Supercritical Fluid Extraction of Polychlorinated Biphenyls and Organochlorine Pesticides From Mussel Tissue, (*Mytilus edulis*)

By

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ABSTRACT

The supercritical fluid extraction (SFE) of twenty one polychlorinated biphenyls (PCBs) and eight organochlorine pesticides (OCPs) from freeze dried mussel tissue is discussed, demonstrated, and compared to traditional Soxhlet extractions. The aim of this study was to determine the efficacy of SFE for the extraction of these lipophilic compounds which involved a spike study to determine the feasibility of on-line sample clean-up by an inclusion of activated alumina within the extraction vessel. These spike extractions from an inert matrix showed that the chemical integrity of the PCBs was not compromised while some OCPs of the DDT derivative were decomposed to other forms.

Preliminary extractions of freeze dried mussel tissue focused on the effect of static extraction time and CO_2 density on recovery of twenty three specific congeners of PCBs. These extractions of freeze dried mussel tissue showed longer static extraction time and higher density/lower temperature would yield quantitative recoveries of PCBs. Triplicate extractions of mussel tissue at the appropriate extraction parameters produced quantitative and similar recoveries to Soxhlet. Finally, an alternative supercritical fluid (CHF_3) yielded non-quantitative recoveries for PCBs and OCPs, but the use of this fluid eliminated the need to use *in situ* alumina to retain co-extracted lipids from the matrix.

This thesis is dedicated to my wife,
Grace, and my parents with love and devotion.

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CHAPTER 1

Introduction

The unique chemical properties of polychlorinated biphenyls (PCBs) and organochlorine pesticides (OCPs) have led to their worldwide production and application in the past. Though their commercial production and use have ceased, their careless disposal and great stability have resulted in contamination of the environment. This fallout has fueled many toxicological studies exploring the harmful effects of PCBs and OCPs and their toxic effects have been well documented. Due to their toxic effects, the analytical chemistry of these compounds have involved their extraction from various environmental matrices.

The environmental analysis of organic lipophilic compounds, like PCBs and OCPs, from various matrices have traditionally involved sampling, extraction, clean-up, and determination. In addition, a concentration of the extract is performed after extraction and clean-up. Traditional extraction techniques rely on tedious and time consuming procedures the require large volumes of expensive and hazardous organic solvents. Also, the analysis is exacerbated by co-extractives, like lipids from biological tissue samples. This requires a lengthy extract purification by gel permeation chromatography or liquid-liquid chromatography to separate these compounds from the co-extracted material. An alternative technique known as supercritical fluid extraction (SFE) can overcome the deficiencies of traditional extraction techniques while yielding similar extraction efficiencies.

The goal of this study is to investigate and develop a method for the extraction of PCBs and OCPs from freeze dried mussel tissue using supercritical fluid and to compare the extraction efficiency between SFE and conventional Soxhlet extraction. An inclusion of activated basic alumina within the extraction vessel is used to perform on-line sample clean-up. First, spike studies involved the extraction of PCBs and OCPs from an inert matrix to determine any possible interactions between these compounds and the adsorbent used for on-line sample clean-up. Second, preliminary extractions were performed from freeze dried mussel tissue to examine the various SFE parameters. These preliminary studies were then used to determine the appropriate extraction parameters. Replicate extractions were then performed to determine the efficacy of SFE versus Soxhlet in terms of recovery. Finally, an alternative supercritical fluid (fluoroform) was used to determine its extraction efficiency in comparison with supercritical fluid CO_2 recoveries.

CHAPTER 2

Supercritical Fluid Extraction

A. Introduction:

The field of analytical chemistry has seen the introduction of novel techniques in the field of sample preparation in the past decade. This growth has stemmed from the need to improve traditional sample preparation techniques (e.g. Soxhlet extraction) which were first developed at the turn of the 20th century. Many areas in analytical chemistry like separation, identification, detection, and quantification of compounds have seen large improvements. However, traditional sample preparation still remains limited by the extensive use of high-grade organic solvents. The extraction process can take several hours to even days to perform. In addition, the result is a dilute extract that requires a concentration step followed by a thorough clean-up procedure prior to quantitative analysis. Overall, traditional sample preparation techniques are slow, difficult, laborious, and expensive to perform.

An ideal sample preparation method should be rapid as well as be selective for quantitative recoveries of target analytes without degradation. Also, the technique ideally must yield an extract solution that requires no further cleanup or concentration before analysis. Lastly, the method should be simple and inexpensive to perform. A sample preparation technique known as supercritical fluid extraction (SFE) has been shown to have the potential to meet most of the above mentioned requirements (1,2). Extensive reduction in the use of toxic and expensive organic solvents as well as a significant reduction in extraction time can be achieved with this technique compared to conventional methods.

B. Principles and Properties of Supercritical Fluids:

Supercritical fluid extraction (SFE) utilizes a fluid phase exhibiting intermediate properties between the gas and liquid phase to extract analytes of interest from matrices. A supercritical fluid can be described as any substance that is above its critical temperature (Tc) and critical pressure (Pc). Tc is the maximum temperature at which a gas can be converted to a liquid with an increase in pressure and Pc is the maximum pressure at which a liquid can be converted to a gas with an increase in temperature. A single component phase diagram is depicted in **Figure 1** to illustrate the supercritical region. This region is above the critical point and can be described as a medium in which both the gaseous and liquid state are indistinguishable.

The unique physical properties of the supercritical fluid are inherently related to the advantages of using SFE as an extraction technique. Supercritical fluids yield rapid extraction times compared to liquid solvents due to their lower viscosity and higher diffusion rates, **Table 1**. In general, supercritical fluids have viscosities an order of magnitude lower and diffusion coefficients an order of magnitude higher than liquids which result in faster extraction and equilibration times. Also, the lower viscosity of the

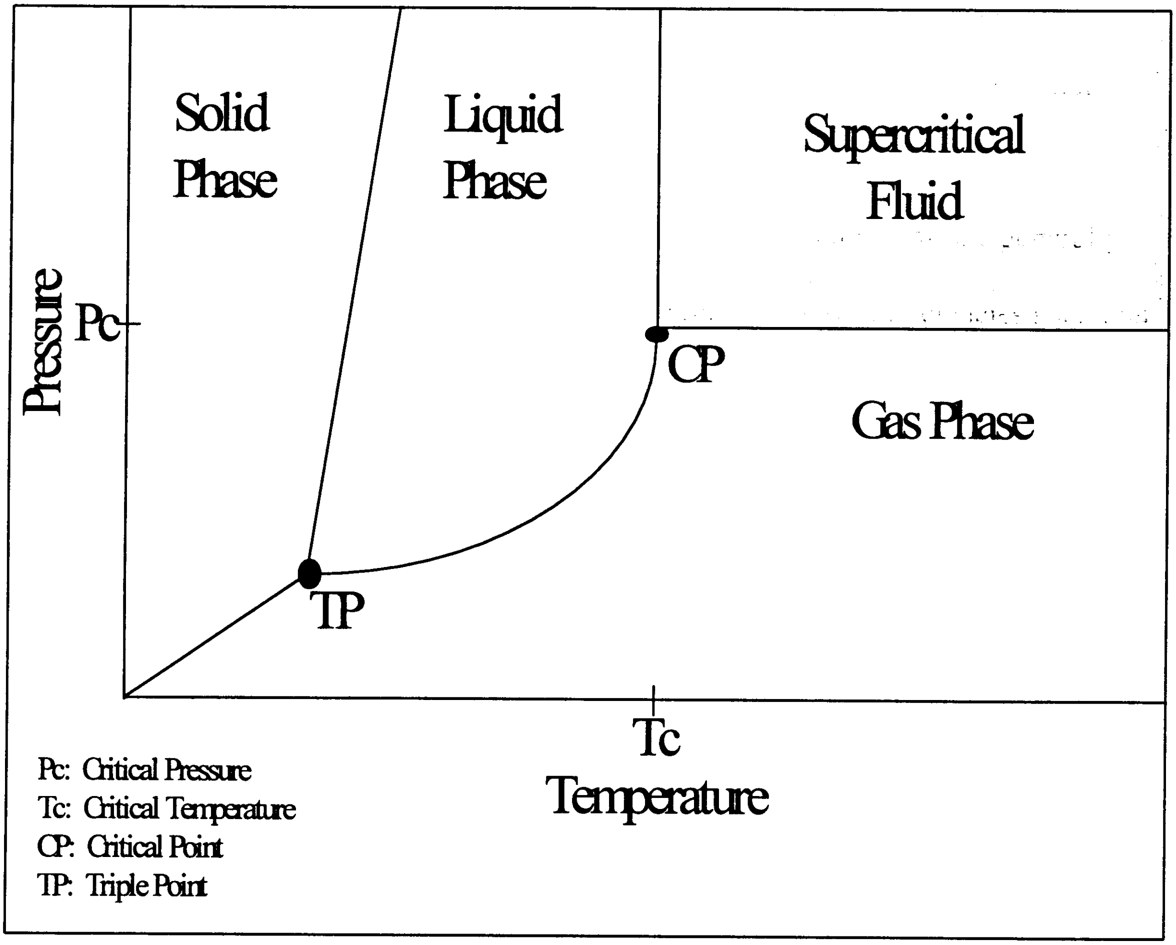


Figure 1: Single component phase diagram

Table 1: General properties of supercritical fluid versus gas and liquid*

Mobile Phase	Density (g/mL)	Viscosity (poise)	Diffusivity (cm^2/s)
Gas	0.001	0.00005-0.00035	0.01-1.0
Supercritical Fluid#	0.2-0.9	0.0002-0.001	0.00033-0.00001
Liquid	0.8-1.0	0.003-0.024	0.000005-0.00002

Typical range of values

* Reference 3

supercritical fluid compared to a liquid improves the mass transfer of analytes between the matrix and the fluid. Overall, this results in a more efficient mass transfer of solutes from the sample matrix.

Secondly, the solvent strength of the supercritical fluid depends primarily on its density. The property of density is directly associated with the concept of solvating power, so the higher the density of the fluid the higher the solvating power. The three properties: viscosity, diffusivity, and solvating power are all functions of the density of the supercritical fluid. The solvating power or density is adjusted in SFE by varying the temperature or pressure of the extraction. A positive adjustment is made by increasing pressure and/or decreasing the extraction temperature with the largest changes being observed for changes that occur close to the critical point of the fluid. By varying the solvating power of the supercritical fluid in SFE, the extraction medium has the ability to be selective for specific target analytes assuming adequate differences in analyte polarity and molecular weight. In addition, the use of toxic and expensive organic solvents is significantly reduced with SFE which employs supercritical fluids that are gases at ambient temperature. Since the supercritical fluid is allowed to vent into the atmosphere upon decompression and precipitation of the analytes, the need for time-consuming concentration steps prior to analysis of the extract is eliminated. Finally, supercritical fluids possess no surface tension. This property allows the supercritical fluid to spread and permeate the surface of the sample matrix which facilitates the removal of analytes not found on the surface.

C. Common Supercritical Fluids

The ideal extraction solvent should have the following characteristics: (1) readily attainable critical parameters, (2) relatively non-toxic and inert, (3) does not support combustion, (4) must be commercially available in highly pure form, and (5) the solvent must be environmentally friendly. The compounds listed in **Table 2** generally meet these qualifications. However, many of the compounds have deficiencies such that their use in SFE is limited. From the list of compounds in the Table, water has the largest dipole moment, but its extreme critical parameters inhibits its use as a supercritical fluid. Alternative fluids like ammonia and trifluoromethane have significant dipole moments, 1.47 and 1.62 debye respectively. However, the use of ammonia as a routine extraction solvent is hampered by its high activity, toxicity, and corrosiveness to pumping systems. On the other hand, trifluoromethane has recently spurred interest and research into its applicability for the extraction of polar compounds from various matrices (4,5). Next, nitrous oxide which also has a permanent dipole moment has been shown to produce good results in SFE even though it supports combustion, but due to safety concerns nitrous oxide cannot be used for routine analysis (6). Xenon which has mild critical parameters with no dipole moment is too expensive and highly pure supplies of the compound are not readily available. Finally, Freon®-12, a chlorofluorocarbon is too harmful to the environment for use in SFE.

The most commonly used supercritical fluid in SFE has been carbon dioxide for

Table 2: Physical constants of common supercritical fluids*

Fluid	Critical Temp. (°C)	Critical Pressure (atm)	Dipole Moment (debye)
CO_2	31.3	72.9	0.00
N_2O	36.5	72.5	0.17
NH_3	132.5	112.5	1.47
SF_6	45.5	37.1	0.00
Xe	16.6	58.4	0.00
CHF_3	25.9	46.9	1.62
CCl_2F_2	111.8	40.7	0.17
H_2O	374.1	217.6	1.85

* Reference 1

several reasons. First, highly purified carbon dioxide is readily and commercially available as well as being very safe (does not support combustion). Second, the mild critical parameters of carbon dioxide allow easy implementation to SFE. Finally, it is relatively inert with most analytes and matrices.

D. Modes of Operation in Supercritical Fluid Extraction

The previous sections addressed the properties of supercritical fluids. One clear advantage was the variable features of supercritical fluids. SFE can be accomplished in the static or dynamic mode of operation. These two modes directly control the amount of interaction between the supercritical fluid and the analyte/matrix.

The static mode incorporates an extraction with a fixed amount of supercritical fluid to interact with the matrix and the analytes of interest. This mode of operation is analogous to making tea where the addition of the hot water to a tea bag causes diffusion of "tea" into the hot water. A recirculating pump may be used to continuously penetrate the matrix with the same supercritical fluid. Static extraction takes advantage of the high diffusivity of the SF to access the analyte/matrix. The advantages of using the static mode with SFE are as follows: (1) conservation of expensive SFE grade extraction fluid and (2) contaminants in the fluid are seldom a problem unless analytes are in trace levels. However, the static mode may not yield exhaustive extractions due to total saturation of the SF with the analyte of interest and co-extractives from the matrix.

Dynamic extractions are performed using a continuous flow of fresh SF to pass and penetrate through the matrix. This mode of operation is similar to a coffee maker percolating water through the coffee grounds to extract the "coffee". In comparison to the static mode, the dynamic mode yields a more exhaustive extraction since fresh SF is always introduced into the sample for solvating and transporting of extracted analytes to the collection device. However, a major concern with dynamic extractions are the impurities in the extraction fluid. Trace amounts of impurities in the extraction fluid will become concentrated at the collection device when large amounts of extraction fluid are used in the dynamic mode. Ultimately, the impurities may interfere with the extract analysis, especially when monitoring analytes at trace levels (7). Also, enhanced matrix mobility is associated with the dynamic mode due to the greater amount of SF which mean more extractable components are removed from the matrix.

These two modes of operation can be coupled together to produce a static-dynamic extraction. The coupling allows the SF to diffuse into the surface of the matrix in the static mode (no fresh flow of SF), after which the solvated analytes are removed and transported to the collection device in the dynamic mode (fresh continuous flow SF). This technique is very useful for extractions that are dependent upon diffusion for the removal of the analytes from the sample matrix. Therefore, the above mentioned operations in SFE allow different strategies for disrupting the analyte/matrix interaction to effectively remove the target analytes from the sample.

E. Collection Strategies in Supercritical Fluid Extraction

The other aspect of SFE which needs to be understood prior to extraction is the collection strategy. The collection or trapping process is probably the most important part of SFE since the effectiveness of the extraction can only be properly measured with a valid trapping scheme. The most common trapping schemes used in SFE are liquid-solvent traps and solid phase adsorbent traps.

Liquid-solvent traps are by far the most popular and simplest means of collection in SFE. It involves the fixed diameter restrictor from the extraction vessel being placed in a vial containing a liquid solvent. The extraction fluid is allowed to decompress into the solvent trap while depositing the extracted effluents into the solvent prior to being vented to the atmosphere. Several different types of organic solvents like methanol, methylene chloride, chloroform, acetone, and hexane have been used as liquid traps (8). The type of solvent used must be compatible with the analytes as well as any modifier being used during the extraction. An in depth study of trapping efficiencies involving different liquid trapping solvents with several analytes ranging in polarity has been conducted (9). Also, the rapid expansion of the CO_2 causes an aerosol effect upon decompression which cools the solvent trap to minimize the loss of solvent in the vial. Flow rates ranging from 1- 2 mL/min of compressed fluid will produce approximately 500 - 1500 mL/min of gas after decompression. Such rapid expansion of gas can diminish the efficiency of the trap by violent bubbling of the collection solvent. The end result may be a lower recovery due to purging of the analyte from the liquid trap or the formation of aerosols that escape to the atmosphere. Overall, liquid traps can yield quantitative recoveries depending upon the analyte and the experimental conditions. However, due to its inherent design flaws volatile compounds are difficult to trap efficiently. In addition, the rapid expansion of compressed gas and extracted materials from the sample can cause restrictors to plug. The liquid trap, while simple, poses certain limitations for trace analysis.

A different approach to trapping is the solid phase trap which utilizes adsorbents to collect extracted effluents. Different solid phases can be used like stainless steel or glass beads, silica gel, Florisil®, ODS (C-18), or any other chromatographic bonded packing material. Upon collection, the trap is subsequently rinsed with the appropriate trap rinse solvent to remove the extracted components from the trap. The nature of the solid phase trap can be altered to match the polarity of the target analytes by simply changing the sorbent material. Also, the solid phase trap can be externally cooled to aid the trapping process of extracted analytes, especially volatile compounds. Trapping of analytes occur on the solid sorbent as the compressed gas is allowed to expand which also gives rise to cryogenic trapping. This trapping scheme is limited to the extent that one must have the appropriate solid sorbent material and the proper rinse solvent. Trapping efficiencies can decrease due to improper selection of the trapping material and inefficient rinsing of the trap. This aspect of solid phase traps was clearly demonstrated by Mulcahey et al. in a study involving different types of solid traps (OH, SiO_2, CN, NH_2) for phenols and *n*-alkanes (10). The study also addressed the importance of the trap temperature to obtain quantitative recoveries. When modifiers were used with solid phase traps, the temperature of the trap had a pronounced effect on recovery. At high modifier levels (greater than 3% by volume) the temperature of the trap had to be elevated above the boiling point of the modifier to avoid condensation and subsequent elution of the trap by the condensed modifier during the extraction. As a trapping strategy, the solid phase trap introduces more complexity to the SFE system and the number of conditions that must be optimized prior to extraction increases relative to liquid traps. However, the potential to perform trace analysis, sample clean up, and concentrate the extract with one extraction procedure warrants further investigation into the feasibility of solid phase trapping in the extraction of environmental samples.

F. Off-Line Supercritical Fluid Extraction

Numerous SFE parameters must be investigated to achieve quantitative recoveries of the analyte(s) of interest with this technique. The extraction temperature in SFE impacts the selectivity of the supercritical fluid. Extractions performed at elevated temperatures under constant pressure result in a decrease of the supercritical fluid density which suggests a decrease in the solvating power. On the other hand, the increase in diffusion resulting from an increase in extraction temperature can aid in the extractibility of analytes by the supercritical fluid. In addition, extractions performed at high temperatures can also affect the matrix-analyte interaction. Despite the decrease in the density at high temperatures, a recent SFE study has shown that elevated temperatures were in most cases sufficient to disrupt the matrix-analyte interaction to achieve quantitative recoveries (63). In terms of kinetics, temperatures near the critical point of the extraction fluid should be used when extracting bulk analytes with weak matrix-analyte interactions (rapid extraction kinetics) (13). Whereas high extraction temperatures should be used to overcome strong matrix-analyte interactions (not solubility limited) (14).

Disruption of the matrix-analyte interaction is dependent upon several other variables like the extraction time required to achieve quantitative recovery of the analyte of interest. This time can vary considerably depending upon the nature of the analyte and the matrix. Extraction times would be considerably longer for analytes with strong matrix-analyte interactions relative to analytes with weak matrix-analyte interactions. The option to incorporate static-dynamic extraction time periods can often overcome strong matrix-analyte interactions. The static extraction time produces a greater contact time between the analyte and the supercritical fluid resulting in higher diffusion of the target analytes from the matrix. As a general guideline the dynamic extraction time should be equivalent to a minimum of three to ten void volumes of SF to obtain a 100% extraction.

The disruption of the matrix-analyte interaction is not sufficient to obtain quantitative extractions. Target analytes must also be soluble in the extraction solvent. However, solubility alone cannot achieve quantitative results, the target analytes must also have a preference for the supercritical fluid. As previously discussed, the most often used fluid for SFE is CO_2 which is non-polar and in many cases cannot overcome these matrix-analyte interactions. The addition of a co-solvent or modifier to the extraction fluid (to increase the solubility of the target analytes) can often overcome the solubility barrier . The specific use and type of modifier would also be dependent upon the chemistry of the analyte of interest. A number of different organic solvents like methanol, methylene chloride, hexane, benzene, and toluene have been used as modifiers with CO_2 (15). The addition of modifiers is achieved by adding the modifier directly to the sample or in-line to the extraction fluid prior to SFE. Recently, Ashraf-Khorassani and Taylor showed that higher extraction efficiencies could be obtained for PCBs from river sediment by adding the modifier directly to the matrix prior to SFE (16). Other than increasing the solvating power of the supercritical fluid, the exact role of the modifier is unclear. However, it is currently believed that the solvent molecules compete for active sites on the sample matrix causing displacement of the analyte from the matrix (17). Finally, trapping conditions must be carefully considered when using modifiers since the modifier is trapped along with the analytes.

Next, having considered the various parameters involved in off-line SFE to achieve quantitative results. This technique can be adapted to perform on-line sample clean-up (i.e. lipids from biological tissue). A simple addition to the extraction cell of a solid adsorbent material like alumina, Florisil®, or silica gel can be used as an *in situ* sample clean up procedure (11). The solid adsorbent material is added in-line and after the sample. Another approach is to modify the solid phase material in the off-line trap to collect all extracted analytes and then to selectively rinse the trap using different organic solvents (12). One prime example is the extraction of lipophilic organic pollutants from fatty biological tissue samples (11). The major advantage of this technique is the significant reduction in the sample processing time by eliminating the laborious sample clean-up step before the assay method. Also, less sample handling is required with this technique which results in better reproducibility of analysis. When on-line SFE sample clean-up is applied to biological samples efficient retention of co-extractives is observed with results which are quantitative (11,12). Overall, this technique efficiently produces a clean extract while reducing the volume of organic solvents, time, and sample handling.

CHAPTER 3

Polychlorinated Biphenyls and Organochlorine Pesticides

A. Properties, Nomenclature, and Toxicity:

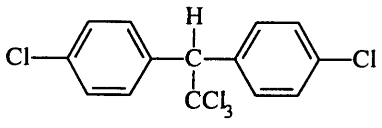
Polychlorinated biphenyls (PCBs) were first discovered before the turn of the century. This class of 209 chemical compounds, called congeners, were extensively used in industry due to their physical properties. The vast industrial applications of PCBs were soon recognized and commercial production began in 1929 in the United States (18). Although PCBs were manufactured world wide by numerous producers the most prominent ones include Monsanto (sold under the tradename "Aroclor®", USA) Bayer AG ("Clophen®", Germany), Prodelec ("Phenochlor®", France), and Kanegafuchi ("Kanechlor®", Japan) (19). The many desirable chemical and physical properties of PCBs relate to their general inertness, thermal and chemical stability, and resistance to acids and bases (20). They also exhibit excellent dielectric properties (electrically insulating), low solubility in water, and low vapor pressure. The viscosity of the PCBs can vary depending on the chlorine content (higher viscosity with increasing chlorine content) (21). Therefore, PCBs were utilized in many areas where these characteristics were desirable, for example, as heat exchange and hydraulic fluids, lubricants, dielectric fluids in transformers and capacitors, and as additives in plastics (21).

A different class of organochlorine compounds has been used as a pesticide.

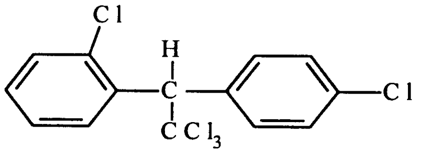
OCPs (organochlorine pesticides) consist of two different major groups based on their molecular structures, namely, the cyclodiene (or diene) group and the DDT group. The cyclodiene pesticides are cyclic compounds with the characteristic “endomethylene bridged” structure and contain six or more chlorine atoms in the molecule. These compounds are the products of a Diels-Alder reaction involving hexachloropentadiene with a suitable unsaturated compound, with one exception (Toxaphene) (26). Chlordane, heptachlor, isodrin, and aldrin are some examples of cyclodiene OCPs. The DDT group is comprised of DDT and its analogs that contain two aromatic rings (i.e. Methoxychlor, DDD, perthane, and keltane). The synthesis of DDT was first reported by Zeidler in 1874 but, the insecticidal properties of the *p,p'*-isomer were not discovered until 1939 by P. Muller in Basle Laboratories of Giegy (27). Worldwide distribution of DDT formulations began a couple years later by J.R. Geigy under the trademarks Gesarol® (Europe), Gesapon® (USA), Guesarol® and Guesapon® (England), and Neocid® (27). There are a few OCPs that do not belong to either of these groups most notably the BHC or HCH (hexachlorocyclohexane) isomers and HCB (hexachlorobenzene). **Figure 2** illustrates the various types of OCPs.

The universal application of OCPs as an insecticide stemmed from their desirable physical and chemical properties. Most OCPs have a low vapor pressure (non-volatile at room temperature) except for the BHC isomers, heptachlor, HCB, and chlordane (26). Also, these compounds, except one (lindane), are practically insoluble in water but are readily soluble in organic solvents. Their continued persistence in the environment

Structures of OCPs*



4,4'-DDT



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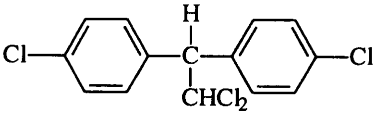

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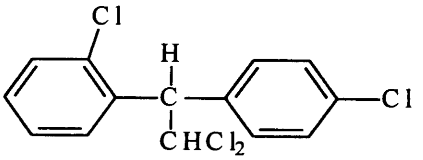

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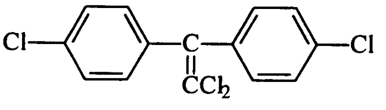

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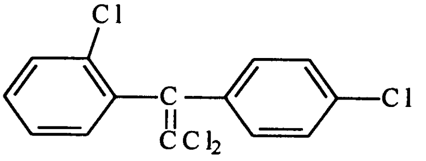

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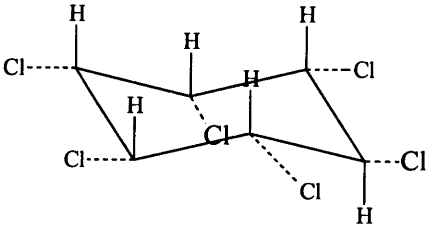
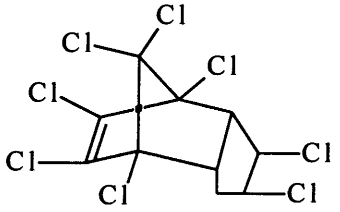
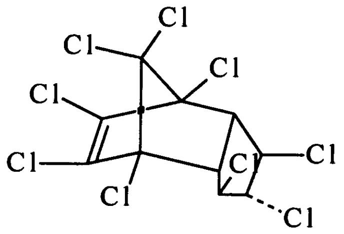


***beta*-BHC or HCH**



***cis*-Chlordane**



***trans*- Nonachlor**

Figure 2: Molecular structures of various organochlorine pesticides

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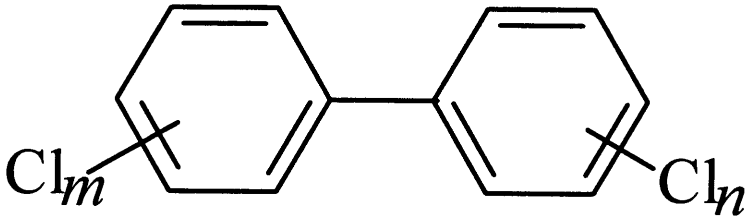
* Reference 26

relates to their inertness towards acids, bases, water, air, and sunlight under mild conditions compared to other pesticides (26). In general, fully chlorinated polycyclic OCPs exhibit greater stability than nonchlorinated or unsaturated acyclic compounds. The nomenclature of OCPs is inconsistent such that it is beyond the scope of discussion here.

On the other hand, PCBs are produced from the reaction of biphenyl with anhydrous chlorine in the presence of iron fillings or ferric chloride as a catalyst ($C_{12}H_{10} + n\,Cl_2 \rightarrow C_{12}H_{10-n}Cl_n + n\,HCl$). The substitution of hydrogen atoms on the biphenyl skeleton with chlorine atoms can vary from one to ten. The basic structure of a PCB is depicted in **Figure 3**. PCBs are subdivided into ten groups, called homologues, depending on the number of chlorine substituents. Each homologue group contains isomers that differ from each other in the substitution pattern of the chlorine atoms. For example, there are three isomers for monochlorobiphenyl and twelve for dichlorobiphenyl (See **Table 3**) (20). Overall, there are 209 individual polychlorinated biphenyls or congeners. In order to simplify the nomenclature of PCBs, a systematic numbering has been introduced (22). The systematic numbering of PCB congeners that has been adopted by the IUPAC is shown in **Figure 4** (23-24).

A different numbering system is used by PCB manufacturers, since commercial products of PCBs are mixtures of a large number of congeners. This system utilizes the trademark name followed by a four digit number in which the first two digits correspond to the starting material (i.e. 12 indicates biphenyl) and the last two indicate

Structure of PCB*



$m + n = 1$ to 10

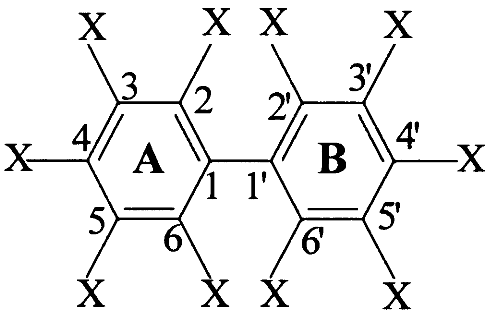
Figure 3: Basic structure of polychlorinated biphenyl

* Reference 18

Table 3: PCB homologues and their corresponding number of isomers, molecular weight, and chlorine content.*

Parent Biphenyl	Number of Isomers	Molecular Weight	Chlorine (%)
Monochloro-	3	188.7	18.8
Dichloro-	12	223.1	31.8
Trichloro-	24	257.5	41.3
Tetrachloro-	42	292.0	48.6
Pentachloro-	46	326.4	54.3
Hexachloro-	42	360.9	58.9
Heptachloro-	24	395.3	62.8
Octachloro-	12	429.8	66.0
Nonachloro-	3	464.2	68.7
Decachloro-	1	498.7	71.2
Total	209		

* Reference 18



	0	2	3	4	2,3	2,4	2,5	2,6	3,4	3,5	2,3,4	2,3,5	2,3,6	2,4,5	2,4,6	3,4,5	2,3,4,5	2,3,4,5	2,3,4,5	2,3,4,5,6
0																				
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4	3	8	13	15																
2,3	5	16	20	22	40															
2,4	7	17	25	28	42	47														
2,5	9	18	26	31	44	49	52													
2,6	10	19	27	32	46	51	53	54												
3,4	12	33	35	37	56	66	70	71	77											
3,5	14	34	36	39	58	68	72	73	79	80										
2,3,4	21	41	55	60	82	85	87	89	105	108	128									
2,3,5	23	43	57	63	83	90	92	94	107	111	130	133								
2,3,6	24	45	59	64	84	91	95	96	110	113	132	135	136							
2,4,5	29	48	67	74	97	99	101	102	118	120	138	146	149	153						
2,4,6	30	50	69	75	98	100	103	104	119	121	140	148	150	154	155					
3,4,5	38	76	78	81	122	123	124	125	126	127	157	162	164	167	168	169				
2,3,4,5	61	86	106	114	129	137	141	143	156	159	170	172	174	180	182	189	194			
2,3,4,6	62	88	109	115	131	139	144	145	158	161	171	175	176	183	184	191	196	197		
2,3,5,6	65	93	112	117	134	147	151	152	163	165	177	178	179	187	188	193	201	200	202	
2,3,4,5,6	116	142	160	166	173	181	185	186	190	192	195	198	199	203	204	205	206	207	208	209

Positions on Phenyl Ring **B** (Number Prime Positions)

Figure 4: IUPAC numbering system for PCB nomenclature.*

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* Reference 23, 24

the average percent of chlorine by weight in the final product (21). Thus, Aroclors® 1221, 1232, 1242, 1248, 1254, 1260, 1262, and 1268 produced by Monsanto correspond to chlorinated biphenyls with a chlorine content ranging from 21 to 68 percent. The only exception is Aroclor® 1016 which is similar to Aroclor® 1242 in chlorine content but has less penta- to hepta-chlorobiphenyl (25).

The widespread environmental occurrence and hazardous properties of PCBs and OCPs were recognized in the 1960's. These contaminants can be transported by the atmosphere from their original source to remote regions of the earth. For example, PCBs and OCPs have been detected in the air of Enewetak Atoll (28) and Antarctica (29), in marine mammals from Antarctica (30), and in surface water from the major oceans and seas (31). The toxic effects associated from exposure to these compounds is well documented. Animals exposed to high dosages of PCBs show body weight loss, lesions and dysfunction of the skin (chloracne), liver (hepatomegaly, hemorrhage, porphyria), bile duct, gall bladder, urinary tract, endocrine system, and reproductive system (20). They have also been known to cause teratogenesis and carcinogenesis. However, relatively little is known on the toxicity of individual congeners since most studies of PCB toxicity in animals and humans report "total PCBs". The toxic effects of OCPs are similar to that of PCBs. In fact, the literature on the effects of OCPs on wild birds is dominated by papers on eggshell thinning phenomenon caused by DDT and its metabolite DDE (32). Although, commercial production of PCBs and OCPs have ceased and their use now curtailed and limited, they still continue to be a hazard to the environment which warrants continued and improved methods of monitoring them in the ecosystem.

B. Analysis of PCBs and OCPs:

The analysis of PCBs and OCPs in environmental samples is similar to the general analytical procedure used for the trace analysis of other lipophilic organic substances. The procedure in most instances consists of the following steps: (1) sampling, (2) extraction, (3) clean-up, and (4) determination and evaluation. A concentration step usually follows steps 2 and 3. The particular execution of the procedure depends strongly on the type of sample to be analyzed and on the expected range of PCB and OCP levels. An extensive overview on the methods of analysis can be found in Lang's review (20), Erickson's book (18), and in Chau's and Afghan's book (26). The methods of extraction can be categorized by the type of environmental matrix. PCBs and OCPs are extracted from water, sediment, and biota matrices. The following sections describe various methods of extraction used for the analysis of these organic pollutants.

(1) Water

The isolation of PCBs and OCPs from water is generally straightforward (solubility in water is low) and fall into three categories: (1) liquid-liquid extraction (LLE) with organic solvents, (2) adsorption using columns packed with various adsorbents, and (3) steam distillation. There have been several articles describing these various techniques. The classic liquid-liquid extraction technique involves a simple shaking of water with solvent in a separatory funnel. The preferable solvents for this procedure are hexane, methylene chloride, or a mixture of hexane and methylene chloride (18). Oliver and Nicol used LLE to obtain recoveries of chlorinated benzenes and toluenes, hexachlorobutadiene, and PCBs from water (33). The spiked water sample (one gallon in a solvent bottle) was extracted with 75 mL of hexane by stirring overnight with a Teflon® stir bar. The extraction efficiency of the hexane LLE procedure from spiked distilled water ranged from 74 to 98% (33). Also, a large volume (200 L) LLE device was designed by McCrea et al. with reports of lower detection limits of organics in ambient waters (34,35). Briefly, the extractor consisted of a 200 L stainless steel drum equipped with a solvent recirculating pump. The pump sprayed and continuously recycled dichloromethane through the water sample as fine droplets (35). This extractor was successfully field tested for recovering organics in natural waters by Oliver and Nicol (36). Recovery data from spiked Lake Ontario water for PCBs and OCPs ranged from 62 to 79% using this device. It also reduced the detection limits of the target compounds by one or two orders of magnitude compared to conventional small volume samples (36).

Next, various types of adsorbent materials have been used to isolate PCBs and OCPs from water samples. These materials vary from activated carbon, graphitized carbon, polyurethane foam (PUF), Tenax®, and Amberlite polymeric resins (XAD-2 and XAD-4). First, graphitized carbon black (GCB) was used to extract trace organic pollutants from spiked water samples. The GCB successfully adsorbed twelve chlorinated pesticides from spiked drinking water. The recoveries were dependent upon the eluant used to remove the pesticides from the GCB (37). A 25 mL mixture of hexane and diethyl ether (1:1) yielded the best recoveries ranging from 75 to 95% while poor recoveries were obtained for PCBs (30-40%) (37). On the other hand, porous polyurethane foams were used by Musty and Nickless to extract OCPs and PCBs from spiked water samples. The adsorbed compounds were eluted from the PUF using 50 mL of acetone and 100 mL of *n*-hexane (38). Overall, quantitative recoveries were obtained for the OCPs while the recoveries of PCBs ranged from 40 to 90% (38). Also, extraction of pesticides from water by adsorption on Tenax® was studied by Leoni et al. with results equivalent to those obtained by the LLE procedure (39). Finally, XAD resins have been proven to efficiently extract PCBs and OCPs from water. Coburn et al. used XAD-2 resins to extract OCPs and PCBs from distilled and natural water samples (fortified). The recoveries of OCPs averaged 96% and 85% for distilled and natural water samples respectively, while the recoveries of PCBs ranged from 78 to 86% from fortified natural water samples (40).

The final category involves the use of steam distillation to extract PCBs and OCPs from water. First, Veith and Kiwis reported that boiling 2.5 L water sample in a steam distillation apparatus for 60 minutes resulted in greater than 89% recovery for all PCBs and OCPs that were monitored (41). While Godefroot et.al. reported nearly 100% for OCPs and more than 80% for PCBs using a micro steam distillation apparatus (42). The major advantage associated with this technique is that the target analytes are extracted into a small volume of solvent with minimal interference from other compounds (i.e. no cleanup procedure required).

(2) Sediment

Sediment samples are usually extracted (after homogenization and drying) with a solvent mixture of acetone and a light aliphatic hydrocarbon in a Soxhlet apparatus. This technique is considered the most exhaustive procedure for the extraction of chlorinated contaminants from sediments. A hexane/acetone solvent mixture of 41:59 (v/v) was used by Oliver and Nicol to extract chlorinated contaminants from sediment. The sample was extracted for 24 hours with recoveries of PCBs greater than 95% (33). Next, PCBs and chlorinated pesticides were successfully extracted with acetone-light petroleum (1:9) for 8 hours to yield 100% recovery for the PCBs (Arochlor 1254) and recoveries ranging from 81 to 112% for the OCPs (43).

For trace level determination of PCBs, an on-line narrow-bore column liquid chromatography/capillary gas chromatography set-up was used to analyze an extract obtained from sediment. This extraction involved 150 mL of *n*-hexane for 4 hours per 30 g of sediment (44). The results from this system produced semi-quantitative information concerning the level of PCBs and/or related organochlorine compounds in the sediment. The National Institute of Standards and Technology (NIST) used Soxhlet extraction for the certification of organic contaminants in marine sediment (SRM 1941a). The sediment samples were extracted using hexane/acetone (1:1 v/v) or methylene chloride for 20 hours. The certified concentrations of PCBs ranged from 0.93 to 17.6 μg/kg dry weight and 0.73 to 70 µg/kg dry weight for the OCPs (45).

Ultrasonic extractions with various solvent combinations have been successfully employed to isolate organic pollutants from sediments. The extraction is performed by immersing an ultrasonic probe into a sediment/solvent (similar to Soxhlet) slurry and sonicating. After sonicating for a set time period the solvent is removed and replaced with fresh solvent and the procedure repeated. This method was used by Johnson and Starr to extract insecticides from various sediment matrices. Quantitative recoveries of OCPs (>90%) were obtained from clay soil and the technique showed no significant differences in recoveries when compared to Soxhlet (46). More recently, the method of sonication extraction was compared to Soxhlet extraction for hydrocarbons and PCBs from aquatic sediments. The results from the study indicated that sonication is a suitable and comparable technique for the extraction of organic pollutants from this matrix while offering less time and solvent consumption (47). This extraction technique was also applied by Dunnivant and Elzerman to determine the level of PCBs in sediment. The sonication procedure reported in the article extracted equal, and in some cases higher quantities of PCBs, compared with Soxhlet extraction technique, from native sediment samples (48).

A number of alternative extraction techniques have been employed for sediments, but one technique has recently received much attention. This technique utilizes a supercritical fluid (i.e. CO_2) to extract contaminants from sediment and is better known as supercritical fluid extraction (SFE). Numerous studies have investigated different applications of SFE in environmental trace analysis. A simple and quantitative trapping scheme aimed for PCB and OCP trace analysis was used in off-line SFE to obtain recoveries of over 90% for compounds with boiling points higher than 120 °C (49). Subsequent extractions of Lindane, p,p'-DDT, and PCBs at low ppb levels were performed from spiked soil samples. Quantitative recoveries of PCBs were obtained at three different spike concentrations (100, 50, and 20 ppb) whereas the recoveries of the two OCPs decreased from >90% to 73-75% as the spike concentrations were decreased (150, 75, and 15 ppb) (49).

Another study compared SFE extraction efficiencies with two other techniques (solvent and Soxhlet) used for PCBs and OCPs in soil. Two types of soil were spiked with 16 PCBs and OCPs at a level of 5 ng/g dry matter and extracted by the three techniques (50). The SFE recoveries ranged from 85 to 105% for PCBs and OCPs with reproducibility of 5% for each component for both types of soil (50). The SFE recoveries were comparable to those obtained by solvent and Soxhlet while providing rapid, clean, and reproducible extractions. Also, Lee and Peart developed an SFE method for the simultaneous extraction of PCBs and chlorinated benzenes from real world sediment samples. The best recoveries were obtained with non-modified CO_2 at 35 Mpa with an extraction temperature of 100 °C (51). The extract required a simple clean up procedure with Florisil® column followed by vigorous shaking of the effluent with a drop of mercury to remove sulfur interferences. SFE recoveries at the optimized conditions and an extraction time of only 21 minutes produced PCB results comparable to 7 hour

Soxhlet extractions (51). Meanwhile, SFE recovery of chlorinated benzene was up to 50% higher than Soxhlet (51).

(3) Biota

The conventional extraction of organic contaminants from biota have utilized similar techniques as those used for extraction from water and sediment. The sample is first homogenized in a blender and dried with anhydrous Na_2SO_4 before Soxhlet, column, or batch extraction. The most often used technique has been Soxhlet extraction for periods of several hours using various solvent systems. Recently, Schantz and Parris et.al. extracted organics in mussel tissue (SRM 1941) and other standard reference materials using Soxhlet apparatus. The mussel tissue sample was extracted for 18 hours using methylene chloride (52). Upon clean up and concentration, the extract was assayed by three GC columns with differing selectivity and two detectors (ECD and MS). The results provided an extensive compilation of concentrations of PCB congeners and pesticides in the reference materials while offering complementary separations obtained by the different columns (52).

Other techniques like column extractions, carried out in glass columns loosely packed with dried biota samples have been performed in the past. One study compared the extractability of PCBs, DDT, and DDE with four solvent systems (diethyl ether, diethyl ether/pentane (1:1), hexane/acetone/diethyl ether/petroleum ether (2.5:5.5:1:9), and methanol/chloroform (1:1)) with cold column extraction and hot Soxhlet extraction technique from fish samples (53). The results showed significant differences between the two extraction techniques for which lower results were obtained for the cold column extraction method. The best results were obtained with Soxhlet extraction of the fish samples with methanol/chloroform (1:1) for 6 hours (53).

Meanwhile, extraction of PCBs and chlorinated pesticides via off-line SFE have been investigated for its potential applicability with biota matrices. One such article describes the use of supercritical carbon dioxide as an extraction medium for the determination of polychlorinated organics in biological tissue samples (54). A simple SFE system devoid of pumps or compressors was used to extract various OCPs and Aroclor 1260 from spiked biological samples as well as incurred fish samples. The static extraction was carried out under isothermal conditions and at three different pressures (100, 122, 143 atm) for periods ranging from 0.5 to 4 hours (54). The extracted contents were then transported to a 25-50 mL ice-cold hexane trap. Also, the extract was subsequently concentrated to approximately 2-5 mL which was fractionated on silica gel to separate the PCBs and OCPs prior to assay by GC-Electron Capture Detection (GC/ECD) (54). The results from the spike biological samples indicated good extraction efficiencies at the three different pressures with recoveries greater than 80% at 100 atm and increasing to greater than 90% at 143 atm for all OCPs and Aroclor 1260 with a static period of only one hour at 50 °C (54). The results also indicated that the extraction efficiency was not significantly affected by the analyte concentration and was similar to Soxhlet recoveries with methylene chloride. In addition, qualitative data obtained from incurred fish tissue via SFE and Soxhlet were quite similar. Generally, the extraction of highly lipophilic compounds (OCPs and PCBs) from biological tissue samples is believed to be directly linked to the extraction of the fatty components (53,54). The end result is that the extractability of these compounds depends upon the level and extractability of the lipid components. The results showed that a 40 fold increase in the lipid level had little or no affect on the extractability of the OCPs and PCBs (54). The data clearly demonstrated the applicability of SFE with CO_2 for the analysis of polychlorinated organics from biological tissue samples.

The extracts obtained in the previous study required a cleanup procedure via silica gel or alumina to remove the co-extracted lipid materials prior to GC analysis. France et.al. demonstrated the feasibility of supercritical fluid as a cleanup technique for the separation of organochlorine pesticides from fats (55). Incurred poultry fat samples from chicken tissues and spiked lard samples containing several OCPs were used for conventional and supercritical fluid cleanup. A supercritical fluid chromatograph (SFC) was modified to perform preparative chromatography with fractions being collected in 5 mL of hexane (55). Two different cleanup columns (1.4 g of neutral alumina, 0.5 g of silica) were used at a column temperature of 40 °C for alumina and 50 °C for silica. The SFC column pressure was 190-270 atm with a liquid CO_2 flow rate of 1 mL/min for 20 minutes (55). Results from the incurred chicken fat indicated that the supercritical fluid cleanup with alumina and CO_2 was not statistically different at the 95% confidence limit, when compared to the conventional method with a glass column filled with deactivated alumina and eluting with hexane or petroleum ether (55). A second approach utilized a silica column with 2.5% (v/v) methanol-modified CO_2 as the mobile phase for the cleanup of spiked lard samples. The mean recoveries and standard deviations obtained with the supercritical fluid technique agreed fairly well with the conventional method except for heptachlor at the 99% confidence limit (55). Overall, the results showed that the supercritical fluid cleanup technique (recoveries: 93%-111% and %RSD: <8.5%) is comparable to conventional cleanup methodology.

The fundamentals of supercritical fluid cleanup investigated by France et al. were used by Johansen et al. to determine PCBs in biological samples with on-line SFE/GC. An on-line system was built by coupling an SFE unit with a chromatographic oven through a thermostatted interface. Samples of cod liver, cod fillet, crab claw meat or crab hepatopancreas were mixed, homogenized, dried, and spiked with Aroclor 1260 or individual PCB congeners. The spike concentration ranged from 50 ppb to 0.6 ppm (56). The extraction cell was loaded with sample sizes ranging from 450 mg to 5 g. Next, the lipid material was retained with basic alumina placed either at the outlet end of the extraction cell or in a separate column placed after the extraction cell (56). Best recoveries were obtained with the basic alumina in the extraction cell with a CO_2 pressure of 14.5 Mpa at 60 °C (density of 0.69 g/mL) for 45 minutes (56). A decompressed flow rate of 133 mL/min was regulated by a 20 cm fused silica restrictor (28 μm i.d.) kept at 250 °C which was connected to a 11 cm long cold trap maintained at -20 °C to -30 °C (56). The results showed that this system successfully performed on-line lipid cleanup while providing recoveries ranging from 80-96% and relative standard deviations between 3 and 6% for all spiked sample matrices. The limit of detection under the optimized conditions was 10-15 pg from extractions of crab claw meat spiked with 25 pg of PCB 52 and 38 pg of PCB 180 (56). The data from this study showed the feasibility of on-line SFE/GC as a sensitive and rapid method for the extraction, cleanup, and analysis of PCBs from biological tissue samples.

A similar study isolated PCBs from milk by a combination of SFE and SFC. Mills and Jefferies investigated and optimized SFE conditions for the extraction of PCBs from freeze-dried milk mixed with Florisil® using supercritical CO_2. All analytes were collected in 1 mL of heptane with a liquid CO_2 flow rate of 3 mL/min (57). The simplex optimization produced an optimum pressure range of 19.6 to 23.52 Mpa at extraction temperatures ranging from 45 to 50 °C with the Aroclor 1242 spiked freeze-dried skim milk (fat content 0.1%, w/w) mixed with Florisil® (57). A similar approach was used to extract fat from cow's milk (fat content 4%, w/w) mixed with Florisil® to determine if the selectivity of SFE was sufficient to separate the fat from the PCBs. However, the fat optimization study yielded extraction conditions similar to the optimal extraction conditions for skim milk. A second step using SFC was required to separate the PCBs from the fat. Because the SFC was being utilized as a semi-preparative step to separate the PCBs from fat, SFC conditions were optimized by injecting Aroclor 1242, fat, and a mixture of the two under various parameters. The best results were obtained with a 15 cm polymeric PRP-1 column with a mobile phase of CO_2/2-propanol (80:20) at 50 °C with a pressure of 13.72 Mpa at a flow rate of 1 mL/min (57). Successful separations of

PCBs from fat were obtained and collected which was indicated by GC/MS chromatograms. The data showed that it was possible to separate PCBs from fat by SFC using a PRP-1 column.

Recently, Snyder et al. used SFE to extract incurred pesticides from poultry tissues. The paper describes and compares the extraction efficiencies of SFE to both conventional solvent extraction and thermal rendering of fat. Various poultry tissues were obtained from chickens fed with rations of heptachlor epoxide, dieldrin, and endrin (58). Supercritical CO_2 was used to extract peritoneal fat (25-30 g), ground meat tissue (20-30 g), and liver samples (7-10 g) at a pressure of 10,000 psi and 80 °C (58). The extraction fluid flow rates and extraction time for the peritoneal samples ranged from 10-20 L/min (30 to 40 minutes), 2-4 L/min for the ground meat samples (2 hours), and 2 L/min for the liver samples (45 minutes) measured under ambient conditions (58). The extracted fat, containing the incurred OCPs were all collected in glass round-bottom flasks. Next, the OCPs were separated from the fat using a micro alumina column method (Food Safety and Inspection Service method 5.002) (58). More than 96% crude fat was extracted by SFE with concentrations ranging from 0.8-1.56 ppm for heptachlor epoxide, 2.21-3.08 ppm for dieldrin, and 1.75-2.41 ppm for endrin from the three poultry tissue samples (58). Also, no significant differences ($P < 0.05$) were found by an analysis of variance for SFE compared to the conventional extraction methods for any of the three pesticides from peritoneal fat and meat tissue (58). However, SFE consistently extracted more pesticide residue from liver tissue than solvent extraction due to its greater permeability of the tissue matrix. The data show that the extraction of these three OCPs by SFE is as effective as conventional thermal rendering and solvent extraction.

A follow up study by Johansen et al., to the on-line SFE-GC (56), describes the interfacing of SFE-HPLC to determine co-planar PCBs from three different biological matrices. The interfacing for the on-line SFE-HPLC system was similar to the system used for the on-line SFE-GC study except for the addition of two switching valves used to switch from the SFE to the HPLC mode. Biological samples of blood, human milk, and crab hepatopancreas were prepared for extraction with the addition of PCB 159 (mono-ortho-substituted) and ^{13}C analogs for the co-planar non-ortho-substituted PCBs (Cl in 3, 4, 5 positions) as internal standards (59). Basic alumina (0.5 g) was placed near the outlet end of the extraction vessel and in a separate cell coupled to the extraction vessel to retain the co-extracted lipid material. All extractions were performed in the dynamic mode (40 minutes), and a pressure of 14.5 Mpa at 60 °C with a flow rate of 133 mL/min gaseous CO_2 (59). The extracted PCBs were trapped using steel tubing with a 0.5 mm i.d. and a length of 20 cm. The average recoveries of spiked non-ortho-substituted (PCB 77, 126, 169) PCBs extracted with SFE and fractionated on-line with HPLC ranged from 71 to 101% for crab hepatopancreas, 35 to 57% for blood, and 76 to 87% for human milk (59). The relative standard deviations of this technique, extraction to analysis, were determined to be 5-16% (59). Also, this method was compared to conventional solvent extraction and off-line HPLC. Similar results were obtained for the crab hepatopancreas samples, while slightly higher recoveries of coplanar PCBs were obtained from blood serum using SFE-HPLC (59). Overall, the data showed rapid extraction, cleanup, and selectivity prior to on-line SFE-HPLC as a sample preparation technique prior to GC analysis for the determination of non-ortho-substituted PCBs.

Recently, Bowadt et al. developed an SFE method for the interference free analysis of PCBs from lyophilized fish tissue. The extractions were performed with 2 g portions of lyophilized tuna fish powder containing a lipid content of 2.8% (dry mass based) and known to be contaminated with PCBs at easily detectable levels (3-84 ng/g) (12). The fish sample was then mixed with 7 g of anhydrous Na_2SO_4 and packed into 7 mL extraction vessels. A solid phase trap consisting of approximately 1 mL Florisil® (0.16 - 0.25 mm particle size) was kept at 20 °C with pure CO_2 and 65 °C when methanol was used as a modifier (12). Upon extraction, the trap was selectively rinsed with 2 x 1.5 mL *n*-heptane, then 1 x 1.5 mL dichloromethane followed by 2 x 1.5 mL *n*-heptane to elute the PCBs separately from the co-extracted fatty material (12). The most reproducible results, when compared to Soxhlet recoveries, were obtained with a 10 minute static extraction using pure CO_2 at a density of 0.75 g/mL (218 bar) at 60 °C followed by a 30 minute dynamic extraction with a flow rate of 1 mL/min (12). However, slightly higher extraction efficiencies were obtained when the extraction temperature was raised to 97 °C which also resulted in greater interferences and standard deviations. The above SFE method at 60 °C was then used to extract PCBs and OCPs from several contaminated species of lyophilized fish with varying degrees of fat content (25.5 - 6.1%). The average recoveries from the SFE experiments in comparison to

Soxhlet gave significantly higher recoveries under the above SFE conditions (12). The data showed the potential of SFE to perform rapid, interference free, and quantitative extractions of PCBs and OCPs from native lyophilized fish tissues.

In a similar study, Lee et al. describes an SFE method for the determination of PCBs and OCPs from fish tissues. Contaminated fish samples (1 g) and fortified fish with PCBs were mixed with 3 g of anhydrous sodium sulfate before being transferred to the extraction cell whereupon 4 g of activated basic alumina was used to fill the vessel (downstream of CO_2 flow path) to retain the lipids (60). The optimal extraction efficiencies was obtained at 100 °C with CO_2 at a density of 0.71 g/mL (5000 psi) incorporating a 0.5 minute static and 20 minute dynamic extraction with a flow rate of 2.5 mL/min (60). The extracted analytes were trapped using a octadecylsilyl-bonded silica (ODS) trap maintained at 15 °C and was subsequently eluted with 1.2 and 0.8 mL isooctane in two fractions (60). The whole SFE procedure was completed in only 40 minutes per sample. Recoveries of PCBs were $\geq 95\%$ for both naturally contaminated and spiked fish samples when compared to Soxhlet recoveries (60). The procedure yielded very low percent standard deviations (<5%) from the fortified trout samples. The extraction efficiency of three commonly found OCPs (*p,p'*-DDE, hexachlorobenzene, mirex) were nearly identical to the Soxhlet results. The data indicated that the incorporation of activated alumina in the SFE methodology of fish produced a clean extract free of lipid interferences while producing quantitative and rapid extractions.

In addition, Hale and Gaylor examined the extraction efficiency at high temperature (150 °C) and high CO_2 pressure (350 atm) for the removal of PCBs from lyophilized fish tissue (61). Stainless steel extraction vessels (10 mL) were loaded with 1 g (dry weight) aliquots of lyophilized fish tissue and then were filled with 6 g of neutral alumina (150 mesh) activated at 150 °C near the exit end of the vessel. The highest recoveries were obtained at 150 °C and 350 atm with an initial 10 minute static extraction followed by a 30 minute dynamic extraction at a liquid flow rate of 3 mL/min CO_2 (61). The extracted PCBs were collected on a C18-modified silica trap at -30 °C which was later rinsed with 2 mL of isooctane to recover the PCBs (61). The effectiveness of this procedure was compared to values obtained by NIST and a multi-laboratory intercomparison exercise for SRM fish homogenates. The mean PCB recoveries obtained via SFE were not significantly different (paired *t*-test, 0.05 level) from those values obtained by NIST or from the multi-laboratory consensus (61). Overall, the data showed the effectiveness of SFE in isolating PCBs from lipid rich fish samples without laborious cleanup procedures.

The major aim of this study was to develop a quantitative supercritical fluid extraction method for PCBs and OCPs from freeze dried mussel tissue and to compare the extraction efficiency between SFE and conventional Soxhlet extraction. Furthermore, the effect of extraction density and temperature upon extraction efficiency was investigated to obtain a near optimal SFE method. On-line sample clean-up with activated basic alumina (i.e. retain lipid co-extractives) was utilized to decrease the total analysis time and investigate any possible interactions of alumina with PCBs and OCPs.

CHAPTER 4

Supercritical Fluid Extraction of Freeze-Dried Mussel Tissue

A. Introduction:

The quantitative analysis of lipophilic organic pollutants, like polychlorinated biphenyls (PCBs) and organochlorine pesticides (OCPs), in biological tissue samples relies on tedious and laborious procedures that involve large volumes of expensive and hazardous organic solvents. In general, these conventional methods (i.e. Soxhlet extraction, etc.) are sufficient in separating the PCBs and OCPs from the biological matrix. However, the analysis of extracts is exacerbated by co-extracted lipids from the biological tissue. This requires lengthy extract purification by gel permeation or liquid-liquid chromatography to separate the PCBs and OCPs from the co-extracted lipids. In addition, more sample handling is required to concentrate the clean extract prior to the assay method. The use of supercritical fluid extraction (SFE) can overcome the deficiencies of traditional extraction techniques while yielding similar extraction efficiencies.

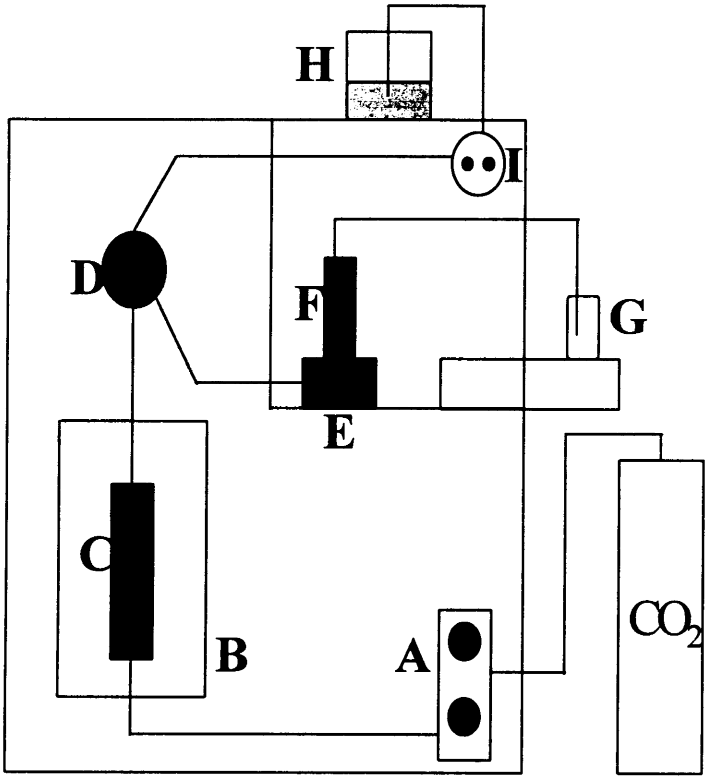
The objective of this study was to develop a method for the extraction of PCBs and OCPs from freeze dried mussel tissue (Mytilus *edulis*) using supercritical fluid and to compare the extraction efficiency between SFE and conventional Soxhlet extraction. The SFE method development includes the effect of supercritical fluid density, extraction temperature, static extraction time, and on-line clean-up for PCBs and OCPs from mussel tissue. The efficacy of SFE in comparison to Soxhlet was determined via replicate extractions of freeze dried mussel tissue in terms of recovery. Past studies have concentrated on the feasibility of SFE for environmental pollutants from sediments. However, only a small number of studies have been performed on biological tissue matrices, mainly poultry and fish, using the selective extraction properties of SFE-CO_2 and on-line purification. Finally, the present study examines the applicability of supercritical fluoroform as an alternate fluid compared to CO_2 and its ability to produce lipid free extracts without the use of *in situ* adsorbents.

B. Experimental:

(1) Extraction

All extractions were performed using a Hewlett-Packard (Wilmington, DE) Model 7680T supercritical fluid extractor employing either CO_2 or CHF_3 as the supercritical fluid. The SFE/SFC grade CO_2 without helium headspace and SFE/SFC CHF_3 with helium headspace (2000 psi) were both obtained from Air Products and Chemicals, Inc. (Allentown, PA). A schematic of the Hewlett-Packard 7680T SFE module is shown in **Figure 5**. This SFE system utilizes a cryogenically cooled (4 °C), dual head, reciprocating pump to deliver the extraction fluid as a liquid which subsequently becomes supercritical upon entering the heated extraction chamber. As the supercritical fluid (SF) passes through the extraction vessel the SF and extracted effluents enter and pass through

SFE Instrumentation



A: Pump
B: Heated Chamber
C: 7 mL Extraction Vessel
D: Valve
E: Nozzle
F: Trap (Solid Sorbent)
G: Trap Rinse Vial
H: Rinse Solvent Reservoir
I: Rinse Solvent Pump

Figure 5: Schematic of the Hewlett-Packard 7680T SFE Module

a variable restrictor which is connected to a solid phase trap (6 cm x 0.45 cm). The solid phase trap consisted of 40 μm octadecyl silica (C_{18}) particles. This zone of decompression and collection was temperature regulated during extraction-trapping and trap-rinsing. The trapped analytes were eluted from the trap using HPLC grade isooctane (EM Science) and collected in 2 mL amber autosampler vials.

An initial spike study of PCBs and OCPs from Hydromatrix® was performed at a CO_2 pressure of 354 bar (0.65 g/mL) and an extraction temperature of 120 °C using a ODS trap maintained at -10 °C. A 10 minute static extraction was followed by a 30 minute dynamic extraction with a liquid CO_2 flow rate of 1 mL/min. The variable restrictor or nozzle was maintained at 70 °C during the extraction and rinse step. Also, the trap was rinsed at 70 °C with isooctane (3 x 1.5 mL). The three trap rinse volumes were all mixed together and then concentrated to 0.5 mL by blowing N_2 gas over the liquid surface.

The incurred PCBs and OCPs were preliminarily extracted from mussel tissue (which had been mixed with activated basic alumina) with supercritical CO_2 at four different densities (0.45, 0.55, 0.65, and 0.75 g/mL), two different temperatures (97 and 120 °C), and two different static extraction times (10 and 20 minutes) while consistently maintaining the same vessel volume sweeps. The nozzle temperature was maintained at 70 °C during all the extractions and rinse steps. Isooctane (3 x 1.5 mL) was used to rinse the trap at 70 °C for the eight different SFE methods. The three trap rinse volumes obtained from each extraction were then mixed together and concentrated to 0.5 mL by blowing N_2 gas over the liquid surface prior to assay. No additional sample cleanup was performed. A list of extraction conditions that were investigated are shown in **Table 4.**

In addition, trifluoromethane or fluoroform (CHF_3) was used as the extraction fluid without activated alumina in the extraction vessel at a pressure of 378 bar (0.909 g/mL) with an extraction temperature of 97 °C. A CHF_3 liquid flow rate of 1 mL/min was used after a 20 minute static extraction followed by a 40 minute dynamic extraction yielding 7.8 vessel volume sweeps. The same nozzle, trapping, and rinsing conditions used in the CO_2 extractions was used for the CHF_3 extractions.

(2) Sample preparation

The Hydromatrix® spike study involved a standard spiking solution of PCBs and OCPs prepared at approximately 0.75 µg of analyte per gram of solvent. A 250 µL (0.13 µg/component) spike of the standard solution was placed onto a Hydromatrix® bed in the extraction cell. The first set of triplicate extractions were performed with the 7 mL extraction vessels loaded with approximately 2 g of Hydromatrix® and 5 g of activated alumina placed on top of the spiked Hydromatrix® bed. A second set of triplicate extractions were performed under the same conditions with additional Hydromatrix® instead of alumina to fill the remaining dead volume on top of the spiked bed. The granular basic alumina was obtained from EM Science (dp < 850 µm) which was activated by heating it at 150 °C in a convection oven for one day. This activated alumina was continuously activated at 150 °C and used in all CO_2 extractions of freeze dried mussel tissue.

Table 4: SFE parameters used in the extraction of PCBs and OCPs from mussel tissue using supercritical CO_2

SFE Parameter	Method #1	Method #2	Method #3	Method #4	Method #5	Method #6	Method #7	Method #8
Density (g/mL)	0.45	0.45	0.55	0.55	0.65	0.65	0.75	0.75
Pressure (bar)	221	221	276	276	354	354	378	378
Extraction Temp. (°C)	120	120	120	120	120	120	97	97
Flow Rate (mL/min)	1	1	1	1	1	1	1	1
Static Time (min.)	10	20	10	20	10	20	10	20
Dynamic Time (min.)	30	30	37	37	40	40	49	49
Vessel Volume Sweeps	7	7	7	7	7	7	7	7

The freeze dried mussel tissue was obtained from NIST as a recently certified standard reference material SRM-2974 (Organics in Mussel Tissue). A 7 mL extraction vessel was used for this study with supercritical CO_2. The sample was prepared for extraction by mixing approximately 1 g of freeze dried mussel tissue (SRM 2974) with activated alumina (1:3). A layer of activated alumina was then introduced to the extraction vessel just above the mussel tissue (**Figure 6**). This procedure was followed each time while investigating extraction efficiency at several SFE parameters. From this effort, the method that produced the best recovery of the PCBs and OCPs would be ascertained. The supercritical CHF_3 study involved the addition of only the freeze dried mussel tissue (1 g) into the 7 mL extraction vessel. The extraction vessels contained no activated alumina and the void volume was compensated with the addition of Hydromatrix®. All collected extracts were concentrated prior to assay by GC-Electron Capture Detection (GC/ECD) or GC-Mass Spectrometry (GC/MS).

(3) Extract Analysis

After the extraction was completed, a 100 µL internal standard solution containing three compounds was introduced to the combined rinse fractions (3 x 1.5 mL) before being concentrated to 0.5 mL. The internal standard solution contained PCB 103 (3.77 µg/g), PCB 198 (3.29 µg/g), and 4,4'-DDT-d_8 (1.34 µg/g); therefore, the amount of each standard added to each combined trap rinse was 377 ng (PCB 103), 327 ng (PCB 198), and 134 ng (4,4'-DDT-d_8). Next, the concentrated extracts obtained from the spike study and the preliminary SFE parameter investigation were analyzed by a Varian 6000

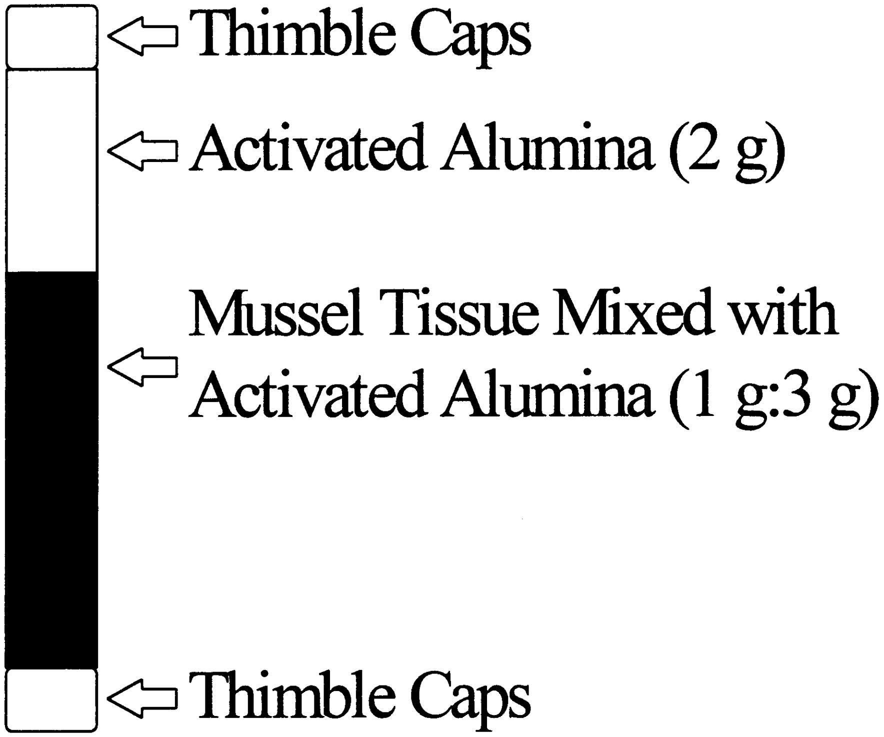


Figure 6: Diagram of extraction vessel filled with freeze dried mussel tissue and activated alumina to retain lipid coextractives

Vista Series GC equipped with an ECD. The extracts obtained from triplicate extractions using supercritical CO_2 and CHF_3 were analyzed by a Hewlett-Packard (Wilmington, DE) 5680 Series II GC equipped with an Hewlett-Packard 7673 autoinjector. This GC system employed a Hewlett-Packard 5972 Mass Selective Detector (MSD). A DB-5MS (5%-phenylmethylsiloxane) column (60 m long x 0.25 mm i.d.) with a film thickness of 0.25 μm (J & W Scientific, Folsom, CA) was used in both GC systems. Also, a retention gap (2.5 m long x 0.25 mm i.d.) was butt connected to the analytical column to aid in focusing the 2 μL cool on-column injections. The column was held isothermally at an initial temperature of 60 °C for 1 minute and then temperature programmed at 45 °C/min to 200 °C and held for 30 minutes. A final temperature program of 2 °C/min from 200 °C to 270 °C with a final hold time of 20 minutes was used to perform the analytical separation of the PCBs and OCPs.

Quantitation involved the use of a five point calibration curve ranging from 11 ng to 250 ng with a correlation coefficient ranging from 0.998 to 0.999 for all analytes monitored. The calibration curve was determined by the ratio of analyte peak area to the peak area of the internal standard. Each extracted analyte peak was identified from the specific retention times of the PCBs and OCPs which were earlier obtained from repeated injection of standards. In addition, the MSD was operated in the single ion monitoring mode (SIM) with multiple ions being monitored at different time spans during the chromatographic separation. **Table 5** lists the specific ions and the time span in which they were monitored during the separation. In addition, a list (**Table 6**) of all monitored

Table 5: Multiple ions monitored in the SIM mode during gas chromatography/mass spectrometry

Time (min.)	Ions Monitored (m/z)
10 -22	181, 222, 256
22 - 33	290, 324
33 - 42	235, 246, 324, 371, 373, 409
42 - 53	235, 243, 246, 324, 360, 409
53 - 71	235, 243, 360, 394, 426
71 - 89	464, 498

Table 6: Monitored ions correlated to the PCB and OCP of interest and corresponding internal standard employed for quantitation

Ions (m/z)	Analyte	Internal Standard
181	beta-BHC	PCB 103
222	PCB 8	PCB 103
256	PCB 18, 28, 31	PCB 103
290	PCB 52, 49, 44	PCB 103
324	PCB 103, 95, 101, 99, 87, (110, 118, 105)	PCB 103 (PCB 198)
235	DDD, DDT	4,4'-DDT-d8
246	DDE	4,4'-DDT-d8
371	cis-Chlrodane	4,4'-DDT-d8
373	cis-Chlordane	4,4'-DDT-d8
409	trans-Nonachlor	4,4'-DDT-d8
243	4,4'-DDT-d8	4,4'-DDT-d8
360	PCB 151, 149, 153, 126, 128, 156	PCB 198
394	PCB 182, 183, 180, 170	PCB 198
426	PCB 198	PCB 198
464	PCB 206	*
498	PCB 209	*

* Not detected by mass spectrometry and therefore not quantifiable
PCBs and OCPs are tabulated with the specific internal standard used for quantitation.

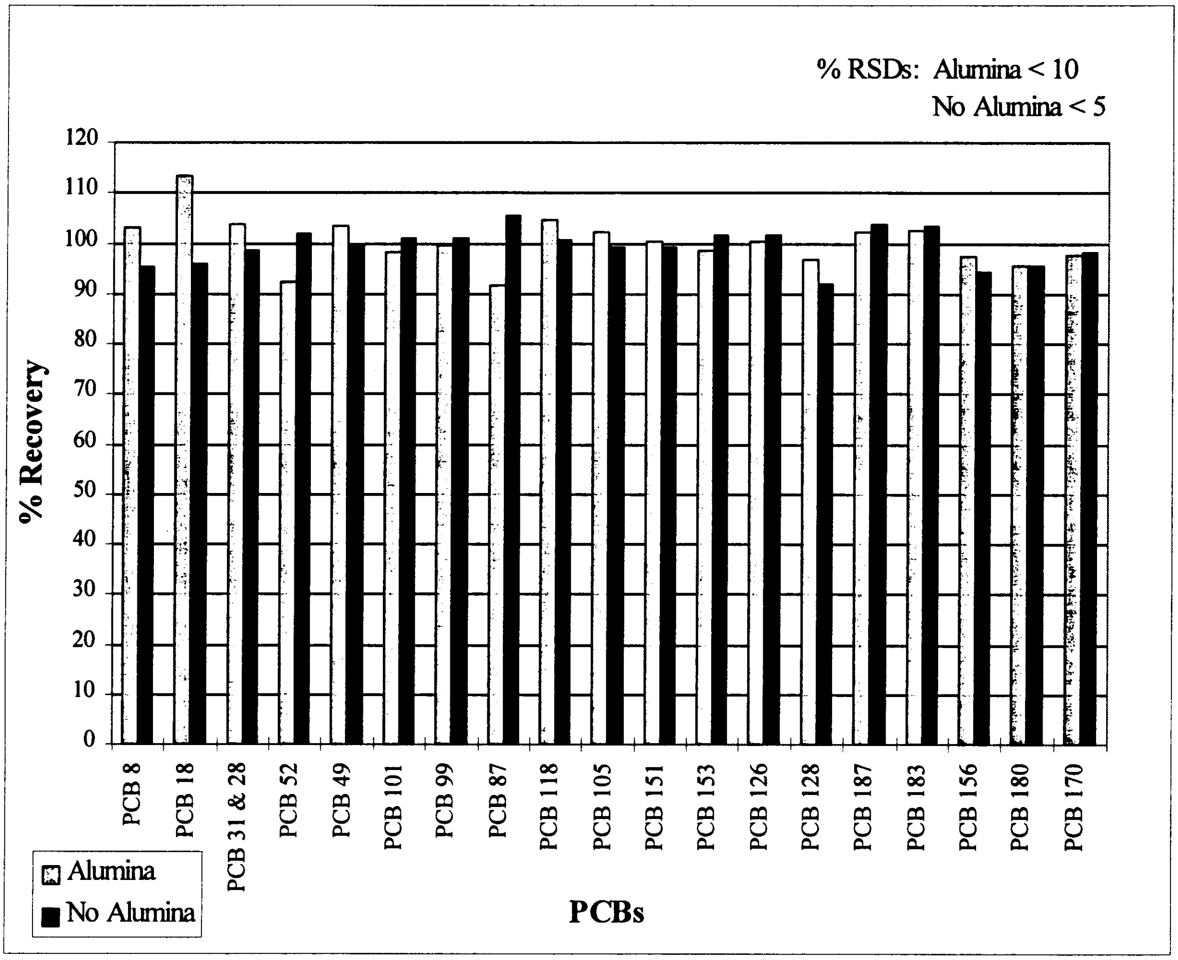
PCBs and OCPs are tabulated with the specific internal standard used for quantitation.

The ratio of extracted analyte peak area to internal standard peak area was then used to determine the concentration of each monitored PCB and OCP (i.e. ng of analyte per g of mussel tissue). This value was converted to percent recovery by taking the ratio of the extracted analyte concentration obtained via SFE to the extracted analyte concentration obtained by Soxhlet. The Soxhlet extractions of mussel tissue which involved 250 mL of dichloromethane for 24 hours, were performed by the National Institute of Standards and Technology (NIST, Gaithersburg, MD). Soxhlet concentrations used for comparison with SFE were the certified concentration values for the mussel tissue standard reference material.

C. Results and Discussion:

(1) SFE of PCBs & OCPs from spiked Hydromatrix® with CO_2

Initially, studies were performed on spiked Hydromatrix® in order to determine if the presence of alumina in the extraction vessel affected the chemical integrity of the PCBs and OCPs. The extraction and extract analysis were performed according the procedure outlined in the Experimental section. The data shown in **Figure 7** indicate that the chemical integrity of the PCBs were not compromised. Also, it showed the SFE unit was working properly and that the collection scheme was sufficient to retain the PCBs. Very little difference was observed in the percent recovery of each PCB with and



Density of CO_2	0.65 g/mL
Extraction Temperature	120 °C
Static Extraction Time	10 minutes
Dynamic Extraction Time	30 minutes
Liquid CO_2 Flow Rate	1 mL/min
ODS Trap Temperature	-10 °C
Trap Rinse (70 °C)	Isooctane (3 x 1.5 mL)

Figure 7: Percent recovery of PCBs from spiked Hydromatrix® with and without activated alumina (analysis by GC/ECD)

without activated alumina in the extraction vessel. Slightly higher relative standard deviations (% RSDs) were obtained with alumina which could be due to co-extraction of low-level impurities in the alumina thus resulting in a higher background which yielded inconsistent GC/ECD peak integration. This was verified by a blank extraction of Hydromatrix® and activated alumina. The GC/ECD chromatogram of the blank extract is shown in **Figure 8**. From the chromatogram four significant peaks were detected by ECD, three internal standards and one unknown (retention time of 27.1 min.). The retention times of 25.4, 48.9, and 62.5 min. corresponded respectively to PCB 103, 4,4'-DDT-d_8, and PCB 198. Also, the baseline showed evidence of low-level impurities in the blank SFE extract. These blank extractions were performed several times and on different days with all extracts showing similar chromatograms.

Similar percent recoveries of several OCPs were also obtained with and without activated alumina except for 4,4'-DDE, 4,4'-DDD and 4,4'-DDT (**Figure 9**). These three compounds were significantly affected by the presence of alumina. Lower recoveries were obtained for 4,4'-DDD and 4,4'-DDT; while, elevated percent recovery was seen for 4,4'-DDE. The low recovery of 4,4'-DDT and the high recovery of 4,4'-DDE were probably due to the basicity of the alumina as well as the high extraction temperature used which apparently caused 4,4'-DDT to degrade to its degradate (4,4'- DDE). This reasoning can also be applied to 4,4'-DDD (analogue of DDT) which has also been shown to degrade under similar conditions to DDMU (2,2-bis(p-chlorophenyl)-1-chloroethane). The DDMU was not monitored during any of the chromatographic

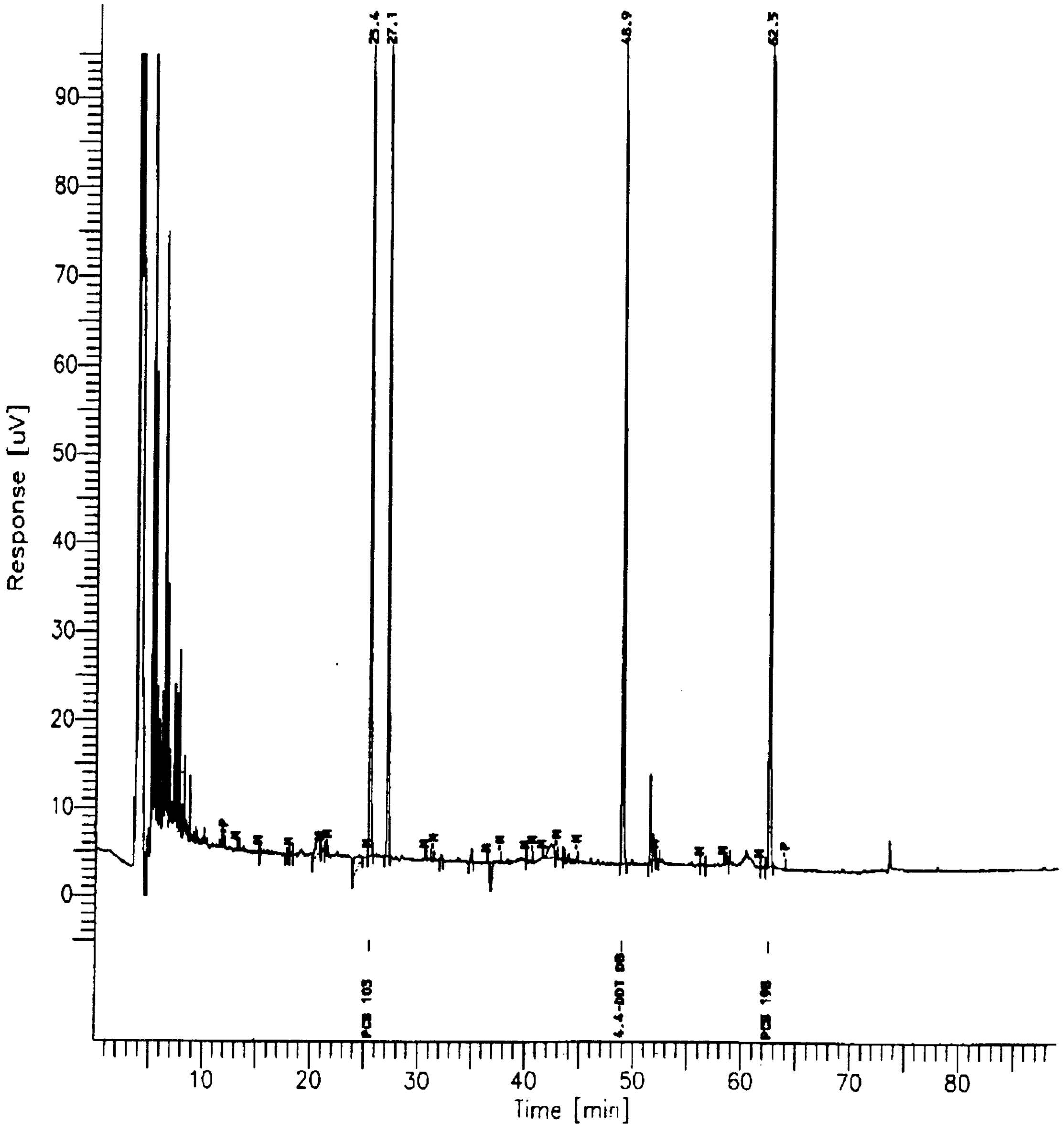
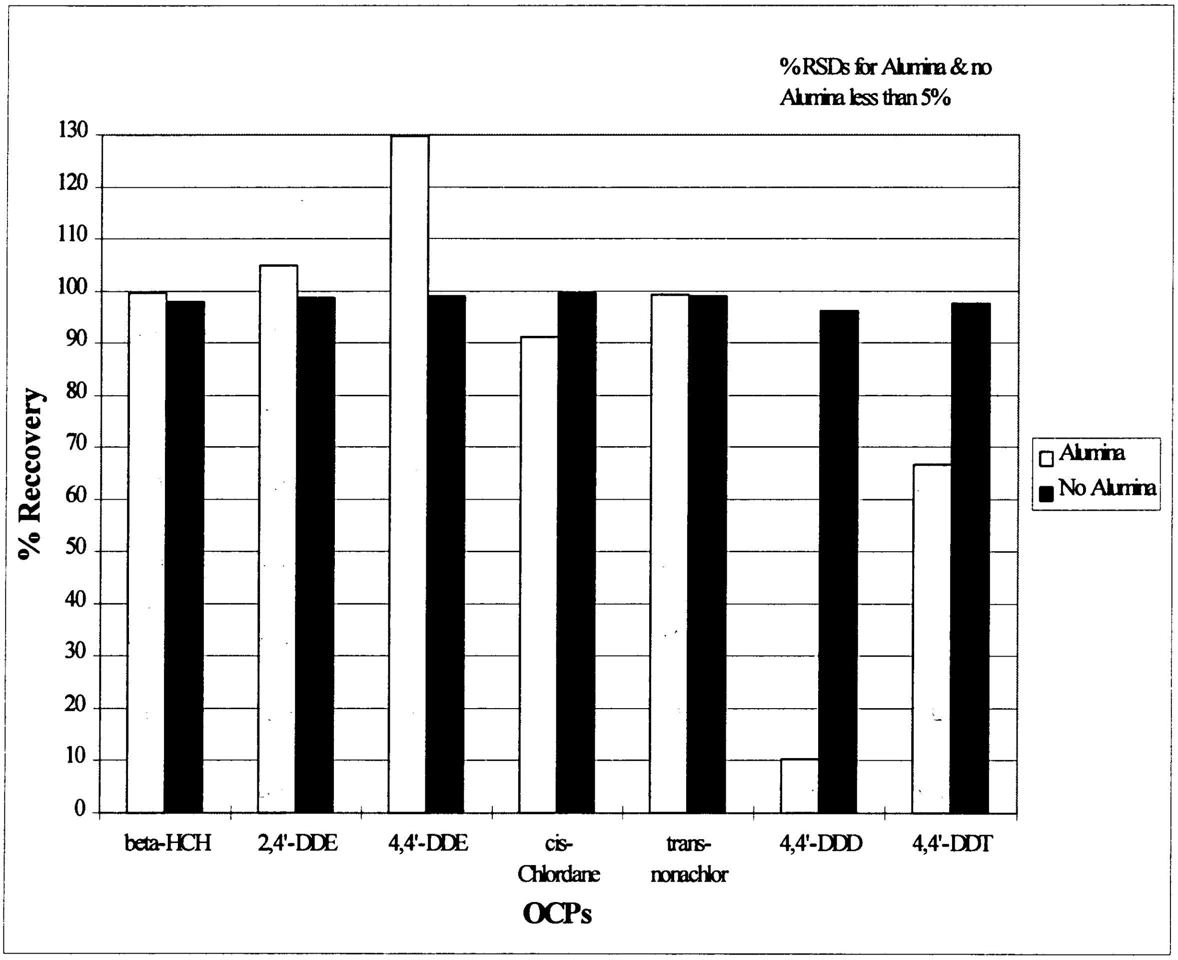


Figure 8: GC/ECD chromatogram of blank SFE extraction with internal standards, Hydromatrix®, and activated alumina in the extraction vessel

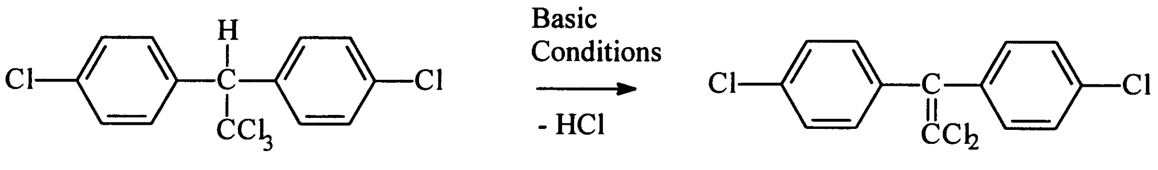


Density of CO_2	0.65 g/mL
Extraction Temperature	120 °C
Static Extraction Time	10 minutes
Dynamic Extraction Time	30 minutes
Liquid CO_2 Flow Rate	1 mL/min
ODS Trap Temperature	-10 °C
Trap Rinse (70 °C)	Isooctane (3 x 1.5 mL)

Figure 9: Percent recovery of OCPs from spiked Hydromatrix® with and without activated alumina (analysis by GC/ECD)

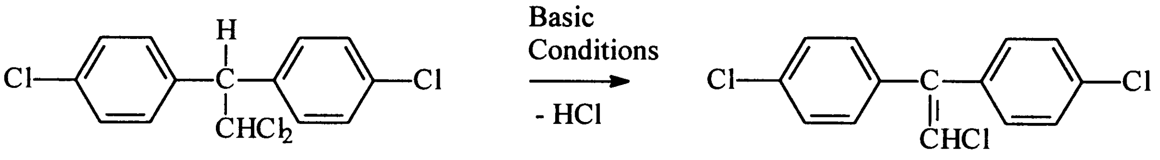
separations involving either the GC/ECD or GC/MS. The degradation process has been reported in the literature as a dehydrochlorination of DDT and DDD to their degradate forms in the presence of basic alcoholic alkali (26, 62, 64-66). This reaction can be described as an E_2-type elimination which results in the loss of HCl and the formation of a carbon-carbon double bond (**Figure 10**). Furthermore, the dehydrochlorination reaction is readily catalyzed by traces of iron, aluminum, and chromium salts (67). As for temperature induced degradation, DDT is quite stable and does not decompose below 195 °C, but it will decompose at approximately 100 °C in the presence of impurities. Various catalysts like anhydrous ferric oxide, anhydrous ferric chloride, aluminum chloride, and iron metal will facilitate the elimination of HCl from DDT (and several DDT analogues) (64). Also, these compounds have been reported to decompose in a hot GC injection port, a process often catalyzed by "active" glass or metal surfaces (68). This problem can be overcome by the use of cool on-column injections and electronic pressure programming of the GC column head pressure to minimize degradation during split and splitless injections (69).

The recovery of DDD from the spike study was significantly lower than DDT which can not be fully explained because the rate of degradation should be much greater for DDT than DDD since DDD exhibits greater stability than DDT (65). In terms of extraction reproducibility the % RSDs were all less than 5% with and without the activated basic alumina in the extraction vessel. Overall, the spike study showed the validity of our instrumentation and the denaturing effect of basic alumina and high



4,4'-DDT **4,4'-DDE**

(A) Dehydrochlorination of 4,4'-DDT to its metabolite 4,4'-DDE



4,4'-DDD **DDMU**

(B) Dehydrochlorination of 4,4'-DDD to DDMU

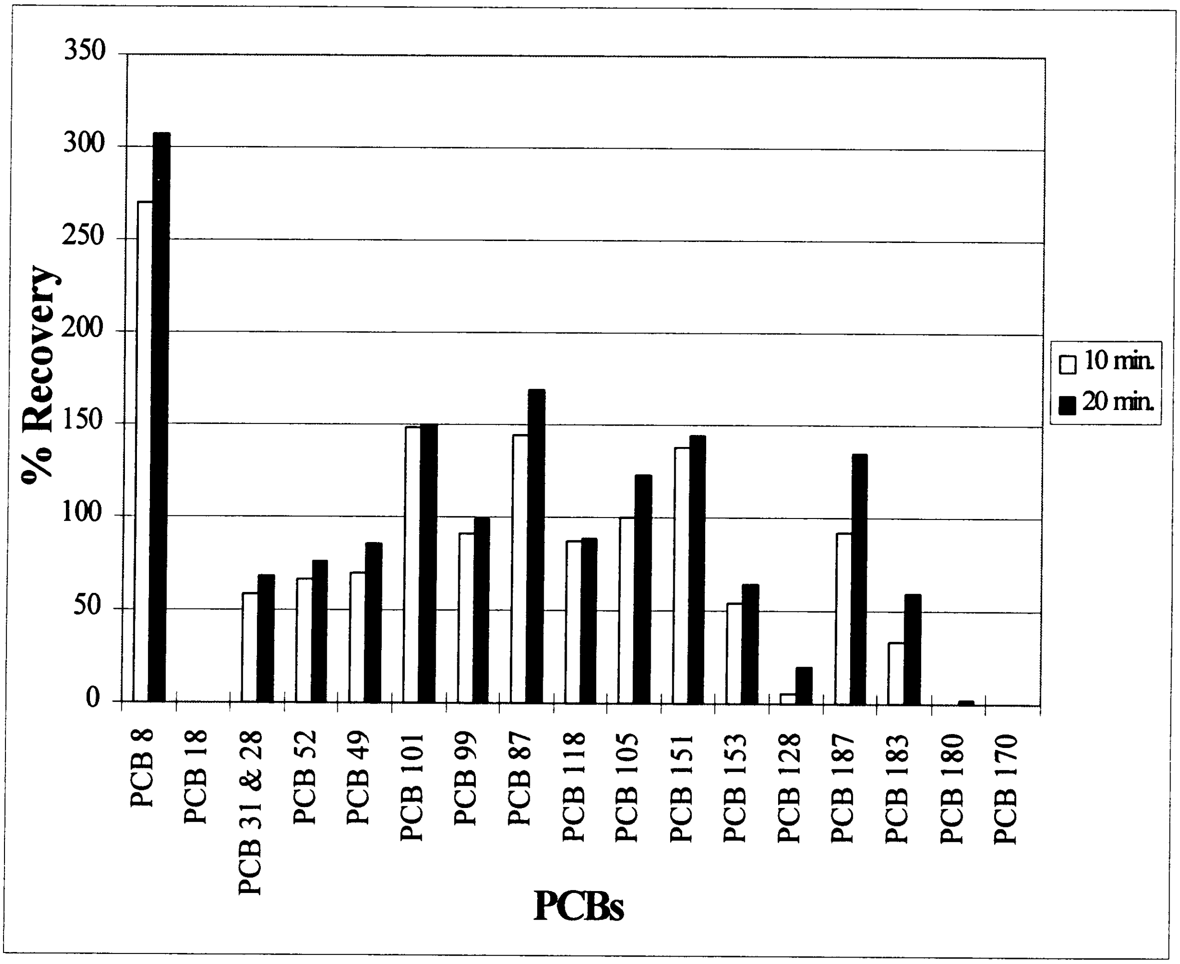
Figure 10: Degradation of 4,4'-DDT and 4,4'-DDD to their respective metabolites*

* Reference 26, 62, 64-66

temperature on several OCPs.

(2) SFE of PCBs from freeze dried mussel tissue (SRM 2974) with CO_2

Having established the inertness of activated alumina toward PCBs, preliminary extraction (n = 1) of the incurred freeze dried mussel tissue was attempted to determine which SFE method would produce the best recovery of the PCBs. The SRM, freeze-dried tissue was prepared and extracted according to the eight methods described in the Experimental. Extractions were initially performed at 120 °C at 0.45 g/mL, 0.55 g/mL, and 0.65 g/mL CO_2 and two different static extraction periods (10 and 20 minutes). The effect of a longer static extraction period upon percent recovery is clearly seen in **Figure 11**. The data indicate that the recovery of every PCB was higher employing a 20 minute static time as opposed to a 10 minute static time. This trend was seen regardless of the CO_2 density used. Recovery higher than 100% relative to the Soxhlet SRM value was obtained for six PCBs (PCB 8, 101, 87, 105, 151, 187). This was attributed to the co-extracted material co-eluting with the PCB peak as well as an increased background which resulted in inconsistent integration of several peaks from the GC/ECD chromatogram (**Figure 12**). It is also conceivable that SFE may be more efficient than conventional Soxhlet extraction for removal of PCBs from the mussel tissue which may account for recoveries greater than 100%. In addition, extremely low recoveries, below 30% were obtained for PCB 18, PCB 128, PCB 180, and PCB 170 which may be due to insufficient solvating power of the supercritical fluid as well as their poor separation from the background noise and baseline in the GC/ECD.



Density of CO_2	0.65 g/mL
Extraction Temperature	120 °C
Static Extraction Time	10 or 20 minutes
Dynamic Extraction Time	40 minutes
Liquid CO_2 Flow Rate	1 mL/min
ODS Trap Temperature	-10 °C
Trap Rinse (70 °C)	Isooctane (3 x 1.5 mL)

Figure 11: Effect of static extraction time on percent recovery of PCBs from freeze dried mussel tissue (assayed by GC/ECD)

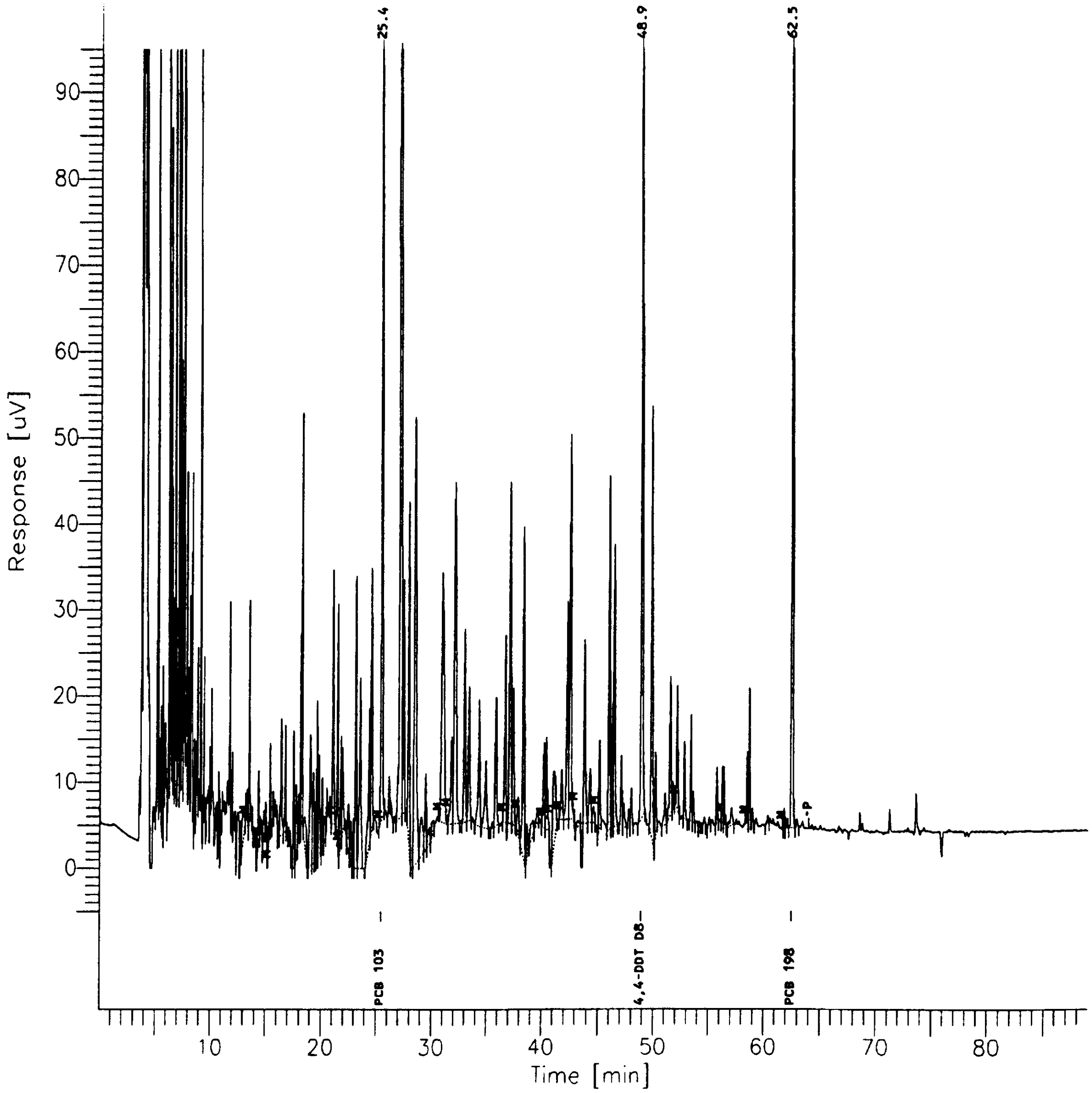
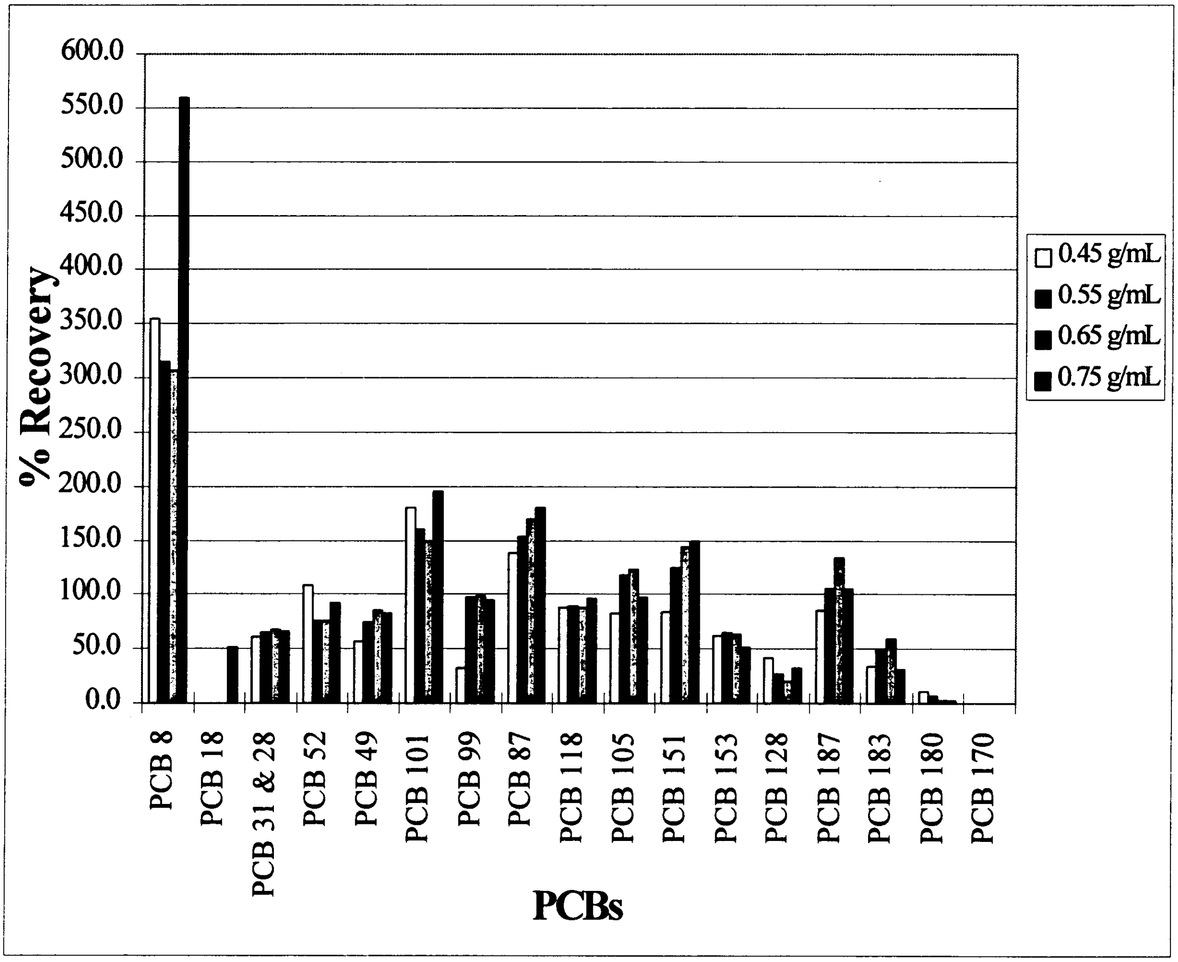


Figure 12: GC/ECD chromatogram of freeze dried mussel tissue extract with activated alumina in the extraction vessel using SFE method #6

SFE extraction efficiencies at high temperature/low density (SFE methods #1- #6) were compared with extraction at lower temperature/high density (SFE methods #7, #8) using the same trapping and analysis procedure. It has been shown in a previous study that the extraction temperature (high temperature to aid diffusion) is more important than the pressure for achieving high extraction efficiencies when extracting organic pollutants from environmental matrices (64). However, it was discovered that the lower temperature/higher density gave higher percent recoveries for approximately half of the PCBs that were quantified (**Figure 13**). For PCB 49 and PCB 99 the recoveries were slightly higher at the lower density/higher temperature, but the differences were generally too low to be significant. Percent recoveries of greater than 100% (PCBs 8, 101, 87, 105, 151, and 187) were again obtained due to reasons addressed in the previous static extraction results. Significant differences in recoveries were obtained for PCB 52, PCB 105, PCB 153, PCB 128, PCB 182, PCB 183, and PCB 180 with higher recoveries at the lower density/higher temperature. This was probably due to the high extraction temperature which resulted in disruption of unwanted matrix-analyte interaction. The result was an increased amount of interfering compounds in the SFE extract and inconsistent integration of peaks due to the higher background.

After evaluating the preliminary extraction results, SFE method #8 was assumed to produce the best recovery of PCBs from the tissue matrix (**Table 7**). Coincidentally, this SFE method was similar to the one investigated by Bowadt et al. with lyophilized fish tissue (60). Triplicate extractions were subsequently performed to determine the



Density of CO_2	0.45, 0.55, 0.65, 0.75 g/mL
Extraction Temperature	120 °C (97 ° C for 0.75 g/mL)
Static Extraction Time	20 minutes
Dynamic Extraction Time	30, 37, 40, or 49 minutes
Liquid CO_2 Flow Rate	1 mL/min
ODS Trap Temperature	-10 °C
Trap Rinse (70 °C)	Isooctane (3 x 1.5 mL)

Figure 13: Effect of density on percent recovery of PCBs from freeze dried mussel tissue (assayed by GC/ECD)

Table 7: Percent recovery of PCBs from freeze dried mussel tissue with various SFE methods with 20 minute static extraction times

PCB Congener	Method #2	Method #4	Method #6	Method #8
8	354.4 %	315.3 %	306.9 %	559.5 %
18	0 %	0%	0 %	52.2 %
31 & 28	61.7 %	65.4 %	67.9 %	66.9%
52	108.3 %	75.7 %	76.6 %	92.2 %
49	56.8 %	74.7 %	85.7 %	82.7 %
101	180.5 %	160.4 %	149.8 %	196.0%
99	32.6 %	98.3 %	99.2 %	95.3 %
87	137.8 %	152.9 %	169.1 %	181.1 %
118	87.8 %	90.1 %	88.7 %	96.5 %
105	82.8 %	118.4 %	123.0 %	97.9 %
151	84.5 %	124.2 %	144.4 %	149.7 %
153	61.9 %	65.5 %	64.1 %	51.1 %
128	42.2 %	26.7 %	20.1 %	32.5 %
182	85.9 %	105.7 %	134.7 %	105.6 %
183	34.5 %	49.7 %	59.3 %	31.2 %
180	11.0 %	7.0 %	2.6 %	2.9 %
170	0 %	0 %	0 %	0 %

reproducibility and the sturdiness of the method. In this case mass selective detection (MSD) was used rather than electron capture detection (ECD) due to interferences seen in the chromatographic separation which were caused by co-extractives from the mussel tissue matrix and the alumina. A GC/MS chromatogram from the triplicate extractions is shown in **Figure 14**. As previously stated in the Experimental section (**Table 5**) the MSD was operated in the SIM mode with multiple ions being monitored. The MSD proved to be a better means of detecting and quantifying the PCBs than ECD since any co-extractives and impurities from the alumina and mussel tissue could be for the most part excluded from the chromatographic separation. The excellent reproducibility, below 10% RSD (i.e. number above the bars in **Figure 15** is % RSD) for the majority of the PCBs except PCB 187, PCB 183, and PCB 170, exemplifies the lack of interferences in the analysis as well as the consistency of the SFE technique. The results shown in **Figure 15** also suggest that SFE was able to quantitatively extract all but one of the PCBs using method #8 relative to the Soxhlet data obtained by NIST. The certified Soxhlet concentration for the monitored PCBs ranged from 5 ng/g to 136 ng/g. According to EPA standards quantitative is defined as recoveries 100% ± 30% which means that only PCB 151 was not quantitatively removed from the tissue sample.

The certified Soxhlet concentrations obtained by NIST are shown in **Table 8** as well as the concentrations obtained by SFE and the relative percent recoveries. Soxhlet extraction concentration levels were obtained from as received freeze dried mussel tissue (SRM 2974) with analysis performed via GC/MS using a DB-5MS column. The certified

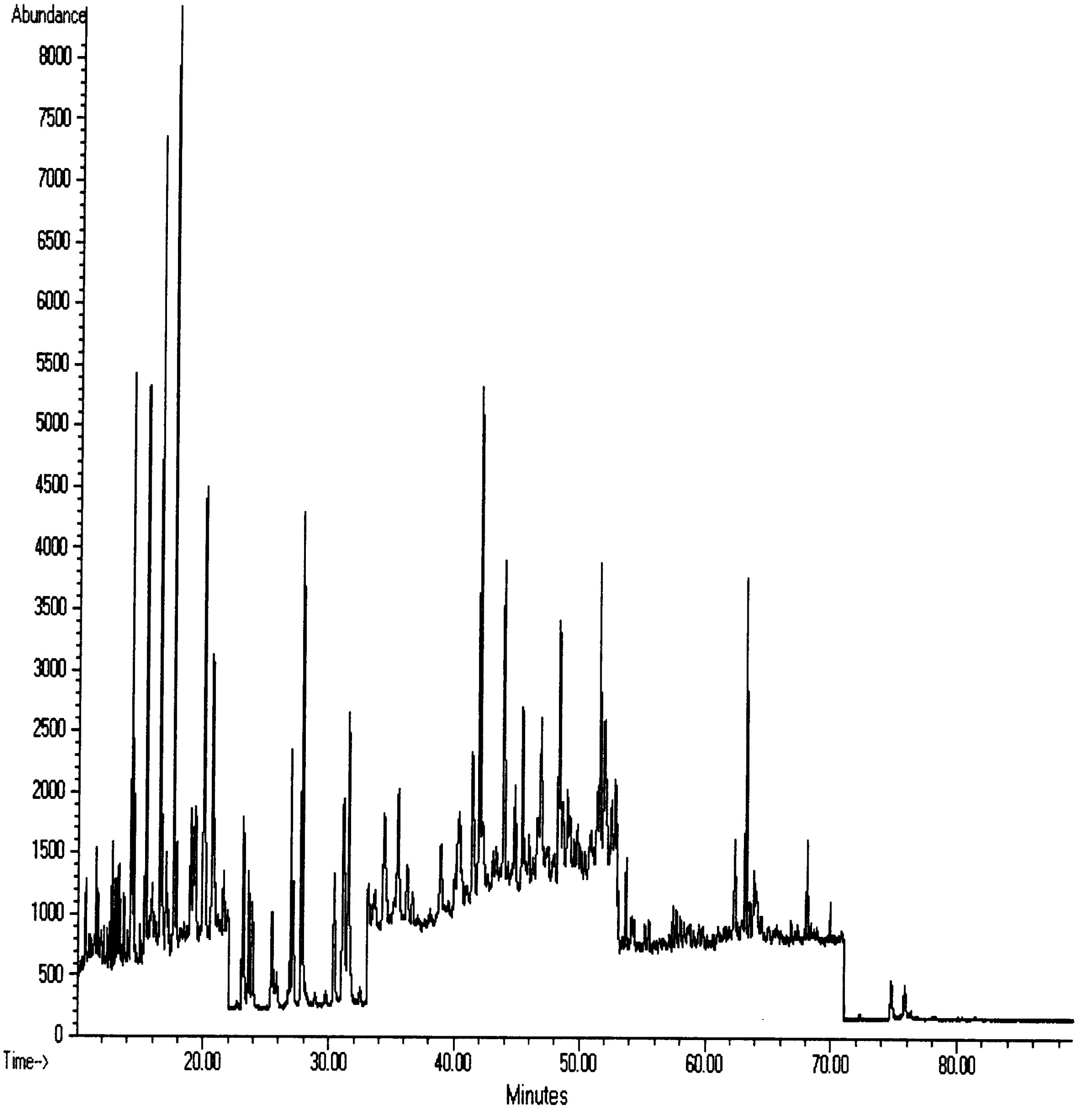
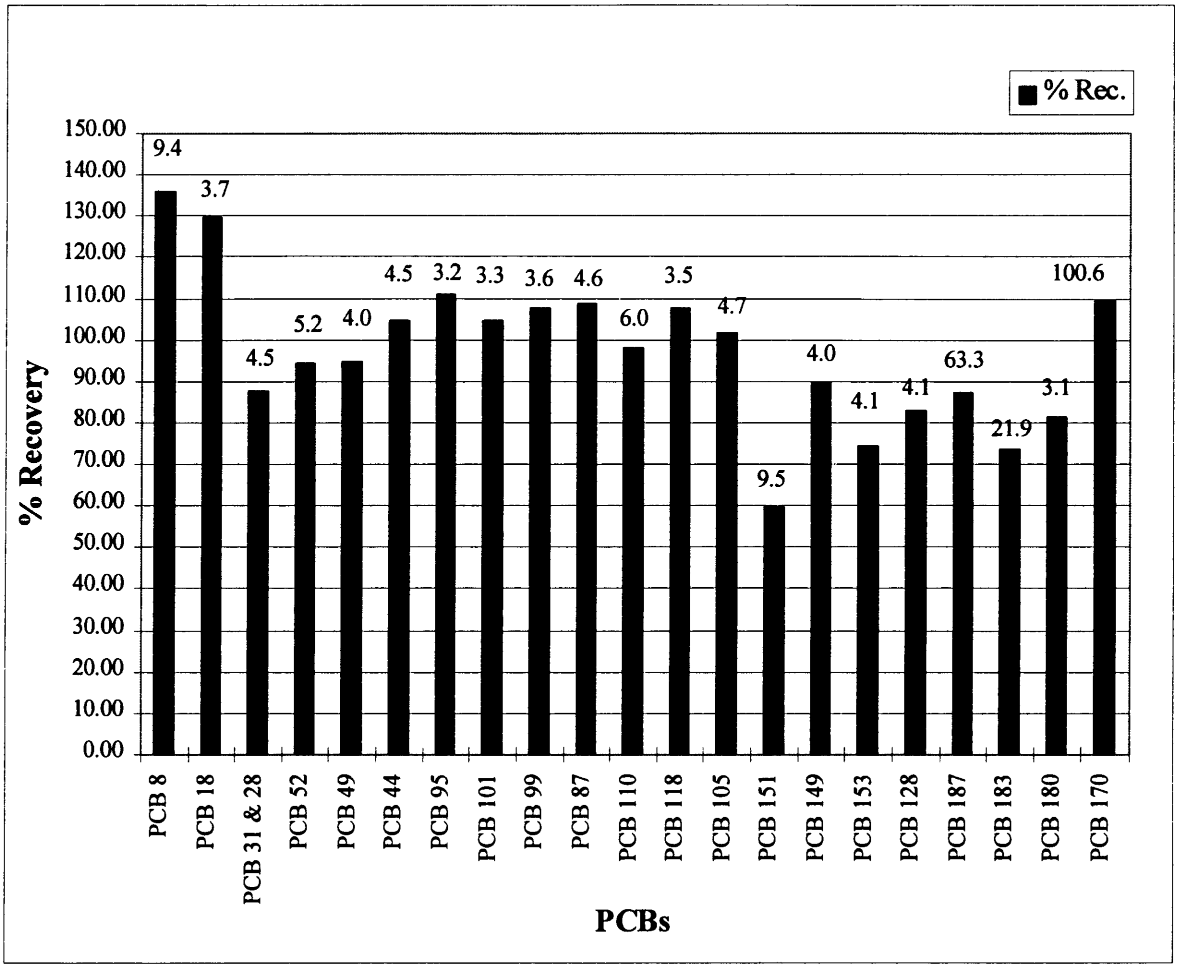


Figure 14: Total ion chromatogram of PCBs and OCPs extracted from SRM 2974 with SFE method #8



Density of CO_2	0.75 g/mL
Extraction Temperature	97 °C
Static Extraction Time	20 minutes
Dynamic Extraction Time	49 minutes
Liquid CO_2 Flow Rate	1 mL/min
ODS Trap Temperature	-10 °C
Trap Rinse (70 °C)	Isooctane (3 x 1.5 mL)

Figure 15: SFE percent recoveries and percent RSDs (above bar) of PCBs from triplicate extractions of SRM 2974 (assayed by GC/MSD)

Table 8: NIST certified Soxhlet concentrations versus SFE obtained concentrations and percent recoveries (SRM 2974 as received)

Compound	Certified NIST Soxhlet Concentration (ng/g)	SFE Concentration (ng/g)	% Recovery
PCB 8	6.63 ± 0.14	9.0	136.0
PCB 18	22.2	28.8	129.8
PCB 31 & 28	136 ± 4	119.4	87.8
PCB 52	109 ± 3	102.7	94.2
PCB 49	74.1 ± 1.2	70.2	94.7
PCB 44	66.6 ± 3.6	69.8	104.8
PCB 95	64.3	71.4	111.1
PCB 101	111 ± 3	116.1	104.6
PCB 99	56.8 ± 1.6	61.2	107.7
PCB 87	45.4 ± 2.3	49.3	108.6
PCB 110	105 ± 5	102.8	97.9
PCB 118	107 ± 7	114.9	107.4
PCB 105	43.3 ± 1.3	44.1	101.7
PCB 151	20.2 ± 0.9	12.0	59.6
PCB 149	70.5 ± 1.2	63.2	89.7
PCB 153	134 ± 2	99.9	74.6
PCB 128	16.3 ± 0.4	13.5	82.9
PCB 187	26.2 ± 0.6	22.9	87.4
PCB 183	14.8 ± 0.5	10.9	73.9
PCB 180	15.2 ± 0.3	12.4	81.6
PCB 170	5.9 ± 0.33	6.5	109.6
2,4’-DDE	5.2 ± 0.71	0	0
4,4’-DDE	37.1 ± 1	25.9	69.8
cis-Chlordane	14.3 ± 0.6	8.7	60.9
trans-Nonachlor	13.4 ± 0.4	8.1	60.1
2,4’-DDD	10.9 ± 0.6	16.5	151.5
4,4’-DDD	41.5 ± 1.8	40.3	97.2
2,4’-DDT	9.5 ± 0.33	10.7	112.3
4,4’-DDT	3.7 ± 0.32	0	0

concentrations for the more volatile PCBs (e.g. PCB 8 and PCB 18) was a lot better with SFE than Soxhlet which indicates that the reduced sample handling with SFE resulted in minimization of the loss of these compounds. However, the percent recovery of PCB 151 was significantly lower with SFE than Soxhlet which may be attributed to experimental error. As for the elevated % RSDs for PCB 187, PCB 183, and PCB 170 was due to the low concentrations of these PCBs (26.2, 14.8, and 5.9 ng/g) which caused differentiation and integration of these peaks from the baseline difficult and rather inconsistent.

Next, a paired t-test was performed to determine if there were any significant differences in terms of recovery between the SFE and Soxhlet extraction methods. A t-critical at P=0.05 is 4.30 which means that if the t-calculated value is greater than 4.30 then the recoveries were deemed to be significantly different. The results are shown in **Figure 16** and one can see that there were no significant differences between the two extraction techniques for approximately half of the PCBs. The highest significant differences were seen for PCBs 18, 151, 163, 128, and 180. Overall, the SFE method proved to be efficient in extracting the PCBs with similar results obtained by Soxhlet.

(3) SFE of OCPs from freeze dried mussel tissue (SRM 2974) with CO_2

The extraction recoveries of OCPs were mostly non-quantitative and exhibited poor reproducibilities with method #8. **Figure 2** in Chapter 2 illustrates the molecular structure of the OCPs. Percent RSDs ranged from 16.3% to 25.5% with recoveries ranging from 60% to 150% relative to Soxhlet. Certified Soxhlet concentration ranges of 3.7 ng/g to 41.5 ng/g were listed for the OCPs. The results shown in **Figure 17** indicate

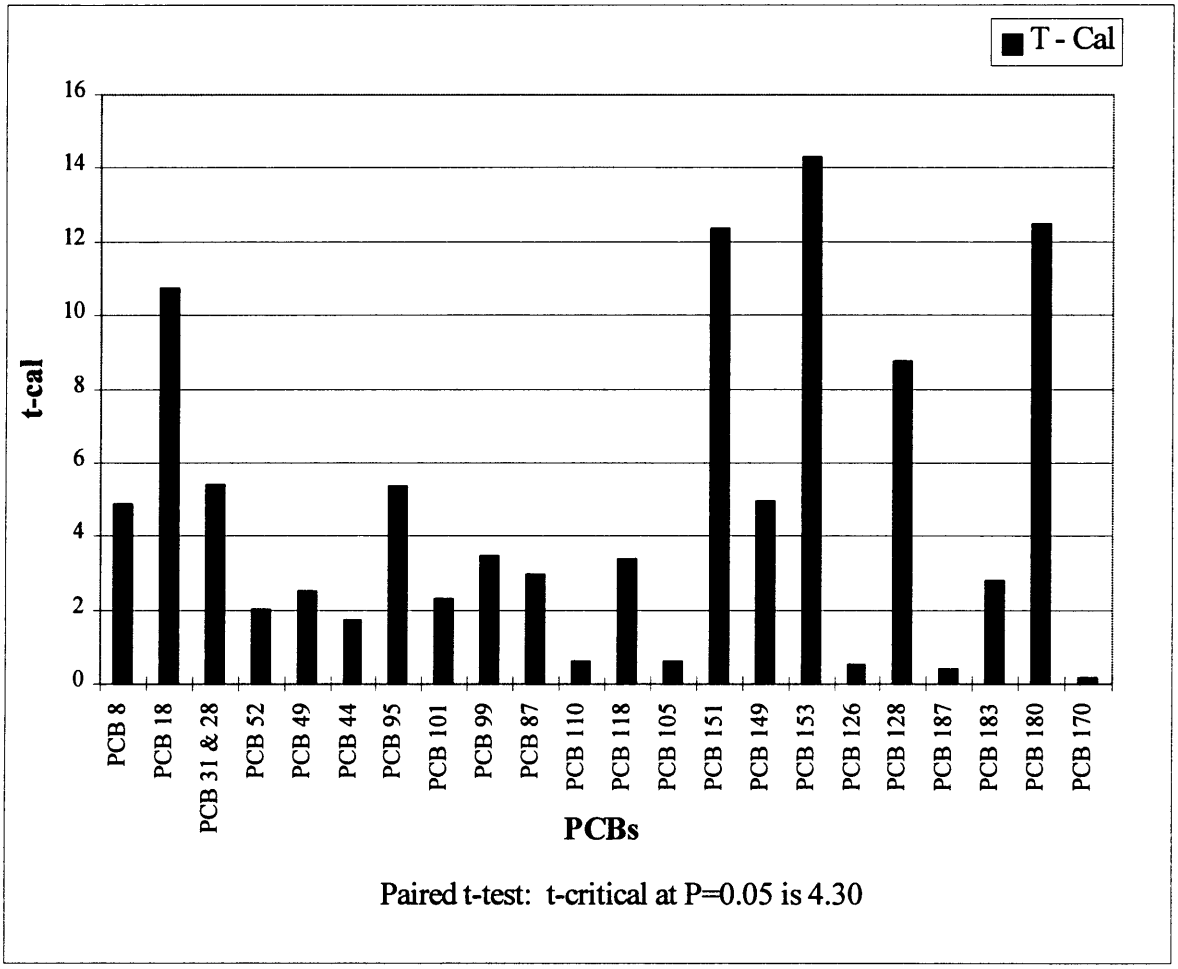
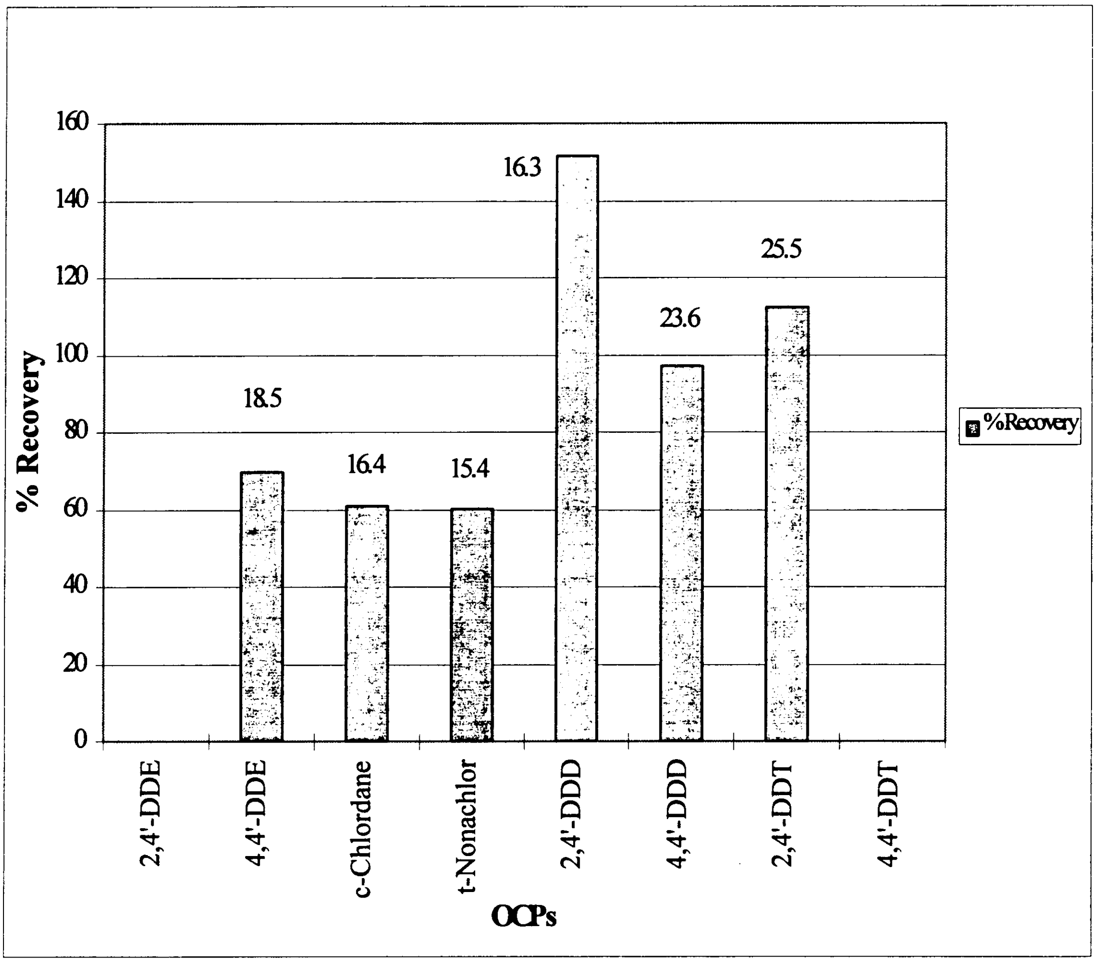


Figure 16: Paired t-test results comparing recoveries obtained by SFE and Soxhlet



Density of CO_2	0.75 g/mL
Extraction Temperature	97 °C
Static Extraction Time	20 minutes
Dynamic Extraction Time	49 minutes
Liquid CO_2 Flow Rate	1 mL/min
ODS Trap Temperature	-10 °C
Trap Rinse (70 °C)	Isooctane (3 x 1.5 mL)

Figure 17: SFE percent recovery and percent RSDs (above bar) of OCPs from triplicate extractions of SRM 2974 (assayed by GC/MS)

percent recoveries of zero for 4,4'-DDT and approximately 70% for 4,4'-DDE. The absence of 4,4'-DDT was expected since the spike study showed that the 4,4'-DDT would degrade to its complementary degradate 4,4'-DDE in the presence of basic alumina and high extraction temperature. This should have resulted in recoveries greater than 100% for 4,4'-DDE. The lower recoveries, however, of 4,4'-DDE, cis-chlordane, and trans-nonachlor seem to indicate that the SFE method was not exhaustive for these compounds. On the other hand, high recoveries were obtained for the DDD derivatives and 2,4'-DDT. In relation to the spike study, the recovery of 4,4'-DDD should have been much lower. A review of the chromatographic separation at the most concentrated standard showed that the retention time of 4,4'-DDD peak (46.83 min.) was not completely resolved from the 2,4'-DDT peak (47.03 min.). **Figure 18** depicts an extracted ion chromatogram for the DDT analogues with the relative positions of the 4,4'-DDD and 2,4'-DDT. The large recovery of 2,4'-DDT may have influenced the quantitation of 4,4'-DDD and thus affected the measured concentration of the 4,4'-DDD. The high degree of variability exemplified by the percent RSDs was due to the non-quantitative recoveries of the OCPs as well as the low concentrations of the OCPs. The concentrations were below 15 ng/g for all monitored OCPs with the exception of 4,4'-DDE (37.1 ng/g) and 4,4'-DDD (41.5 ng/g). In the case of 4,4'-DDE and 4,4'-DDD poor reproducibility may have been due to the varying degrees of analyte degradation which occurred during the extraction.

The results from a paired t-test showed that there were significant differences

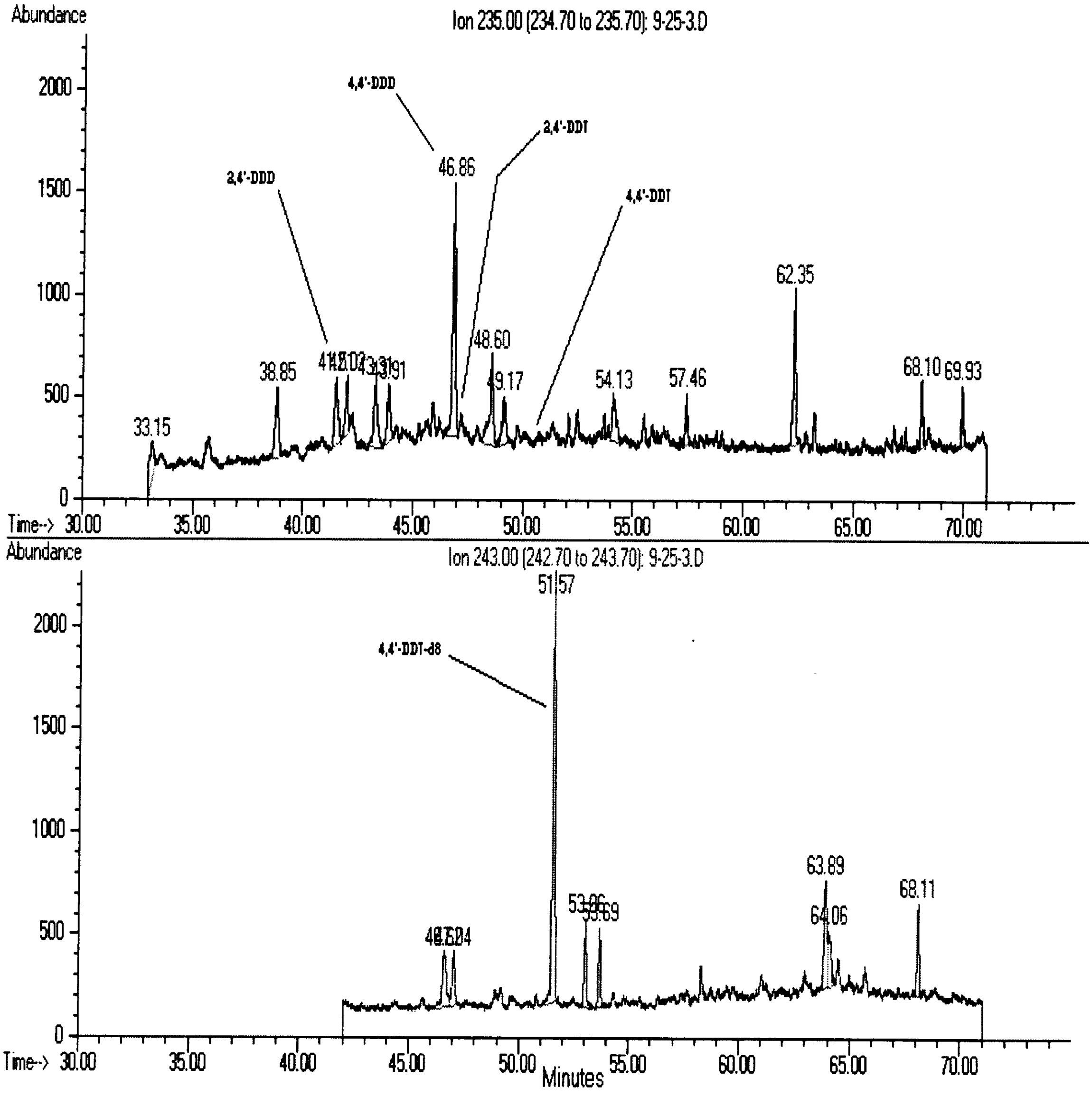


Figure 18: GC/MS extracted ion chromatogram of DDT and its analogues

between the SFE and Soxhlet extraction methods for two of the OCPs. A t-critical at $P=0.05$ is 4.30 which means that if the t-calculated value is greater than 4.30 then the recoveries were deemed to be significantly different. The results are shown in **Figure 19** and one can see that there were no significant differences between the two extraction techniques for 4,4'-DDE, 2,4'-DDD, 4,4'-DDD, and 2,4'-DDT. The highest significant differences were seen for c-Chlordane and t-Nonachlor. Their respective t-calculated values (6.8 and 7.4) were higher than the t-critical value of 4.30. Overall, inferences as to the effectiveness of SFE for the extraction of the DDT analogues cannot be made due to the degradation effects promoted by alumina and the high extraction temperature. As for the recovery c-Chlordane and t-Nonachlor the SFE method proved to be inexhaustive and highly variable due to their low recoveries and concentrations.

(4) SFE of PCBs and OCPs from freeze dried mussel tissue (SRM 2974) with CHF_3

Next, SFE extractions using fluoroform (CHF_3) as the supercritical fluid were performed. The properties of fluoroform as a supercritical fluid has been discussed in the previous chapter. It has been reported that co-extracted fat or lipid material is significantly reduced using this particular fluid as opposed to CO_2 (70). The study showed that there was a 100 fold decrease in the amount of fat extracted when using fluoroform instead of carbon dioxide. The use of this fluid would result in the elimination of activated alumina in the extraction cell. Replicate extractions ($n = 3$) with fluoroform were performed at significantly higher reduced density than CO_2. The GC/MS chromatogram shown in **Figure 20** is similar to those obtained from extracts

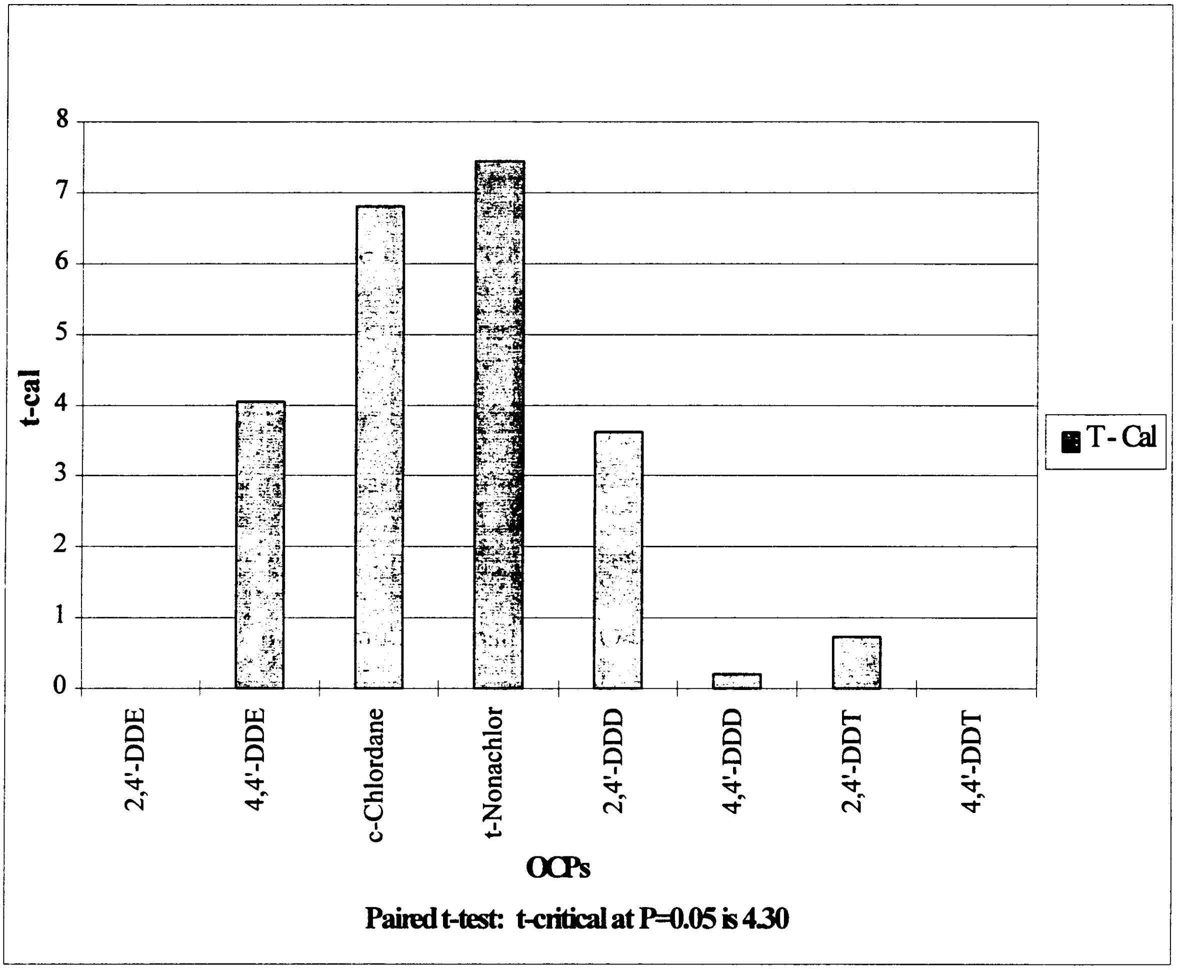


Figure 19: Paired t-test results comparing OCP recoveries obtained by SFE and Soxhlet

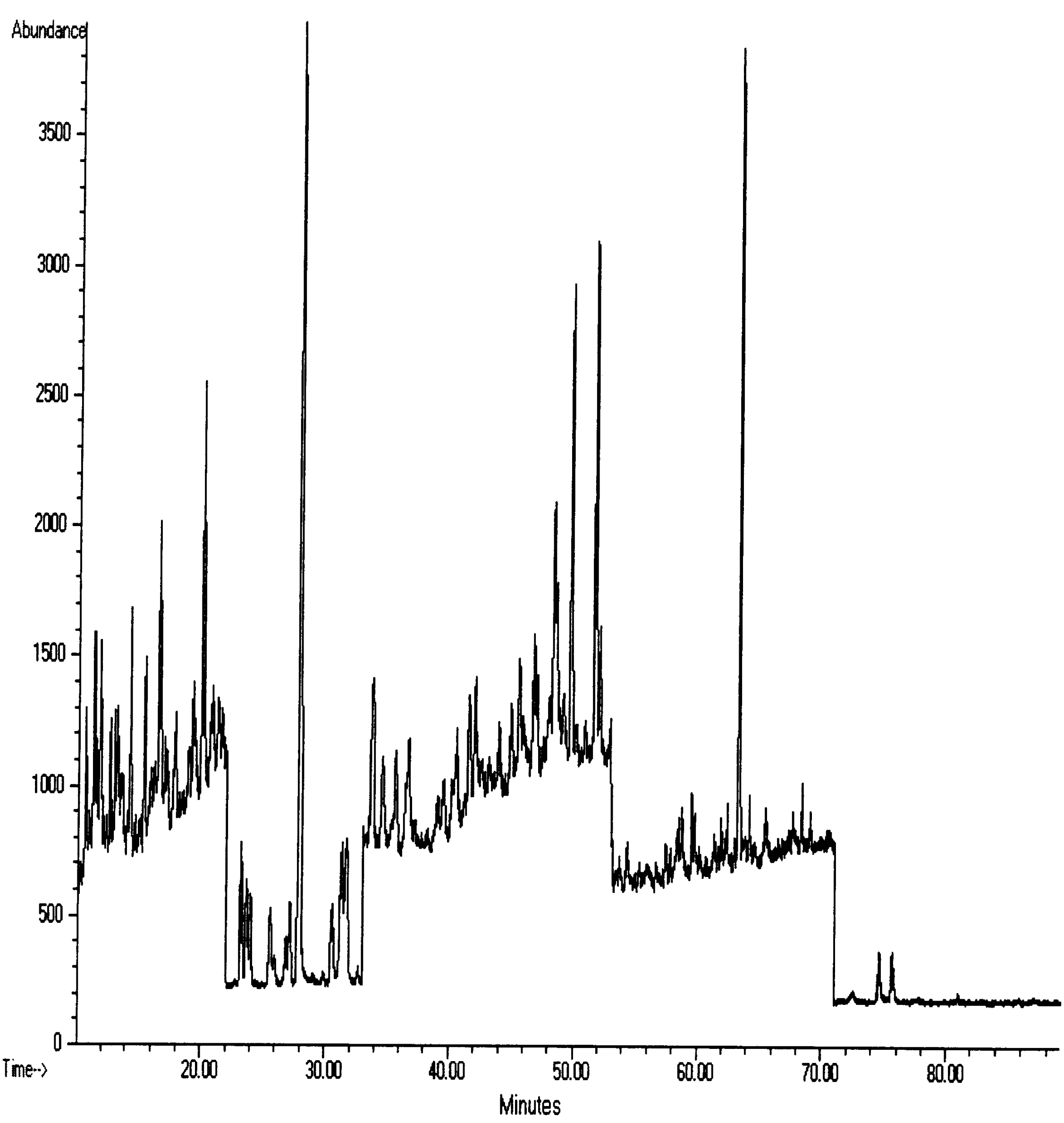
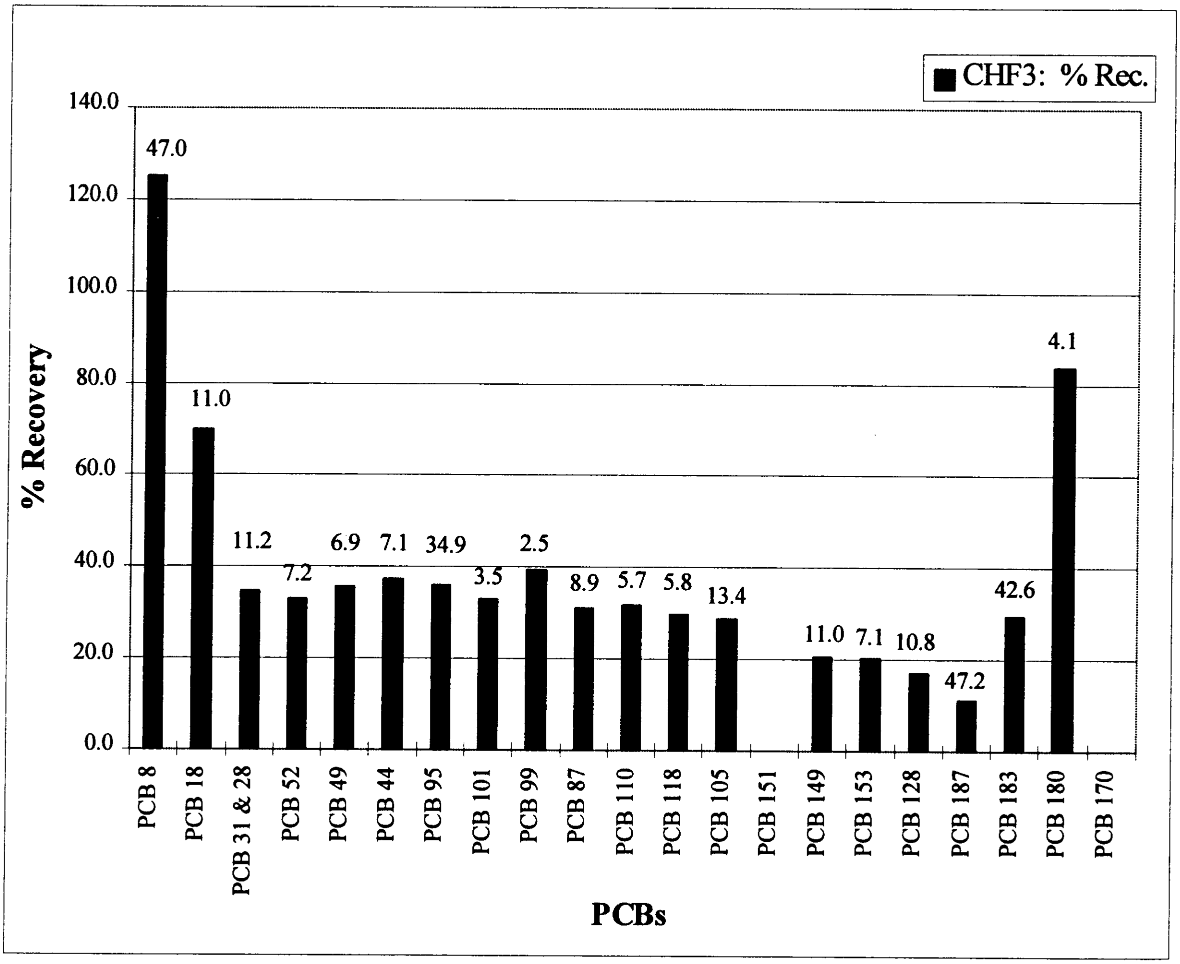


Figure 20: Total ion chromatogram of PCBs and OCPs extracted from SRM 2974 with SFE method #8 using fluoroform (CHF_3)

using supercritical CO_2 except for the peaks being smaller due to lower recoveries of the target analytes. Other than this the chromatogram showed that fluoroform selectively extracted some target analytes without any significant co-extracted lipid material. Next, it was assumed that a higher solvating power was used with the fluoroform extractions. However, the results (**Figure 21**) indicate that fluoroform was a poorer extraction medium than carbon dioxide for all PCBs. Overall the percent recoveries were significantly lower and exhibited higher variability than values obtained with supercritical CO_2 due to their low extracted concentrations. Two PCBs (PCB 151 and PCB 170) were not even detected by the mass selective detector. Upon review of the solubility isotherms for CO_2 and CHF_3 it was determined that even though the SF density of fluoroform was higher than SF density of CO_2, the solvating power of this fluid was much lower than carbon dioxide. As shown in **Figure 22** the solubility of supercritical fluoroform is significantly lower than supercritical carbon dioxide at the extracted temperature of 97 °C and a pressure of 378 bar. The solvating power of fluoroform is only greater than carbon dioxide at the lower pressures. Therefore, at lower pressures (below 261 bar), fluoroform is a better extraction medium than CO_2 and at higher pressure (higher than 261 bar) the opposite is true, with the CO_2 becoming the better extraction medium. Alternatively, the lipid material may have to be extracted from the tissue matrix before the PCBs can be removed. In other words, PCBs may favor the lipid matrix over the polar fluoroform phase which means that with insufficient supply of fluoroform, the bulk of the PCBs may remain with the fat.



Density of CHF_3	0.909 g/mL
Extraction Temperature	97 °C
Static Extraction Time	20 minutes
Dynamic Extraction Time	49 minutes
Liquid CO_2 Flow Rate	1 mL/min
ODS Trap Temperature	-10 °C
Trap Rinse (70 °C)	Isooctane (3 x 1.5 mL)

Figure 21: SFE percent recovery and percent RSDs (above bar) of PCBs from replicate extractions (n = 3) using supercritical fluoroform (CHF_3)

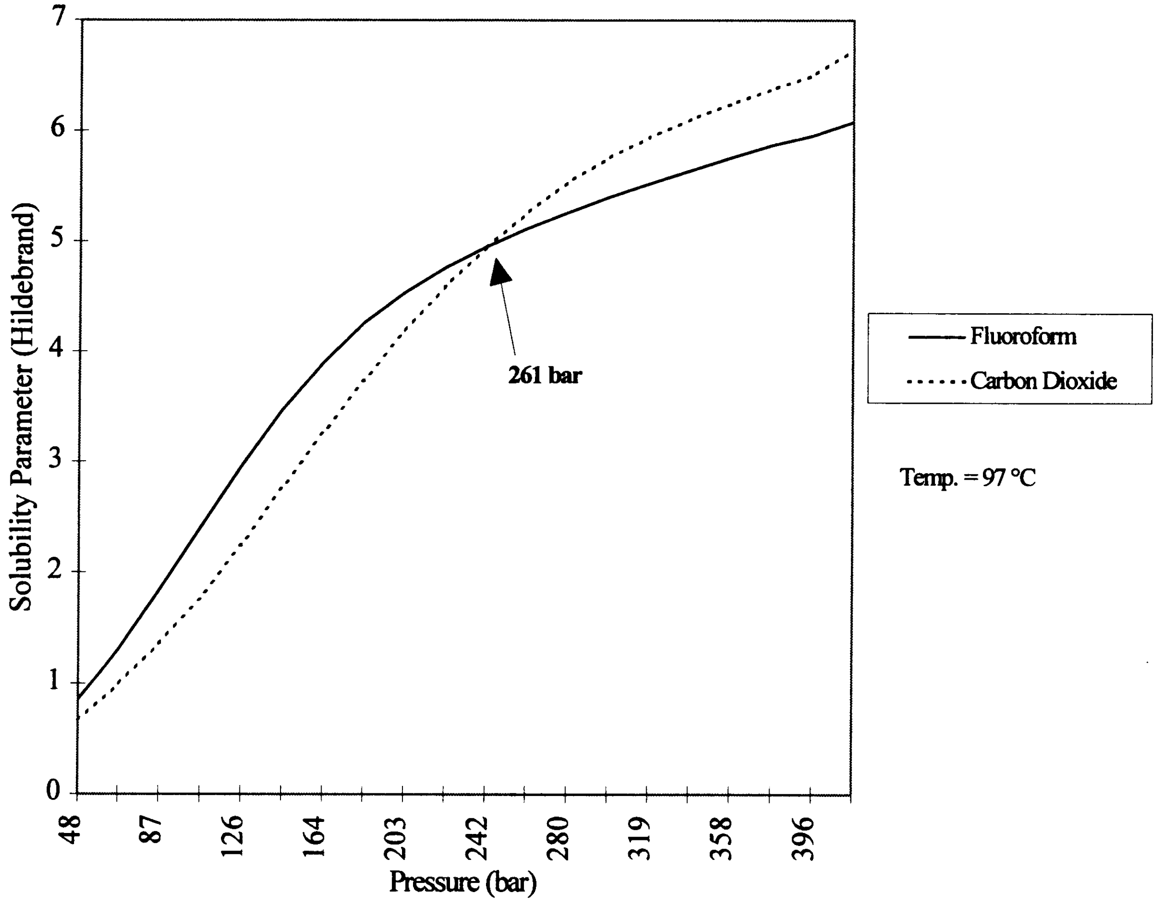
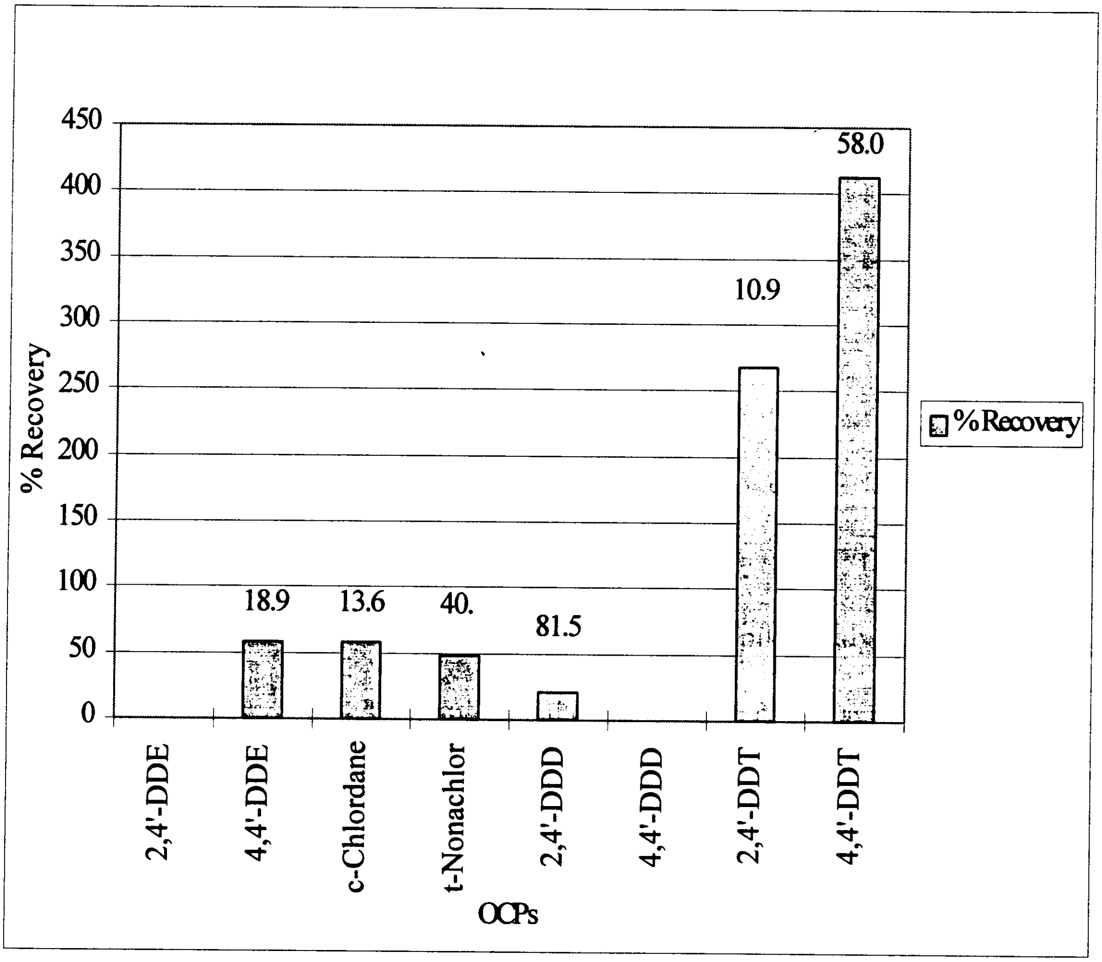


Figure 22: Solubility parameter versus pressure for CO_2 and CHF_3

* Reference 71

This trend of low recoveries was also seen with OCPs (**Figure 23**) except for 2,4'-DDT and 4,4'-DDT, percent recoveries of 20% to over 400% was observed with %RSDs ranging from 10.9% to 58.0%. Again the recoveries of 4,4'-DDE, cis-chlordane, trans-nonachlor, and 2,4'-DDD with fluoroform was lower than carbon dioxide due to the lower solvating power of CHF_3. A slightly lower recovery of 4,4'-DDE was obtained due to the lack of degradation of 4,4'-DDT to 4,4'-DDE due to the absence of alumina in the extraction vessel. This resulted in the recovery of 4,4'-DDT to exceed 400%. According to the replicate extractions with CO_2, a lower recovery of 2,4'-DDT should have been observed due to the poorer solvating power of fluoroform than CO_2 at the extraction pressure. However, the recovery of 2,4'-DDD was lower with fluoroform which may be due to the lack of degradation of 2,4'-DDT to 2,4'-DDD. This is probably the most probable reason since the recovery of 2,4'-DDD was 151.5 % and 112.3 % for 2,4'-DDT with CO_2 and approximately 20.6 % and 267.7 % respectively with fluoroform. The difference in total additive recovery for these two OCPs are only 34.5 % and are within experimental error. This reinforces the idea of degradation caused by basic alumina and high extraction temperature to degrade various types of OCPs. The absence of recovery for 4,4'-DDD was not expected since the spike study and replicate extractions with CO_2 showed significant recoveries. Relatively high recoveries of 4,4'-DDD should have been observed since degradation of 4,4'-DDD to DDMU was eliminated by the absence of basic alumina with fluoroform. The details of degradation for the DDT analogues was discussed in the spike study section. The absence of DDD with fluoroform cannot be



Density of CHF_3	0.909 g/mL
Extraction Temperature	97 °C
Static Extraction Time	20 minutes
Dynamic Extraction Time	49 minutes
Liquid CO_2 Flow Rate	1 mL/min
ODS Trap Temperature	-10 °C
Trap Rinse (70 °C)	Isooctane (3 x 1.5 mL)

Figure 23: SFE percent recovery and percent RSDs (above bar) of OCPs from replicate extractions (n = 3) using supercritical fluoroform (CHF_3)

concretely explained. However, speculation into the large recovery of 4,4'-DDT may be correlated with the recovery of 4,4'-DDD. The recovery of 4,4'-DDD may be dependent upon some degradation of 4,4'-DDT. Under the right conditions 4,4'-DDT can undergo transformation to 4,4'-DDD. A combination of poor solvating power and the lack of degradation of 4,4'-DDT may have attributed to the absence of 4,4'-DDD in the fluoroform extractions. Again, exhaustive extraction of lipophilic compounds like PCBs and OCPs may require the total extraction of the lipid material from the tissue matrix.

D. Conclusions:

In conclusion, the spike PCB study showed no interaction of the PCBs with the activated basic alumina in the extraction cell during SFE with CO_2. However, the spike study with OCPs demonstrated the degradation effect of activated basic alumina towards 4,4'-DDT and 4,4'-DDD. Higher recoveries of PCBs from mussel tissue were obtained with the longer static extraction times regardless of the density of the supercritical carbon dioxide. Furthermore, higher recoveries were obtained at higher densities even at the sacrifice of higher temperature with the benefit of decreased amounts of co-extractives from the mussel tissue and the activated alumina. Replicate extractions with CO_2 showed the effectiveness of SFE to quantitatively extract PCBs from the freeze dried mussel tissue. The results also indicate that recoveries obtain via SFE were similar to those obtained by more conventional techniques like Soxhlet. In addition, the use of activated alumina to perform on-line extract purification during SFE was effective in eliminating lipid co-extractives from the final extract solution. This eliminated the need to perform laborious and time consuming lipid clean-up procedures which have plagued conventional extraction techniques.

On the other hand, the extraction of OCPs proved to be non-quantitative and highly variable caused by the basic alumina in the extraction vessel. Extractions performed with CO_2 showed evidence of degradation of 4,4'-DDT caused by the alumina in the extraction cell and the high extraction temperature. Over 100 % recoveries were obtained for 2,4'-DDD which may be due to the degradation of 2,4'-DDT. However, the data for 4,4'-DDD contradicted the results obtained in the spike study. A low recovery for 4,4'-DDD was not obtained and the actual concentration of 4,4'-DDD may have been influenced by the large recovery of 2,4'-DDT during quantitation of the peaks.

Next, recoveries obtained with fluoroform provided further evidence for the degradation caused by the alumina in the extraction vessel. Slightly lower recoveries than CO_2 were obtained for several OCPs. The results also seem to indicate a relationship between 2,4'-DDD and 2,4'-DDT in terms of recovery and degradation. The lack of 4,4'-DDD in the extract was unexpected and seems to indicate that the recovery of this OCP may be related to the degradation of 4,4'-DDT. Overall, the recoveries were significantly lower in comparison to Soxhlet values.

The study with fluoroform demonstrated the applicability of this alternative fluid to extract PCBs and OCPs without the need to add any adsorbents in the extraction vessel to retain the lipid material. The high recovery of 4,4'-DDT in the absence of basic alumina during SFE proved that the degradation of 4,4'-DDT was caused by the presence of the basic alumina. Although, percent recoveries for all PCBs and most OCPs were lower in comparison to those obtained using CO_2, which was attributed to the lower solvating power of fluoroform than CO_2 at the extraction pressure, but further study is needed to concretely determine the efficacy of fluoroform.

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