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Supercritical fluid extraction of toxic organics from soils

Cai, Jane Yizhi, Ph.D.

City University of New York, 1990

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**SUPERCRITICAL FLUID EXTRACTION OF TOXIC
ORGANICS FROM SOILS**

by

Jane Yizhi Cai

**A dissertation submitted to the Graduate Faculty in Chemistry in
partial fulfillment of the requirements for the degree of Doctor of
Philosophy, The City University of New York.**

1990

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The manuscript has been read and accepted for the Graduate Faculty in Chemistry in satisfaction for the dissertation requirement for the degree of Doctor of Philosophy.

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Dedicated to
my lovely daughter, Elizabeth Y. Wei

ABSTRACT

Supercritical Fluid Extraction of Toxic Organics from Soils

by

Jane Yizhi Cai

Advisor: Professor David C. Locke

Supercritical fluid (SCF) extraction of six representative pesticides and two polychlorinated biphenyls (PCBs) from four types of soil samples was investigated in comparison with the conventional Soxhlet extraction technique. Supercritical carbon dioxide, nitrous oxide, and modified carbon dioxide were employed as the extraction fluids. The pesticides and PCBs include aldrin, parathion, malathion, 2,4-D methyl ester, amitrol, aldicarb, and Aroclors 1254 and 1260. The soil samples including sand, loam, top-soil and clay with various grain sizes were spiked with the pesticides or PCBs at a variety of spiking levels (e.g. from ca. 1 ng to ca. 1 mg per gram of soil sample). The extraction conditions were optimized. The extracts were analyzed by gas chromatography or by high performance liquid chromatography.

In general, the supercritical fluid extractions (SFE) of most of the compounds studied give satisfactory (>80%), and in many cases quantitative (>95%), recoveries. A low recovery (ca. 10%) was usually observed for the SFE of amitrol even under optimized conditions, which is attributed to the possible binding between amitrol and the soil components. An increase in the system pressure significantly enhances the SFE recoveries, which is attributable to the increase in the density of

the SCF and, therefore, the solubility of the compounds in the SCF. At the highest pressures studied (e.g. >9000 psi), the increase in the recoveries in some cases becomes less significant because of the inherent limit of the solubilities of the organics in the SCF. The effect of the system temperature on the recoveries is more complex owing to the existence of competing factors such as the vapor pressure of the solutes and the density of the SCF. In most cases, the recovery goes through a maximum as the temperature increases at the temperatures above the critical point. The effect of a modifier (e.g. methylene chloride, methanol, benzene, toluene, or water) depends on the nature of the modifier and the extraction system. For the SFE of aldrin and PCBs, modification of the supercritical CO₂ with methylene chloride greatly increases the recoveries, whereas methanol does not affect the recovery and benzene reduced the recovery. For the SFE of 2,4-D methyl ester, parathion and malathion, methanol enhances the recoveries. Water increases the recoveries of amitrol and aldicarb. These results are qualitatively interpreted in relation with the polarities and polarizability of the modifiers. The extraction time required for SFE is generally in a range of 10 to 30 min, which is shorter than that for the Soxhlet extractions. For the SFE of PCBs, almost quantitative recoveries were achieved within 20 min. The efficiency of SFE varies with the type and grain size of soil samples. The recoveries were found to be in the following order: sand > loam > top-soil > clay. And the larger the grain size the higher the recoveries. All the results demonstrated that the SFE of the pesticides and PCBs except amitrol presents a superior method over the conventional Soxhlet extraction.

ACKNOWLEDGMENTS

Many years of hard work have been invested in completing this thesis. During that time, there have been numerous individuals who have share their time, knowledge, patience, and love with me. At this opportunity, I would like to particularly thank several of them.

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1.0 INTRODUCTION

Approximately 500 million kilograms of pesticides are used in the United States each year to control pest arthropods, plant pathogens, and weeds [USDA, 1982]. Most of these pesticides are synthetic organic compounds. In the first analysis, we seem to be winning our war against pests with these remarkably successful pesticides. For example, for the \$3 billion invested annually in pesticidal control, nearly \$12 billion are gained as increased crop yield, which is an excellent return on the investment based on a common business standard [Headley, 1971; Pimentel, 1978]. However, we have been and are still paying far heavier prices for the pesticide damages to the environment and to society resulting from both handling and application of these toxic chemicals. Of the half billion kilograms of pesticides, only a small fraction actually reaches the target pests [Pimentel, 1980]. The majority of these pesticides enter the environment, contaminating the soil, water, air, and biota in ecosystems and perhaps poisoning or adversely affecting nontarget organisms including humans for many years or even decades to come.

Many of these pesticides have already been demonstrated to be harmful, dangerous, or even deadly, to human health and have been banned from production and applications (e.g. aldrin, parathion, etc.). However, the residues of the pesticides are still widespread and persist in the environment owing to the great chemical and physical stabilities of these

organic compounds. Knowledge of the extent of the toxic organics in the environment depends upon the advancement of the analytical science and technology. In particular, when toxic organic compounds are adsorbed onto soil particles or chemically bonded to other compounds in the soil via ion-ion interactions or hydrogen bonding, they become difficult to extract by normal organic solvent techniques, leading to negative analytical errors and subsequently to a false sense of public security. Therefore, it is a great challenge to the analytical chemist to develop faster, more effective and powerful extraction techniques and more sensitive detection methods for the analysis of these toxic compounds in the environment.

In recent years, research on the properties of supercritical fluids has become very important because of the great promise of supercritical fluid extraction techniques. There are many advantages of using supercritical fluid extraction over conventional extraction techniques. The densities of supercritical fluids are comparable to those of typical liquids but the viscosities of supercritical fluids are similar to those of typical gases. The diffusivities of supercritical fluids are an order of magnitude higher than those of liquids. Many molecular solids with low volatility show greatly enhanced solubilities in supercritical fluids. Solvent recovery can be readily achieved by manipulating the density, and therefore the solvating power, of the supercritical fluid to precipitate the solid. The applications of supercritical fluid extraction range from analysis of organic isomers to separation of natural products, from coffee decaffeination to edible-oils extraction, and from extraction of oligomers

from polymers to monomer purification, etc.

In this research, the supercritical fluid extraction of toxic organic compounds from soil has been studied in comparison with the conventional solvent extraction techniques. Eight representative toxic organic compounds have been selected in this study from the most heavily-used and widespread pesticides or environmental pollutants. They include aldrin, an organochlorine insecticide; parathion and malathion, organophosphorus insecticides; aldicarb, a carbamate insecticide; amitrol and 2,4-D methyl ester, widely-used herbicides; and Aroclor 1254 and Aroclor 1260, polychlorinated biphenyls (PCBs), which are most infamous as hazardous, persistent and ubiquitous environmental pollutants. The methodology has been successfully established for the supercritical fluid extraction and the subsequent determination of these compounds from various types of soil samples. The influences of the extraction parameters such as temperature, pressure, extraction time, concentration of the organic compounds, nature of the supercritical fluids employed, and addition of modifiers to the supercritical fluids, on the extraction efficiency have been investigated systematically in comparison with the conventional Soxhlet extraction method. The quantitative determination of the toxic organic compounds extracted from soil samples has been carried out using capillary gas chromatography (GC) and/or high performance liquid chromatography (HPLC) as well as mass spectroscopy.

2.0 BACKGROUND

2.1 Toxic Organic Compounds: Pesticides and Polychlorinated Biphenyls (PCBs)

In almost all the industrialized and in many developing countries, people are exposed to pesticides on a regular basis. Food, particularly fresh fruits and vegetables, drinking water, flowers and trees, even beaches, rivers and oceans, all contain pesticide residues. In 1987, the National Academy of Science issued an alarming report on pesticides in the food supply which concluded that the pesticides in our food might cause more than one million additional cases of cancer in the United States over our lifetimes [Mott, 1988]. In 1986, approximately 140 dairy herds in Arkansas, Oklahoma and Missouri were quarantined owing to contamination by banned pesticides including heptachlor. Some milk contained heptachlor in amounts as much as seven times the acceptable level. A report by the Environmental Protection Agency (EPA) in 1987, entitled "Unfinished Business", stated that pesticides in food is one of the nation's most serious health and environmental problems [EPA, 1987]. Many pesticides such as captan, chlorothalonil, permethrin, acephate, DDT, parathion, and dieldrin, etc., which were widely used in food production have been reported, or suspected, to cause cancer. To date, EPA has identified fifty-five pesticides as carcinogens that could leave residues in food products [Mott, 1988]. Many other pesticides are known to cause birth defects or miscarriages - some of them can produce changes in human

genetic materials that can be passed to future generations. Moreover, some pesticides can cause sterility or impaired fertility [Mott,1988].

The detrimental effects caused by the use and careless disposal of pesticides is not limited to food products but also extend to the environment in general. For instance, the use of chlorinated insecticides, such as aldrin and DDT, etc. significantly reduced populations of various birds, including bald eagles, ospreys, peregrine falcons, and brown pelicans [Mott, 1988]. Most of the organochlorine compounds (e.g. DDT, aldrin and dieldrin, etc.) were banned by EPA between 1972 and 1980 because of their carcinogenicity and environmental persistence, and are no longer used in the United States. However, the residues and accompanying perniciousness are still occurring, which probably result either from the continued uses of these chemicals in some foreign countries that export food to the U.S., or from contamination at trace levels of these chemicals persisting in the U.S. environment [Mott, 1988].

The fact of the existence of pesticides in the environment has been the subject of numerous studies. Because of the potential hazards to living organisms, some of the more persistent pesticides have been replaced by less persistent ones which are said to rapidly "disappear" from environmental components such as soil. However, the "disappearance" of a pesticide from soil may not truly result from its degradation but may result from our inability to determine its residues by conventional separation and analytical procedures. A pesticide which fails to be

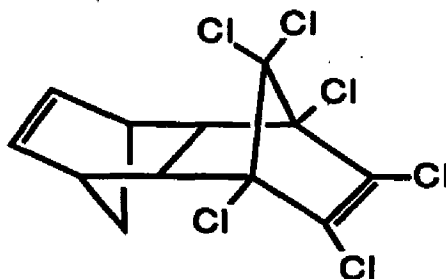
extracted, or gives poor extraction results, may be a consequence of the following two causes: first, its possible conversion to compounds which cannot be extracted, thus forming "bound" residues; and second, the lack of highly efficient and suitable extraction and detection methods. Formation of bound residues was demonstrated in many studies on herbicides such as propanil [Bartha, 1971], glyphosate (*N*-phosphoromethyl glycine) [Miles et al., 1988], 2,4-dichlorophenoxyacetic acid (2,4-D acid) [Smith, 1984], and on insecticides such as parathion [Katan, 1977].

Since the edible portions of many food products grow directly in contact with soil, the soil can be viewed as a major type of vehicle for transport of pesticides into the plants and crops. Plants and crops may also act as sponges absorbing the water-soluble chemical residues from the soils through their roots. Therefore, it is an important and challenging task for synthetic chemists to prepare safer, less toxic to human and more effective pesticides and insecticides, etc., and for analytical chemists to develop faster, highly efficient and more sensitive analytical methods for extraction, separation and quantitative determination of trace amounts of various toxic organic compounds in soil samples. With the above views in mind, this dissertation research has focussed on the development of methods for extraction and determination of eight representative toxic organic compounds in soil samples. Among these compounds, aldrin, Aroclor 1254 and Aroclor 1260 are chlorinated hydrocarbons. Aldrin was used as an insecticide. Both Aroclors belong to the family of polychlorinated biphenyls, PCBs, which are known to be hazardous,

persistent, and the most ubiquitous of environmental pollutants, and were used as pesticide extenders, in addition to their many uses in industry [Erickson, 1986]. Parathion and malathion are typical organophosphorous insecticides. Aldicarb is a carbamate insecticide. Amitrol and 2,4-D methyl ester are widely used as herbicides.

2.2 Organochlorine Compounds and Their Analyses

Aldrin, Aroclor 1254 and Aroclor 1260 were selected as representatives of the toxic organochlorine compounds. Aldrin is an assigned name for an insecticidal product containing not less than 95% of the endo-exo isomers of 1,2,3,4,10,10- hexachloro- 1,4,4a,5,8,8a- hexahydro- 1,4:5,8- dimethanonaphthalene [Kearns, 1949]:



Aldrin

The pure compound is a white crystal with a melting point of 102°C and is very soluble in most of the common organic solvents such as alcohol, acetone, benzene, chloroform, etc. but insoluble in water [Melnikov, 1971]. Aldrin was originally manufactured as a cyclodiene-organochlorine

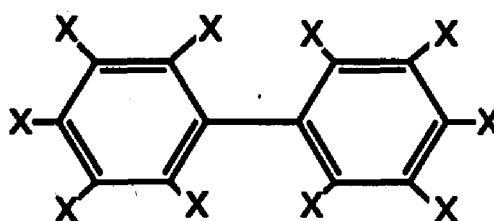
insecticide after World War II (1948) [Ware, 1983]. It was one of the most effective, long-lasting and economical termite control agents known.

Therefore, it had been used to the greatest extent of all the soil insecticides for the control of termites and other soil-borne insects whose immature larvae feed on the roots of plants. Similar to the other persistent cyclodiene insecticides, aldrin is stable in the soil environment and is not readily decomposable upon long exposure to the ultra-violet light from sunlight. Because of their human health hazard and their persistence in the environment, the use of aldrin and other cyclodiene-organochlorine insecticides on crops was restricted or banned [Ware, 1983]. However, the residues from the earlier application of these compounds remains up to the present time.

The toxic effects of aldrin have been long established not only on insects, mammals, birds and fishes but also on human health [Ware, 1983, Stevens, 1971]. Poisoning was believed to occur through ingestion, inhalation or skin absorption. Prolonged exposure to aldrin may cause hepatic damage. Severe symptoms may result from ingestion or percutaneous absorption of 1 to 3 grams, especially in the presence of liver disease [Clayton, 1981]. As a neurotoxicant, aldrin showed toxic effects to all the animals similar to DDT and hexachlorocyclohexane (HCH). It may cause renal damage, tremors, ataxia, convulsions followed by central nervous system depression, respiratory failure, and death.

Polychlorinated biphenyls (PCBs or Aroclors) are highly

environmentally stable industrial products synthesized by direct chlorination of biphenyl. The biphenyl molecule has ten possible sites at which chlorination can take place. Therefore, there are 209 possible polychlorobiphenyl compounds and isomers. Many of these compounds or isomers do not exist at a significant level in the commercial PCB mixtures. Aroclor products are identified by a four-digit numbering code in which the first two digits represent the type of the parent molecule, e.g. the digits 12 for biphenyls and 54 for triphenyls, and the last two digits specify the weight percentage of chlorine content in the mixture.



Polychlorinated Biphenyls
X = H or Cl

Commercial PCBs are distinguished by their high stability and resistance to decomposition by common acids, bases, oxidation, and reduction, their miscibility with numerous organic solvents, their non-flammability, and their excellent electrical insulation properties. Because of these highly desirable physical and chemical properties, PCBs have a wide range of applications in industry and agriculture as flame retardants, polymer-plasticizers, hydraulic fluids, adhesives, inks, lubricants, wax extenders, dedusting agents, cutting oils, and dielectric

fluids for capacitors and transformers, as well as pesticide extenders. However, PCBs, when present at high concentrations, are known to have severe toxic effects in humans and animals. The toxic symptoms in humans include chloracne, pigmentation of skin and nails, excessive eye discharge, swelling of eyelids, distinctive hair follicles, and gastrointestinal disturbances [for representative reviews, see Fishbein, 1974; Kimbrough, 1974]. The PCBs have also been listed as carcinogens by the EPA [EPA, 1981]. Owing to their widespread use, careless disposal practices, and environmental stability, PCBs have been widely identified in a great diversity of environmental matrices including fish, wild and domestic animals, plants, soils, rivers, lakes, oceans and their underlying sediments, aquatic and marine flora, air, rain, and snow [Risebrough, 1968; Fishbein, 1972; Buckley, 1982; Ballschmiter, 1981; Wasserman, 1979; Brady, 1987]. It is also apparent from several analytical studies that PCBs preferentially bioaccumulate in the food chain and that the residues of PCBs are routinely detected in human adipose tissue, blood and milk [Wasserman, 1979; Cordle, 1978; Holdrinet, 1977; Safe, 1982 & 1985]. The factors that would alter the composition of a commercial PCB preparation introduced as a pollutant into an aquatic or marine environment include physical partitioning between the water-sediment and water-air interfaces, sediment desorption processes, and biomagnification and bioconcentration with aquatic forms of life and the food chain. Although the uses of PCBs in many industrial applications have been banned or restricted, PCB pollution in the global environment is still persistent. It is believed that PCBs are the most ubiquitous industrial chemical pollutant

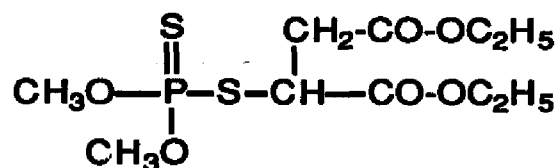
in the global ecosystem [Safe, 1985]. Therefore, analysis of PCBs present in a variety of environmental matrices becomes an important and challenging global research project. The analytical approach requires first the isolation of isomer-specific PCB mixtures from environmental matrices followed by high-resolution separation and accurate quantification.

The current analytical methods for PCBs are similar to those for aldrin and many other organochlorine compounds such as DDT, DDE, dieldrin, endrin, heptachlor, and chlordane, etc. One method recommended by the EPA for the analyses of these organochlorine pesticides and PCBs is Method-608 [Federal Register, 1984]. In this method, an aqueous sample containing the organochlorine analyte is extracted with approximately 200 mL of methylene chloride using a separatory funnel. The methylene chloride solvent is evaporated and the residue dissolved in hexane (ca. 50 mL) which is then concentrated to a volume of 1 to 2 mL. A complete extraction by this procedure takes about one and one-half hours. The extracts are then separated and analyzed by gas chromatography with an electron capture detector. Gas chromatography coupled with a mass spectrometer was also recommended by the EPA for aldrin and PCBs analyses (Method 625) [Federal Register, 1984]. Successful application of the GC/MS method was reported in many studies of environmental samples [Onuska, 1986; Pellizzari, 1985]. Other analytical methods are modified versions of the above methods, including the uses of several GC columns in parallel with different stationary phases to separate PCB congeners

[Albro, 1981; Albro, 1979], GC with a flame ionization detector [Schulte, 1983], multidimensional gas chromatograph which is equipped with two independent sets of ovens and ^{63}Ni electron capture detectors for separation of PCB mixtures [Duinker, 1988], separation prior to the GC analysis by using Florisil cartridge and Silica Sep-Paks for aldrin and PCB quantifications [Lerman, 1982; Sapp, 1989; Chiang, 1987]. High performance liquid chromatography (HPLC) has also been employed for aldrin and PCB analyses either as a final quantification or as a separation technique [Seiber, 1974; Horgan, 1973; Little, 1970; Willmott, 1974]. Similar to the separations by HPLC, thin layer chromatography (TLC) has been demonstrated to be a useful technique either for cleanup or for the final quantitative measurements of aldrin and PCB analyses [Hattula, 1974; Koeniger, 1975].

2.3 Organophosphorus Pesticides and Their Analysis

Malathion, *O,O*-dimethyl- 5- 1,2- di(carboxy)ethyl phosphorodithioate, is a selective, broad-spectrum and currently most widely used organophosphorus insecticide.

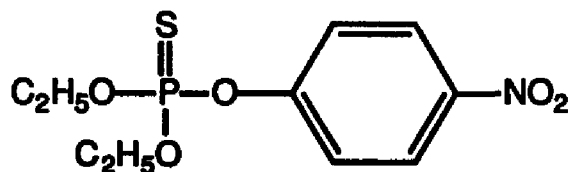


Malathion

After being introduced for public uses in 1950 by American Cyanamid, it quickly received a general recognition in agricultural practices throughout the world as an effective insecticide against the pear psylla, red spider mites, aphids, and many other insects. It has been applied on many vegetables, forage crops and fruits. As implied by its name, malathion has been used to control the vectors of malaria. Considerable quantities of malathion are also employed to protect stored food products [Fest, 1973]. In comparison with parathion, another widely used organophosphorus insecticide, malathion is about one hundred times less toxic to warm-blood animals but only about two to four times less toxic to insects [Merck Index, 1983]. Owing to its generally good insecticidal properties and its low acute mammalian toxicity, malathion has been adopted for uses not only in agriculture but also in various species of livestock [Hayes, 1982]. For example, it is a key components in flea powders for dogs, cats and other domestic animals and is used in dips for control of mange mites [Ware, 1983]. Since 1956, malathion has been employed successfully in the control of Medfly in Florida from 1956 to 1981, in Texas in 1966, and in Los Angeles, California in 1975-1976. In 1981-1982, it was applied again in California against the Mediterranean fruit fly which had invaded the rich fruit-growing areas of California. In the "war" against the Mediterranean fruit fly, malathion was mixed with a protein bait made of molasses and yeast and was then sprayed from ground equipment and from helicopters over the fly-infected and surrounding areas. This war is still going on in the Los Angeles area in 1990. Both male and female fruit flies are attracted to the bait and die a few hours after

feeding on the tasty morsels containing malathion [Ware, 1983].

Parathion is a common name for o,o- diethyl-o-p- nitrophenyl phosphorothioate,



Parathion

which was once the most widely used phenyl organophosphorus insecticide in the United States. It has a broad-spectrum of insecticidal activities.

Parathion was synthesized as the first phenyl derivative of phosphorothioate compounds in 1944 [Fest, 1973] and was suggested by Unterstenhøfer for the control of orchard pests in 1948 [Fest, 1973].

Parathion is one of the oldest synthetic commercial insecticides. As a result of the long history and broad utilities of parathion, the total usage of parathion has been greater than that of all the other organophosphorus (except malathion) insecticides combined. Parathion has a higher environmental stability than aliphatic phosphorothioate compounds (e.g. malathion) and has been used in large quantity as an active, non-systemic contact, and stomach insecticide and as an ovicide against mosquitoes, red spider mites, potato aphids, citrus fruit spiders, greenhouse thrips, and house flies [O'Brien, 1961]. It is noteworthy, however, that parathion

shows a "depth action", i.e. it penetrates into the plants to certain extent but without any true translocation [Fest, 1973]. On the living plants, parathion decomposes at a relatively high rate (within 2 to 8 days) owing largely to the effects of sunlight and enzyme system of the plants. Because of its high toxicity, parathion has not been permitted for use on domestic animals and in the home.

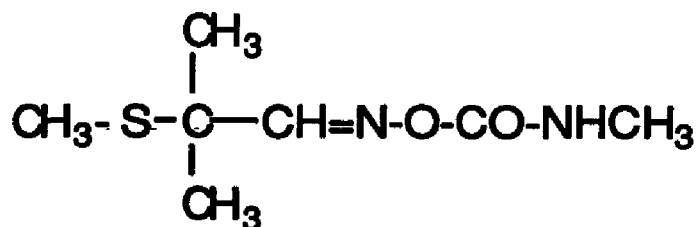
The persistence of parathion and malathion residues in the environment have been studied for many years. It has been known that to a great extent parathion does not actually degrade in the soil environment but is irreversibly bound mainly to the organic fractions of soils [Miles, 1978; Stewart, 1971; Katan, 1976; Lichstemstein, 1977]. The experiments performed by Katan et al. [Katan, 1976], using ^{14}C - labeled parathion, revealed that 49% of aminoparathion and 1.6% of parathion itself were adsorbed on a loam soil within only two hours. They suggested that the binding of ^{14}C - labeled parathion residue to soil was the result of the activity of soil microorganisms which degraded ^{14}C parathion to the amino compounds that bound to soil rapidly and tightly.

Many methods for the determination of malathion and parathion in various samples have been reported [Hladka, 1975]. Most of the published results deal with the analyses of the residues of these pesticides in fruits, vegetables and certain other biological materials, usually at, or above, the part per million (ppm) level. The analytical instruments include gas-liquid chromatography equipped with a phosphorus- specific detector

or a mass spectrometer for the analysis of malathion in milk and blood [Muan, 1986], high performance liquid chromatography with a variable-wavelength UV/vis detector [Cabras, 1988], paper, thin-layer chromatography [Racke, 1988], and gel-permeation or adsorption chromatography for the sample clean-up prior to the quantification by gas chromatography with a nitrogen- phosphorus or a flame-photometric or flame ionization detection system [Ault, 1979; Adachi, 1984; Kjølholt, 1985].

2.4 Analysis of Carbamate Insecticides

Aldicarb, [2-methyl-2-(methylthio)propionaldehyde- α -(methylcarbamoyl)oxime] is one of the most active carbamate pesticides, which was manufactured by Union Carbide (1962) and was first commercially sold in the United States under the tradename Temik in 1970 as a cotton insecticide and in 1974 as a potato insecticide. It was one of the first major "systemic" pesticides (hence named "Temik") and has insecticidal, nematocidal and acaricidal properties [Marshall, 1985].



Aldicarb

Aldicarb has been registered as an insecticide in thirty-three countries for potatoes and in twenty-eight countries for cotton. In addition to the uses for potatoes and cotton, aldicarb is currently employed in the United States as a broad-spectrum soil-applied systemic pesticide for control and protection of peanuts, sugar beets, sugarcane, sweet potatoes, bananas, green maize, tobacco, orange groves, and several ornamental and nursery plants from attack by mites, insects, and nematodes, etc. [Balayannis, 1983; Marshall, 1985; Iwata, 1977; Krause, 1986]. It is also used virtually everywhere in the world where citrus is grown. Field studies indicate that aldicarb can effectively protect the young seedlings and mature citrus trees against aphids and other sucking insects such as thrips tabaci, which are the main vectors of virus diseases [Tashiro, 1969; Shaw, 1970]. The pesticidal activity of aldicarb is due to its toxins which are highly mobile in moist soil and are taken up by the plant roots and carried throughout the entire plant. Aldicarb is such a strong insecticide that two or three months after it was applied near the roots it will kill the insects biting the leaves of the plant. Aldicarb is at least 250 times more deadly to mammals than DDT and 850 times stronger than serin (i.e. carbaryl, 1-naphthyl methylcarbamate), and is the most extensively used carbamate pesticide to date [Marshall, 1985].

The toxicity and its harmful effects to humans have been observed and are well-documented. In the summer of 1985, nearly one thousand people in several western states and Canada were poisoned by residues of

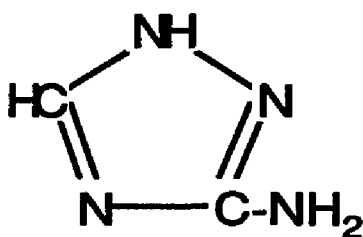
the pesticide Temik in watermelons. Within two to twelve hours after eating the contaminated watermelons, these people suffered from nausea, vomiting, blurred vision, muscle weakness and other symptoms [Mott, 1988; Green, 1987]. Aldicarb has also been found in drinking water wells in many locations throughout the country. The EPA announced that Temik was identified as a harmful contaminant in ground water in fifteen states including New York, Wisconsin, and Florida, etc. [Rothschild, 1982; Miles, 1985]. The most severe contamination appeared in eastern Suffolk County of Long Island, New York [Zaki, 1982]. The extensive monitoring program showed that the level of contamination of aldicarb in 1121 (13.5%) of the 8404 wells examined exceeded 7 parts per billion (ppb), the New York State recommended guidelines. Of all the contaminated wells, 52 percent contained aldicarb between 8 and 30 ppb, 32 percent between 31 and 75 ppb, and 16 percent more than 75 ppb.

The serious problems of environmental contamination by aldicarb have attracted great attention. Many studies have been devoted to the determination of aldicarb residues from environmental samples. Reported methods for the analysis of aldicarb include thin-layer chromatography (TLC) [Kneak, 1966; Metcalf, 1966], HPLC with various detection techniques such as mass spectrometer [Wright, 1982], UV-vis [Lawrence, 1978; Muszkat, 1983], and post-column derivatization and fluorometric detection [Krause, 1979], and GC with a variety of detectors such as the Hall detector [Galoux, 1979], mass spectrometer [Muszkat, 1983], flame ionization detector [Kneak, 1966], and esterification and detection with an

electron capture detector [Moye, 1975]. However, the apparent thermal instability of aldicarb presents a difficult problem to the methods involving gas chromatography. Rapid degradation of aldicarb in the GC injection port or in the column frequently occurs resulting in a small GC peak or no peak at all. Some limited success has been achieved using short columns and relatively low operating temperatures [Cook, 1969; Riva, 1969; Trehy, 1984]. The HPLC technique should be ideally suitable for aldicarb analysis because of the minimal effect on the stability of aldicarb and the plethora of highly efficient columns currently available.

2.5 Analysis of Herbicides: Amitrol and 2,4-D

Amitrol (3-amino-5-triazole) is a water-soluble heterocyclic nitrogen compound composed of a five-member ring having three nitrogen and two carbon atoms as well as one amino group substituted at the 3 position of the ring.

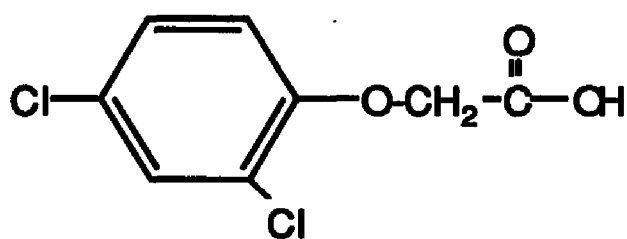


Amitrol

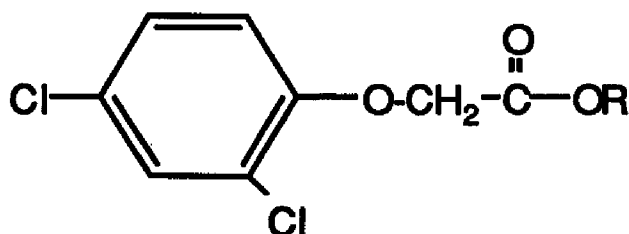
Amitrol was first introduced as a herbicide by the Am. Chemical Paint

Co. in 1954. Because of its rapid absorbability by both roots, and the above ground parts of plants, and its high efficiency in inhibition of growth of plants, amitrol has been extensively used for direct foliage application to eradicate poison ivy, poison oak, scrub oaks, and thistles, to suppress grass in roadsides and orchard floors, and to act as a cotton defoliant [Bailey and White, 1971]. Strong herbicidal activity has been observed when amitrol is used in a mixture with a long-lasting soil-active herbicide, ammonium thiocyanate. The addition of ammonium thiocyanate increases the foliar activity of amitrol [Melnikov, 1971; Ashton, 1973]. Owing to poor selectivity, amitrol herbicide is used only in non-crop land. Amitrol has been listed as a carcinogen by the EPA [EPA, 1981]. Analysis for amitrol is generally achieved using high performance liquid chromatography.

2,4-Dichlorophenoxyacetic acid (2,4-D acid or 2,4-D) and its derivatives such as alkyl esters (2,4-D esters) and salts (2,4-D salts) are a family of phytotoxic substances that are used as effective phenoxy acid herbicides. The most widely used ones, on which a great deal of herbicide research has been conducted, are 2,4-D free acid, 2,4-D methyl, isopropyl, butyl or octyl ester, and 2,4-D sodium, potassium or ammonium salt.



2,4-D (Free Acid Form)



2,4-D esters:
R= Me, C₃H₇, C₄H₉, etc.

Since 1944 when 2,4- D compounds were first employed as herbicides [Hamner, 1944], these compounds have received considerable attention as as outstanding, broad-leaved weed regulator, and have been continuously applied in a very large volume worldwide. More than 32 million kilograms are used each year in the United States alone in 1500 formulated products and in 35 ester and salt forms. 2,4-D compounds are generally applied on agricultural fields such as on cereal, millet, grain crops, sorghum, sugarcane, potatoes for control of broad-leaved weeds, on siding, right-of-way, turf and lawns, and in forest conservation programs [Thompson, 1984; Meagher, 1966; Smith, 1984].

The foliar action of 2,4-D compounds involves penetration of leaves, stems or roots, absorption into the symplast, migration across

parenchymatous tissue to the vascular channels, translocation from source to sinks of plant foods with the assimilate stream and finally the herbicidal response. Death of the plant tissues may result from contact action, most characteristic of the 2,4-D ester on foliage [Ashton, 1973]. The effectiveness of 2,4-D esters in controlling various weeds is considerably higher than that of 2,4-D salts. The same effect in the eradication of dicotyledonous weeds can be achieved when the 2,4-D esters are used at the dosages one-half to one-third (in terms of the acid) of the dosage of the sodium salt of 2,4-D acid [Melnikov, 1971]. Many 2,4-D esters, such as methyl, isopropyl, butyl and octyl esters, are relatively volatile and their vapors may damage susceptible plants located next to the treated areas. 2,4-D causes irritation of eyes and gastrointestinal disturbances.

Numerous analytical approaches for the determination of 2,4-D and its derivatives in a variety of environmental samples have been reported. The methods include gas-liquid chromatography with an electron capture detector [Sell, 1983; Thompson, 1984; Smith, 1979; Nash, 1983], with a nitrogen-phosphorous detector [Solomon, 1988], with the Coulson conductivity detector (CCD) [Cook, 1983], and with the mass spectrometer [Bristol, 1982; Lopez-Avila, 1986], and reverse-phase high performance chromatography [Drinkwine, 1979; Wulf, 1976].

The extraction of soil samples containing 2,4-D is generally performed by Soxhlet extraction or by mixing and shaking the soil samples

with single or multiple solvent systems, e.g. with calcium hydroxide solution [Smith, 1972], sulfuric acid-acetone [Khan, 1975], acetone-phosphoric acid [Gutenmann, 1964], or acetone-hexane followed by acidification [Lee, 1983]. Recently, solid phase extraction (SPE) [Hoke, 1986] and supercritical methanol extraction [Capriel, 1986] have been applied for the extraction of many phenoxy acid herbicides including 2,4-D. Both SPE and supercritical methanol extraction have demonstrated many advantages over the conventional methods which will be discussed in detail in the following sections.

2.6 Supercritical Fluid Extraction

2.6.1 An Overview of Conventional Extraction Methods

For quantitative analysis of the pesticides discussed in the previous sections, the analytical chemist first has to isolate the pesticides from their matrixes. When the pesticides are contained in soil samples, an efficient and quantitative extraction of these organics is a formidable task. At the present time, extraction of organic compounds including pesticides from soils is usually performed using a liquid solvent extraction technique with a Soxhlet apparatus, sonication or flask shaker [Smith, 1984; Kjølholt, 1985]. These traditional extraction techniques are still in use and are valuable for the extraction of many organic compounds from soils. However, their limitations in many cases render them unable to meet the challenge of the needs of modern society. Among the limitations of the traditional extraction techniques are the time required, i.e. several hours to days; the use of relatively large volumes of ultra-pure solvents which are expensive to purchase and to dispose of, and which must be evaporatively condensed after the extraction; and incomplete recovery and/or degradation of thermally labile analyte species because the extraction process often involves elevated temperatures [Krause, 1986; Lemley, 1988]. Furthermore, the drastic extraction conditions may in some cases destroy the structure of soil by solubilizing the constituents of the soil materials which lead to possible interference and complication in the subsequent chromatographic analysis; and hydrolysis

in the presence of a strong acid or base often results in the destruction of the identity of bound pesticide residues in soils [Khan, 1982; Huber, 1983].

For decades, attempts have been made to overcome these limitations. For example, gel-permeation chromatography [Hopper, 1981] and adsorption chromatography involving alumina [Holder, 1969], silica gel [Steinwandter, 1982] and treated soil [Mangani, 1981] as adsorbents have been studied. Although these new methods have eliminated some of the limitations, they have inherent drawbacks, such as relatively poor column efficiency, slow elution, high consumption of solvents, and use of labor-intensive procedures [Petrick, 1988]. A high-temperature-distillation (HTD) technique was developed by Khan and Hamilton [Khan, 1980] and was used to release bound pesticide residue and/or metabolites from soil samples [Zhang, 1984; Capriel, 1986]. Although the HTD technique provides a possible means for releasing certain pesticides, the thermal decomposition of the residues of many pesticides during the process leads to an unsatisfactory recovery. Recently, solid-phase extraction columns, such as Silica Sep-Paks and Florisil Cartridges, have been applied to the isolation of pesticides [Gorder, 1981; Chiang, 1987]. The solid phase extraction offers some advantages over the conventional liquid solvent extraction and the column chromatography separation in the speed of the isolation process, and prevention of unstable pesticides from decomposition. However, the technique still requires relatively large volumes of solvent.

In the search for more effective extraction methods, we have demonstrated that supercritical fluid extraction is a rapid, powerful and quantitative separation technique. The strong solvating ability and other special properties make supercritical fluids attractive and useful solvents in this new extraction technology. The major goal of this dissertation research was to develop a methodology for the supercritical fluid extraction of pesticides and other toxic organic compounds from soils. Since supercritical fluid extraction is a new technology, little is known about a number of parameters involved in the extraction. The effects of these parameters, such as nature of the supercritical fluid, pressure, temperature, extraction time, and additives to the fluid, on the efficiency of the extraction were investigated in comparison with the conventional liquid solvent extraction method using a Soxhlet apparatus.

2.6.2. Basic Principles of Supercritical Fluid Extraction

When a gas or a liquid is brought to a condition at which both temperature and pressure have reached or exceeded the critical points of the gas or the liquid, it becomes a supercritical fluid (SCF). Supercritical fluid exists in a single fluid phase possessing characteristics intermediate between those of gases and liquids. **Figure 2.6.1** illustrates a pressure-temperature diagram in the critical region for a pure component [Eckert, 1986]. Curve AB represents a path of changing from liquid to vapor phase without the presence of two separated phases.

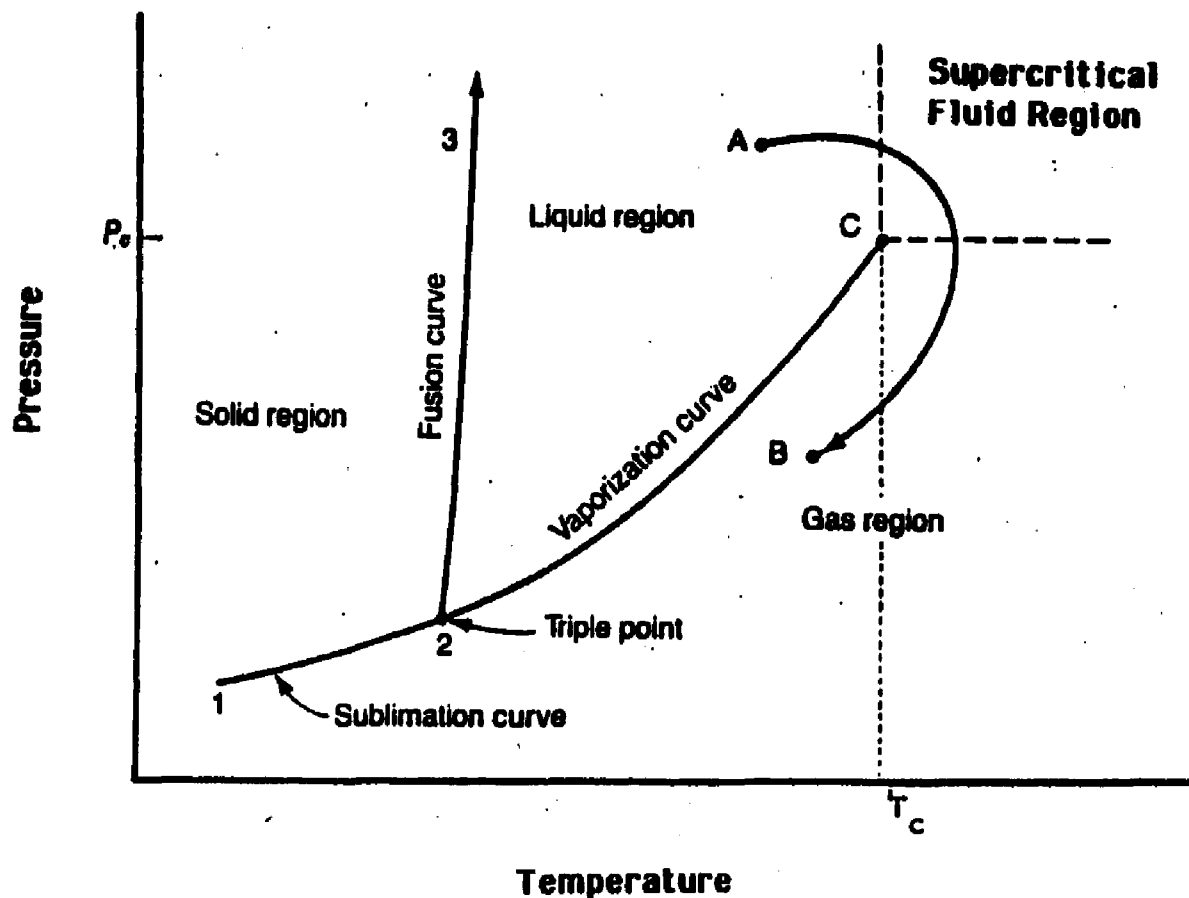


Figure 2.6.1. A pressure-temperature diagram in the critical region for a pure component. Line A-B is the path for changing a liquid to a vapor without two separation phases being present. [Eckert, C. A.; Van Alsten, J. G.; and Stoicos, T., *Environ. Sci. Technol.*, **20**, 319 (1986)]

Point C is the critical point. For example, the critical temperature (T_c) and critical pressure (P_c) of carbon dioxide are 31.1°C and 72.8 atm, respectively. The critical temperatures and pressures of some common gases are listed in Table 2.6.1 [McHugh, 1986].

Research on the properties of SCF has intensified in recent years because of the great promise of technological applications of SCF as solvents for chromatography, chemical reactions, fractionation and separation, coal liquefaction, etc. [Squires, 1987]. To capitalize fully on the unique solvent characteristics of the supercritical fluids, it is necessary to understand the physicochemical properties of SCFs. In Table 2.6.2, some important properties of SCFs are compared in order of magnitude with those of a typical gas or liquid.

The most interesting and useful property is the high density of SCFs which is close to that of a liquid. A high density generally implies a strong solvating power. It has been established that the solvent strength of a SCF is related to the density of the SCF in the critical region. Giddings and his co-workers developed empirical methods of applying the solubility parameter concept to dense supercritical solutions [Giddings, 1969; Czubyt, 1970]. They concluded that the solvent strength of a SCF is directly related to the density of the SCF by the following empirical equation:

$$\delta_2 = \delta_{liq} (\rho_2 / \rho_{liq})$$

Table 2.6.1. Critical Constants for Various Supercritical Solvents
[McHugh, 1986].

Solvents	Critical Temperature (°C)	Critical Pressure (atm)
Ammonia	132.5	111.3
Benzene	289.0	48.3
Carbon dioxide	31.1	72.8
Chlorotrifluoromethane	28.9	38.7
Cyclohexane	280.3	40.2
Ethane	32.3	48.2
Ethylene	9.3	49.7
Isopropanol	235.2	47.0
Propane	96.7	41.9
Propylene	91.9	45.6
<i>p</i> -Xylene	343.1	34.7
Toluene	318.6	40.6
Trichlorofluoromethane	198.1	43.5
Water	374.2	217.6

Table 2.6.2. Order of magnitude comparison of some physicochemical properties of a supercritical fluid (SCF) with a typical gas or liquid [Gouw, 1972; Groves, 1985]

Property	Gas	SCF	Liquid
Density (g/cm ³)	10 ⁻³	0.6	1
Diffusivity (cm ² /sec)	10 ⁻¹	10 ⁻³ -10 ⁻⁴	10 ⁻⁵ -10 ⁻⁶
Viscosity (g cm ⁻¹ sec ⁻¹)	10 ⁻⁴ -10 ⁻⁵	10 ⁻⁴	10 ⁻² -10 ⁻³

where δ_2 is the Hildebrand solubility parameter; δ_{liq} is the characteristic solubility parameter of the parent liquid when the gas is compressed to the reduced liquid density, ρ_{liq} ; and ρ_2 is the reduced density of supercritical fluid. When δ_{liq} is approximated by $1.25P_c^{1/2}$, where P_c is the critical pressure in atmospheres, the equation becomes:

$$\delta_2 = 1.25P_c^{1/2} (\rho_2 / \rho_{liq})$$

The solubility in terms of molar fractions is related to the solubility parameters by:

$$RT \ln(x_1/x_2) = \Phi_2^2 V_1 (\delta_1 - \delta_2)^2$$

where x_1 and x_2 are the molar fractions of solute and solvent, respectively; Φ_2 is volume fraction of the solvent; V_1 is molar volume of the solute; and δ_1 and δ_2 are the solubility parameters for the solute and solvent, respectively. The highest solvency occurs when $\delta_1 = \delta_2$. Since the solubility parameter δ_2 of a SCF depends on its density which in turn depends on the pressure and temperature of the system, the value of δ_2 , therefore the solvating strength of the SCF, can be manipulated to be close to that of δ_1 for a maximum solubility by control of the pressure and

temperature of the system. As shown in Figure 2.6.2, for a reduced temperature ($T_R = T/T_c$) range of 0.9 to 1.2 and at reduced pressures ($P_R = P/P_c$) greater than 1.0, the reduced density ($\rho_R = \rho/\rho_c$) of the SCF solvent can change from about 0.1, a gas-like density, to about 2.5, a liquid-like density. As the liquid-like density is approached, the supercritical fluid begins to act as a liquid. For example, pentane, carbon dioxide and ammonia at the liquid density have Hildebrand solubility parameters of 7.0, 10.6 and 13.2 (cal/cm³)^{1/2}, respectively [Hawthorne, 1987]. However, as the reduced temperature is increased above the value of 1.2, the reduced density decreases. In order to reach liquid-like density, the reduced pressure should be greater than a value of 5 at this T_R . It is apparent that when operating in the supercritical region, both pressure and temperature can be used to regulate the density and therefore the solvating power of the fluid. For instance, the solubility of phenanthrene in supercritical ethylene changes by three orders of magnitude with only a 150-bar change in the operating pressure [Eckert, 1986]. This important property of the SCFs makes solubility-programming possible. Thus, a desired solubility of a solute can be designed by a proper selection of the operating temperature and pressure of the system.

In addition to their unique solubility characteristics, the SCFs also have most favorable transport properties. The diffusivity of a SCF is substantially higher than that of a typical liquid, which therefore allows rapid mass transport in the supercritical fluid media. In comparison with

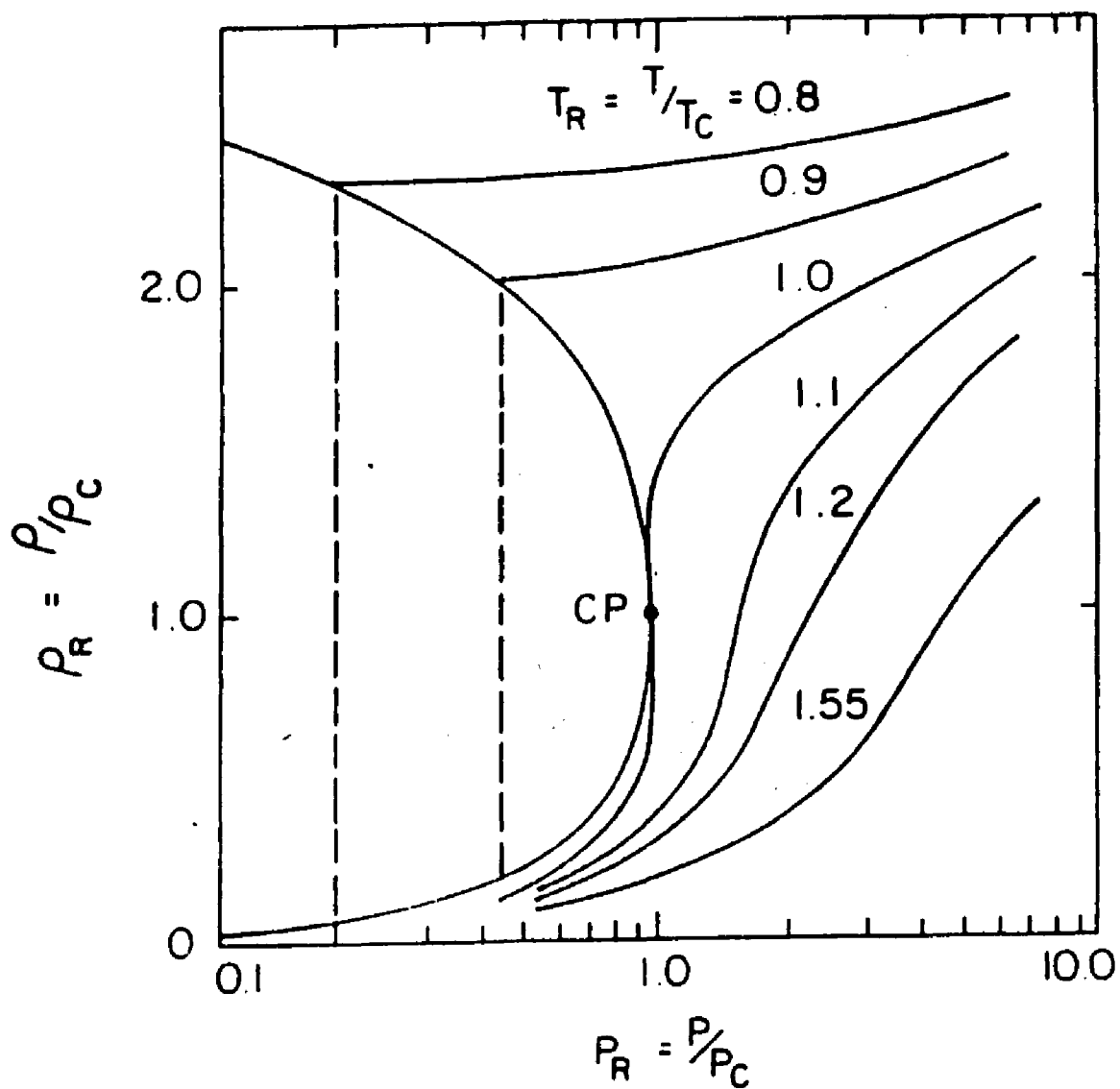


Figure 2.6.2. Variation of the reduced density (ρ_R) of a pure component in the vicinity of its critical point. [McHugh, 1986]

conventional liquid-liquid or liquid-solid extractions, the higher diffusivity in supercritical fluid extraction means that analytes can relatively easily diffuse out of a complex matrix, and that the efficiency of extraction per unit time is higher and the total time for extraction is shorter. The diffusivity of a SCF is affected by the temperature and pressure of the system as shown in Figure 2.6.3 [Paulaitis, 1983]. Even at high pressures (e.g. 200 atm), the self-diffusion coefficient for the supercritical carbon dioxide is still about one order of magnitude higher than that of a normal liquid. Diffusion plays a particularly important role in the SCF extraction of pesticides and other toxic organic compounds from porous carriers such as solids, soils and sludges. To demonstrate the significance of diffusion in these kinds of carrier matrices, the characteristic diffusion time (Δt , in sec) is an important parameter which is directly proportional to a characteristic squared length R (in cm) and inversely proportional to an effective diffusivity (D_{eff} , in cm^2/sec) [Groves, 1985]:

$$\Delta t \sim R^2 / D_{\text{eff}}$$

For a spherical particle, R could be considered as the radius of the matrix particle and D_{eff} can be modified to include the effects of porosity (ϵ) and tortuosity (τ) within the spherical pellet. Thus,

$$\Delta t \sim R^2 \tau / \epsilon D_{12}$$

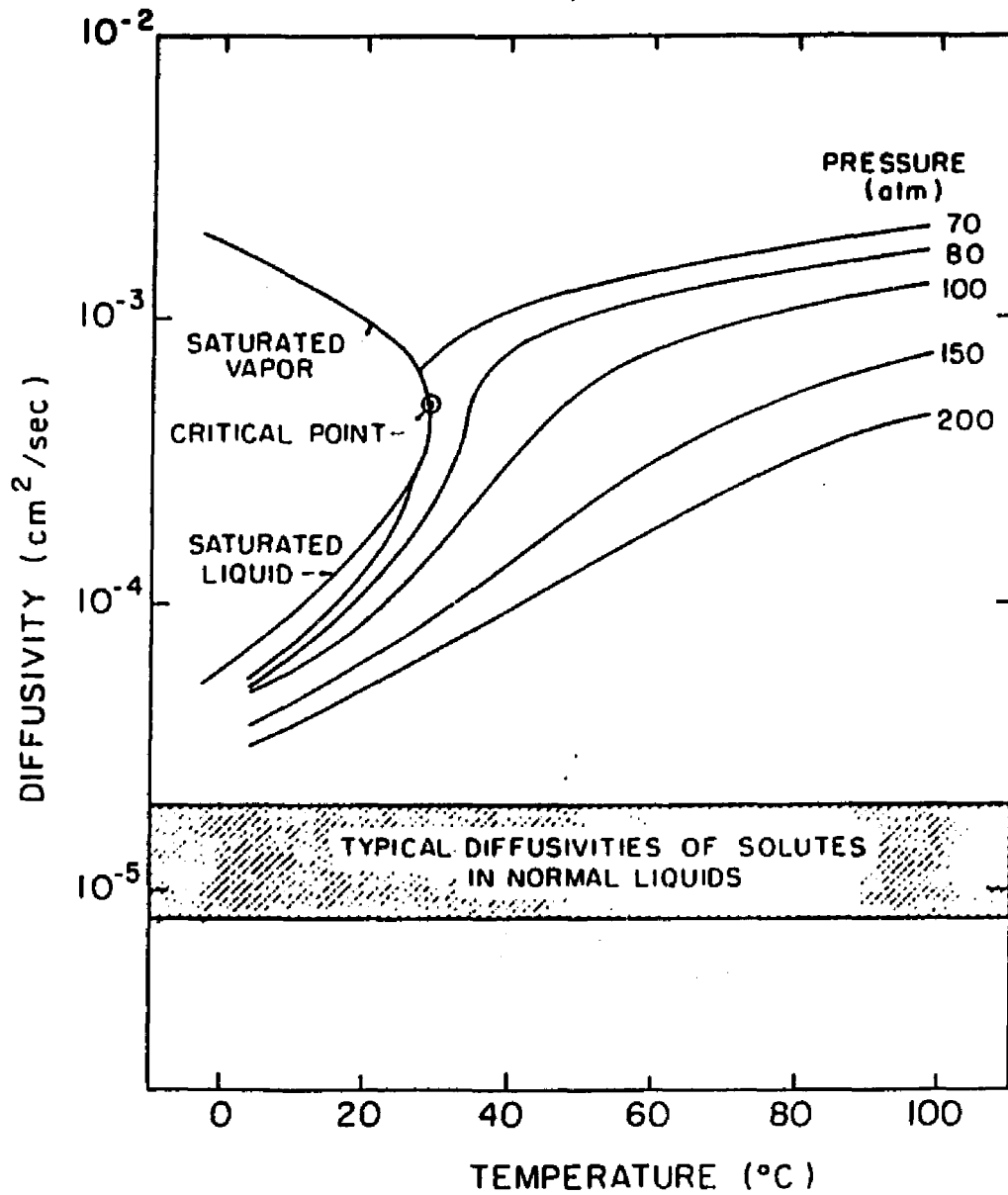


Figure 2.6.3. Diffusivity behavior of carbon dioxide [Paulaitis, 1983].

where D_{12} is the molecular diffusion coefficient. The values for tortuosity and porosity are normally in the range of 2 to 20 and 0.4 to 0.5, respectively, and D_{12} has a magnitude of 10^{-4} cm²/sec [Wilsch, 1983]. Hence, for a small particle of 1 mm radius, the characteristic diffusion time Δt is in the range of 8 min to 1.5 hours depending on the value of tortuosity. The high diffusivity makes the SCF extraction a fast and effective technique.

In addition to high diffusivity, another interesting and important property of SCFs is their low viscosity. As shown in Table 2.6.1, the viscosity of a SCF can be as low as that of a gas. Figure 2.6.4 [McHugh, 1986] demonstrates that despite the rapid change in the viscosity of carbon dioxide in the supercritical region at the high pressure levels (300-400 atm), the viscosity of the SC-CO₂ (ca. 0.1 cps) is still one order of magnitude lower than a typical liquid (ca. 1 cps) [Gouw, 1972; Groves, 1985]. With gas-like viscosities, supercritical fluids permit the rapid penetration into porous solid matrices and facilitate natural convection.

As shown in Table 2.6.1, there are many common chemical substances having low critical temperatures ranging from room temperature to ca. 100°C such as CO₂, nitrous oxide, ethane and fluoroform. This low T_c is especially attractive for the application of these fluids in the safe extraction of many thermally labile organic compounds without

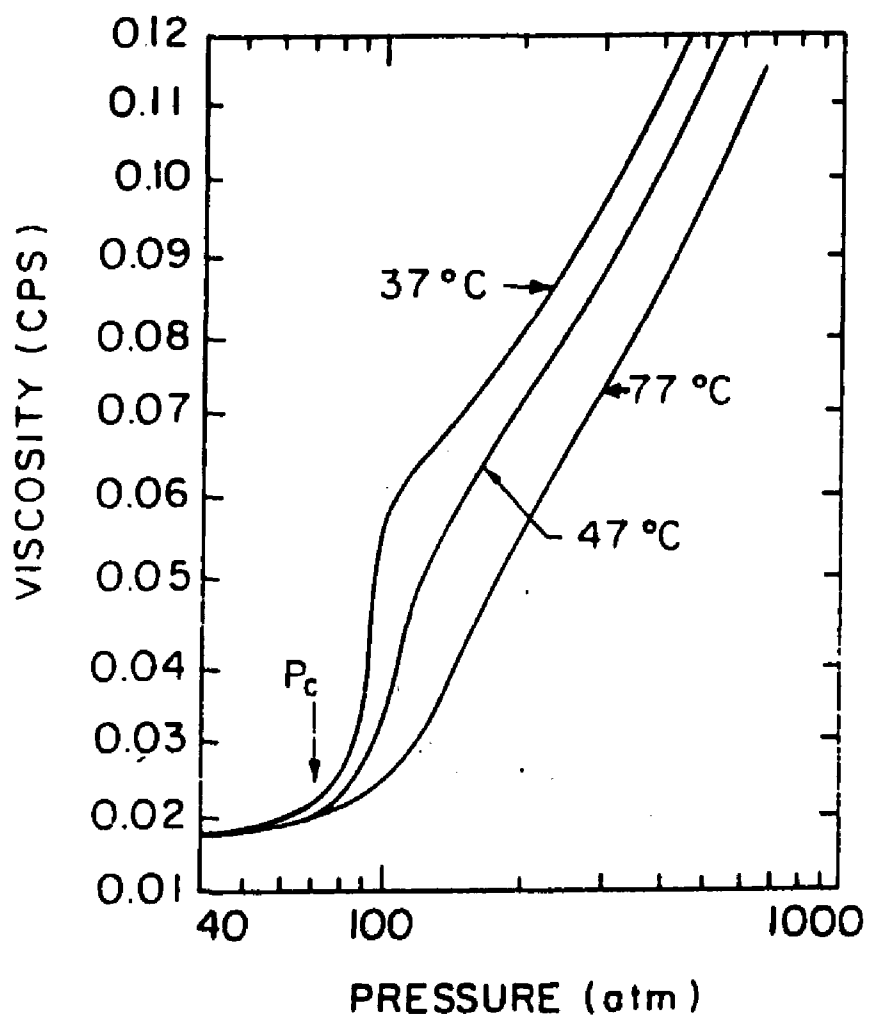


Figure 2.6.4. Viscosity behavior of carbon dioxide [McHugh, 1986].

degradation. Carbon dioxide is one of the most popular and widely used supercritical fluids because of its readily achievable critical conditions (31°C and 73 atm), its low cost and high purity, and its chemical inertness. It is also attractive for industrial applications because it is nontoxic, nonflammable, and environmentally safe in general.

A significant cost factor for many separation processes is the recovery of the extraction solvents which is usually the most time-consuming and energy-intensive step in the process. For example, in liquid-liquid extraction processes, the solvent in the extract is separated and recovered by distillation, which consumes a large amount of energy and time. However, in the supercritical fluid extraction process, the solvent (e. g. SC-CO₂) can be completely evaporated and removed from the extracts by simply reducing the system pressure to ambient while collecting the extracted materials or transferring the extraction effluent to an analytical chromatograph. Therefore, supercritical fluid extraction can improve productivity by reducing the cost in energy and time.

Changing solvent strength through the use of different SCFs and the addition of modifiers can result in an enhancement of extraction selectivity for specific solutes or specific classes of compounds. Compared to conventional extraction techniques, another advantage of SCF extraction is the capability of direct on-line qualitative or quantitative analysis. Since the transfer of the extracts to the chromatograph can be accomplished quantitatively [Hawthorne, 1988; Hedrick, 1989; Wheeler and

McNally, 1989], the SCF extractor can be interfaced to a gas chromatograph (SFE/GC), a supercritical fluid chromatograph (SFE/SFC) or a high performance liquid chromatograph (SFE/HPLC). As a means of GC injection, supercritical fluid extraction is a viable alternative to headspace injection, thermal desorption purge-and-trap, and pyrolysis, depending on the analytical needs. Unlike GC, there are no sample volatility limitations in SFE/SFC, therefore this coupled technique can be extended for application to compounds with a much wider range of molecular weights. Furthermore, many detection techniques are available for SFE/GC and SFE/SFC, such as flame ionization detection [Nomura, 1989; Smith, 1986; Chester, 1985], UV-vis detection [Gere, 1982; Mori, 1989], Fourier transform infrared detection [Hellgeth, 1986; Zerda, 1986], and mass spectrometric detection which has been also demonstrated to be a nearly universal detection system for SFC with both high sensitivity and selectivity [Wright, 1985; Smith, 1982, 1983 and 1984].

2.6.3 Applications of Supercritical Fluid Extraction

Recognition of the above superior properties of supercritical fluids and the advantages of supercritical fluid extractions have been well documented in the recent literature for a variety of applications. All these reports demonstrate that supercritical fluid extraction is a powerful alternative to conventional liquid-liquid or Soxhlet extraction for recovery of many organic substances from various matrices. These

applications include the following representative examples:

(A) In the field of environmental studies, supercritical fluid has been used to extract diesel fuel components from silica gel and marine sediments [Smith, 1985]. Supercritical CO₂, N₂O and methane with or without a methanol modifier were used for the quantitative recovery of polycyclic aromatic hydrocarbons (PAHs) from diesel exhaust, Tenax-GC, urban dust, flyash, cigarette smoke, wood smoke, and river sediment [Hawthorne, 1986, 1987; Campbell, 1986; Schantz, 1986]. Directly coupled SFE with gas chromatography (SFE/GC) was employed in analysis of organic pollutants such as PAHs, PCBs, *n*-alkanes, and heteroatom-containing PAHs (e.g. fluorenone and quinoline, etc.) from polyurethane foam sorbents (PUF) [Hawthorne, 1989].

(B) In agricultural product development, supercritical CO₂ was employed to remove sulfonylurea herbicides and their metabolites from complex matrixes [McNally and Wheeler, 1988] and to remove s-triazine herbicide from sediment [Janda, 1989]. Knopf and co-workers have investigated the SC-CO₂ extraction of toxaphene and 2,2-bis(*p*-chlorophenyl)-1,1,1-trichloroethane (DDT) from contaminated soils [Brady, 1987]. Bound pesticide residues such as atrazine and carbofuran, etc. were removed from soil and plant samples using supercritical methanol [Capriel, 1986].

(C) In food and bioproduct analysis, The coupled SFE/SFC was applied

for the extraction and analysis of caffeine from coffee beans [Sugiyama, 1985] and fat tissue from meat products [King, 1989]. Determination of menadione (Vitamin K₃) in an animal feed and Vitamin K₁ in powdered infant formulas have been achieved using supercritical CO₂ extraction and HPLC with an electrochemical detector [Locke, 1988]. Application of SFE in other fields such as in pharmaceutical industry were also reported [Larson, 1986].

However, the supercritical fluid extraction of pesticides and PCBs has not been systematically investigated previously, which makes the present research immensely interesting and important not only in terms of the development of methodology for the analysis of those globally concerned organic chemicals but also in gaining further insights into the basic understanding of the supercritical fluid extraction process.

3.0 EXPERIMENTAL SECTION

3.1 Materials, Reagents and Preparation of Solutions

Aldrin (purified grade) was purchased from Supelco, Inc., PA. Three standard solutions of aldrin with concentrations ranging from 14 µg/mL to 2.0 mg/mL in acetone were prepared by dissolving 0.14 to 2.0 mg of aldrin in 10.0 mL acetone (ACS grade, J.T. Baker Chemical Co., Phillipsburg, NJ) in a 10-mL volumetric flask. Another standard, 1.1 µg/mL solution of aldrin, was prepared by dissolving 0.11 mg of aldrin in 100 mL acetone. Other aldrin solutions at lower concentrations were prepared by dilution of the above four standard solutions. For example, dilution of the 41 µg/mL aldrin solution with acetone gave solutions with aldrin concentrations of 41, 84 and 164 ng/mL.

PCBs: Concentrated standard solutions of PCB-1254 (1.362 mg/mL) and PCB-1260 (1.143 mg/mL) in hexane were prepared by weighing 0.1362 and 0.1143 g of Aroclor (PCB) 1254 and 1260 (Monsanto Co., St. Louis, MO), respectively, followed by dissolving the samples in 100 mL of hexane (HPLC grade, Fisher Scientific, Fair Lawn, NJ) in a 100-mL volumetric flask. Other less concentrated solutions of PCB 1254 or PCB 1260 were prepared by diluting the above standard solutions with hexane.

2,4-D-Methyl ester was purchased from Sigma Chemical Co., St. Louis, MO and was also synthesized by esterification of 2,4-D acid. The

esters obtained from both sources gave essentially the same analytical results. The esterification of the free 2,4-D acid to 2,4-D methyl ester was accomplished according to the following procedure [Metcalf, et al. 1961]: To an 18x150-mm test tube containing 100 mg of 2,4-D acid, 3 mL of boron trifluoride-methanol ($\text{BF}_3\text{-CH}_3\text{OH}$) reagent (Aldrich Chemical Co.) was added. The mixture was boiled in a water-steam bath for 2 minutes. The contents were then transferred to a 60-mL separatory funnel. The completion of transfer was ensured by washing the test tube with 3 portions of 20 mL of diethyl ether. To the separatory funnel, 20 mL of distilled water were added. After thorough mixing, the contents were allowed to settle and the phases separated. The lower aqueous layer was discarded; the organic layer was collected and dried with anhydrous magnesium sulfate powder for about 4 hours. After removal of magnesium sulfate powder by filtration and evaporation of diethyl ether solvent, the product 2,4-D methyl ester was recrystallized in a white crystalline form. The standard solution of 2,4-D methyl ester was prepared by dissolving 0.5420 g of the ester in 100 mL of methylene chloride (Spectrophotometric grade, Aldrich Chemical Co., Milwaukee, WI).

Malathion and parathion: The standard solutions of malathion and parathion were prepared by dissolving 0.5558 g of malathion (99% purity, from Ultra Scientific, Hope, RI) and 0.1136 g of parathion (98%, from Ultra) in 100 mL of methylene chloride, respectively.

Amitrol and aldicarb: Concentrated stock solutions of amitrol (2.871 mg/mL) and aldicarb (1.683 mg/mL) were prepared by weighing 0.1436 g of amitrol (Sigma) and 0.0842 g of aldicarb (ChemService Inc., West Chester, PA) followed by dissolving each in 50.00 mL of methanol (HPLC grade, Fisher) in volumetric flasks, respectively.

3.2 Soil Sampling

Four different types of soils, i.e. sandy, clay, loamy, and top soils, were selected as test matrices. Sandy soil and clay were collected from the upper 1 to 10 cm of a playground on the campus of the Queens College, Flushing, New York, in Summer 1986 and Fall 1987, respectively. Loamy and top soil samples were collected from a garden in Brooklyn, New York, in Fall, 1987. Each of the samples collected from the fields was allowed to dry in the laboratory environment at a temperature about 25 °C and a relative humidity of about 30 to 40 % in air for two weeks. After removal of big stones, grass, roots, and parts of organic debris by passing through a 1-mm mesh stainless steel sieve, the soil samples were then ground into a fine powder in a glass mortar which was precleaned with distilled water and dried in air. In order to evaluate the influence of particle size on the efficiency of extraction, sandy, loamy and top soil samples were fractionated through the stainless steel sieves with 350, 250 and 177 μ m openings, giving three size-fractions in the range of 45-60, 60-80, and 80-120 mesh for each of the soil samples. After the size-fractionation,

the soil samples were further dried in an oven at 50°C for one hour and then kept in a desiccator ready for later uses. The entire soil sampling and handling processes followed well-established standard procedures and techniques whenever applicable.

3.3 Extraction Techniques

3.3.1 Soxhlet Extraction

Approximately 10 g of soil sample were weighed to ± 0.001 g into a glass extraction thimble which was 6.6 cm in length and 1.8 cm in internal diameter. Direct soil spiking was done about one hour prior to the extraction by pipetting 1.00 mL of the standard solution of the organic compounds to be extracted to the thimble. The thimble containing the soil sample and the standard was then placed into a standard Soxhlet extraction apparatus and the contents were extracted with 70 mL of appropriate solvent under atmospheric pressure for 4 to 16 hours. Acetone was employed as the solvent for the extraction of aldrin from spiked soil samples; hexane was used for PCBs; methylene chloride for 2,4-D methyl ester, parathion and malathion; methanol for amitrol and aldicarb samples. These extraction experiments were performed on each of the four types of the soil (i.e. sandy, loamy, clay, and top soil) samples spiked with the organic compounds. For comparison, control experiments were performed under identical conditions on each of the four types of unspiked soil (i.e. blank) samples. Particular attention was given to check for the

possibility of any contamination of the soil samples by pesticide and/or PCBs by running blanks, since all of the soil samples were collected from the metropolitan area of New York City.

3.3.2 Supercritical Fluid Extraction

A schematic diagram of the supercritical fluid extraction apparatus is shown in **Figure 3.3.1**. Carbon dioxide (Linde bone-dry grade, Union Carbide, Long Island City, NY) or nitrous oxide (Airco, Madison, WI) (A), supplied through a single stage tank regulator (B), was first filtered through an Autoclave Engineers (AE) 5-microcup-type line filter (AE Model # CXF4-5), and was then fed to a motor-driven single-ended diaphragm compressor (Model #46-13411-2, Newport Scientific, Jessup, MD) (C) where the gas was compressed various pressures up to a maximum pressure of 10,000 psi, depending on the critical pressure of the gas involved. The pressure of the supercritical fluid in the extraction system was monitored by a Bourdon-tube pressure gauge (Ashcroft) (D). Constant temperature for the extraction was achieved by placing the extraction chamber in an oven (E). For the aldrin-extraction experiments, the extraction apparatus was held isothermal at various temperatures (in the range of 45°C to 60°C) in a Labline Instruments Imperial II circulating radiant heat oven (Model # 3505 M). A LC oven, which was purchased later from Scientific Systems, Inc., State College, PA and which is more convenient and accurate for temperature control ($\pm 0.1^\circ\text{C}$) and occupies less

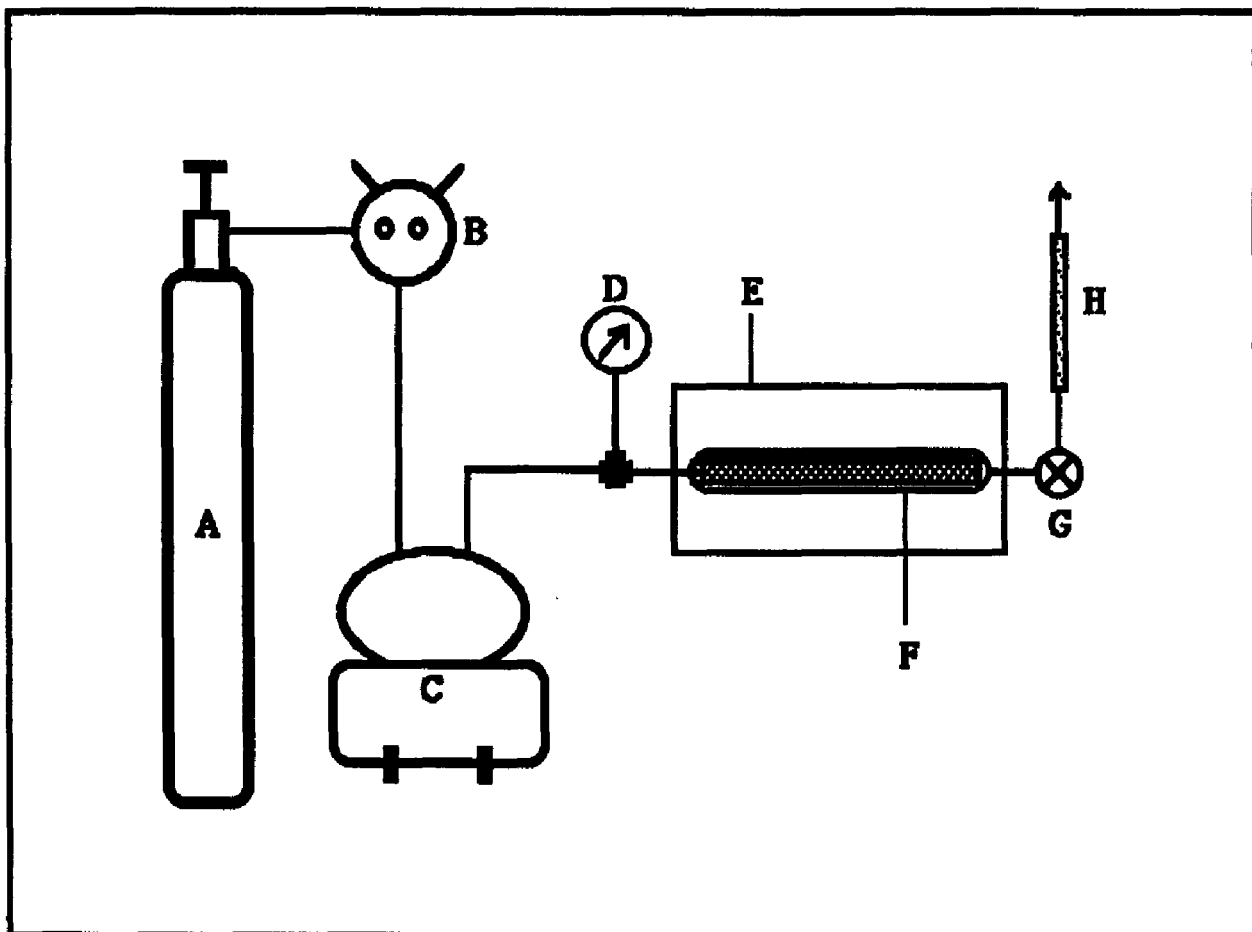


Figure 3.3.1. Schematic diagram of the supercritical fluid extraction apparatus. (A) Gas Tank; (B) Regulator; (C) Motor-driven single-ended diaphragm compressor; (D) Pressure gauge; (E) Temperature-control oven; (F) Extraction chamber; (G) Exit valve; (H) Trap tubing.

space, was employed for the extraction of PCBs and other organic-containing soil samples. The temperatures used for extractions were controlled and varied between 35 and 99 °C. One Autoclave Engineers' medium pressure metering valve was employed as the exit valve (G), to control the flow rate of the supercritical fluid and to depressurize the system to atmospheric pressure in a controlled fashion. The extraction chamber (F) was a stainless steel tube of 12 inches in length, 0.375 inch o.d. and 0.236 inch i.d., respectively. The volume of the empty chamber was calculated to be 9.33 cm³.

A typical operating procedure for an extraction experiment is given as follows: First, the whole extraction apparatus was washed with an appropriate solvent such as hexane, methylene chloride, acetone or methanol and was air-dried. The clean extraction chamber was then packed with 10.00 g of soil sample with gentle tapping, followed by pipetting 1.00 mL of the standard solution containing pesticide or PCB. Glass wool, precleaned with an appropriate solvent (e.g. hexane, methylene chloride, acetone, or methanol), was put into both ends of the chamber to secure the packing. After equilibrating for one hour at ambient temperature and pressure, the packed extraction chamber was introduced into the extraction oven, where the chamber was maintained isothermal at the desired working temperature until completion of the extraction experiment. Upon installation of the extraction chamber, a low pressure of carbon dioxide or nitrous oxide was used to purge the entire apparatus for about 0.5 to 1 minute to remove any air. The exit valve was then

closed, the system was pressurized, and sample in the chamber was allowed to be extracted for the desired period of time, generally 5 to 30 minutes. After completion of the extraction, the compressor was turned off and the exit valve opened slowly to allow a linear depressurization of the system. The extracts were depressurized through the trap tube (H), a stainless steel tube 6 inches long x 1/4 inch o.d. packed with silica gel (60-200 mesh, T.J. Baker, Inc.) or Tenax-TA (60-80 mesh, Allech Associates Inc., Applied Science Labs, Deerfield, IL). One end of the trap (H) was connected to the exit valve through a Swagelock elbow union while the other end was connected to a frit which was employed to prevent the absorbent material in the trapping tube from being blown out. A backup solvent trap was connected to the frit in order to collect any possible escaped analytes. On depressurization below P_c , the solubility of the extracts drops to essentially zero. The extracts were therefore adsorbed by the trapping materials in the trap where the pressure gradient was the lowest in the system. When the depressurization was completed, the trap and the exit valve were washed with about 60 mL of appropriate solvent to dissolve the extracts. The solution was then dried in a rotary evaporator (Buchi Model 101) with the water-bath at about 40°C, under slightly reduced pressure (water aspirator). The residue was dissolved in 1.00 mL of organic solvent, ready for further analyses by GC, HPLC and GC/MS, etc. The extraction equilibration time reported in the results and discussion section does not include the time required for pressurization (ca. 5 min) and depressurization (ca. 10 min).

Both silica gel and Tenax-TA were used as the trapping materials and their adsorption efficiencies were compared. Tenax-TA is a porous polymer based on 2,6-diphenyl-*p*-phenylene oxide which has been specially designed to function as an adsorbent for volatile organics from air and for trapping organics from liquids [Alltech, 1987]. It has low levels of potentially interfering substances such as aromatic hydrocarbons. However, because of its exceptionally good adsorption properties, Tenax-TA must be precleaned before use by the following method [Alltech, 1987]: The trap tube packed with Tenax-TA (about 1 gram) was washed with 200 mL acetone and dried with purified nitrogen gas at a flow rate of ca. 20 mL/min at room temperature. The material was then washed with about 200 mL of methanol and was dried in 20 mL/min purified nitrogen in an oven at 50°C for ten minutes followed by heating to 325-340 °C for 2 hours. The material was cooled to ambient temperature with continuous flow of nitrogen gas.

In order to evaluate the adsorption efficiency of the trap materials, a solvent trap which contained about 60-70 mL of organic solvent was connected in series to the frit (Nylon-66 filter, 0.45 µm) at the end of the trap tube (H) through a 2-ft length of 1/4-inch o.d. Tygon tubing. Upon completion of the extraction, the solvent in the trap combined with that eluted from the Tygon tubing was concentrated in a rotary evaporator and the residue, if any, was analyzed by gas or liquid chromatography. Control experiments were also conducted on blank samples in order to evaluate possible interference from the Tygon tubing. No contamination caused by

the tubing was observed.

3.4 Chromatographic, Spectroscopic and Chemical Analyses

The samples obtained from the extraction experiments were analyzed by gas chromatography (GC), high performance liquid chromatography (HPLC) and mass spectroscopy (MS). The total content of organic substances in the soil samples was determined by chemical analyses.

3.4.1 Gas Chromatography and Mass Spectrometry

Two sets of instruments were employed for GC studies. Analyses of aldrin, 2,4-D methyl ester, parathion, and malathion were performed on a Hewlett-Packard Model 5880A GC, equipped with a flame ionization detector (FID) and a 30m x 0.25 mm (i.d.) DB-5 bonded phase (Durabond 5% phenyl-95% methyl with a 0.25 μ m film thickness) fused silica open tubular column (J & W Scientific Inc., Folsom, CA). Generally, 1 μ L of standard solution or extracted sample solution was injected into the GC with helium as carrier gas at a flow rate of 1 mL/min. For the determination of aldrin, temperatures of the injection port and the FID detector of the GC were kept at 250 and 300°C, respectively. The temperature of the column was initially held at 150°C for 2 min, increased to 220°C at a programmed heating rate of 8°C/min, and held at 220°C for 2 min. All the data was collected and processed with a Hewlett-Packard

5880A integrator.

To prevent overload of the column and yet retain good detection limits, both the split ratio and the injection volume were considered in the analyses. The split ratio is the ratio of flow through the splitter valve to the column flow. The split flow can be adjusted to a desired value with the flow controller. The column flow can be measured at the outlet of the column or can be calculated from the following equation:

$$\text{Column flow} = \pi r^2 L / t$$

where r and L are radius and length of the column in cm, respectively, and t is the retention time of an unretained component (CH_4). High split ratios (50:1 to 500:1) are usually employed for high resolution, low capacity columns. The split ratio of 200: 1 was used for the determination of aldrin, parathion, malathion and 2,4-D methyl ester in this work unless otherwise specified.

Determination of aldrin and PCBs at low concentrations (4.1 μg per gram of soil to 16.4 ng per gram of soil) was performed on a Shimadzu Model Mini-2 Gas Chromatograph equipped with a constant current, pulse-frequency modulated ^{63}Ni electron capture detector (ECD), and a glass column (6-ft x 3 mm i.d.), packed with 1.5% Supelco Phase (SP) -2255 + 1.95% SP-2401 on 100/200 mesh Supelcoport. SP-2255 is a methyl/phenyl (50:50) silicone which has a lower viscosity and better

thermal stability than the commonly used OV-17 packing material [Supelco,1987]. The low viscosity permitted preparation of high efficiency columns. SP-2401 is a fluoropropyl silicone with low viscosity and high thermal stability as well. The temperatures of the packed column for isothermal analyses of aldrin and PCBs were 170°C and 200°C, respectively. The temperature of the injection port and detector was 220°C for the analysis of aldrin and 250°C for PCBs. Nitrogen gas (99.99%, Linde) used as the carrier gas was maintained at a flow rate of 60 mL/min during the GC analyses of both aldrin and PCBs. Electronic integration of peak areas of the gas chromatograms was obtained from a Shimadzu Model C-R#A integrator. All the standard solutions and the solutions prepared after the extractions (1 µL) were injected into the GC at least four times to examine the reproducibility.

Good column conditioning is especially important with the electron capture detector because of the susceptibility of the detector to contamination. New columns, and columns which had not been used for a period of time, frequently contain many different chemical substances. In order to obtain stable baselines, these substances must be expelled from the columns before use for analysis. Column conditioning was achieved by the following procedure: (a) the column was connected to the injection port only to prevent contaminating the detector during the conditioning; (b) nitrogen gas was purged through the column for 30 minutes at a flow rate of 60 mL/min at ambient temperature; (c) while maintaining constant flow rate, the initial temperature was then set at 50°C for 1 hour and was

raised to 200°C which was the highest operating temperature in the experiments; (d) the column was conditioned at 200°C while purging with nitrogen overnight (approximately 16 hours); (e) after conditioning, the column was allowed to cool to ambient temperature, while purging with nitrogen to prevent oxygen from entering the column and damaging the packing; (f) the nitrogen gas was then turned off and approximately 15 minutes were allowed for the column pressure to equilibrate before disconnecting the column (because the rubber O-ring in the column must be replaced after conditioning), otherwise expulsion of packing material might occur which would result in a poor performance of the column in analysis; and (g) finally, the conditioned column was reinstalled with injection port and detector and with replacement of the ferrules, and was ready for use.

3.4.2 High Performance Liquid Chromatography (HPLC)

Qualitative and quantitative analyses of the samples containing Amitrol and Aldicarb were carried out using HPLC on a Perkin-Elmer LC 410 HPLC equipped with an LC-235 diode array detector or on a Perkin-Elmer LC-400 HPLC equipped with an LC-90 UV detector. The UV absorption spectra of the samples were recorded using an LC-235 diode array UV detector and an LCI-100 integrator. The wavelengths of maximum absorption ($\lambda_{\max}$) of each analyte were determined from the UV spectra and were utilized for the UV detection using the LC-90. Deionized water and acetonitrile (HPLC grade, Aldrich) were used as mobile phases. In general, 5% by volume methanol (HPLC-grade) was added to deionized water as preservative. Before turning on the HPLC pump, the solvents were degassed with helium. A Partisil 50DS-3 (Beckman) reversed-phase C₁₈ column (4.6 mm x 25 cm) was used with a C₁₈ guard column. Generally, a flow rate of 1 mL/min was applied. Introduction of the sample solutions to the system was achieved by a rotary valve with a 6- μ L sample loop. All the determinations were undertaken at ambient temperature.

GC/MS of some extracts were studied on a Hewlett-Packard 5988A GC/quadrupole MS system coupled with a Hewlett-Packard 1000 data system. A 30m x 0.025 mm i.d. DB-5 fused silica capillary column (J & W Scientific) was used.

3.4.3 Determination of Total Organic Substances in the Soil Samples

The amount of organic substances in the soil samples can be determined by chemical oxidation methods, including (a) direct oxidation using chromic acid with applied heating [Schollenberger, 1927] and (b) oxidation using dichromate salt as oxidant with spontaneous heating (Walkley-Black method, [Walkley, 1947]). The assumption made in these methods is that all of the organic compounds in soil samples are oxidized completely under the experimental conditions. Therefore, the total organic content determined by these methods represents oxidizable materials in soil samples. In the direct oxidation, the meq of chromic acid consumed in the oxidation of organic substances is measured by chemical titration or by monitoring the color change {Cr (III) green color formation} of the solutions. This can be related to the total carbon content or total organic content by a calibration based on measurements with other methods. Determination of oxidizable substances by the Schollenberger method is a rapid and popular type of analysis.

The Walkley-Black method could be considered as a modified Schollenberger method and generally offers more reliable results because it avoids excessive heating and therefore the formation of charcoal in the system. In our experiments, this method was utilized to determine the total oxidizable organic substances in the soil samples.

A typical analytical procedure is given as follows: A soil sample (0.5 g, weighed to ± 1 mg), which had passed through a 0.25 mm (60 mesh) stainless steel sieve, was placed in a 250-mL Erlenmeyer flask and exactly 10.00 mL of a standard 1.00 N potassium dichromate ($\text{K}_2\text{Cr}_2\text{O}_7$) solution was added to the soil sample. (The standard solution was prepared by dissolving exactly 4.904 g of primary standard grade $\text{K}_2\text{Cr}_2\text{O}_7$ in about 20 mL of distilled water followed by dilution of the solution to 100.0 mL with distilled water.) The soil sample and the solution were mixed by swirling the flask. Concentrated sulfuric acid (20 mL) was added to the flask and was mixed by gentle rotation for one minute to insure complete contact of the reagent with soil. After about 20 to 30 minutes standing, the mixture was diluted to 200 mL with distilled water followed by a sequential addition of 10 mL of 85% H_3PO_4 , 0.2 g of NaF, and 30 drops of diphenylamine indicator solution which was prepared by dissolving approximately 0.5 g of diphenylamine (Aldrich) in 20 mL of distilled water and 100 mL of concentrated sulfuric acid (Fisher Scientific). The contents were then back titrated with a standard 0.5 N ferrous ammonium sulfate solution delivered from a 50-mL burette. The standard ferrous ammonium sulfate solution was prepared by dissolution of 196.1 g of $\text{Fe}(\text{NH}_4)_2(\text{SO}_4)_2 \cdot 6\text{H}_2\text{O}$ in 800 mL of water containing 20 mL of concentrated H_2SO_4 and dilution to 1 liter. The color of the solution in the mixture was dull green at the beginning and shifted to a turbid blue as the titration proceeded. At the end point, the color sharply changed to a bright

green. Control experiments (i.e. the blank system without soil) were also performed using the identical procedure for comparison. The percentage of total organic matter (OM) in the soil sample was calculated from the following equation:

$$\% \text{ OM} = 10 (1 - T/S) \times f$$

where S is the volume in mL of ferrous ammonium sulfate solution used in the blank titration without the soil sample; T is the volume in mL of the ferrous solution used in the sample titration; and f is a factor expressed as:

$$f = (1.00 \text{ N}) \times (12/4000) \times (1.72/0.77) \times (100/W)$$

where 1.00 N is the concentration of $\text{K}_2\text{Cr}_2\text{O}_7$; W is the weight of the soil sample; (12/4000) is the meq weight of carbon; 1.72 is the conventional factor for organic matter from carbon; and 0.77 is a conventional recovery factor established by Walkley et al. [1947].

4.0 RESULTS AND DISCUSSION

Eight representative toxic organic compounds including the pesticides aldrin, parathion, malathion, aldicarb, amitrol, 2,4-D methyl ester, and environmental pollutants Aroclor 1254 and Aroclor 1260 were introduced into four types of soils including sand, clay, loam and top soil at various quantities in the range of nanogram (ng) to microgram (μg) per gram soil. The concentrations of these compounds were so selected that they are quite similar to or lower than the actual levels of these compounds existing in the contaminated soil environment. The general methodology for analysis of these compounds consists of two basic components: (1) separation of the compounds from the spiked soil samples by supercritical fluid extraction with supercritical carbon dioxide and organic solvent (e.g. methanol, methylene chloride, and benzene)- modified SC- CO_2 as solvents, and by conventional liquid-solid extraction in a Soxhlet apparatus for comparison, and (2) quantitative determination of the compounds as extracted from the soil samples by gas or liquid chromatography with various detection techniques and by mass spectroscopy. Since the efficiency of the extractions is expressed as the recovery of each extraction, based on the chromatographic analysis of the extract, the accuracy of quantification of the analytes becomes of the utmost importance. Therefore, the first task of this research was to establish reliable and appropriate quantification methods. The choice of chromatography and detection techniques for each analyte in a standard solution is discussed in the first part of the results-and-discussion

section.

Despite extensive studies in recent years, supercritical fluid extraction remains more or less an empirical technique. Thus, solubility of an organic compound in the supercritical fluid cannot be precisely predicted on theoretical grounds alone. In particular, in order to establish an effective analytical methodology, it is essential to develop an empirical understanding of the effects of experimental parameters such as temperature, pressure, extraction time, nature of the supercritical fluid employed, and concentration of the analytes, etc. on the efficiency of SFE. The results related to the extractions and their theoretical implications are discussed in the latter part of the results-and-discussion section.

4.1 Analysis of the Standard Solutions and Instrument Calibration

4.1.1. Packed column GC with electron-capture detector

The Electron-Capture Detector (ECD) is known to be very selective toward halogenated organic compounds. This high selectivity, coupled with its great sensitivity, has made ECD very popular and useful for analysis of residue levels of pesticides and PCBs. It has been shown that the ECD response for halogenated compounds is very high, but the sensitivity differs with the type of compound and even with slight

structural changes, as observed between homologous compounds [Erickson, 1986]. A comparison of the relative molar responses of electron-capture and flame ionization detector to some isomeric chloro-biphenyls is given in Table 4.1.1. It is evident that the electron-capture detector is more sensitive and that the relative molar response is both a function of the degree of chlorination and the position of the substituent. Therefore, the GC with ECD was chosen for determination of aldrin and PCB samples in this research.

For most organic compounds, quantitation is relatively straightforward. The instruments used are generally required to be calibrated using standard solutions of known concentrations of the analytes. The amount of unknown is then measured by comparison of the signals such as peak areas or peak heights for the unknown with the calibration factor or curve. Quantitation of PCBs is however not so simple since PCBs are not a single compound but rather a complex mixture of 209 possible isomers. In addition, a precise calibration cannot be made because most of the isomers are not readily available. Therefore, the quantitation of the complex chromatograms can only be an estimated value based on the similarity of the sample PCB mixture to a commercial product (e.g. Aroclor 1254 or 1260). If the "fingerprints" of the sample and standard are similar, the quantitative confidence is better. The most appropriate calibration method uses a set of four available isomers for calibration and quantitation of the PCB mixtures. It is, therefore, usually obtained by measuring the peak areas or peak heights of selected key diagnostic peaks

present in the environmental PCB extract and comparing this results with the areas or heights obtained with the appropriate commercial PCB mixture (e.g. Aroclor 1254 and Aroclor 1260). Figures 4.1.1 and 4.1.2 are typical gas chromatograms of standard solutions of Aroclor 1254 (2.72 µg/mL) and Aroclor 1260 (0.457 µg/mL), respectively. The GC operating conditions for both PCB mixtures are the same: input and output attenuation were set at range 1 and 32, respectively, and the ECD reference current was 1.0 nA. Calibration for each Aroclor product was carried out using five diagnostic peaks which are indicated in Figures 4.1.3 and 4.1.4, respectively. The electron capture detection limit was found to be 0.109 ng for Aroclor 1254 and 91.4 pg for Aroclor 1260 using a signal to noise ratio (S/N) of 3.

The quantitation of aldrin standard solutions was carried out using both electron capture detection and flame ionization detection (FID). The injection volume was 1 µL. The lowest detectable quantity (LDQ) using the ECD was 42 pg with the reference current set at 1.0 nA, and input and output attenuations range 1 and 16, respectively. With the FID, the LDQ was 1 ng at an attenuation of 2^{-2} . Both LDQ values were based on a S/N of 3, and well satisfy the requirements for the actual environmental analyses.

Table 4.1.1. Relative Molar Responses of Electron Capture and Flame Ionization Detectors to Some Chlorobiphenyls. [Hutzinger, 1974]

Chlorobiphenyl	Relative Molar Response	
	Electron Capture	Flame Ionization
2-	1.00	1.00
3-	0.20	0.92
4-	1.10	0.87
2,2'-di	5.16	0.99
2,4-di	17.7	0.86
2,6-di	32.0	0.91
3,3'-di	6.10	0.94
3,4-di	15.2	0.86
4,4'-di	5.97	0.81
2,4,4'-tri	135	0.78
2,2',4,4'-tetra	106	0.87
2,2',6,6'-tetra	20.6	0.90
3,3',4,4'-tetra	396	0.87
3,3',5,5'-tetra	320	0.85
2,3,4,5-tetra	367	0.87
2,3,5,6-tetra	259	0.71
2,2',4,4',6,6'-hexa	347	
3,3',4,4',5,5'-hexa	726	
2,2',3,3',4,4',6,6'-octa	1,180	
2,2',3,3',5,5',6,6'-octa	1,150	
deca	1,410	

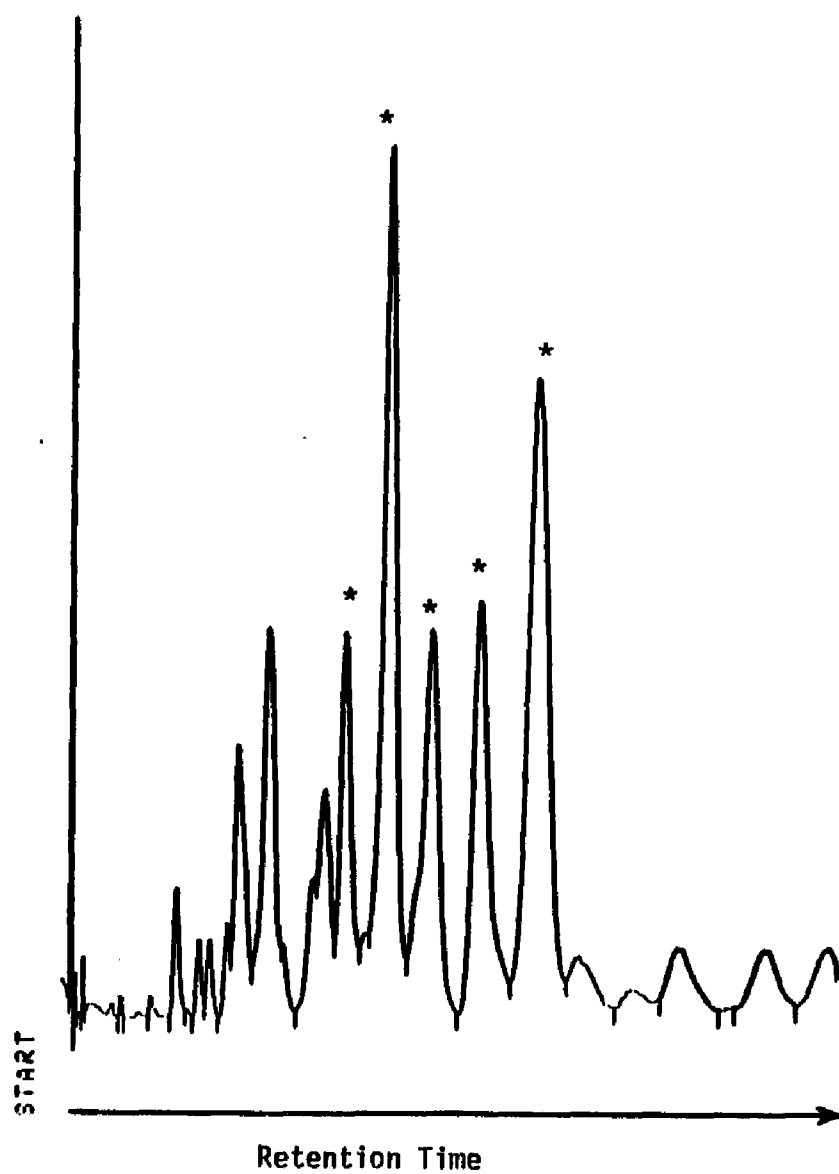


Figure 4.1.1. GC chromatogram of Aroclor 1254. Five diagnostic peaks are marked with *. Standard solution: 2.724 $\mu\text{g/mL}$. GC Conditions: Column temperature: 200°C; Injection port temperature: 250°C; Injection volume: 1 μL ; Range: 1; I: 0.5 nA; ATTN: 2-5.

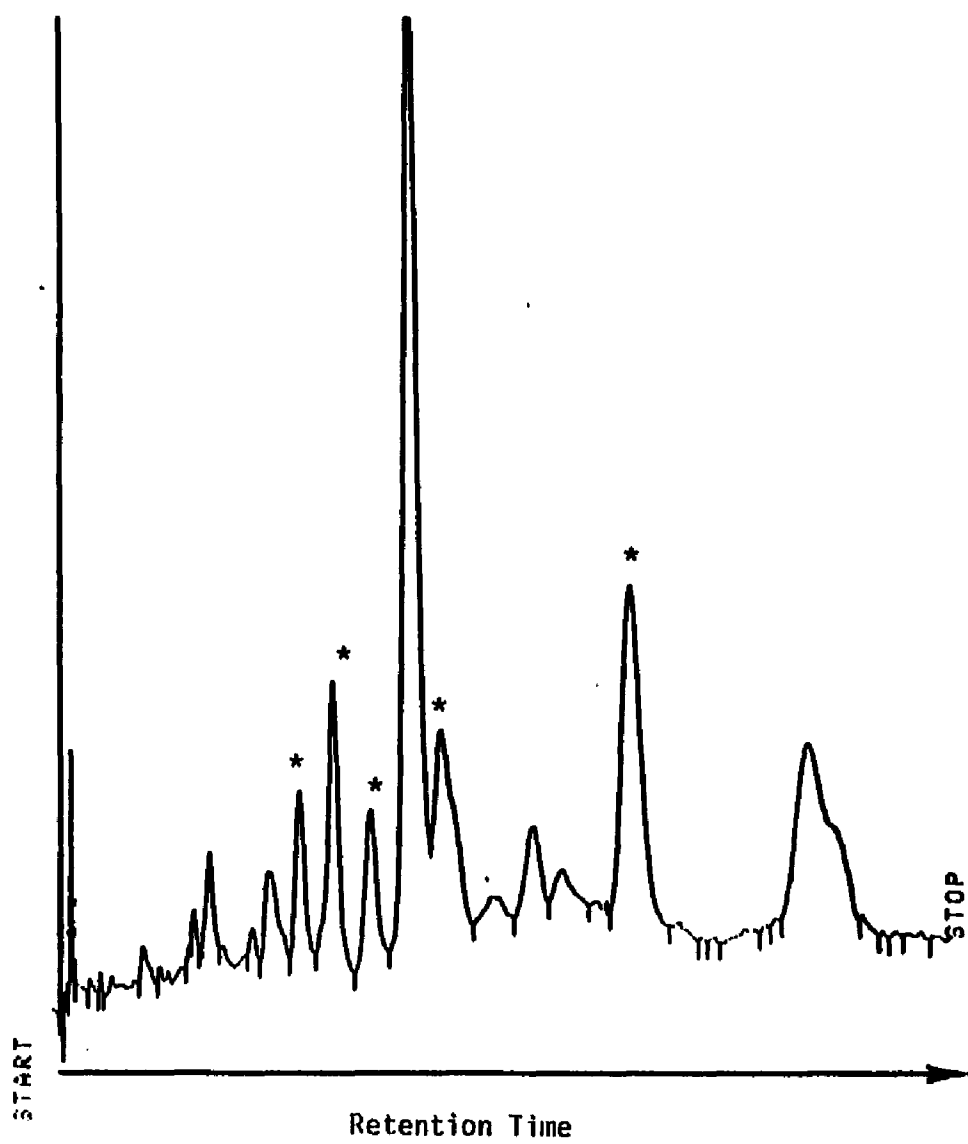


Figure 4.1.2. GC chromatogram of Aroclor 1260. Five diagnostic peaks are marked with *. Standard solution: 0.457 $\mu\text{g/mL}$.

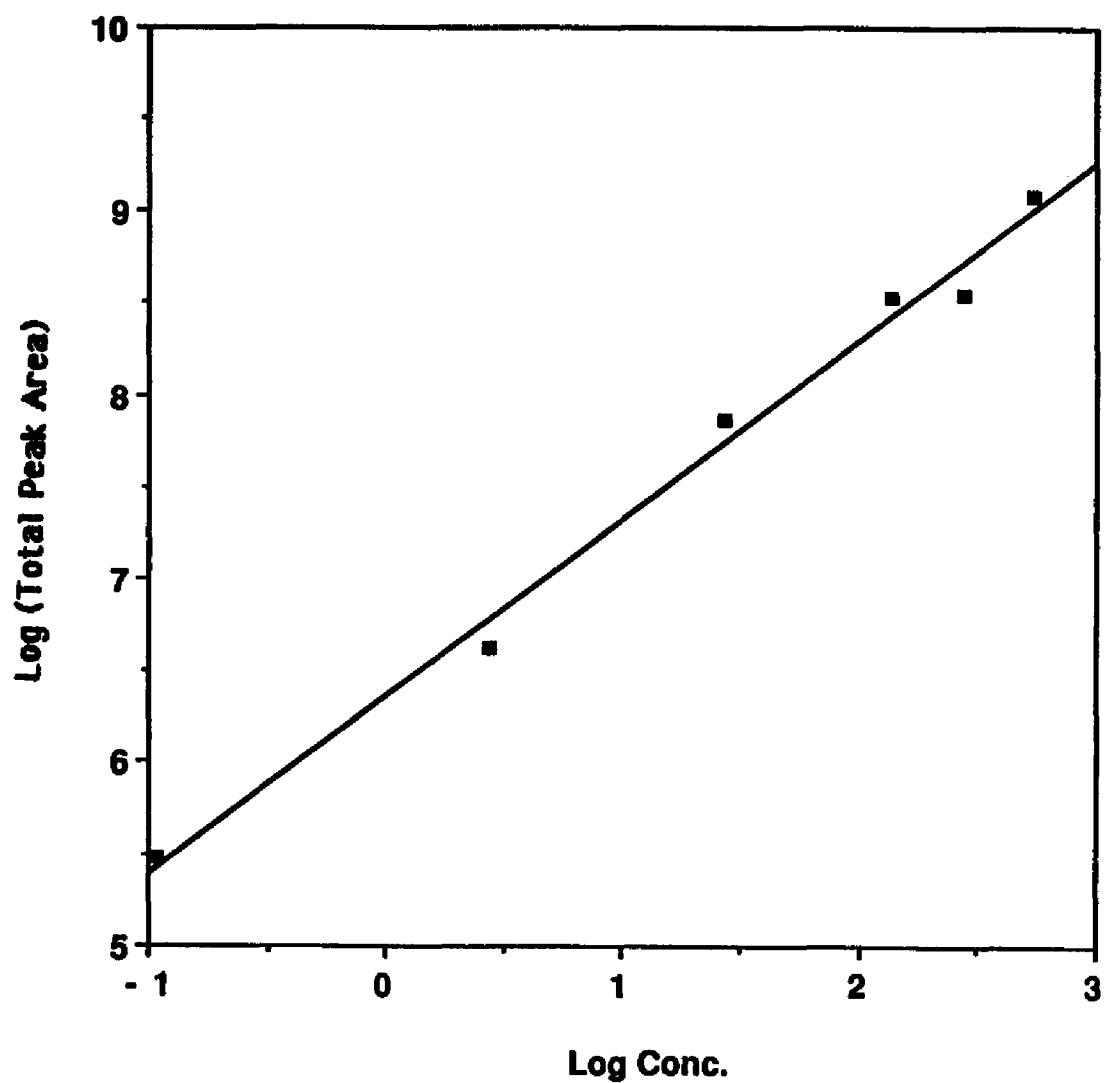


Figure 4.1.3. Calibration curve for Aroclor 1254: a logarithmic plot of the sum of the areas of all five diagnostic peaks versus the concentration of Aroclor 1254 in $\mu\text{g/mL}$.

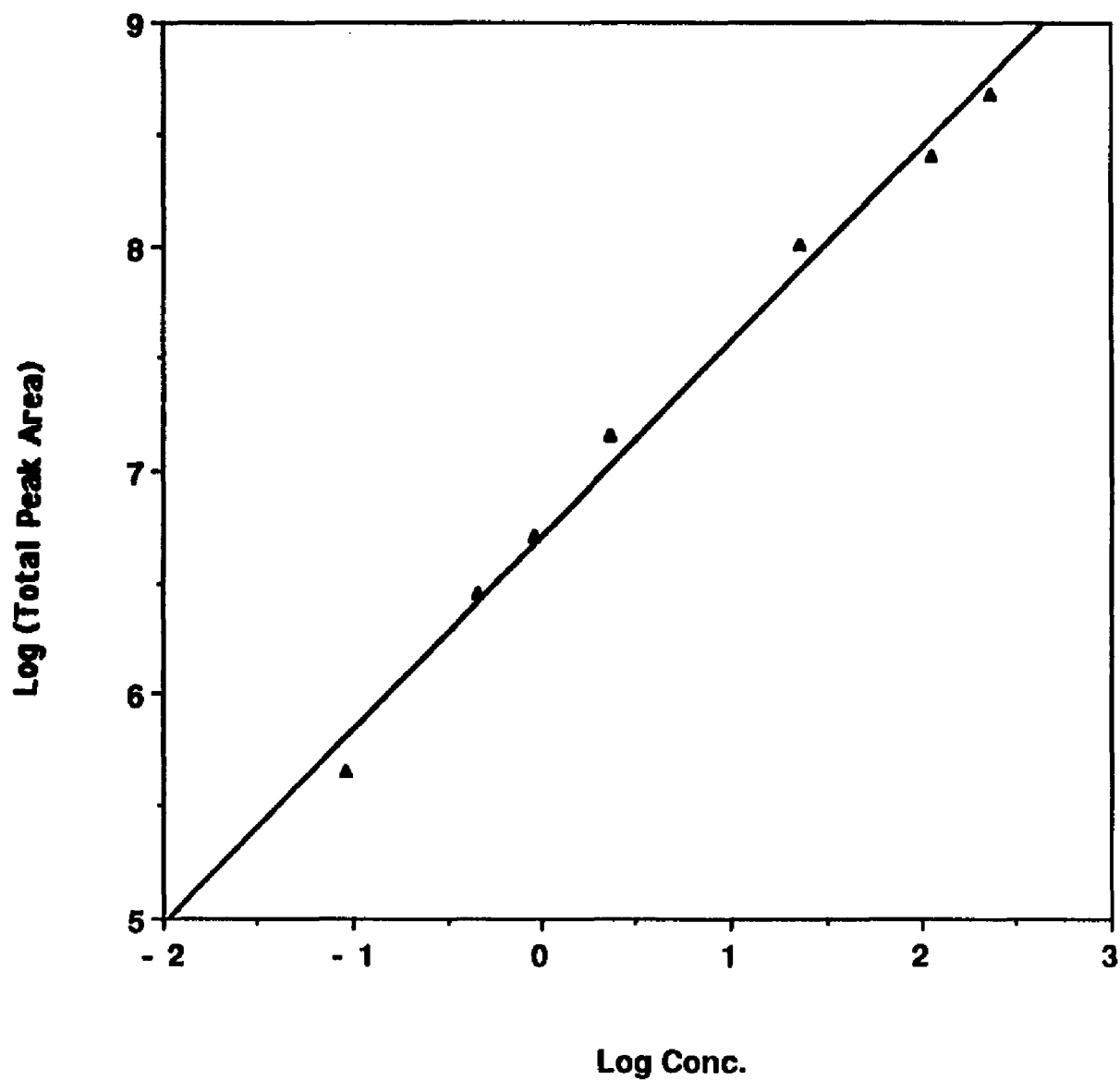


Figure 4.1.4. Calibration curve for Aroclor 1260: a logarithmic plot of the sum of the areas of all five diagnostic peaks (T.P.A.) versus the concentration of Aroclor 1260 in $\mu\text{g/mL}$.

4.1.2. Capillary column with flame ionization detector

The GC chromatogram with a flame ionization detector for 1 μ L-injection volume of a standard solution containing 2.12 μ g of 2,4-D methyl ester, 2.18 μ g of malathion and 0.45 μ g of parathion is given in **Figure 4.1.5** in which the peaks corresponding to the three compounds are labeled a, b and c, respectively. The calibration curves obtained from five standard mixture solutions of 2,4-D methyl ester, malathion and parathion are shown in **Figure 4.1.6**. The LDQ for these three compounds were 0.31 ng, 0.38 ng and 0.64 ng, respectively, with the GC attenuation of 2^{-3} and S/N of 3. The slope of the curve for malathion (**Figure 4.1.6**) is relatively smaller than those for parathion and 2,4-D methyl ester, which might be due to the inherent smaller detector response. It should not be attributed to the lower solubility of malathion because the solubility tests for malathion in various solvents showed no significant difference between the solvents employed. For example, the GC-peak area counts per microgram (μ g) of malathion in three different solvents, methylene chloride, methanol and toluene, were found to be quite similar; 6×10^3 , 5×10^3 and 5×10^3 , respectively.

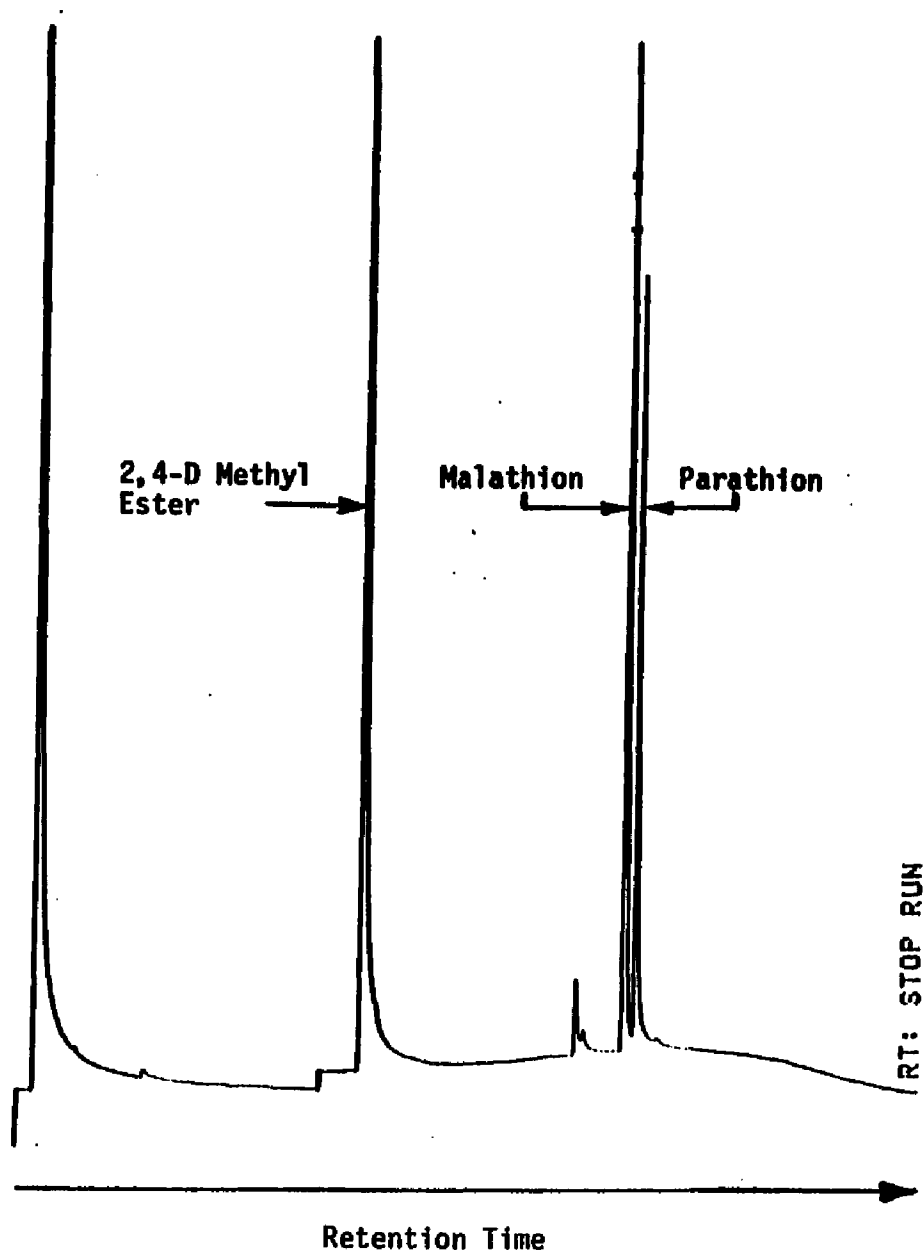


Figure 4.1.5. The GC chromatogram of a standard solution containing 2.12 μg of 2,4-D methyl ester, 2.18 μg of malathion and 0.45 μg of parathion. Injection volume: 1 μL .

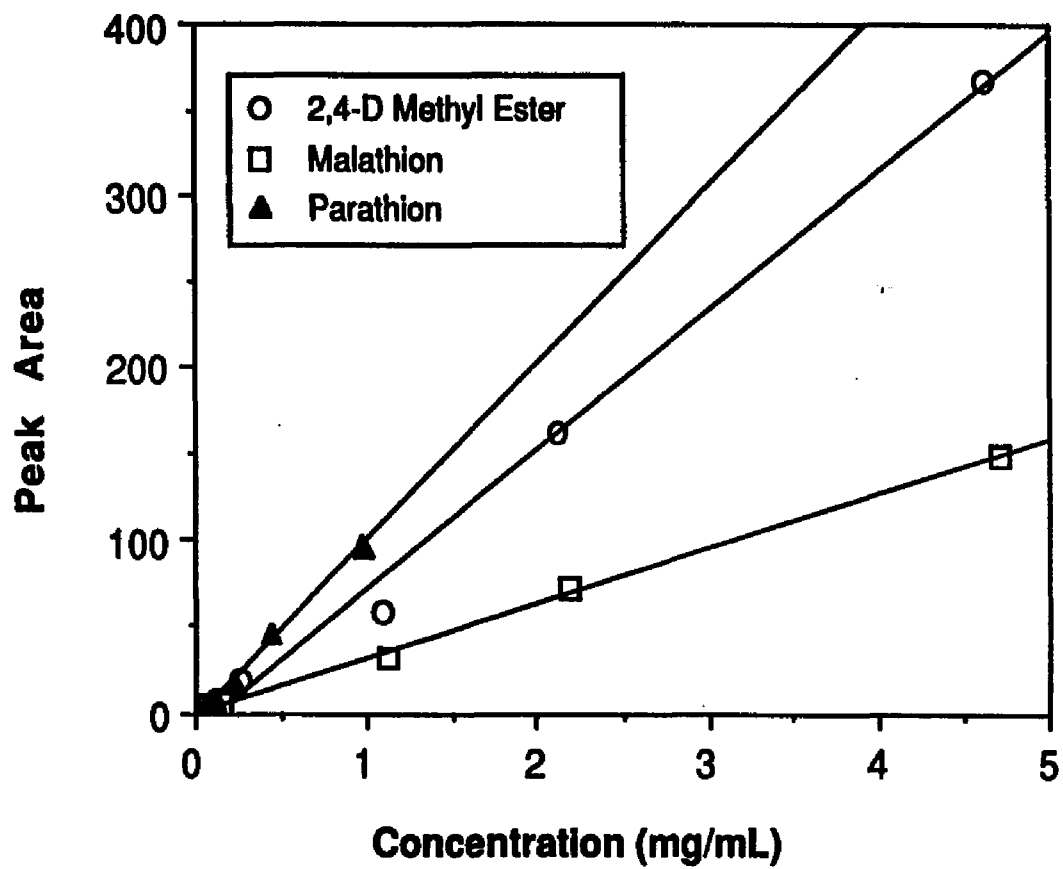


Figure 4.1.6. Calibration curves for standard mixture solution of 2,4-D methyl ester, malathion and parathion.

4.1.3. HPLC with ultraviolet detector

Reversed- phase liquid chromatography (RPLC), generally carried out on chemically bonded octadecylsilanes such as C₁₈ / Corasil, Permaphase ODS, and Vydac reversed-phase, has shown excellent promise for analysis of many pesticides with high resolution and sensitivity [Lawrence, 1978]. Therefore, reversed-phase LC was employed for the analysis of methanol solutions of aldicarb and amitrol with acetonitrile/water (40/60 by volume) as mobile phase. A Perkin-Elmer LC-410 equipped with a Model-235 diode array detector was employed for determination of the wavelengths at which amitrol or aldicarb has maximum UV absorbance. The rapid scanning photodiode array spectrophotometer offers a wide range of options to aid in structural identification without the requirement of interrupting solvent flow and without encountering all of the frustrations inherent in stopped-flow procedures. The spectrum of amitrol showed that maximum absorption occurred at a wavelength of 211 nm, which agrees well with the literature [Hoodless et al.,1978], where the wavelength of maximum absorption ($\lambda_{\max}$) was reported at 207 nm with molar absorptivity (ϵ) of 5.0×10^3 . When the wavelength was changed from 210 to 254 nm, the value of ϵ dropped from 4.5×10^3 to less than 0.05×10^3 .

Figure 4.1.7 shows the UV spectrum of aldicarb recorded in acetonitrile/water (40/60) solution. There are two strong absorption

bands, one below ca. 220 nm and another at ca. 248 nm. The results are similar to those reported by Sparacino and Hines (1976). The molar absorptivities were reported to be 40.8×10^3 and 1.9×10^3 for the two maxima at λ_{max} of 193 and 248 nm, respectively. The other ϵ ($\times 10^3$) values were reported to be 5.5, 2.5, 1.7, and 0.9, at the λ values of 210, 220, 254 and 280 nm, respectively [Sparacino, 1976].

Thus, a change in detection wavelength from the λ_{max} results in a reduction of the HPLC peak areas of both amitrol and aldicarb. When the detection wavelength was changed from 210 nm to 220 nm, the HPLC peak areas of amitrol and aldicarb decreased by 42 and 33 percent; and when changed from 210 nm to 230 nm, both peak areas reduced by 70 and 48 percent, respectively. Therefore, the wavelength for the UV-detector was selected as 210 nm for analysis of both amitrol and aldicarb in order to obtain the large peak areas and hence the high sensitivities.

Acetonitrile/water was employed as the mobile phase in the HPLC study of amitrol and aldicarb. For a number of reasons such as the relatively low viscosity, intermediate solvent strength, and the lowest UV background interference, acetonitrile/water mobile phases generally give the best overall results. Different proportions of acetonitrile / water were investigated for a given concentration of the standard solution of amitrol and aldicarb. The best resolution for both compounds was achieved using 40% acetonitrile in water (Figure 4.1.8).

Calibration for both compounds was carried out using 40/60 acetonitrile/water as shown in **Figure 4.1.9**. The LDQ's for both amitrol and aldicarb were determined at 210 nm to be 7.3 ng and 6.8 ng , respectively, with attenuation being set at 8 and a signal to noise ratio (S/N) of 3 at an injection volume of 6 μ L. These minimum detection limits should generally satisfy the requirements for the analyses of actual environmental samples of amitrol and aldicarb.

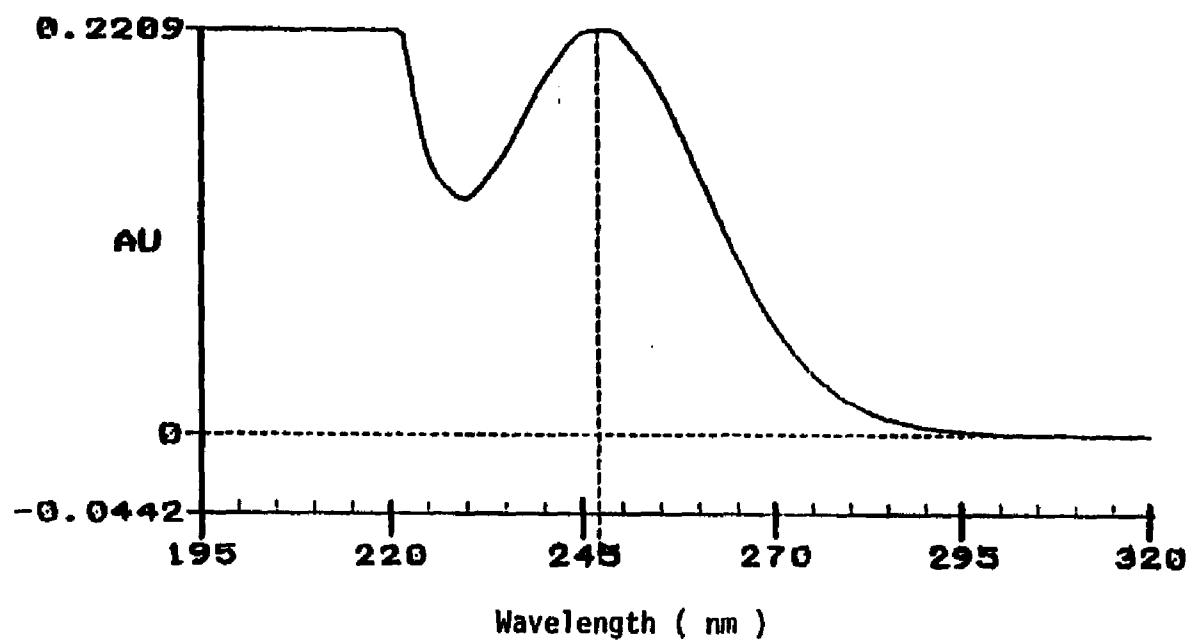


Figure 4.1.7. UV spectrum of aldicarb in acetonitrile/water (40/60) solution.

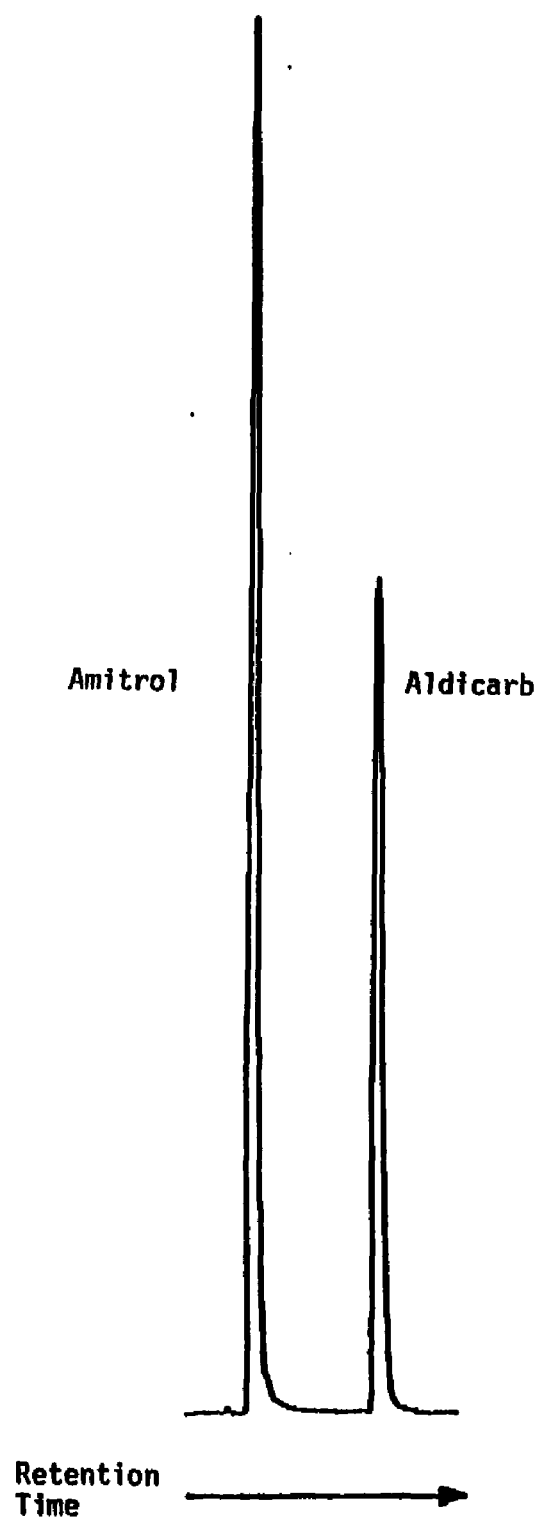


Figure 4.1.8. HPLC of amitrol and aldicarb. Mobile phase: a mixture of acetonitrile (40% v) and water (60% v). Injection volume: 6 μ L.

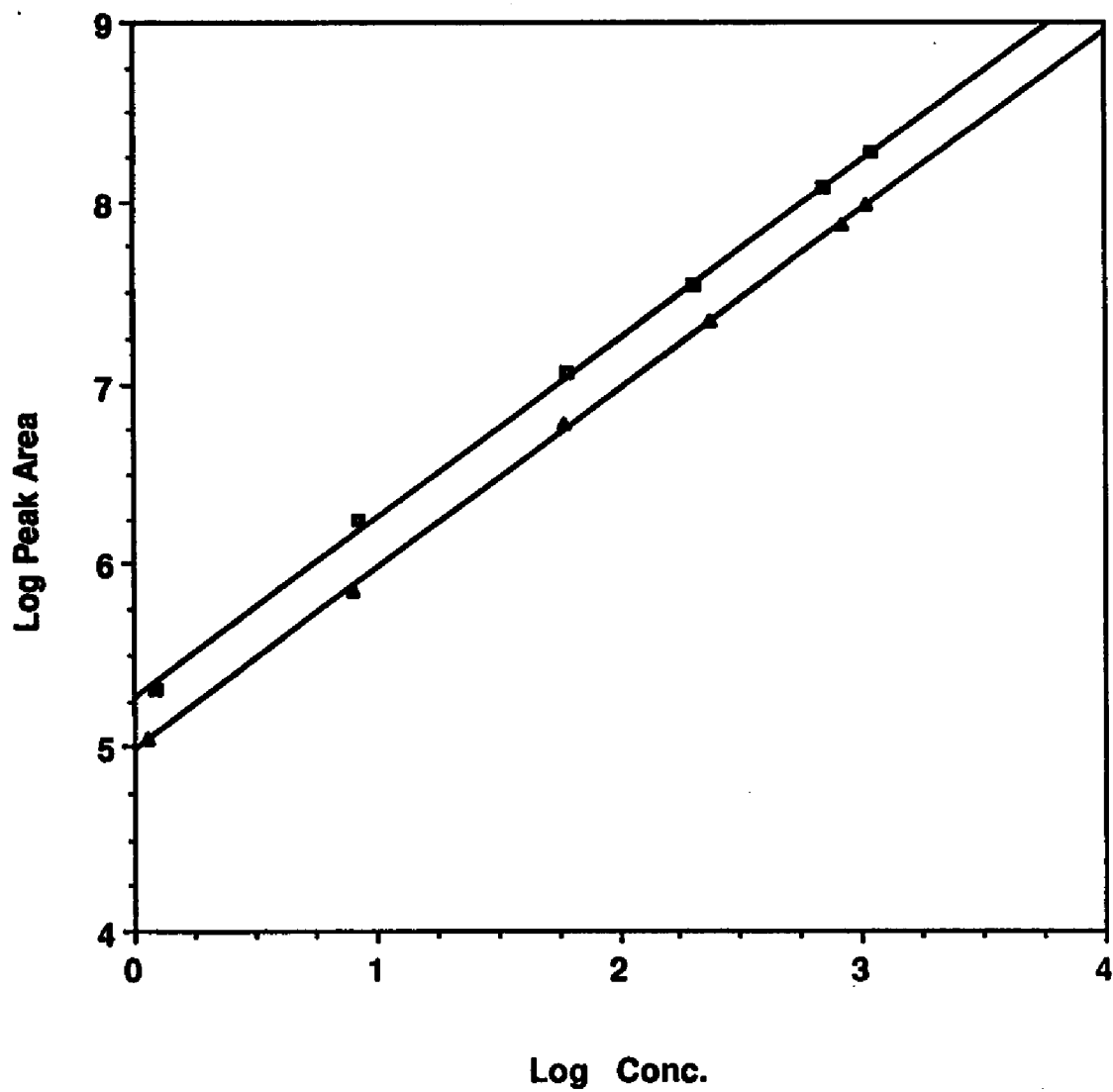


Figure 4.1.9. Calibration curves for (□) amitrol and (Δ) aldicarb. Concentration in mg/mL.

4.2. Effects of Pressure and Modifiers on the Efficiency of Supercritical Fluid Extraction

There are several experimental parameters which must be optimized for a successful supercritical fluid extraction. These variables include pressure, extraction fluids and their modifiers, temperature, and extraction time. A change in conditions may alter remarkably the extraction efficiency. Selection and control of the extraction conditions can also guide analytical chemists in the analysis of unknown samples containing compounds similar to those in known samples.

The dependence of the extraction efficiency for selected pesticides and PCBs on CO₂ pressure and organic modifiers including methylene chloride, methanol and benzene is discussed in the following sections. It should be noted that a change in the system pressure alters the density of CO₂ and that the effect of pressure could be, therefore, better presented as the effect of density. The pressure is used only for the sake of convenience and clarity of discussion in the following section.

4.2.1. Effect of Pressure

Table 4.2.1 shows the effect of the system pressure on the recoveries of aldrin in the SC-CO₂ extractions from an aldrin-spiked sandy soil (4.1 µg/g) at 45°C for 20 min. It is apparent that the recovery of

aldrin increases significantly as the system pressure is increased from 5000 psi to 8000 psi. It is well-established that the solvating power of a supercritical fluid is directly related to its density, which in turn is extremely sensitive to the pressure of system [McHugh and Krukonis, 1986]. High density of the fluid means that the molecular interactions increase because of the shorter intermolecular distances. The observed increase in the recovery of aldrin with pressure can be well interpreted by the increase in density, and therefore the solvating power, of the supercritical fluid.

To evaluate the effects of higher pressures on recovery, the SC-CO₂ modified with 5% (in vol.) of methanol (the effect of modifier will be discussed in section 4.2.2.) extractions of top soil spiked with a mixture of 2,4-D methyl ester, malathion and parathion were performed in the pressure range of 4000 to 9000 psi at 45°C for 20 minutes. The results are given in Table 4.2.2 and graphically presented in Figure 4.2.1. In general the recovery increases with the system pressure, similar to aldrin. It should be noted that the almost quantitative recoveries (e.g. ca. 97%) can be achieved in a short period of time (e.g. 20 min). Up to a pressure of 8000 psi, the recoveries of analytes increase rapidly with pressure. However, when higher pressures were applied, the recoveries of the pesticides seem to level off. This could be explained in terms of the solubility limits of 2,4-D methyl ester, malathion and parathion in the supercritical fluid. Thus, the density of the supercritical fluid increases when the pressure is raised to 9000 psi but the solubilities of the

Table 4.2.1 Effect of System Pressure on the Recovery of Aldrin from Sandy Soil.^a

Extraction Pressure^b		P_R^c	ρ_R^d	Extraction Recovery (n = 3)
in psi	in atm.			
5,000	340.2	4.67	1.23	68 ± 3.5 %
6,500	442.3	6.08	1.59	74 ± 4.2 %
8,000	544.4	7.48	1.96	86 ± 3.9 %

a. SFE conditions: SC-CO₂ at Temperature = 45°C ($T = 318$ K and reduced temperature $T_R = T/T_c = 1.05$); Equilibration time = 20 min.

Samples: Sandy soils (60/80 mesh) spiked with 4.1 µg/g. The recovery of aldrin was determined by GC/ECD.

b. Conversion factor: 1 atm = 14.696 psi.

c. Reduced pressure $P_R = P / P_c$.

d. Reduced density $\rho_R = \rho / \rho_c$

Table 4.2.2. Recovery of 2,4-D Methyl Ester, Malathion and Parathion from Spiked Top Soil. ^a

Pressure (psi)	% Recovery and %RSD (n=3)		
	2,4-D M.E.	Malathion	Parathion
4000	37 ±5.8	37 ±6.1	44 ±5.1
5000	50 ±4.2	47 ±4.8	57 ±3.8
6000	57 ±5.0	62 ±5.0	66 ±4.6
7000	77 ±4.4	77 ±3.5	83 ±4.1
8000	97 ±3.1	95 ±4.0	97 ±3.3
9000	98 ±4.0	90 ±6.2	95 ±4.1

a. SFE conditions: SC-CO₂ + Methanol (5% v/v), Temperature = 45 °C, Equilibration time = 20min. The recovery of the pesticides were determined by GC/FID method in a methanol solution.
 Samples: Top soils (60/80 mesh) spiked with levels of (mg/g soil) 0.14, 0.14 and 0.028 for 2,4-D methyl ester, malathion and parathion, respectively.

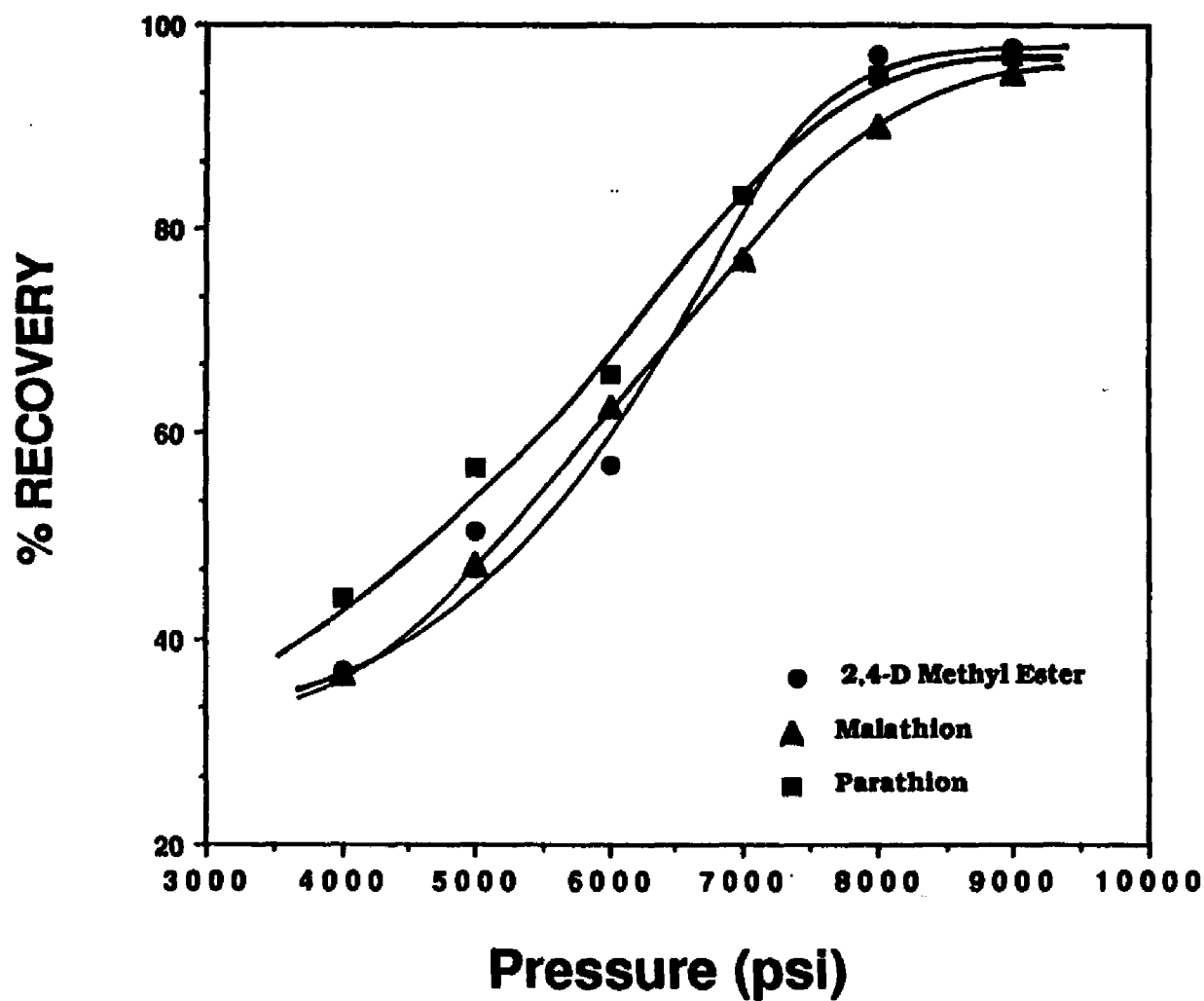


Figure 4.2.1. Effect of pressure on recovery of 2,4-D methyl ester, malathion and parathion. Temperature: 45°C; time: 20 min.

pesticides probably have already reached their own limits at around 8000 psi. The dependence of recovery on the pressure as discussed above is similar to the well-documented dependence of the solubility of solid naphthalene on the system pressure [Tsekhanskaya, 1962; Diepen, 1948]. The solubility of naphthalene in supercritical ethylene ($T_c = 9.3^\circ\text{C}$, $P_c = 49.8 \text{ atm}$) at 35°C increases rapidly with an increase in pressure in the range of 50 to 100 atm. However, at pressures greater than 100 atm, the solubility rapidly reaches a limiting value of ca. 5 mole-%. Similar pressure effects on recoveries have also been generally observed in the extractions of amitrol and aldicarb (**Figure 4.2.2**) and of Aroclor 1254 and 1260 (**Figures 4.2.3** and **4.2.4**, respectively) by SC- CO_2 in the absence or presence of organic modifiers which will be discussed in detail in the next section.

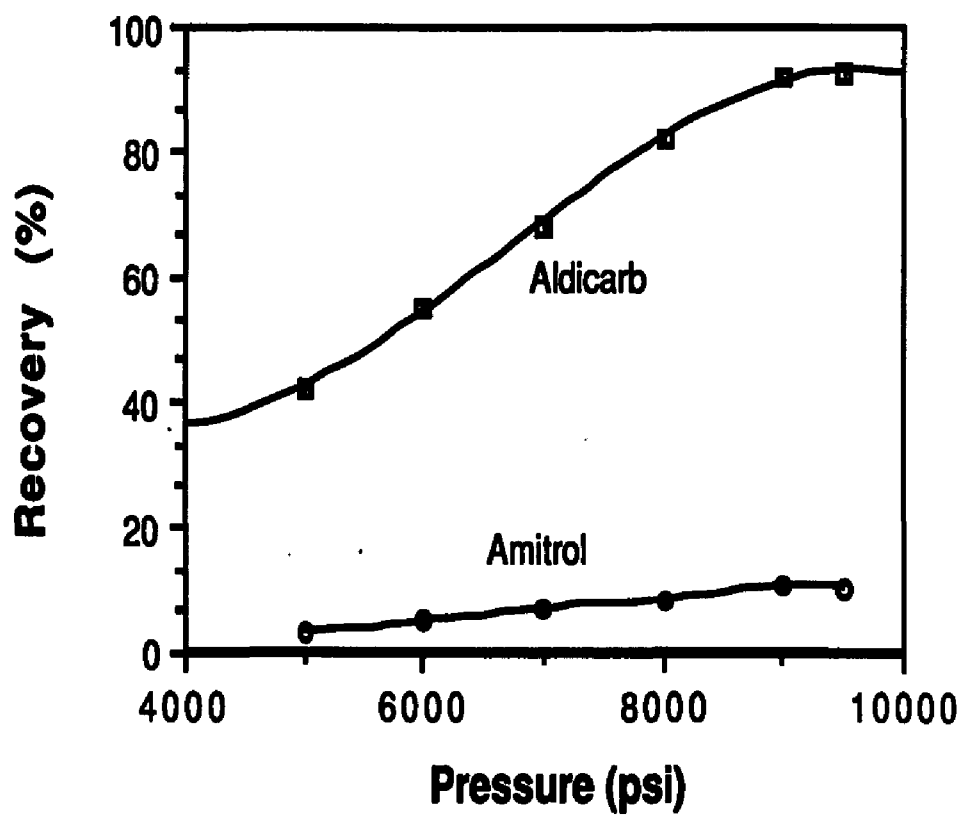


Figure 4.2.2. Effect of pressure on recovery of amitrol and aldicarb in the presence of 10% water modifier. Temperature: 90°C; time 30 min.

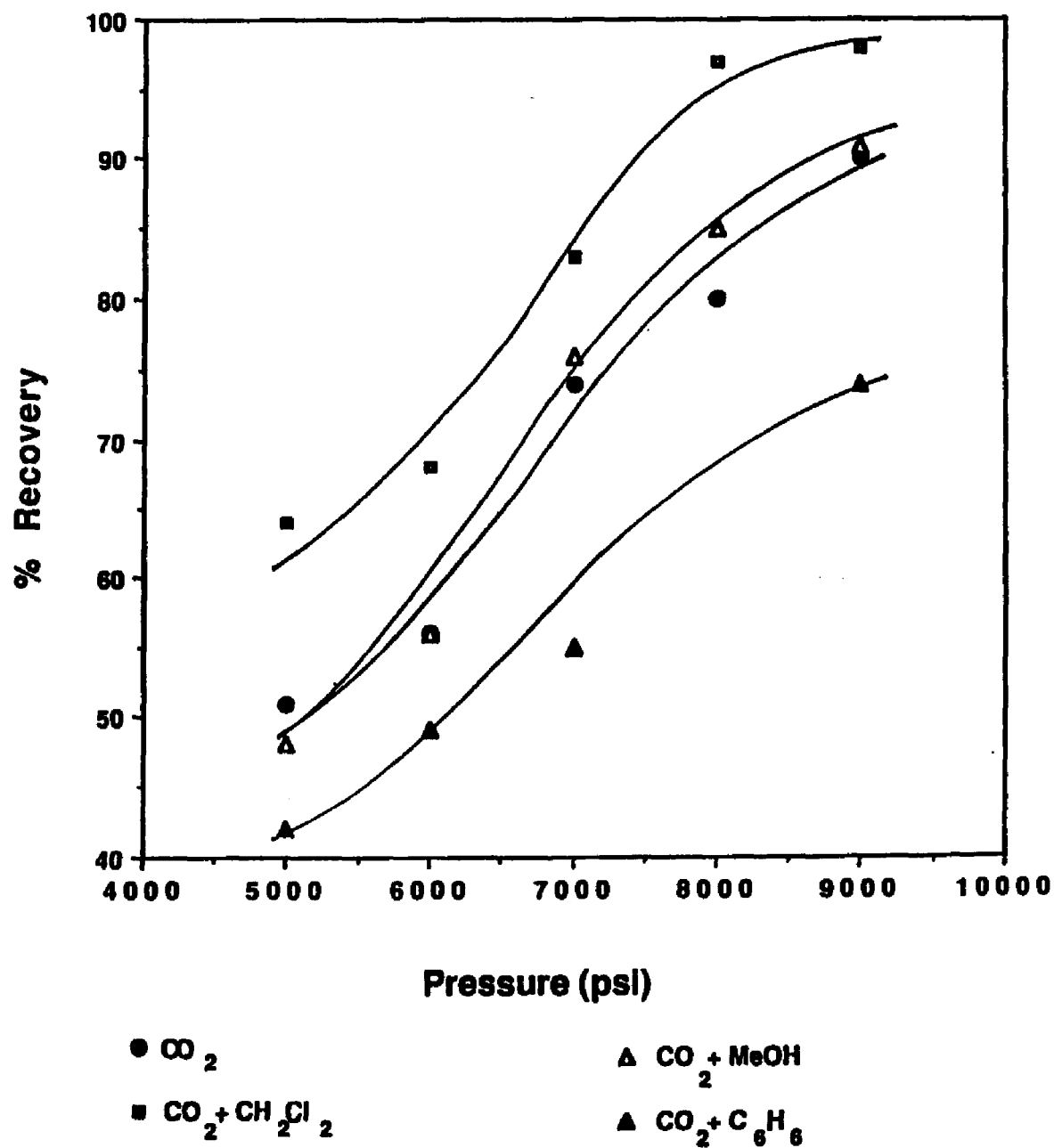


Figure 4.2.3. Effect of pressure and modifiers on recovery of Aroclor 1254.

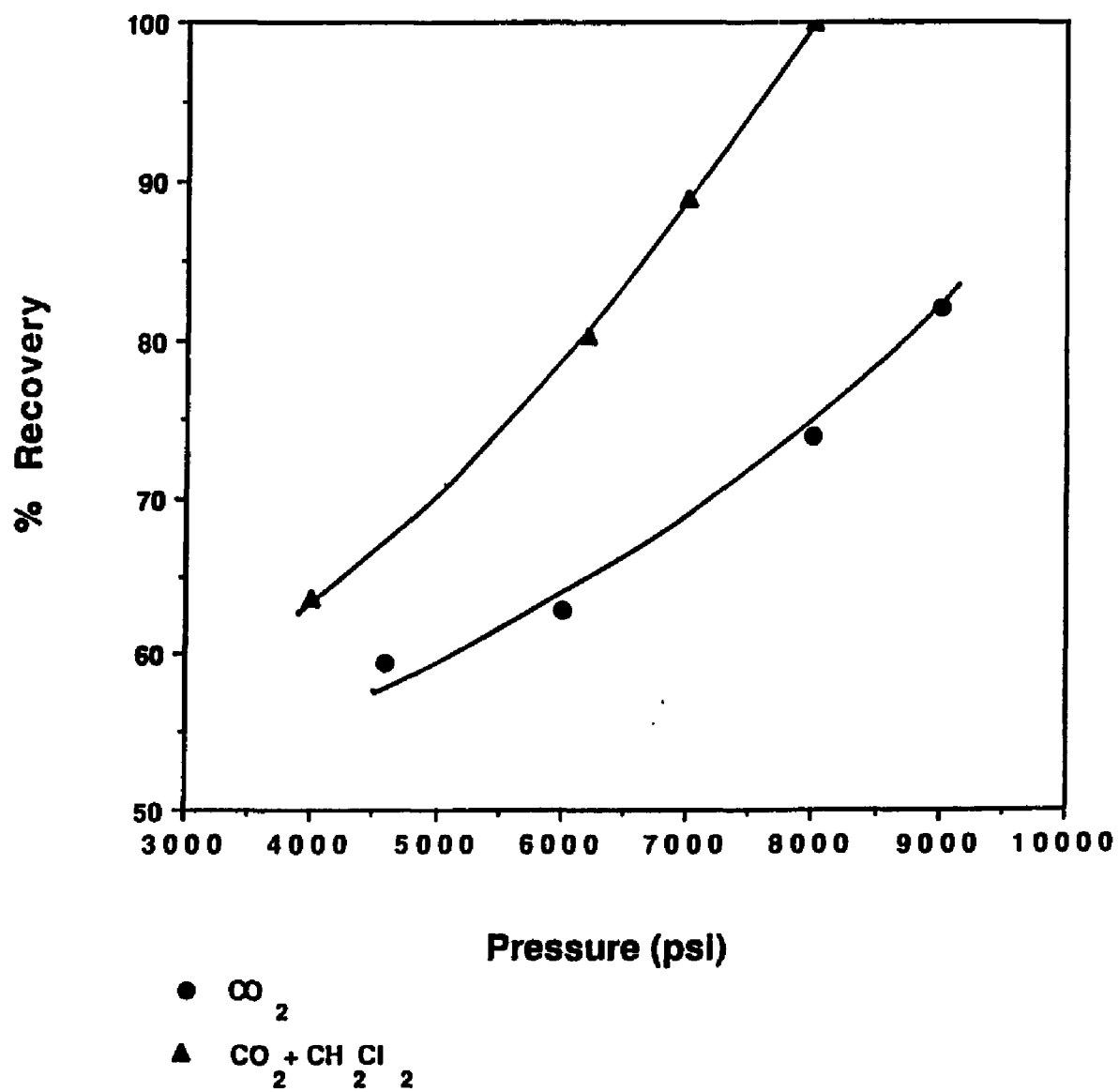


Figure 4.2.4. Effect of pressure and modifier on recovery of Aroclor 1260.

4.2.2. Modifier Effect

An interesting phenomenon observed in our experiments is that the efficiency of the supercritical carbon dioxide extractions of pesticides and PCBs is significantly improved by using appropriate organic modifiers. The introduction of a modifier to the extraction system was accomplished simply by adding the desired amount of liquid organic solvent (modifier) through a pipet into the packed extraction chamber prior to pressurization. Since supercritical CO₂ equilibrates in the extraction chamber during the extraction, a uniform CO₂-modifier mixed-fluid phase can be obtained.

Figure 4.2.3 shows a typical example of a comparison of the extraction efficiencies obtained for Aroclor 1254 using CO₂ alone and CO₂ plus three different modifiers, i.e. CO₂ with 10% (v/v) methylene chloride, 10% (v/v) methanol and 10% (v/v) benzene. Samples were extracted from sandy soil in triplicate for 20 min with each of the modified supercritical fluids at five different pressures varied from 5000 to 9000 psi. Temperature was kept constant at 45°C for all the extractions. As shown in **Figure 4.2.3**, supercritical CO₂ modified with 10% methylene chloride seems to give the best extraction recoveries for Aroclor 1254. These recoveries are generally improved by about 10% throughout the pressure range of 4000 to 9000 psi in comparison to those without methylene chloride modifier. On the other hand, methanol asserts little or no influence on the recovery of Aroclor 1254; and benzene appears to have a negative effect on the recoveries. The percent relative standard deviation (% RSD) for these

studies are also listed in **Table 4.2.3**. The %RSD data further demonstrate clearly that there are significant effects of the modifiers on the recoveries of Aroclor 1254. The use of methylene chloride as a modifier also increases the recovery of Aroclor 1260 in the supercritical CO₂ extractions as shown in **Figure 4.2.4**. The extent of the increase in recoveries appears to be even greater than that in the case of Aroclor 1254. For example, at a pressure of 6000 psi, the improvement of the recovery of Aroclor 1260 was about 20% for the system with methylene chloride-modified supercritical carbon dioxide as extraction fluid. This could be attributed to the higher average number of chlorine atoms per molecule (6.30) for Aroclor 1260 than that (4.96) for Aroclor 1254 [Hutzinger,1974], which could result in a stronger interaction between Aroclor 1260 and chlorinated organic modifier (i.e. CH₂Cl₂) than that between Aroclor 1254 and CH₂Cl₂. Again, the effect of pressure on the extraction is also observed clearly; recovery increases with system pressure.

The effects of different modifiers were also investigated for the SC-CO₂ extraction of aldrin from sandy soils as well. The results and the general trend of the influences of the modifiers are comparable with those for the extractions of PCBs. The data summarized in **Table 4.2.4** for the SC-CO₂ extraction of aldrin at 45°C and 8000 psi demonstrate that the addition of methylene chloride to the system greatly enhances the recovery of the extraction by about 30 to 40 %, while methanol gives a

Table 4.2.3. Effect of Modifiers on Recovery of Aroclor 1254 at Various System Pressures.^a

Pressure (psi)	<u>% Recovery of the extraction with supercritical fluid</u>			
	CO ₂	CO ₂ +CH ₂ Cl ₂	CO ₂ +MeOH	CO ₂ +C ₆ H ₆
5000	51 ±1.9	64±4.3	48±5.1	42±3.0
6000	56±2.4	68±2.3	56±3.3	49±3.7
7000	74±2.1	83±2.6	76±2.9	55±2.8
8000	80±2.4	97±2.6	85±3.0	-
9000	90±1.8	98±2.0	91±3.4	74±3.6

a. Supercritical carbon dioxide modified with 10 % by volume of the organic solvents: methylene chloride, methanol and benzene. The extractions were performed in triplicate (n=3) at 45°C and for 20 min. Samples: 0.272 µg/g spiked sandy soils (60/80 mesh).

Table 4.2.4. Influence of Modifiers on Recovery of Aldrin^a

Supercritical Fluid Employed	% Recovery	% RSD^b
SC-CO ₂	51	6.7
SC-CO ₂ + 10% (v/v) Toluene	49	5.9
SC-CO ₂ + 10% (v/v) Methanol	66	2.0
SC-CO ₂ + 10% (v/v) Methylene Chloride	87	3.5

- a. Extractions were performed at 45°C, 8000 psi for 20 min.
Samples: 4.1 µg/g sandy soils (45/60 mesh). Trapping material:
Silica gel.
- b. Relative standard deviation estimated from the results of three
independent extraction experiments (n=3).

minor increase in the recovery, and toluene has little or no effect on the extraction of aldrin.

The above observations are quite consistent with those reported by Dooley et al. [1987]. They studied supercritical CO₂ extraction of DDT, an organochlorine compound similar to PCBs and aldrin, and found that recovery of DDT was essentially the same (about 70%) in the absence or in the presence of 5 percent toluene (by weight) under otherwise the same experimental conditions. Hence, the recovery-enhancement effect of the modifiers in the SC-CO₂ extraction of the organochlorinated compounds such as PCBs and aldrin could be ranked in the following general order:

Methylene chloride > methanol > toluene ~ benzene.

However, the effects of these modifiers on the recoveries appear to vary from one system to another. In the extraction of Aroclor 1254 (Table 4.2.3), the addition of methanol had no significant effect on the recovery and that of benzene resulted in a decrease in the recoveries. On the other hand, in the extraction of aldrin (Table 4.2.4), methanol improved the recovery slightly but toluene showed almost no effect at all. This behavior appears to be quite reproducible based on the triplicate experiments (see the %RSD in Tables 4.2.3 and 4.2.4). Hence, it is apparent that the effects of modifiers on SFE are more complicated and such systems are less predictable in comparison with those of pure supercritical fluid solvent systems. The solubilities of solutes in a given

particular extraction conditions depend on the nature of sample matrix and the properties of solutes and also on the characteristics of a modifier, i.e. the critical parameters of the binary SCF/modifier fluid mixture. Since these critical parameters as well as the phase diagrams of these supercritical fluid mixtures are not well defined or described in the literature, it is only possible at the present time to give a qualitative comparison and explanation for the behavior of these binary supercritical fluid mixtures.

Some physical properties of the organic modifiers under investigation including dielectric constant, polarizability and critical parameters, are listed in Table 4.2.5. The dielectric constant qualitatively represents the polarity of an organic solvent. Based on the dielectric constant data in Table 4.2.5, methylene chloride should be more polar than benzene and toluene. When methylene chloride was applied to supercritical carbon dioxide as a modifier, the polarity of extraction fluid mixture should be increased from a nonpolar to a slightly polar fluid phase. The polarity of this modified supercritical fluid could be comparable to those of Aroclor 1254, Aroclor 1260 and aldrin which are all weakly polar compounds. Thus, the solubilities of these three organochlorine compounds should be greater in the methylene chloride-modified SC-CO₂ than in pure, nonpolar CO₂, resulting in better recoveries in the extractions. Presumably, the extraction efficiency of relatively polar compounds should also be related to the polarity of extraction fluid. Polar components could be expected to be extracted in a greater extent by a polar than a nonpolar solvent.

Table 4.2.5 Selected Physical Properties of the Organic Modifiers Studied.^a

Modifier	Dielectric Constant (ϵ)	Polarizability (10^{-24} cm^3) ^b	Density (g/cm^3)	<u>Critical Properties</u>	
				T_c ($^{\circ}\text{C}$)	P_c (Atm)
Methanol	32.63	3.29	0.791 ²⁰	240	78.5
CH_2Cl_2	9.08	6.48	1.327 ²⁰	237	60.0
Benzene	2.27	10.32	0.879 ²⁰	289	48.6
Toluene	2.38	12.3	0.867 ²⁰	321	41.6

a. Data from the *Handbook of Chemistry and Physics*, 68 th Ed. CRC Press, West Palm Beach, FL, 1988.

b. Average electric dipole polarizability in Units of 10^{-24} cm^3 .
Polarizability of CO_2 is 2.91.

However, polarity cannot be the only factor involved in extraction recovery because methanol reduced the recoveries in the extractions of the PCBs (Table 4.2.3) or showed a smaller improvement in the recovery of aldrin in comparison with methylene chloride (Table 4.2.4). However, the dielectric constant of methanol is much larger than that of methylene chloride (Table 4.2.5). Such an inconsistency might be expected because of the complexity of the properties of a modified supercritical fluid system. Although the exact explanation for the above observations will depend on further theoretical and experimental studies of these binary solvent systems, we suggest that these observations might be interpreted in terms of polarizabilities or solubility parameters of CO₂, the chlorinated hydrocarbon solutes, and the modifiers. It has been suggested that the solvent strength of supercritical fluids should approach that of liquids since the densities are the same. This is clearly an oversimplification [Dobbs, 1986]. Dobbs et al. [1986] found that the solubility of hydrocarbons (e.g. hexamethylbenzene) in SC-CO₂ increases significantly with the addition of small amount of alkane modifiers. The addition of methanol (3.5 mol %) has no effect on the solubility of hexamethylbenzene. They argued that even when the densities are the same, the polarizability of CO₂ is lower than that of most hydrocarbons. The enhancement of the solubility of nonpolar hexamethylbenzene by nonpolar alkane modifiers was attributed to the similar polarizabilities (therefore, solubility parameters). Since the polarizability of methanol is

not much greater than that of CO₂, no, or less, enhancement of the solubility should be expected [Dobbs, 1986]. This argument is quite reasonable on the basis of the dependence of solubility on the difference in the solubility parameters of solvent and solute, i.e. $(\delta_1 - \delta_2)^2$. PCBs and aldrin are weakly polar compounds. The polarizabilities of PCBs and aldrin should be quite different from that of CO₂ or methanol (Table 4.2.5), resulting in low solubilities. In contrast, the polarizability of methylene chloride might be very close to that of PCBs and aldrin, leading to higher solubilities. McNally and Wheeler [1988] found that in SC-CO₂ extractions of the herbicides diuron and linuron, a drastic increase in extraction efficiency was exhibited when 10% ethanol was used as the modifier as opposed to methanol. This was attributed to the lower polarity of ethanol ($\epsilon = 25$) in comparison to that of methanol ($\epsilon = 33.6$) [McNally, 1988].

As modifiers, benzene and toluene would not be expected to alter the polarity of SC-carbon dioxide to any great extent because of their own low polarities, as represented by the low dielectric constants (Table 4.2.5). The experimental results for the extraction of aldrin (Table 4.2.4) indeed showed little or no effect of toluene on the recovery of aldrin, which is consistent with this expectation. However, with benzene, the recovery of Aroclor 1254 (Table 4.2.3) was actually reduced. This phenomenon has never been observed previously and could be of great theoretical or experimental importance. The causes of the reduction of the extraction efficiency are still unknown and should be the subject of future research.

In SFE of DDT with SC-CO₂, Dooley [1987] reported that toluene as a modifier does not increase the recovery of DDT. From the phase behavior of the ternary system of CO₂-DDT-toluene at 80°C and 100 atm, it is concluded that the solubility of DDT in pure CO₂ is 0.3×10^{-4} but the solubility of DDT in a mixed solvent of 95% (wt) CO₂ and 5% (wt) toluene is well below 0.4×10^{-4} [Dooley, 1987]. Since DDT is also a chlorinated hydrocarbon similar to aldrin, the lower recovery of aldrin obtained with benzene as modifier might be, therefore, similarly explained in terms of the solubility of aldrin. This should be further examined by studies of the phase behavior of the CO₂-aldrin-benzene system. On the other hand, the polarizabilities of benzene (10.32) and toluene (12.3, see Table 4.2.5) are higher than those of methylene chloride, CO₂ and methanol, which may not match with the polarizability of aldrin or PCBs, resulting in low solubilities as well. We hypothesize that both the polarizability (or solubility parameter) and the polarity (as represented by ϵ) of the supercritical fluid mixture should be optimized to match those of the solute, in order to achieve a high extraction efficiency.

The efficiency of supercritical fluid extraction has been found to depend also on the quantity of modifier employed. In Figure 4.2.5, the recoveries of the methylene chloride-modified SC-CO₂ extraction of Aroclor 1254 from the spiked sandy soil is plotted against the volume of methylene chloride introduced into the extraction system. As the volume

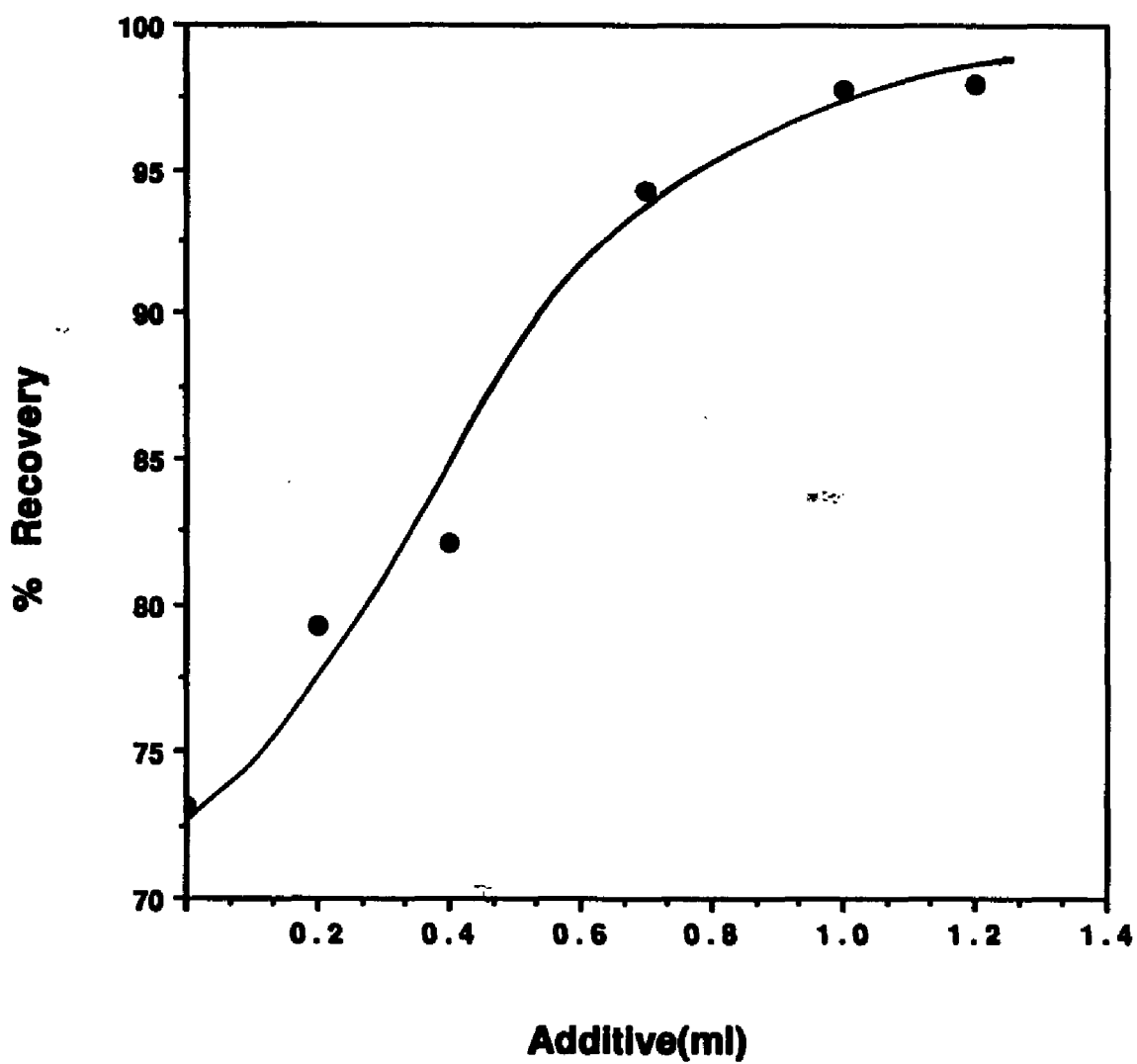


Figure 4.2.5. Effect of the amount of methylene chloride modifier on the recovery of Aroclor 1254.

of methylene chloride was increased from 0 to 0.8 mL, the recoveries increased rapidly from ca. 73% to ca. 95%. Further increase in the volume of methylene chloride did not significantly affect the recovery because almost quantitative recoveries were already obtained when 1 mL of the modifier was introduced. For this specific system, the results indicated that a modifier volume of 1.0 mL is enough for a nearly quantitative recovery. However, there should not be any generalization that an increase in the volume of methylene chloride would always lead to a higher recovery. Depending on the quantity of sample matrix, application of an excess amount of modifier might reduce the apparent recovery because solutes were eluted out of the extraction chamber with the excess modifier during the sample loading process. Furthermore, an excess amount of modifier has been found to saturate the carbon dioxide with the modifier [McNalley et al., 1989].

Modifier effects were also investigated for the supercritical fluid extraction of amitrol and aldicarb. Extraction of amitrol (21 $\mu\text{g/g}$) and aldicarb (24 $\mu\text{g/g}$) from spiked sandy soil was performed at 9500 psi and 45°C for 30 min using both supercritical carbon dioxide alone and 10% (by volume) water-modified CO_2 . Figures 4.2.6a and 4.2.6b are the HPLC chromatograms recorded for the extracts from the unmodified SC- CO_2 and the water-modified SC- CO_2 extractions, respectively. It is apparent that the peak heights for both amitrol and aldicarb in Figure 4.2.6b (water-modified CO_2) are significantly larger than those in Figure 4.2.6a

(unmodified). Water induces higher solubilities of amitrol and aldicarb in the supercritical fluid phase, which presumably reflects the high solubilities of amitrol and aldicarb in water. The proper quantity of water applied to extraction chamber was found to be 0.7 to 1.0 mL. The maximum recoveries of amitrol and aldicarb were obtained when using 0.7-1.0 mL of water as modifier under the extraction conditions of 9500 psi, 90°C and equilibrated for 30 min. Recoveries varied with modifiers as well as with the extraction gas, as listed in Table 4.2.6. The pesticides in the soil samples after supercritical fluid extraction were further extracted for 6 h on a Soxhlet extraction apparatus with methanol. The results from the Soxhlet extractions are also listed as "% unextracted" after SFE in Table 4.2.6. The generally low recoveries obtained for the SC-CO₂ extraction of

amitrol might be attributed to the difference in polarities between solutes and the nonpolar carbon dioxide. Both amitrol and aldicarb are quite soluble in water; about 28 grams of amitrol can be dissolved in 100 mL water at 23°C, and about 0.6 gram of aldicarb in 100 mL water at 25°C [Melnikov, 1971] (Table 4.2.7). Carbon dioxide, however, being a nonpolar solvent, might thus be expected to be a poorer solvent for both aldicarb and especially amitrol. Nitrous oxide exhibited similar extraction efficiency as carbon dioxide and no significant improvement in recovery was observed, although N₂O is more polar than CO₂. The methanol or water-modified SC-CO₂ extractions significantly improved the recoveries

Table 4.2.6. Effect of modifiers and extraction gases on the efficiency of supercritical fluid extraction of Amitrol and Aldicarb from spiked sandy soil.^a

Extraction Fluid	<u>Amitrol</u>		<u>Aldicarb</u>	
	%Recovery	%Unextracted	%Recovery	%Unextracted
SC-CO ₂	0	95	60	30
SC-N ₂ O	0.3	94	-	-
SC-CO ₂ +(10% v) CH ₃ OH	5	94	82	10
SC-CO ₂ +(10% v) H ₂ O	10	82	92	5

a. SFE Conditions: 9500 psi, 90°C and 30 min.; Sample: 10 g sandy soil spiked with 2.1×10^2 µg amitrol and 2.4×10^2 µg aldicarb. The %unextracted was determined by a Soxhlet extraction after SFE.

Table 4.2.7 Some Physical Properties of Amitrol and Aldicarb [Melnikov, 1971]

Compound	Molecular Weight	Solubility in Water
Amitrol	84.08	28 g/100 mL (23°C)
Aldicarb	190.26	0.6 g/100 mL (25°C)

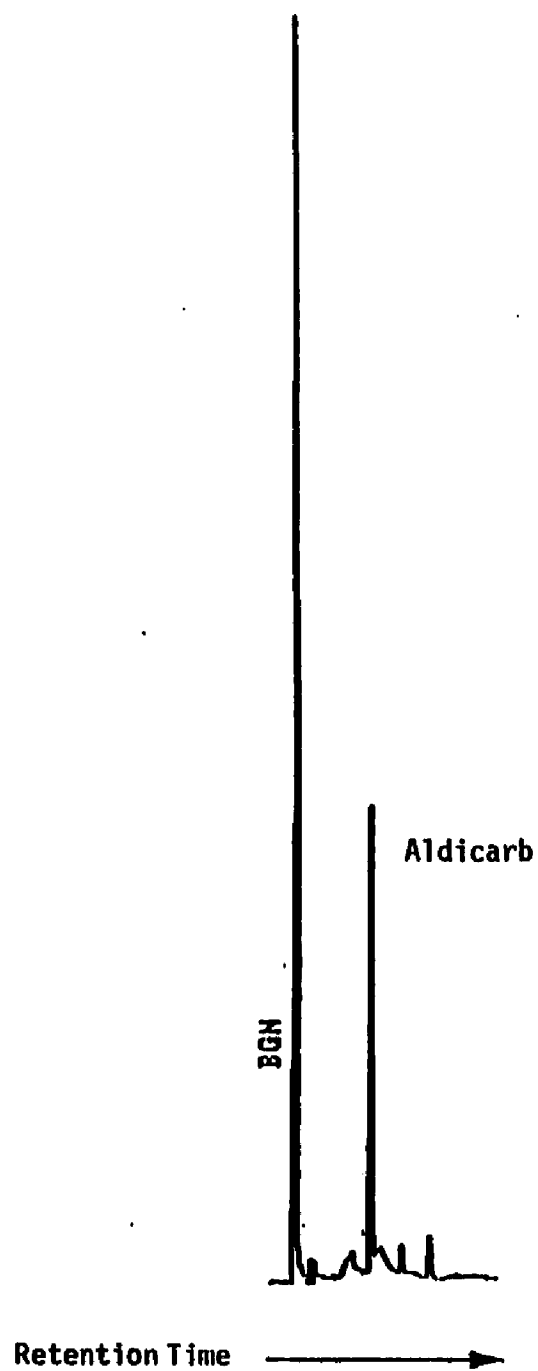


Figure 4.2.6a HPLC of the extracts obtained from SFE of amitrol and aldicarb with SC-CO₂ as solvent in the absence of modifier.

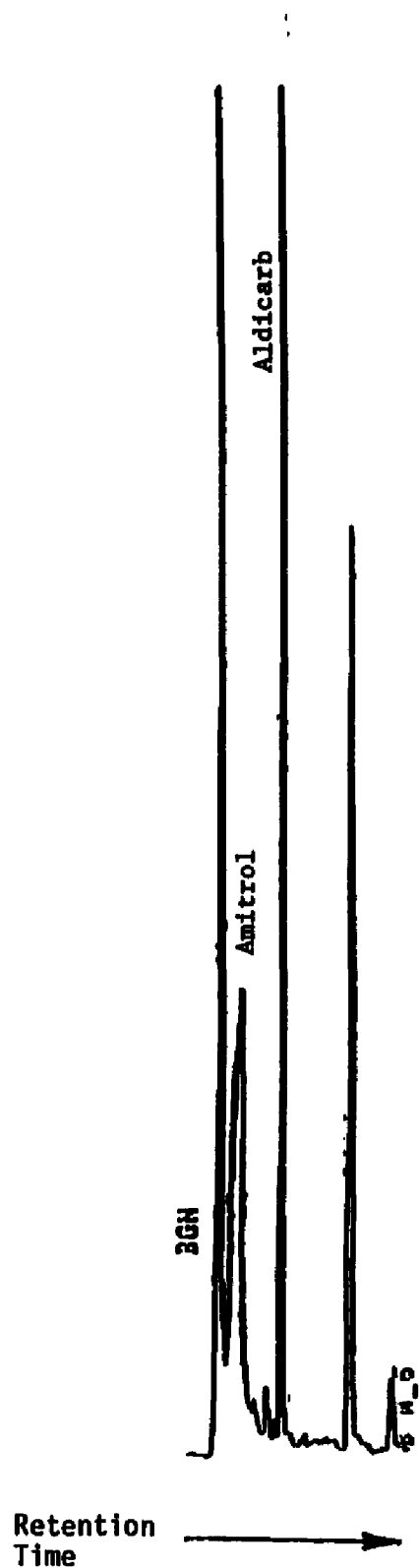


Figure 4.2.6b. HPLC of the extracts obtained from SFE of amitrol and aldicarb with SC-CO₂ as solvent in the presence of 10%(v/v) H₂O.

of both aldicarb and amitrol. In comparison with methanol, the water modified solvent system gave even higher recoveries, which can be attributed to the greater solute-solvent interactions. However, it should be noted that even when the water-modified SC-CO₂ was employed in the extraction, the recovery of Amitrol was still only 10% (Table 4.2.6).

The specific interactions of polar modifiers with certain polar solutes have been observed by several researchers. For example, Dobbs et al. [1987] demonstrated that the addition of 3.5 mole-percent methanol to carbon dioxide increased the solubility of 2-aminobenzoic acid by a factor of 6. This enhancement was explained by specific acid-base interactions between methanol and 2-aminobenzoic acid. In the case of Amitrol and Aldicarb, both compounds are polar and Amitrol is in fact a base. Carbon dioxide becomes a weak acid in the presence of water because CO₂ is an acid anhydride, which affects its solvent properties and hence extraction selectivity. Thus, it should be expected (and as has been demonstrated in the results presented in Table 4.2.6) that the addition of water can both favor the solubility and assist the desorption of these pesticides from soil sites. The adsorption of Aldicarb onto soil is a function of soil pH, and increases as soil pH increases [Zhong, 1986; Lemley, 1988; and Richey, 1977].

To summarize modifier effects briefly, the extraction efficiency is a strong function of the chemical nature of the modifier. A modifier can not only change the fluid properties, such as polarity, density and therefore

solvent power, it can also displace adsorbed solute. The modifiers may also change the pore diffusional situation because modifiers are capable of capillary condensation or adsorption into the soil or a colloidal matrix, thereby changing the pore environment [Dooley, 1987]. Further research efforts are required to develop a full understanding of the nature of the modifier effects.

4.2.3. Efficiency of Adsorbents and Solvent Traps

Two solid adsorbent materials, silica gel and Tenax TA, were used to collect the extracts after depressurization of the SFE. The trap tube was connected to a solvent backup trap containing an organic solvent such as acetone or hexane. The extractions for aldrin and PCB 1254 were carried out respectively under the same conditions: 8000 psi pressure, 45°C and 20 min equilibration. After each supercritical carbon dioxide extraction was finished, the soil sample was re-extracted in a Soxhlet apparatus to determine the percentage of analytes remaining in the samples. The amount of aldrin and Aroclor 1254 found on the solid adsorbent, in the trapping solvents, and the residual in the original soil samples are listed in **Table 4.2.8**. The extraction results showed that Tenax TA exhibited better adsorbing ability for aldrin than silica gel did, and that no aldrin was detected in the acetone backup trap when Tenax TA was used; whereas 25 percent of aldrin was collected by the solvent trap when silica gel was employed as the solid adsorbent.

In the case of Aroclor 1254 (**Table 4.2.9**), no significant difference between silica gel and Tenax TA was observed. Both trapping materials gave nearly 100 percent efficiency; i.e., whatever was extracted was adsorbed. Silica gel is preferred because it has good adsorbent properties, it does not need lengthy cleaning process, and it is more economical than Tenax TA.

Moreover, the experimental results showed that most of extract was trapped within the first two inches of the collecting tube, implying that a longer tube was not necessary. However, in order to prevent any possibility of losing the extracts and for safety consideration, a 6-inch long stainless steel tube was generally used, which could be packed with up to 1 g silica gel or 0.8 g Tenax TA. It was also found that a small amount of extract was precipitated in the joint section between the exit of the extraction chamber and the inlet of the collecting tube during the depressurization. This required a careful rinsing of this section including the exit valve and the union elbow after each operation. Another suggestion which should be made based on our results is that the distance between the outlet of the chamber and the trapping tube should be minimized to ensure the maximum adsorption of the extracts in the trapping tube.

Table 4.2.8. Distribution of Aldrin and Aroclor 1254 following SFE.^a

<u>Distribution of Extracts in</u> (%)	<u>Aldrin</u> % Recovery	<u>Aroclor 1254</u> % Recovery
Silica Gel (adsorbent)	51	80
Acetone (solvent trap)	25	-
Hexane (solvent trap)	-	0
Unextracted Residual ^b :	17	18
Total recovered analyte:	93	98
Tenax TA (adsorbent)	85	80
Acetone (solvent trap)	0	-
Hexane (solvent trap)	-	0
Unextracted Residual ^b :	10	20
Total recovered analyte:	95	100

a. Supercritical fluid extraction conditions: SC-CO₂ at 8000 psi and 45°C for 20 min.

b. Determined by Soxhlet extraction of the SFE'd sandy soil samples. 4 Hour with 100 mL of acetone (for aldrin) and hexane (for PCB). Original soil samples spiked with: 4.1µg Aldrin/g and the 0.27 ng Aroclor 1254 /g.

Table 4.2.9 Evaluation of Silica Adsorbent for Trapping Aroclor 1254.

Adsorbent in	% Recovery of Aroclor at the Pressure (psi)^b				
	9000	8000	7000	6000	5000
Silica gel adsorbent	90	80	74	56	52
Solvent trap (hexane)	0	0	0	0	0
Remained in the soil: ^a	5	18	18	33	34
Total Recovered:	95	98	92	89	86

- a. Obtained after the SFE by the Soxhlet extraction of the soil samples for 4 Hour with 100 mL of hexane.
- b. SC-CO₂ extraction the sandy soils (60/80 mesh) spiked with 0.272 µg Aroclor 1254/g at 45°C for 20 min.

4.3. Effect of Temperature

The selection of a proper pressure and temperature for a supercritical fluid extraction is of the highest importance since both can affect significantly the efficiency of extraction. As described earlier, a solute typically exhibits a threshold pressure above which solubility increases significantly; in general, the region of maximum increase in solubility is near the critical pressure of the extraction fluid, where the change in density is greatest [Gitterman, 1983].

In contrast, the effect of temperature on the fluid phase solubility is often more complicated than that of pressure, and is much more difficult to predict [Smith, 1983; Yonker, 1985]. The complications arise from the fact that not only the density of a supercritical fluid, but also the vapor pressure of solute are related to the temperature. Nash [1983] has given an excellent literature review on the volatilization of pesticides from soils. The potential for pesticide volatilization is related to pesticide vapor pressure. One of the primary environmental factors affecting the vapor pressure of a compound on the surface of its matrix is temperature. It is quite clear that increasing the temperature will lead to an increase in vapor pressure of a solute. In the case of supercritical fluid extraction, the solubility of a solute increases significantly at sufficiently high temperatures where the solute vapor pressure may also become large. For example, Figure 4.3.1 shows that the solubility of solid naphthalene in supercritical ethylene ($P_C = 49.7$ atm, $T_C = 9.3$ °C) increases approximately

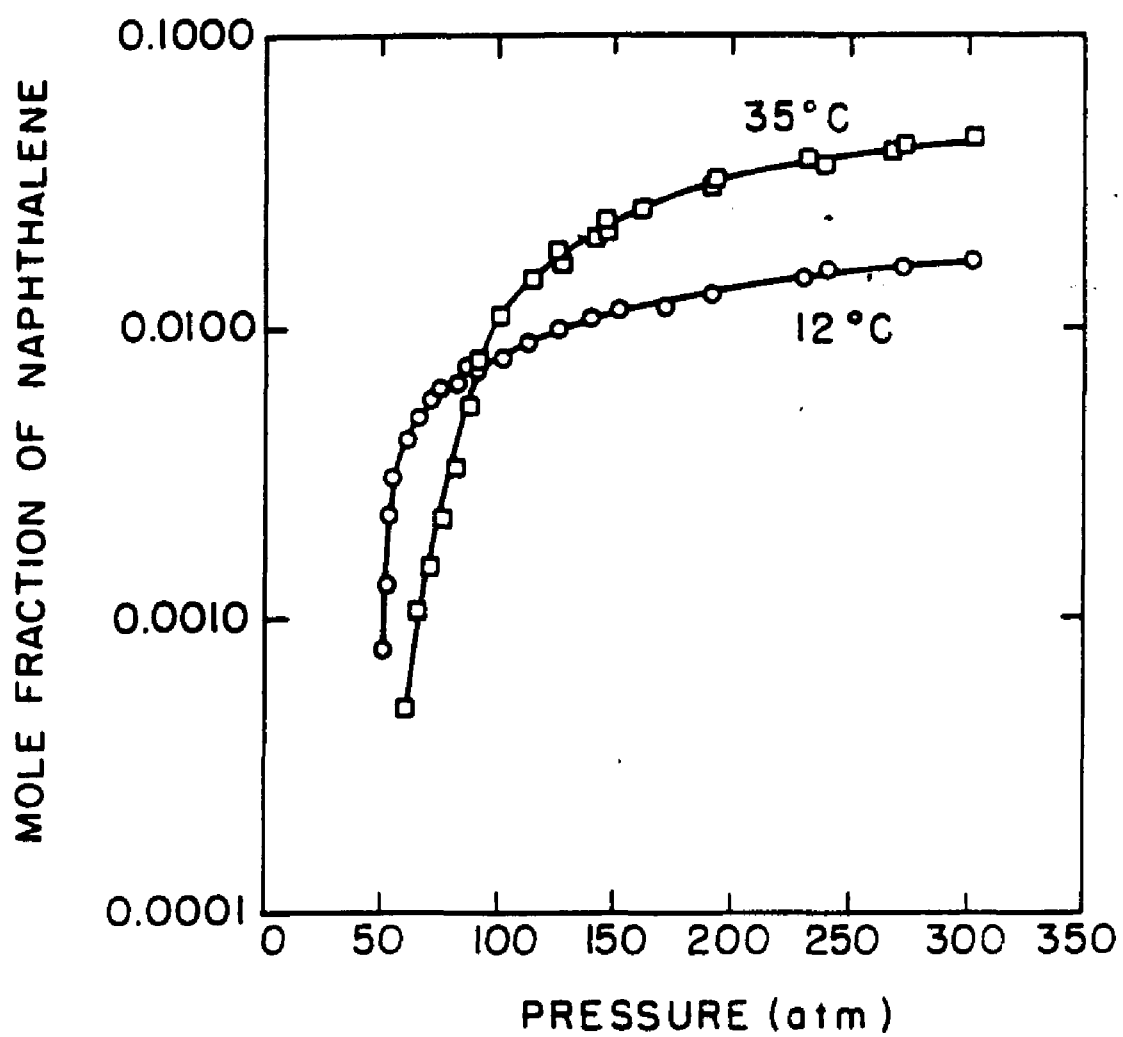


Figure 4.3.1. Solubility behavior of solid naphthalene in supercritical ethylene. [Tsekhanskaya, 1962; Diepen, 1948]

5 times when the temperature is raised from 12°C to 35°C at pressures greater than about 150 atm. This higher naphthalene solubility at 35°C is attributed to the increase in the sublimation pressure of naphthalene upon heating [Tsëkhanskaya, 1962; Diepen, 1948].

The efficiency of supercritical fluid extraction of the pesticides and PCBs under investigation from soil was studied as a function of temperature. The behavior of the systems at different temperatures are quite different and are of great interest. Figure 4.3.2 presents the temperature dependence of the percent recoveries of methylene chloride-modified SC-CO₂ extraction of the PCBs at 9000 and 8000 psi. The recoveries of the PCBs increased and reached almost quantitative recovery as the temperature was increased from 35°C to 45°C. Apparently such increases in the recoveries in the vicinity of the critical temperature of carbon dioxide ($T_c = 31^\circ\text{C}$) are consistent with the general property of a supercritical fluid that the solubility of a solute increases greatly when the system temperature just exceeds the critical temperature. The increase in the recoveries of the PCBs in this temperature range could also be interpreted in terms of the effect of temperature on the vapor pressure of the PCBs. Hutzinger et al. [1974] and Brady et al. [1987] reported that the vapor pressure of liquid Aroclor 1254 was more than one order of magnitude greater at the temperature of 56°C than that at 25°C (Table 4.3.1). However, a significant decrease in the recoveries of both PCBs Aroclor 1254 and Aroclor 1260 was observed at temperatures higher than 45°C in our studies (Figure 4.3.2). As the

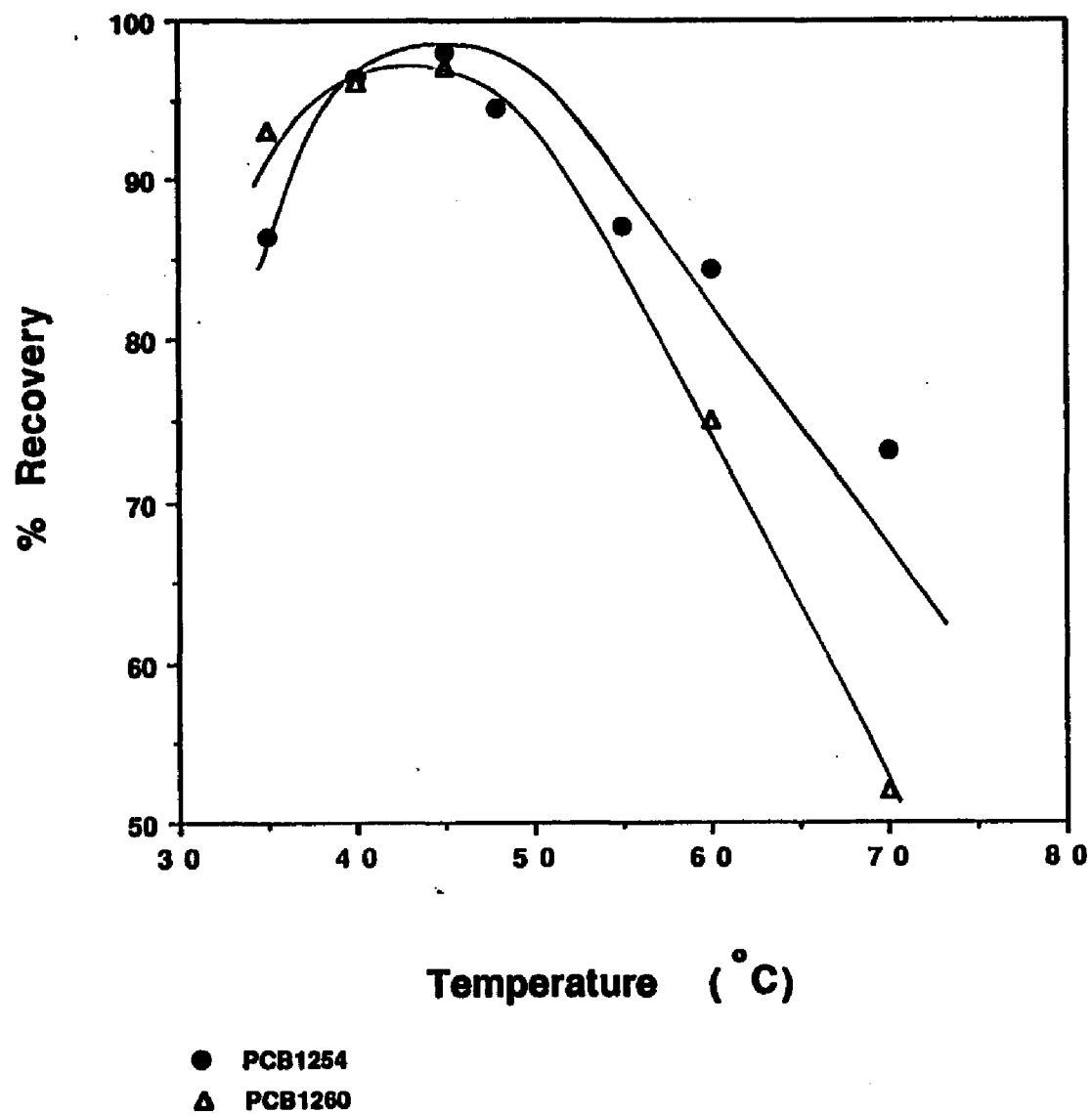


Figure 4.3.2. Temperature effect on the recovery of Aroclor 1254 and Aroclor 1260.

Table 4.3.1. Vapor Pressures (*P*) of Aroclor 1254.^a

<i>T</i> , (°C)	Calculated <i>P</i> , (mmHg)	Experimental <i>P</i> , (mmHg)
25	8.25 x10 ⁻⁵	7.71x10 ⁻⁵
56	1.74x10 ⁻³	1.0x10 ⁻³
75	0.832x10 ⁻²	1.0x10 ⁻²
112	1.07x10 ⁻¹	1.0x10 ⁻¹
144	0.639	1
200	7.49	10
270	70.8	100

a. Data from Hutzinger, et al., 1974 and Brady et al., 1987.

temperature increased from ca. 45°C to ca. 70°C, the recoveries decreased from more than 95% to ca. 70% and ca. 50% for Aroclor 1254 and Aroclor 1260, respectively. These results indicate that the increase in the vapor pressure of the PCBs with temperature cannot be the sole factor responsible for the increase in the recoveries. Another, and probably more important factor is the density of the supercritical fluid. Our results (Figure 4.3.2) are in good agreement with the estimated solubility in carbon dioxide as a function of temperature (Figure 4.3.3) [Brady, 1987]. At constant pressure, the lower densities of the SCF at higher temperatures decrease the solvation of the solute in the supercritical phase and consequently lead to a lower solubility and a poorer recovery of the solute.

In addition to the above two competing factors, i.e. the volatility of solute which always increases with temperature, and the density of the supercritical fluid which decreases with temperature at temperatures above the T_C , the following should also be considered to enable one make an intelligent guess of the temperature effect on extraction efficiency.

First, the structure and properties of the analytes such as molecular weight and vapor or sublimation pressure should be considered to predict and select the proper extraction conditions. For volatile compounds, it can be expected that a greater increase in solubility can be achieved when the temperature is increased slightly. However, when the volatility of a compound is small, an increase in extraction temperature may not favor

good recovery. Second, the nature of sample matrices, for example the chemical and physical properties of soils, (e.g. total organic matter content, water content, soil humidity, etc.) could also be important in the selection of the extraction temperature. The vapor pressure of an organic compound in a soil environment may differ from the vapor pressure of the pure compound. This difference can be attributed to adsorption of the compound on the soil, which reduces the activity relative to that of the pure compound. Thus, a change in temperature might affect the interaction between an analyte and its matrix, and so affect the rate of adsorption and desorption of the species. Third, the properties of a pure supercritical fluid, a supercritical fluid mixture, or organic-modified supercritical fluid, will be different from each other in response to temperature changes, since the solvent strength and the strength of interactions between the analyte and fluid depend on the properties of the fluid.

The dependence of recovery of aldrin on the temperature in a SC-CO₂ extraction of aldrin is demonstrated in **Table 4.3.2**. More than a 20% decrease in the recovery of aldrin is observed as the temperature is increased by only 15°C. These results suggest that the effect of temperature on density (therefore the solvating power) of supercritical carbon dioxide is more important than the effect on the vapor pressure of aldrin.

The temperature effects observed for the extraction of 2,4-D methyl ester, parathion and malathion (**Figure 4.3.4**) are quite similar to those

of the PCBs. Sandy soil spiked with 0.14 mg/g of 2,4-D methyl ester, 0.14 mg/g of malathion and 2.78×10^{-2} mg/g of parathion were extracted with methanol-modified SC-CO₂ at 9000 psi, and almost quantitative recoveries were achieved for all of these pesticides at 45°C in 20 min. However, the recoveries dropped at temperatures higher than 50°C. At 70°C, only about 50 percent recoveries were obtained for these three compounds. The results are also summarized in Table 4.3.3.

Amitrol and aldicarb gave quite different temperature dependencies as compared to the other pesticides and PCBs. As shown in Table 4.3.4, the recoveries of the water-modified SC-CO₂ extractions increased continuously from approximately 5% and 80% to about 11% and 93% for amitrol and aldicarb, respectively, as the temperature increased from 45°C to 99°C. The dependence of the recoveries on temperature for these two systems seems to be weaker than those for the PCBs, aldrin, 2,4-D methyl ester, parathion and malathion. However, it should be noted that no decrease in the recoveries was observed when temperature was increased up to 99°C. As discussed earlier, the effects of the extraction temperature are also related to the properties of supercritical fluid employed. When the fluid is modified, the properties of the mixture have to be considered. Water was selected as the modifier for amitrol and aldicarb because it is highly polar and effectively dissolves these two pesticides. Thus, there will be a stronger interaction between the polar solutes such as amitrol and aldicarb and the supercritical carbon dioxide-water solvent mixture, which in turn leads to the higher

recoveries observed. In this case, the effect of the increase in vapor pressure dominates over that of the decrease in the density of the fluid, which probably did not change much since the extractions were all undertaken at pressure up to 9500 psi.

Table 4.3.2. Effect of the Extraction Temperature on the Recovery of Aldrin, Aroclor 1254 and Aroclor 1260.

Temperature (°C)	<u>Aldrin</u>		<u>Aroclor 1254</u>		<u>Aroclor 1260</u>	
	%Recovery	%RSD	%Recovery	%RSD	%Recovery	%RSD
35	-	-	86	2.4	93	2.0
40	-	-	96	2.1	96	1.2
45	86	2.9	98	1.8	97	3.2
48	-	-	94	2.0	-	-
55	-	-	87	2.3	-	-
60	6.0	4.1	84	1.9	75	2.2
70	-	-	73	3.6	52	1.6

SFE conditions: SC-CO₂ + Methylene Chloride (10% v/v) for PCBs and SC-CO₂ for aldrin. Pressure: 8000 psi for aldrin and Aroclor 1260, and 9000 psi for Aroclor 1254. Equilibration time: 20 min for aldrin and Aroclor 1254 and 10 min for Aroclor 1260. All the extractions were performed in triplicate . Adsorbent: Tenax TA.

Samples: Sandy soil (60/80 mesh) spiked with aldrin (4.1 µg/g), Aroclor 1254 (0.272 µg/g) or Aroclor 1260 (0.091 µg/g).

Table 4.3.3. Effect of Extraction Temperature on the Recoveries of 2,4-D Methyl Ester, Malathion and Parathion.

Temperature(°C)	2,4-D methyl ester	Malathion	Parathion
40	90±4.1	85±5.1	92±3.4
45	93±3.5	98±3.3	100±3.1
50	78±3.7	89±3.0	82±4.3
60	57±4.6	63±3.2	67±3.8
70	45±3.3	42±4.1	52±2.9

SFE conditions: SC-CO₂+ Methanol (5% v/v), Pressure = 9000 psi,

Equilibration Time=30 min. Triplicate extractions (n = 3).

Samples: Sandy soil (60/80 mesh) spiked with 2,4-D methyl ester (0.14 mg/g), malathion (0.14 mg/g), and parathion (2.8x10⁻² mg/g).

Table 4.3.4. Temperature Effect on Extraction of Amitrol and Aldicarb

Temperature(°C)	% Recovery Amitrol	% Recovery Aldicarb
45	5±3.8	79±4.0
80	6±4.2	86±2.9
85	7±4.7	86±4.8
90	10±4.0	92±3.3
95	10±3.6	93±3.1
99	11±2.7	94±3.0

SFE conditions: SC-CO₂ + H₂O (10%v/v); Pressure = 9500 psi;

Equilibration Time = 30 min. Triplicate extractions (n=3).

Sample: Sandy soil (60/80 mesh) spiked with Amitrol = 20.5 µg/g ;

Aldicarb = 24.0 µg/g.

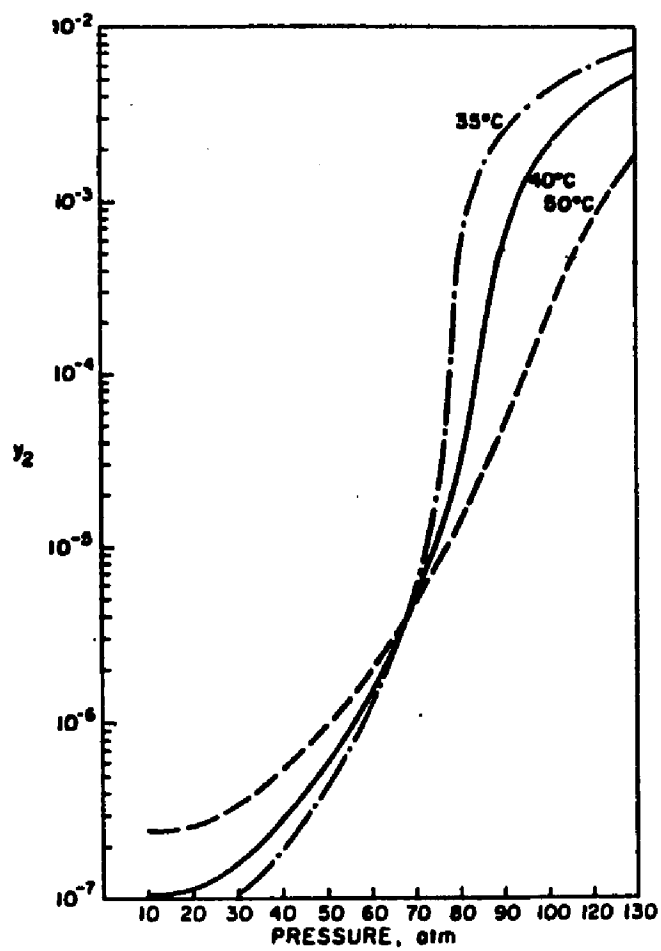


Figure 4.3.3. Estimated PCB ($C_{12}H_5Cl_5$) solubility in CO_2 [Hutzinger, 1974; Brady, 1987].

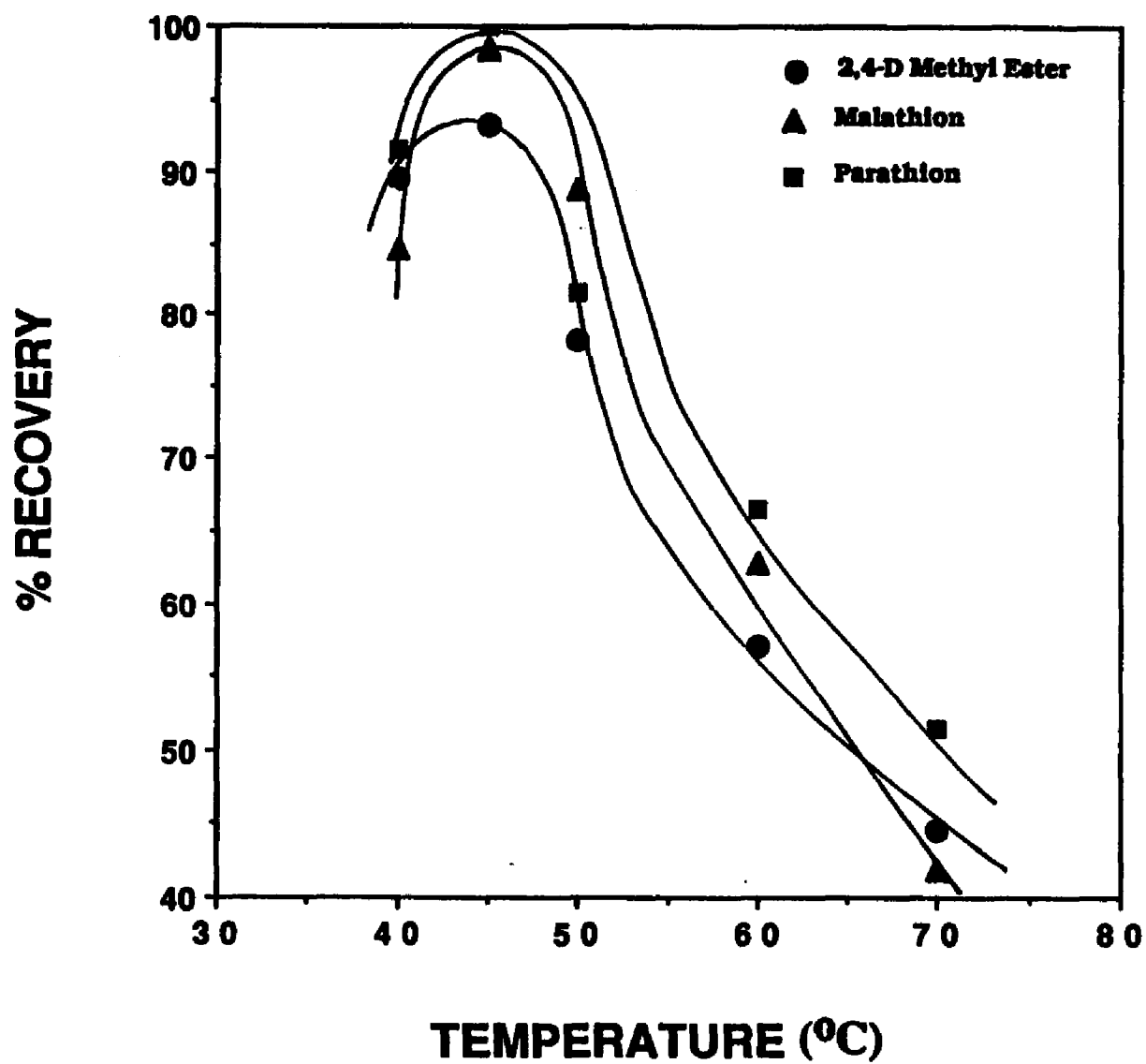


Figure 4.3.4. Effect of temperature on the recovery of 2,4-D methyl ester, malathion and parathion.

4.4. Optimization of the Extraction Time

The extraction time is another important analytical variable in supercritical fluid extraction. In general, it is preferred that in any analytical method, the time required for completion of an analysis should be as short as possible without sacrificing the accuracy of the analysis for several obvious reasons, such as higher work efficiency and fast analytical results. This is particularly important for extraction techniques. Thus, different extraction equilibration times were investigated for the supercritical fluid extraction of the pesticides and PCBs in order to determine how to obtain the best extraction recovery of a given sample within a minimum period of time.

Table 4.4.1 lists the recovery in the SC-CO₂ extraction of aldrin from the spiked sandy soil at three different equilibration times, 10, 20 and 30 minutes, with other extraction parameters being constants. Thus, at 8000 psi and 45°C, aldrin was almost quantitatively extracted by SC-CO₂ in only 20 min. When the extraction time was decreased from 20 to 10 min, the recovery of aldrin decreased drastically from ca. 90% to ca. 46%. However, when the extraction time was increased to 30 min, triplicate extraction results showed no significant improvement in the recovery. These observations suggest that under the experimental conditions, all of the extractable aldrin had been separated from the soil sample by the supercritical carbon dioxide within 20 minutes. The extraction of aldrin from the sandy soil samples, therefore, requires 20

min as the optimum equilibration time.

The effects of extraction time on the recoveries of PCBs were studied using supercritical carbon dioxide modified with methylene chloride (10% v/v) and the results are also summarized in **Table 4.4.1**. Both Aroclor 1254 and Aroclor 1260 could be readily extracted from spiked sandy soils with more than 90 percent recoveries after only about 10 min.

The SC-CO₂+ 5% methanol (v/v) extractions of 2,4-D methyl ester, malathion and parathion from spiked sandy soil samples were performed at different lengths of time at 9000 psi and 45°C, and the results are shown in **Figure 4.4.1**. It is apparent that from 5 min to 20 min, the recoveries of these pesticides increases greatly and that more than 96 percent recoveries are achieved in the time length as short as 20 min. At equilibration times longer than 20 min, the recoveries do not increase significantly. Therefore, similar to the results obtained for aldrin, an extraction time of more than 20 min does not seem to be necessary for these pesticides.

In order to reach higher recoveries, both amitrol and aldicarb seem to need a longer equilibration time (**Table 4.4.2**) than the other compounds discussed above. By extraction with water-modified SC-CO₂, even at a pressure of 9500 psi and a temperature of 90°C, it required at least 30 min to obtain a 92 percent recovery of aldicarb from sandy soils. The effect of extraction time on the recovery of amitrol was not as significant

as those of aldicarb. When the extraction time changed from 5 to 30 min, only about a 6-percent increase in recovery of amitrol was observed. The difficult in extraction of amitrol might be due to the tight binding between amitrol and certain components of the soil.

The extraction equilibration time effect on the recovery also depends on the nature of sample matrix, i.e. on the type of soil samples. A comparison of the extraction recoveries in three types of soils is given in Table 4.4.2. At 9500 psi and 90°C, 30 min was required for SC-CO₂+H₂O extraction of 10% amitrol and 92% aldicarb from sandy soils. However, under the same operation conditions, at least 40 min was needed to recover 8% amitrol and 74% aldicarb from loamy soil samples, and to recover only 4.5% amitrol and 65% aldicarb from top soil rich in organic content. These results further suggest that there are strong adsorptive interaction forces or so called tight bonding between the analytes and soils having higher organic content, which means, a longer time is needed to extract them. In fact, amitrol as a herbicide has been known to be absorbed rapidly and efficiently by the roots of plants [White, 1971].

Table 4.4.1. Effect of Extraction Equilibration Time on the Recoveries of Aldrin, Aroclor 1254 and Aroclor 1260.

Extraction Time* (min)	<u>Aldrin</u>		<u>Aroclor 1254</u>		<u>Aroclor 1260</u>	
	% Recovery	RSD	% Recovery	RSD	% Recovery	RSD
2.5	-	-	-	-	49	2.7
5	-	-	63	4.0	54	5.5
10	46	7.4	90	3.6	97	3.2
15	-	-	98	1.4	-	-
20	90	3.4	98	1.8	100	2.5
30	90	4.5	-	-	-	-

SFE conditions: SC-CO₂ + methylene chloride (10% v/v) for PCBs and SC-CO₂ for aldrin. Extraction Temperature : 45°C. Pressure: 8000 psi for aldrin and Aroclor 1260; 9000 psi for Aroclor 1254. %RSD values were obtained from triplicate extractions.

Sample: Sandy soil (60/80 mesh) spiked with aldrin (0.14 µg/g), Aroclor 1254 (0.272 µg/g) or Aroclor 1260 (0.0913 µg/g).

* The extraction equilibration time does not include the time required for pressurization (ca. 5 min) and depressurization (ca. 10 min).

Table 4.4.2. Effect of the Extraction Equilibration Time and Type of Soil Matrices on the Recoveries of Amitrol and Aldicarb.

Type of Soil	Extraction Time* (min.)	<u>Amitrol</u>		<u>Aldicarb</u>	
		% Recovery	% RSD	% Recovery	% RSD
Sandy soil	5	4	3.2	32	2.9
	10	6	4.4	41	3.5
	15	7	4.0	57	4.1
	20	8	4.6	72	3.8
	25	9	3.9	85	4.2
	30	10	4.6	92	5.0
Loamy soil	40	8	5.4	74	4.3
Top soil	40	4.5	4.8	65	5.6

SFE conditions: SC-CO₂+H₂O (10% v/v); Pressure = 9500 psi; Temperature = 90°C.

Samples: Sandy soil (60/80 mesh) spiked with Amitrol (20.5 µg/g soil), Aldicarb (24.0µg/g soil). The RSD values were obtained from triplicate extractions.

* The extraction equilibration time does not include the time required for pressurization (ca. 5 min) and depressurization (ca. 10 min).

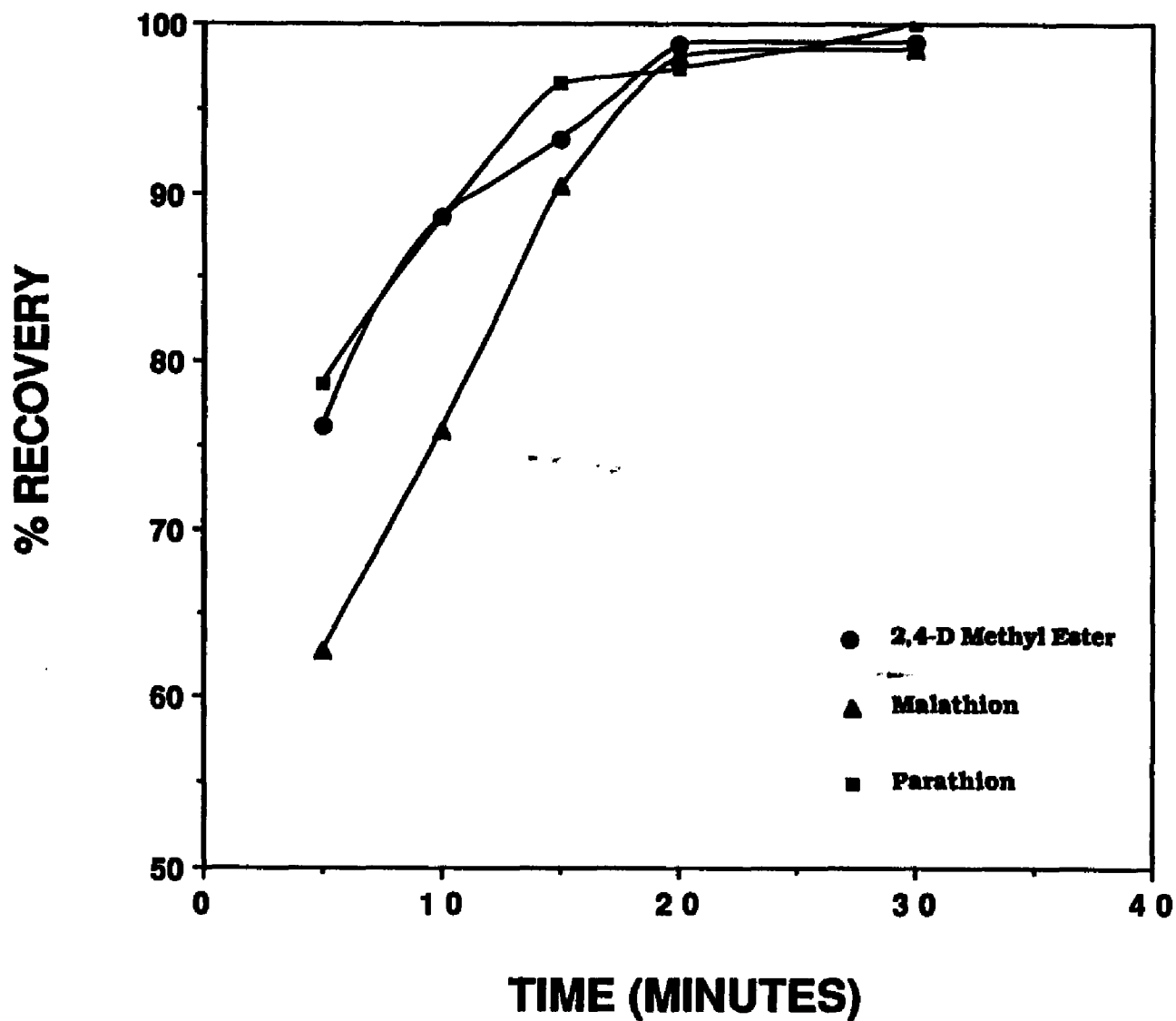


Figure 4.4.1. Effect of extraction time on the recovery of 2,4-D methyl ester, malathion and parathion from a spiked sandy soil sample at the SC-CO₂ pressure of 9000 psi and 45°C.

4.5 Effect of Type and Particle Size of Soil

In this section, extraction by both conventional Soxhlet and supercritical fluid methods of the toxic organics from different types of matrices was studied. Factors influencing the supercritical fluid extraction processes include soil type, the content of organic matter in the soil, and the size of the soil particles.

4.5.1 Effect of Soil Type and Organic Content

The efficiency of supercritical fluid extraction of the pesticides and PCBs was studied with variations of the types of soils and the organic matter content of the soil samples. The results are summarized in **Tables 4.5.1 through 4.5.5**. The general trend of these studies shows that the recoveries decrease as the organic matter content of the soil samples increases.

The total organic content of the soil samples was determined by the Walkley-Black method via the oxidation of the organic compounds in the soils with potassium dichromate [Walkley, 1947]. The values obtained for the total organic content represents the total oxidizable organic materials present in the samples. The total organic content in the soil samples was found to have the following general order:

top soil > loamy soil > clay > sandy soil.

Table 4.5.1 Effect of Soil Type on Extraction Recovery of Aldrin

Soil Type	% Organic Matter	Soil Particle Size (mesh)	%SFE Recovery	% Soxhlet Extraction Recovery
Sandy	0.066	60/80	90±3.4	87±3.4
Loamy	5.54	60/80	75±3.5	-
Clay	1.16	-	57±4.1	69±3.1

SFE Conditions: SC-CO₂ at Pressure = 8000 psi, Temperature = 45°C, and

Extraction equilibration time = 20 min. Additional 15 min are needed for pressurization and depressurization. Adsorbent: Tenax TA.

Sample: 1.4 µg/g aldrin spiked soils (60/80 mesh).

Soxhlet Extraction Conditions: 24 hrs with 100 mL of acetone.

All the extractions were performed in triplicate (i.e. n = 3).

Table 4.5.2 Effect of Soil Type and Particle Size on Extraction Recovery of Aroclor 1254.

Soil Type	% Organic Matter	Soil Particle Size (mesh)	% SFE recovery ^a	% Soxhlet Extraction Recovery
Sandy	0.066	<45	98±2.8	-
Sandy	0.066	60/80	98±2.4	86±2.8 ^b
Sandy	0.066	80/100	85±2.0	-
Loamy	5.54	60/80	89±3.8	87±4.0 ^c
Topsoil	11.7	60/80	79±3.4	85±2.7 ^c
Clay	1.16	-	69±3.1	89±2.7 ^c

- a. SFE conditions: SC-CO₂ + methylene chloride (10% v/v) at Pressure = 9000 psi, Temperature = 45°C, and Extraction equilibration time = 20 min. Additional 15 min are needed for pressurization and depressurization.

Sample: 0.27 µg/g PCB (Aroclor 1254) spiked soils (60/80 mesh).

Triplicate extractions were performed (n=3).

- b. Soxhlet extraction for 4 h with 100 mL of hexane; n = 3.
- c. Soxhlet extraction for 15 h with 100 mL of hexane; n = 3.

Table 4.5.3. Effect of Soil Type and Particle Size on Extraction Recovery of Aroclor 1260.

Soil Type	% Organic Matter	Soil Particle Size (mesh)	% SFE Recovery^a	% Soxhlet Extraction Recovery
Sandy	0.066	60/80	98±2.1	99±2.2 ^b
Sandy	0.066	80/100	91±3.2	-
Loamy	5.54	60/80	85±2.6	90±3.0 ^c
Topsoil	11.7	60/80	72±2.9	81±2.9 ^c
Clay	1.16	-	65±2.4	82±3.3 ^c

- a. SFE conditions: SC-CO₂ + methylene chloride (10 % v/v) at Pressure = 8000 psi, Temperature = 45°C, and Equilibration time = 10 min.
Sample: 45.6 ng/g spiked soils (60/80 mesh). n = 3.
- b. Soxhlet extraction for 5 hrs. with 100 mL of hexane; n = 3.
- c. Soxhlet extraction for 15 hrs. with 100 mL of hexane; n = 3.

Table 4.5.4. Effects of Soil Type and Particle Size on Extraction Recovery of 2,4-D Methyl Ester (0.14 mg/g Soil), Malathion (0.14 mg/g) and Parathion (0.028 mg/g).

Soil Type	Organic Matter,%	Soil Particle Size(mesh)	<u>% SFE Recovery^a</u>			<u>% Soxhlet Recovery^b</u>		
			2,4-D	Mala.	Para.	2,4-D	Mala.	Para.
Sandy	0.066	60/80	93	98	100	95	96	96
Sandy	0.066	80/100	90	93	95	-	-	-
Loamy	5.54	80/100	86	89	92	90	95	97
Top soil	11.1	80/100	82	84	90	91	93	94
Clay	1.37	-	78	74	81	86	82	90

a. SFE conditions: SC-CO₂ + methanol (5% v/v) at Pressure = 9000 psi,

Temperature = 45°C, and Equilibration time = 30 min.

b. Soxhlet extraction for 5 hrs. with 100 mL of methylene chloride.

Table 4.5.5. Effect of Soil Type and Particle Size on Extraction Recovery of Amitrol and Aldicarb.

Soil Type	Organic Matter(%)	Soil Particle Size (mesh)	<u>% SFE Recovery^a</u>		<u>% Soxhlet Recovery^b</u>	
			Amitrol	Aldicarb	Amitrol	Aldicarb
Sandy	0.066	60/80	10	92	94	96
Loamy	5.54	60/80	6	68	66	90
Top soil	11.1	60/80	4	63	-	-
Clay	1.37	-	4	40	63	82

a. SFE conditions: SC-CO₂ + water (10% v/v) at Pressure = 9500 psi,

Temperature = 90°C, and Equilibration time = 30 min.

b. Soxhlet extraction for 20 hrs with 100 mL of methanol.

A direct comparison of the influence of the soil type on recovery of pesticides and PCBs can be made from the data in **Tables 5.5.1 - 5.5.5** based on the same amount of soil (e.g. 10 g) and the same soil particle size (e.g. 60 mesh, except the clay soils because the particle size of the clay samples are too small to be measured accurately). As the data demonstrate, all the tested pesticides and PCBs (with the exception of amitrol) appear to be well released from sandy soil; the recoveries of the supercritical fluid extractions are high, ranging from at least 85% to almost quantitative recovery. The recovery from the loamy soil ranks second and that from the top soil and clay the least. This general tendency indicates that the liberation of the organic compounds from the soil matrices depends on the extent of adsorption, owing to the possible strong binding of these pesticides to the soils, which is strongly related to the total content of soil organic matter. Soils with a low content of organic matter have been normally considered to contain sites that preferentially adsorb polar and ionic species. This explains in part why more than 90 percent of the weakly polar compounds such as PCBs (**Tables 4.5.2 and 4.5.3**) could be recovered in 10 min, while the more polar amitrol needed ca. 30 min to reach only about 10 percent recovery (**Table 4.5.5**). The low recovery for the extraction of amitrol from all the soil matrices can be attributed to its strong adsorption on or even bonding to the soils. A soil with a higher organic matter content is more complex and interactions might take place between the components of the soils and the analytes which might not be limited with the strongly polar compounds but may include weakly polar compounds as well [Brady,1987]. Moreover, the soils

with higher organic matter contents have more possible reactive sites and relatively higher surface areas [Brady 1987]; therefore, they appear to have a higher potential adsorption capacity and to behave as chemically active adsorbents for the pesticides studied. The significantly lower recoveries obtained from loamy and top soil samples are consistent with these expectations that there is a certain portion of the pesticides which is bonded onto the chemically active sites of the soils and is more difficult to liberate from their host matrix under the supercritical fluid extraction conditions used.

Clay usually gives the lowest supercritical extraction efficiencies as indicated by the results in Tables 4.5.1-4.5.5. Although the total content of organic matter in clay is significantly lower than the top soil, its relatively high surface area resulting from the smaller soil particles, leads to a greater adsorption capacity, which should be expected to give lower recoveries. Furthermore, it was found that after each extraction performed on a clay sample it was more difficult to transfer the clay out of the extraction chamber and to clean the chamber, because the clay particles seem to adhere together and stick to the wall of the extraction chamber. Thus, penetration and diffusion of the fluid in the clay matrix are more difficult because of this tight packing of clay particles, resulting in reduced interactions between solutes and supercritical fluid. This should be responsible for the low recoveries observed in the extraction of clay.

4.5.2 Effect of Particle Size of Soil

Supercritical extraction is also affected by particle size of soil. In the above sections, the particle size of soil was always 60/80 mesh. Extraction of the organic compounds from the sandy soil with particle sizes of > 45 mesh, 60/80 mesh, and 80/100 mesh were selected as typical examples to demonstrate the effect of the particle size on the efficiency of the extractions. Thus, Aroclor 1260-spiked (91.3 ng/g) sandy soil of 60/80 and 80/100-mesh particle sizes were extracted with supercritical CO₂ modified by 10 % methylene chloride (v/v) at 45°C for 10 min at various pressures. As shown in **Figure 4.5.1**, there is a small increase in the recovery as the particle size is increased from 80/100 to 60/80 mesh although the increase is not as large as resulted from an increase in the system pressure. Little is reported in the literature about the effects of the soil particle size on the efficiency of a supercritical fluid extraction. The factors responsible for this increase are still to be investigated. In accord with the observation made in the cases of extraction of the clay matrices (Section 4.5.1), a possible explanation could be offered based on the surface area of the samples. Thus, both the particle size and the empty space between the particles are smaller in the 80/100 -mesh soil samples than those in the 60/80-mesh samples, which might lead to a greater surface area and possibly greater adsorption capacity for the organic compounds, and therefore to the relatively lower extraction recoveries.

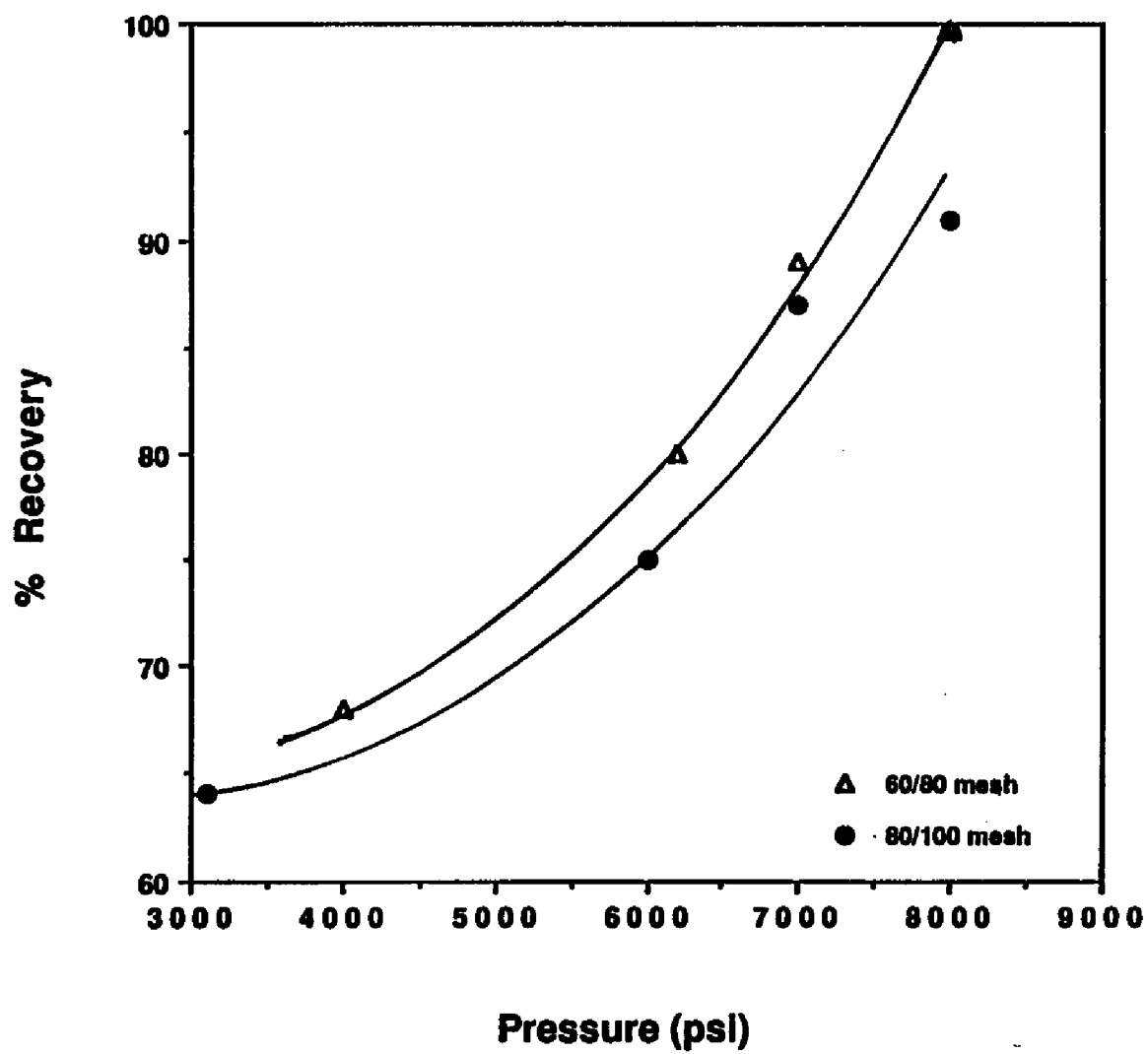


Figure 4.5.1. Effect of particle size of sandy soil on the recovery of Aroclor 1260.

4.5.3 Effect of Spiking Levels on the SFE Recovery

The efficiency of supercritical fluid extraction was also investigated with respect to the spiking level. The soil samples were spiked with various concentrations of the pesticides and PCBs followed by supercritical fluid extraction at the optimized conditions as discussed in the previous sections. The results are summarized in **Tables 4.5.6 through 4.5.9**.

For the extraction of aldrin, satisfactory recoveries were generally obtained at spiking levels ranging from 200 µg to 16 ng per gram of soil which are quite comparable with those obtained from the Soxhlet extractions. Only when the spiking level was further reduced to 8.4 ng/g soil, the recovery seemed to decrease significantly (**Table 4.5.6**). For the supercritical fluid extractions of the PCBs samples, the recoveries were found to be excellent (**Table 4.5.7**). Even when the spiking level was reduced to ca. 10 µg/g soil, the recoveries were almost quantitative for Aroclor 1260 (99%) and very good for Aroclor 1254 (84%). For 2,4-D methyl ester, malathion and parathion, the supercritical fluid extraction also gives excellent recoveries. In all the cases, almost quantitative recoveries were obtained. At low spiking levels (ca. 10 µg/g soil), more than 90% recoveries were observed (**Table 4.5.9**). The supercritical extraction of aldicarb also demonstrated high recoveries (**Table 4.5.8**). At a spiking level as low as 0.8 µg/ g soil, the recovery of aldicarb was still quite satisfactory (86%). However, the recovery of amitrol as usual

was low, regardless of the spiking level. Generally, only 10% of the amitrol could be recovered from the spiked soil samples. The low recoveries of amitrol should be quite reasonable because of the possible interaction or bonding between the amitrol molecules and certain components in the soils as discussed in the previous sections.

Table 4.5.6 Effect of Spiking Level on the SC-CO₂ Extraction and Soxhlet Extraction of Aldrin from Sandy Soil (Particle Size: 60/80 mesh).

Spiking Level (µg/g soil)	% Recovery	
	Supercritical CO ₂ ^a	Soxhlet ^b
200	84	-
11	85	75
4.1	86	81
1.4	90	87
0.1	86	-
0.016	80	-
0.0084	48	-

a. SFE conditions: Pressure = 8000 psi; Temperature = 45°C; Extraction time = 20 min. Adsorbent: Tenax TA.

b. Solvent: 100 mL of acetone; Extraction time = 20 hrs.

Table 4.5.7. Effect of the Spiking Level on the Extraction of PCBs from 60/80 Mesh Sandy Soil.

Spiking Level ($\mu\text{g/g}$ soil)	% PCB Recovery	
	Aroclor 1254 ^a	Aroclor 1260 ^b
0.00912	-	99
0.0109	84	-
0.0456	-	97
0.0913	-	100
0.135	91	-
0.229	-	99
0.272	97	-
2.29	-	95
3.31	92	-
11.4	-	95
13.6	94	-

a. SFE conditions: $\text{SC-CO}_2 + \text{CH}_2\text{Cl}_2$ (10% v/v); Pressure = 8000 psi;

Temperature = 45°C; Equilibration time = 20 min.

b. $\text{SC-CO}_2 + \text{CH}_2\text{Cl}_2$ (10% v/v); Pressure = 8000 psi; Temperature = 45°C;

Equilibration time = 10 min.

Table 4.5.8. Effect of the Spiking Level on SFE of Amitrol and Aldicarb from Sandy Soil

Amitrol		Aldicarb	
Spiking Level($\mu\text{g/g}$)	% Recovery	Spiking Level($\mu\text{g/g}$)	% Recovery
110.4	11	103.6	93
70.8	11	84.2	90
20.5	10	24.0	92
6.2	9	6.0	89
		0.8	86

SFE conditions: SC-CO₂+Water (10% v/v) at Pressure = 9500 psi,

Temperature = 90°C ,and Equilibration time = 30 min.

Samples: 60/80-mesh sandy soil.

Table 4.5.9. Effect of Spiking Level on the Recovery of SFE of 2,4-D Methyl Ester, Malathion and Parathion from Sandy Soil

<u>2,4-D Methyl Ester</u>		<u>Malathion</u>		<u>Parathion</u>	
Spiking Level($\mu\text{g/g}$)	% Recovery	Spiking Level($\mu\text{g/g}$)	% Recovery	Spiking Level($\mu\text{g/g}$)	% Recovery
462	95	470	97	97.5	97
212	94	218	95	44.5	98
108	93	111	96	22.7	97
25.5	93	15.1	98	12.4	100
12.8	90	7.5	90	6.2	94

SFE conditions: SC-CO₂+ Methanol(5% v/v) at Pressure = 9000 psi,

Temperature = 45°C, and Equilibration time = 20 min.

Samples: 60/80 -mesh sandy soil.

4.5.4. Comparison of SFE with Soxhlet Extraction

Soxhlet extractions of each of the pesticides and PCBs under investigation from the different matrices were performed to compare with the supercritical fluid extraction technique. The results are also summarized in Table 4.5.1 through Table 4.5.5. Although the recoveries of the Soxhlet extractions in some cases, particularly when the top soil and clay were involved, were slightly higher, a significant saving of extraction time was clearly achieved in the supercritical fluid extractions. Almost quantitative recoveries in the supercritical extraction of the PCBs and the pesticides except Amitrol have been obtained within 30 min of extraction time, and the extraction efficiencies are quite comparable with the Soxhlet method which in contrast usually requires several hours or even overnight in order to reach high recoveries.

Besides speeding up the extraction process, relatively clean (implying a higher selectivity) extracts could be obtained using supercritical fluid extractions. This advantage over the conventional Soxhlet method can be demonstrated directly by comparing the chromatograms of extracts. Figures 4.5.2a, 4.5.2b and 4.5.2c are the gas chromatograms obtained with a FID detector for aldrin in a standard solution, and for aldrin from a supercritical carbon dioxide extraction and a Soxhlet extraction of 4.1 µg/g of aldrin-spiked sandy soil, respectively. The extracts from the supercritical extraction seem to be neater and have fewer interfering signals than those obtained from the Soxhlet extraction. The results

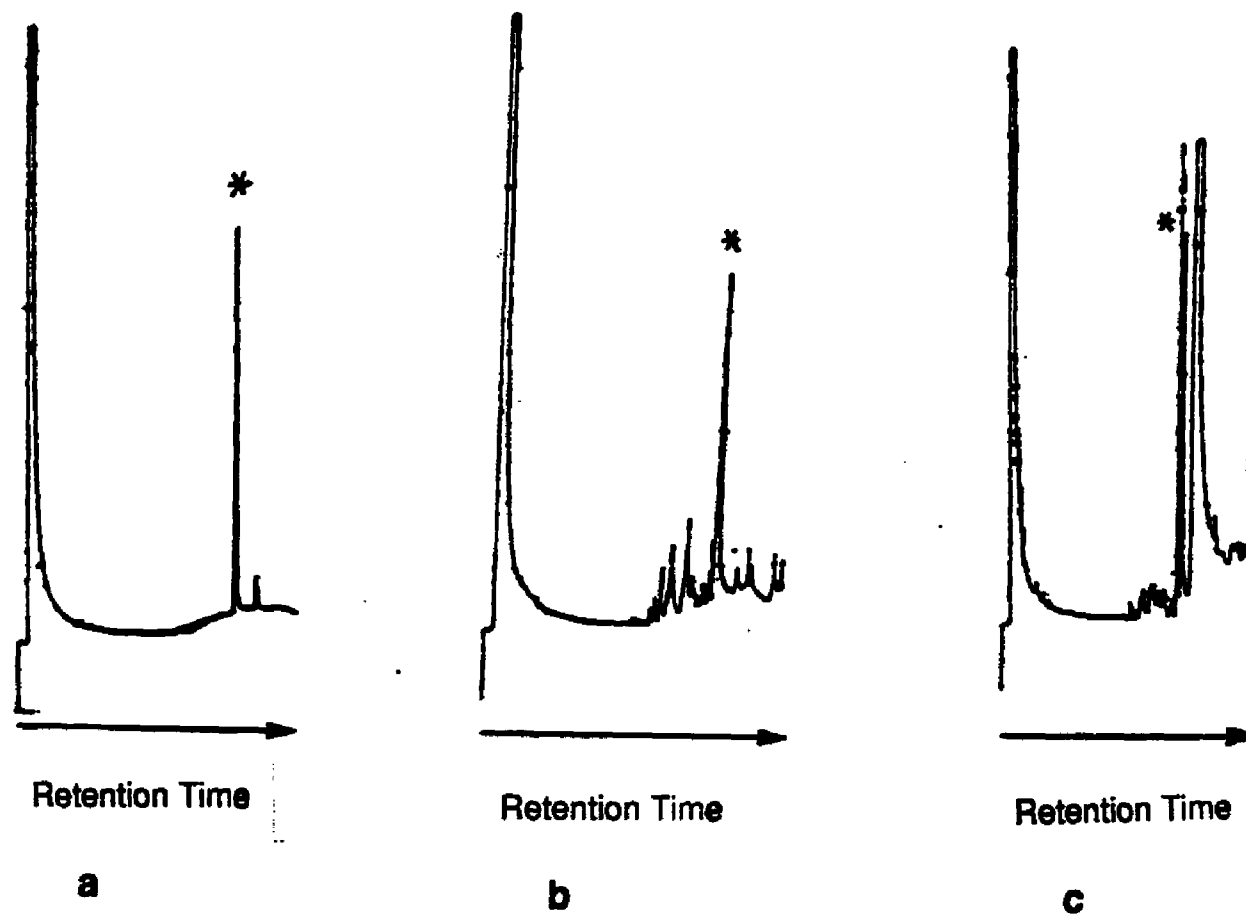


Figure 4.5.2. GC chromatogram of aldrin (0.041 mg/mL): (a) standard solution; (b) SFE extracts; (c) Soxhlet extracts.

suggest that supercritical carbon dioxide exhibits selectivity in solvating the analytes under investigation. Furthermore, excessively long extraction time in the Soxhlet method might conceivably cause some undesirable reactions between the components and the solvent, leading to decomposition or reaction products from the soil components and therefore to more interference signals. On the other hand, since the gas (e.g. CO₂) used as the supercritical fluid is of higher purity than common organic liquid solvents, the supercritical fluid introduces fewer impurities into the analytes, also resulting in the neater chromatograms. Another example of cleaner extracts using supercritical fluid extraction technique is given in **Figures 4.5.3 and 4.5.4**, which are the GC/MS chromatograms for the extracts from a supercritical carbon dioxide extraction and a Soxhlet extraction of an unspiked top soil blank sample. It is apparent that the extracts obtained from the SFE process contain fewer peaks and are much cleaner than those from the Soxhlet extraction.

Many other advantages of the supercritical fluid extraction over the conventional Soxhlet extraction are also self-evident throughout this dissertation. In short, the major advantages of the supercritical fluid extraction versus the conventional Soxhlet extraction techniques for the analysis of the pesticides and PCBs might be summarized as follows:

- Faster
- Comparable recoveries
- Lower operating temperatures

- **No sample contact with boiling solvents**
- **Sample-solvent contact time minimized**
- **No exposure to light during the extraction**
- **Solvent removal by simple depressurization**
- **Gases in use are generally purer than common liquid solvents**
- **Gases are generally cheaper than liquid solvents**
- **Easier solvent disposal**
- **Common gases are nontoxic and nonflammable**
- **SCFs have lower viscosities and higher diffusivities**
- **SCFs have high densities which are regulatable by the control of the experimental conditions (e.g. the system pressure)**
- **Solvency increases with density**
- **Entrainers may facilitate selectivity**
- **Possible temperature-programming in the extraction.**

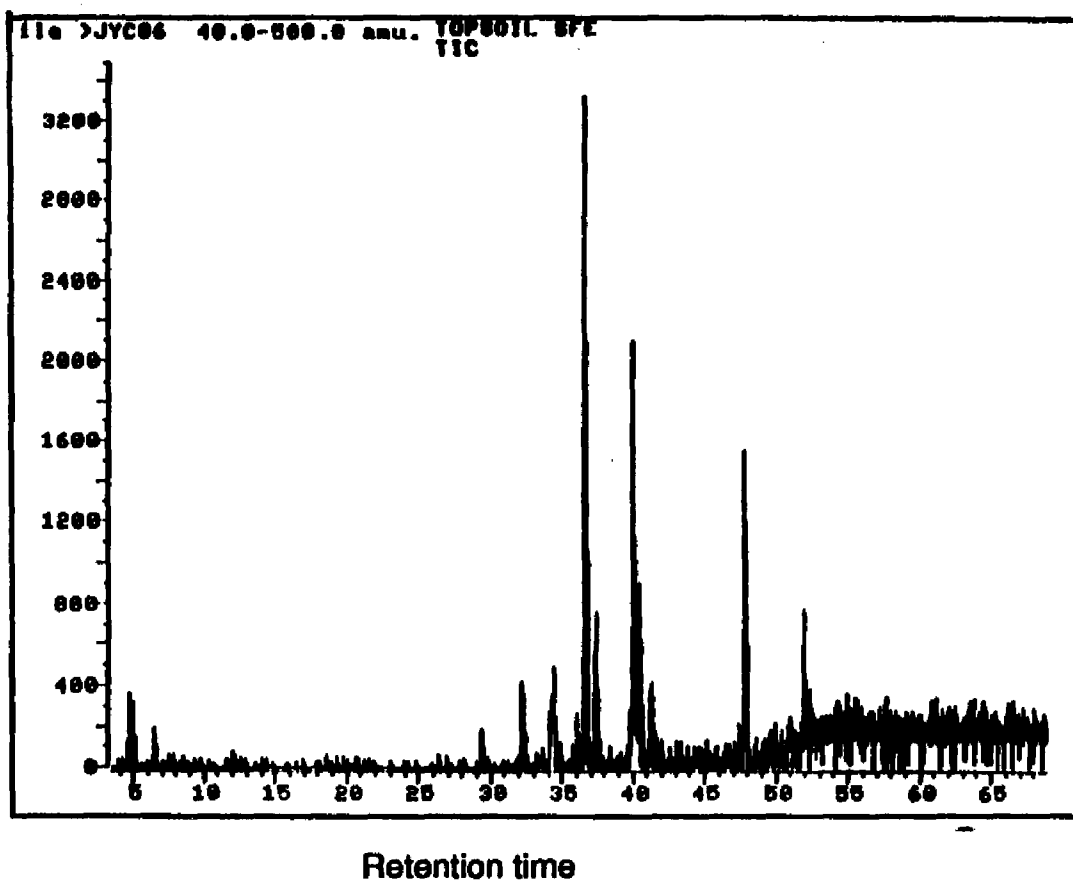


Figure 4.5.3. GC-MS of SC-CO₂ extracts from a blank (unspiked) top soil sample.

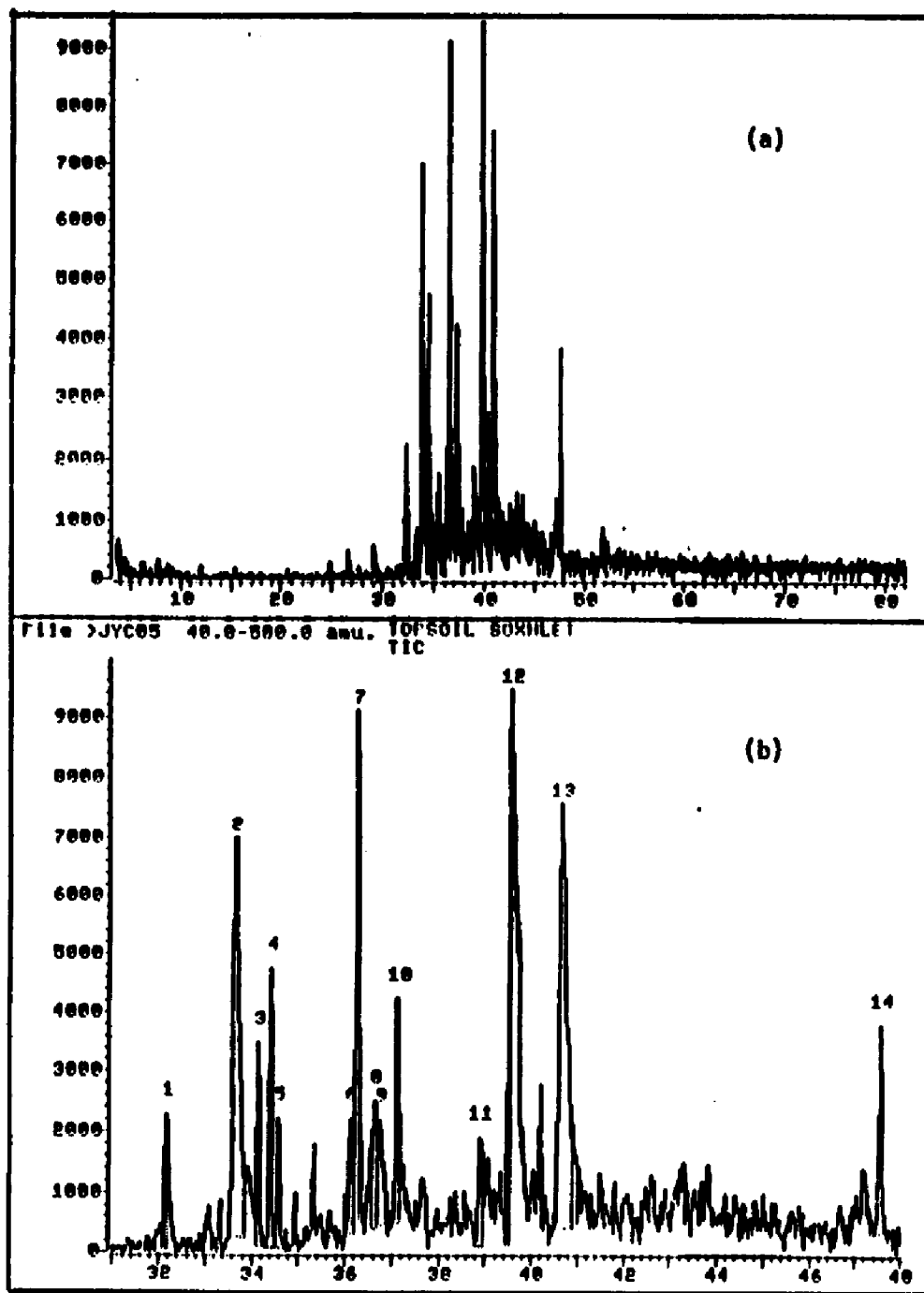


Figure 4.5.4. GC-MS of Soxhlet extracts from a blank (unspiked) top soil sample. (b) is an expansion of (a).

5.0 CONCLUSIONS

We have systematically studied the supercritical fluid extraction of six representative pesticides and two commercial PCBs from four types of soil samples. Supercritical carbon dioxide, nitrous oxide, and modified carbon dioxide were employed as the extraction fluids. The pesticides and PCBs include aldrin, parathion, malathion, 2,4-D methyl ester, amitrol, aldicarb, and Aroclors 1254 and 1260, which are all related to the human health and environmental problems in our society. The soil samples including sand, loam, top-soil and clay with various grain sizes were collected from the metropolitan area of New York City, and were spiked with the pesticides or PCBs at a broad range of spiking levels (e.g. from ca. 1 ng to ca. 1 mg per gram of soil sample). The extracts were analyzed by gas chromatography with an electron-capture, flame ionization or mass spectroscopic detector or by high performance liquid chromatography with a diode-array ultraviolet-visible spectroscopic detector.

The effects of the extraction pressure, temperature, and time; supercritical fluid modifier; and the type and grain size of the soils, on the recoveries of the extractions were investigated; and the extraction conditions for each of the pesticides and PCBs were optimized. The supercritical fluid extractions of all the compounds studied except amitrol give quite satisfactory (>80%), and in many cases quantitative (>95%), recoveries. A low recovery (ca. 10%) was usually observed for the SFE of amitrol even under optimized conditions, which is attributed to the

possible binding between amitrol and the soil components.

An increase in the system pressure was found to enhance significantly the recoveries of the supercritical fluid extractions, which is attributable to the increase in the density of the SCF and, therefore, the solubility of the compounds in the SCF. The recoveries level off in most cases when the pressure further increases to ca. 8000 to 9000 psi or higher, because of the inherent limit of the solubilities of the organics in the supercritical fluid. The effect of the system temperature on the recoveries was found to be more complex owing to the existence of several competing factors which includes the vapor pressure of the solutes and the density of the SCF. In most cases, the recovery increases and reaches a maximum as the temperature increases at the temperatures above the critical point. This could be attributed to the increase in the vapor pressure of the solutes. Further increases in the temperature result in a decrease in the recovery because the density and, therefore, the solvating power of the supercritical fluid are lower at the higher temperatures.

The recoveries of the SFE of the pesticides and PCBs are significantly improved by using appropriate modifiers. The effect of modifiers, such as methylene chloride, methanol, benzene, toluene, and water, depends upon the nature of the modifier, the extraction system and the soil type. It has been found that the SFE recovery is affected particularly by the nature of the modifiers. For the SFE of PCBs, modification of the supercritical CO₂

with methylene chloride greatly increases the recoveries, whereas methanol shows essentially no effect on the recovery and benzene reduces the recovery. The recovery-enhancement effect of the modifiers in the SC-CO₂ extraction of the organochlorine compounds such as PCBs and aldrin can be ranked in a general order: methylene chloride > methanol > toluene ~ benzene. These observations of the modifier effects on the recoveries could be qualitatively interpreted in relation with the polarities and polarizability of the modifiers. In comparison with methanol, methylene chloride has a higher polarizability which might be comparable to the solutes PCBs and aldrin, leading to higher solubilities and recoveries. Benzene and toluene have much lower polarities than methanol or methylene chloride, which could account for their small or negative effects on the recoveries. For the SFE of 2,4-D methyl ester, parathion and malathion, an enhancement of the recoveries was observed when methanol was employed as a modifier. Water was found to increase the recoveries of amitrol and aldicarb. In these cases, the polarity of the modifiers might have played a more important role.

The extraction equilibration time required for the SFE is generally much shorter comparing to conventional Soxhlet extractions. The optimum extraction time is in the range of ca. 10 to 30 min. For the SFEs of PCBs, aldrin, 2,4-D methyl ester, malathion, and parathion, almost quantitative recoveries were obtained within 20 min of the equilibration time. The efficiency of SFE also varies with the type and particle size of soil samples. The recoveries have been found to be in the following order:

sand > loam > top-soil. This order appears to correlate with the total organic content of these soil samples. The high recoveries were obtained when a large particle size of the soil sample was employed, which could be attributed to the small surface area and probably great organics-adsorption capacity of the large particle size. The SFE of the pesticides and PCBs from a clay matrix gives the lowest recoveries because clay has smallest grain size among all the types of soil samples.

The Soxhlet extraction of the pesticides and PCBs from soils were also investigated and the extraction recoveries are compared with those from the SFE. All the results demonstrated that the supercritical fluid extraction of the pesticides and PCBs except amitrol presents a superior method over the conventional Soxhlet extraction.

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