INNOVATIVE TECHNIQUES FOR THE ANALYSIS OF CHLOROPHENOLS AND ACID HERBICIDES IN SOILS AND WATER

BY

DAVID C. HAY

A Thesis
Submitted to the Faculty of Graduate Studies
in Partial Fulfilment of the Requirements
for the Degree of

MASTER OF SCIENCE

Department of Soil Science
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ABSTRACT

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Regulatory and enforcement agencies require rapid, inexpensive analytical techniques for contaminants containing phenolic and carboxylic acid functional groups. Conventionally, these molecules have been difficult to analyze. A lengthy cleanup process has often been required following extraction and before the extract containing the analyte could be analyzed quantitatively. The new method of analyzing soils and water utilizes the solid-phase microextraction (SPME) technique which allows the derivatization of the analytes on the polyacrylate fibre coating eliminating the traditional cleanup process when analyzing acid herbicides and chlorophenols. Confirmation of the chlorophenols was performed by derivatization with diazomethane and comparison with commercially available chloroanisoles or by GC with ion trap detection. LOD ranged from 0.01 μg/L to 0.05 μg/L whereas LOQ ranged from 0.03 μg/L to 0.07 μg/L for 2,4,5-T and dicamba respectively. Reproducibility expressed as % RSDs for the derivatization method ranged from 2.7 - 31.4%.

The use of the SPME technique has been optimized to allow for the analysis of chlorophenols and acid herbicides in soils and water through decreased pH, addition of NaCl, sample agitation and derivatization of the analyte. Sample agitation was achieved through the development of the 'Woodpecker' apparatus in conjunction with the conventional static autosampler with equilibrium being reached in approximately 20 min. However, this was not as effective as the more vigorous stirring with the insertion of magnetic stir bars into each sample vial used in manual SPME. The SPME technique enables the calculation of partition coefficients (K_{SPME}) which closely parallel log K_{OW} values.

Conventional extraction techniques allow for the quantification of the total residue contained within the sample. However, due to the inherent nature of the fibre coating and the gentler extraction conditions, this new method allows the analyst to measure only the portion of the analyte which is able to partition into the fibre coating, *viz.*, the freely dissolved analyte. Dissolved organic carbon in environmental samples sorbs a portion of the analyte which will not then be available for SPME. However, these results were confounded with analyte degradation. The SPME technique thus provides a method of biomimetic extraction and is able to measure the fraction of the contaminant which is potentially bioavailable.

Dedicated in memory

of my father,

Aubrey H. Hay

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FOREWORD

This thesis has been prepared in the traditional format in accordance with the Department of Soil Science guidelines. The referencing style used does not follow that of the Canadian Journal of Soil Science as research reported herein will not be submitted for publication in the aforementioned journal. To assist in publication purposes the referencing style used by the Journal of Agricultural and Food Chemistry was selected and adopted throughout this thesis. Up to this date, a paper has been prepared for submission to the Journal of Agricultural and Food Chemistry for publication entitled, 'SPME-Alkylation-GC of Residues of Chlorinated Phenols and Acid Herbicides in Water'.

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LIST OF ABBREVIATIONS AND CHEMICAL FORMULAE

α	degree of dissociation of the acidic functional groups
Å	Angstrom
ACS	American Chemical Society
Ah	uppermost mineral horizon darkened with organic matter
amu	atomic mass unit
ANOVA	analysis of variance
atm	atmosphere
BAP	benzo[a]pyrene
BC(k)	mineral horizon which can not be classified either as B or C horizons but having carbonate present within
Bm	mineral B horizon having a different structure from parent material
BSA	bovine serum albumin
BTEX	benzene, toluene, ethyl benzene, xylenes
C_{Aq}	analyte concentration in solution
°C	Celsius
C	mineral horizon unaffected by pedogenic processes
CCME	Canadian Council of Ministers of the Environment
CH_2N_2	diazomethane
CLOT	carbon layer open tubular
cm	centimetre(s)
C_{OC}	chemical concentration in organic matter of particles (ng/kg OM)
C_P	chemical concentration in the particulate matter (ng/kg of particle)
C_{WD}	freely dissolved chemical concentration in water (ng/L)
d	day(s)
2,4-D	2,4,-dichlorophenoxyacetic acid
DCM	dichloromethane
2,3-DCP	2,3-dichlorophenol
2,4-DCP	2,4-dichlorophenol
2,6-DCP	2,6-dichlorophenol
3,4-DCP	3,4-dichlorophenol
3,5-DCP	3,5-dichlorophenol
DIG	distilled in glass
DOC	dissolved organic carbon
DOM	dissolved organic matter
dS	deciSiemens

DT_{50}	degradation time for 50% analyte
FID	flame ionization detector (detection)
ECD	electron capture detector (detection)
ELISA	enzyme linked immunosorbent assay
EPA	Environmental Protection Agency (USA)
g	gram (grams)
GC	gas chromatograph (chromatography)
GF/C	glass microfibre filters
GI	gastrointestinal tract
H_2SO_4	sulphuric acid
h	hour(s)
H_2	hydrogen
He	helium
HPLC	high pressure liquid chromatography
Hz	Hertz
id	inner diameter
ITD	ion trap detector (detection)
K	Kelvin
KCl	potassium chloride
kg	kilogram (kilograms)
km	kilometre(s)
K_{OC}	organic carbon normalized partition coefficient
K_{OW}	octanol-water partition coefficient
KOH	potassium hydroxide
K_P	sorption coefficient to particulate matter (L water/kg particle)
K_{SPME}	partition coefficient of analyte between coating and sample matrix
L	litre (litres)
LC_{50}	lethal concentration to 50% of population
LD_{50}	lethal dose to 50% of population
LOD	limits of detection
LOQ	limits of quantitation
m	metre (metres)
M	molar
M	magnetic stirring
2-MCP	2-monochlorophenol
4-MCP	4-monochlorophenol
MeOH	methanol
mm	millimetre(s)
mg	milligram (milligrams)
min	minute(s)
mL	millilitre (millilitres)
mol	moles
mPa	milliPascals
mS	milliSiemens
MS	mass spectrometer (spectrometry)

n	amount sorbed by fibre (moles)
N_2	nitrogen
NaCl	sodium chloride
NaOH	sodium hydroxide
ng	nanogram (nanograms)
NPD	nitrogen phosphorus detector (detection)
OC	organic carbon
OM	organic matter
PAHs	polycyclic aromatic hydrocarbons
PCA	pentachloroanisole
PCBs	polychlorinated biphenyls
2,3,4,5,6-PCP	2,3,4,5,6-pentachlorophenol
PDMS	polydimethylsiloxane
pg	picogram (picograms)
pH	negative logarithm hydrogen concentration
pK_a	dissociation constant (negative logarithm)
ppm	parts per million
QSPRs	quantitative structure-property relationships
r^2	regression coefficient
RSD	relative standard deviation (%)
rpm	revolutions per minute
s	second(s)
S	static
S/N	signal/noise ratio
SPE	solid phase extraction
SPME	solid phase microextraction
t	tonne(s)
TCDD	tetrachlorodibenzo-*p*-dioxin
TFA	trifluoroacetic acid
2,4,5-T	2,4,5-trichlorophenoxyacetic acid
t'_R	retention time
2,3,4-TriCP	2,3,4-trichlorophenol
2,4,6-TriCP	2,4,6-trichlorphenol
2,3,4,6-TetCP	2,3,4,6-tetrachlorophenol
2,3,5,6-TetCP	2,3,5,6-tetrachlorophenol
µg	microgram (micrograms)
µL	microlitre (microlitres)
µm	micron (micrometres)
V	volt (s)
VOCs	volatile organic compounds
vp	vapor pressure
V_s	volume of the fibre coating
W	'Woodpecker'
y	year (s)

1. INTRODUCTION

Pesticide contamination of soils, sediments, and water is an ever increasing problem. The literature contains numerous reports of pesticides in groundwater (Barker, 1987; Leng, 1995; Miller *et al.*, 1995*a*; Miller *et al.*, 1995*b*), and the effects of contaminants on aquatic habitats (Heckman, 1982). The fact that approximately 90% of rural people are reliant on groundwater in the Canadian Prairies (Hill *et al.*, 1996) indicates the importance of conducting research on the transport of organic contaminants in groundwater (Mackay *et al.*, 1985; Krawchuk and Webster, 1987; Reynolds *et al.*, 1995). This is a difficult task as one of the major contributors of pollution is that of agriculture through predominantly non-point sources (Cooper, 1993).

Researchers, industry, and regulatory and enforcement agencies require rapid, inexpensive, analytical techniques which will enable increased throughput of samples. Currently liquid extraction, solid phase cartridge extraction, or supercritical fluid extraction techniques are most commonly used in quantification procedures for contaminants. The desire to eliminate or at least reduce organic solvent usage prompted the development of the solid phase microextraction (SPME) technique at the University of Waterloo by Dr. Janusz Pawliszyn (Belardi and Pawliszyn, 1989).

The SPME technique combines quick specific extraction with convenient thermal desorption of the analyte within the injection port of a gas chromatograph. The advantages of SPME include the virtual elimination of solvent use and disposal costs, reduced amount of time required for analyte extraction, and the simplicity of the technique itself. Such a technique is suitable for field sampling, screening tests, and usage in contract laboratories.

The major funder for this research is Manitoba Hydro. Manitoba Hydro is a public utility which generates hydroelectricity for consumers in the province of Manitoba. In doing so, Manitoba Hydro applies herbicides within rights-of-way to control excessive vegetation growth. In compliance with current environmental legislation, pesticides applied to rights-of-way must be monitored. A new method for the quantification of these polar analytes was further developed using the SPME technique. The methodology developed herein will assist in providing quality analytical data in shorter turnaround times than have been previously possible.

The objective of this thesis was to develop a new analytical technique which could be applied to chlorophenols and acid herbicides. In order to achieve this goal, derivatization of the analytes is desirable in order to increase their vapor pressure and reduce their thermal labilities making residues more amenable to GC analysis. The polyacrylate SPME fibre had been previously used to extract chlorophenols and other polar analytes;

however, these analyses had been performed without derivatizing the phenols and had required special GC columns (SPB-608, Sigma-Aldrich Canada Ltd., Mississauga, ON, Cat. # 2-5312). Such columns were specifically designed for the analysis of compounds which do not have suitable GC characteristics. The technique developed over the course of the thesis work used vapor phase diazomethane to methylate extracted polar analytes on the SPME fibre, thus simplifying the analysis.

Varian Canada Ltd. (Mississauga, ON) has the exclusive rights for the development of autosampler capabilities in conjunction with the commercially available SPME fibres from Sigma-Aldrich Canada Ltd. (Mississauga, ON). The goals of the study were more readily achieved using the autosampler system on the GC. Factors optimizing the SPME technique including equilibration, ionic strength, pH, agitation and rinsing were examined in detail. These factors were incorporated into a 'stand-alone' agitation apparatus to expedite the extraction of the analytes compared to static autosampler analysis. The analyses were applied to a variety of matrices ranging from HPLC water, and natural river waters, to aqueous soil extracts.

Using the SPME technique developed, partition coefficients were calculated from the amount of analyte sorbed to the fibre coating. Previous literature has shown that these partition coefficients can be related to log K_{ow} values (Dean *et al.*, 1996). SPME only extracts the analyte which is freely dissolved while leaving the fraction bound to organic matter unextracted (Vaes *et al.*, 1996*b*). The methodology used in this project is different

from other analytical methods in that total residues are not measured; but rather, only a portion of the total residue is extracted, the fraction of the contaminant which is freely dissolved. This technique shows promising results as environmental toxicologists increasingly wish to determine the quantity of analyte which is actually bioavailable.

Biomimetic extraction is the partitioning of an analyte within the natural environment so that valuable information regarding harm to organisms can be obtained. The concept of biomimetic extraction is different from that of conventional exhaustive extractions. The analyst is not concerned about completely removing the analyte from the matrix but rather selectively partitioning the freely dissolved analyte into a surrogate lipid phase to simulate the bioconcentration process under natural conditions. If the amount of analyte sorbed to the surrogate lipid phase exceeds the critical body residue, narcotic toxicity is elicited (Parkerton and Stone, 1997). Biomimetic extraction is an important technique in determining bioavailability of certain xenobiotics and allows measurement of the net narcotic toxicity of mixtures such as hydrocarbons and PAHs. Solid phase microextraction fibres allow the analyst to perform biomimetic extraction and to measure the bioavailable fraction or freely dissolved concentration of single compounds or mixtures (Vaes *et al.*, 1996*b*).

2. LITERATURE REVIEW

New analytical methods for organics in a wide variety of complex matrices are constantly being required. The presence of acidic moieties, such as, phenolic and carboxylic acid functional groups, further compounds the challenge inherent in developing new methods of analysis. Two such examples of these compounds were studied: chlorophenols and acid herbicides. Their chemical and physical properties have been briefly reviewed to provide background information on the scope of this project.

2.1 Chlorophenols

2.1.1 General description

Chlorophenols can be classified as broad spectrum pesticides but are used in a variety of ways as wood preservatives, in paints, in photographic solutions, in tanning agents for hides and leathers, in textiles, and as antimicrobial agents in industrial applications. Disinfection of drinking water by chlorination yields chlorinated phenol byproducts (Coutts *et al.*, 1979). However, their greatest use lies in wood preservation. In 1976, 95% of the Canadian production of chlorophenols was used for wood preservation activities (Jones, 1981). Chlorophenols also have found their niche in the manufacturing

of health care products including dental products as well as in home, farm and hospital disinfectants.

Of the 19 possible chlorophenol congeners, only seven chlorophenols have commercial importance. These include 2-chlorophenol, 4-chlorophenol, 2,4-dichlorophenol, 2,4,5-trichlorophenol, 2,4,6-trichlorophenol, 2,3,4,6-tetrachlorophenol, and 2,3,4,5,6-pentachlorophenol. The lower chlorinated phenols are used as biocides while other chlorinated phenols are used as precursors in the synthesis of other pesticides. Many countries have either banned or restricted the use of polychlorinated phenols since the late 1980s (Hale *et al.*, 1994). The worldwide production of chlorophenols has been estimated at about 181 000 tonnes of which pentachlorophenol (PCP) represents approximately 82 000 tonnes (Hale *et al.*, 1994).

The more highly chlorinated phenols contain varying degrees of impurities such as dioxins and furans (Jones, 1981). The presence of these impurities, created during the synthesis process, makes accurate toxicity data for chlorophenols difficult to determine. However, it has been established that chlorophenols are only mildly toxic to terrestrial organisms; whereas, they are highly toxic to aquatic organisms, increasingly so as the number of chlorine substituents on the phenol ring increases (Jones, 1981). As the chlorophenol becomes increasingly chlorinated, degradation in the environment is affected: the importance of aerobic decomposition decreases and of anaerobic degradation increases (Hale *et al.*, 1994). In terms of environmental fate, chlorophenols may also be removed from the aquatic environment by volatilization, photodegradation,

or sorption to sediments (Hale *et al.*, 1994). Half-lives in soil range from 14 d for 2-chlorophenol to > 72 d for pentachlorophenol (PCP). However, these compounds can persist in soil systems for up to 5 y (Hale *et al.*, 1994). Chlorophenols, especially pentachlorophenol (PCP), are toxic to many forms of aquatic life and can bioaccumulate in the tissues of organisms exposed to contaminated ecosystems (Jones, 1981; Cruz and Wells, 1992).

Pentachlorophenol (PCP) was introduced in 1936 as a timber preservative and later used as a general disinfectant (Cui and Ruddick, 1994). Pentachlorophenol is used to control termites and to protect wood from fungal rot and wood-boring insects, in addition to its use as a general herbicide (Cruz and Wells, 1992). In many ways, dichlorophenols and trichlorophenols are chemically similar, and behave similarly to the more highly substituted chlorophenols (Table 2.1). Dichlorophenols and trichlorophenols are the degradation products of pentachlorophenol produced predominantly through photochemical and biochemical degradation reactions (Cui and Ruddick, 1994). The chemical structures of chlorophenols and pentachlorophenol are shown in Figure 2.1.

Figure 2.1 Chemical structure of chlorophenols containing up to 5 chlorine atoms and the chemical structure of pentachlorophenol (PCP)

The Canadian maximum acceptable concentration for phenols in drinking water is 2 μg/L. Total phenol concentration greater than 5 μg/L results in unacceptable tastes and odours as a result of phenol chlorination during water treatment (CCME, 1994).

Table 2.1 Physical and chemical properties of chlorophenols

Analyte	Molecular Weight (g/mol)	pK_a	Water Solubility (mmol/L) (25 °C)[3]	Vapor Pressure (Pa) (25 °C)[3]	log K_{ow}[2]
2-MCP	128.56	8.49	168	249	2.15
4-MCP	128.56	9.18	208	32.1	2.53
2,3-DCP	163.00	6.44			2.95[1]
2,4-DCP	163.00	7.68	29.3	14.3	3.15[1]
2,6-DCP	163.00	6.80	16.1	12.4	2.92
3,4-DCP	163.00	7.39	56.7		3.47
3,5-DCP	163.00	6.92			3.43[1]
2,3,4-TriCP	197.45	7.66	3.59	2.24	3.80
2,4,6-TriCP	197.45	7.42	3.28	2.67	3.75
2,3,4,6-TetCP	231.98	5.38	0.604	0.643	4.45
2,3,5,6-TetCP	231.98	5.48			
2,3,4,5,6-PCP	266.34	4.92	0.0627	0.0849	5.02

(Source: Jones, 1981)

[1] National Institute of Health Sciences, 1997, Internet URL: http://db.nihs.go.jp/cgi-bin/sybgw/KEMI/E1a/pp. 428, 427, 430, respectively.

[2] Shake flask method (Mackay *et al.*, 1995)

[3] Mean values (Mackay *et al.*, 1995)

Chlorophenols are highly polar and chemically reactive and exhibit low vapor pressures which causes adsorption and tailing problems in their analysis by GC (Drozd, 1981). Therefore, derivatization into acetate esters using acetic anhydride (Coutts *et al.*, 1979) or methyl ethers using diazomethane (Cruz and Wells, 1992) or other methylating agents is used to generate compounds which are more amenable to GC analysis. The production of these derivatives is necessary to increase the vapor pressure, make the molecule less thermally labile, less sensitive to pH, and assist in enhancing GC peak symmetry (Drozd, 1981).

2.2 Acid Herbicides

2.2.1 General description

Acid herbicides are a broad classification of herbicides widely used to control broad-leaved weeds in lawns, crops, and rights-of-way. Acid herbicides as a group contain a carboxylic acid functional group which, when alkylated, produce the corresponding alkyl esters. Four such acid herbicides were chosen, namely, dicamba, 2,4-D (2,4-dichlorophenoxyacetic acid), triclopyr, and 2,4,5-T (2,4,5-trichlorophenoxyacetic acid). All continue to be used with the exception of 2,4,5-T which was eliminated in much of the world because of the production of chlorinated dioxins as contaminants during its synthesis (Esposito, 1980). Despite the fact that 2,4,5-T is not currently produced in Canada, it was included because it shows that the new derivatization methodology developed is capable of application with other herbicides containing similar functional groups. In 1980, commercial 2,4,5-T guaranteed to contain less than 0.05 ppm

tetrachlorodibenzo-*p*-dioxin (TCDD) was available from non US producers (Esposito, 1980).

Nevertheless, monitoring of such residues continues to be of importance to regulatory agencies. The European Countries Drinking Water Directive states that individual pesticides should not exceed 0.1 μg/L (Tolosa *et al.*, 1996). Analysis of such chlorinated organics is usually performed by derivatization and GC-ECD analysis (Waliszewski and Sedas, 1992). Many analyses also use solid phase extraction (SPE) methods in quantifying pesticide residues in water (Thompson *et al.*, 1995; Tolosa *et al.*, 1996).

Acid herbicides have striking similarities to chlorophenols in that they do not have convenient gas chromatographic properties. The presence of polar moieties causes these compounds to have low vapor pressures, to be chemically labile and to have tailing problems on the GC column. They are rather water soluble, have low vapor pressures and relatively low log K_{ow} values. The acid herbicides generally exhibit a low acute toxicity hazard in mammals. Table 2.2 shows the physical and chemical properties of the acid herbicides used in this study.

Table 2.2 Physical and chemical properties of acid herbicides

Analyte	Molecular Weight (g/mol)	pK_a	Water Solubility (mmol/L)	Vapor Pressure (mPa)	log K_{ow} (acid)
triclopyr[1]	256.5	3.97	31.6[5,7]	0.2[4]	2.63[3]
2,4-D[1]	221.0	2.64	1.41[4,6]	0.011[5]	2.81[3]
2,4,5-T[2]	255.5	2.88	0.587[4]	0.0007[4]	3.13[3]
dicamba[1]	221.0	1.87	29.4[4]	0.0045[4]	2.21[3]

[1] (Tomlin, 1994)
[2] (Worthing and Hance, 1991)
[3] (Howard, 1991)
[4] 298 K
[5] 293 K
[6] pH 1
[7] pH 7

2.2.2 Dicamba

Dicamba (Figure 2.2) is a selective systemic herbicide which acts as an auxin-like growth regulator. It was originally reported in 1961 and is presently sold under the trade name Banvel. Dicamba is used to control annual and perennial broad-leaved weeds and brush species in rights-of-way (Tomlin, 1994). The acute oral LD_{50} for rats is 1700 mg/kg and the LC_{50} (96 h) for rainbow trout and bluegill sunfish is 135 mg/L (Worthing and Hance, 1991). In mammals, dicamba is subject to glycine conjugation and is rapidly eliminated in the urine. Degradation rates in plants vary widely. In soil, microbial degradation is possible with DT_{50} values ranging from 10-20 d (Howard, 1991). Approximately 6818 tonnes of dicamba was utilized in 1993 in the United States for both agricultural crop production and through non-agricultural sectors (Aspelin, 1994).

Water quality guidelines reflect how pristine the water must be according to how the water will be utilized. The Canadian drinking water quality guideline for dicamba has been set at 120 μg/L (CCME, 1991). This is considerably higher than the European Countries Drinking Water Directive of 0.1 μg/L. An interim guideline for the protection of freshwater aquatic life in Canada has been established at a concentration of 10 μg/L (CCME, 1991). A water quality guideline for livestock for dicamba has been placed at 69 μg/L (Miller *et al.*, 1995*a*).

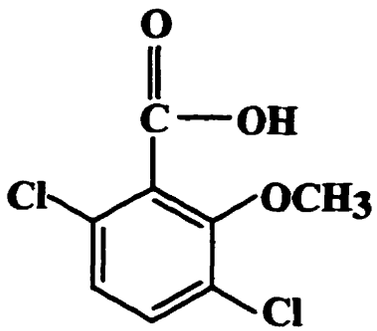


Figure 2.2 Chemical structure of dicamba (3,6-dichloro-2-methoxybenzoic acid)

2.2.3 2,4-D

2,4-dichlorophenoxyacetic acid (2,4-D) (Figure 2.3), an aryloxyalkanoic acid, was first reported in 1942. 2,4-D is used as a selective systemic herbicide, acts as a growth inhibitor, and can be used in the post-emergent control of annual and perennial broad-leaved weeds in cereals, grasslands, forests, and rights-of-way. 2,4-D can also be used to control broad-leaved aquatic weeds (Tomlin, 1994). 2,4-D is one of the most widely used herbicides ever developed. Approximately 20 454 tonnes of 2,4-D was used in the

United States in 1993 for agricultural crop production and in non-agricultural sectors (Aspelin, 1994).

Extensive research has been conducted on the mammalian toxicology; acute oral LD_{50} for 2,4-D has been reported for rats to be 375-805 mg/kg depending on the formulation (Worthing and Hance, 1991). In the case of fish, ester formulations are more toxic. The LC_{50} (96 h) for 2,4-D ester in rainbow trout is 0.5-1.2 mg/L (Worthing and Hance, 1991). Elimination of unmetabolized 2,4-D is rapid. For doses up to 10 mg/kg, complete excretion is possible in 24 h. Metabolism in plants, soil, and water involves hydroxylation, decarboxylation, cleavage of the acid side-chain, and ring opening. Half-lives in soil are of the order of 10-50 d (Howard, 1991).

Sorption to soil depends on soil type and organic content (Jones, 1981; Howard, 1991). In aquatic systems, half lives of 10 to >50 d have been reported (Howard, 1991). Breakdown products include 2,4-dichlorophenol, other hydroxylic aromatics and polymeric acids. Canadian interim livestock and human drinking water quality guidelines for 2,4-D have been established at 100 µg/L (Hill *et al.*, 1996; Miller *et al.*, 1995*a*).

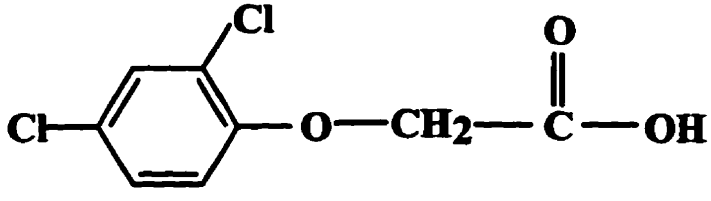


Figure 2.3 Chemical structure of 2,4-D (2,4-dichlorophenoxyacetic acid)

2.2.4 Triclopyr

Triclopyr (Figure 2.4) is an aryloxyalkanoic acid manufactured by Dow Elanco registered for post-emergent use in Europe and North America and marketed under the trade name Garlon (Johnson and Hall, 1996). Triclopyr is a selective systemic herbicide which is rapidly absorbed by the foliage and roots. Triclopyr accumulates in the meristematic tissue and elicits an auxin-type response in plant species affected. It can be applied to control woody plants and many broad-leaved weeds in grassland ecosystems. Triclopyr has also proven useful in uncultivated lands, industrial areas, coniferous forests, plantation crops, and rice fields (Tomlin, 1994).

Acute oral LD_{50} have been reported to be 577 mg/kg for female rats and 692 mg/kg for male rats; whereas, the LC_{50} (96 h) for rainbow trout is 117 mg/L (Tomlin, 1994). Triclopyr is predominantly excreted unmetabolized in the mammalian urine. In plants, the DT_{50} is approximately 3-10 d whereas the average half-life in soil is 46 d depending on the soil and climatic conditions (Tomlin, 1994). Triclopyr quickly degrades through photolysis and hydrolysis with an approximate half life of 4 d in natural waters (Johnson and Hall, 1996). Triclopyr may be used as an aquatic herbicide if environmental fate and distribution studies prove favorable (Johnson and Hall, 1996).

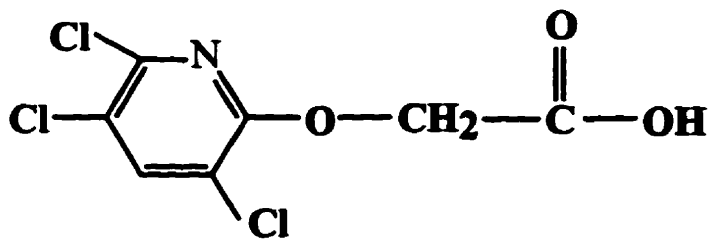


Figure 2.4 Chemical structure of triclopyr (3,5,6-trichloro-2-pyridyloxyacetic acid)

2.2.5 2,4,5-T

2,4,5-Trichlorophenoxyacetic acid (2,4,5-T) (Figure 2.5) is similar to 2,4-D but with the addition of a further chlorine atom. It was originally reported by Hamner and Tukey in 1944 (Worthing and Hance, 1991). 2,4,5-T was used in post emergent applications or in combination with 2,4-D for the control of shrubs and trees. Following application of the herbicide either as a spray formulation or through injection into the bark, absorption may occur through the plant roots, foliage or bark. Oral LD_{50} for rats is 300-1700 mg/kg indicating that its mammalian toxicity is rather low (Worthing and Hance, 1991).

2,3,7,8-Tetrachlorodibenzo-*p*-dioxin is a common contaminant produced during the synthesis of 2,4,5-T (Esposito, 1980). The LC_{50} for rainbow trout has been calculated to be 350 mg/L (Worthing and Hance, 1991). 2,4,5-T was a major component (about 50%) of the product Agent Orange used extensively as a military defoliant in Vietnam in the late 1960s and early 1970s. Most of the toxicological problems associated with the use of Agent Orange were associated with the presence of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) in the defoliant (Esposito, 1980). The association of 2,4,5-T with Agent Orange prompted a vast amount of research leading to its elimination in much of the world.

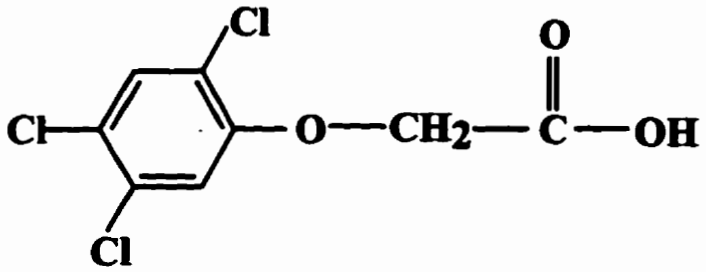


Figure 2.5 Chemical structure of 2,4,5-T (2,4,5-trichlorophenoxyacetic acid)

2.3 Conventional Total Residue Analysis of Organic Contaminants

The main objective with conventional total residue analysis of organic contaminants is to completely remove the analytes from the matrix without differentiating the ease with which the analyte can be removed from the matrix. Recently, with an increased awareness in environmental consciousness, analytical procedures are moving in the direction of measuring only the freely dissolved fraction as this is the portion of the analyte which is bioavailable. When assessing bioavailability of gill breathing organisms, the freely dissolved portion is of utmost importance. However, other variables are also important especially with particle feeding organisms in that they have a close association with analytes potentially sorbed to organic phases.

2.3.1 Extraction techniques

In order to perform quantitative analytical chemistry, the analyte must be extracted from the matrix of interest. This task of extracting an analyte from the matrix can be a difficult and time consuming process. The extraction process proceeds to the extent permitted by the extraction technique and traditionally Soxhlet extraction has been the benchmark in residue analysis laboratories. However, this procedure is time consuming requiring samples to be extracted for many hours. Large quantities of organic solvents must be used in this technique and they require expensive disposal after the extraction process is complete. The Soxhlet extraction technique achieves some of the best possible recoveries because residues associated with the matrix are removed from the matrix itself and into

the extracting solvent. An analyst using this technique seeks to measure total residue as opposed to the portion which is biologically available.

Methodology for the analysis of organics from water has usually involved liquid-liquid extraction (United States Environmental Protection Agency, 1992) or solid phase extraction (SPE) discs (Bolygó and Atreya, 1991; Font *et al.*, 1993; Crespo *et al.*, 1994; Mußmann *et al.*, 1994) followed by capillary gas chromatography (GC) using an appropriate detector, such as flame ionization detection (FID), nitrogen phosphorus detection (NPD), electron capture detection (ECD) or mass spectrometry (MS). In the case of volatile organic contaminants (VOCs), some form of cryotrapping has often been involved to focus the more volatile analytes on the capillary column prior to analysis.

With the knowledge of conventional extraction techniques, we can devise an extraction technique which measures only the dissolved fraction of the analyte. This is important in that the fraction is the most important fraction biologically and has the ability to move vast distances. Compounds may be so strongly sorbed to soil that only a small portion of each can re-enter solution drastically decreasing the toxicity imparted onto organisms. Solid-phase microextraction is a technique thought to measure the freely dissolved fraction (Vaes *et al.*, 1997*b*); that fraction which is most important from a toxicological standpoint.

2.4 Solid-Phase Microextraction (SPME) Theory

Solid-phase microextraction (SPME) is a relatively new solventless extraction technique originally developed by Pawliszyn *et al.* (Belardi and Pawliszyn, 1989). The SPME technique combines quick specific extraction with convenient thermal desorption of the analyte within the injection port of a gas chromatograph. The SPME extraction technique involves partitioning of the analyte between the matrix being extracted and a fused silica fibre coated with a polymeric stationary phase such as polydimethylsiloxane (PDMS) or polyacrylate (Louch *et al.*, 1992) (Figure 2.6). SPME can be viewed as being analogous to solid phase extraction (SPE) in which the analyte partitions onto a solid phase cartridge and is later eluted for analysis. However, because of the small amount of sorbent used to coat each fibre, SPME is not an exhaustive extraction technique, rather, it is based on equilibrium partitioning (Belardi and Pawliszyn, 1989).

2.4.1 General principles

Several fibre coatings are now commercially available from Supelco Canada, Inc., (Sigma-Aldrich Canada Ltd., Mississauga, ON). The choice of fibre coating depends upon the analyte to be extracted. In the case of non-polar compounds, the 100 μm polydimethylsiloxane fibre is often the coating of choice; whereas, in the case of more polar analytes, the 85 μm polyacrylate fibre is recommended (Shirey, 1994*b*). Thick films perform best for compounds with lower distribution constants; whereas, thin films are preferred for compounds with higher distribution constants (Arthur *et al.*, 1993). Other fibre coatings exist for volatiles (Robacker and Bartelt, 1996), and semi-volatiles

(Horng and Huang, 1994; Shirey and Wachob, 1994); pencil lead has also been used successfully as a sorbent for SPME work (Wan *et al.*, 1994).

The polymer coated fibre is retracted into the septum piercing needle of the SPME device to confer protection and gain the ability to insert it through the septa of sample vials. Upon piercing the septa, the fibre is extended into the liquid or exposed to the headspace in the vial depending on the method of extraction used. The fibre is maintained in this position for a fixed period of time or until equilibrium is reached (Figure 2.7). The amount of time required to reach equilibrium is determined by the partition coefficient of the analyte and the fibre coating used. The time required to reach equilibrium can be minimized by vigorous magnetic stirring of the sample.

Following analyte extraction, the fibre can be retracted and inserted into a heated GC injection port. At the initial portion of the temperature program, the column is maintained at a temperature which allows the analyte to be condensed onto the head of the capillary column. This desorption step usually takes less than five minutes which leaves the fibre cleaned and ready to undergo the next extraction. The amount of analyte which is sorbed to the SPME fibre is dependent on the volume of stationary phase coating on the glass fibre. Fibre coating thicknesses of 7, 20, 30 and up to 100 μm are available (30 and 100 μm commercially) in the case of the polydimethylsiloxane coating.

An exhaustive extraction does not occur in the SPME technique; however, a linear relationship exists between the amount of analyte sorbed to the fibre coating and the concentration in solution (Zhang *et al.*, 1994). This relationship can be expressed by the following equation:

$$n = K_{SPME} V_S C_{Aq} \qquad \text{(Equation 2.1)}$$

where:

n	=	amount sorbed by fibre (moles)
K_{SPME}	=	distribution constant of the analyte
V_S	=	volume of the fibre coating
C_{Aq}	=	analyte concentration in solution

2.4.2 Applications of SPME

The new polydimethylsiloxane coated solid phase microextraction (SPME) fibre technique first reported by Belardi and Pawliszyn (1989) was successfully used to extract 2-naphthol from aqueous solution and has been further described in a series of recent papers (Arthur and Pawliszyn, 1990; Arthur *et al.*, 1992*a*; Arthur *et al.*, 1992*b*; Arthur *et al.*, 1992*c*; Hawthorne *et al.*, 1992; Louch *et al.*, 1992; Potter and Pawliszyn, 1992; Zhang *et al.*, 1994; Webster *et al.*, 1994*a*; Webster *et al.*, 1994*b*). Thermal desorption of the sorbed BTEX within the GC injector coupled with cryofocussing has been used in conjunction with conventional capillary GC and flame ionization detection (FID), or ion-trap detection (ITD) (Potter and Pawliszyn, 1992). The SPME technique has also been combined with laser desorption/ionization in an ion trap mass spectrometer (Cisper *et al.*, 1994), element-selective detection by GC-atomic emission detection (Eisert *et al.*, 1994),

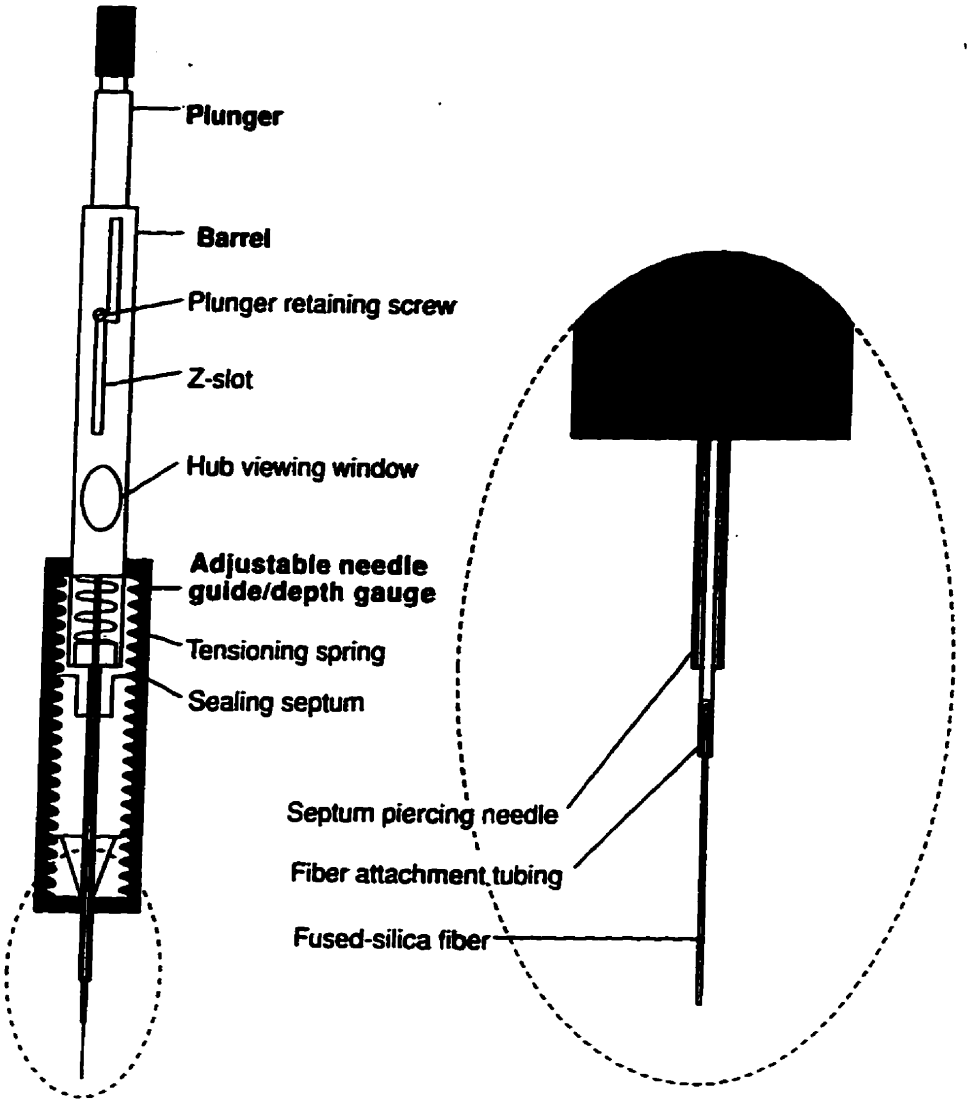


Figure 2.6 **Solid phase microextraction (SPME) apparatus: size comparable to that of a normal GC syringe (Source: Zhang *et al.*, 1994, p. 847A)**

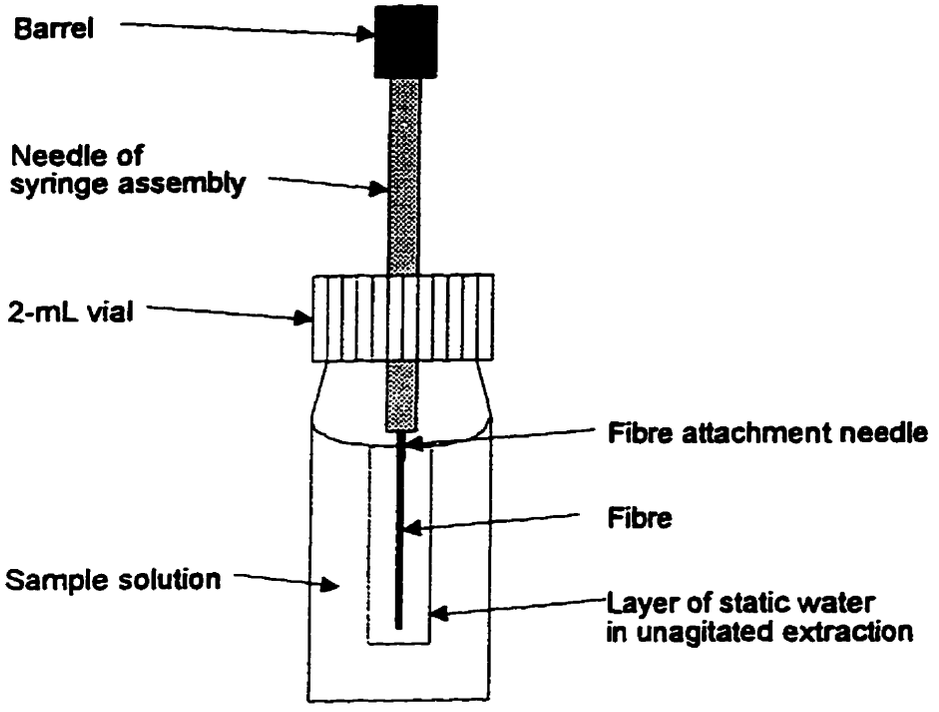


Figure 2.7 **Septum-cap vial with SPME assembly illustrating position of the fibre and septum-piercing needle during direct solid phase micro-extraction showing layer of static water in unagitated extraction (Source: Thomas *et al.*, 1997, In Publication)**

infrared spectroscopy (Heglund and Tilotta, 1996) and Raman spectroscopy (Wittkamp and Tilotta, 1995).

The SPME technique has also demonstrated potential application to the extraction of hexachlorocyclohexanes in soil solutions (Popp *et al.*, 1994; Webster *et al.*, 1994*a*), chlorobenzenes, PCBs, and PAHs (Arthur and Pawliszyn, 1990; Potter and Pawliszyn, 1994), caffeine and fragrances (Hawthorne *et al.*, 1992), ethanol extraction in wine (Urruty and Montury, 1996), US EPA priority pollutants (Arthur *et al.*, 1992*b*), nitrogen and phosphorus containing pesticides (Choudhury *et al.*, 1996), chlorinated herbicides (Webster *et al.*, 1994*b*; Graham *et al.*, 1996) and organophosphates (Fort *et al.*, 1995). SPME has also recently been applied to inorganic ions (Otu and Pawliszyn, 1993) and a SPME/HPLC interface for analysis of explosives and PAHs in water is currently available (Supelco, 1995; Supelco, 1996).

Conventional capillary GC has usually been carried out using nonpolar columns, *e.g.*, DB-1 and DB-5 columns (from J & W Scientific). As mentioned above, use of these columns to separate hydrocarbon analytes thermally desorbed in the injector of the GC has required cryofocussing before the initiation of the GC analysis. Recently, GC analysis of BTEX split-injected in the conventional manner has been reported using the newly developed carbon layer open tubular (CLOT) capillary column (Spock *et al.*, 1992) from Supelco (Canada) Ltd. (Mississauga, ON). A new simple, quick method for analysis of BTEX has been developed from aqueous samples in which the convenience of solventless fibre extraction is mated with the separating power of the CLOT column

without the need for expensive cryofocussing (Sarna *et al.*, 1994). A new fast GC analysis of BTEX has been also been reported (Shirey, 1994*a*; Górecki and Pawliszyn, 1995).

2.4.3 Benefits of SPME

The SPME technique requires less time for extraction than conventional analysis, an important feature in that results can be made available at a fraction of the cost while allowing for faster analysis-dependent decisions. For example, SPME has the potential to be applied in the site assessment process to determine the extent of contamination using a portable GC with minimal capital cost. Work crews would not need to remain idle for long periods while awaiting laboratory analyses. The SPME technique requires no additional equipment except for the SPME sampling device itself and a conventional gas chromatograph. These attributes lend themselves well for applications such as field sampling and preliminary screening work. SPME can be applied to a wide variety of analytes making it suitable for quantitative analysis in the modern analytical laboratory.

SPME has a wide variety of applications and positive attributes. The SPME technique utilizes no solvents except for standard preparation; thus disposal costs associated with the solvents are also drastically decreased. In a world of ever increasing concern for the environment, minimizing solvent usage is both economically and ethically attractive (Pawliszyn, 1995; Webster *et al.*, 1996).

2.4.4 SPME enhancement

The speed with which extraction is carried out is determined by the mass transport of the analytes from the sample matrix to the fibre coating. In the case of direct SPME involving a liquid, the mass transfer rate is determined largely by diffusion rates. Poor sensitivity results if the matrix adsorbs analytes more strongly that the SPME fibre coating does. On the other hand, if the fibre has a stronger ability to adsorb analytes than the matrix does, the analytes begin to partition onto the fibre (Zhang *et al.*, 1994).

Analytical results may be obtained through SPME either through direct extraction as in the case of liquids or headspace analysis for more volatile analytes. Soil samples and other environmental samples are difficult to analyze with SPME as with other more conventional techniques. However, headspace analysis may be conducted for volatile analytes in complex environmental matrices. The interactions between the analyte and the matrix from which the extraction is being carried out is minimal in the case of sand samples in part due to the smaller surface area compared to clays and the lower organic matter content. Sands, however, have relatively weak analyte/matrix interactions which maximizes the efficiency of headspace sampling if the analytes are sufficiently volatile. If the analyte is thermally stable, the sample vial can be heated and/or water added to the matrix to increase the vapor pressure of the analyte and make it more conducive to extraction while increasing sensitivity. Zhang and Pawliszyn (1993*a*) determined that the extraction efficiency of volatile hydrocarbon compounds from soils could be increased if the sample matrix contains 10% water content and the extraction was carried out at higher temperatures (*e.g.*, 50 °C).

The coatings used in SPME technology have a strong affinity for organic compounds (the K_{SPME} values are quite large) allowing the SPME technique to achieve a high concentrating effect (K_{SPME} values refer to the fibre/matrix partition coefficient). The large K_{SPME} values assist in maximizing the effect of 'pulling' the analyte out of the matrix. Exhaustive removal of the analyte is not achieved unless either the K_{SPME} value is large enough and/or the volume of the sample is unusually small. When analyzing solid matrices such as clays, which contain oils, greases, and humic material, sampling from the headspace may be possible by SPME as long as the analyte is sufficiently volatile to be released from the matrix into the headspace (Zhang and Pawliszyn, 1993*a*; Zhang and Pawliszyn, 1993*b*; Zhang and Pawliszyn, 1993*c*). In cases where the analyte is only semi-volatile, the mass transfer rate from the matrix into the headspace above can be impeded and subsequently increase the extraction time required.

In addition to heating the sample, Zhang and Pawliszyn (1993*a*) found that the addition of modifiers to complex matrices such as soils aided in the amount of material that was released to the headspace to be absorbed by the fibre. Modifiers are added to facilitate the release of the analyte into the headspace by affecting the Henry's law constant. In all cases, with BTEX analyses, the mass of the analyte which is absorbed by the SPME fibre significantly increases when adding a saturated aqueous salt solution to the matrix. Heating the sample plus the addition of modifiers to the matrix can minimize the interactions between the matrix and the analyte creating a condition where a larger recovery of the analyte from the matrix is achieved.

There are also instances where the matrix adsorbs analytes more strongly than the fibre coating thereby preventing the analyte from partitioning into the coating of the fibre. The problem can be counteracted by heating the sample which will in turn increase the vapor pressure of the analytes. The kinetic energy input may be enough to dissociate the analytes from solids into the headspace at an increased mass transfer rate. Stirring a liquid within the sample vial achieves a similar effect in that the transfer rate into the headspace increases. However, if the temperature is increased sufficiently, the partitioning into the fibre begins to decrease because the analyte molecules have sufficient kinetic energy to counteract the sorption process and the fibre coating loses its ability to adsorb analytes. Therefore, there is an optimum temperature at which one can increase the sorption of the analytes onto the fibre but at higher temperatures, the kinetic energy of the analyte cause the fibre coating to lose its affinity to retain the molecules.

SPME has applications in headspace analysis of compounds of volatiles (Robacker and Bartelt, 1996) such as substituted benzenes (MacGillivray *et al.*, 1994), volatile chlorinated hydrocarbons (Chai *et al.*, 1993), halogenated volatiles in food and beverages (Page and Lacroix, 1993), flavor volatiles (Steffan and Pawliszyn, 1996), and volatile organic sulfur aromatic compounds in fungi (Pelusio *et al.*, 1995).

2.4.5 Problems associated with SPME

Originally there were reports of possible inconsistencies among fibres produced commercially (Arthur *et al.*, 1992*a*); however, the manufacturer has since resolved complaints associated with the manufacturing process. Fibres manufactured according to new quality control guidelines are more reproducible. The analytical reproducibility expressed as % relative standard deviations (% RSDs) of metolachlor residues in runoff and tile drainage water ranged from 1-10% in 40 environmental samples analyzed (Graham *et al.*, 1996). Similar reproducibility results were published by Buchholz and Pawliszyn (1993) for phenols which had % RSD values of 5 or less. Precision can even be as low as 1% using an autosampler (Zhang *et al.*, 1994). In instances where % RSDs increased as a result of sampling 'dirty' matrices, Tugulea *et al.*, (1997) developed a fibre cleaning technique to rejuvenate fibres where extraction is impeded.

A commonly reported problem in the literature is that of carryover from previously run samples (Young *et al.*, 1996). Longer desorption times are required to desorb analytes from thicker films for analytes which have high K_{SPME} values (Arthur *et al.*, 1992*d*). Problems with carryover for these analytes appear to be much less with the polyacrylate fibre than the polydimethylsiloxane fibre especially with analytes which have high partition coefficients (Webster, 1997, unpublished results). Carryover from previous injections using the SPME fibre can cause later injections to yield inflated results. Carryover also depends on fibre thickness, and the concentration of the analyte, and is more prevalent for the late-eluting compounds (Young *et al.*, 1996).

However, even with current fibre technology, there exists a 1% limit for the presence of a cosolvent (Arthur *et al.*, 1992*b*; Thomas *et al.*, 1996). Organic solvent concentrations above this limit may cause the 100 μm polydimethylsiloxane (PDMS) fibre coating to become loose and slip off the underlying silica fibre. Arthur *et al.*, (1992*b*) reported that the accuracy of SPME becomes affected because as the solvent concentration increases, the distribution constant decreases, and less analyte is able to be sorbed to the fibre coating. Urruty and Montury (1996) showed that the time required to reach equilibrium was a function of ethanol concentration when sampling for residues in wines using the PDMS fibre. They also showed behavior of some analytes towards this technique was not similar in all matrices.

When analyzing soil samples by SPME, external calibration may not be appropriate due to matrix interactions with the analyte (Yang and Peppard, 1994). For this reason, internal standards may be required providing that they are sufficiently similar to the target analytes. However, since SPME is a non exhaustive technique, compounds which have similar partition coefficients need to be used in order to be of any usefulness. This means that spiking isotopically labeled analogues of the target analytes is a logical choice (Zhang *et al.*, 1994). Labelled analogues will have similar chemical and physical characteristics and partition coefficients with respect to the soils and sediments. However, caution is advised because spiking the analyte onto the soils and sediments may result in behavior unlike native sorbed analytes.

2.4.6 Analysis of phenols by SPME

Phenols have been successfully analyzed by SPME at acidic pH and with the addition of NaCl (Buchholz and Pawliszyn, 1993). The decrease in pH protonates the analyte causing a greater affinity for the fibre coating. The 'salting-out' phenomenon decreases the solubility of the analyte in water and forces more of the analyte into the fibre (Buchholz and Pawliszyn, 1994). Buchholz and Pawliszyn (1994) found that Henry's law constants of 2,4-dinitrophenol (1.6×10^{-8} atm m^3/mol) and 2,4-dichlorophenol (4.2×10^{-5} atm m^3/mol) were not high enough to enable the analysis of phenols to be performed by headspace SPME.

2.4.7 *In situ* derivatization using SPME

In situ derivatization using SPME fibres was initially reported by Boyd-Boland *et al.* (1994) by using a derivatizing reagent first and then reacting that with the analytes. Similar work was done using acetic anhydride prior to extracting phenols to form phenol acetates (Buchholz and Pawliszyn, 1994) using the polydimethylsiloxane coating. The derivatizing agent was exposed to the fibre and then analytes were allowed to partition (sorb) to the fibre coating. The method developed were for those phenols regulated by EPA wastewater method 604 Phenols and the acid-extractable section of EPA method 625 which is based on liquid-liquid extraction. Using the method developed, it appeared that 2,4-dinitrophenol and 2-methyl-4,6-dinitrophenol were not derivatized. The formation of phenol acetates resulted in better peak shape and less tailing than the underivatized phenols due to hydrogen bonding with the GC column stationary phases

(Buchholz and Pawliszyn, 1994). Chromatographic separation of phenol and 2-chlorophenol can be difficult when analyzing free phenols; however, derivatization to produce the acetate forms solves this problem (Buchholz and Pawliszyn, 1994).

2.4.8 Calculation of partition coefficients

One of the numerous benefits of SPME is the ability to analyze environmental samples if only a small volume is available. However, analytes which have high distribution constants sampled from small volumes can be significantly depleted after only one injection (Arthur *et al.*, 1992*a*). For example, Langenfeld *et al.* (1996) calculated that the amount of analyte at a concentration of 100 ng/mL with a partition coefficient (K_{SPME}) of 50 extracted from a 1 mL vial would have a mass of 3.34 ng. Since the K_{SPME} value is low, a 2 mL vial would mean that only 3.40 ng of material would be extracted by the fibre. Conversely, an analyte which has a K_{SPME} of 5000 extracted from a 1 mL vial would extract a mass of 77.6 ng versus 126.8 ng if there were 2 mL of sample (Langenfeld *et al.*, 1996). It is important to use similar size aliquots when analyzing environmental samples and standards especially for analytes with high K_{SPME} values. The greater the K_{SPME} value, the lower the detection limits for that compound (Buchholz and Pawliszyn, 1993).

Several authors have correlated log K_{SPME} values with octanol-water partition coefficients (log K_{OW}) (Parkerton and Stone, 1997). Arthur *et al.* (1992*b*) estimated and used log K_{OW} values to predict the linear range and limits of quantification prior to analysis using the polydimethylsiloxane fibre. Both values were found to be in the same order of magnitude

with a trend in a similar direction. A similar study was completed by Dean *et al.* (1996) to estimate log K_{ow} values using the 85 micron polyacrylate fibre. These correlations were found to be useful even in 'dirty' samples (Potter and Pawliszyn, 1994). However, others have questioned this predictive ability between log K_{SPME} and log K_{OW} on the premise that polar compounds have higher log K_{SPME} than non polar compounds with the same degree of hydrophobicity because hydrogen bonding increases the interaction with the polyacrylate fibre (Vaes *et al.*, 1996*a*). Nonetheless, characteristic relationships will likely be valid for individual classes of compounds.

2.4.9 SPME measures available residue

SPME measures the concentration of analyte which is freely available (Vaes *et al.*, 1996*b*). Vaes *et al.* (1996*b*) reported that protein neither sorbs to the fibre nor influences the amount of analyte which sorbs to the fibre in comparisons between SPME and equilibrium dialysis. Analytes used in this study included aniline, nitrobenzene, 4-chloro-3-methylphenol and 4-*n*-pentylphenol and were added to buffered bovine serum albumin (BSA) solutions. These results using a polyacrylate fibre showed no statistically significant difference between the free concentration on the inside and outside of the dialysis tube in the equilibrium dialysis experiment. These results show that the protein is not adsorbed to the fibre nor does it influence the amount of phenolic analyte which is sorbed by the fibre.

However, it has been reported by Vaes *et al.* (1996*b*) that the possibility exists for equilibrium with organic matter to become upset when sampling from vials as the freely

available analyte is sorbed to the fibre causing bound residues previously unavailable to become freely available. As the analyte is taken up by the SPME fibre, there will be a shift and subsequent rebalancing in the matrix/water equilibrium possibly increasing analyte concentration providing the rate of desorption is great enough (*i.e.*, ≥ rate of sorption by the fibre). Similar results were reported by Wan *et al.* (1994) who determined that extraction of 2-chlorophenol, lindane, and methyl parathion on pencil lead was not affected by the presence of dissolved humic substances at a concentration of 10 mg/L. The amount of each analyte extracted was the same in distilled water as was extracted in water which had a humic acid concentration of 10 mg/L.

Appreciable decreases in aqueous phase concentrations when performing biomimetic extractions are possible as a result of utilizing the SPME technique. Parkerton and Stone (1997) calculated that using the polydimethylsiloxane fibre to extract various hydrocarbons (log $K_{ow} < 7$), less than 10% of the contaminant would be removed from the aqueous phase. A 10% removal of the analyte would not cause a significant shift in the equilibrium thereby causing additional analyte to move into the aqueous phase. Significant removals of the analyte from the aqueous phase causes the equilibrium to shift and cause more analyte to become freely dissolved. This would not simulate biomimetic extractions and cause an increased amount of analyte to be sorbed to the fibre.

2.4.10 Comparison of SPME to other extraction techniques

SPME has been compared to a variety of other extraction techniques. Liquid-liquid extraction gave a lower analytical result than SPME but % RSDs were much better for liquid-liquid extraction on work with organochlorine pesticides in water compared to extraction using a PDMS fibre (Young *et al.*, 1996). SPME has been compared favorably to results of SPE and ELISA for metolachlor in surface runoff and tile drainage water (Gaynor *et al.*, 1996). Slopes of regression lines and intercept values comparing SPE-GC, and two different immunoassay techniques did not differ from unity compared to SPME-GC analysis using the 100 μm PDMS fibre.

MacGillivray *et al.* (1994) compared headspace SPME for BTEX (benzene, toluene, ethylbenzene, and the xylenes) in water versus purge and trap technology using the PDMS fibre for headspace extractions. A strong correlation was seen between the SPME headspace method and the purge and trap method. A consistently high bias among the analytes was noticed towards headspace SPME as illustrated by slope values being greater than one (1.05 to 1.07) when comparing headspace SPME to purge and trap. However, unity values for the calculated linear regression equations were within confidence intervals.

2.4.11 Equilibration times

Arthur *et al.* (1992*b*) reported long equilibration times as a result of large distribution coefficients. This was often further compounded by inefficient mixing which produced a thin unstirred layer of water adjacent to the fibre limiting the rate of diffusion by analytes across this static layer (Arthur *et al.*, 1992*c*). A more efficient means of agitating solutions was required to minimize the static layer so that mass transport of the analyte into the fibre coating was only limited by diffusion (Louch *et al.*, 1992). The stationary layer of water next to the fibre was found to be minimized by using cross shaped stir bars as opposed to hexagonal stir bar which left a thicker stationary layer (Arthur *et al.*, 1992*d*). Thomas *et al.* (1997) used an agitation device to vibrate samples to reduce equilibration times and improve limits of detection for BTEX and polycyclic aromatic hydrocarbons (PAHs) using a 100 μm PDMS fibre and GC-FID detection. Limits of detection for the compounds studied ranged from 48-526 pg/mL for vibration enhanced versus 117-661 pg/mL for static extraction (Thomas *et al.*, 1997).

2.4.12 Further developments of SPME

Future developments in the area of SPME include further automation of the SPME technique (Berg, 1993), new coatings with molecular recognition capabilities and therefore more specific extraction abilities (Zhang *et al.*, 1994), on-line monitoring of flowing samples (Motlagh and Pawliszyn, 1993) and further development of internally cooled SPME fibres by heating the matrix while simultaneously cooling the fibre coating (Zhang and Pawliszyn, 1995).

2.5 Bioavailability and Toxicity

An analytical result representing the contaminant concentration in sediment does not reflect the bioavailable fraction of the xenobiotic which is associated with sediments as current extraction techniques aim to liberate 100% of the analyte regardless of how it is held within the matrix. Therefore, a more accurate measure is required to define the fraction of total contaminant available for biological uptake. Bioavailability is dependent on many variables such as the nature of the contaminant, the organism involved and the feeding patterns. However, with organisms whose only route of exposure is through the freely dissolved portion of the analyte (*i.e.* gill breathing organisms), the SPME technique may provide a means of determining bioavailability. As SPME is a non exhaustive extraction technique and operates on the basis of partitioning, it offers a better choice providing methods *viz.*, volatilization into the sample headspace or dissolution into soil or sediment water are available to transfer the residues to the fibre. Soils, sludges, and other complex matrices such as biological tissue provide the analyst with analytical challenges, some of which may be addressed conveniently through applications of SPME.

The concentration and nature of dissolved organic material (DOM), or humic substances, contained in an environmental sample help to determine the extent to which a given contaminant is held (see also p. 39). The moieties of significance in DOM include the carboxylic and phenolic functional groups. The formation of covalent bonds between the

xenobiotic and the humic material or penetration into the humic material matrix may account for the fact that some residues can not be released by Soxhlet extraction with methanol or by supercritical fluid extraction techniques. The only way to break these covalent bonds or to free the entrapped residue is by using chemical methods such as hydrolysis or degradative oxidation to liberate the previously bound xenobiotic (Scheunert *et al.*, 1992). Therefore, if the xenobiotic is bound tightly enough to the DOM, the bioavailability of the xenobiotic is substantially decreased.

Bioavailability of contaminants in sediment can be defined as "the fraction of the total contaminant in the interstitial water and on the sediment particles that is available for bioaccumulation" (Landrum and Robbins, 1990, p. 238). The magnitude of this decrease is directly proportional to the extent of binding between the organic contaminant and the organic matter (Kukkonen *et al.*, 1991) and this complexation will reduce the amount of xenobiotic which is bioavailable because in order for a xenobiotic to be bioavailable to gill breathing organisms, it must first be freely dissolved (Landrum *et al.*, 1985). The degree of toxicity a given compound will have in an aquatic system is dependent on the extent to which it is affected by the binding affinity of the DOM in natural waters. Compounds with smaller K_{ow} values are less strongly sorbed to DOM and therefore more bioavailable because less of the residue will be sorbed and desorption of the sorbed residue from the matrix will be easier (Landrum and Robbins, 1990).

The sorption of xenobiotics by dissolved humic material increases with increasing dissolved organic carbon (DOC) (Carter and Suffet, 1982; Chin *et al.*, 1991). The

binding of xenobiotics to organic matter reduces the toxicity to organisms, reduces leaching and transportability of pollutants (Bollag and Meyers, 1992). The importance of the functional groups contained within the organic matter such as phenolic and especially carboxylic acid moieties dictates how strongly such xenobiotics will be held (Maqueda *et al.*, 1993). The variable nature of DOM means that total concentration of DOM alone is not a good predictor of the sorption capacity (Kukkonen *et al.*, 1991) (see also p.38).

The predicted degree of binding between a contaminant and the humic material present in the system can be expressed as an organic carbon normalized partition coefficient K_{OC}. These K_{OC} values can be used to predict the bioavailability in the presence of DOM (Kukkonen and Pellinen, 1994). Xenobiotics bound to the organic material are consequently not bioavailable to zooplankton at the lower end of the food chains. Contaminants can also be transferred higher up the aquatic food chain to larger organisms such as fish if a xenobiotic is bioavailable (Kukkonen and Pellinen, 1994).

Gobas (1997) concludes that adsorption (*i.e.*, on surfaces) and absorption (*i.e.*, into particles) of a xenobiotic in an aquatic system makes the sorbed compound unavailable for uptake via the gills in fish. Large particles are not believed to be able to cross gill membranes thereby reducing the amount of a particular chemical which is actually bioavailable for gill uptake. Biological organisms, especially fish and *Daphnia* species are especially useful in measuring the bioavailable chemical concentration in aquatic ecosystems. Some contaminants may be sorbed to particulate matter and ingested eventually passing through the gastro-intestinal (GI) tract wall (Gobas, 1997). However,

the analyte must be freed from the food particle into solution to make it bioavailable before it can be absorbed.

The fate of organic compounds in an aquatic environment is largely controlled by the association that the xenobiotic has with the organic matter present in the system. Dissolved organic matter (DOM) has also been viewed as providing a 'cosolvent effect' (Gobas and Zhang, 1994). The presence of DOM in a system and this 'cosolvent effect' affects the extent to which organic chemicals are freely dissolved and either sorbed or bound in water to the humic material in a partition-like process. The cosolvent approach suggests that there will be little or no effect on the bioavailability of xenobiotics because the fraction that is freely dissolved in natural waters remains unchanged (Gobas and Zhang, 1994).

Kukkonen *et al.* (1991) found that the accumulation of benzo[a]pyrene by *Daphnia magna* was reduced by increasing DOM concentration. They further found that DOM concentration is not only important but also the quality of DOM has an impact on the bioavailability of organic xenobiotics. The water sample was fractionated into the following components, namely, hydrophobic acids, hydrophobic neutrals, and hydrophilic compounds. With respect to the benzo[a]pyrene (BAP), the hydrophobic acid component had the greatest binding affinity while the hydrophilic component had the least. Not surprisingly, the hydrophobic acids played the greatest role because of the carboxylic and phenolic functional groups interacting with the xenobiotic (Kukkonen *et*

al., 1991). However, the hydrophilic acids still had a certain amount of interaction with the xenobiotic and should not be discounted.

If a pollutant is sorbed to sediments, the ability of the organism to take up the sorbed pollutant will be impeded (Karickhoff and Morris, 1987). The biological uptake of hydrophobic xenobiotics depends to a large extent on the organic carbon content of the sediment, the lipid content of the organism and the relative affinities of the compound for organic carbon versus animal lipid. However, in instances where there has been a depletion of sorbed compounds, the extent to which bio-uptake proceeds will be limiting. Whether or not a chemical is sorbed to the matrix and remains so, relates to the sorption theory of xenobiotics to organic matter. The xenobiotic may not be bioavailable simply because it is physically inaccessible and incorporated deep within the sediment particle or DOM. If a xenobiotic is taken up through the gill membranes of a fish, the freely dissolved portion in the water column decreases and therefore desorption from sediments may be kinetically favored by this upset in the steady-state equilibrium.

The interaction of organic contaminants with naturally occurring organic matter plays an important role in the environmental fate of such compounds (Gobas and Zhang, 1994). There are numerous sources in the literature of the sorption phenomena to organic matter mostly in reference to the more lipophilic compounds. However, this was also found to be true for decreasing the quantity of freely dissolved dehydroabietic acid with increasing DOC (Kukkonen *et al.*, 1991). Piccolo (1996) reported adsorption of glyphosate by

humic substances reaching more than 7500 μg of glyphosate/g adsorbent at a 250 μg/mL concentration. Rochette *et al.* (1996) concluded that 2,4-D extractability was affected by the organic matter in their SFE method. Recoveries were as low as 11 ± 2% of spiked 2,4-D using sodium humate as a humic material.

The binding of the analyte to dissolved organic matter (DOM) and the way in which such bound analyte might be freed by conventional solvent extraction is directly related to the nature of the DOM. A reduction in extraction efficiency caused by the presence of DOM in river water means that the chemical loading to aquatic ecosystems may be significantly underestimated (Driscoll *et al.*, 1991). However, it is important to note that total concentration is not the only criteria but also chemical variability of the DOM. The wide range of partition coefficients for individual xenobiotics can be attributed to the variability of DOM from natural waters sampled at different locations (Kukkonen *et al.*, 1991).

Driscoll *et al.* (1991) found that the recoveries of chlorinated hydrocarbons from river water by conventional solvent extraction decreased with increasing log K_{ow} values. Recovery of the organics decreased with increasing exposure time in the natural water containing between 2-7 mg/L DOC. The addition of dissolved commercially available humic acid extracts to solutions of chlorinated hydrocarbons make these lipophilic compounds less bioavailable and therefore less toxic towards organisms (Driscoll *et al.*, 1991). Similar results were also found using straight chain alkanes and polycyclic

aromatic hydrocarbons by Maguire *et al.*, (1993). Maguire (1994) found that commercially available humic acids markedly increased the binding efficiency of the analyte to the humic acid material.

Driscoll *et al.* (1991) also reported that as time to equilibration with the DOM increased, the extraction efficiencies by conventional extraction decreased with the exception of the method incorporating destruction of DOM with chromic acid oxidation. The use of chromic acid oxidation/extraction enabled quantification of the total analyte found within the sample in all forms (Maguire, 1994). The chlorinated hydrocarbon analytes were not destroyed when employing this analysis method. The results obtained with the chromic acid digestion extraction technique were also found to be more reproducible than those for the conventional solvent extraction. Similar conclusions have been drawn with respect to supercritical fluid extractions which yield larger values than traditional solvent-based methods for a variety of organic compounds which have thus underestimated many residues (Hawthorne *et al.*, 1993; Hawthorne and Miller, 1994; Khan, 1995).

The kinetics behind the sorption-desorption process for the acid herbicide picloram and soil have been described by McCall and Agin (1985). Desorption from external binding sites of organic matter in soil occurs relatively quickly, whereas desorption from the more tightly bound internal sites can take many hours before equilibrium is attained (two-step sorption-desorption mechanism). The longer a contaminant is associated with the environmental sample, the greater the fraction sorbed to the internal sites.

The humic and fulvic material contained in environmental samples provides an opportunity for the analyte to be trapped on the surface of the organic matter via chemisorption or physisorption processes. In order for the analyte to be analyzed it must be desorbed from the organic matter surface and diffuse though the matrix. The external sites permit sorption to occur much more quickly that the internal sites. They appear to accommodate a constant amount of analyte whereas the internal sites continue to sorb more of the analyte over a much longer period of time.

This process is known as mass transport as the analyte moves from the matrix to the matrix-fluid interface (Langenfeld *et al.*, 1995). The external sites may represent soil surfaces or humic materials contained within the soil matrix while the internal sites may represent areas in the soil particles that are more resistant to diffusion because of size restraints. If an analyte becomes associated with dissolved organic matter, the effect on the degradation and ultimately the bioavailability of the analyte is decreased, however, the actual mechanism in which this occurs is somewhat unclear.

Maqueda *et al.* (1993) determined that the number of carboxyl and phenolic groups could be correlated with increasing sorption of the analyte using spectroscopic determination. Their modelling of the number of binding sites always predicted that the total observed was less than the predicted values. This was explained by a steric hindrance effect not permitting binding of the analyte in all locations that are chemically suited at a given time (Maqueda *et al.*, 1993).

Li *et al.* (1992) proposed a model to account for the binding of organic contaminants with humic materials. This model was a discrete binding site model which accounts for the limited binding capacity of Laurentian fulvic acid for the herbicide atrazine. Li *et al.* observed that where the fulvic acid carboxyl group deprotonation occurs, the binding to the fulvic acid was inversely related to pH. As the pH decreases, the degree of protonation of the fulvic acid carboxylic groups increase. With respect to atrazine binding capacity of humic materials, there is a strong correlation with the number of protonated carboxyl groups, but with a participation of those sites of 1% or less. This was explained by the formation of structure-specific binding sites on the humic substances analogous to those encountered in enzyme biochemistry models as a result of the effects of tertiary structure. Competition between the sorption of hydroxyatrazine versus atrazine was not seen to exist within these structure-specific binding sites (Li *et al.*, 1992).

A soil associated pollutant which follows Karickhoff's sorption theory (partition model) is assumed to be dissolved in a solid solution form in the organic matter of the soil particle. The pollutant partitions itself between the water and the organic content of the soil particulate matter. This can be represented by the organic carbon-based sorption coefficient K_{oc} which is equal to the sorption coefficient to particulate matter divided by the fraction of organic carbon (McCall and Agin, 1985).

$$K_{OC} = \frac{C_{OC}}{C_{WD}} = \frac{C_P}{(OC \times C_{WD})} = \frac{K_P}{OC} \qquad \text{(Equation 2.2)}$$

where: C_{OC} = chemical concentration in organic matter of particles (ng/kg OM);
C_{WD} = freely dissolved chemical concentration in water (ng per litre);
C_P = chemical concentration in the particulate matter (ng/kg of particle);
OC = fraction of OM in the particle (kg of OM per kg of particle); and
K_P = sorption coefficient to particulate matter (L of water per kg of particle).

Humic substances are influential in the adsorption and desorption of analytes on and off of the matrices involved. Chiou *et al.* (1986) determined that the adsorption, complexation, or binding of dissolved humic material enhanced the apparent water solubility of certain xenobiotics. Increased true water solubility would result in increased bioavailability and toxicity of the xenobiotic in the aquatic ecosystem because the freely dissolved concentration would also increase.

There are few conclusions on this subject matter in the scientific literature. However, it is clear that the actual quantity of analyte which is bioavailable is of increasing importance. Parkerton and Stone (1997) utilized the approach of biomimetic extraction which is the process in which analytes are allowed to partition to a surrogate lipid phase mimicking bioconcentration. The time to reach sorption to the point of equilibrium using semi-permeable membrane devices may be weeks. A similar much more convenient biomimetic extraction technique has been developed through the use of SPME using a wide variety of extracting fibres.

3. MATERIALS AND METHODS

3.1 Chemicals and Equipment used

3.1.1 Chemicals

Chlorophenols and chloroanisoles with one to five chlorine substituents were obtained from Ultra Scientific, Hope, RI, USA. Herbicide standards of 2,4,5-T (EPA-Research, Triangle Park, NC, USA), triclopyr (Riedel-de Haën, AG, Seelze, Germany), 2,4-D and dicamba were used as received from Agriculture Canada, Ottawa, ON. Distilled-in-glass (DIG) methanol was obtained from Anachemia Science, Winnipeg, MB. Precleaning of reagent grade salts was achieved by baking in a muffle furnace overnight at approximately 500 °C in order to eliminate any interferences which might be caused by volatile impurities in the reagent grade material.

3.1.2 Diazomethane preparation

The diazomethane (CH_2N_2) was prepared according to Stanley (1966). Potassium hydroxide (KOH) (2.3 g) (Aldrich Chemical Company, Inc., Milwaukee, WI, USA) was dissolved in 2.3 mL of distilled water in a 125 mL Erlenmeyer flask and cooled. A 25 mL aliquot of ethyl ether (Mallinckrodt Canada Inc. Pointe-Claire, QC) was added and the solution was cooled prior to adding 1.5 g of precursor (*N*-methyl-*N*'-nitro-*N*-

nitrosoguanidine) obtained from Aldrich Chemical Company, Inc. (Milwaukee, WI, USA). The precursor was added slowly and the flask was shaken vigorously after each addition of precursor. The ether layer (*ca.* 16 mL) was decanted and stored at -20 °C for up to one week in a tightly closed container.

Caution

> *Diazomethane is carcinogenic and mutagenic and its precursor is a potent mutagen. Care must be taken in the preparation and handling of diazomethane in the laboratory. Do not use ground glass joints (risk of explosions). All diazomethane procedures must be carried out behind a safety shield in a fume cupboard. Store excess diazomethane solutions in a freezer when not being used. The use of gloves, face shields, and other personal protective equipment is imperative* (Stanley, 1966; Cruz and Wells, 1992).

3.1.3 Instrumentation

Supelco manual and autosampler SPME fibres with 85 μm polyacrylate coating (Catalogue #5-7304) and 100 μm polydimethylsiloxane (Catalogue #5-7300) were used (Sigma-Aldrich Canada Inc., Mississauga, ON). A Varian 3400 GC-ECD and a Varian 3400 GC-FID (Varian Canada Inc., Mississauga, ON) equipped with split-splitless injection systems were used in conjunction with Varian Star 4.02 software. A Varian 8200 autosampler modified for SPME was used for autosampler applications.

Confirmatory work was performed using a Hewlett-Packard 5890 GC (Hewlett-Packard Canada Ltd., Mississauga, ON) equipped with a Finnigan 806 ion trap detector (Technical Marketing Associates, Vancouver, BC).

3.2 Experimental Procedures

Experimental procedures are described in the order encountered by the analyst.

3.2.1 Preparation of solutions in methanol

Individual stock solutions of the chlorophenols and chloroanisoles were prepared in distilled-in-glass (DIG) methanol as were standards of 2,4,5-T, triclopyr, 2,4-D and dicamba. These were further diluted with methanol for manual liquid injection purposes. Mixtures of the chlorophenols, chloroanisoles, and acid herbicides were prepared having a final concentration of 20 ng/μL. These mixtures were further diluted with methanol.

3.2.2 Preparation of solutions in water

Using the stock solutions prepared in methanol, dilutions with HPLC grade water were made to achieve the following concentrations: 0.01, 0.05, 0.1, 0.25, 0.5, 0.75, and 1.0 μg/L. A 4 *M* NaCl (233.8 g NaCl in 1 L HPLC grade water) solution was adjusted to pH 1.7 and used as the diluent for the chlorophenols and acid herbicides. Aliquots (1.5 mL) of the spiked solutions were placed in autosampler vials with Teflon coated septa. Each vial contained a small Teflon-coated magnetic stir bar (7 x 2 mm) which was used to

agitate the solution. A standard curve based on triplicate analysis was constructed from these standards.

3.2.3 Direct liquid injection

The chloroanisole standards were diluted with methanol to concentrations less than 10 ng/μL. A 1 μL aliquot was used for quantitative GC in the preparation of standard curves. The purge time for the GC method was adjusted to 0.45 min for manual work. See Appendix II for chloroanisole and acid herbicide calibration curves.

3.2.4 Fibre conditioning procedure

The 85 μm polyacrylate fibre was conditioned in the GC injection port for at least 3 h at a temperature of 250 °C. This was sufficient to remove any interferences that might appear in the chromatogram. Periodically, blanks were run to establish that complete desorption and no carryover or extraneous contamination was occurring.

3.2.5 Equilibration and ionic strength study

In order to determine equilibration times, a 1.5 mL aliquot of a solution containing 0.5 μg/L of the acid herbicide mixture was analyzed as described earlier with varying extraction time. Fibre extraction times of 2, 5, 10, 15, 20, 25, and 30 min were conducted and reported. The variation in partition coefficients of the acid herbicides at a variety of ionic strengths ranging from 0.1, 0.25, 0.5, 1, 2, and 4 *M* NaCl were also examined. The

acid herbicides mixture was acidified to pH 1.7 and an aliquot of 1.5 mL having a concentration of 0.5 μg/L was placed in each vial.

3.2.6 Manual SPME without derivatization

The vials were placed on a magnetic stir plate and the fibre was extended through the Teflon-coated septa into the solution, allowing for sorption of the analytes. After a 20 min sorption period, the fibre was retracted into the SPME device. The syringe needle was then inserted into the injection port of the GC to thermally desorb the analytes into the chromatographic system.

3.2.7 Fibre rinsing procedure (manual SPME)

In order to avoid salt buildup on the polyacrylate fibre, an additional rinsing step was employed to remove the salt residues. The fibre was extended into a vial containing HPLC grade water and stirred for approximately 1 min. The fibre was retracted into the SPME sampling device and thermally desorbed into a hot injection port for three min. Periodically, blanks were run to establish that complete desorption and no carryover or extraneous contamination were occurring.

3.2.8 Direct liquid injection incorporating derivatization step

For manual liquid injection purposes, the corresponding methyl esters and ethers were prepared by adding enough of the diazoalkane-ether solution until the solution remained yellow. After a period of a few minutes, the ether was evaporated to less than the original

volume under a gentle stream of nitrogen. The sample extract was then made up to a predetermined volume and an aliquot (1 μL) used for quantitative GC. The purge time used for the GC method was 0.45 min for manual injection. See Appendix II.

Table 3.1 shows examples of analyte methylation. All compounds referred to in text were methylated in a similar fashion.

3.2.9 SPME extraction and derivatization procedure

Approximately 100 μL of diazomethane solution was transferred into a 2 mL vial fitted with a Teflon®-lined septum screw cap and kept in a freezer until required. The SPME fibre containing the extracted analyte was then inserted into the vial and fibre exposed to the headspace over diazomethane solution. The fibre was held in this position for a period of one minute following which it was retracted into the protective sheath and desorbed in the heated GC injection port. Calibration curves were constructed for seven concentrations, 0.01, 0.05, 0.1, 0.25, 0.5, 0.75, and 1 μg/L, prepared in the 4 *M* NaCl solution (Section 3.2.2).

3.2.10 Autosampler considerations

The chloroanisole mixtures were run using the 48 x 2 mL autosampler vials in conjunction with Varian Star 4.02 software and the Varian 8200 autosampler modified for SPME work. Similar conditions applied as for manual SPME work with the exception of agitation. Acid herbicides could not be analyzed using the autosampler as it

would not permit their analysis using the SPME-alkylation technique. The SPME-alkylation technique was performed manually.

3.2.11 Agitation mechanism

An agitating device was designed and subsequently constructed in association with the Department of Biosystems Engineering to be incorporated with the Varian 8200 autosampler. This simple yet innovative agitation device, coined the 'Woodpecker', was mounted on the GC adjacent to the autosampler and worked by agitating the sample carousel of the autosampler as seen in Figure 3.1 while the SPME fibre was immersed in the liquid. Two optical sensors controlled the beginning and ending of vibration by activating an on/off switch. These sensors ensured that agitation occurred only while the fibre was immersed in the liquid. This equipment was run externally to the Star 4.02 software but did not interfere with the extraction process.

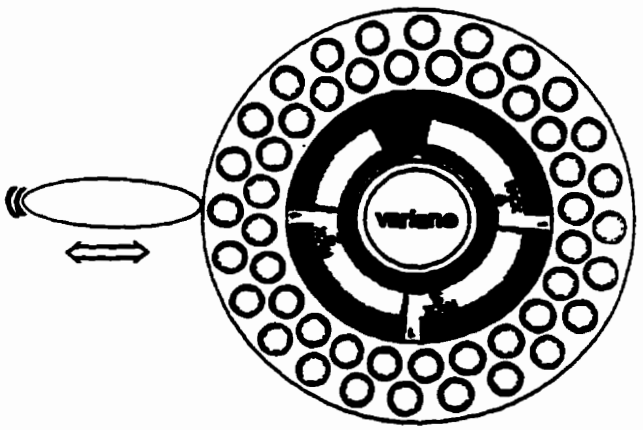
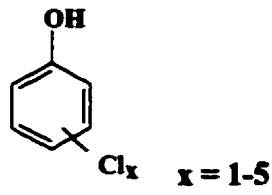
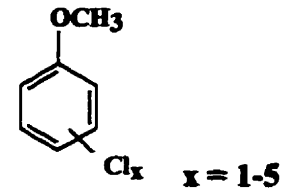
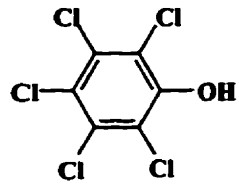
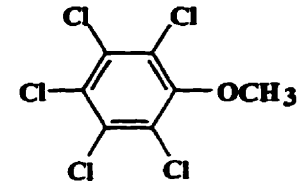
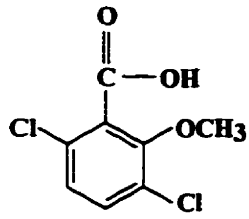
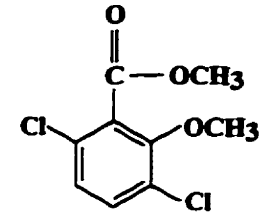
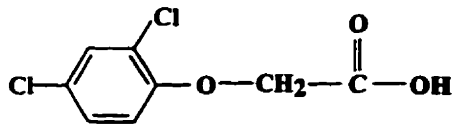
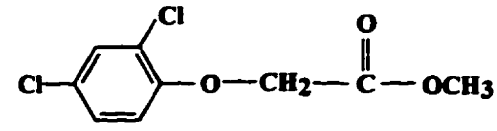
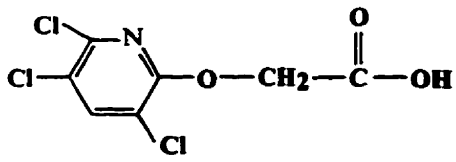
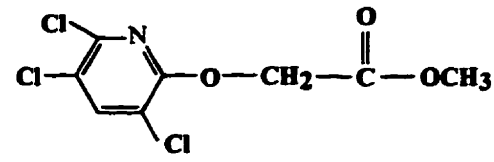


Figure 3.1 'Woodpecker' apparatus to agitate sample vials contained in Varian 8200 autosampler sample carousel

Table 3.1 Conversion of extracted analyte to methyl derivative using diazomethane

Extracted Analyte	Derivatized Product
 chlorophenol	 chloroanisole
 pentachlorophenol	 pentachloroanisole
 dicamba	 dicamba methyl ester
 2,4-D	 2,4-D methyl ester
 triclopyr	 triclopyr methyl ester

In order to compare equilibrium curves, chloroanisole standards of equal concentration were run with three different treatments, using manual stirring, the conventional static autosampler, and the 'Woodpecker' enhanced autosampler. Authentic chloroanisole standards were used to test the agitation device because these were the products produced using the methylation process.

3.2.12 Fibre rinsing procedure (automated SPME)

Samples were analyzed using only the outer ring of the sample carousel. In the inner row, HPLC water blanks were used to perform a fibre rinse. The fibre was desorbed in between samples to avoid contamination. The additional fibre rinsing step was added because salt buildup on the fibre was anticipated to reduce sorption capacity of the fibre. The salt was easily removed using this fibre rinsing protocol prior to analysis of the remaining vials. The performance of the 85 μm polyacrylate fibre was impeded neither by salt concentration nor by pH adjustment.

3.3 GC Methods

3.3.1 GC-ECD method

Analyses were performed using a 30 m 0.25 mm id DB-5 capillary column with a 0.1 μm film thickness (J & W Scientific, Inc., Folsom, CA, USA) in splitless mode.

Chromatographic conditions used on the GC-ECD were as follows: carrier flow rate (He)

3 ± 0.2 mL/min, make-up flow rate (N_2) 28 ● 2 mL/min. All gases were of pre-purified grade and obtained from Welders Supplies Limited (Winnipeg, MB). The detector conditions were as follows: signal to noise ratio 5:1, range 1, attenuation 4, tangent height percentage 10%, detector bunch rate 4 points (10.0 Hz), and monitor length 64 bunched points (6.4 s).

The use of SPME fibres necessitated the frequent replacement of injection port septa because of the tendency for the SPME septum-piercing needle to cause septum coring. When performing automated work, the septum was replaced every 48 samples regardless of whether or not injection port pressure indicated replacement was required.

3.3.2 GC-FID method

Analyses were performed using a 30 m 0.25 mm id DB-5 capillary column with a 0.1 μm film thickness (J & W Scientific, Inc., Folsom, CA, USA) in splitless mode. Chromatographic conditions used on the GC-FID were as follows: carrier flow rate (He) 30 mL/min, make-up flow rate (H_2) 30 mL/min and air 300 mL/min. The detector conditions were as follows: signal to noise ratio 5:1, range 1, attenuation 4, tangent height percentage 10%, detector bunch rate 4 points (10.0 Hz), and monitor length 64 bunched points (6.4 s).

3.3.3 GC-MS method

Analyses were performed using a 60 m 0.25 mm id DB-5 capillary column with a 0.1 μm film thickness (J & W Scientific, Inc., Folsom, CA, USA) in splitless mode. Chromatographic conditions used on the GC-MS were carrier flow rate (He) 1 mL/min. The ion detector was run in full scan mode with a scan range of 50-350 amu (scan time 1.0 s). The multiplier was maintained at 1700 V with a delay of 180 s. Temperatures of the transfer line were maintained at 275 °C, manifold 225 °C, open split 270 °C, and exit nozzle at 260 °C.

3.3.4 Temperature programs

The temperature programs used for the chlorophenols, chloroanisoles, and acid herbicides are described in Table 3.2.

Table 3.2 **GC methods for chlorophenol and acid herbicide analysis** (Analytes in 1.6 mL solution were extracted by direct SPME for 20 min with stirring. Solutions were prepared in 4 *M* NaCl solution and acidified to pH 1.7. All temperatures in °C.)

Method Parameter	Chlorophenol[1] GC-ECD/FID	Acid Herbicide GC-ECD/FID	Validation Method GC-MS
Injector	220°	220°	220°
Detector	300°	300°	300°
Temperature Program	40° (4 min), 12°/min to 260°, (2.67 min)	100° (0 min), 7.5°/min to 200° (2 min), 20°/min to 280° (0.67 min)	60° (0 min), 4°/min to 300°, (0 min)
Purge On	3 min	3 min	3 min
Total Run Time	25 min	20 min	60 min
Derivatization	Optional	Yes	Yes

[1]and methyl derivatives (chloroanisoles)

3.4 Analysis of Fortified Natural Waters and Soils

3.4.1 Water samples

Water samples (1 L) were collected on May 27, 1996 from the La Salle River (NE¼ 25-11-5 W1) near Oakville, MB approximately 50 km west of Winnipeg, MB. In addition to the La Salle River water samples, well water was sampled at a domestic well located at NW¼ 19-11-4 W1 near Oakville, Manitoba. A Red River sample was collected from the boat launch in St. Vital Park on the east side of the Red River.

Water samples were stored in clean amber glass bottles with Teflon®-lined caps which had been triple rinsed with hexane and acetone. Samples were collected without any headspace in order to minimize any volatile losses and stored at 4 °C. The acid herbicide mixture was also spiked into water obtained from the La Salle River to demonstrate the potential of SPME as a viable extraction technique for environmental water samples containing from 0.1 μg/L to 1.0 μg/L of these analytes.

3.4.2 Soil samples

The Osborne clay was collected approximately 2 km south of Brunkild, MB. The legal land description for this parcel is NW¼ 17-7-1 W1. The Miniota sand was collected from the W½ 35-9-17 W1 approximately 10 km southwest of Douglas, MB under native vegetation. In both cases the soils were ground and sieved (28 mesh) to aid handling in the laboratory studies. More information regarding the two soils can be found in Appendix I (Ehrlich *et al.*, 1953; Ehrlich *et al.*, 1957; Michalyna *et al.*, 1976; Michalyna *et al.*, 1988).

3.4.3 Analysis of water and soils

Soil extracts were prepared using a 2:1 ratio of 4 *M* NaCl solution to air-dried soil. The slurry was shaken for 1 h followed by centrifugation at 1500 rpm for 15 min. The supernatant was prepared by passing the extract through a 0.45 μm GF/C filter. Extracts were analyzed for dissolved organic carbon (DOC) at the Water Chemistry Laboratory at the Freshwater Institute, Canada Department of Fisheries and Oceans, Winnipeg, MB

(Stainton *et al.*, 1977). Samples were oxidized using potassium persulphate to form carbon dioxide which was trapped onto a molecular sieve column and quantified using an infrared detector.

Organic carbon measurements were determined using the Yeomans and Bremner (1988) method. Conductivity and pH measurements were determined in soils on a 2:1 ratio (air-dried soil). Particle size analysis was performed and determined using the Gee and Bauder (1986) method. Particle size determination was used to classify the soil according to texture.

3.4.4 Trace analysis of water

Environmental water samples (250 mL) were spiked with 2,4,5-T, dicamba, triclopyr, and 2,4-D at three concentrations namely, 0.1, 0.5 and 1.0 μg/L. A 1.5 mL aliquot was placed in 2 mL autosampler vials along with a Teflon-coated magnetic stir bar. The polyacrylate fibre was exposed to the stirred solution for 20 min. The SPME fibre was retracted and derivatized for 1 min using diazomethane as mentioned earlier. Following derivatization, the fibre was thermally desorbed in the GC injection port in the usual way.

3.4.5 Trace analysis of soils

Three matrices were used when studying the analysis of soils. These were glass beads, Miniota sand, and Osborne clay. Twenty mL of 4 *M* NaCl was added to 10 g of each matrix. The vial was shaken for 1 h, and then centrifuged at 2000 rpm for 15 min. The

supernatant recovered (approximately 15 mL) was then acidified to pH 1.7 before extracting with the SPME fibre and followed by derivatization.

3.5 Examination of SPME Method

3.5.1 Calculation of partition coefficients (K_{SPME})

Using Equation 2.1 describing the relationship between the amount of analyte sorbed to the fibre coating and the concentration in solution, partition coefficients (K_{SPME}) were calculated. In order to calculate the value, three variables are required. These are the amount of analyte sorbed by the fibre (n) in moles, the concentration of the analyte in solution being sampled (C_{Aq}) in moles per litre and the volume of the fibre (V_S) in litres. Rearrangement of Equation 2.1 and knowing the above three values allows one to calculate the partition coefficient (K_{SPME}) for the analytes used in this study.

The amount of analyte sorbed by the fibre (n) was calculated using liquid manual injection calibration curves and converted to moles of analyte. In each case, standard solution concentrations of 0.5 μg/L of each analyte were converted to molarity using their respective molar masses to calculate C_{Aq}. Analytes were extracted under similar conditions to those described in section 3.2.2.

SPME fibres are constructed by applying a polymer coating over the fibre core. This fibre core has a diameter of 112 μm (Belardi, 1994). The polyacrylate fibre coating has a

thickness of 85 μm coating the fibre core of one cm length (Belardi, 1994). Calculation of the volume of the fibre core and polymer coating (6.2×10^{-7} L) and subtraction of the volume of the underlying fibre core (9.8×10^{-8} L) yields the volume of the polymer coating (V_S) alone (5.2×10^{-7} L).

3.5.2 Comparison between polyacrylate and polydimethylsiloxane fibre

Chloroanisole standards were independently analyzed using a 100 μm polydimethylsiloxane fibre and a 85 μm polyacrylate fibre at three concentrations; 1, 10, and 100 μg/L. This similar procedure was repeated for three different waters. These were HPLC grade water, La Salle River water, and soil extract water. The soil extract water was prepared by adding 10 g Miniota sand to 20 g 4 *M* NaCl solution, stirring overnight and centrifuging at 2000 rpm for 15 min. Sodium chloride (NaCl) was added to the HPLC grade water and La Salle river water to achieve an ionic strength of 4 *M*. This was measured by determining the electrical conductivity of each sample. Acidification of the matrix did not occur. Each matrix was spiked with the chloroanisole mixture and allowed to sit overnight. Each treatment was performed in triplicate. The results obtained using the polyacrylate and polydimethylsiloxane fibres were compared with respect to fibre type and matrix over the concentration range.

4. RESULTS AND DISCUSSION

This project served to further develop the SPME technique and explore areas such as *in situ* derivatization on the SPME fibre, agitation of solutions undergoing SPME, and the effect of dissolved organic carbon on SPME of pesticide residues in water and soil.

4.1 Forms of Herbicides Detected and Effect of pH

At pHs normally encountered in the natural environment, the analytes used in this study exist predominantly in their dissociated (anionic) form. Acidification results in the equilibrium being driven to the right in Equations 4.1 and 4.2. At pH 1.7, which was used in the study, these analytes existed largely in their protonated (neutral and extractable) forms. Acidification was used to enhance the fraction of the herbicide which existed in the unionized form, thus enhancing extractability using the polyacrylate SPME fibre. However, under these conditions, the analyst is no longer measuring concentrations which are typically experienced by organisms in the natural environment.

$$\underset{\text{pH 7}}{\text{R-O}^-} \overset{H^+}{\rightleftharpoons} \underset{\text{pH 1.7}}{\text{R-OH}} \qquad \text{(Equation 4.1)}$$

$$\underset{\text{pH 7}}{\text{R-COO}^-} \overset{H^+}{\rightleftharpoons} \underset{\text{pH 1.7}}{\text{R-COOH}} \qquad \text{(Equation 4.2)}$$

Using the Henderson-Hasselbalch Equation (McBride, 1994) (Equation 4.3), the degree of protonation at varying pHs was calculated (Tables 4.1 and 4.2) using pK_a values (Jones, 1981). When pH is equal to the pK_a of the herbicide, equal amounts exist in ionized and unionized forms. Decreasing the pH of the solution increased the proportion which was associated (protonated) and therefore extractable; at pH 1.7 a considerable portion of the analyte was present in the uncharged form and was amenable for extraction by SPME.

$$pH = pK_a + \log\left\{\frac{\alpha}{(1-\alpha)}\right\} \qquad \text{(Equation 4.3)}$$

where: α = degree of dissociation of the acidic functional groups

Table 4.1 Percent protonation of acid herbicides at acid pHs (Equation 4.3)

Analyte	pH						
	5.0	4.0	3.0	2.0	1.7	1.0	0.5
triclopyr	8.5	48.3	90.3	98.9	99.4	99.5	99.9
2,4,5-T	0.8	7.1	43.1	88.4	93.8	98.7	99.6
2,4-D	0.4	4.3	30.4	81.4	89.7	97.8	99.3
dicamba	0.1	0.7	6.9	42.6	59.7	88.1	95.9

Table 4.2 **Percent protonation of chlorophenols at pHs from 9.0 to 3.0 (Equation 4.3)**

Analyte	pH						
	9.0	**8.0**	**7.0**	**6.0**	**5.0**	**4.0**	**3.0**
2-MCP[1]	23.6	75.5	96.9	99.7	99.9	100.0	100.0
4-MCP	60.2	93.8	99.3	99.9	100.0	100.0	100.0
2,3-DCP[2]	0.2	2.7	21.6	73.4	96.5	99.6	100.0
2,4-DCP	4.6	32.4	82.7	98.0	99.8	100.0	100.0
2,6-DCP	0.6	5.9	38.7	86.3	98.4	99.8	100.0
3,4-DCP	2.4	19.7	71.1	96.1	99.6	100.0	100.0
3,5-DCP	0.8	7.7	45.4	89.3	98.8	99.9	100.0
2,3,4-TriCP[3]	4.4	31.4	82.0	97.9	99.8	100.0	100.0
2,4,6-TriCP	2.6	20.8	72.5	96.3	99.6	100.0	100.0
2,3,5,6-TetCP[4]	0.0	0.3	2.9	23.2	75.1	96.8	99.7
2,3,4,6-TetCP	0.0	0.2	2.3	19.3	70.6	96.0	99.6
2,3,4,5,6-PCP[5]	0.0	0.0	0.8	7.7	45.4	89.3	98.8

MCP[1] monochlorophenol
DCP[2] dichlorophenol
TriCP[3] trichlorophenol
TetCP[4] tetrachlorophenol
PCP[5] pentachlorophenol

The pH of the solution is an important variable when extracting chlorophenols and acid herbicides by SPME. In accordance with the Henderson-Hasselbalch equation, the amount extractable depends to a large extent on the pH of the solution and the pK_a value of the analyte. The lower the pH, the greater the proportion which is capable of

partitioning as a neutral molecule and therefore capable of being extracted using this technique. The mean values of two extractions at pHs ranging from 1.7 to 6.1 are plotted in Figure 4.1. At the pH corresponding to the pK_a of the analyte, a rapid increase in protonation occurs resulting in the steep slope at the left.

Increasing chlorine substitution of the chlorophenols decreases the pK_a value. At pHs which are of environmental significance (pH 6-8), which would include the majority of natural water systems, the percent of analyte which is extractable using the SPME method should also decrease with increasing chlorine substitution.

4.2 Enhanced Extraction

Since residue analysis usually seeks to quantify total residues, the SPME extraction method used in this project was optimized using a combination of two variables (Buchholz and Pawliszyn, 1993 & 1994) and tested using 2,4-dichlorophenol as a model compound. Acidification to pH 2 increased the quantity of analyte extracted *cf.* that from HPLC water without pH adjustment. Acidifying the sample to pH 2 ensured that the analytes were largely in their undissociated forms (Table 4.1). Partitioning to the fibre coating was further increased by saturating the 1.7 μg/mL solution of 2,4-dichlorophenol with NaCl ('salting-out effect'). Optimizing the extraction conditions allowed a significant increase in the partition coefficient of 2,4-dichlorophenol (Figure 4.2).

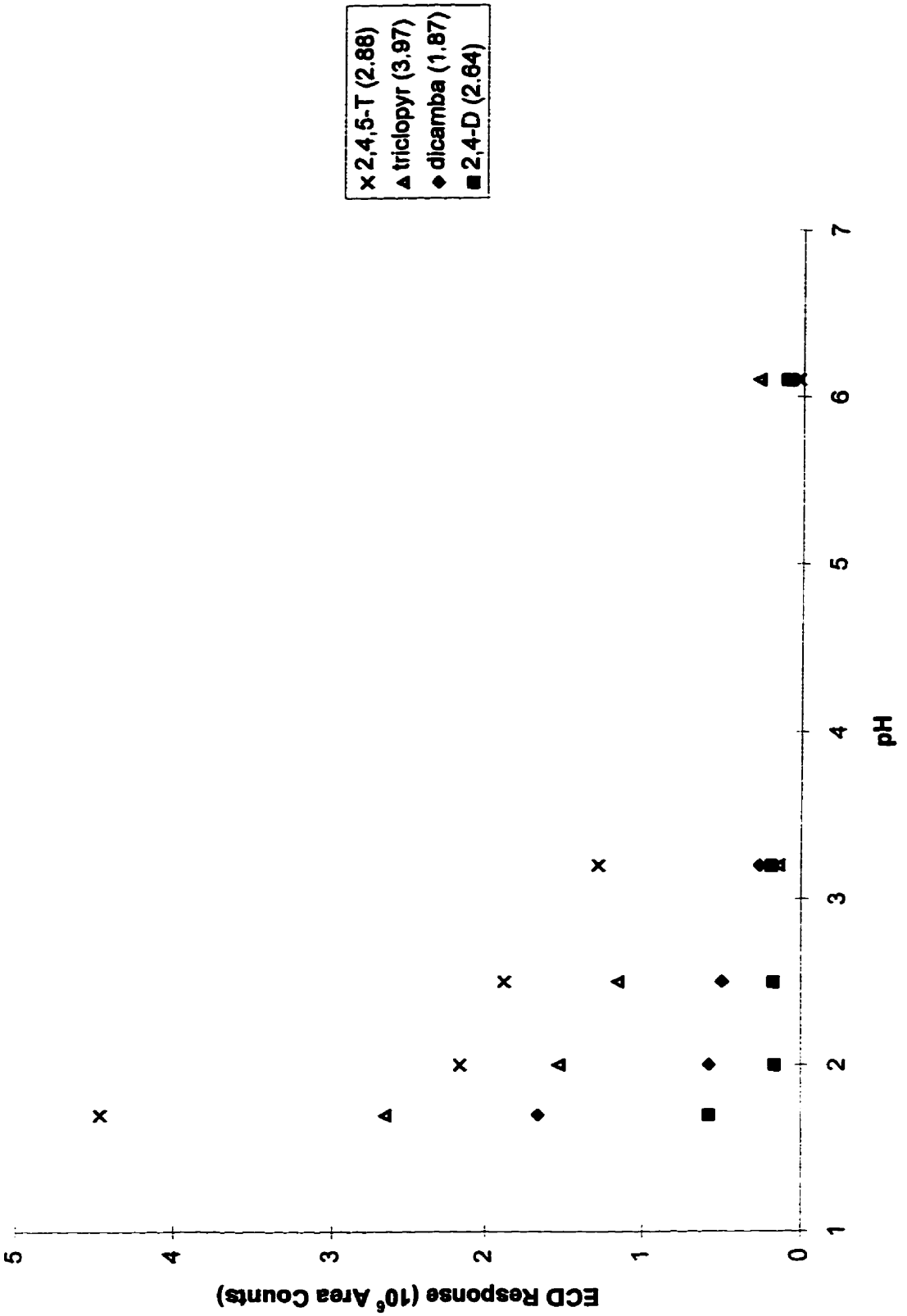


Figure 4.1 **Effect of pH on extractable portion of herbicide using 1.0 μg/L standards prepared in HPLC grade water (pK_a values of each analyte indicated in parentheses)**

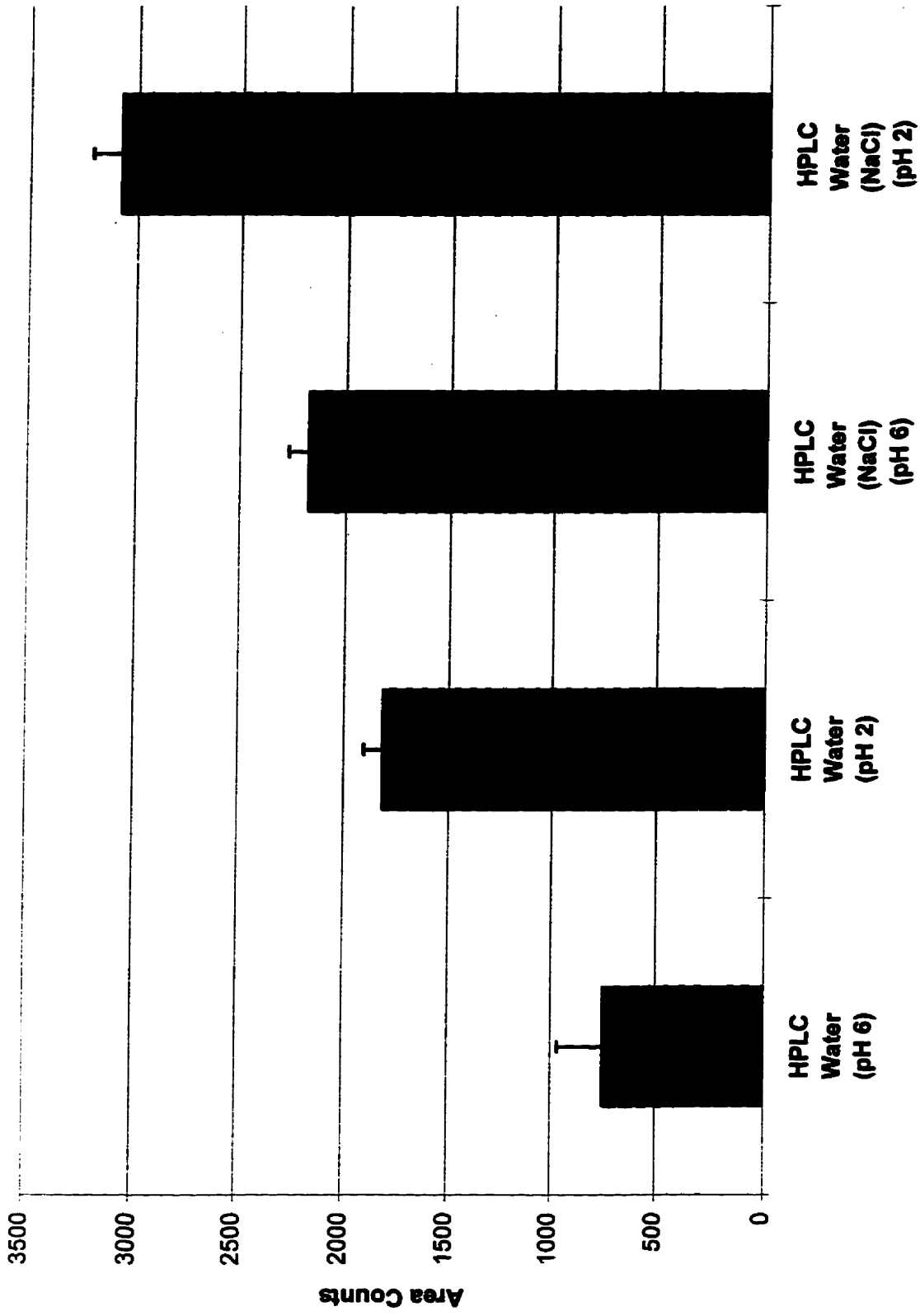


Figure 4.2 **Effect of the addition of hydrochloric acid and 4 *M* NaCl to 1.7 μg/mL solution of 2,4-dichlorophenol using an 85 μm polyacrylate SPME fibre**

Acidification to pH 2 increased the partition coefficient by a factor of 2.4 *cf.* solutions in HPLC water causing a decrease in the water solubility of the 2,4-dichlorophenol and increasing the affinity of the analyte for the fibre coating. The presence of 4 *M* NaCl in the standard solution had a similar effect (factor of 2.9) despite the fact that the pH was also unadjusted. When 4 *M* NaCl was added to the solution in combination with adjustment to attain pH 2, the partition coefficient for 2,4-dichlorophenol was increased by a factor of 4.1. A 4.1 fold increase in extraction over HPLC grade water had the effect of decreasing detection limits and/or shortening the extraction time required to yield a given GC detector response. Error bars are plotted in Figure 4.2 representing one standard deviation plus and minus the mean values. Mean values were calculated based on a minimum of three replicates.

4.3 Derivatization

Chlorophenols and acid herbicides are not ideal analytes with respect to capillary gas chromatography and their GC analysis can be difficult. The presence of phenolic and carboxylic acid functional groups is not only responsible for the polarity and low vapor pressures of these groups of compounds but also makes them chemically reactive. Analysis of chlorophenols directly on capillary columns is possible but is complicated by adsorption effects and tailing of chromatographic peaks caused by partial degradation. Analysis of carboxylic acids is more difficult (Drozd, 1981). However, these problems can be overcome through the use of derivatization techniques (Shafik and Enos, 1969; Drozd, 1981), which have the effect of reducing the polarity of the molecule and increasing its volatility.

In conventional residue analysis, environmental samples must first undergo an extraction procedure yielding an extract which requires a clean-up procedure to remove coextractives. Before analysis by GC, the concentration of the extract must be increased by reducing the volume of solvent. Each step allows the opportunity for errors and increases in variability prior to final analysis by capillary gas chromatography. Pentachlorophenol (PCP) analysis reported by Cruz & Wells (1992) used 500 mL of pentane to extract PCP from 30 L of seawater. The pentane was then reduced to about 5 mL after the pentane layer had been separated from the water. A second extraction step was then undertaken using 0.01 *M* NaOH. The extract was then acidified to < pH 3 using H_2SO_4 and saturated with NaCl, followed by a second extraction into hexane prior to derivatization and addition of an internal standard (Cruz and Wells, 1992). At this point, analysis proceeded by GC-ECD. Each step in this procedure introduced the possibility of sample extract loss or contamination and the production of flawed analytical data.

In contrast, the method developed in the current work used a simple two-step procedure utilizing the SPME technique. The chlorophenol or acid herbicide was extracted by SPME and derivatized on the fibre coating eliminating the requirement for a cleanup step. Polar analytes were extracted on the polyacrylate fibre and then derivatized to less polar analogues which were amenable to GC analysis. Methylating the chlorophenol to yield the methyl ether, or the acid herbicide to yield the methyl ester, enhanced the chromatography over direct GC analysis of chlorophenols and made GC of the acid herbicides possible. The SPME derivatization process is straightforward, minimizing the

number of steps required and therefore reducing the potential for analyte loss and contamination, compared to conventional methods. This methodology has been applied in this study to both soil and water matrices.

The methylation process appeared to occur virtually instantaneously. Short time intervals to which the fibre was exposed to the diazomethane were difficult to replicate reproducibly, therefore a fixed time period was selected to ensure that the methylation reaction went to completion. Since methylation with diazomethane normally takes place readily at room temperature, it is likely that the process was complete before entry into the heated injection port. To maintain consistency in analysis and ensure reproducibility, the fibre was suspended above the diazomethane solution for one minute. If the fibre remained in this position for longer than one minute, the solvent tended to cause the coating to expand and jam during the fibre retraction into the septum piercing needle. Any possibility of contamination from previous derivatizations was avoided by using the aliquot of diazomethane solution once only. However, no evidence of contamination was witnessed upon solution reuse.

The advantage of performing the extraction prior to derivatization using the polyacrylate fibre is that the extraction is more selective for the polar compounds of interest. During the derivatization process, only the compounds sorbed to the fibre are methylated and not the others which did not partition into the fibre coating. Therefore the non polar interferences associated with methylation of coextractives in traditional liquid-liquid

extraction procedure extracts are minimized because these analytes have significantly lower partition coefficients as compared to the more polar analytes.

A number of fibre coatings are available to the analyst depending on the nature of the compound. Polar fibre coatings such as polyacrylate or Carbowax are useful for polar compounds such as phenols and carboxylic acids, whereas the polydimethylsiloxane fibre coating is useful in the extraction of hydrocarbons (Zhang *et al.*, 1994). The polydimethylsiloxane fibre did not respond well with the more polar analytes such as the acid herbicides. Partition coefficients were lower and thus less analyte was extracted to be methylated. Other techniques which were found to be unsuitable included suspending the polyacrylate fibre in the diazomethane headspace first followed by direct SPME extraction of the phenol/carboxylic acid solution. Using this technique, the derivatizing agent tended to desorb from the fibre coating and did not remain to methylate the acid herbicide being extracted.

It is not unreasonable to assume that another herbicide, imazapyr (Figure 4.3), which Manitoba Hydro routinely uses for vegetation control in rights-of-way, could also be analyzed using the SPME-alkylation-GC method in conjunction with a NPD detector or MS. Imazapyr, more commonly known as Arsenal, is very effective against annual and perennial broad-leaved weeds, grasses, sedges and woody plants (Worthing and Hance, 1991). Other acid herbicides including imazapyr, although not tried, also have possibilities of being analyzed using this SPME-based method.

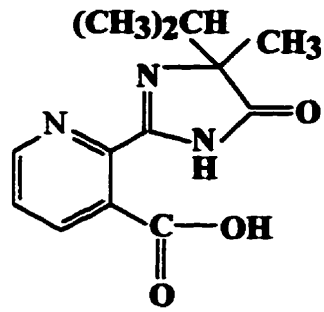


Figure 4.3 Chemical structure of imazapyr (2-(4-isopropyl-4-methyl-5-oxo-2-imidazolin-2-yl)nicotinic acid)

4.4 Retention Times

Retention times for the chlorophenols, chloroanisoles, and acid herbicides (methyl derivatives) are shown in Table 4.3. Relative retention times (t'_R) were calculated for the chlorophenols and chloroanisoles relative to 2,4-dichlorophenol. In the case of the acid herbicides, dicamba was used as the reference to calculate the relative retention times (t'_R) for the methyl esters.

Table 4.3 Relative retention times (t'_R) of phenols, anisoles and acid herbicide methyl esters

Compound	t'_R (min)	
	Phenol	**Anisole**
2,4-dichlorophenol	1.00	1.16
2,3-dichlorophenol	1.01	1.20
2,3,4-trichlorophenol	1.25	1.40
3,5-dichlorophenol	1.28	1.10
2,3,4,5,6-pentachlorophenol	1.64	1.61

Compound	t'_R (min)
	Methyl Ester
dicamba	1.00
2,4-D	1.21
triclopyr	1.34
2,4,5-T	1.51

Method alterations such as increasing carrier gas flow and raising the rate of temperature increase were utilized in an attempt to minimize tailing and sharpen peak shape. This allowed for virtual baseline separation of triclopyr (10.3 min) and pentachlorophenol (10.6 min). Figure 4.4 shows a typical chromatogram obtained using the SPME-alkylation-GC method on a 0.75 µg/L mixture of acid herbicide methyl esters and PCP (methyl ether) in water. Note that the EC detector specific response is much greater for PCP than for the acid herbicides. The PCP methyl ether peak is thus off-scale.

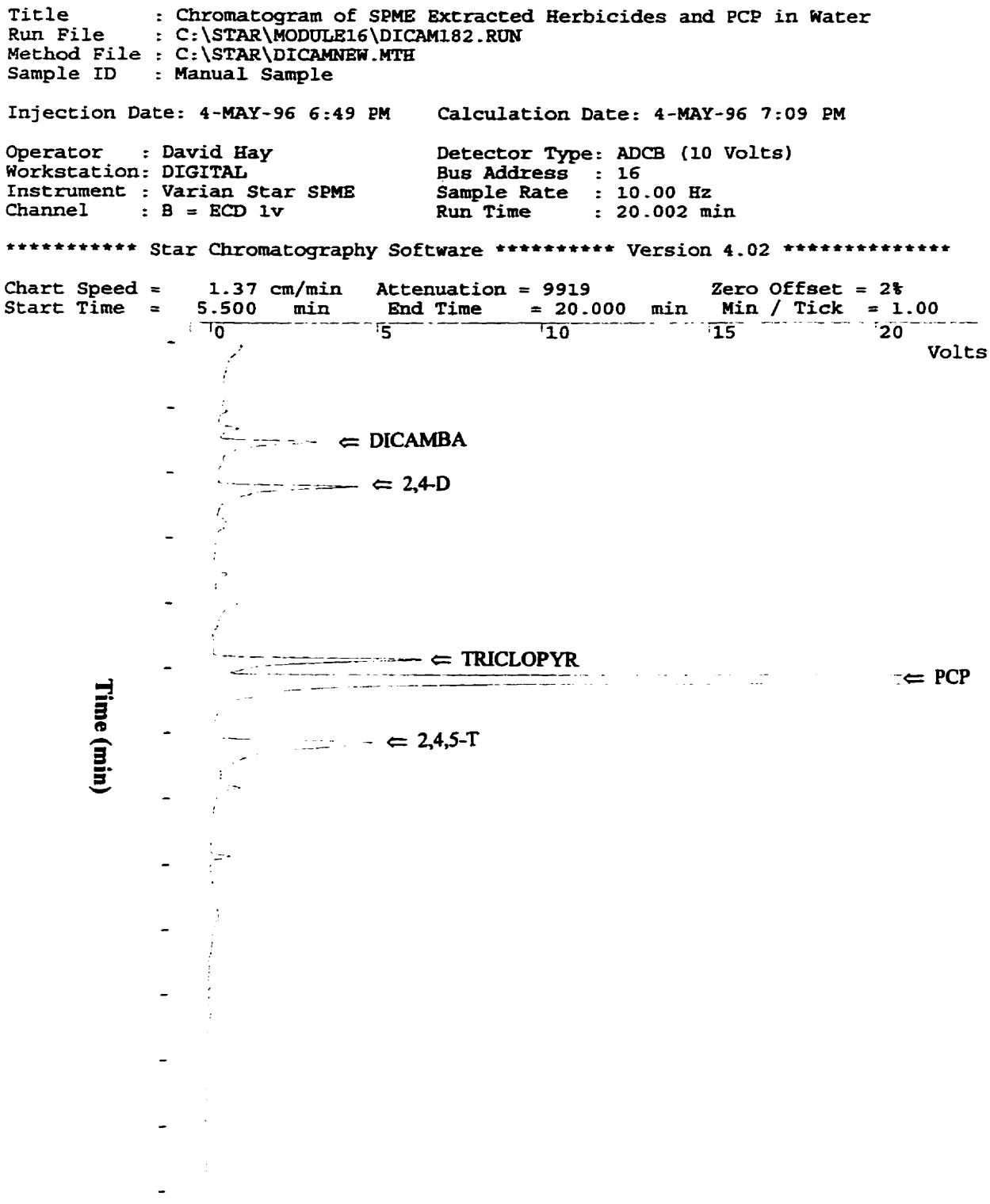


Figure 4.4 Typical chromatogram of acid herbicide mixture (methyl esters) including PCP (methyl ether) in water analyzed by SPME containing 0.75 μg/L

4.5 Equilibration Time

Equilibration times were determined to be in the order of 15-20 min for each of the acid herbicides included in the derivatization study. The equilibration times were determined based on a 0.5 μg/L solution (1.6 mL) which was prepared in 4 *M* NaCl, acidified to pH 1.7 and magnetically stirred. The rate of partitioning was greatest at time periods ranging from 0-10 min but plateaued for all compounds at 20 min (Figure 4.5). At approximately 20 min, equilibrium was attained between the analyte in solution and the polyacrylate fibre coating. The steep slopes in the 0-10 min portion of Figure 4.5 introduced the opportunity for errors when performing SPME extractions and therefore a longer time interval was selected. It is also important to have extractions at equilibrium if one is maximizing limits of detection or calculating partition coefficients.

It has been shown for analytes which have high distribution constants that the static water layer immediately surrounding the fibre coating can significantly impede the rate of sorption by the polymeric coating (Louch *et al.*, 1992). This thin static water layer can be minimized by agitating the solutions (*e.g.*, by magnetically stirring each sample) minimizing diffusion problems and hastening the extraction process. An agitation device was constructed to be used in conjunction with the Varian 8200 autosampler and used to decrease equilibration times (Section 4.13). It was not controlled using the Varian Star 4.02 software but contained optical switches to regulate vibration during the extraction.

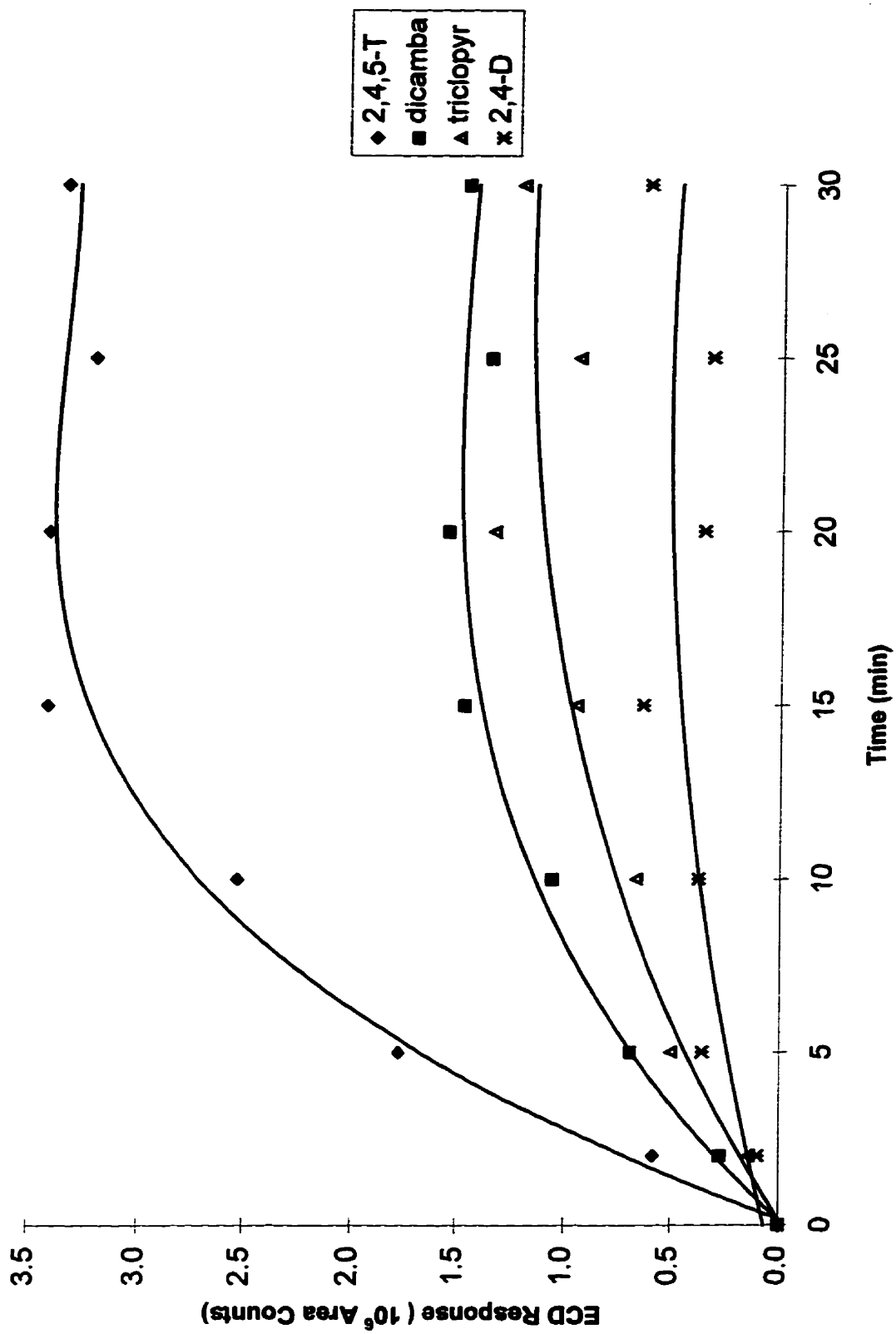


Figure 4.5 **Time to reach equilibrium for a 1.5 mL aliquot of 0.5 μg/L acid herbicide mixture (methyl esters) using an 85 μm polyacrylate SPME fibre**

4.6 Calibration Curves

Linear regression calibration curves were calculated for each of the acid herbicides over the range of 0.01 μg/L to 1.0 μg/L (Figure 4.6). The points on Figure 4.6 represent the mean of three replicates. Error bars have been plotted which represent one standard deviation on either side of the mean. The r^2 values for each of the acid herbicides ranged from 0.923 for dicamba to 0.964 for 2,4,5-T. This corresponds to probabilities of significant F values ranging from 0.0005 for dicamba to 0.00009 for 2,4,5-T (Table 4.4).

Previous SPME work has resulted in the calculation of two slope calibration curves (Graham *et al.*, 1996; Webster *et al.*, 1994*a*). Since the calibration curve had a slightly different slope at the lower concentrations compared to the upper portion of the curve one would overestimate concentrations at the lower concentrations by applying a single slope calibration. Louch *et al.* (1992) attributed the change in slopes to surficial sorption onto (as opposed to partition into) the fibre coating followed by the second slope which includes intracellular movement of the analyte into the organic matrix (solvent behavior). Two slope calibration curves could only be calculated if there were enough data points on each regression line to maintain their statistical significance.

4.7 Limits of Detection and Quantitation

The ACS published guidelines (MacDougall *et al.*, 1980; Keith, 1992) define the limit of detection (LOD) to be the signal of the blank (μg/L) + 3σ which is the smallest peak which can confidently be judged as a chromatographic peak. The limit of quantitation

(LOQ) is defined as the signal of the blank (μg/L) + 10σ. The LOQ has been determined to be the smallest peak area which can be measured with acceptable precision and therefore reportable (MacDougall *et al.*, 1980). Values for the blank (μg/L) were calculated to be as follows: dicamba (0.04), 2,4-D (0.01), triclopyr (0.02), 2,4,5-T (0.006). These values were then added to the respective standard deviations (σ) at the 0.01 μg/L concentration. The LOD and LOQ values for the acid herbicides were calculated and reported in Table 4.4.

LOD values varied from 0.01 μg/L (2,4,5-T) to 0.05 μg/L (dicamba) for all compounds studied. Similarly, LOQ values ranged from 0.03 μg/L for 2,4,5-T and 0.07 μg/L for dicamba (Table 4.4). The LOD values are less than those reported for EPA Method 8150B for water which are reported as being 0.27 and 1.2 μg/L for dicamba and 2,4-D respectively (United States Environmental Protection Agency, 1992). 2,4,5-T is not included in EPA Method 8150B for water as it is no longer registered for use in the USA. Triclopyr is also not included in Environmental Protection Agency (EPA) Method 8150B; however, an ELISA technique for the detection of triclopyr has a LOD of 0.1 μg/L for water (Johnson and Hall, 1996). In all cases, the SPME-alkylation-GC method can attain LOD which surpass EPA standards.

The LOD and LOQ values are environmentally significant as background concentrations of phenoxy acid herbicides in soils on the Canadian prairies are less than 60 ng/g, less than 1 μg/g for phenols, and less than 0.4 μg/g for PCP (Webber and Singh, 1995).

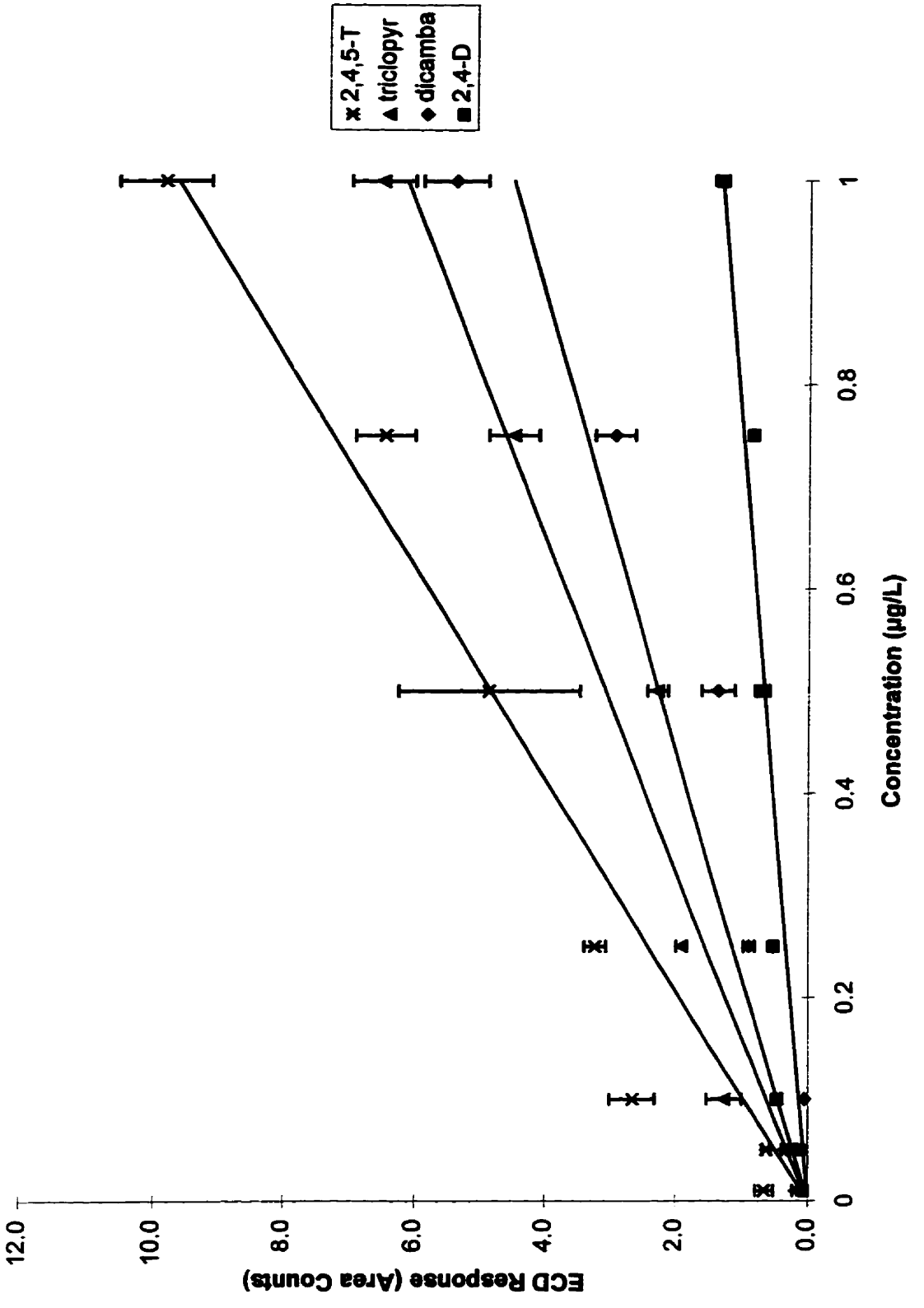


Figure 4.6 **SPME calibration curves for herbicide mixture ranging in concentrations from 0.01 µg/L to 1.0 µg/L with polyacrylate fibre**

Table 4.4 Calibration curve data for acid herbicide derivatives

Analyte	Slope (10^6)	r^2	Probability of Significant F value	LOD (µg/L)	LOQ (µg/L)
2,4,5-T	9.7	0.964	0.00009	0.01	0.03
triclopyr	6.2	0.961	0.0001	0.03*	0.05
dicamba	1.3	0.923	0.0005	0.05†	0.07
2,4-D	4.5	0.924	0.0005	0.02§	0.04

* LOD 0.1 µg/L (ELISA, Johnson and Hall, 1996)
† LOD 0.27 µg/L (EPA Method 8150B water)
§ LOD 1.2 µg/L (EPA Method 8150B water)

4.8 Reproducibility

The precision of standards run for the calibration curve was measured as a percent relative standard deviation (% RSD) value as calculated by Equation 4.4 and are expressed in Table 4.5.

$$\% \text{ RSD} = \frac{\text{standard deviation } (\sigma)}{\text{mean concentration}} * 100 \qquad \text{(Equation 4.4)}$$

Table 4.5 Percent relative standard deviation expressed at varying concentrations for acid herbicide mixture and pentachlorophenol

	% Relative Standard Deviation (Concentrations expressed as µg/L)						
Analyte	0.01	0.05	0.10	0.25	0.50	0.75	1.0
2,4,5-T	21.1	9.7	13.3	5.2	28.5	6.9	7.1
triclopyr	28.5	11.9	20.8	4.5	7.0	8.6	7.3
dicamba	31.4	5.1	13.2	9.0	18.4	10.5	9.3
2,4-D	27.2	28.6	13.8	3.0	16.1	10.7	12.0
pentachlorophenol	25.7	20.3	23.8	8.8	10.3	2.7	7.3

% RSD values tend to be larger at the lower concentrations because of interferences from excessive noise to signal become larger. The % RSD values were calculated based on three replicates at various concentrations. The % RSD values were acceptable at concentrations greater than 0.25 μg/L for most analytes with the exception of 2,4,5-T at 0.50 μg/L. The % RSDs were rather large at the 0.01 μg/L concentration and would presumably get larger as one decreased the concentration of the analyte in solution. The % RSD values could possibly be improved through more exact timings of the extraction such as in the use of the autosampler, performing extractions at times permitting attainment of full equilibrium rather than just on the shoulder of the equilibrium curve, or via increased number of replicates.

4.9 Effect of Ionic Strength

The addition of salt had no effect with respect to the partitioning of acid herbicides into the fibre unlike the situation with chlorophenols; partition was unaffected and no salting-out effect was seen. This was tested at various molar strengths (0.1, 0.25, 0.5, 1, 2, and 4 *M*) of NaCl for the acid herbicides. The 'salting-out' effect appears to be more important for the less polar chlorophenols but not for the more polar acid herbicides. 'Salting-out' only works for uncharged species of molecules which exhibit low water solubilities. This means that ionic strength of the soil is of little concern when analyzing soils for acid herbicide residues using this analytical methodology.

4.10 Confirmation by GC-MS

Confirmation of chlorophenols was performed by derivatization with diazomethane and comparison with commercially available chloroanisoles by GC-EC or GC-ITD (ion trap detection). Confirmation of the derivatization of acid herbicides was performed via ITD. The acid herbicides were derivatized to form their methyl esters (see GC-MS data in Figures 4.7 and 4.8). The concentrations of the analytes which were run in this study spanned three orders of magnitude (0.1 to 10 μg/L). The acid herbicide methyl esters were compared to total ion chromatograms and mass spectra to dicamba and 2,4-D methyl esters contained within the reference library standards. The fragmentation patterns observed between the injected analyte and that of the reference library yielded a fit of 961 and 929 out of a possible 1000 for dicamba and 2,4-D methyl esters respectively (Figures 4.7 and 4.8).

Figure 4.7 shows the fragmentation of dicamba methyl ester with a base peak of 203 being created through the loss of a methoxy group in the interpretation of the mass spectra. Figure 4.8 shows the fragmentation of 2,4-D methyl ester as a loss of a chlorine atom resulting in a base peak of 199 (Finnigan Mass Spectral Library, 1988). The natural isotopic abundance of chlorine at M + 2 can be seen in the mass spectra yielding characteristic Cl_n peak clusters (McLafferty, 1980).

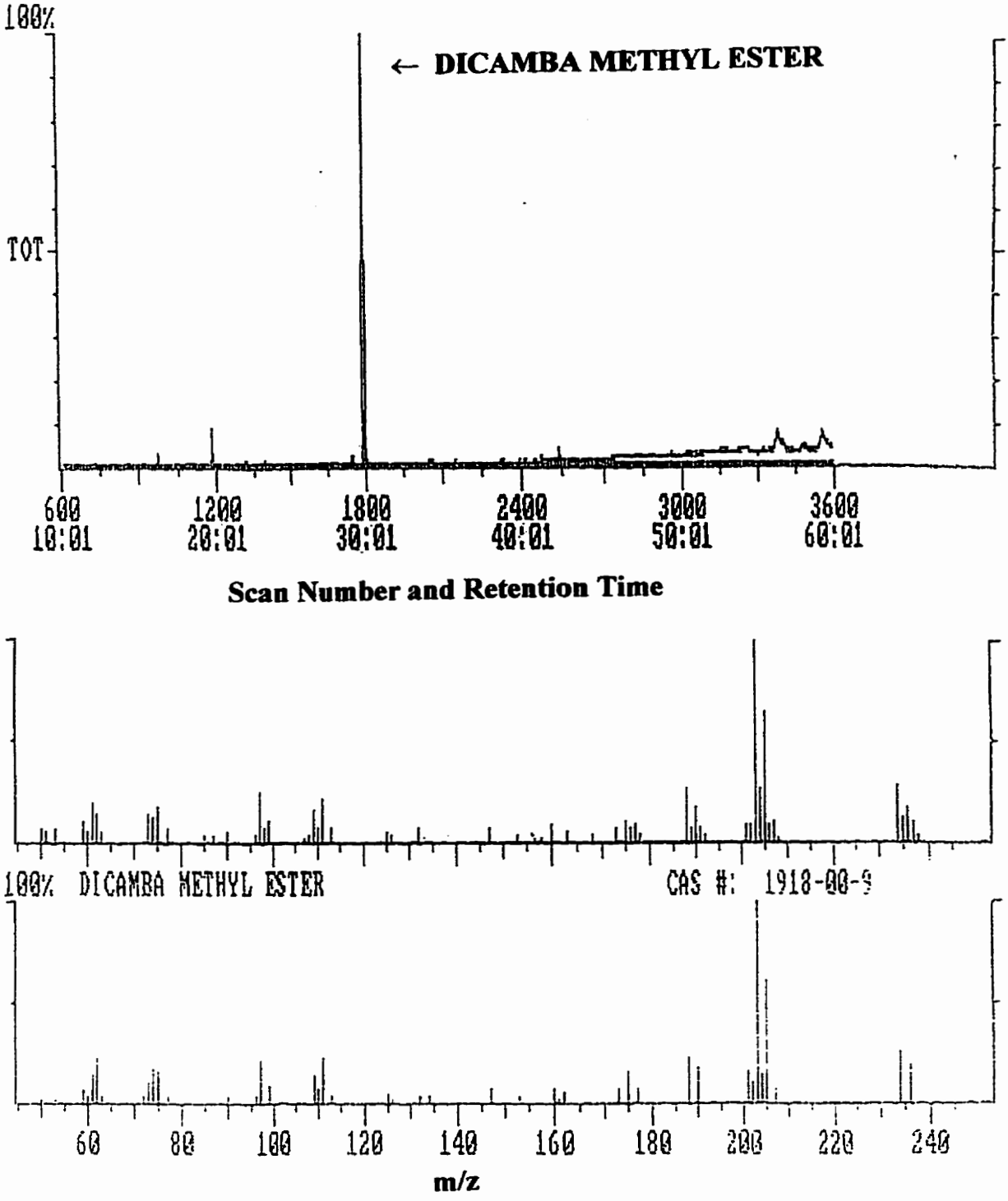


Figure 4.7 **Total ion chromatograph and mass spectra from reference library showing dicamba methyl ester**

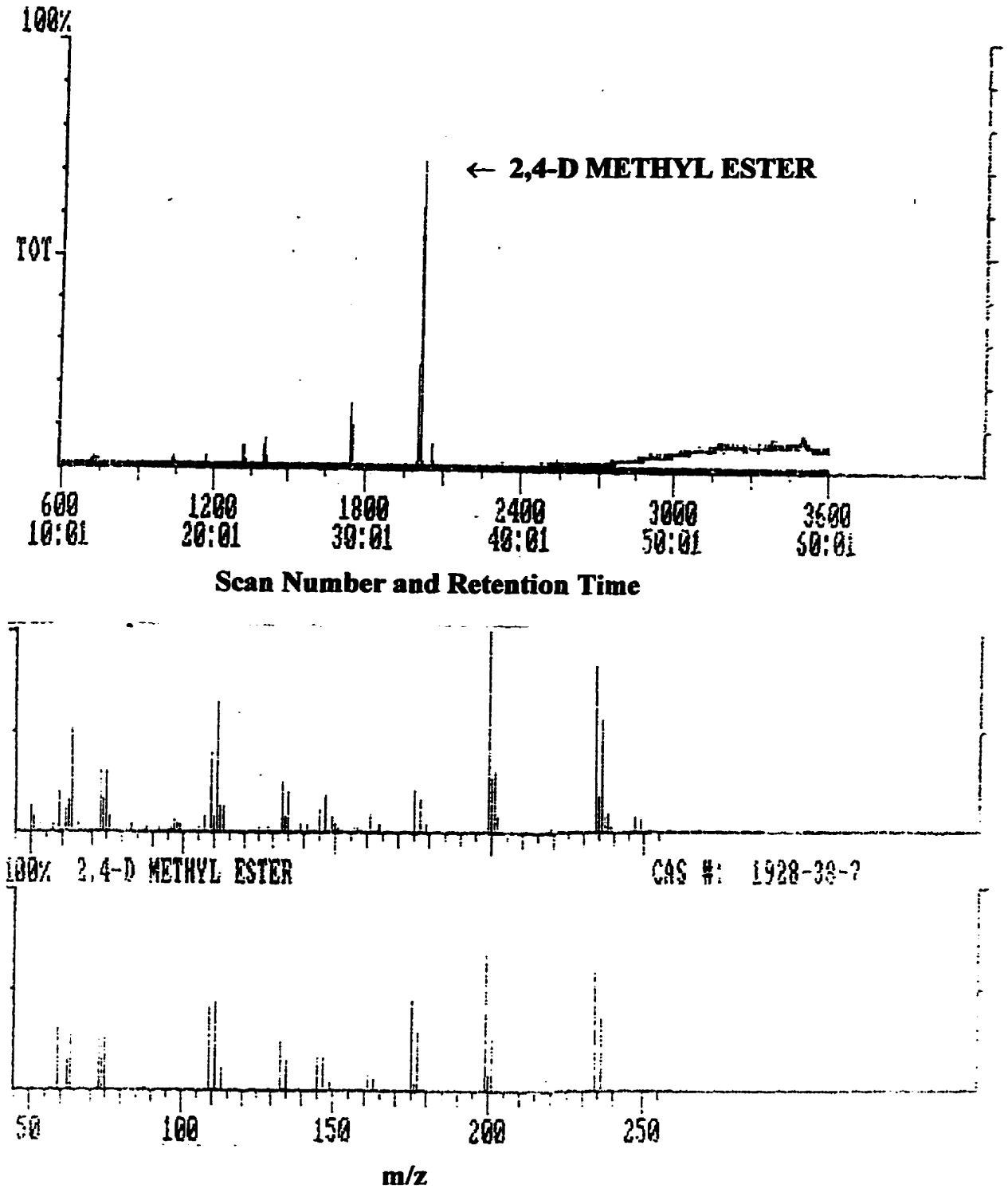


Figure 4.8 **Total ion chromatograph and mass spectra from reference library showing 2,4-D methyl ester**

4.11 Extraction from Natural Waters

The mixture of acid herbicides was spiked into La Salle River water to demonstrate the potential of SPME as a viable extraction technique for environmental water samples. Upon performing the analysis on samples taken on two different dates, a peak was detected at 10.6 min (Figure 4.9). This matches exactly the retention time determined for a coinjection with pentachloroanisole. Definitive confirmation could be obtained by reanalysis by GC-MS. Assuming the analyte to be pentachlorophenol, peak integration resulted in detection of PCP at a concentration of 0.12 μg/L in two separate samples from the La Salle River.

The spiked La Salle River water data was compared to the HPLC water standards (Figure 4.10). A 1:1 correlation represents that the measured amount of analyte in each matrix is the same. However, with each analyte, the measured concentration in La Salle River water was less than in the HPLC water. The measured concentrations were in the order of 40-85% that of the standards in HPLC water and can be explained by a combination of factors namely; sorption to organic matter decreasing the amount of analyte which is freely dissolved and also degradation of the analyte prior to analysis. Biodegradation could be greatly reduced if the water samples were spiked with sodium azide or mercuric chloride prior to spiking. The background noise was approximately two times greater in the La Salle River water than for HPLC grade water which increased the LOD and LOQ values. The slope data and increased LOD and LOQ values are included in Table 4.6.

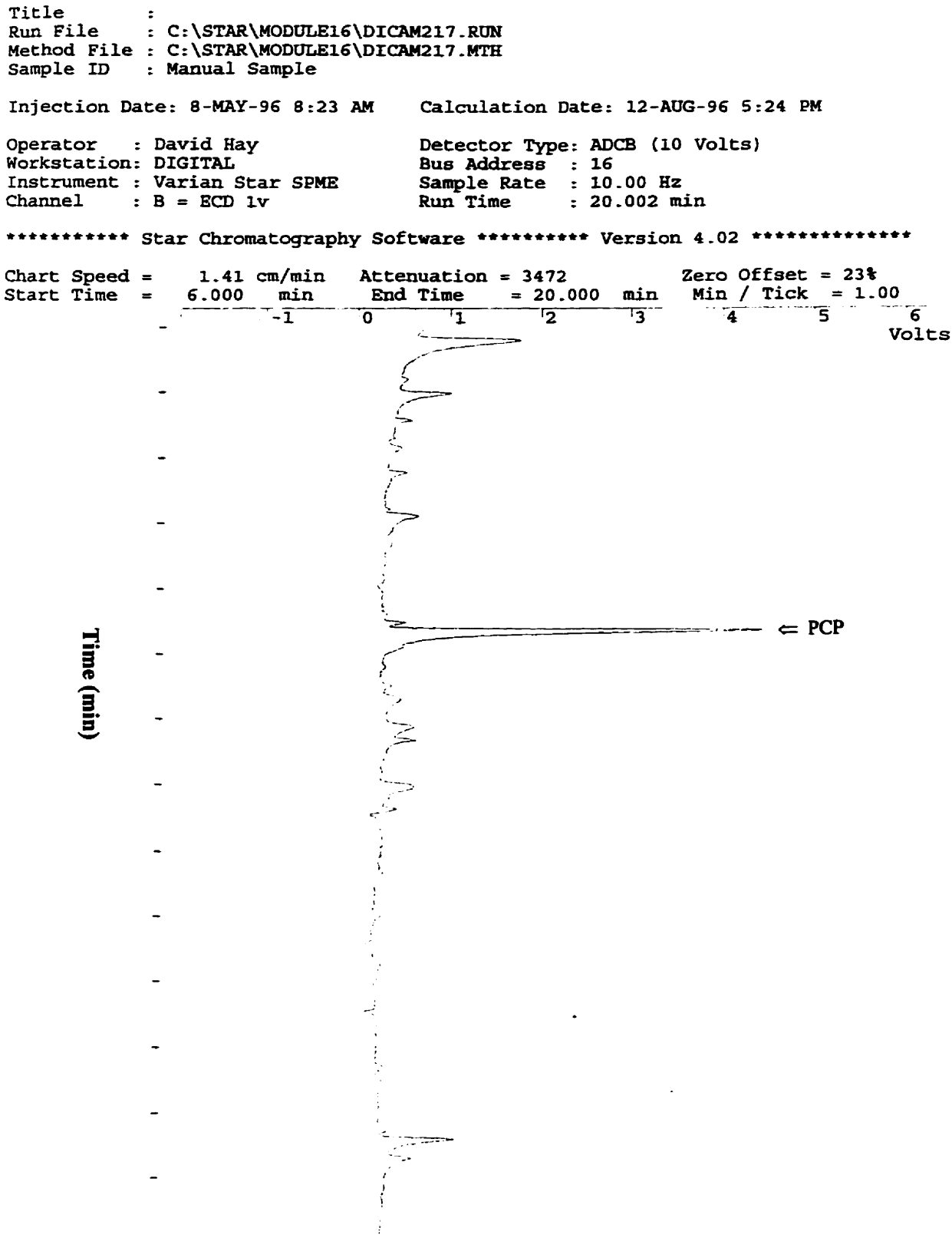


Figure 4.9 **Chromatogram showing suspected pentachlorophenol at a concentration of 0.12 μg/L (peak eluting at 10.6 min) extracted from La Salle River water by SPME**

Table 4.6 Linear slope data from pesticide spike into La Salle River water

Analyte	Slope (10^6)	LOD (μg/L)	LOQ (μg/L)
2,4-D	0.85	0.03	0.06
2,4,5-T	0.78	0.02	0.05
dicamba	0.67	0.06	0.1
triclopyr	0.39	0.04	0.08

The organic carbon/water partition coefficient (K_{OC}) is derived using the soil sorption partition coefficient (K_p) and normalizing it according to the fraction of organic carbon (Chiou *et al.*, 1986). The soil sorption partition coefficient is defined as the ratio of concentration of sorbed chemical to the concentration in solution in a soil-water suspension at equilibrium. K_{OC} values are important parameters when discussing mobility and bioavailability of compounds in environmental fate studies. K_{OC} literature values for dicamba, 2,4-D, and 2,4,5-T are 4.4, 19.6, and 52 respectively (Howard, 1991). Tomlin (1994) reports the K_{OC} value for triclopyr to be 59 mL/g. Technically, K_{OC} values are not unitless because the density of soil or sediment is not equal to the density of water, however, many authors report it as a dimensionless constant. Karickhoff (1981) reported that a near linear relationship exists between K_{OC} and K_{OW} values. This can be expressed in Equation 4.5.

$$K_{OC} = 0.411\ K_{OW} \qquad \text{(Equation 4.5)}$$

Figure 4.10 compares the measured concentration of the acid herbicides in La Salle River water. The effects of sorption to organic carbon roughly parallels the literature K_{OC} values. Literature K_{OC} values follow the order; dicamba, 2,4-D, 2,4,5-T, and triclopyr, from smallest to largest. Figure 4.10 illustrates the effects of sorption over a range of three concentrations with varying slopes. Triclopyr, presumably because of the pyridine ring (Figure 2.4), accounts for the higher K_{OC} value than the other acid herbicides. The order of increasing sorption of the acid herbicides used in this study was in the order (smallest to largest); 2,4-D, 2,4,5-T, dicamba, and triclopyr. It should be noted that the first three analytes were relatively close together and that DOM in La Salle River water is not necessarily the same as Aldrich humic acid standards which are often used in the determination of literature K_{OC} values.

Schwarzenbach *et al.* (1993) reported Equation 4.6 which can be used to determine the fraction of analyte associated with the water phase. If K_p values are small, the fraction of analyte which is associated with the water phase is large. Table 4.7 compares the amount of analyte which is calculated to be associated with the dissolved organic carbon in La Salle River water (15 mg/L) and soil extract water (200 mg/L) as a function of K_{OC}. An increased DOC concentration measured in the soil extracts as compared to the river water allows for greater sorption of the analytes studied. Results measured and reported in Figure 4.10 show greater sorption to DOC as compared to calculations reported in Table 4.7. The difference between the results calculated and those measured are probably due to degradation of the analyte during the sorptive equilibrium time period.

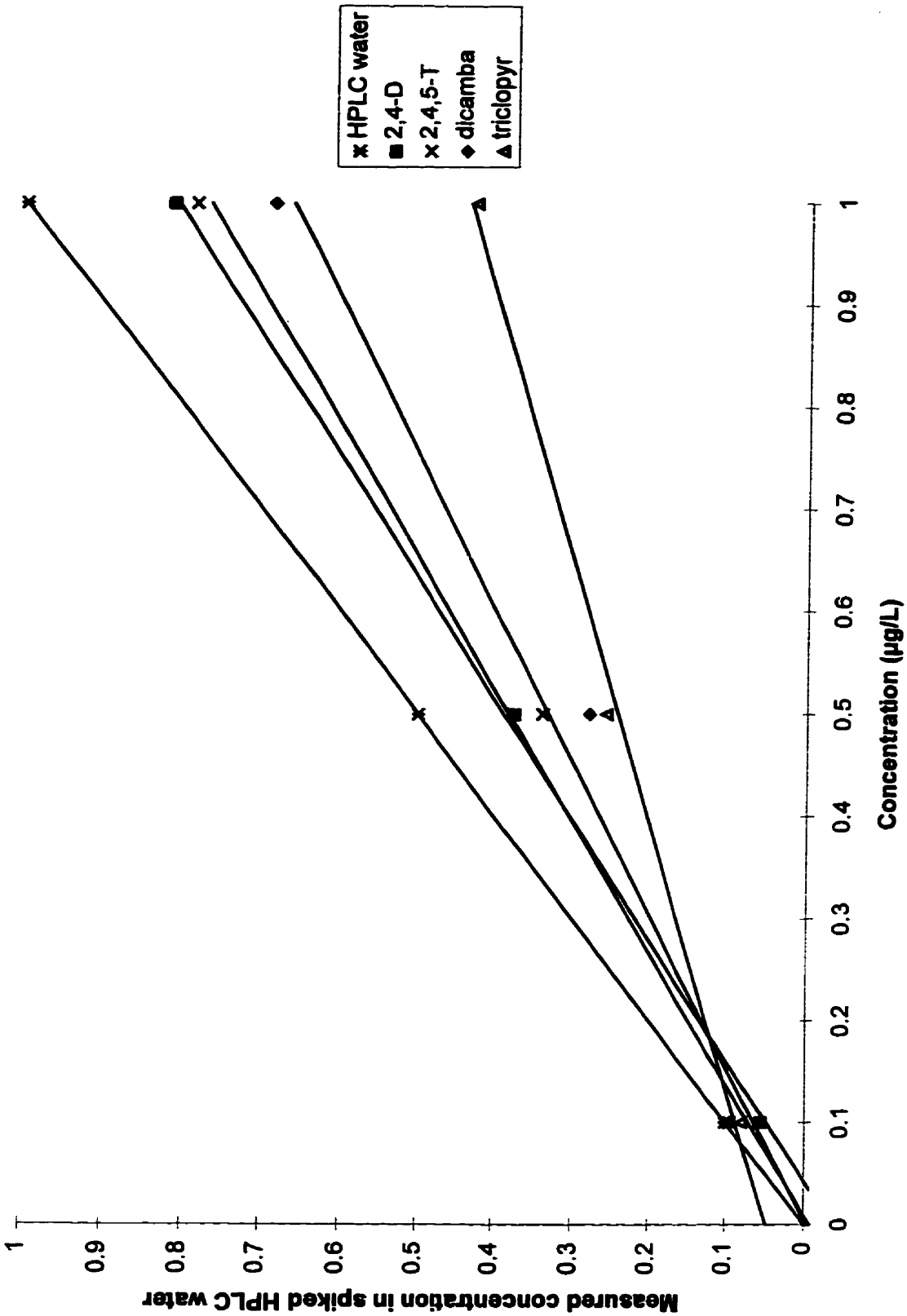


Figure 4.10 **Concentration measured in La Salle River water compared to HPLC grade water**

$$f_w = \frac{1}{1 + \frac{M_s}{V_w} K_p} \qquad \text{(Equation 4.6)}$$

where: f_w = fraction of analyte associated with the water phase
M_s = mass of solids (kg)
V_w = water volume (L)
K_p = soil sorption partition coefficient (L/kg)

Table 4.7 Amount of analyte sorbed (%) as a function of dissolved organic carbon

Analyte	Amount of analyte sorbed to DOC (%)	
	La Salle River water (15 mg/L DOC)	Soil extract water (200 mg/L DOC)
dicamba	0.006	0.09
2,4-D	0.03	0.4
2,4,5-T	0.08	1.0
triclopyr	0.09	1.2

4.12 Usage of other Diazoalkanes

PCP is metabolically converted to pentachloroanisole (PCA) in nature which means that detection of PCA may be indicative of PCP contamination but transformed through biological processes (Cruz and Wells, 1992). The fact that chloroanisoles may be contaminants themselves suggests that another derivatizing agent (*e.g.*, diazoethane, diazopentane) should be employed for methodology to be used for the analyses of environmental waters. If diazomethane is used, the analysis would have to be performed twice, once using an underivatized sample and secondly utilizing the SPME derivatization procedure. The difference between the underivatized sample (amount of

PCA present originally) and the derivatized sample (produced via derivatization in the lab) is the amount of PCP to be reported.

4.13 Agitation of Solution during SPME

The Varian 8200 autosampler is capable of handling samples for extraction by SPME and injection into the GC. The possibility of utilizing the autosampler was explored in conjunction with agitation. The effect of agitation on the attainment of equilibrium can be tested by agitating the vial during extraction. Under conventional autosampling, the vial was static, and over 600 min were required for the sample to reach equilibrium with the fibre in the case of 2,4-dichlorophenol (Figure 4.11). Only two samples per day could be analyzed by this method; hence, an agitation device which worked in conjunction with the Varian 8200 autosampler was developed to decrease equilibration times and increase the amount of analyte being sorbed to the fibre in comparable time periods.

The effect of stirring the sample speeds the partition process, thereby decreasing the amount of time to reach equilibrium. Stirring also aids in achieving homogeneity and therefore a more representative analysis can be performed. The amount of analyte extracted is limited by the rate of diffusion through the water matrix. Up to the present, agitation had only been accomplished through the use of magnetic stir bars and a magnetic stir plate. In order to automate the method, an agitation device was constructed to vibrate the sample carousel of a Varian 8200 autosampler. This agitation device ('Woodpecker' apparatus) served to increase the amount of analyte extracted compared to static extraction.

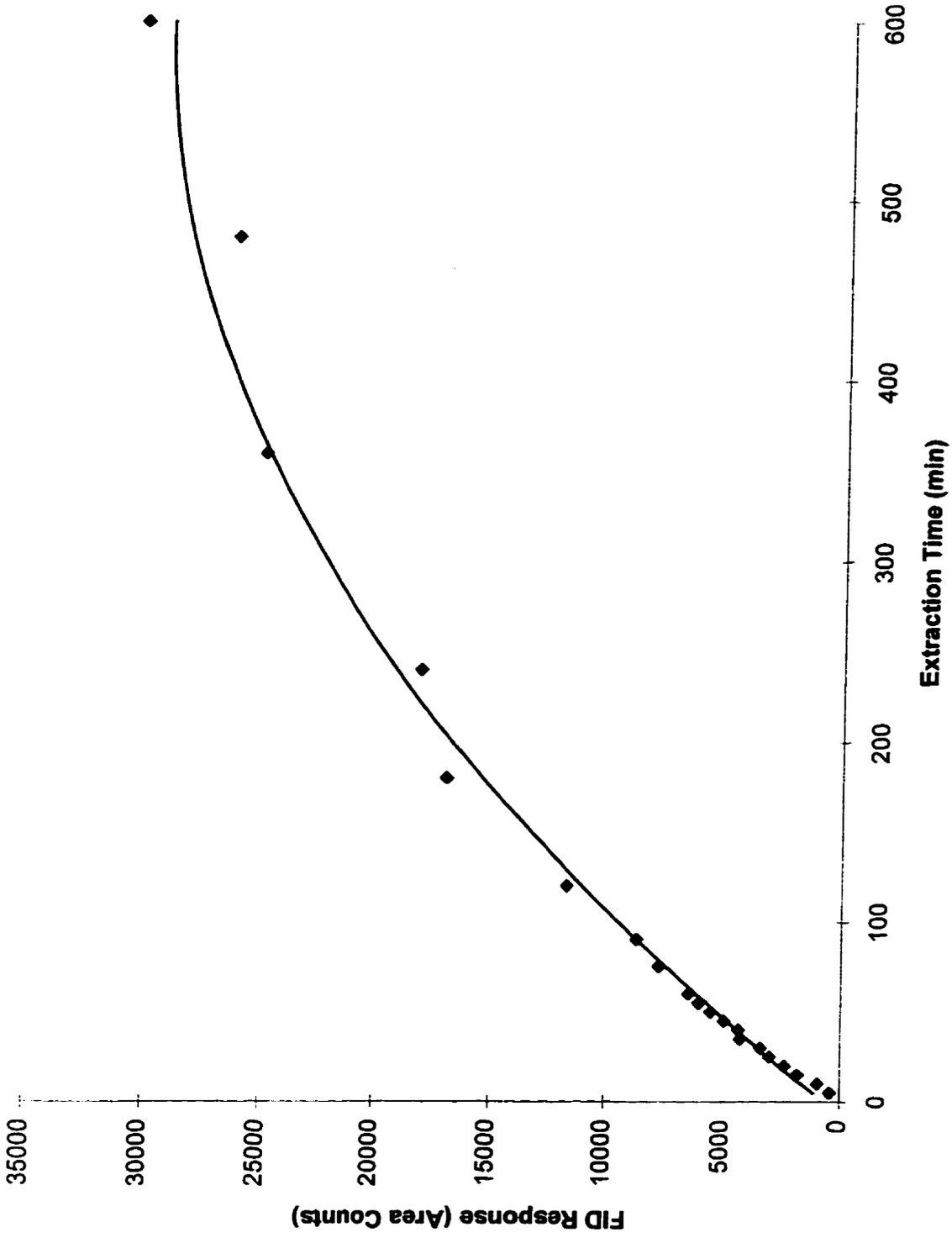


Figure 4.11 **Equilibrium curve for a 1.7 mg/L solution of 2,4-dichlorophenol using an 85 μm polyacrylate SPME fibre**

The agitation caused the movement of the sample within the vial and decreased the problem of retarded diffusion across the boundary layer surrounding the fibre (Zhang *et al.*, 1994). Subsequent times to reach equilibrium were reduced; alternatively, the GC response measured in area counts was increased at any given time by agitation (Figure 4.12). Longer periods of time to reach equilibrium have been shown for analytes which have high distribution constants because the static water layer immediately surrounding the fibre coating can significantly impede the rate of sorption by the polymeric coating (Louch *et al.*, 1992). This static water layer was minimized by stirring each sample minimizing diffusion problems and hastening the extraction process.

The 'Woodpecker' apparatus rapidly tapped the sample carousel and agitated the sample vials externally minimizing the static water layer in a fashion similar to magnetic stirring. The amplitude of agitation diminished towards the middle of the carousel; samples were thus run only in the outer ring with vials in the inner ring being utilized to contain water to wash salt accumulation off the fibre. Sample contamination was negated because the samples were being externally agitated rather than being altered by the introduction of a magnetic stir bar into the sample vial.

The mean values of triplicate analyses are reported in Figure 4.12 comparing conventional static autosampler (S) versus 'Woodpecker' enhanced extraction (W). Without the use of the 'Woodpecker' apparatus, the times required to reach equilibrium were substantially longer for 2,3-dichloroanisole, 2,4-dichloroanisole, and 3,5-dichloroanisole; equilibrium was attained in *ca.* 20 min using the agitation apparatus.

Table 4.8 shows the times required to reach equilibrium without agitation and the time factors achieved using agitation ranging from 5.8 to 6.9. These numbers drastically reduced the amount of time required for extraction and enabled increased throughput of samples over the conventional method.

The rate of attainment of equilibrium of the three analytes was enhanced as a result of using the 'Woodpecker' apparatus, which was analogous to that experienced in the case of magnetic stirring versus static extraction for manual SPME. Figure 4.13 compares extraction time required to reach equilibrium using manual stirring and results obtained using the 'Woodpecker' apparatus. The introduction of the magnetic stir bar to each sample could result in contamination being introduced; this difficulty was not encountered during the thesis work, but could be a problem at ultratrace levels. At the time of writing, there was no mechanism to enable magnetic stirring during operation of the autosampler. Using the 'Woodpecker' apparatus, excellent detection limits and equilibration times were achieved. Computer control of the sampling carousel allowed for much better reproducibility than could have been obtained through human control. Despite the fact that improvements could possibly be made over the prototype, it achieved the goals of decreasing times to reach equilibrium. Towards the end of the thesis work, Varian Canada Inc. released their patented agitation device (SPME Shake) in conjunction with the Varian 8200 autosampler to shorten equilibration times. The agitation is provided in this case by shaking the extraction fibre during extraction.

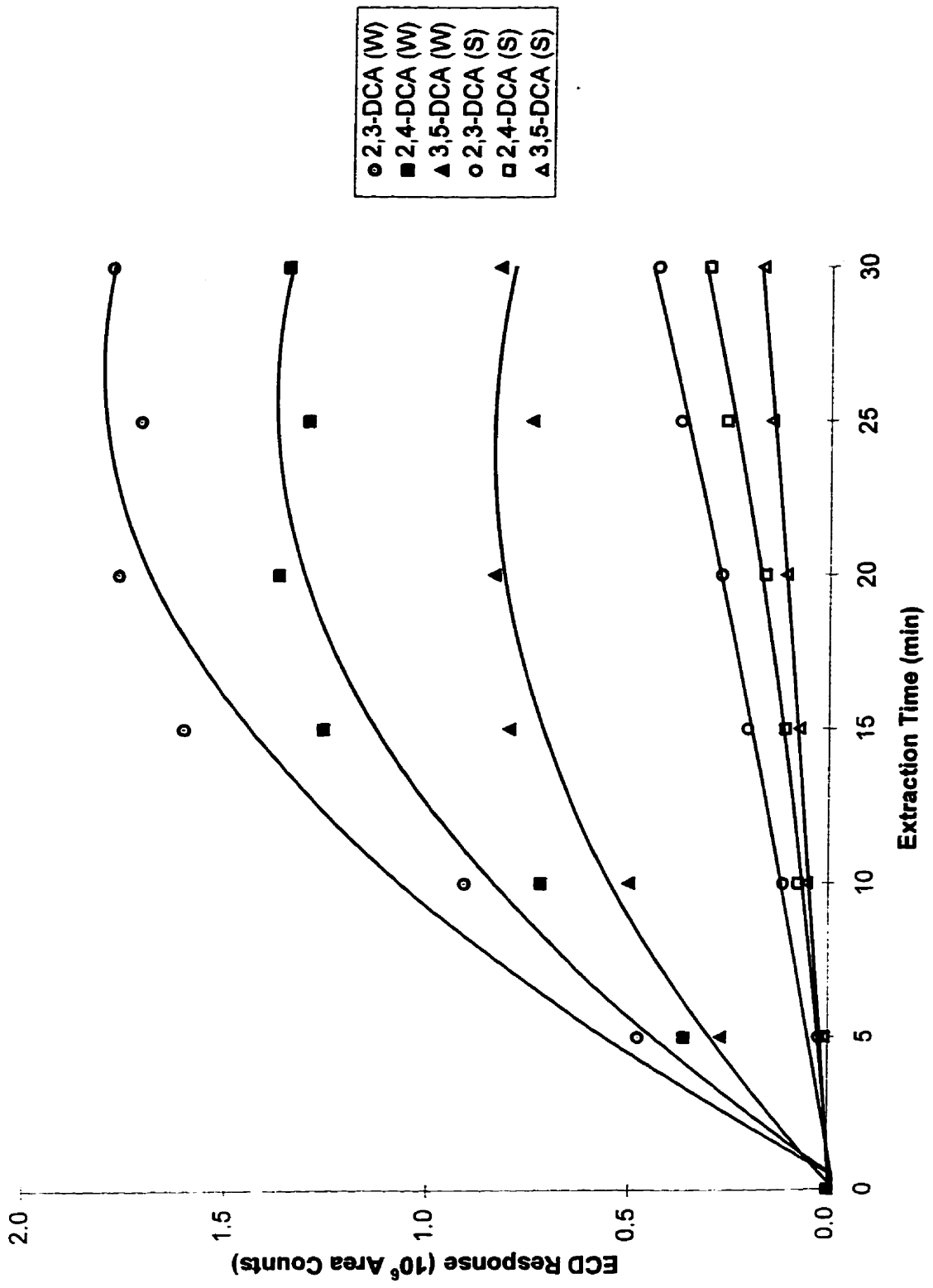


Figure 4.12 **Extraction time required to reach equilibrium: static autosampler (S) *cf.* use of 'Woodpecker' (W)**

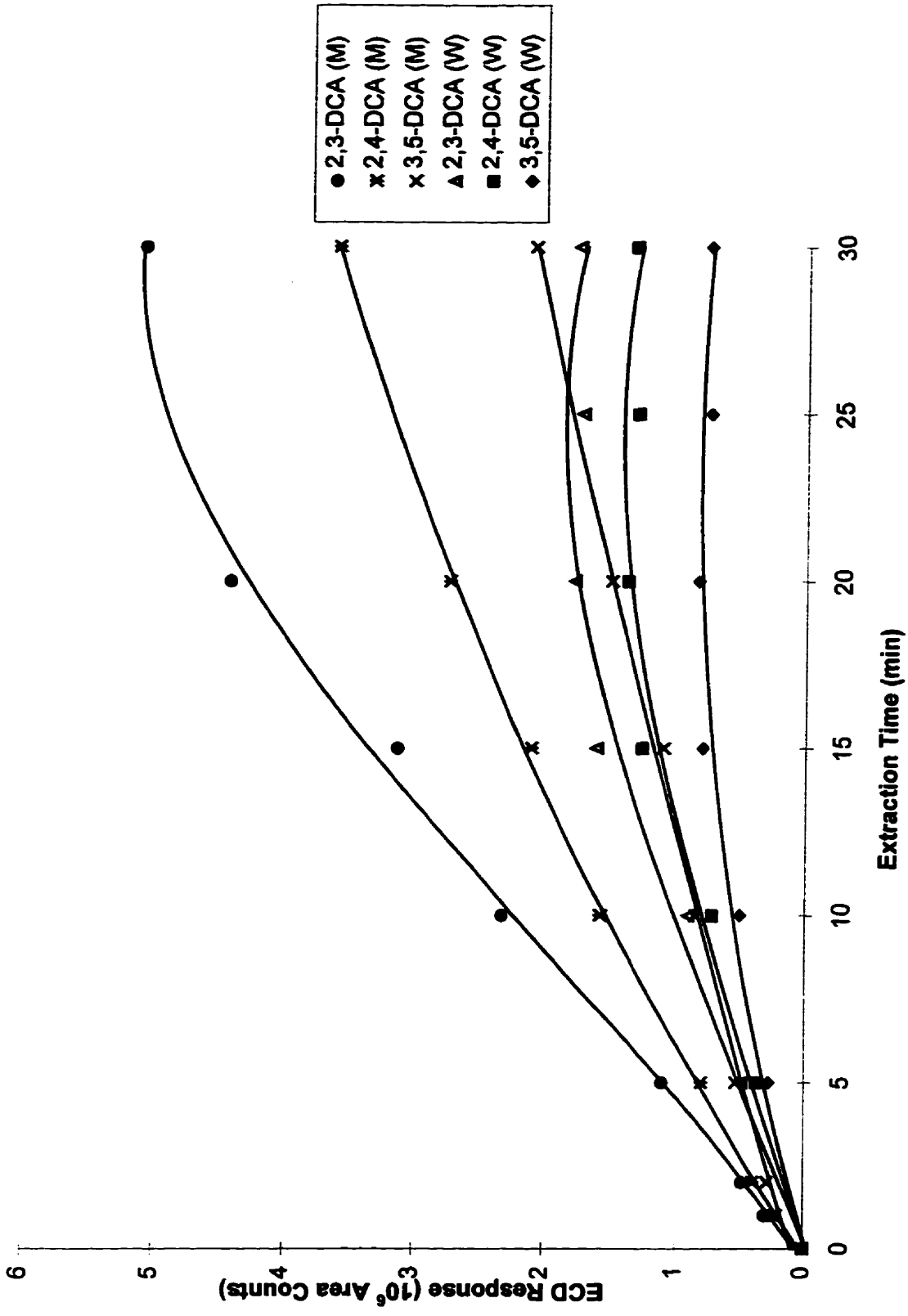


Figure 4.13 **Extraction time required to reach equilibrium: manual stirring (M) *cf.* use of 'Woodpecker' (W)**

Table 4.8 Times required to reach equilibrium (min) and time factors gained through the usage of the 'Woodpecker' apparatus

Analyte	Equilibrium Time without 'Woodpecker' Apparatus (min)	Equilibrium Time using 'Woodpecker' Apparatus (min)	Time Factors Gained using 'Woodpecker' Apparatus
2,3-dichloroanisole	115	20	5.8
2,4-dichloroanisole	128	20	6.4
3,5-dichloroanisole	138	20	6.9

The use of magnetic stirring for manual SPME injection increased the rate of partitioning into the polyacrylate fibre compared to the use of autoinjection with the 'Woodpecker' enhanced method (Figure 4.13). Area count comparisons of these two methods were substantially different despite the fact that both methods had reached equilibrium. This phenomena can be accounted for due to the fact that the 'Woodpecker' enhanced method increased the speed at which the analyte in the immediate region of the fibre partitioned into the fibre. However, in the case of the magnetic stirring method, a greater amount of analyte was available to partition much more readily into the fibre due to the vigorous stirring. Redesigning this prototype agitation apparatus might result in attainment of similar area counts as compared to magnetic stirring. However, this was not investigated as the 'Woodpecker' enhanced method provided adequate detection of the chloroanisoles while improving the partitioning seen in the conventional static autosampler.

In Figures 4.12 and 4.13, the trend in partitioning from highest to lowest, ranged from 2,3-DCA, 2,4-DCA, to 3,5-DCA. 2,3-DCA is the most polar of the three chloroanisoles studied therefore having the largest partition coefficient and being very suitable for analysis using the 85 μm polyacrylate fibre. Calculation of partition coefficients were calculated and reported in section 4.16 (Calculation of Partition Coefficients). The ability to agitate samples using the automated system greatly increased the number of samples which could be analyzed during a day. However, chlorophenols and acid herbicides could not be analyzed using the automation method as the autosampler software would not permit their analysis in conjunction with the SPME derivatization procedure.

4.14 Analysis of Soils

Each soil was analyzed and reported in Table 4.9 in order that comparisons could be made between the two soils used in the laboratory study. The two soils were chosen to represent opposite ends of the particle size spectrum of agricultural soils in Manitoba.

The Miniota soil is composed of 86% sand with only 9% clay with silt making up the remaining 5%. At the other extreme, the Osborne clay is 67% clay and 1% sand with the remaining fraction being composed of 32% silt. Based on the particle size analysis and using the textural triangle with these measurements, the Miniota sand was classified as a loamy sand whereas the Osborne clay was designated to be a heavy clay.

pH measurements showed the Miniota sand to have pH 6.8 and the Osborne clay pH 7.5. Conductivity expressed in dS/m varies between 0.054 for the sand and 0.65 for the clay. Despite the more than order of magnitude difference between the conductivities of these two soils, the Osborne clay is not considered to be a saline soil. Organic carbon measurements were determined using the Yeomans and Bremner (1988) method. Organic carbon values are very similar between the two sites ranging from 4.7% for the sand and 5.6% for the clay. The organic carbon results for the Miniota sand are rather high for a sandy textured soil but may be attributed to the fact that the soil was obtained from uncultivated land and to the presence of native tall grasses which may have resulted in the inclusion of a small amount of fine root material in the soil sample.

Table 4.9 Physical and chemical characteristics of soils used in laboratory[†]

Parameter	Miniota Sand	Osborne Clay
% Sand	86	1
% Silt	5	32
% Clay	9	67
pH	6.8	7.5
Conductivity (dS/m)	0.054	0.65
% Organic Carbon	4.7	5.6
Soil Texture	Loamy Sand	Heavy Clay

[†] More detailed information can be found in Appendix I for the Miniota Association (Ehrlich, 1957; Michalyna *et al.*, 1976) and the Osborne Association (Ehrlich, 1953; Michalyna *et al.*, 1988).

4.15 Effect of Dissolved Organic Carbon on Extraction of Total Residue in Water

Water from four sources, *viz.*, HPLC grade water, well water from a domestic well near Oakville, MB, the La Salle River, the Red River at St. Vital Park in Winnipeg, MB as well as aqueous extracts of Miniota sand and Osborne clay were spiked with 2,4-D, dicamba, 2,4,5-T, and triclopyr. Soil extracts were analyzed for dissolved organic carbon (DOC) at the Water Chemistry Laboratory at the Freshwater Institute, Canada Department of Fisheries and Oceans, Winnipeg, MB (Stainton *et al.*, 1977). These four water samples ranged from 0.6 mg/L DOC for the HPLC grade water up to 15.0 mg/L DOC from the La Salle River. Results are reported in Table 4.10.

Table 4.10 Dissolved organic carbon (DOC) values for water and soil extracts

Sample Name	Dissolved Organic Carbon (DOC)	
	Mean DOC (mg/L)	Standard Deviation (±)
HPLC grade water	0.6	0.4
Well	11.1	1.2
La Salle River	15.0	0.84
Red River	14.7	2.0
Miniota Sand	198	6.8
Osborne Clay	219	6.6

Analytes were spiked into these matrices and allowed to equilibrate for 72 h and the samples were analyzed as previously described. The analyte concentration extracted as determined by this procedure was correlated with the amount of DOC present in the

system. No attempt was made to control biodegradation of the analytes and therefore results are confounded with the addition of this extra variable.

The SPME technique only measures the freely dissolved fraction in a protein solution (Vaes *et al.*, 1996*b*). The measured concentration (freely dissolved) of each analyte in Table 4.11 appears to decrease as DOC increases. This trend is apparent in Figure 4.14. Comparisons should be made between acid herbicides, phenols, and chloroanisoles thus spanning a range of polarities. One would expect to see this effect to the least extent for analytes containing carboxylic groups. The presence of ether linkages (chloroanisoles) should have the greatest amount sorbed to DOC with phenols in between. Table 4.11 gives the relationship between dissolved organic carbon and measured concentration (μg/L) as a function of linear regression lines along with statistical output.

Table 4.11 Relationship between dissolved organic carbon (mg/L) and measured concentration (μg/L) (See Figure 4.14)

Analyte	Slope	Y-Intercept	r^2	Probability of significant F value
dicamba	-0.00068	0.5	0.301	0.259
2,4,5-T	-0.00079	0.5	0.477	0.129
triclopyr	-0.00083	0.5	0.594	0.073
2,4-D	-0.0012	0.5	0.812	0.014

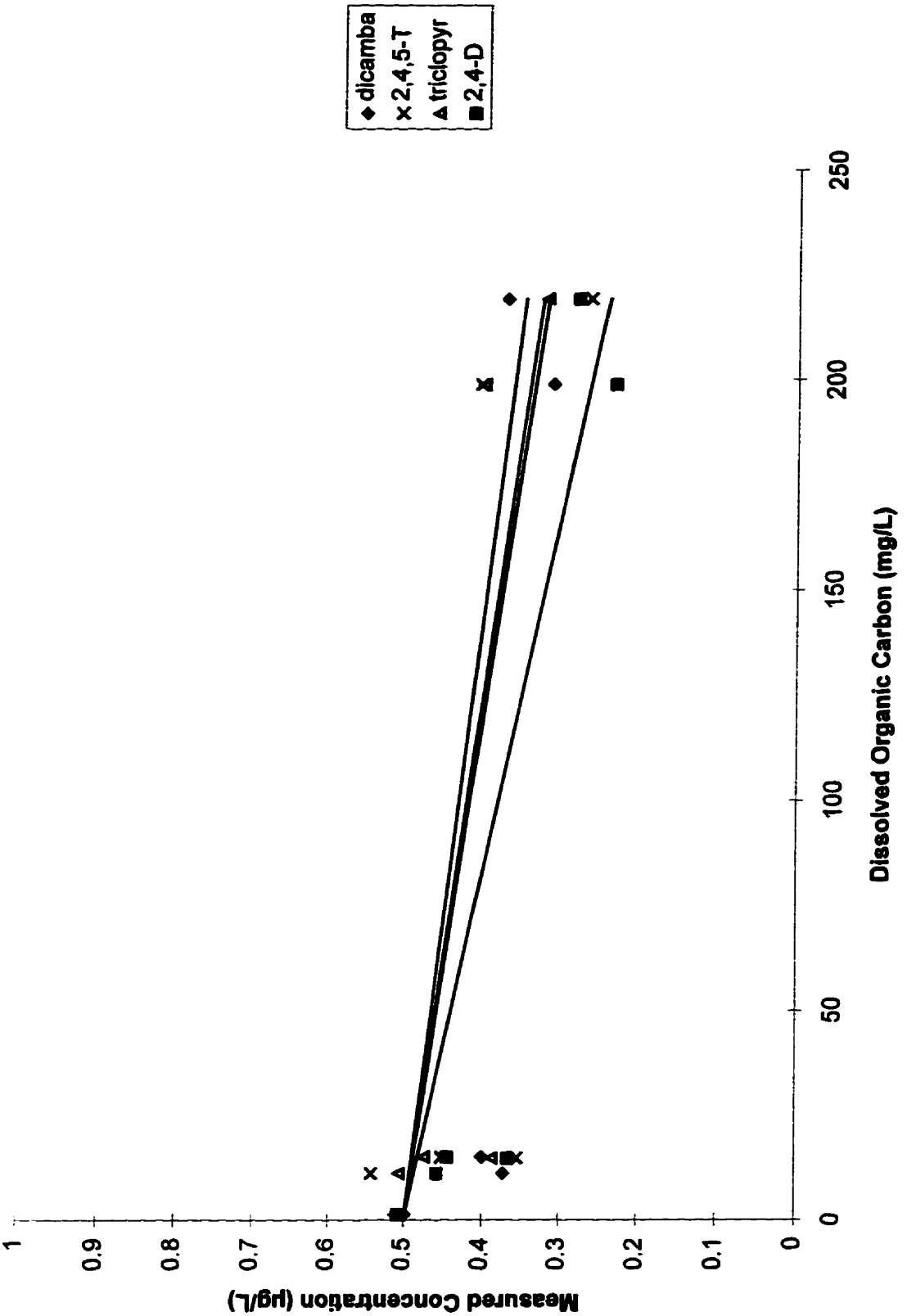


Figure 4.14 **Effect of dissolved organic carbon (Table 4.10) on extractability of acid herbicide mixture as analyzed by SPME (see Table 4.11) (Order in legend is in order of increasing slopes)**

The r^2 values for the lines of best fit indicate that there is a weak correlation ranging from 0.301 (dicamba) to 0.812 (2,4-D) between the measured concentration of the analyte and the concentration of dissolved organic carbon. However, due to the variability of the DOC from one source to another and potential biodegradation, r^2 values were not expected to be extremely high. Nonetheless, the probabilities of a significant F value ranged from 0.014 (2,4-D) which indicates a high degree of significance to less significant results of 0.259 (dicamba). The only statistically significant relationship ($p < 0.05$) was observed in the case of 2,4-D.

The organic carbon found within each matrix has a major role in the way interactions between herbicides and organic carbon behave. The results were also plotted as the concentration detected (% freely dissolved) for the three water sources compared to HPLC grade water. Figure 4.15 shows the results from natural water samples fortified to 0.5 μg/L. Error bars are plotted as ± one standard deviation in Figures 4.15 through 4.17. Even though biodegradation could not be accounted for, it appears that in general less biodegradation of 2,4,5-T and triclopyr is occurring as compared to dicamba and 2,4-D in Figure 4.15. Biodegradation half-lives are on the order of two weeks for dicamba and 2,4-D and approximately six weeks for 2,4,5-T (Howard, 1991).

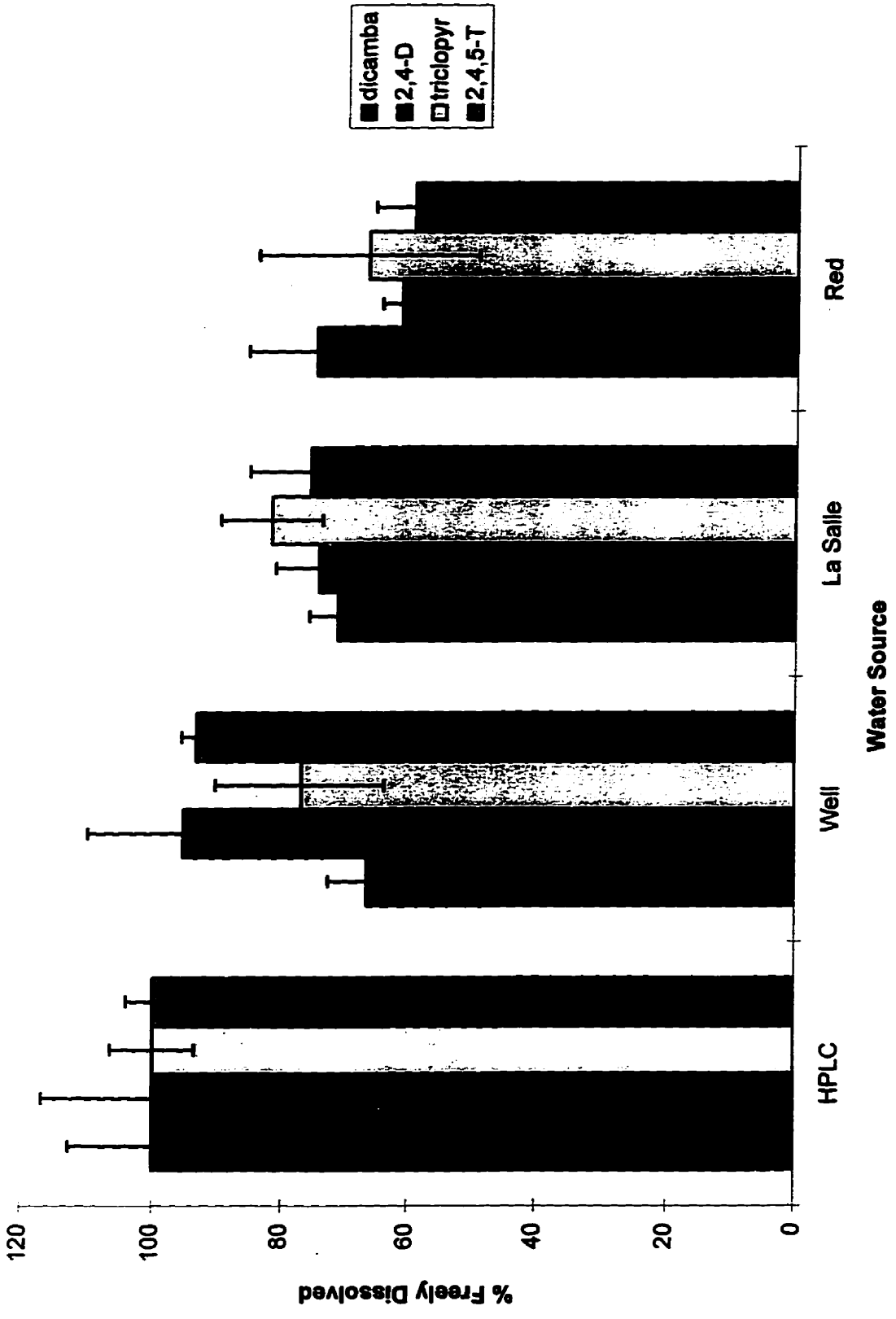


Figure 4.15 **Effect of various water sources on spiked extractability of herbicides (0.5 μg/L)**

As was the case with % organic carbon in air-dried soil (Table 4.8), the soil extracts also followed a similar trend to that of dissolved organic carbon (DOC). The Miniota sand extract contained a concentration of 198 mg/L DOC while the DOC concentration of the Osborne clay soil extract was determined to be 219 mg/L. The soil extracts were analyzed as if they were water samples containing large amounts of dissolved organic carbon. As with the DOC contained in the water samples, the amount of DOC in the soil extracts can be used in part to explain the differences in extractability with respect to the two soils utilized.

The 28-80 mesh glass beads were used to illustrate the role of the matrix in determining sorption differences. Glass beads are unlike soil in that the specific surface area to which the analyte can sorb is much lower. The sorptive capacity of the matrix determines extractability from the soil extract if harsh extraction techniques are not employed. The SPME technique overestimates the amount of analyte which is freely dissolved in the system as the extractant is 4 *M* NaCl and acidified to pH <2. Therefore, the concentration detected by SPME can be considerably lower than the total residues attained from conventional techniques because only the fraction dissolved in the water is detected. If there is little sorption to DOC or particulate matter, then there will be small differences. With SPME, analytical results reflect potentially bioavailable residues assuming the method is performed under natural conditions. The premise of this argument is that for a given analyte to be bioavailable, it must first be in solution (Parkerton and Stone, 1997; Gobas, 1997). This is similar to the concept of biomimetic extraction in which an analyte selectively partitions onto a surrogate lipid phase

simulating bioconcentration. Toxicity will result if partitioning is favorable and the amount of analyte sorbed exceeds the critical body residue generating narcotic toxicity. Analytical results are obtained through a similar partitioning process using SPME.

Therefore, it is reasonable to assume that without performing toxicity testing, the SPME extraction technique could provide valuable information giving an indication of narcotic toxicity as the SPME process works on the basis of partitioning of analytes. The analyte which is not freely dissolved in the water fraction is assumed to be unavailable to the organisms, thereby not able to cause harm (Gobas, 1997). The xenobiotic may be present in high quantities but is not readily extractable and therefore would not possess an appreciable risk to organisms within the environment unless it desorbed at a later date.

Conventional extraction techniques attempt to quantify the total residue contained within the sample. However, due to the inherent nature of the fibre coating and the gentler extraction conditions, this new method allows the analyst to measure only a portion of the total residue. Despite the fact that the matrix may contain substantially higher residue concentrations, they may be bound and unavailable to cause harm to organisms in the environment. Speciation of compounds within the environment is an important variable when considering levels of environmental contaminants and the SPME technique may be used as a surrogate in measuring the fraction of the contaminant which is potentially bioavailable similar to biomimetic extractions providing natural conditions are used.

In soil, originally KCl was used when preparing standards of the acid herbicides and chlorophenols. However, NaCl was later used as sodium has a greater hydrating radius than that of potassium (Skoog and West, 1976). The effective diameter of K^+ is 3 Å whereas Na^+ has an effective diameter of 4-4.5 Å (Skoog and West, 1976). The presence of the sodium ion versus the potassium ion served to increase the extractability by separating the clay plates to a greater extent, thereby allowing the herbicide to move into solution. Maximizing the amount of residue which moves into solution while still minimizing the 'harshness' of the extraction process is important because the centrifugate is the portion which was being analyzed.

The Miniota loamy sand and the Osborne heavy clay soils have very similar organic carbon contents. Clay soils usually contain higher % carbon than sandy textured soils. The similar organic carbon contents can be explained by the fact that the Miniota loamy sand was sampled from a native prairie setting as opposed to the Osborne heavy clay which was sampled from a cultivated site. The Miniota soil has an organic carbon content of 4.7% while the Osborne clay has an organic carbon content of 5.6%. Acid herbicides do not appreciably bind to clay particles but do bind to DOC. This would account for the similar detectable concentrations between the sand and clay matrices. However, the difference in % organic carbon content is reflected in normalized area counts for three matrices; namely, glass beads, Miniota sand, and Osborne clay. More analyte was sorbed to the organic carbon in the Osborne clay extract compared to the Miniota sand extract (5.6% versus 4.7% organic carbon) respectively (Figure 4.16). However, biodegradation of the analytes is possible in soil water extracts as was observed

in the La Salle River water. However, as the DOC in these matrices is significantly greater, more sorption can occur in these matrices ranging from 0.09% - 1.2%. These results are tabulated in Table 4.7.

The sorption by these matrices was in the order glass beads < Miniota sand < Osborne clay reflecting the nature and number of active sites in the matrix largely due to the presence of organic matter and clay content and is seen in Figure 4.17 for three chloroanisoles. The results have been normalized for the glass beads artificially representing 100% of the analyte being in the dissolved fraction. SPME proceeds by partition of the dissolved analyte into the fibre coating. The analyte bound to the soil or dissolved organic matter are different chemical species and will not be able to partition into the fibre coating of the SPME fibre in the same way.

4.16 Calculation of Partition Coefficients

The calculated log K_{SPME} values obtained from the use of the polyacrylate fibre were greater than the literature log K_{OW} values in all instances (Table 4.12). This degree of overestimation ranged from 6% in the case of 3,5-DCP to 43% in the case of triclopyr. As there were only seven analytes used in this study, an additional six phenolic compounds were pooled to form a larger data set (Dean *et al.*, 1996). Dean *et al.* (1996) prepared the phenols in a pH 2 buffer solution (250 mL of 0.2 *M* KCl and 65 mL of 0.2 *M* HCl in 1 L of HPLC grade water) whereas the results from this study were prepared in 4 *M* NaCl and acidified to pH 1.7 which may account for some deviation between data sets.

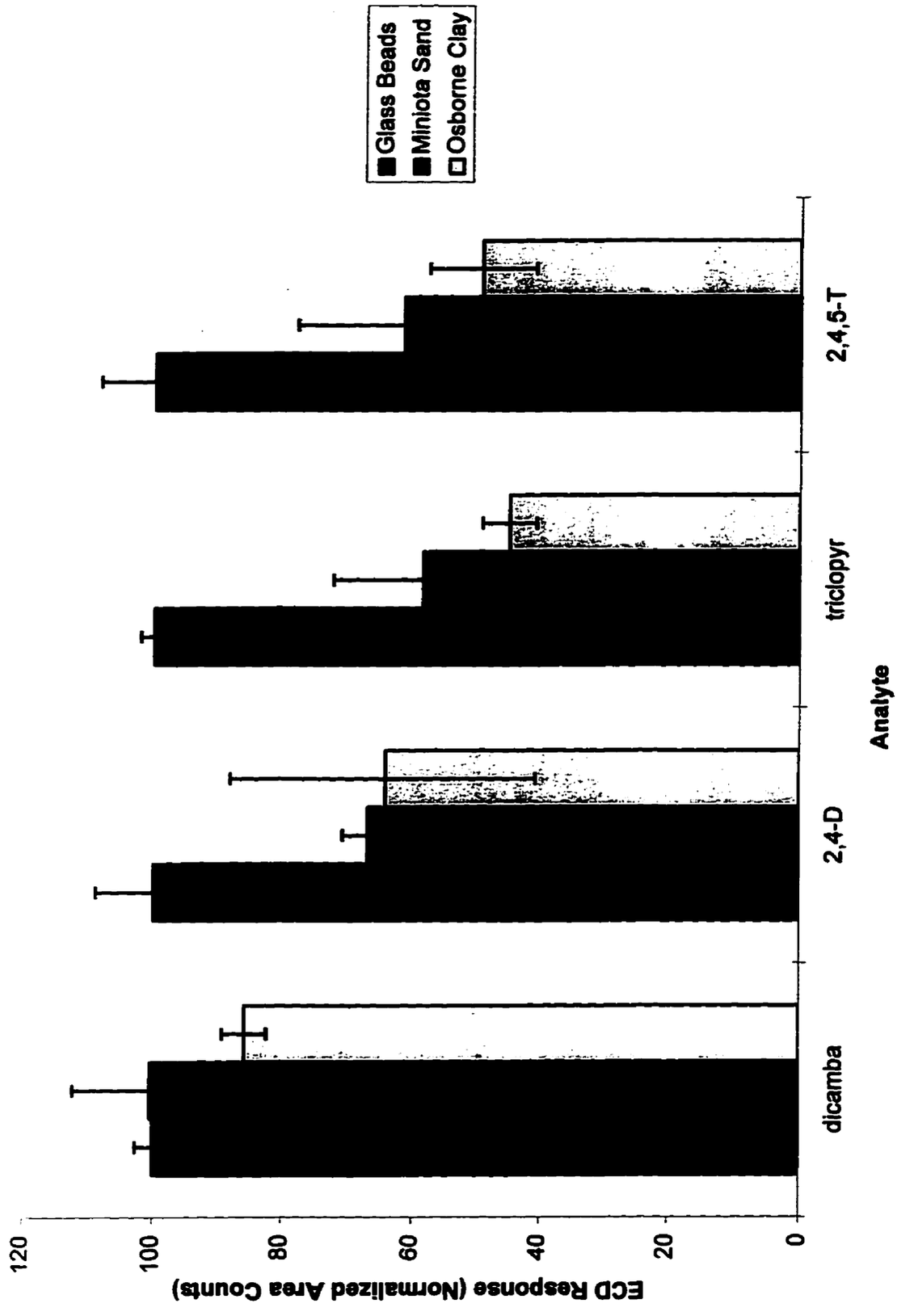


Figure 4.16 **Extractability of Miniota and Osborne Soils after spiking acid herbicides into the soil at 100 ng/g soil**

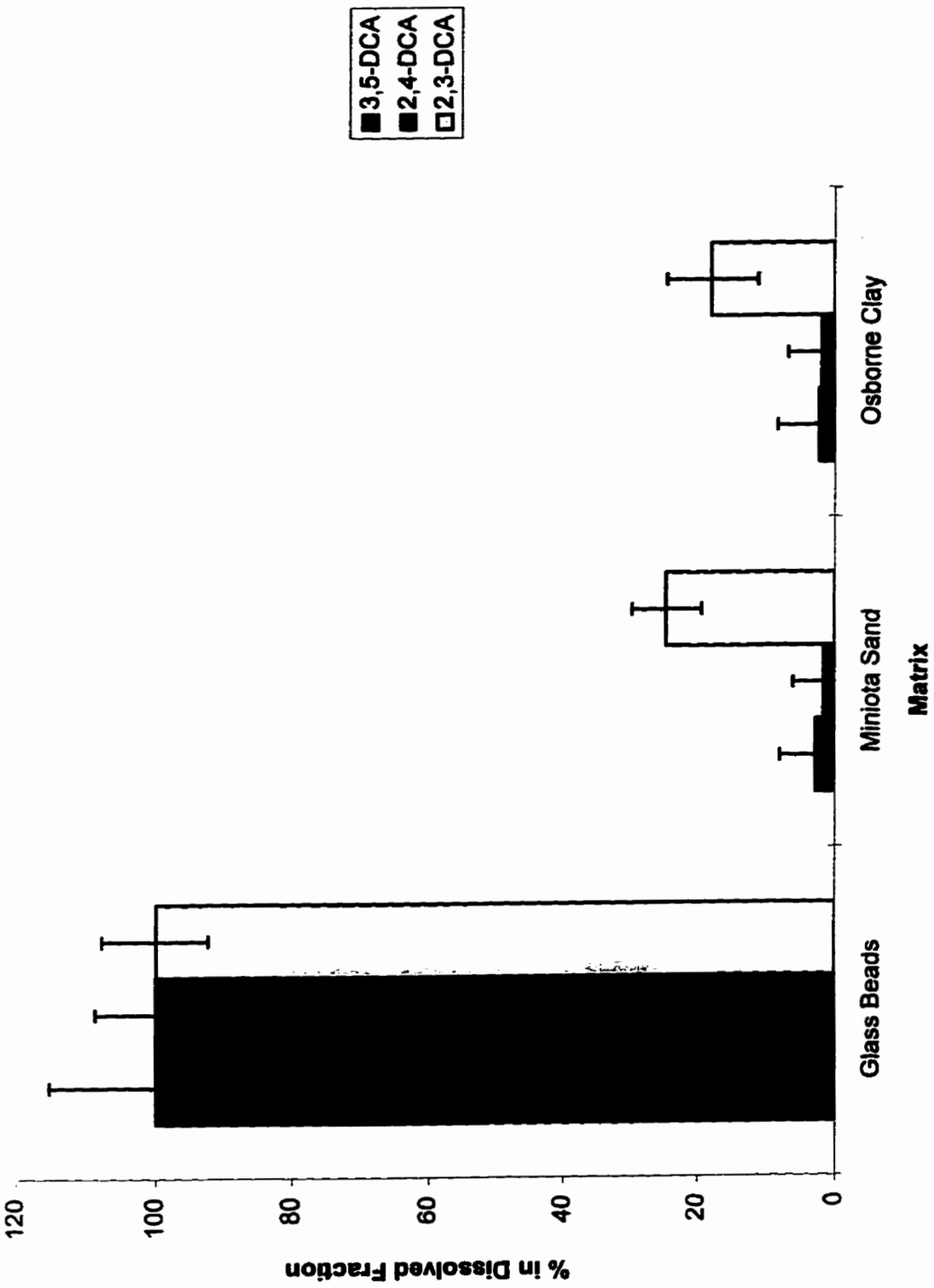


Figure 4.17 Percent chloroanisoles in dissolved fraction following spiking

Nonetheless, using the pooled data set, a linear regression was calculated to be $y = 1.13 + 0.015$ ($r^2 = 0.68$). The correlation between log K_{OW} values and log K_{SPME} values is graphically shown in Figure 4.18. The probability of a significant F value is 0.0005. This allows one to use the polyacrylate fibre to predict log K_{OW} values for compounds which contain phenolic and carboxylic acid functional groups using the SPME technique. Previous findings with the PDMS fibre showed that K_{OW} values could be estimated within an order of magnitude (Arthur *et al.*, 1992*b*).

Table 4.12 Comparison between calculated K_{SPME}, log K_{SPME}, and literature log K_{OW} values

Analyte	Calculated K_{SPME}	Calculated log K_{SPME}	Literature log K_{OW}
2,4-D[†]	4.4×10^3	3.6	2.8
dicamba[†]	2.6×10^2	2.4	2.2
triclopyr[†]	5.9×10^3	3.8	2.6
2,4,5-T[†]	4.0×10^3	3.6	3.1
2,3-DCP[§]	7.5×10^3	3.9	3.0
2,4-DCP[§]	6.0×10^3	3.8	3.2
3,5-DCP[§]	4.2×10^3	3.6	3.4

[†] quantified as methyl ester
[§] quantified as methyl ether (chloroanisole)

Since the K_{SPME} values are all above 10^3 with the exception of dicamba, this means that significant depletion of the analyte could occur after just one sampling from a 2 mL vial. Arthur *et al.* (1992*a*) reported that using a PDMS fibre, an analyte having a distribution

constant of 10^2 required 10^3 injections to remove 95% of the analyte whereas an analyte having a distribution constant of 10^3 required only 11 injections to remove 95% of the analyte. This further decreased to 2 injections required to remove 95% of the analyte if the analyte had a distribution constant of 10^4. The main reason for this depletion is that the volume of the autosampler vial is under 2 mL and only contains a few ng of analyte. The concentrating power of the SPME technique makes it attractive in situations where there is little sample available to be analyzed while not compromising detection limits. However, to avoid depletion during sampling, a larger vial and a larger volume of solution could be used.

This study only included analytes which had log K_{OW} values that ranged from 2.2 to 3.4. In order to get a more accurate picture of the trend that was occurring, additional compounds with higher log K_{OW} values would need to be extracted using the polyacrylate fibre and would serve to extend the relationship between log K_{SPME} and log K_{OW} values in a direction further away from the origin.

Partition coefficients as determined by the SPME fibres are useful in the determination of method detection limits. As log K_{SPME} values can be used as a surrogate to log K_{ow} values which are easily located in the scientific literature, an analyst has the ability to predict the range of which analytes may be quantified using a particular fibre coating.

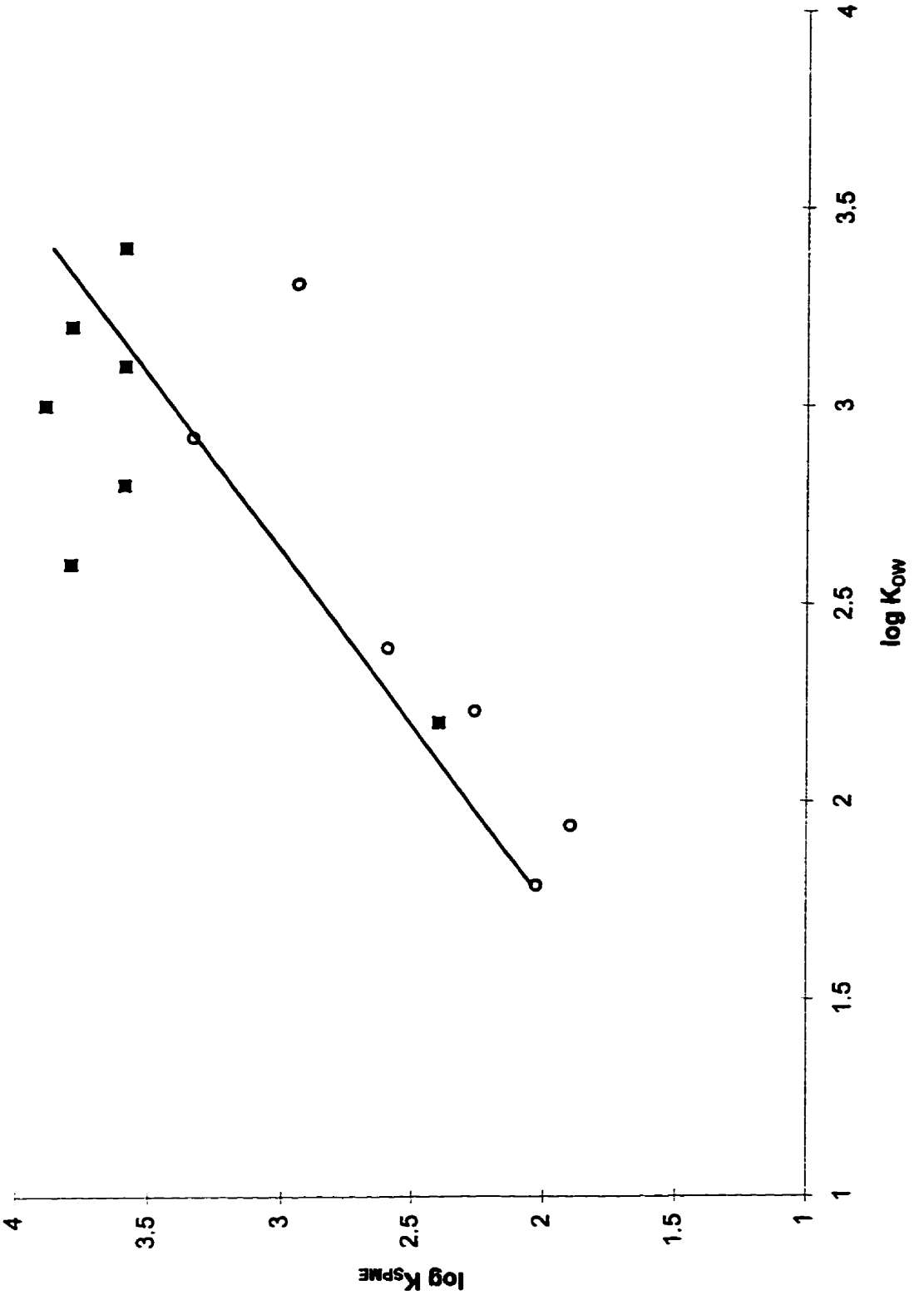


Figure 4.18 **Correlation between log K_{OW} and log K_{SPME} values (Unfilled circles are data points from Dean *et al.*, 1996; solid points are from calculations reported in Table 4.12)**

A problem with the estimation of log K_{OW} values using K_{SPME} values may be associated with the 'salting-out' procedure which was used to decrease the water solubility and increase the partitioning of the analytes into the polyacrylate fibre. This may account for the overestimation using the polyacrylate fibre as one is measuring the freely dissolved analyte under conditions which are altered from the natural environment. K_{SPME} values were calculated in this study and those reported in Dean *et al.* (1996) using acidified samples.

4.17 Comparison between polyacrylate and polydimethylsiloxane (PDMS) fibre

Partition coefficients were determined to be higher using the polyacrylate fibre for chloroanisoles as compared to the polydimethylsiloxane (PDMS) fibre. The actual number of ng of analyte sorbed by the fibre was actually greater using the PDMS fibre. One would expect a greater amount of material to be sorbed to the PDMS fibres as compared to the polyacrylate fibre based on the decreased polarity of the chloroanisoles relative to the corresponding chlorophenols. However, the polyacrylate fibre partition coefficients (K_{SPME}) appear to be larger because of the smaller volume of fibre coating on the polyacrylate fibre (5.3×10^{-4} mL) as compared to the PDMS fibre (6.7×10^{-4} mL). The PDMS fibre coating volume is roughly 26% larger than the polyacrylate fibre coating volume. However, the trend in increasing polarity (3,5-DCA < 2,4-DCA < 2,3-DCA) is not well represented in log K_{SPME} values because of the small difference in partition coefficients between these three compounds Table 4.13.

Table 4.13 Calculated log K_{SPME} values for chloroanisoles using polydimethylsiloxane and polyacrylate SPME fibres

Analyte	log K_{SPME}	
	Polydimethylsiloxane	**Polyacrylate**
3,5-DCA	2.81	3.06
2,4-DCA	2.84	3.05
2,3-DCA	2.92	3.07

In the analysis using soils, the soil extracts were analyzed to obtain electrical conductivity values to determine the amount of soluble salts which were in the soil extracts prior to the addition of 4 *M* NaCl. The 4 *M* NaCl in HPLC grade water, 4 *M* NaCl in La Salle River water, and in Miniota soil water had electrical conductivity values of 392, 386, and 397 mS/cm, respectively. Manitoba Environment (1997) records indicate that at the sampling location the mean electrical conductivity values were measured to be 0.905 mS/cm prior to the addition of NaCl. Therefore, the contribution of soluble salts in the 'salting-out' at such low levels compared to 4 *M* NaCl will be negligible.

As a general trend at both the 10 and 100 μg/L concentrations, the amount of extractable chloroanisoles decreased moving from HPLC grade water, to La Salle River water and finally to Miniota soil water spiked at a constant level (Table 4.14 and 4.15). There is a slight aberration in the La Salle River water using the PDMS fibre. However, these values are within one standard deviation. Statistically significant differences (ANOVA) at $p \leq 0.05$ are indicated in boldface type comparing La Salle River water to HPLC grade

water and Miniota soil water to HPLC grade water, respectively. Using the polyacrylate fibre, the measured freely dissolved portion of the analyte decreased more than with the PDMS fibre and thus the concentration measured using the polyacrylate fibre was less.

Polyacrylate SPME fibres are useful in extracting polar analytes such as chlorophenols while the polydimethylsiloxane fibre is more suitable for less polar analytes such as chloroanisoles. The conclusion drawn from this data is that the chloroanisoles are better able to partition to the PDMS fibre than to the polyacrylate fibre. One possibility to account for the reason why the polyacrylate fibre measures less analyte than the PDMS fibre is that there is competition occurring between the polar functional groups of the dissolved organic carbon and the polyacrylate fibre. The difference in polarity using the PDMS fibre and the organic matter does not interfere with the extraction process. However, these results require closer scrutiny to validate this hypothesis. The time to equilibrium may not have been long enough as solutions were only allowed to sit overnight. Ideally, solutions should be prepared and allowed to stand for a much longer time period to allow DOC sorption to proceed to equilibrium. However, the increased time interval also increases the chances of chloroanisole degradation.

Table 4.14 Concentrations at spiking level of 100 μg/L determined using PDMS and polyacrylate SPME fibres as a function of matrix

Polydimethylsiloxane	HPLC grade water	La Salle River water	Miniota soil water
3,5-DCA	100	103	**85.5**
2,4-DCA	100	105	**95.1**
2,3-DCA	100	106	**97.7**
Polyacrylate (PA)	**HPLC grade water**	**La Salle River water**	**Miniota soil water**
3,5-DCA	100	**78.9**	**70.7**
2,4-DCA	100	82.4	**75.9**
2,3-DCA	100	**85.0**	**69.4**

Boldface type indicates significance at $p \leq 0.05$ level (ANOVA).

Table 4.15 Concentrations at spiking level of 10 μg/L determined using PDMS and polyacrylate SPME fibres as a function of matrix

Polydimethylsiloxane	HPLC grade water	La Salle River water	Miniota soil water
3,5-DCA	10	10.2	**3.65**
2,4-DCA	10	12.8	**4.97**
2,3-DCA	10	12.8	**5.98**
Polyacrylate (PA)	**HPLC grade water**	**La Salle River water**	**Miniota soil water**
3,5-DCA	10	**6.38**	**4.57**
2,4-DCA	10	8.02	**3.26**
2,3-DCA	10	8.40	**3.94**

Boldface type indicates significance at $p \leq 0.05$ level (ANOVA).

5. SUMMARY AND CONCLUSIONS

Chlorophenols, unlike the acid herbicides, can be directly analyzed using the 85 μm polyacrylate SPME fibre. However, detection limits can further be decreased through derivatization using diazomethane using the SPME-alkylation-GC-ECD technique. The acid herbicides, including dicamba, 2,4-D, triclopyr, and 2,4,5-T, and all chlorophenols tested can be analyzed by conducting a direct SPME sorption onto the polyacrylate fibre followed by a second sorption step in the headspace of a vial containing an ether solution of diazomethane in under 1 min. The carboxylic acid methyl esters, are formed in the case of the acid herbicides and methyl ethers (chloroanisoles) in the case of chlorophenols. Confirmation of the identity of the methyl esters was performed by GC-MS or via comparison of t'_R with those of authentic material (chloroanisoles).

Extraction conditions are improved if the samples being analyzed are saturated with NaCl and acidified to pH <2 compared to analysis in HPLC grade water. The analytes used in this study had equilibrium times on the order of 20 min provided a small magnetic stir bar was placed in each 2 mL glass vial. The incorporation of agitation provided by the 'Woodpecker' apparatus in conjunction with the Varian 8200 autosampler served to increase the sensitivity of the method over that of static autosampling. Times to reach

equilibrium using this agitation device were on the order of 20 min and increased the number of samples which could be analyzed in a day.

LOD ranged from 0.01 μg/L to 0.05 μg/L whereas LOQ ranged from 0.03 μg/L to 0.07 μg/L for 2,4,5-T and dicamba, respectively. These limits are less than those reported for EPA Method 8150B for water. Spiking standards into natural waters was conducted to demonstrate the potential of SPME as a viable extraction technique for environmental water samples. The concentration range of analytes tested was 0.01 μg/L to 1.0 μg/L. Reproducibility expressed as % RSDs for the derivatization method ranged from 2.7 - 31.4%.

The fact that chloroanisoles may be contaminants themselves suggests that perhaps another derivatizing agent should be employed. Use of a homologous diazoalkane such as diazoethane or diazopentane would produce a convenient molecule not found in the natural environment. Other diazoalkanes were not utilized in this study.

All derivatization work was performed manually. The 8200 Varian Autosampler might be utilized in the future for this type of work; however, the required software had not yet been released by Varian. However, with a minor amount of reprogramming, it would be possible to do the extraction from one vial and move to another vial without the desorption step to perform a headspace sorption of diazomethane. This procedure would accomplish the same end result without the necessity to perform the work manually. As

all events would be time controlled, automated injection might be expected to lessen some of the variability observed with the manual work. The possibility of incorporating such suggestions has been forwarded to Varian Canada Inc. for review.

Analysis of soils was completed by adding 20 mL of 4 *M* NaCl to each 10 g sample of air-dried soil, shaking for 1 h, followed by centrifugation at 2000 rpm for 15 min. A 1.6 mL aliquot of the supernatant was then acidified to pH <2 before extracting with the polyacrylate fibre and prior to the derivatization step.

As SPME measures only the dissolved portion of the analyte and none of the analyte which is sorbed to organic matter, it has important applications in the area of biomimetic extraction provided unaltered extraction conditions are utilized. These types of techniques are important in that they are able to calculate only the fraction of analyte which is potentially bioavailable assuming sorbed analytes are unavailable for uptake by organisms. As a mathematical relationship exists between the amount of analyte sorbed to the fibre coating and concentration of the analyte in solution, the calculation of partition coefficients (log K_{SPME}) can be determined. These have been found to closely parallel log K_{ow} values.

A comparison between extraction using the polyacrylate and PDMS fibres found that with both coatings as a result of increased amount of DOM, the amount of analyte which was freely dissolved and able to partition to the fibre coating decreased. However, the polyacrylate fibre measured less analyte than the PDMS fibre and may be accounted for

as a result of competition occurring between the polar functional groups of the dissolved organic carbon and the polyacrylate fibre.

Recommendations for further study include other comparisons with other analytical methods to establish SPME as a valid analytical technique. In conjunction with this, a standardization procedure is required so that analysts using this technique can document the number of extractions which can be performed with each fibre and what cleaning procedure if necessary is required to rejuvenate the fibre. This would ultimately depend on the matrix being sampled. This area needs to be examined in more detail prior to large scale usage of SPME in commercial laboratories.

Further work is required to compare the fractions recovered by the polyacrylate and polydimethylsiloxane fibres in different matrices. The effect of organic matter on the sorption kinetics of various compounds must be more clearly understood. This could be determined using a series of compounds ranging in polarity such as chlorophenols, chloroanisoles, and acid herbicides over a wider range of DOC concentrations. Analyses of freely dissolved residues in natural waters are nevertheless possible at present, but the kinetics of the interaction with DOC need further study. When conducting research in this area, it is necessary to inhibit biodegradation with the addition of sodium azide or mercuric chloride.

An agitation device which more closely represents the effect of magnetic stirring needs to be investigated for compounds which require either decreased limits of detection or

decreased time to reach equilibrium. This agitation unit should be coupled to the 8200 autosampler using existing software. More analytes over a much larger range of log K_{ow} values need to be examined to determine whether a true linear relationship exists between log K_{ow} values and log K_{SPME}. SPME has countless applications and can further be applied to a variety of matrices such as air, soils, and water using different fibre coatings to preferentially extract certain analytes. Interfacing the SPME technique with other instrumental analyses techniques also remains a possibility.

6. CONTRIBUTION TO KNOWLEDGE

The SPME technique has been applied to a wide variety of analytes ranging from non polar organics such as hydrocarbons to polar compounds such as phenols. The research reported herein explores the possibility of extending usage for application in the area of chlorophenols and acid herbicides and proves that *in situ* derivatization directly on the polyacrylate fibre using diazomethane is possible. The analytical methodology developed will enable environmental samples including water, soil, and sediments from field sites to be analyzed in Manitoba, perhaps by Manitoba Hydro itself. The turnaround time for such analyses and the cost of analyses should be much reduced from the current cost involved in sending the samples out of province for custom analyses.

It is quite conceivable that this work could be applied to a much wider number of analytes that require derivatization if coupled with the appropriate detector. In conducting preliminary work, the calculation of partition coefficients using the polyacrylate fibre can predict the range over which certain analytes can be quantified and possibly even to predict log K_{ow} values. SPME measures only the freely dissolved portion of the analyte which may be of greatest environmental and toxicological concern with certain organisms. This research has been conducted on a variety of waters ranging from 'pristine' (HPLC grade water) to environmental to soil waters.

The benefits of the SPME technique are numerous ranging from simplicity, speed with which extraction occurs, cost savings associated with there being no need for solvent disposal, small required sample size, specific extractions, and superb detection limits. This research also illustrated the need for an agitation unit in conjunction with the current Varian 8200 static autosampler. Since this research was initiated, Varian Canada Inc. has introduced an agitation unit of another design to satisfy this need in collaboration with Supelco Canada Ltd.

The derivatization procedure was not able to be performed using the Varian 8200 autosampler. However, with a minor amount of reprogramming, a sample could be extracted onto the SPME fibre and derivatized using an aliquot of diazomethane prior to thermally desorbing the analyte in the GC injection port. The ability to program the current SPME software would rapidly increase the number of samples which could be analyzed with excellent precision.

The freely dissolved portion is not the same as the total residue measured using other analytical techniques. As the freely dissolved analyte may be of toxicological significance it makes sense that this is the portion which should be quantified and used when implementing soil and water quality guidelines. If a significant portion of the analyte is unavailable to an organism, the guidelines should reflect this difference for these organisms.

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8. APPENDICES

APPENDIX I

Additional Information

Miniota Sand and Osborne Clay

APPENDIX II

Manual Liquid Injection Calibration Curves for

Chloroanisole Mixture

and

Acid Herbicide Mixture

APPENDIX I

Additional Information

Miniota Association

The Miniota sand was collected from the W½ 35-9-17W approximately 10 km south of Douglas, MB under native vegetation. Soils belonging to the Miniota Association are developed on sandy and coarse sandy outwash deposits which tend to become coarser deeper into the solum. Under the Canadian Soil Classification System devised by Agriculture Canada Expert Committee on Soil Survey (1987), this soil can be classified as an Orthic Black. Three horizons predominate including a thick 12-20 cm Ah horizon, a 10-18 cm thick dark brown Bm horizon, and a BC(k) horizon. Parent material is moderately to strongly calcareous, medium sand to gravelly textured deposits.

The topography is level to slightly undulating and drainage in the area is good to excessive due to the coarseness of the soil. Native vegetation in the area includes mixed prairie grasses and other herbaceous plant species. Organic carbon was relatively high (4.7%) because of the fact that the area was under native vegetation and cultivation practices had not disturbed the organic matter equilibrium. Nonetheless, if cultivation were to occur, soil moisture would be a problem due to the texture of the soil and organic carbon in the soil would begin to decrease. The soil texture precludes it from being extensively used for agricultural production because of potentially severe wind erosion deposits (Ehrlich, 1957; Michalyna *et al.*, 1976).

APPENDIX I

Additional Information

Osborne Association

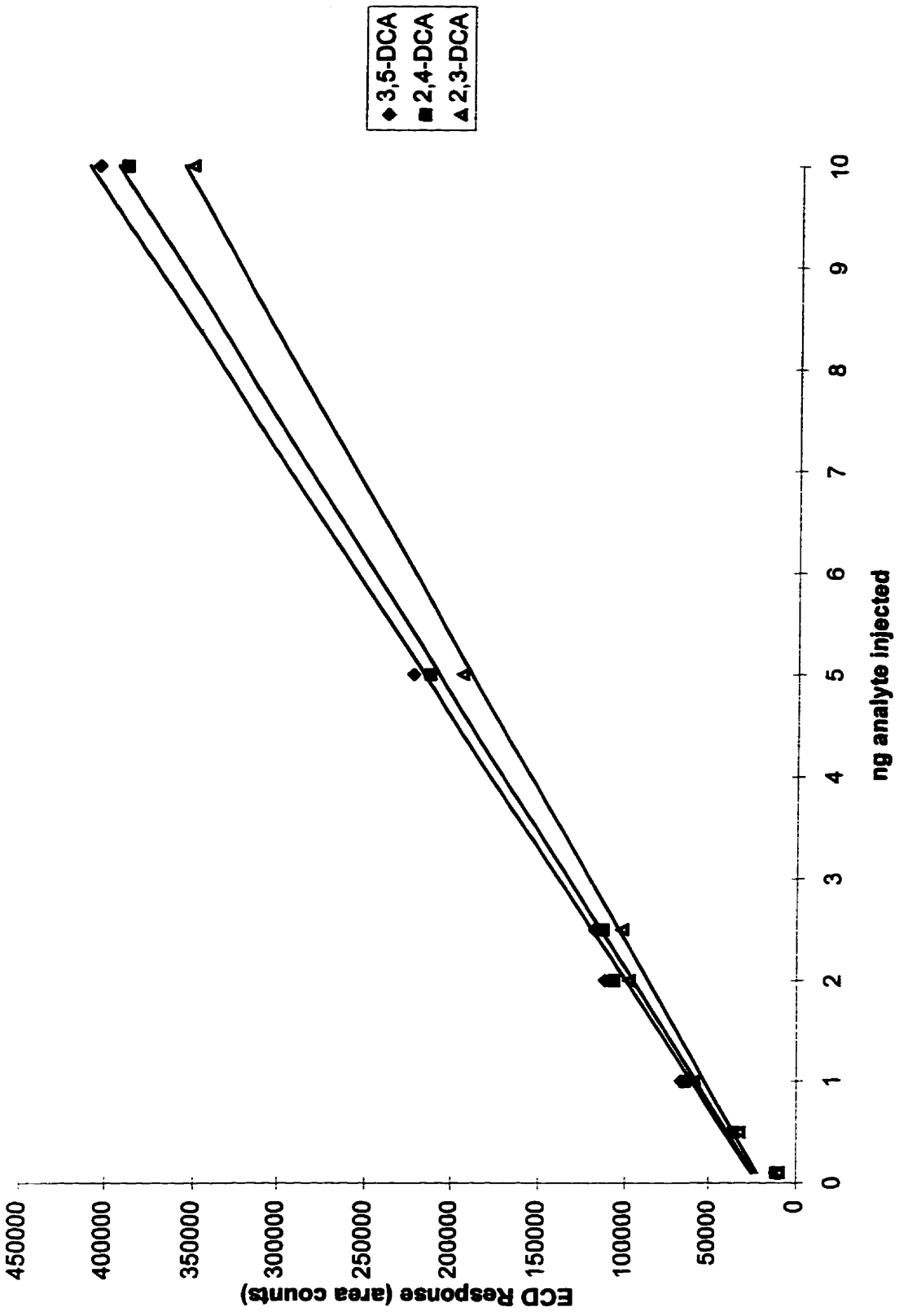
The Osborne clay which belongs to the Red River Association was collected approximately 2 km south of Brunkild, MB. Soils belonging to this association have a thick Ah horizon (up to 30 cm) which is rich in organic matter overlying a calcareous C horizon. Parent materials in the area include clayey fluvial deposits. The presence of distinct mottling indicative of gleization classifies this soil as a Rego Humic Gleysol under the Canadian Soil Classification System (Agriculture Canada Expert Committee on Soil Survey, 1987). Particle size analysis indicates this soil is predominantly clay (67%). Not surprisingly, this heavy clay soil suffers from impeded drainage problems (Ehrlich, 1953; Michalyna *et al.*, 1988).

Topography in this area range from flat to slightly depressional. Native vegetation included meadow grasses, sedges, cattails and willows prior to the advent of modern agriculture. Modern agricultural practices have improved surface drainage substantially. Organic carbon values from this site were determined to be 5.6%. Soils in this area are best suited for grain or grass hay production. Periodically wet conditions account for the mottling which is located deeper into the solum. These periods of wetness preclude the production of root crops such as sugar beets and potatoes (Ehrlich, 1953; Michalyna *et al.*, 1988).

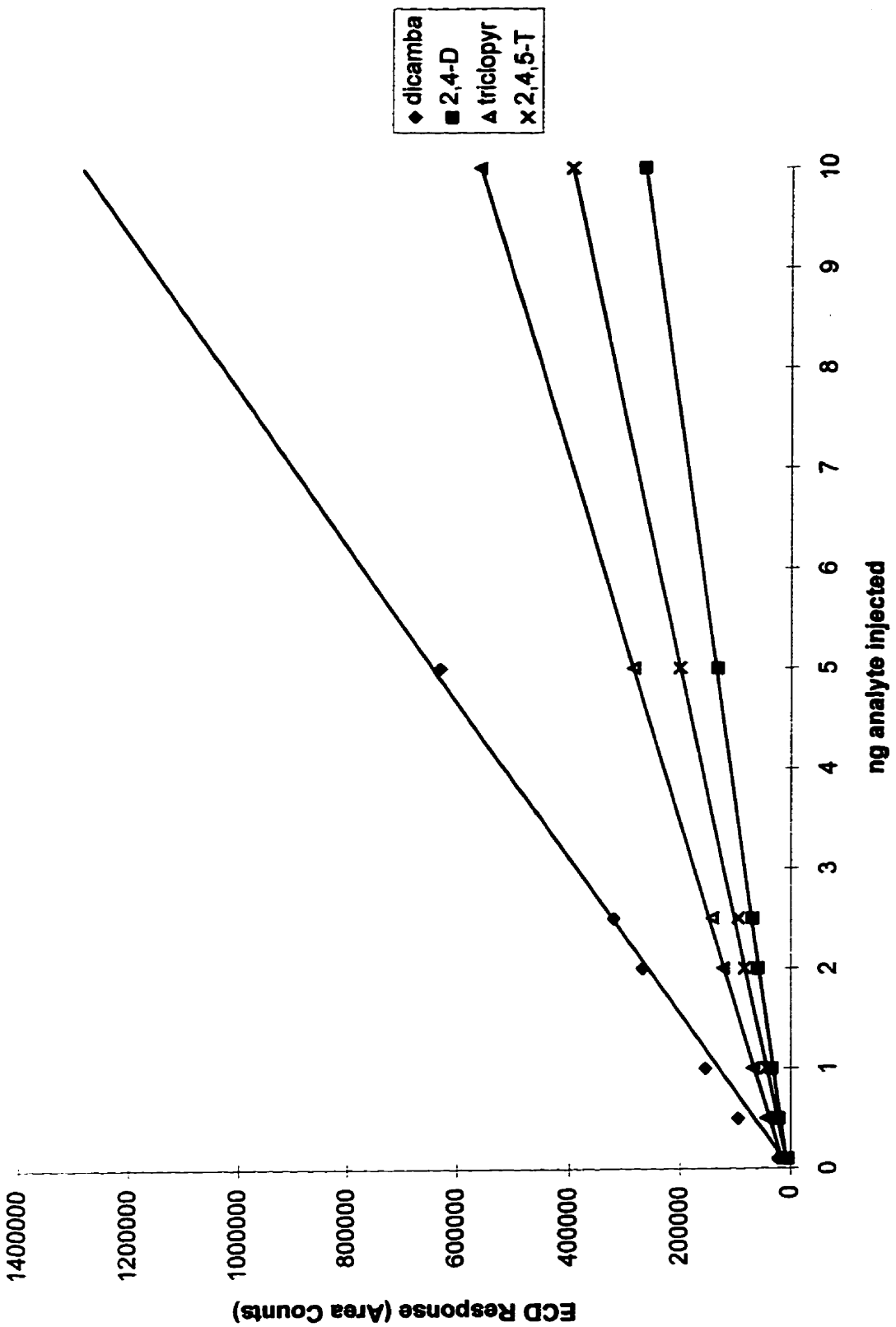
APPENDIX II

Manual Liquid Injection Calibration Curves for

Chloroanisole Mixture and Acid Herbicide Mixture



Appendix II Liquid Injection Calibration Curves for Chloroanisoles



Appendix II Liquid Injection Calibration Curves for Acid Herbicides