°C only supplied enough thermal energy to desorb the weakly bound analytes, while SFE at 200°C supplied thermal energy to desorb both weakly and strongly sorbed species. As a result, the apparent rate constants at 200°C deviate from the true site-specific rate constants because the mole fraction distribution shifted so that $X_1$, $X_2$ and $X_3$ appeared to be extracted simultaneously under the "fast" apparent rate constant $k_{d1}$. Nevertheless, increasing the extraction temperature has been shown to be effective at increasing the elution rate of species from soil samples, as well as increasing the rate of analyte release from the surface, and the modeling approach was useful for studying analyte-matrix interactions in SFE. Increasing the temperature may be a useful approach to improve SFE efficiencies regardless of the rate-limiting step.

However, it is apparent that elevated temperature may not be applicable to those compounds that are thermally labile such as diuron and bensulfuron methyl. Thermal degradation would occur if high temperature was applied during the SFE extraction.

Two methods of introducing modifier during analytical-scale supercritical fluid extraction have been routinely used, One method is to

saturate the matrix in the extraction cell with liquid modifier and then conduct the extraction with the supercritical fluid. The second method is to use modified supercritical fluids as the extractant phase (either premixed in the supercritical fluid or mixed and delivered by pumping during extraction) at or above the modified phase's critical pressure and temperature. These two separate techniques of introducing the modifier into the system were evaluated.

It should be noted that the addition of large amounts of modifier will considerably change the critical parameters of the mixture. As a result, binary mixtures of carbon dioxide and organic solvent are often used in a subcritical state, where the diffusion coefficients are smaller than in a supercritical state.

For aged residues as discussed earlier, the significance of variables is found to be different from what have been obtained using an optimization scheme for freshly fortified analysis. It is therefore necessary to conduct an independent optimization scheme experiment to define the importance and significance of variables to SFE extraction efficiency. According to the preliminary trials using different modifiers (methanol/water; acetonitrile/water; acetonitrile/surfactant/water), temperature, pressure, preaddition of modifier in vessel, and soaking time, an approximate range of variables could be selected for further surface response design experiments.

To accomplish rapid or optimum extraction, the model outlines the optimization process as consisting of two discrete steps. First, the solubility of the analyte in the extraction fluid over the matrix should be improved. This is

accomplished using pressure, temperature, or co-solvents to reduce the retention of analyte on the matrix. Second, to ensure rapid mass transfer of the analytes from the matrix to the fluid, experimental conditions should be optimized, perhaps not to the same point that maximum solubility is achieved. The physicochemical phenomenon responsible for slow mass transfer is different from the elution process of a solubilized analyte in the extraction fluid. The possibility of ensuring rapid mass transfer could necessitate a static extraction step.

Solubility considerations are basically correlated with the molecular structure of the analyte, and solvent polarity. The use of supercritical fluids allows solubility to be adjusted for a choice of solvent and variations in pressure and temperature (i.e., density) of the solvent used. A commercially available software program (Isco, Lincoln, Neb.) may be used to equate the solvent polarity (strength) of a supercritical fluid via the Hildebrand solubility parameter and to enable one to calculate this parameter for mixtures of modifier(s) with $CO_2$ or other common SFE phases.

Matrix considerations are least understood at present. Variability of matrix type is wide, and the physical and chemical complexity of matrices make extractions difficult, especially with heterogeneous soils. By using the SFE process and the higher precision result it provides, one can now experimentally interpret the effects of the individual matrix components. This capacity opens a complex arena of understanding and control that has not been previously investigated in depth. In method development, controlling parameters are often determined first, then conditions are optimized to

enhance their effects. A better knowledge of the individual matrix contributions will allow optimal control of the process.

Extraction fluid polarity should be selected based upon the target solute-extraction fluid as well as solute-matrix interactions. In general, the latter one may be more complex, since these interactions are variable, especially with heterogeneous soils. Addition of modifier(s) may minimize or overcome potential interactions that have taken place between the solute molecule and the soil matrix. This technique was first illustrated in the extraction of primary aromatic amines from soil by SFE (Oostdyk et al., 1993). The soil, depending on the type and its constituents, is likely a mixture of polar and nonpolar portions, so this example can be considered a non-ideal case. The addition of polar modifiers, most commonly methanol, for the extraction of polar solutes has been shown to be effective by several researchers (Oostdyk et al., 1993; Hawthorn and Miller, 1987; Lopez-Avila et al., 1990). However, in certain cases, the addition of a methanol modifier is not sufficient for the quantitative removal of analyte, particularly for aged or native analytes. Conflicting theories on the effects of water or moisture on extraction recoveries appear in the literature. For nonpolar analytes, the presence of water appears to be detrimental, whereas for polar analytes, the effect seen thus far is beneficial (McNally et al., 1992). Methanol and water were the modifiers of choice in these experiments, in which the solid matrix phase is saturated with the modifier and then pressurized in the extraction cell along with the supercritical fluid. It was also observed that the use of methanol or other polar additives as modifiers for supercritical fluid

extraction would be less efficient than the multi-modifier as indicated in the analytical results above.

Based upon the preliminary experiments, the modifier with Triton® X-100 and the dynamic mode of extraction were chosen for surface response design to conduct the multivariate optimization experiments (Chapter 2).

Both dynamic and repeated static modes of extraction were conducted for the aged atrazine soil samples, to determine which one is better than the other. Three modifiers were used for the evaluation. The modifier with surfactant (0.5% Triton® X-100) gave better extraction efficiency than either acetonitrile or methanol as a modifier. It was also noted that preaddition and prewetting of the modifier in extraction vessel enhanced the initial extraction rate considerably, but subsequent extraction rate and efficiency were almost the same as the ones without preaddition of the modifier. Within five and six times of sequential extraction with SFE (30 minutes), more than 89% of bound atrazine could be recovered. This suggested that multiphase SFE extraction is better than the other approaches tested. With elevation of temperature, extraction rate accelerated even more significantly than other SFE parameters changed. It was assumed that during relatively higher temperature, most of the modifier would be partially vaporized, and the liquid phase would be minimum. It is probably more favorable to the supercritical fluid phase. As a result, the solvating power of combined vaporized modifier and supercritical fluid could be enhanced during the extraction.

In the dynamic extraction, the flow rate of the supercritical fluid with modifier was evaluated. In a practical sense, the extraction rate behavior exhibited by a particular sample at different flow rates will yield efficient recoveries. Flow rate studies are also useful to determine the reasonable upper sample size that can be extracted (i.e., larger samples have larger associated void volumes in interstitial spaces, and therefore require higher flow-rates simply to sweep the sample void volume every few minute). Extractions that are controlled primarily by the partitioning kinetics (rather than having large amounts of available extraction fluid for the solvating step) can potentially be efficiently extracted in the static mode (that is, with no continual flow of the extraction fluid) provided that a short dynamic extraction is performed after the static extraction step simply to flush the extracted analytes out of the extraction cell. When extraction rates are controlled mostly by the fluid flow-rate, the use of extraction cell volumes (or any measurement of the total volume of fluid passed through the sample) is a useful parameter to describe an SFE method. It was observed, however, for the majority of the heterogeneous environmental samples, the extraction rate does not depend significantly on the fluid flow-rate and total volume of extraction fluid passed through a sample has little relevance to extraction efficiency since the contact time of the sample and the fluid is more important than the amount of extraction fluid that is used, particularly for the case of aged residues.

The least understood step that controls the SFE efficiencies obtained from heterogeneous environmental samples is the partitioning of the analyte

molecules from the active sites in the sample matrix into the supercritical fluid (step 1). Because of the large number of possible interactions that might occur between the analyte molecules and an environmental matrix, a fundamental understanding of these partitioning processes has been impossible to attain. However, a preliminary description of the processes that control SFE rates can be useful in developing quantitative SFE methods for complex environmental samples. The consideration of the three general issues of analyte solubility, kinetic limitations, and analyte-matrix-extraction fluid interactions is useful for attempting to understand the extraction process in support of the development of quantitative SFE conditions.

Many mechanisms have been proposed to explain the effect of modifiers on the extraction of solutes from various substrates. The first of these is the consideration of the solubility of the solute in modified supercritical fluids. Enchanted extraction rate and efficiency is achieved when solute-modifier interactions are examined. Molecular group interactions between modifiers and solutes that give rise to hydrogen bonding have been suggested as a potential influence of modified supercritical fluids in SFE (Sunol and Chen, 1985). Matrix swelling resulting from the interaction of the modifier with the matrix and the matrix has been proposed as the predominate interaction in SFE. A relationship between the interaction of the modifier with the matrix and rate of mass transfer of the solute from the matrix can be developed. The rate of solute mass transfer is known to change with varying matrix compositions, for example, soil versus plant materials. In fact, mass-transfer differences have been exhibited in the supercritical fluid

extraction of the same solute molecule from soils of varying compositions (Hawthorne and Langenfeld, 1992). Additionally, solute extraction from the matrix can vary with the amount of contact time that has transpired. In laboratory studies of field samples, pesticides and degradation products are well-known to become bound to the matrix with time and field weathering. These aged samples have been difficult to extract using supercritical fluid conditions that yielded acceptable recoveries for freshly fortified and short-term field samples. However, experimental results where the sample matrix was saturated with water prior to supercritical fluid extraction yielded acceptable recoveries for these bound residues. The use of water as a modifier was prompted by the use of water in the extraction of caffeine from coffee (Kruckonis and McHugh, 1986). Water was added as a swelling agent to obtain better recoveries. Liquid modifiers added to the point of matrix saturation cause more matrix swelling than supercritical fluid. With swelling, the interior volumes of the matrices experience increased exposure to the total mobile-phase mixture, consisting of the modifier and the supercritical fluid. Because of this increased exposure, sample matrix swelling enhances the rate of mass transfer of desired analyte into the extraction fluid. Then, provided the modifier or the supercritical fluid-modifier mixture is a good solvent for the solute, higher extraction efficiencies result.

Dynamic (slow flow rate) and/or repeated static mode of extraction was a better approach for aged residues than single extended static mode. Higher recoveries were received from the repeated static extraction, than those from the dynamic extraction, possibly due to a longer interaction time in the

process of collecting extract, which was not counted for 5 minutes, ranging from 5-10 additional minutes. Using surfactant modifier and dynamic and repeated static extractions, more than 85% of aged residues can be easily extracted from the Alliston soil within 30-40 minutes (Figures 3.6-3.7).

Temperature seemed to have significant effects on the recovery, especially for the aged residues in soils. Recovery increased rapidly from 69 to 94% while elevating temperature from 40°C to 100°C, particularly for aged diuron in the case of surfactant addition (Triton® X-100) (Figure 3.8). Similarly, recovery was enhanced from 76 to 91% at an elevated temperature from 100°C to 150°C for aged atrazine samples with an addition of surfactant (Figure 3.3). As reported, it would take at least 60-90 minutes to extract most of the bound residue from the soil using the dynamic mode (Khan, 1995). Elevation of temperature became very crucial to recovering the "bound atrazine". Addition of modifier would also play an important role in the extraction. Among the tested modifiers, surfactant yielded the highest recovery, especially with a significant increase in recovery when temperature increased to 150°C, while other modifiers such as acetonitrile and methanol do not give as much recovery as the surfactant-modifier.

For aged residues, SFE extraction kinetics seemed to heavily relate to mass transfer or diffusion controlled process, therefore solubilization of analyte in the supercritical fluid is no longer an important parameter for better extraction rate and efficiency. However, extraction rate is determined by mass transfer. It has been found that dynamic and/or repeated static modes of extraction would be the best approach for native residue recovery.

Acceptable recoveries were obtained using similar extraction with elevation of temperature from 40° to 100°C. Aged diuron in Danish soil has been extracted using optimum experimental parameters for SFE, yielding recovery more than 98% for day 0 soil and more than 95% for day 100 soil within 30 minutes extraction (Figures 3.8-3.9).

It was observed that the supercritical fluid extraction rate was faster for the day 0 soil sample than the one for the day 100, although these two samples were stored frozen for over two years since the samples were taken from the field. This suggested that the residence time in freezer should not be proportionally counted for aging, but time in field would attribute to "bound" residues. Using SFE methodology, energies of activation values for diuron desorption from the Danish soil were 11.8 kJ/mol and 16.8 kJ/mol for day 0 and day 100 samples, respectively. These two soils were almost identical in terms of their characteristics. The sample with aged residue of longer residence time (day 100) yielded a higher value of energy of activation than that for the day 0. This is probably associated with additional energy that is required to desorb the more bound residues.

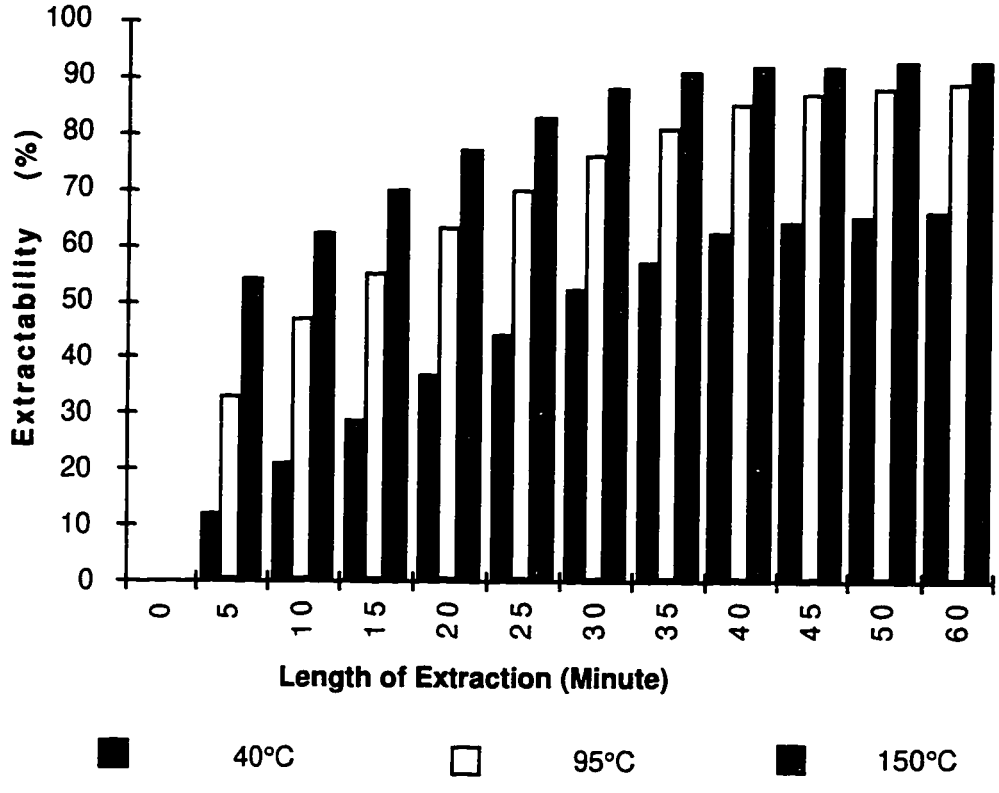



**Figure 3.3 Extraction rate/efficiency of atrazine from aged Alliston soil using Triton® X-100 modifier (30%)**

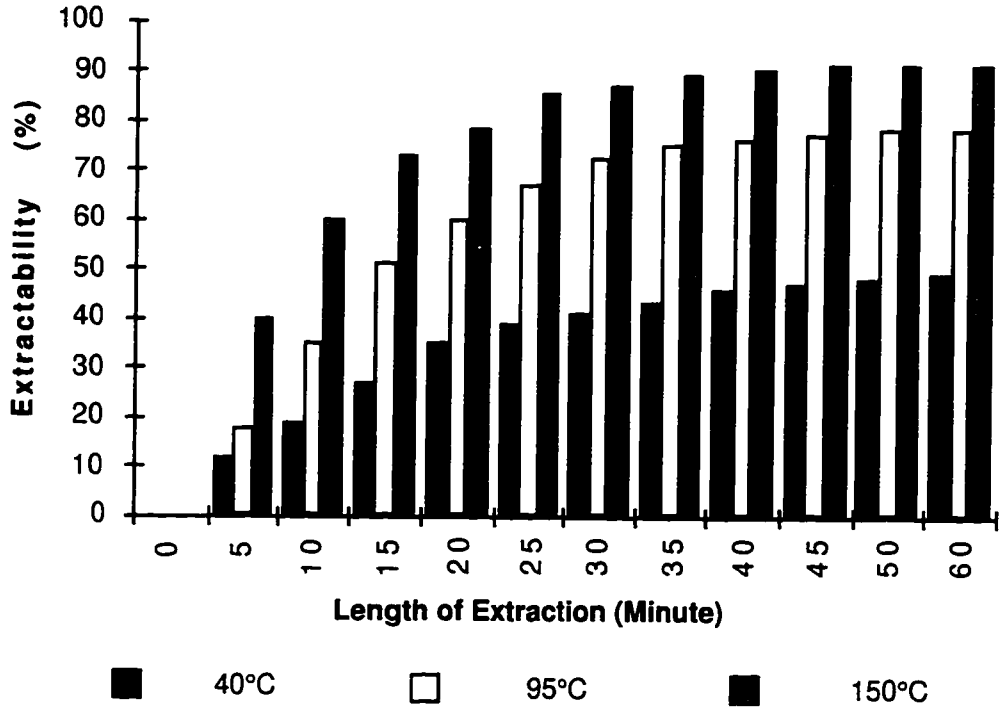



Figure 3.4 Extraction rate/efficiency of atrazine from aged Alliston soil using methanol modifier (30%)

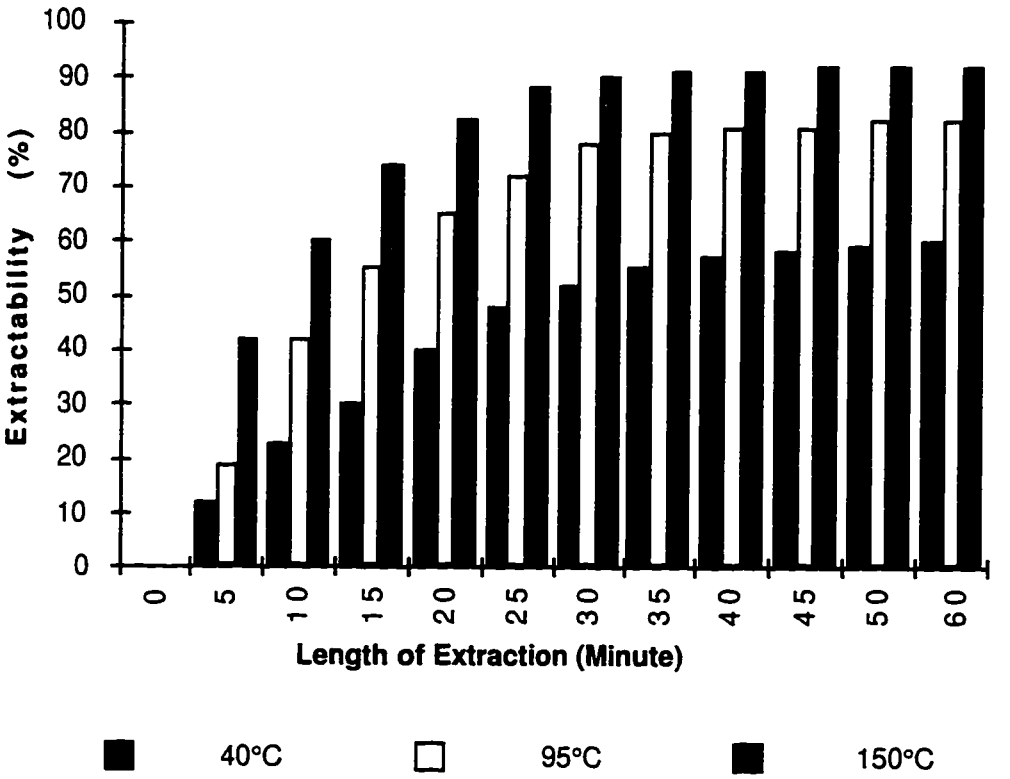



**Figure 3.5 Extraction rate/efficiency of atrazine from aged Alliston soil using acetonitrile modifier (30%)**

It would be advantageous to introduce modifier and supercritical fluid separately because the modifier could, for some matrices, act as a swelling agent. This example illustrates a nonideal SFE matrix/analyte system. The matrix is polar, as is the analyte. The choice of $CO_2$ as the solvent phase would not be experimentally or theoretically predicted as optimum without the modifier. Fahmy et al. (1993) investigated the effects of polar modifiers on the matrix swelling of environmental samples under supercritical fluid conditions. In the study, samples were examined via a high pressure sapphire view cell, and the extent of swelling was measured. Thus a correlation between extraction rate and efficiency and swelling of the matrix was illustrated and discussed. Interestingly, swelling reached a maximum and then dropped at higher pressures. Optimum pressures for both extraction and swelling were illustrated in the systems examined. It was hypothesized that water acted as a swelling agent for the matrix while other modifiers such as methanol or acetonitrile were acting as solubilizing agents for the analyte of interest once the matrix had swollen. Several different modifiers were reported to impart different degrees of swelling on the matrices.

Kinetic models have been proposed to explain extraction rates in SFE processes, but experimental results supporting these theories are scattered throughout a variety of papers. Bartle and co-workers (1990) described SFE extraction kinetics using the hot-ball model, which assumes that the solute diffuses out of a homogeneous spherical particle (a matrix particle) into a medium (the supercritical fluid) in which the extracted species is infinitely dilute. This model assumes that the matrix particles are all of the same size,

the particles are spherical, the analyte of interest is uniformly distributed throughout the matrix before the extraction is conducted, the rate of flow is so rapid that the concentration of the analyte remains at or close to zero, and the analytes move through the matrix by a process similar to diffusion. The authors comment that the model performs reasonably well despite the nonideal nature of the samples that were compared. Variances from the model predictions are probably related to the first three assumptions. For example, solute mass transfer is not totally a result of diffusion from a homogeneous medium; it also involves diffusion out of pores, migration from one adsorption site to another, and displacement of analyte molecules on adsorption sites by the supercritical fluid.

It was also found that neither the flow-rate nor the cell geometry had a significant influence on the extraction efficiency. In some particular instances, the extraction recovery can also be improved by decreasing the extraction cell diameter (i.e., by increasing the linear velocity). This result can be explained by a better mass transfer within the cell due to an increase in turbulence in the fluid flow pattern inside the extraction cell. Polar solutes generally require modified carbon dioxide as the extractant. In some instances they can be derivatized in situ by the addition of a reagent directly to the sample matrix prior to extraction (Hills et al., 1991; Hawthorne et al., 1992; Lee et al., 1992).

Another consideration is the way in which analytes are incorporated within the matrix. For analytes at or near the surface of the matrix, the high diffusivity of supercritical carbon dioxide ensures adequate contact between

solute and solvent. If the analyte is entrained within crevices, the extraction will certainly require more time.

Some proponents of the static method believe that the long exposure to solvent allows the matrix to swell, thus improving the penetration of carbon dioxide into its interstices and increasing analyte recovery. Those who prefer the dynamic method claim that continual exposure of analyte to fresh solvent enhances partitioning of the analyte into the mobile phase. As with other SFE operating criteria, selection should be based on actual performance.

Our studies suggested that prewetting the matrix sample with modifier, proceeding with an initial static extraction, and then following with dynamic extraction yielded a faster extraction rate than that from either static or dynamic extraction results. Prewetting at the beginning of the extraction simply allows the matrix to swell resulting in better exposure to the supercritical fluid and modifier, especially in the case of extracting native or bound residues. Extraction rate and efficiency of aged diuron in Danish and Swedish soils were also evaluated using elevated temperatures (40-100°C).

SFE not only offered the best extraction efficiency, but also provided selectivity for a particular analyte of interest using a multivariate optimization scheme. Optimum SFE was applied to sample extraction of diuron degradation and lysimeter studies. It was found that extracts obtained from SFE were much cleaner than those from conventional approaches (i.e., Soxhlet) and surfactant extraction. Extensive cleanups were required for the

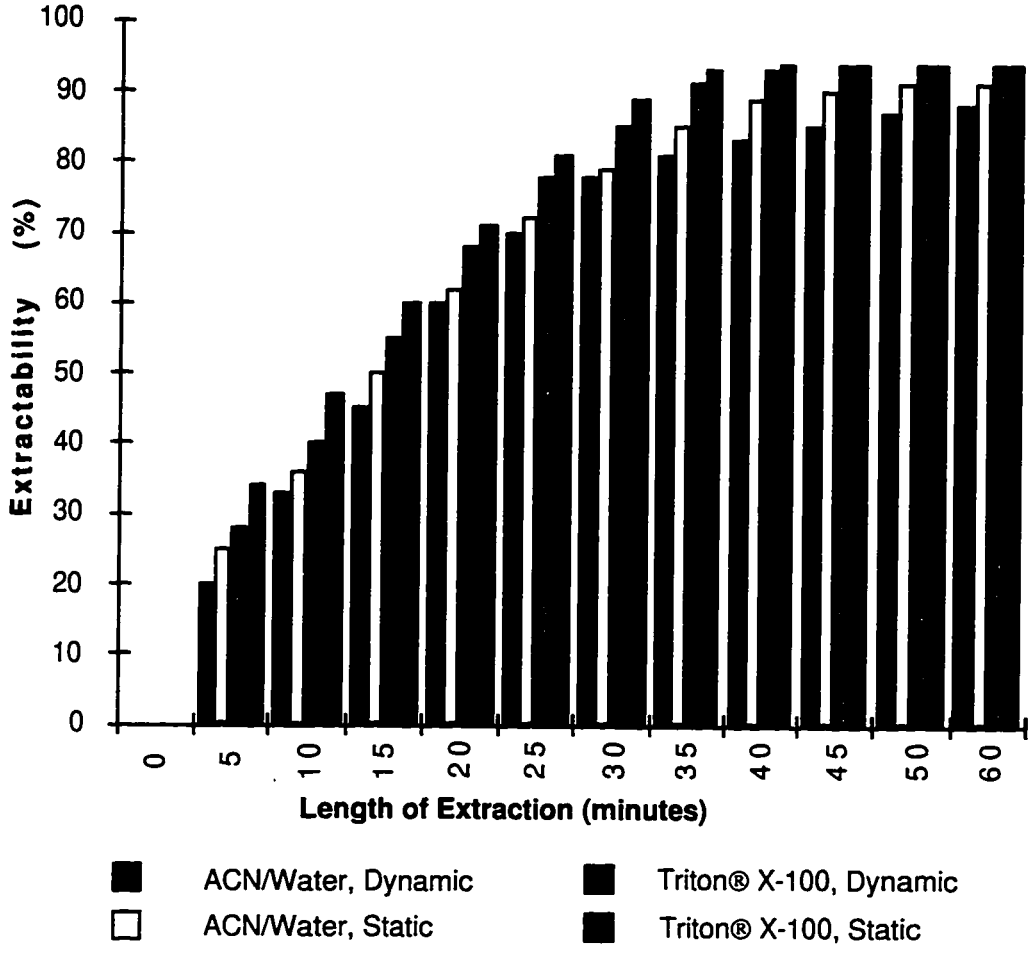



**Figure 3.6 Extraction rate/efficiency of atrazine from aged Alliston soil using different modifiers (preadded) and modes**

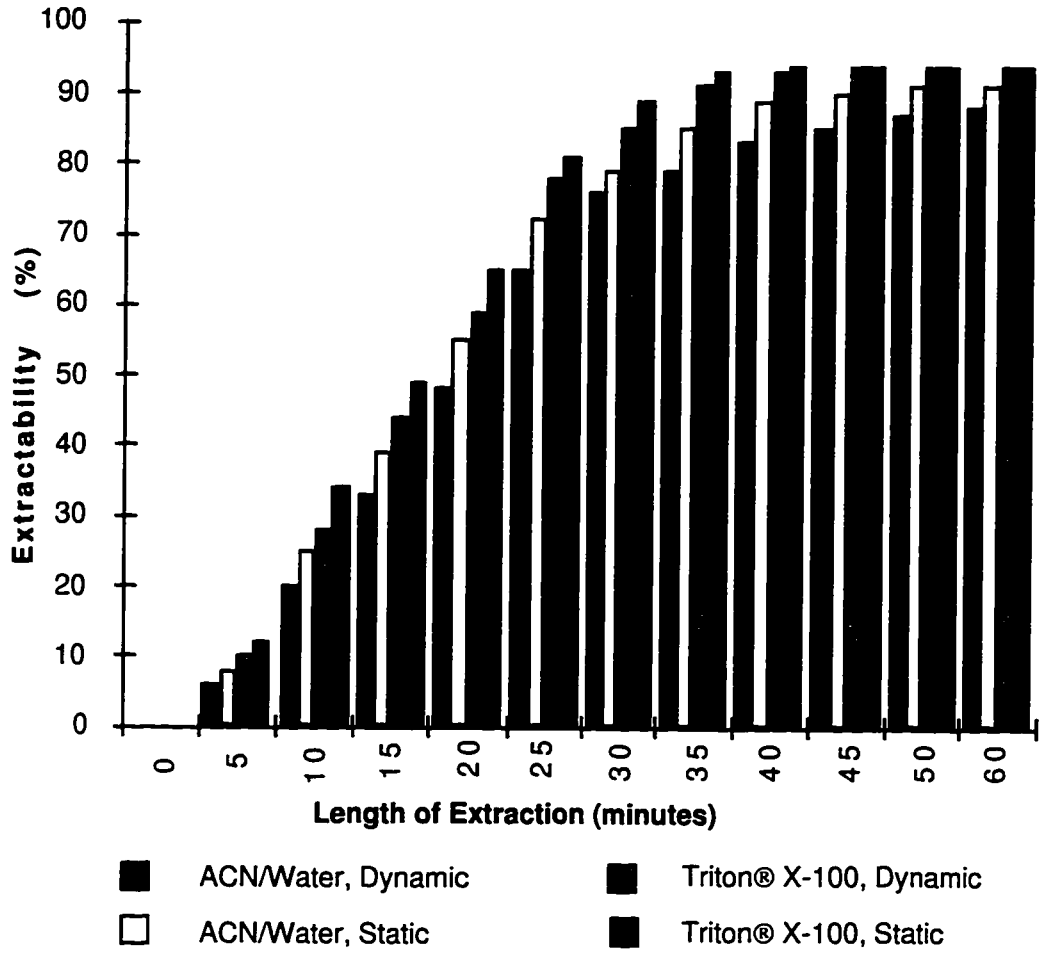



**Figure 3.7 Extraction rate/efficiency of atrazine from aged Alliston soil using different modifiers (pump-delivery) and modes**

conventional methods to enable quantification and analysis of analytes by LC-RadioChem and LC-UV.

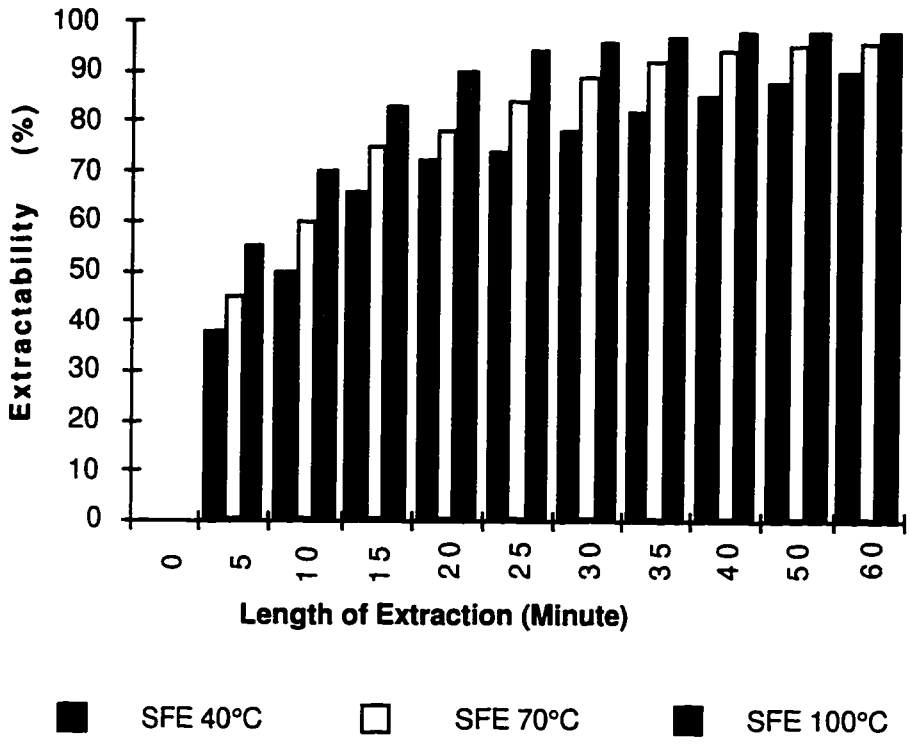



**Figure 3.8 Extraction rate/efficiency of diuron from Danish soil (day 100) using different temperatures**

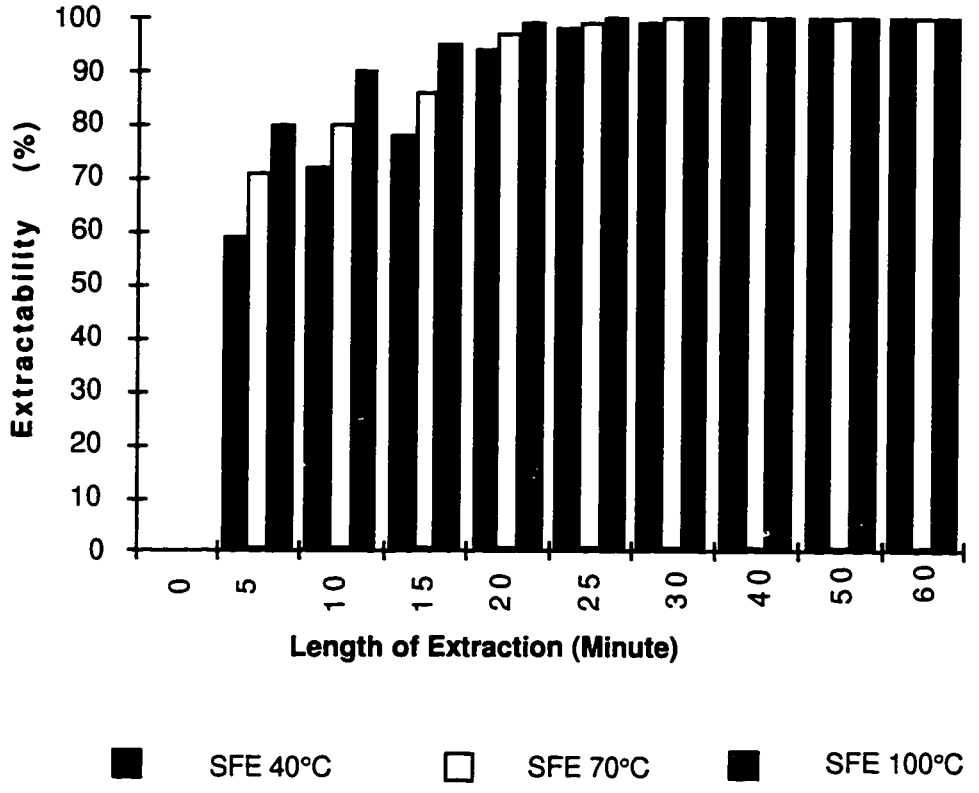



**Figure 3.9 Extraction rate/efficiency of diuron from Danish soil (day 0) using different temperatures**

### 3.3.5 Identification and Semiquantification of Potential Degradation Compounds from the Field Degradation Studies

The field samples from the diuron degradation study were extracted with surfactant extraction and SFE extraction. The radioactive concentration (dpm/g) determined for the extracted soil residues were used to calculate the extraction efficiencies (refer to Table 3.1). The calculations used to determine extract recovery (dpm/g), residue recovery (dpm/g), extraction efficiency (% of the bound residue), the % AR in surfactant extracts, and LC recoveries (% of total dpm, and % AR) were discussed earlier within this chapter. These results indicated that extraction efficiencies decrease with increased sampling intervals. Recoveries ranged from 41.9% to 98.0% of the bound residues.

The recovery data obtained from LC analyses of surfactant extracts are listed in Table 3.3. The UV, Ramona, and Foxy fraction collection chromatograms from the analysis of the Swedish soil are shown in Figure A.3.

Test samples were 100-day samples of the Swedish loamy sand, Swedish sand, and Danish sandy loam test groups, respectively. Test samples from Danish sandy loam test group were representative of all sampling intervals. Diuron, DCPMU, and DCPU were the major, isolated components identified in the extracts. These analytes were also the major extract components found in a similar study.

The recovery of significant diuron, DCPMU, and DCPU residues in the surfactant extractions indicate these compounds are sorbed strongly to the soils. Loamy sand, sandy loam, and sand were the soil types of these samples.

The distribution pattern for the degradation of $^{14}C$-diuron and appearance of DCPMU and DCPU in the surfactant extracts over the 100-day test period for the Danish sandy loam soil test group shows the decline of diuron (85% to 17% AR), the appearance of DCPMU (0% to 18% AR), and the appearance of DCPU (0% to 14% AR).

**Table 3.3 Recovery results of diuron and degradation compounds from a field degradation study[a]**

| Compound | Recovery (%) | | | | | |
|---|---|---|---|---|---|---|
| | Swedish 100 Day | Danish 100 Day | Danish 0 Day | Danish 32 Day | Danish 64 Day | Danish 100 Day |
| Diuron | 4 | 30 | 94 | 55 | 18 | 10 |
| DCPMU | 24 | 39 | 1 | 26 | 34 | 26 |
| DCPU | 25 | 4 | 0 | 3 | 12 | 14 |

[a] Refer to Table 3.1 for individual soils.

Aerobic soil metabolism of $^{14}$C-bensulfuron methyl (DPX-F5384) in selected soils were studied (unpublished research). Flanagan and Keyport silt loam soils treated with $^{14}$C-bensulfuron methyl (DPX-F5384) at rates of 0.6, 0.05 ppm (equivalent to 500 and 50 g ai/ha) were aged aerobically in darkness at 25°C. On these soils in the presence of viable aerobic microbes, $^{14}$C-bensulfuron methyl (DPX-F5384) degraded with a first half-life of 4-20 weeks. The major degradation product was $^{14}CO_2$. Three nonvolatile intermediate degradation products have also been identified (unpublished research). Soil bound residues which could not be extracted accounted for about 12-20% of the applied radioactivity after 52 weeks (collaborative studies within DuPont).

In sterilized soils, the degradation rate of $^{14}$C-bensulfuron methyl (DPX-F5384) was slower, indicating that microbes facilitate, but are not essential for the degradation of this compound in soils. Degradation under sterile conditions transformation resulted in increased amounts of the nonvolatile products, while less than 1% mineralization was observed.

Identification of metabolites was conducted using electron impact (EI) mass spectrometry. The mass spectrum and TLC $R_f$ values and LC retention times matched the ODM-DPX-F5384 reference standard. The rapid conversion of DPX-F5384 to ODM-DPX-F5384 is an example of metabolism by mixed function oxidases. Identification of DPX-F5384 and the other radiolabeled soil metabolites was based on coelution of the radiolabeled compounds with authentic standards of suspected metabolites by reversed-phase LC.

Sulfonamide, homosaccharin, and DPX-F5384 were major radiolabeled compounds in the acetonitrile/chloroform wash. DPX-F5384 and sulfonamide were the major compounds in the methylene chloride extract from the pH 7 solution. ODM-DPX-F5384 and additional homosaccharin were recovered in the methylene chloride extract from the pH 3 aqueous solution.

Results from the SFE process showed similar degradation products in the aged samples. It was assumed that these transformation compounds were primarily formed prior to binding to the soils, as observed in the sorption/desorption studies.

Atrazine was relatively stable over the period of aging in the soils. No significant (quantifiable) metabolites were found in the aged soil samples, except for hydroxylated atrazine (HAT), which was relatively low compared to atrazine. Supercritical fluid extraction of atrazine and polar metabolites from sediment was reported recently (Papilloud et al., 1996). Similarly, no major metabolites were found in the aged samples except for the hydroxylated atrazine, which was confirmed by the LC/MS technique.

## Findings and Future Research

### Findings

Sorptive and desorptive behavior of pesticides basically depended on their physiochemical characteristics and the nature of heterogeneous soil systems. These ultimately determine the environmental fate of the pesticides after their direct and indirect applications.

The studies indicated that the content of organic matter was the major variable contributing to diuron and bensulfuron methyl sorption. The low $1/n_d$ (isotherm slope for desorption) values showed that both pesticides were not readily desorbed from the soils tested. The rates of sorption were more rapid than those of desorption for atrazine and bensulfuron methyl, especially in the case of bensulfuron methyl on the Belhaven soil containing high organic matter (57.5%). In this soil sorption was extremely fast, compared to other soils that contained high clay (56.4%) or high sand (91.6%). The slow rates of desorption were presumably associated with the heterogeneous nature of the soil, and potential hysteresis phenomena. Hysteresis was observed, at various degrees, depending on the pesticides and soils tested. As the residence time of the sorbed pesticide in the soils increased, less desorption occurred.

The sorption and desorption of the three herbicides could be described by an initial fast followed by a rather slow approach to an apparent equilibrium. The rates of sorption and desorption were dependent on the type of sorbent (different soil systems), pesticide, and temperature.

The energy of activation values for both sorption ($E_a$ = 11-25 kJ/mol) and desorption ($E_d$ = 18-38 kJ/mol) suggested that transport or diffusion control is rate-limiting for both processes. The study also showed that the desorption of bensulfuron methyl was almost irreversible, particularly with high content of organic matter in the soil. It was correlated to the $K_d$ values obtained from the isotherm equilibrium experiments. The stronger sorption of bensulfuron methyl than diuron on a similar soil suggested a different mechanism, possibly an ion exchange mechanism.

These preliminary data may assist in macroscopically understanding and interpreting potential mechanisms and/or binding strengths. This could be especially useful in learning about "bound residues".

The MOS offered the opportunity to systematically and simultaneously examine the interaction and effects among important soil environmental variables and extraction parameters. MOS is a highly efficient technique for studying a large number of variables and identifying optimal extraction conditions for the SFE.

Pesticide residence time had a major influence on binding processes for all tested pesticides. Extractability as a function of soil composition was greatly dependent on the particular pesticide examined. Bensulfuron methyl was extracted with the greatest difficulty from soils containing both high levels of organic matter and clay. Atrazine was extracted more easily than diuron. High organic matter and high clay content led to strong binding for diuron and atrazine, respectively.

Effects of SFE parameters on extractability were apparently related to the nature of residues (e.g., freshly fortified versus aged residues). For freshly fortified samples, analyte solubility in supercritical fluid and/or modified supercritical fluid was the critical factor as indicated by the strong influence of pressure on extraction efficiency. For aged samples, temperature was an important determinant of extraction efficiency, indicating that mass transfer or diffusion processes were rate-limiting. The presence of modifier and extraction duration also significantly impacted extractability of aged pesticides. These data clearly indicated that commonly used spiking methods are often not valid for determining quantitative extraction conditions for heterogeneous matrices using SFE.

Among the Soxhlet, sonication, surfactant extraction, accelerated solvent extraction (ASE), and supercritical fluid extraction (SFE) in the comparison studies, ASE provided better extraction efficiency than those conventional methods. This is probably associated with elevation of temperature and pressure that create a subcritical phase for the extraction resulting in the increase of solvating power and solubility of analyte into the extraction liquid. Surfactant extraction yielded better extractability, particularly in the case of diuron and atrazine, than other solvent extractions (i.e., Soxhlet).

SFE was the best approach to recover aged residues from the soils. With the aid of surfactant as a modifier, additional bound residues can be extracted using optimal SFE method, especially for aged atrazine and diuron.

Prewetting of aged samples was found to be effective in accelerating the extraction rate.

For aged atrazine and diuron extraction, elevated temperature appeared to be a significant factor in effectively recovering or extracting the analytes from the soils. However, it seemed to be detrimental in recovering bensulfuron methyl, presumably associated with thermal degradation occurred in the extraction. To optimize the SFE procedures, characteristics of the analyte(s) should be also carefully taken into account. Analytical results suggested that interaction time was important to the extraction, but fresh supercritical fluid using dynamic mode may cause better extraction rate and efficiency of aged residues. The combination of an initial static followed by a dynamic mode extraction apparently enhanced extraction rate and efficiency.

Pesticide degradation occurred mostly in the environment (i.e., soil system), but in some cases, it was also observed under laboratory conditions (i.e., during sorption/desorption, sample aging, analytical procedure, etc.). The rate and extent of the degradation basically depended on the characteristics of the pesticide and the nature of the soil conditions/ composition. In general, bensulfuron methyl degraded very rapidly even in soil-aqueous system (during sorption/desorption and sample aging). Major transformation compounds were identified as sulfonamide, O-desmethyl-bensulfuron methyl (ODM-DPX-F5384), pyridine-amine, and homosaccharin. It also showed thermal degradation in the SFE process at an elevated temperature (> 80°C). Diuron degradation was observed in the field studies (degradation and lysimeter studies), but no apparent degradation took place

under laboratory conditions. 3,4-Dichlorophenylurea (DCPU) and N-(3,4-dichlorophenyl)-N'-methylurea (DCPMU) were present to various extents depending on the length in the field. The percent of DCPU and DCPMU detected increased with the increase in sampling intervals. Atrazine was considered to be the most stable compounds, compared to bensulfuron methyl and diuron. No degradation took place under any laboratory conditions (sorption/desorption, sample aging, and SFE procedures at elevated temperature, i.e., 150°C). It showed some degree of transformation after extended period of time (aging) in the field, resulting in dealkylation and/or hydroxylation.

These results demonstrated that the conditions used in the SFE were appropriate and the analytical results regarding degradation for the field aged samples were reliable, accurate, and valid.

**Future Research**

It is without doubt that further research is necessary to better understand the complexity of the heterogeneous nature of the soil systems. As indicated earlier, seven factors are known to affect the fate and behavior of pesticides in soils: (1) chemical decomposition, (2) direct/indirect photochemical decomposition, (3) microbial decomposition, (4) volatilization, (5) movement, (6) plant or organism uptake, and (7) adsorption. The phenomenon of *adsorption/desorption* directly or indirectly influences the magnitude of the effect of the other six factors.

Factors that affect the retention of pesticides are soil organic matter (SOM) content (i.e., number, type, and accessibility of humic substance (HS) functional groups, nature of the pesticides, properties of the soil including types and quantities of clay minerals and other soil components, pH, exchangeable cation, moisture, and temperature. However, the mechanisms by which pesticides are retained by soil are not clearly understood. In most cases, researchers usually hypothesize (macroscopically) a potential mechanism to explain the behavior of pesticide retention and/or binding. To truly reveal their mechanisms, it is vitally important to generate some microscopic information in interpreting the real processes in the complex environment. This will certainly assist to understand the interaction of matrix-analyte and to yield better extraction efficiencies.

Due to the powerfulness of the MOS and SFE techniques, it is worthwhile investigating other important independent variables such as different modifiers (type: i.e., micellar, ion-pairing, chelating reagent, etc.), higher temperature if commercially available, multiresidue optimization (a few pesticides and/or degradation compounds' coexistence), so as to extend the applicability of SFE for other environmental analysis. In addition, accelerated solvent extraction (ASE) was a viable alternative to SFE. Therefore, it may also need further investigation using the MOS approach. In the near future, a bench top LC/MS or LC/MS/MS may be commercially available and should be employed to identify and quantify the analyte(s) of interest for reliable and accurate results.

## REFERENCES


Andersen, M. R.; J. W. King and B. S. Hawthorne (1990), in M. L. Lee and K. E. Markides (Eds) *Analytical Supercritical Fluid Chromatography and Extraction,* Chromatography Conferences, Provo, UT, p. 313

Ahlrichs, J. L. (1972) "The soil environment" in: Goring C. A. I., and J. W. Hamaker (Eds) *Organic Chemicals in the Soil Environment* Marcel Dekker, New York. Vol 1, pp 3-46

Agnihotri, N. P. and A. K. Barooah (1994) "Bound residues of pesticides in soil and plant-A review" *J Sci & Indust Research* **53:** 850-861

Akhtar, M. H. (1987) "The disposition of bound $^{14}C$ agrochemical residues in plants and animal tissues when consumed by animals" In: Greenhalgn R. and T. R. Roberts (Eds) *Pesticide Science and Biotechnology,* IUPAC Proc 6th Int Congr Pestic Chem pp 509-512

Alexander, M. (1965) "Persistence and biological reactions of pesticides in soils" *Soil Sci Soc America Proc* **29**: 1-7

Alexandrou, N. and J. Pawliszyn (1989) "Supercritical fluid extraction for the rapid determination of polychlorinated dibenzo-p-dioxins and dibenzofurans in municipal incinerator fly ash" *Anal Chem* **61(24)** 277--2776

Bailey, G. W. and J. L. White (1964) "Review of adsorption and desorption of organic pesticides by soil colloids, with implications concerning pesticide bioactivity" *J Agric Food Chem* **12:** 324-332

Bailey, G. W. and J. L. White (1970) "Factors influencing the adsorption and desorption and movement of pesticides in soil" *Residue Reviews* **32:** 29-32

Balba H. M., Still, G. G. and E. R. Mansager (1979) "Pyrolytic method for estimation of bound residues of chloroaniline compounds in plants" *J Assoc Off Anal Chem* **62:** 237-240

Baldwin, B. C.; M. F. Bray, and M. J. Geoghegan (1966) "The microbial decomposition of paraquat" *Biochem J* **101:** 15 P

Ball, W. P.; and P. V. Roberts (1991) "Long term sorption of halogenated organic chemicals by aquifer material" *Environ Sci Technol* **25 (7):** 1237-1249

Barcelo, D. (1988) "A review of liquid chromatography in environmental pesticide analysis" *Chromatographia* **25 (10):** 928-936

Barefoot, A. C. and R. W. Reiser (1987) "Packed capillary liquid chromatography-mass spectrometry using both direct-coupling and moving-belt interfaces" *J Chromatogr* **398:** 217-226

Barefoot, A. C. and R. W. Reiser (1989) "Packed capillary liquid chromatography/mass spectrometry using a moving belt interface" *Biomed Environ Mass Spectrom* **18:** 77-82

Barefoot, A. C.; R. W. Reiser, and S. A. Cousins (1989) "Microcolumn liquid chromatography-mass spectrometry using moving belt and continuous-flow fast-atom-bombardment interfaces" *J Chromatogr* **474:** 39-50

Barinova, E. S.; E. S. Brodskii; E. V. Koverzanova; and S. V. Usachev (1995) "Determination of residual quantities of chlorinated pesticides in potatoes by chromatography-mass spectrometry" *J Anal Chem* (Transl of Zh Anal Khim) **50 (3)** 294-297

Barnabas, I. J.; J. R. Dean; W. R. Tomlinson, and S. P. Owen (1995) "Experimental design approach for the extraction of polycyclic hydrocarbons from soil using supercritical carbon dioxide" *Anal Chem* **67:** 2064-2069

Barooah, A. K. (1991) "Persistence of cypermethrin in soil and bioavailability of its bound residues" *PhD Thesis,* IARI, New Delhi

Bartha, H. (1971) "Fate of herbicide-derived chloroanilines in soil" *J Agric Food Chem* **19:** 385-387

Bartha H., and T. S. Hsu (1976) "Spectroscopic characterization of soil organic matter" in: Kaufman D. D.; Still G. G.; Paulson, G. D.; and S. K. Bandal (Eds) *Bound and Conjugated Pesticide Residues* ACS Symp Series **29:** 258-271

Bartle, K. D.; A. A. Clifford; S. B. Hawthorne; J. J. Langenfeld, and R. J. Robinson (1990) "A model for dynamic extraction using a supercritical fluid" *J Supercrit Fluids* **3:** 143-149

Bartle, K. D., and A. A. Clifford (1991) "Determination of the oil content of rapeseed by supercritical fluid extraction" *LC-GC Int,* 4, **No. 10:** 11

Bartle, K. D.; T. Boddington; A. A. Clifford, and S. B. Hawthorne (1992) "The effect of solubility on the kinetics of dynamic supercritical-fluid extraction" *J Supercrit Fluids* **5:** 207-212

Behymer, T. D.; T. A. Bellar, and W. L. Budde (1990) "Liquid chromatography/particle beam/mass spectrometry of polar compounds of environmental interest" *Anal Chem* **62:** 1686-1690

Bellar, T. A. and W. L. Budde (1988) "Determination of nonvolatile organic compounds in aqueous environmental samples using liquid chromatography/mass spectrometry" *Anal Chem* **60:** 2076-2083

Bellar, T. A., T. D. Behymer, and W. L. Budde (1990) "Investigation of enhanced ion abundance from a carrier process in high-performance liquid chromatography particle-beam mass spectrometry" *J Am Soc Mass Spectrom* **1 (1):** 92-98

Bernhard-Bitaud, C., Schiavon, M., and F. Andreux (1994) "Effect of repeated atrazine treatments on its adsorption by brown soil" Proc. Int. Workshop "*Environ Behav Pestic Regul Aspects*" 5th, 79-86. Copin, A (Editor). Publisher: Eur Study Serv Rixensart, Belg.

Bicking, M. K. (1992) "A simplified experimental design approach to optimization of SFE conditions for extraction of an amino hydrochloride" *J Chromatogr Sci* **30:** 358-360

Bicking, M. K.; T. G. Hayes; Kiley, J. C., and S. N. Deming (1993) "An experimental design approach to the optimization of supercritical fluid extraction for the determination of oil and grease in soil" *J Chromatogr Sci* **31:** 171-176

Biggar, J. W., U. Mingelgrin, and M. W. Cheung (1978) "Equilibrium and kinetics of adsorption of picloram and parathion with soil" *J Agric Food Chem* **26:** 1306-1312

Borggaard, O. K., and J. C. Streibig (1988) "Atrazine adsorption by some soil samples in relation to their constituents" *Acta Agric Scand* **38:** 293-301

Brady, B. O.; C.-P. C. Kao; R. D. Gambrell; K. M. Dooley, and F. C. Knopf (1987) "Supercritical extraction of toxic organics from soils" *Ind Eng Chem Res* **26:** 261-268

Breen, C. (1991) "Thermogravimetric and Infrared study of the desorption of butylamine, cyclohexylamine and pyridium from Ni- and Co-exchanged montmorillonite" *Clay Miner* **26:** 487-496

Brian, R. C. (1967) "The uptake and adsorption of diquat and paraquat in soil." *Annl Appl Biol* **59:** 91-99

Bright, F. V. and M. E. P. McNally (1992) (Eds) "Supercritical Fluid Technology" Theoretical and Applied Approaches in Analytical Chemistry *ACS Symposium Series 488*

Browm, M. M.; I. S. Kim; F. I. Sasinos, and R. D. Stephens (1990) in M. A. Brown (Ed): Liquid chromatography/mass spectrometry. Applications in agricultural, pharmaceutical and environmental chemistry, *ACS Symposium Series*, No. **420** p. 198

Brusseau, M. L.; R. E. Jessup, and P. S. C. Rao (1991) "Nonequilibrium sorption of organicals: Elucidation of rate-limiting processes" *Environ Sci Technol* **25:** 134-142

Burford, M. D.; S. B. Hawthorne, and D. J. Miller (1993) "Extraction rates of spiked versus native PAHs from heterogeneous environmental samples using supercritical fluid extraction and sonication in methylene chloride" *Anal Chem* **65(11)** 1497-1505

Burns, R. G., and L. J. Audus (1970) "Distribution and breakdown of paraquat in soil" *Weed Res* **10:** 49-58

Cairns, T.; E. G. Siegmund, and J. J. Stamp (1989) "Evolving criteria for confirmation of trace level residues in food and drugs by mass spectrometry; Part I" *Mass Spectrom Rev* **8 (2):** 93-117

Cairns, T.; E. G. Siegmund, and J. J. Stamp (1989) "Evolving criteria for confirmation of trace level residues in food and drugs by mass spectrometry; Part II" *Mass Spectrom Rev* **8 (3):** 127-145

Calderbank, A (1968) "The bipyridylium herbicides" In: Metcalf R. L. (Ed) *Advances in Pest Control Research, Interscience* New York. Vol 8 pp 127-235

Calderbank, A. (1989) "The occurrence and significance of bound pesticide residues in soil" *Review of Environmental Contamination and Toxicology* Vol **108** pp 71-103

Camel, V.; A. Tambute and M. Caude (1993) "Analytical-scale supercritical fluid extraction: a promising technique for the determination of pollutants in environmental matrices" *J Chromatogr* **642**: 263-281

Capriel, P, and A. Haisch (1983) "Persistenz von atrazin und seiner metaboliten im boden nach einmaliger herbicid anwendungen" *Z Pflanzenernaehr* Bodenk **146:** 474-480

Capriel, P.; A. Haisch, and S. U. Khan (1985) "Distribution and nature of bound (nonextractable) residues of atrazine in a mineral soil nine years after herbicide application" *J Agric Food Chem* **33:** 567-569

Capriel, P; Haisch, A., and S. U. Khan (1986) "Supercritical methanol: an efficacious technique for the extraction of bound pesticide residues from soil and plant samples" *J Agric Food Chem* **34:** 70-73

Chiou, C. T. (1981) "Partition coefficients and water solubility in environmental chemistry" In: J. Saxena and F. Fisher (Eds) *Hazard assessment of chemicals: Current developments.* Academic Press, New York pp 117-153

Chiou, C. T.; L. J. Peters, and V. H. Freed (1979) "A physical concept of soil-water equilibria for nonionic organic compounds" *Science* **206:** 831-832

Chiou, C. T., and T. D. Shoup (1985) "Soil sorption of organic vapors and effects of humidity on sorptive mechanisim and capacity" *Environ Sci Technol* **19:** 1196-1200

Chiu, K. S.; A. van Langenhove, and C. Tanaka (1989) "High-performance liquid chromatographic/mass spectrometric and high-performance liquid chromatographic/tandem mass spectrometric analysis of carbamate pesticides" *Biomed Environ Mass Spectrom* **18:** 200-206

Chester, T. L.; J. D. Pinkston and D. E. Raynie (1992) "Supercritical fluid chromatography and extraction" *Anal Chem* **64:** 153R-170R

Cochrane, W. P.; D. Cahput; and J. Singh (1994) "Analysis of pesticides and PCB in water, soil and plant material" *Sample Prep Biomed Environ Anal* [Proc Chromatogr Soc Int Symp] Meeting date 1991, pp. 191-201

Constenla, M. A.; P. Moza; I. Scheunert; A. Haque, and W. Klein (1984) "Fate of buturon-$^{14}C$ in soil during seven seasons of exposure under outdoor conditions" *J Agric Food Chem* **32:** 208-211

Crow, J.A. and J. P. Foley (1990) "Optimization of separation in supercritical fluid chromatography using a modified simples algorithm and short column" *Anal Chem* **62:** 378-386

Crowther, J. B. and J. D. Henion (1985) "Supercritical fluid chromatography of polar drugs using small-particle packed columns with mass spectrometric detection" *Anal Chem* **57:** 2711-2716

Damanakis, M., D. S. H. Drennan, J. D. Fryer, and K. Holly (1970) "The adsorption and mobility of parapuat on different soils and soil constituents" *Weed Res* **10:** 264-277

Diamond, W. (Ed) (1981) *Practical Experiment Designs for Engineers and Scientists* Lifetime Learning Publications, Belmont, California.

Di Toro, D. M. and L. M. Horzempa (1982) "Reversible and resistant compounds of PCB adsorption-desorption" *Environ Sci Technol* **16:** 594-602

Edwards, A. P., and J. M. Bremner (1967) "Microaggregates in soils" *J Soil Sci* **18:** 64-73

Engelhardt, H. and A. Groß (1988) "On-line extraction and separation by supercritical fluid chromatography with packed columns" *Journal of High Res Chromatogr* **11:** 38-42

Farmer, W. J. and Y. Aochi (1974) "Picloram sorption by soils" *Soil Sci Soc Am Proc* **38:** 418-423

Fahmy, T. M.; M. E. Paulaitis; D. M. Johnson and M. E. McNally (1993) "Modifier effects in the supercritical fluid extraction of solutes from clay, soil and plan materials" *Anal Chem* **65**: 1462-1469

Fraile, R., and V. Sanchez (1993) "Experimental design optimization of the separation of the aromatic compounds in petroleum cuts by supercritical fluid chromatography" *Journal of High Res Chromatogr* **16(3):** 169-174

Fuhr, F. (1987) "Non-extractable pesticide residues in soil" in: Greenhalgh, R.; Roberts, T. R. (Eds) *Pesticides Science and Biotechnology Proc 6th Int Congr Pestic Chemistry*, IUPAC. Blackwell Scientific Publications London. pp 381-389

Fuhremann, T. W., and E. P. Lichtenstein (1978) "Release of soil-bound methyl ($^{14}C$) parathion residues and their uptake by earthworms and oat plants" *J Agric Food Chem* **26:** 605-610

Gamble, D. S. and S. U. Khan (1990) "Atrazine in organic soil: Chemical speciation during heterogeneous catalysis" *J Agric Food Chem* **38:** 297-308

Gamble, D. S. and L. A. Ismaily (1992) "Atrazine in mineral soil: The analytical chemistry of speciation" *Can J Chem* **70:** 1590-1596

Giddings, J. C.; M. N. Myers; L. McLaren and R. A. Keller (1968) "High pressure gas chromatography of nonvolatile species" *Science* **162**: 67-73

Giddings, J. C.; M. N. Myers and J. W. King (1969) "Dense gas chromatography at pressures to 2000 atmospheres" *J Chromatogr Sci* **7**: 276-283

Gilchrist, G. F. R.; Donald S. Gamble; H. Kodama; and S. U. Khan (1993) "Atrazine interactions with clay minerals: Kinetics and equilibria of sorption" *J Agri Food Chem* **41(10)**: 1748-1755

Glass, R. L. (1987) "Adsorption of glyphosate by soils and clay minerals" *J Agric Food Chem* **35**: 497-500

Golab, T.; W. A. Althaus, and H. L. Wooten (1979) "Fate of $^{14}C$-trifluralin in soil" *J Agric Food Chem* **27**: 163-179

Goring, , C. A. L., and W. V. Bartholomew (1952) "Adsorption of mononucleotides, nucleic acids and nucleoproteins by clay" *Soil Sci* **74**: 149-164

Goring, , C. A. L. (1967) "Physical aspects of soil in relation to the action of soil fungicides" *Annu Rev Phytoathol* **5**: 285-318

Graham-Bryce, I. J. (1981) "The behavior of pesticides in soil" *In: The Chemistry of Soil Processes*, D. J. Greenland and M. H. B. Hayes (Eds), Wiley, New York, pp. 621-670

Greenland, D. J. (1965) "Interaction between clays and organic compounds in soils" Part I. Mechanisms of interaction between clays and defined organic compounds. *Soil Fert* **28(5)** 415-425

Gurgial, G. S.; N. R. Foster and J. S. L. Yun, in M. L. Lee and K. E. Markides (Eds) (1991) *Proceedings of the Second International Symposium on*

*Supercritical Fluids*, Boston, MA, May 1991, Chromatography Conferences, Provo, UT p. 66

Hadaway, A. B., and F. Barlow (1952) "Studies on aqueous suspensions of insecticides. III. Factors affecting the persistence of some synthetic insecticides" *Bull Ent Res* **43:** 281-311

Hamaker, J. W., and J. M. Thompson (1972) "Adsorption" in: Goring C. A. I., and J. W. Hamaker (Eds) *Organic chemicals in the soil environment* Vol 1, Marcel Dekker Inc., New York. pp 49-143

Hamaker, A. B., and C. A. I. Goring (1976) "Turnover of pesticide residues in soil" In: Kaufman D. D.; G. G. Still; G. D. Paulson, and S. K. Bandal (Eds) *Bound and Conjugated Pesticide Residues* ACS Symp Series **29:** 219-243

Hance, R. J. (1969) "Influence of pH, exchangeable cation and the presence of organic matter on the adsorption of some herbicides by montmorillonite" *Can J Soil Sci* **49:** 357-364

Hance, R. J.; T. H. Byast, and P. D. Smith (1980) "Apparent decomposition of paraquat in soil" *Soil Biol Biochem* **12:** 447-448

Hance, R. J. (1982) "Herbicide persistence-Is it a problem?" in pesticide chemistry, human welfare and the environment, vol. 4, J. Miyamoto and P. C. Kearney (Eds) *5th Int Congr Pesticide Chemistry Proc* (Kyoto, Japan), pp. 195-200

Hance, R. J.; T. H. Byast; P. D. Smith, and T. M. Weight (1985) "Paraquat persistence-statistical analysis of the WRO long term trial" *Weed Res Org* (Oxford, England) Tech Rep No 73

Haque, R.; F. T. Lindstrom; V. H. Freed; and R. Sexton (1968) "Kinetic study of the sorption of 2,4-D on some clays" *Environ Sci Technol* **2:** 207-211

Hawthorne, S. B. (1990) "Analytical-scale supercritical fluid extraction" *Anal Chem* **62**: 633-636A

Hawthorne, S. B.; D. J. Miller; D. D. Walker; D. E. Whittington and B. L. Moore (1991) "Quantitative extraction of alkylbenesulfonates using supercritical carbon-dioxide and a simple device for adding modifiers" *J Chromatogr* **541**: 185

Hawthorne, S. B.; D. J. Miller; D. E. Nivens and D. C. White (1992) "Supercritical fluid extraction of polar analytes in situ chemical derivatization" *Anal Chem* **64**: 405-412

Hawthorne, S. B.; D. J. Miller; M. D. Burford; J. J. Langenfeld; S. Eckert-Tilotta and P. K. Louie (1993) "Factors controlling quantitative supercritical fluid extraction of environmental samples" *J Chromatogr* **642**: 301-317

Helling, C. S., and A. E. Krivonak (1978) "Physicochemical characteristics of bound dinitroaniline herbicides in soils" *J Agric Food Chem* **26**: 1164-1172

Hills, J. W.; H. H. Hill; and T Maeda (1991) "Simultaneous supercritical fluid derivatization and extraction" *Anal Chem* **63**: 2152-2155

Hinze, Willie L. (1992) "Cloud point extraction and preconcentration procedures for organic and related pollutants of state concern" *Rep-Water Resour Res Inst* Univ. of North Carolina **269**: 36 [CODEN: RWCDT; ISSN: 0078-1525]

Hitchen, S. M.; C. J. Dowle, and R. L. Tranter (1993) "Experimental design approach for supercritical fluid extraction." *Analytica Chimica Acta* **271**: 83-90

Hofler, F., and S. Reindl (1994) "Optimization of the parameters in supercritical fluid extraction of polynuclear aromatic hydrocarbons from soil samples." *Anal Chem* **66**: 1808-1816

Hong, Jongki; Yunwoo, Eo; Jaeseong, Rhee;Taekiae, Kim; Kangjin, Kim (1993) "Simultaneous analysis of 25 pesticides in crops using gas chromatography and their identification by gas chromatography-mass spectrometry." *J Chromatogr* **639(2):** 261-271

Hsu, T. S., and H. Bartha (1976) "Hydrolyzable and nonhydrolyzable 3,4-dichloroaniline-humus complexes and their respective rates of degradation." *J Agric Food Chem* **24:** 118-122

Ishii, Hideki; Junichiro, Miura; and Hiroto, Watanabe (1977) "Extraction of nickel with 1-(2-thiazolylazo)-2-naphthol into a nonionic surfactant as the solvent." *Bunseki Kagaku* (Japan) **26(4):** 252-256

Jackson, P. (1993) "Advantages of combined gas chromatography-mass spectrometry-matrix isolation Fourier transform infrared analysis." *Anal Proc* **30(10):** 394-395

Janda, V.; G. Steenbeke, and P. Sandra (1989) "Supercritical fluid extraction of s-triazine herbicides from sediment" *J Chromatogr* **479**: 200

Jenkinson, D. S., and J. H. Rayner (1977) "The turnover of soil organic matter in some of the Rothamsted classical experiments" *Soil Science* **123:** 298-305

Karasek, F. W. and R. E. Clement (Eds) (1988) "Basic gas chromatography-mass spectrometry: principles and techniques" Elsevier in Amsterdam

Karickhoff, S. W. (1980) "In contaminants and Sediments" Baker, R. A., Ed.; Science/Butterworth: Ann Arbor, MI pp. 193-205

Karickhoff, S. W. (1984) "Organic pollutant sorption in aquatic systems" *J Hydraul Eng* **110:** 707-735

Karickhoff, S. W., and D. S. Brown (1978) "Paraquat sorption as a function of particle size in natural sediments" *J Environ Qual* **7:** 246-252

Karickhoff, S. W.; D. S. Brown, and T. A. Scott (1979) "Sorption of hydrophobic pollutants on natural sediments" *Water Res* **13:** 241-248

Kaickhoff, S. W. and K. W. Morris (1985) "Sorption dynamics of hydrophobic pollutants in sediment suspension" *Environ Toxicol Chem* **4:** 469-479

Katan, J.; T. W. Fuhremann, and E. P. Lichtenstein (1976) "Binding of [$^{14}$C] parathion in soil: A reassessment of pesticide persistence" *Science* **193:** 891-894

Kaufman, D. D.; G. G. Still; G. D. Paulson, and S. K. Bandal (Eds) (1976) "Bound and Conjugated Pesticide Residues" *ACS Symp Series* **29:** pp 1-10

Kearney, P. C. (1976) "Summary of soil bound residues discussion session" In: Kaufman, D. D.; G. G. Still; G. D. Paulson, and S. K. Bandal (Eds) (1976) *Bound and Conjugated Pesticide Residues* ACS Symp Series **29:** pp 378-382

Kearney, P. C. (1982) "Definition of non-extractable pesticide residues in plants and soils" *J Assoc Offic Anal Chem* **65:** 1031-1032

Khan, S. U. (1973) "Interaction of bipyridylium herbicides with organo-clay complex" *J Soil Sci* **53:** 199-204

Khan, S. U. (1980) "Pesticides in the soil environment" *Fundamental Aspects of Pollution Control and Environmental Science,* Ser. 5, R. J. Wakeman (Ed), 5 Elsevier, Amsterdam 240p.

Khan, S. U. and H. A. Hamilton (1980) "Extractable and bound (nonextractable) residues of prometryn and its metabolites in an organic soil" *J Agric Food Chem* **28:** 126-132

Khan, S. U., and W. J. Saidak (1981) "Residues of atrazine and its metabolites after prolonged usage" *Weed Res* **21:** 9-12

Khan, S. U., and K. C. Ivarson (1981) "Microbiological release of unextractable (bound) residues from an organic soil treated with prometryn" *J Agric Food Chem* **29:** 1301-1303

Khan, S. U., and K. C. Ivarson (1982) "Release of soil bound (unextractable) residues from various physiological groups of microorganisms" *J Environ Sci Health* **B17(6):** 737-749

Khan, S. U. (1982a) "Bound pesticide residues in soil and plants" *Residue Reviews* **84:** 1-24

Khan, S. U. (1982b) "Distribution and characteristics of bound residues of prometryn in an organic soil" *J Agric Food Chem* **30:** 175-179.

Khan, Shahmat, U.; Stephane Dupont (1987) "Bound pesticide residues and their bioavailability" *Pesticide Sci Biotechnology, Proc. Int. Congr. Pestic. Chem., 6th;* Meeting Date 1986, 417-20. Editor(s): Greenhalgh, Roy; Roberts, Terence Robert. Publisher: Blackwell, Oxford, UK. (CODEN: 56BKA6)

Khan, S. U. (1995) "Supercritical fluid extraction of bound pesticide residues from soil and food commodities" *J Agric Food Chem* **43:** 1718-1723

King, J. W. (1989) "Fundamentals and applications of supercritical fluid extraction in chromatographic science" *J Chromatogr Sci* **27:** 355-364

Klein, W., and I. Scheunert (1982) "Bound pesticide residues in soil, plants and food with particular emphasis on the application of nuclear techniques" in: *Agrochemicals: Fate in food and environment* Proc. Intern Symp IAEA Vienna. pp 177-205

Knight, BAG, and P. J. Denny (1970) "The interaction of paraquat with soil: Adsorption by an expanding lattice clay mineral" *Weed Res* **10:** 40-48

Knight, BAG, and T. E. Tomlinson (1967) "The interaction of paraquat with mineral soils" *J Soil Sci.* **18:** 233-243

Kovcas, M. F. (1986) "Regulatory aspects of bound residues (chemistry)" *Residue Reviews* **97:** 1-17

Kruckonis, V., and M. McHugh (1986) "Supercritical fluid extraction: principles and practice" Butterworths: Stoneham, MA, 182-187

Laird, D. A.; E. Barriuso; R. H. Dowdy; W. C. Koskinen (1992) "Adsorption of atrazine on smectites" *Soil Sci Soc Am J* **56:** 62-67

Langenfeld, J. J., Hawthorne, S. B., Miller, D. J. and J. Pawliszyn (1995) "Kinetic study of supercritical fluid extraction of organic contaminants from heterogeneous environmental samples with carbon dioxide and elevated temperatures" *Anal Chem* **67:** 1727-1736

Ledwith, A., and H. J. Woods (1970) "Change transfer spectra and reaction intermediates. Part 2. Stable crystalline complexes from phenols and paraquat salts" *J Chem Soc* (C), pp 1422-1425

Lee H. B.; T. E. Peart; and R. L. Hong-You (1992) "In situ extraction and derivatization of pentachlorophenol and related compounds from soils using a supercritical fluid extraction system" *J Chromatogr* **605:** 109

Leenheer, J. A, and J. L. Ahlrichs (1971) "A kinetic and equilibrium study of the adsorption of carbaryl and parathion upon soil organic matter surfaces" *Soil Sci Soc Am Proc* **35:** 700-704

Li, S. F. Y., H. K. Lee, and C. P. Ong (1990) "Optimization of Mobile phase composition for high performance liquid chromatographic separation by means of the overlapping-resolution mapping scheme" *J Chromatogr* **506:** 245-252

Li, S. F. Y.; C. P. Ong, and K. Li (1994) "Systematic multivariate optimization of supercritical fluid extraction" *J Chromatogr Sci* **32:** 53-56

Lichtenstein, E. P.; T. W. Fuhremann, and K. R. Schulz (1971) "Persistence and vertical distribution of DDT, lindane, and aldrin residues, 10 and 15 years after a single soil application" *J Agric Food Chem* **19:** 718-721.

Lichtenstein, E. P.; J. Katan, and B. N. Anderegg (1977) "Binding of 'persistent' and 'nonpersistent' $^{14}C$-labeled insecticides in an agricultural soil" *J Agric Food Chem* **25:** 43-47

Lichtenstein, E. P. (1980) "Bound residues in soils and transfer of soil residues in crops" *Residue Reviews* **76:** 147-153

Locke, M. (1993) "Supercritical $CO_2$ fluid extraction of fluometuron herbicide from soil" *J Agric Food Chem* **41:** 1081-1084

Lopez-Avila, V.; N. S. Dodhiwala, and W. F. Beckert (1990) "Supercritical fluid extraction and its application to environmental analysis" *J Chromatogr Sci* **28:** 468-476

Ma, Liwang, and H. M. Selim (1994) "Predicting atrazine adsorption-desorption in soils: a modified second-order kinetic model" *Water Resour Res* **30(2):** 447-456

Macalady, D. L.; N. L. Wolfe (1984) "Abiotic hydrolysis of sorbed pesticides" *ACS Symp Series* No. **259:** 221-244

McCall, P. J. and G. L. Agin (1985) "Desorption kinetics of picloram as affected by residence time in the soil" *Environ Toxicol Chem* **4:** 37-44

McNally, M. E. P., and J. R. Wheeler (1988a) "Extraction coupled with supercritical fluid chromatography for the separation of sulfonylurea herbicides and their metabolites from complex matrices" *J Chromatogr* **435**: 63-71

McNally, M. E. P., and J. R. Wheeler (1988b) "Increasing extraction efficiency in supercritical fluid extraction from complex matrices: predicting extraction efficiency of diuron and linuron in SFE using SFC retention" *J Chromatogr* **447**: 53-63

McNally, M. E. P, Deardorff, C. M. and T. M. Fahmy (1992) "Supercritical fluid extraction: New directions and understandings" *Symposium Series 488*; ACS, Washington, DC

Means, J. C.; S. G. Wood; J. J. Hassett; and W. L. Barnwart (1980) "Sorption of polynuclear aromatic hydrocarbons by sediments and soils" *Environ Sci Technol* **14:** 1524-1528

Miller, C. T.; and J. A. Pedit (1992) "Use of a reactive surface-diffusion model to describe apparent sorption-desorption hysteresis and abiotic degradation of lindane in a subsurface material" *Environ Sci Technol* **26:** 1417-1427

Morris, M. and T. J. Mitchell (1983) "Two-level multifactor designs for detecting the presence of interactions" *Technometrics* **25(4):** 345-355

Mulcahey, L. J.; J. L. Hedrick and L. T. Taylor (1991) "Collection efficiency of various solid-phase traps for off-line supercritical fluid extraction" *Anal Chem* **63**: 2225-2232

Murray, D. S.; P. W. Santelman, and J. M. Davidson (1975) "Comparitive adsorption, desorption, and mobility of dipropetryn and prometryn in soil" *J Agric Food Chem* **23:** 578-582

Murugaverl, B. and K. J. Voorhees (1991) "On-line supercritical fluid extraction/chromatography systems for trace analysis of pesticides in soybean oil and rendered fats." *J Microcol Sep* **3:** 11-16

Nimmo, W. B., A. G. M. Willems, K. D. Joustra, and A. Nerloop (1986) "The degradation of diflubenzuron and its chief metabolites in soils. Part II. Fate of 4-chlorophenylurea" *Pestic Science* **17:** 403-411

Nomura, N. S., and H. W. Hilton (1977) "The adsorption and degradation of glyphosate in five Hawaiian sugarcane soils" *Weed Res* **17:** 113-121

Ong, C. P.; K. P. Chin; H. K. Lee, and S. F. Y. Li (1991) "Optimization of supercritical fluid chromatographic separation of nitroaromatics by the overlapping-resolution mapping scheme" *J High Res Chromatogr* **14:** 249-253

Oostdyk, T. S.; R. L. Grob; J. L. Snyder, and M. E. McNally (1993) "Optimization of the supercritical fluid extraction of primary aromatic amines" *J Chromatogr Sci* **31:** 177-182

Papilloud, S.; W. Haerdi; S. Chiron, and D. Barcelo (1996) "Supercritical fluid extraction of atrazine and polar metabolites from sediments followed by confirmation with LC/MS" *Environ Sci Technol* **30:** 1822-1826

Pavlostathis, S. G.; and G. N. Mathavan (1992) "Desorption kinetics of selected volatile organic compounds from field contaminated soils" *Environ Sci Technol* **26:** 532-538

Peck, D. E. (1977) "The adsorption-desorption of diuron by fresh water sediments" *M. S. Thesis,* University of California, Riverside

Pignatello, J. J. (1990) "Slowly reversible sorption of aliphatic halocarbons in soils" *Environ Toxicol and Chem* **9:** 1107-1115

Pignatello, J. J.; F. J. Ferrandino; and L. Q. Huang (1993) "Elution of aged and freshly added herbicides from a soil" *Environ Sci Technol* **27:** 1563-1571

Pillmoor, J. B.; J. K. Gaunt, and T. R. Roberts (1984) "Examination of bound (nonextractable) residues of MCPA and flamprop in wheat straw" *Pestic Science* **15:** 375-381

Pinck, L. A., and F. E. Allison (1951) "Resistance of a protein-montmorillonite complex to decomposition by soil microorganisms" *Science* **114:** 130-131

Plackett, R. L. and J. P. Burman (1946) "The design of optimal multifactorial experiments" *Biometrika* **33:** 305-325

Rache, K. D., and E. P Lichtenstein (1985) "Effects of soil microorganisms on the release of bound $^{14}C$ residues from soils previously treated with [$^{14}C$] parathion" *J Agric Food Chem* **33:** 938-943

Raghu, K. and J. Drego (1986) "Bound residues of lindane: Magnitude, microbial release, plant uptake and effect on microbial activities, in quantification, nature and bioavailability of bound $^{14}C$-pesticide residues in soil, plant and food" *(IAEA, Vienna)* 41

Rao, P. S. C. (1974) "Pore-geometry effects on solute dispersion in aggregated soils and evaluation of model" *Ph.D. Dissertation,* University of Hawaii

Reindl, S, and F. Hofler (1994) "Optimization of the parameters in supercritical fluid extraction of polynuclear aromatic hydrocarbons from soil samples" *Anal Chem* **66:** 1808-1816

Richter, Bruce E (1992) "Supercritical fluid extraction methods" in *Emerging Strategies for Pesticide Analysis*, Thomas Cairns and Joseph Sherma, Eds. p. 51-68. CRC Press; Boca Raton.

Ricter, B. E.; J. Ezzell; and D. Felix (1994) "Single laboratory method validation report: extraction of organophosphorous pesticides, chlorinated herbicides, and polychlorinated biphenyls using accelerated solvent extraction (ASE) with analytical validation by GC/NPD and GC/ECD" *Document 101124*, Dionex Corp.

Riley, D, and R. P. Gratton (1974) "Unavailability to plants of diquat residues in soils" *10th Int Congr Soil Sci* **3:** 193-202

Riley, D; B. V. Tucker, and W. Wilkinson (1976) "Biological unavailability of bound paraquat residues in soil" in: Kaufman, D. D.; G. G. Still; G. D. Paulson, and S. K. Bandal (Eds) *Bound and conjugated pesticide residues* ACS Symp Series 29: 301-353

Roberts, T. R. (1984) "Non-extractable pesticide residues in soils and plants" *IUPAC Reports on pesticides (17) Pure Appl Chem* **56:** 945-956

Ruepel, M. L.; B. B. Brightwell; J. Schaefer, and J. J. Marvel (1977) "Metabolism and degradation of glyphosate in soil and water" *J Agric Food Chem* **25:** 517-527

Saltzman, S., and B. Yaron (Eds) (1986) "Pesticides in soils" Van Nostrand-Reinhold, New York

Saar, J. and A. Salomon (1990) "Thermospray mass spectrometry of N-methylcarbamates" *Mass Spectrom* **25:** 209-213

Sauerbeck, D. R. (1980) "Influence of crop rotation, manurial treatment and soil tillage on the organic matter content of German soils" In: Boels D., D. B. Davies, and A. E. Johnston (Eds) *Soil Degradation* Balkema, Rotterdam. pp 163-177

Sawhney, B. L. and K. Brown (Eds) (1989) "Reactions and movement of organic chemicals in soils" *SSSA Special Publication Number 22,* Soil Science Society of America, Inc., American Society of Agronomy, Inc. Madison, Wisconsin, USA

Senesi, N. (1981) "Free radicals in electron donor-acceptor reactions between a soil humic acid and photosynthesis inhibitor herbicides" *Z Pflanzenernaehr Bodenk* **144:** 580-586

Shalaby, L. M. (1985) "Liquid chromatography/mass spectrometry of the thermally labile herbicides, chlorsulfuron and sulfometuron methyl" *Biomed Mass Spectrom* **12:** 261-268

Smith, A. E. and L. J. Milward (1983) "Comparison of solvent systems for the extraction of dicolofop, picloram, simazine and triallate from weathered field soil" *J Agric Food Chem* **31:** 633-637

Smith, S. N., and D. C. G. Muir (1984) "Determination of extractable and nonextractable radioactivity from small field plots 45 and 95 weeks after treatment with $^{14}$C-dicamba, (2,4-dichloro-$^{14}$C-phenoxy) acetic acid, $^{14}$C-triallate and $^{14}$C-triflualin" *J Agric Food Chem* **32:** 588-593

Smith, S. N.; A. J. E. Lyon, and I. B. Sahid (1976) "The breakdown of paraquat and diquat by soil fungi" *New Phytol* **77:** 735-740

Snyder, J. L.; R. L. Grob; M. E. McNally and T. S. Oostdyk (1992) "Comparison of supercritical fluid extraction with classical sonication and Soxhlet extraction for selected pesticides" *Anal Chem* **64:** 1940-1946

Snyder, J. L.; R. L. Grob; M. E. McNally and T. S. Oostdyk (1993) "The effect of instrumental parameters and soil matrix on the recovery of organochlorine and organophosphate pesticides from soils using supercritical fluid extraction" *J Chromatogr Sci* **31:** 183-191

Somasundaram, L. and J. R. Coats (1991) "Pesticide transformation products in the environment" *ACS Symposium Series* No. 459 Pesticide Transformation Products: Fate and Significance in the Environment

Somasundaram, L.; J. R. Coats; V. M. Shanbhag; K. D. Racke (1991) "Mobility of pesticides and their hydrolysis metabolites" *Environ Tox Chem* **10:** 185-194

Sorensen, C. H. (1972) "Role of amino acid metabolites in the formation of soil organic matter" *Soil Biol Biochem* **4:** 245-255

Sparks, Donald, L. (1989) "Kinetics of soil chemical processes" Academic Press, Inc. Harcourt Brace Jovanovich Publishers, USA

Sparks, Donald, L. (1995) "Environmental soil chemistry" Academic Press, Inc., Harcourt Brace Jovanovich Publishers, USA

Sprankle, P., W. F. Meggitt, and D. Penner (1975a) "Rapid inactivation of glyphosate in the soil" *Weed Sci* **23:** 224-228

Sprankle, P., W. F. Meggitt, and D. Penner (1975b) "Adsorption, mobility and microbial degradation of glyphosate in soil" *Weed Sci* **23:** 229-234

Stahl, E. and G. Rau (1984) "Decontamination of insecticides in natural drugs with supercritical carbon dioxide" *Plants Med* **2:** 171-173

Steinberg, S. M., Pignatello, J. J., and B. L. Sawhney (1987) "Persistence of 1,2-dibromethane in soils: Entrapment in intraparticle micropores" *Environ Sci Technol* **21:** 1201-1208

Stevenson, F. J. (1976) "Organic matter reactions involving pesticides in soil." in: Kaufman, D. D., Still, G. G., Paulson, G. D., S. K. Bandal (Eds) *Bound and Conjugated Pesticide Residues* ACS Symposium Series 29 pp 180-207

Stott, D. E.; J. P. Martin; D. D. Focht, and K. Haider (1983) "Biodegradation, stabilization in humus and incorporation into soil biomass of 2,4-D and chlorocatechol carbons" *Soil Sci Soc Am J* **47:** 66-70

Terce, M. (1983) "Effect of the dilution of kaolinite and montmorillonite suspensions on the adsorption of atrazine" *Argonomie* **3:** 883-890

Terce, M., and R. Calvet (1978) "Adsorption of several herbicides by montmorillonite. kaolinite and illite clays" *Chemosphere* **4:** 365-370

Tucker, B. V.; D. E. Pack; J. N. Ospenson; A. Omid, and W. D. Thomas (1969) "Paraquat soil bonding and plant response" *Weed Sci* **17:** 448-451

US Federal Register (1975) Registration of pesticides in the United States-Proposed guidelines. *Fed Regis* **40:** p 123

van Genuchten, M. T.; J. M. Davidson, and P. J. Wienenga (1974) "An evaluation of kinetic and equilibrium equations for the prediction of pesticide movement through porous media" *Soil Sci Soc Am Proc* **38:** 29-35

Vannoort, R. W.; J. P. Chervet; H. Lingeman; G. J. de Jong and U. A. Th. Brinkman (1990) "Coupling of supercritical fluid extraction with chromatographic techniques" *J Chromatogr* **505:** 45-77

Voyksner, R. D.; and J. T. Bursey (1984) "Modification and optimization of source and interface parameters for direct liquid introduction liquid chromatography/mass spectrometry of pesticides" *Anal Chem* **56:** 1582-1587

Voyksner, R. D.; J. T. Bursey; and E. D. Pellizzari (1984) "Postcolumn addition of buffer for thermospray liquid chromatography/mass spectrometry identification of pesticides" *Anal Chem* **56:** 1507-1514

Voyksner, R. D.; J. T. Bursey; and E. D. Pellizzari (1984) "Analysis of selected pesticides by high-performance liquid chromatography-mass spectrometry" *J Chromatogr* **312:** 221-235

Walsh, J. M.; G. D. Ikonomou and M. D. Donohue (1987) "Supercritical phase behavior: the entrainer effect" *Fluid Phase Equilibria* **33:** 295-314

Weber, J. B. (1970) "Mechanisms of adsorption of s-triazines by clay colloids and factors affecting plant availability" *Residue Reviews* **32:** 93-130

Weber, J. B., and S. B. Weed (1968) "Adsorption and desorption of diquat, paraquat and prometon by montmorillonite and kaolinite clay minerals" *Soil Sci Soc Am Proc* **32:** 485-487

Wheeler, J. R. and M. E. P. McNally (1989) "Supercritical fluid extraction and chromatography of representative agricultural products with capillary and microbore columns" *J Chromatogr Sci* **27:** 534-539

Wheeler, R. E. (1972) "Efficient experimental design" Invited address, *Annual Meeting American Statistical Assoc.*

Wheeler, R. E. (1994) "Software and training for engineers and scientists" Invited address, *ECHIP® Reference Manual* Echip, Inc. (Hockessin, DE) USA

White, J. L. (1976) "Clay-pesticide interactions" in: Kaufman, D. D., Still, G. G., Paulson, G. D., and S. K. Bandal (Eds) "Bound and conjugated pesticide residues" *ACS Symposium Series* **29** pp 208-218

Wiersma, D.; van Goor, and G. van der Veen (1986) "Cadmium, lead, mercury and arsenic concentrations in crops and corresponding soils in the Netherlands" *J Agric Food Chem* **34:** 1067-1074

Wright, B. W.; S. R. Frye; D. G. McMinn, and R. D. Smith (1987) "On-line supercritical fluid extraction-capillary gas chromatography" *Anal Chem* **59:** 640-646

Wu, C. H.; N. Buehring; J. M. Davidson; and P. W. Santelmann (1975) "Napropamide adsorption, desorption and movement in soils" *Weed Science* **23:** 454-457

Yaron, B., and S, Saltzman (1972) "Influence of water and temperature on adsorption of parathion by soils" *Soil Sci Soc Am Proc* **36:** 583-586

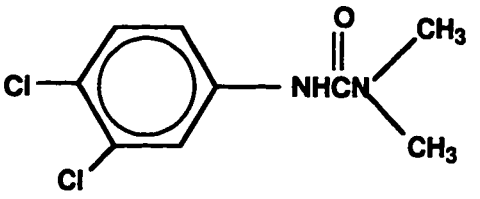
Yuyama, Takeshi; R. C. Ackerson; Shunji, Takeda; and Yoshihisa, Watanabe (1987) "Soil and water relationship in the behavior of bensulfuron methyl under the paddy field condition" *Zasso Kenkyu* **32(4):** 282-291

Zhang, L. Z., Khan, S. U. Akhtar, M. H. and K. C. Iverson (1984) "Persistence, degradation, and distribution of deltamethrin in an organic soil under laboratory conditions" *J Agric Food Chem* **32:** 1207-1211

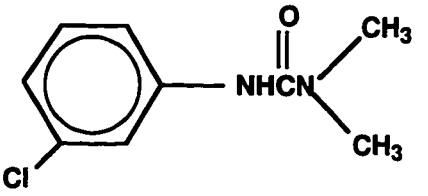
Zunino, H.; F. Borie; S. Afuilera; J. P. Martin, and K. Haider (1982) "Decomposition of derived soils of Chile" *Soil Biol Biochem* **14:** 37-43

# APPENDIX I

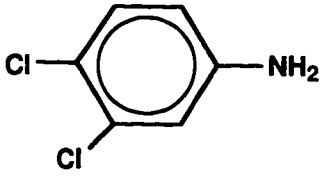
## Chemical Structures and Names



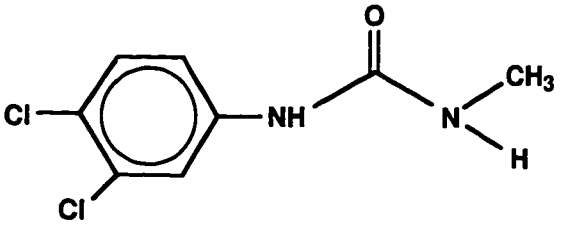

Diuron (DPX-14740)
(N'-(3,4-dichlorophenyl)-N,N-dimethylurea)



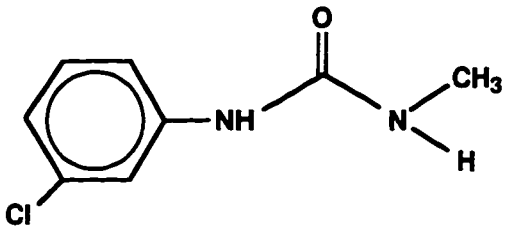

(N'-(3-monochlorophenyl)-N,N-dimethylurea) (mCPDMU)



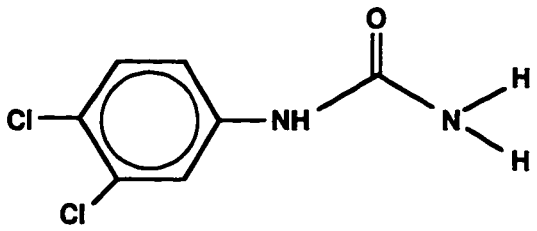

3,4-Dichloroaniline (DCA)



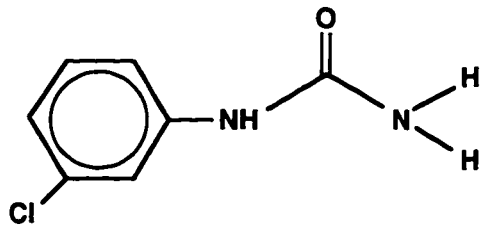

N'-(3,4-dichlorophenyl)-N-methylurea (DCPMU)



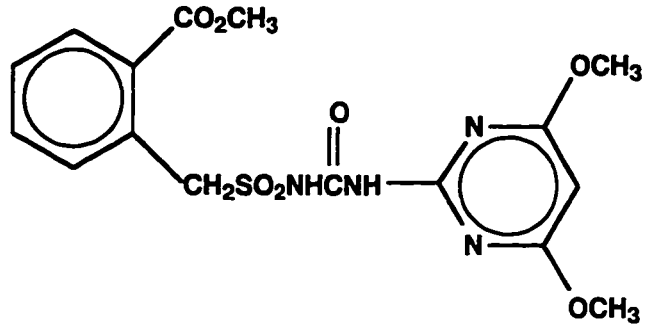

N'-(3-monochlorophenyl)-N-methylurea (mCPMU)



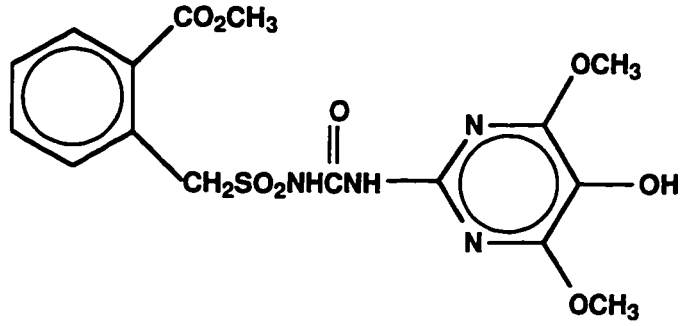

3,4-Dichlorophenylurea (DCPU)



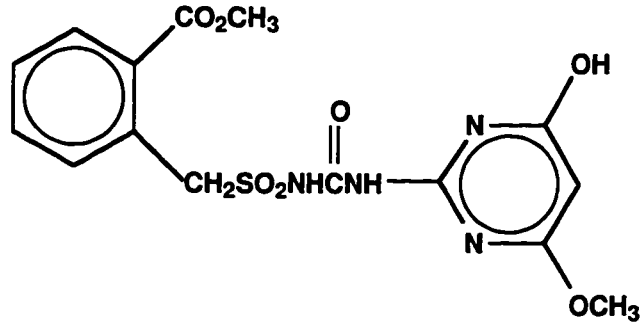

3-monochlorophenylurea (mCPU)



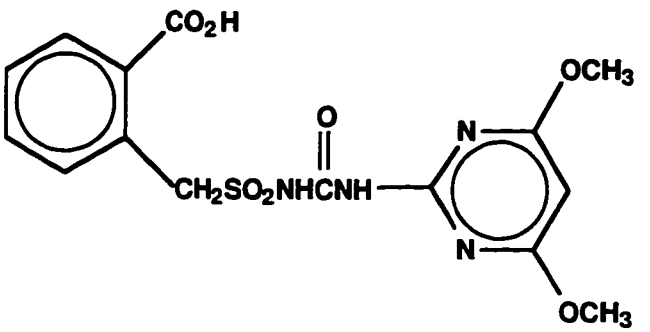

Bensulfuron Methyl (DPX-F5384)



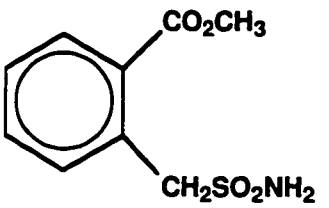

Hydroxypyrimidinyl-bensulfuron methyl (HPY-DPX-F5384)



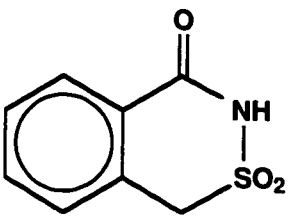

O-desmethyl-bensulfuron methyl (ODM-DPX-F5384)



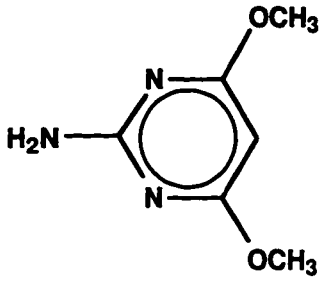

Free Acid Bensulfuron Methyl (FA-DPX-F5384)



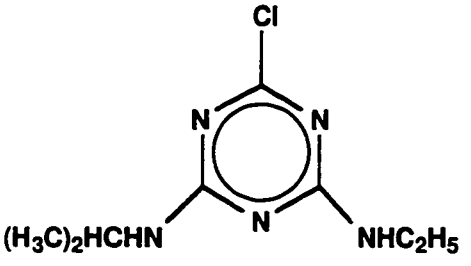

Sulfonamide



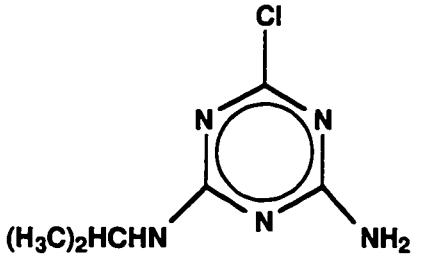

Homosaccharin



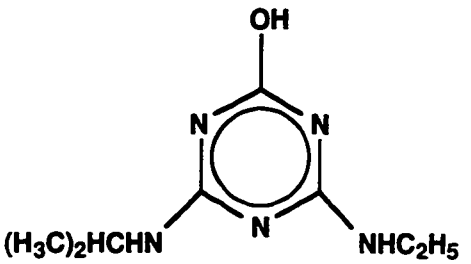

Pyrimidine-amine



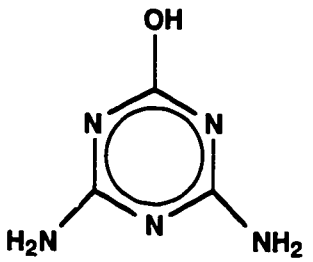

Atrazine



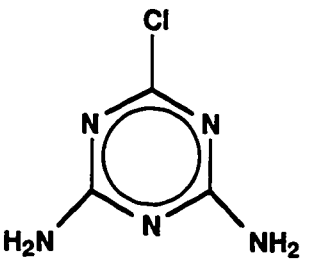

N-De-ethyl-atrazine (DEA)




2-Hydroxy-atrazine (HAT)




2,6-Diamino-4-hydroxy-atrazine (DHAT)




2,6-Diamino-atrazine (DAA)

# APPENDIX II

## Soil Characterization Data

**Soil characterization data for Woodstown, Cecil, Flanagan, and Keyport soils[a]**

| Parameter | Characterization | | | |
|---|---|---|---|---|
| | Woodstown | Cecil | Flanagan | Keyport[b] |
| % Sand (0.05-2.0 mm) | 60 | 61 | 2 | 11(12) |
| % Silt (0.002-0.05 mm) | 33 | 21 | 81 | 78(83) |
| % Clay (<0.002 mm) | 7 | 18 | 17 | 11(5) |
| % Organic Matter | 1.1 | 2.1 | 4.3 | 4.7(7.5) |
| pH | 6.6 | 6.5 | 5.4 | 4.3(5.2) |
| Cation Exchange Capacity, cmol/kg | 5.3 | 6.6 | 21.2 | 14.1(15.5) |
| Type | Sandy loam | Sandy loam | Silt loam | Silt loam |

[a] Soil analyses were performed at the Soil Testing Laboratory, College of Agricultural Sciences, University of Delaware, Newark, Del. Mechanical analyses, to determine the sand, silt, and clay content, were conducted after removal of the organic matter by wet oxidation.

[b] Keyport soils were different for diuron and bensulfuron methyl sorption/desorption studies. Values in () were for bensulfuron methyl, while the others were for diuron studies.

**Soil characterization data for Florida sandy, Spain clay, and North Carolina high organic soils[a]**

| Parameter | Characterization | | |
|---|---|---|---|
| | Miaka (Florida) | Cullera (Spain) | Belhaven (North Carolina) |
| % Sand (0.05-2.0 mm) | 91.6 | 6.8 | 85.2 |
| % Silt (0.002-0.05 mm) | 4.0 | 36.8 | 10.0 |
| % Clay (<0.002 mm) | 4.4 | 56.4 | 4.8 |
| % Organic Matter | 1.0 | 3.4 | 57.5 |
| pH | 6.2 | 8.1 | 4.0 |
| Cation Exchange Capacity, cmol/kg | 3.8 | 22.7 | 31.6 |
| Type | Sand | Clay | Loamy Sand |
| Predominant Clay Mineralogy | Kaolinite | Montmorillonite & Vermiculite | Montmorillonite & Kaolinite |
| Specific Area ($m^2/g$) | 360 | 560 | 720 |

[a] Soil analyses were performed at the Lambda Research, Cincinnati, Ohio.

# APPENDIX III

## Instrumentation and Operational Parameters

**Ramona 92 Radiochem**

| | | |
|---|---|---|
| **Detector:** | Scintillator | Solid |
| | Background (cps) | A: 0.00, B: 0.00 |
| | Spillover (%) | A>B 0.00, B>A 0.00 |
| | Range | A: Manual, B: Manual |
| | Logic Threshold | A: 500, B: 500, C: 500 |
| | Discriminator | Upper.....999 |
| | | Lower 8 |
| | Intensity A (cps) | 10 |
| | Intensity B (cps) | 10 |
| | Smoothing Times A (sec) | |
| | Peak | 10.0 |
| | Background | 10.0 |
| | Monitoring | 5.0 |
| | Smoothing Times B (sec) | |
| | Peak | 10.0 |
| | Background | 10.0 |
| | Monitoring | 5.0 |
| | Flow Rate (μL/min) | 1000 |
| | Cell Volume (μL) | 300 |
| | Efficiency (%) | A: 100.00, B: 100.00 |
| | Splitter (%) | 100.00 |

**Foxy Fraction Collector** Filled with 72 vials and programmed to collect 10 x 1 min fractions followed by 62 x 0.4 min fractions to collect 35 min of HPLC eluate per analysis

**Liquid Scintillation Counting (LSC)** LSC instruments were programmed for a maximum 10-min counting time for radioactivity determination in HPLC fractions

**Date Processing** The LSC results were electronically transferred to VAX system and processed in the RS/1 program #HPLC

**HPLC Conditions**

**Mobile phases**
%A: 0.2% aqueous formic acid
%B: unused
%C: methanol

**Programming**

| Time | %A | %C |
|---|---|---|
| 0 | 98 | 2 |
| 10 | 80 | 20 |
| 14 | 45 | 55 |
| 21 | 45 | 55 |
| 32 | 0 | 100 |
| 35 | 98 | 2 |

Reequilibration 10 min prior to next run

Refer to Appendix III for the HPLC diagram

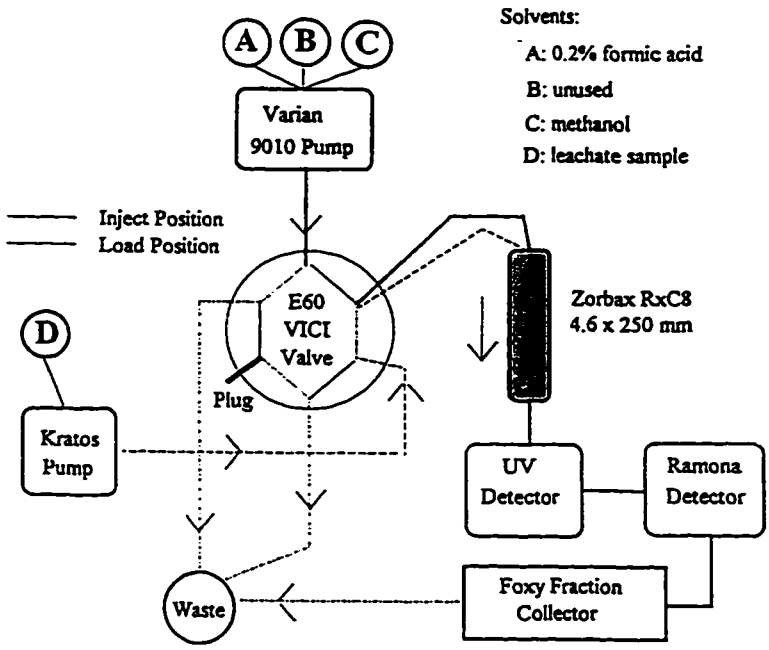



**Diagram for HPLC System**

# APPENDIX IV

## Representative Chromatograms and Mass Spectra

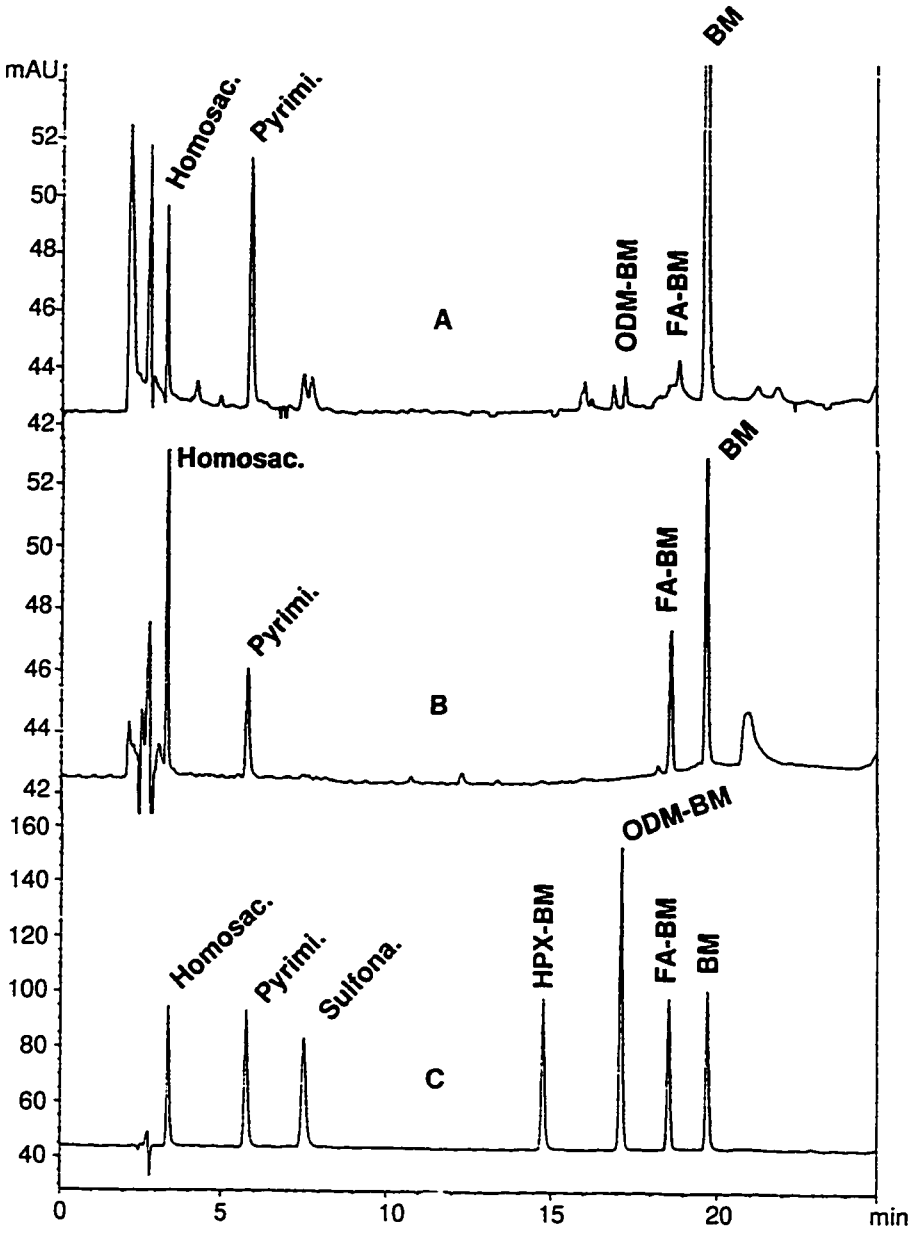



Figure A.1 Chromatograms of soil and silica extracts from bensulfuron methyl sorption/desorption studies (A), SFE extraction (silica) at 80°C (B), and mixed standards (C)

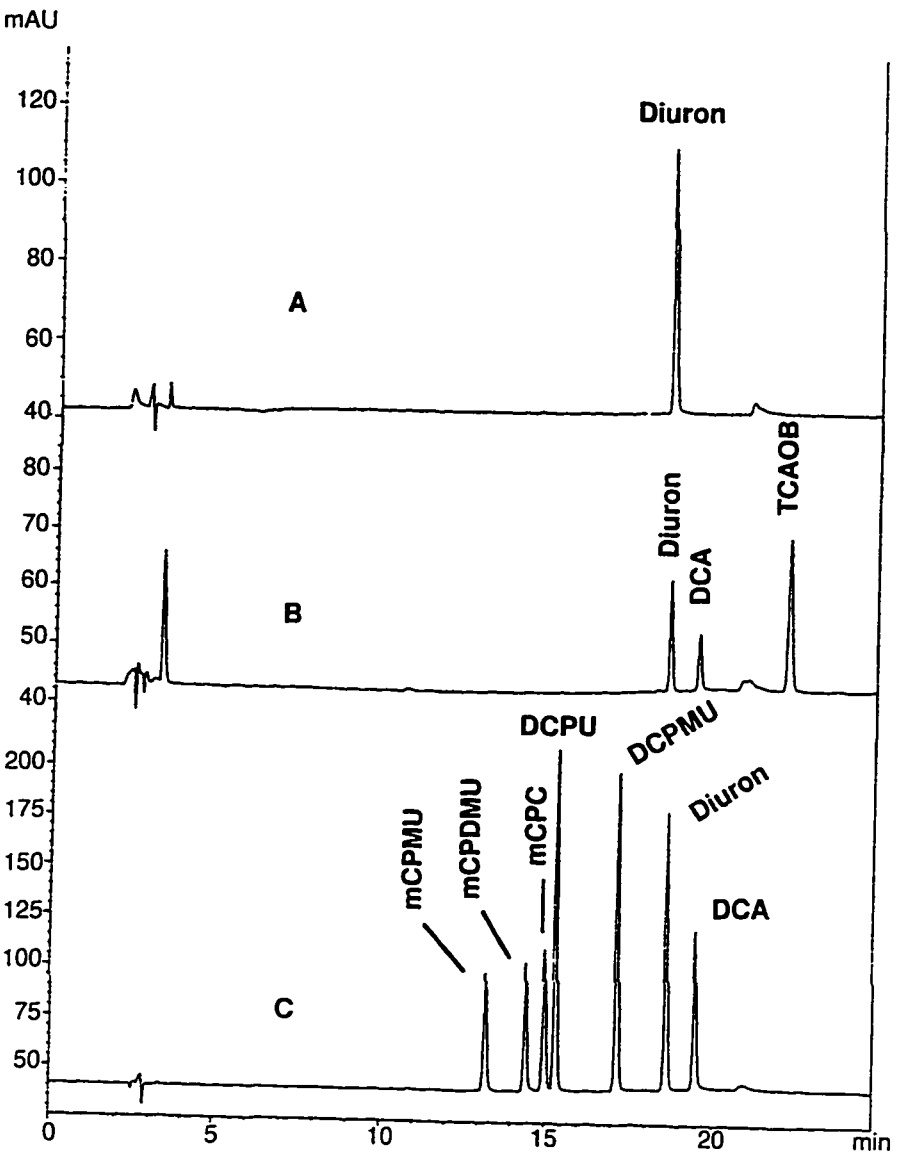



Figure A.2 Chromatograms of soil and Celite® 545 extracts from diuron sorption/ desorption studies (A), SFE extraction (Celite) at 120°C (B), and mixed standards (C)

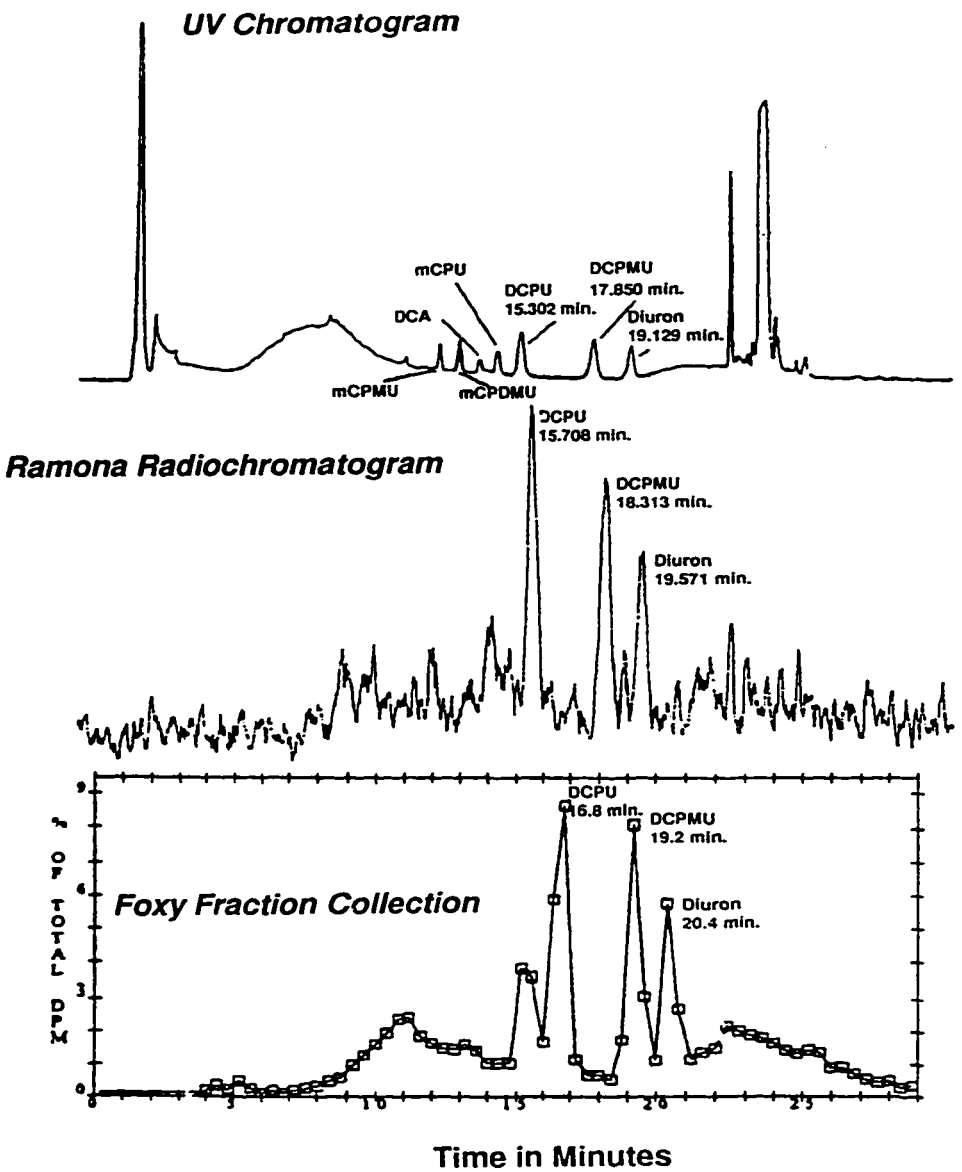



Figure A.3 Chromatograms of Swedish soil extract for UV, Ramona, and Foxy fraction analysis of diuron and potential degradation compounds

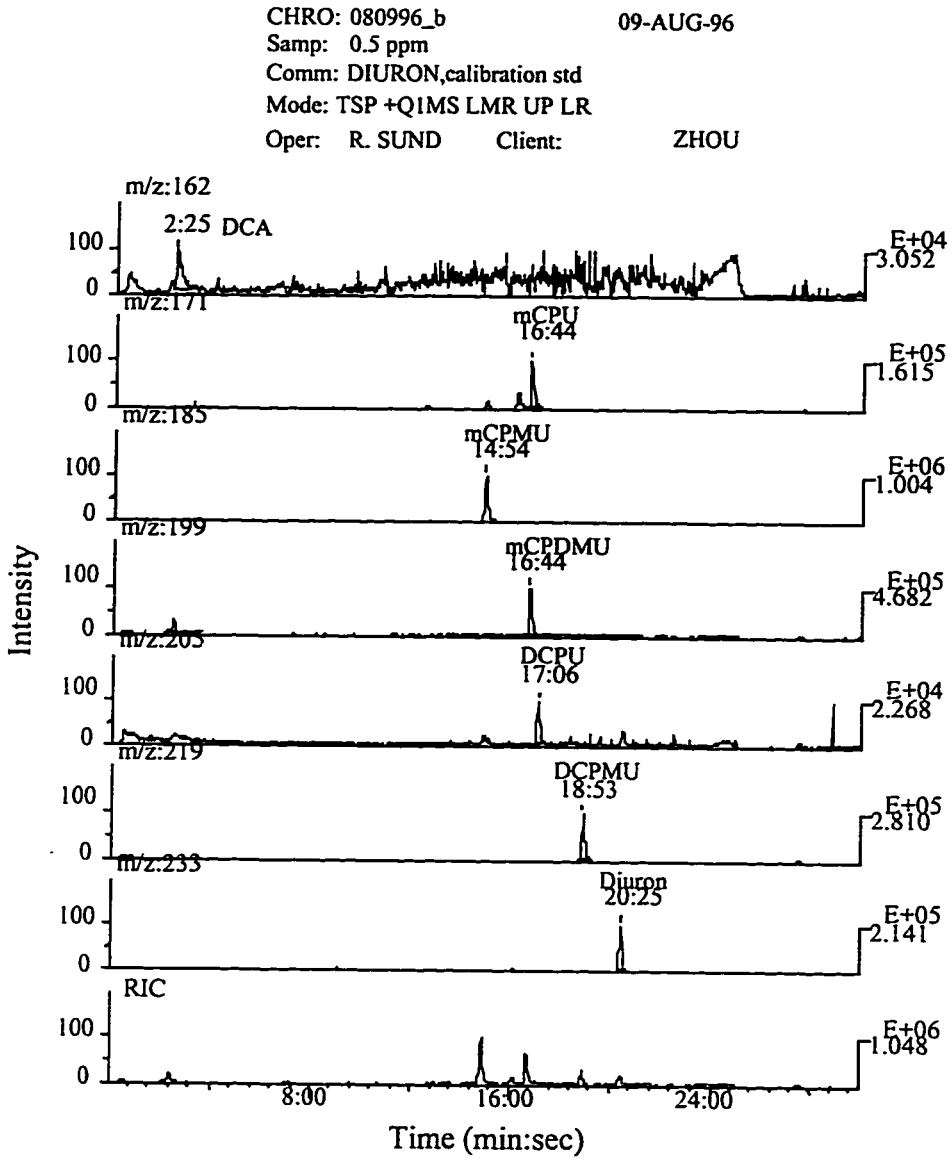



**Figure A.4 Chromatograms and mass selective ions of diuron and its potential degradation compounds**

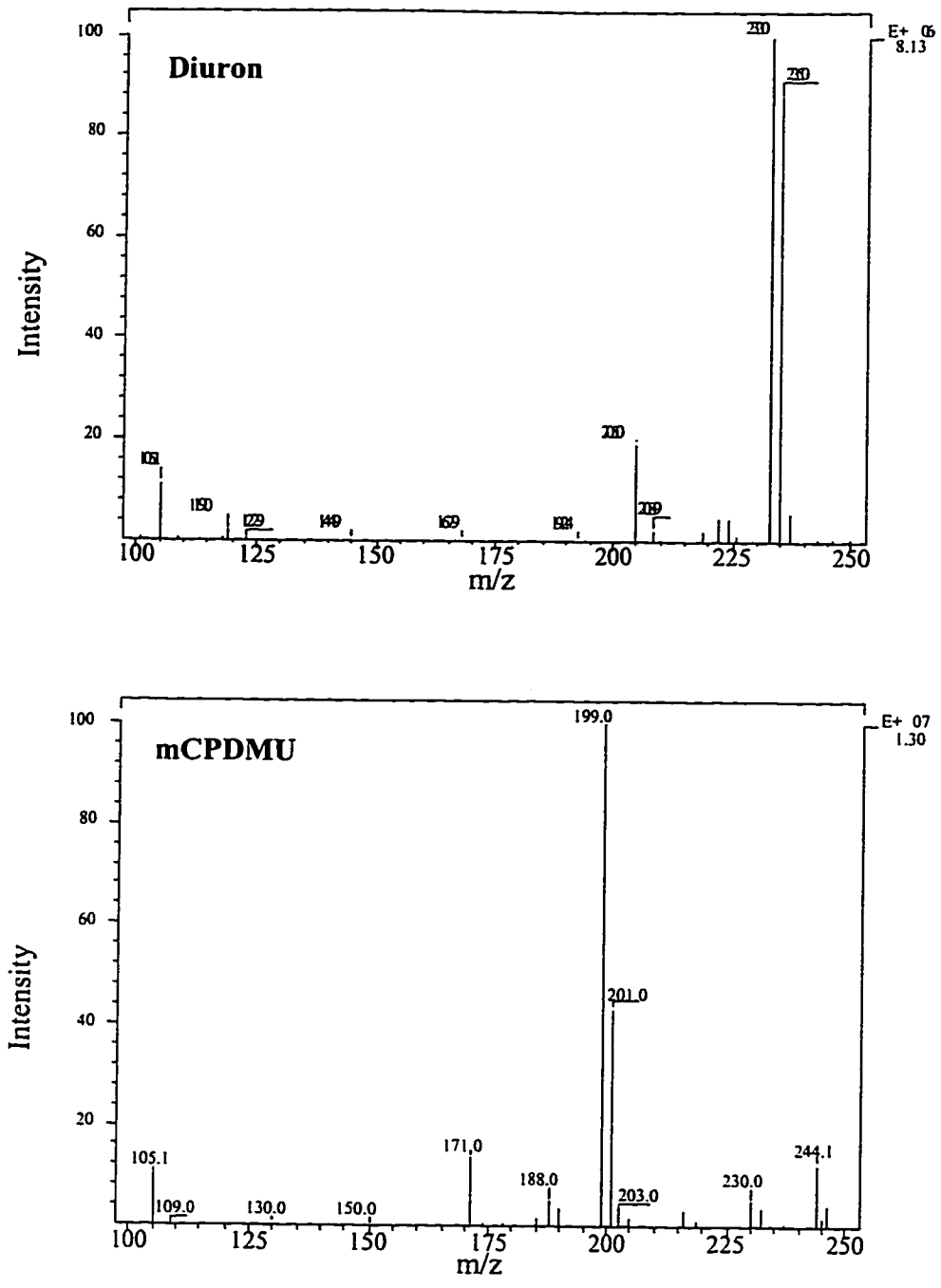



**Figure A.5 Representative mass spectra of diuron and mCPDMU**

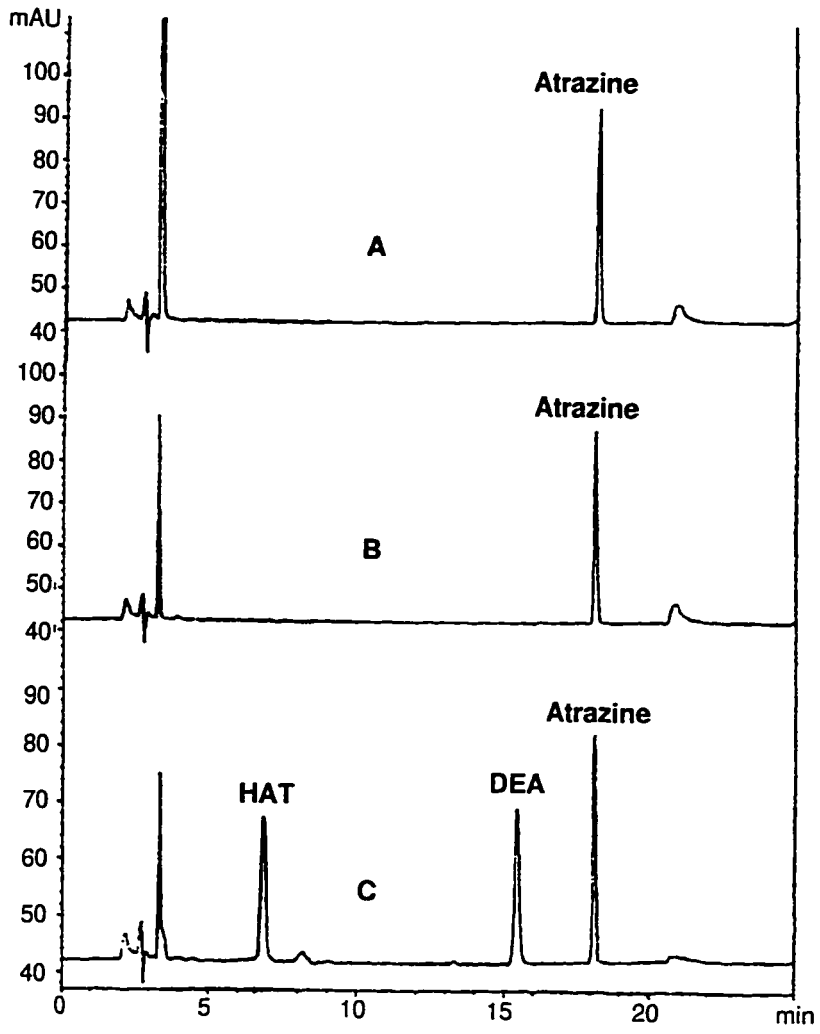



Figure A.6 Chromatograms of soil extract from atrazine sorption/ desorption studies (A), SFE extraction at 150° (B), and mixed standards (C)