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BIOSORPTION OF 1,2,3-TRICHLOROPROPANE AND TRICHLOROETHYLENE BY THE DIATOM THALASSIOSIRA PSEUDONANA

DISSERTATION
Presented in Partial Fulfillment of the Requirements for
the Degree Doctor of Philosophy in the Graduate School of The Ohio State University
By
Bruce William Berdanier, B.S.C.E., M.S.C.E.

The Ohio State University
1995

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I express my sincere thanks to Dr. Robert M. Sykes for the freedom he gave me to pursue my degree objectives and research efforts. Additional thanks go to Dr. Scott Bair for sharing the concepts of hydrogeology to augment and expand my environmental education. Gratitude is expressed to Dr. Robert Stiefel for his support and encouragement in the use of the laboratory facilities at the Water Resources Center. To my wife Melinda, and to my children, Aaron and Reid, I offer my highest thanks and praise for your patience, support, and understanding as I uprooted and disrupted your lives in the pursuit of my dreams for the past six years. I also wish to acknowledge the encouragement and insight provided by Dr. Tom White, Dr. Dan Pardieck, and James Hadley of Ciba-Geigy while I was formulating my initial research proposal.
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CHAPTER I
INTRODUCTION

Goals and Objectives

This study's objective was to determine the potential for algal sorption of 1,2,3-trichloropropane (TCP) and trichloroethylene (TCE) by a specific type of diatom, *Thalassiosira pseudonana*. A number of investigations (5, 6, 10, 12, 15, 17, 19, 22, 23) into the ability of green and blue-green algae to bio-accumulate organic chemicals were found. Most of these studies have focused on bioaccumulation in algae for the purpose of predicting the effects that the concentration of organic chemicals in the food chain at this low level could have on higher trophic level organisms such as fish, birds, and mammals. Some of the studies have shown concentrations in the algae over 1000 times the concentration in the water for certain types of organic compounds.

No reports of studies to quantify the sorption of TCP on any type of algae were found in the literature. One would expect smaller molecules with higher octanol/water coefficients to quickly and effectively sorb into cell lipids. Because TCE and TCP are both small, uncharged molecules, they might be able to penetrate into algal cells and be sorbed by lipids. One would also expect the bioconcentration factor (BCF) to demonstrate a dependency on the initial aqueous concentrations of TCE and TCP. Also, the only work done to
date on the sorption of TCE on algae (6) has been on green algal types, which has provided absolutely no insight into the sorption potential of TCE or TCP on diatoms, which are the dominant fresh water algae in the springtime.

Some studies have investigated the potential of designing unit operations of wastewater treatment around algae as the suspended or fixed growth media (13, 16, 18, 21, 22, 23). Theoretical and laboratory predictions of specific industrial chemical or general nutrient removal have been very promising for these systems. These studies have shown a fair amount of success in the bench-scale units, but have never performed well in pilot or field-scale facilities.

Very little attention has been given to the evaluation of diatoms in past studies. One study (24) was found that evaluated the bioaccumulation of DDT by fresh water diatoms, and another study (25) evaluated the biosorption of PCB by marine diatoms. Only one study (22) was located that compared the sorption of dieldrin by green algae and both living and killed diatoms. A major part of the reason for lack of studies concerning fresh water diatoms can be linked to the difficulty in culturing them in a laboratory setting. A great deal of time, equipment, and laboratory space has to be devoted to the single operation of developing a culture of the diatoms of significant enough concentration to conduct the experiments. On the other hand, several types of green algae cultures are readily available from most university collections and are rather easy to culture in the laboratory.

I conducted bench-scale experiments at Ohio State University to determine the bioconcentration factor for TCP and TCE on a collection of diatoms that were representative of the diatoms occurring in an upground reservoir during different seasons of the year. The diatoms were purchased in a
preserved state and were diluted to the desired cell concentration for each experiment. Although the mechanisms involved in the transport and uptake of the chemicals still remain to be determined, and further investigations need to be undertaken to quantify the reason for the differences in the sorptive capabilities of live and killed diatoms, studies (22, 27) have indicated that sorption is on the same order of magnitude for live or killed diatoms. Also, the fact that the diatoms were killed gave this study control over the actual concentration and cell count throughout each experiment, which investigators dealing with live cultures have not had.

A secondary, but important, objective of this study was to develop a comparatively simple methodology for the identification and quantification of chlorinated hydrocarbons in both raw and finished water supplies. Chlorinated compounds have become a growing concern as an ever increasing number of chemical contamination situations have been identified in even moderately developed and populated areas. Chlorinated compounds have been used successfully and ubiquitously for industrial, municipal, agricultural, and residential applications throughout our country. The U.S. Environmental Protection Agency (EPA) has promulgated regulations on an ongoing basis to set requirements for the identification and quantification of organic chemicals in our water supplies. Most cities, villages, and industries have met the testing and reporting requirements either by developing qualified laboratories in-house and personnel on staff, or by contracting with commercial laboratory concerns to perform the testing and report preparations. Smaller cities and villages which cannot afford a sophisticated laboratory or laboratory staff need to be able to test for chlorinated compounds on an ongoing basis with a minimal staff at a moderate training level.
Motivation for the Biosorption Study

Volatilization and biosorption (bioaccumulation) have been identified as the two principal processes affecting TCP and TCE in water supply reservoirs. The literature (42,44) and simple calculations have indicated that photolysis, hydrolysis, and biodegradation do not cause TCP and TCE degradation at significant rates (62, 65).

A modeling study that I participated in outside of the work done in this laboratory investigation simulated TCP and TCE concentration versus time profiles at a water intake in a water supply reservoir (62). The model was based on VOC concentrations at the intake, pumping rate records, temperature profiles, and other data. The model results indicated that either volatilization or biosorption in combination with vertical reservoir hydrodynamics, could account for TCP and TCE concentration versus time profiles (62, 66). The modeling report recommended that laboratory analyses of the volatilization and biosorption processes, along with field measurements of algal biomass should be completed to quantify the relative importance of the two processes.

Bench-scale experiments to quantify volatilization were reported by Hadley and Pardieck (64). The findings of the volatilization study indicated that the level of biosorption occurring in a drinking water reservoir needed to be considered along with the concentration of algae living in the water in any model for operation or treatment of a reservoir contaminated with organic compounds. Biosorption might confound reservoir management efforts aimed at enhancing volatilization of organic chemicals such as TCP and TCE. Alternatively, biosorption could be used or enhanced to reduce the scale of
efforts necessary to enhance volatilization. Also, although volatilization is currently considered more reliable and easier to facilitate for the removal of VOC's, it may not be the method of choice at some future date. Environmental regulations for controlling the release of VOC's from treatment processes have increased, and biosorption could be enhanced as an alternative removal process. Bench-scale evaluation of biosorption should be carried out so that this process could be included in any reservoir management benefit-cost analysis.

If the BCF's for specific organic chemicals were known for algal populations occurring in a given water supply reservoir, field measurements could be taken to quantify the concentration and distribution of algal populations and organic chemicals throughout the period of a year. A model combining these results could allow prediction of TCP and TCE biosorption in an upground reservoir over the course of the year. Biosorption could then be quantitatively included, along with volatilization and hydrodynamics in a model designed to simulate the effect of reservoir management schemes. To make such predictive models a reality, further studies need to be completed both in the laboratory and in field conditions to understand the processes controlling the biosorption phenomenon in algae at different levels of algal and organic chemical concentration.

There is also a need for the operators of municipal water and wastewater treatment plants in smaller cities and villages to be able to test for the presence of chlorinated hydrocarbons in their raw and finished water regardless of whether they originated from rivers, upground reservoirs, natural lakes and quarries, or wells. Many cities, villages, and small industrial concerns have a laboratory staff which is fairly well versed in the routine water quality
parameters. However, the vast majority of these personnel have had no technical training in the theory or practical operation of a gas chromatograph system that could be used for the identification and quantification of different organic chemicals in their water supply. The result of this situation is that most smaller size cities and villages (<10,000 population) are using commercial labs to meet the annual monitoring required by the EPA for their water supplies.

Performing laboratory analyses to meet the requirements of the national or state regulations may not provide ongoing protection or evaluation of system performance. Especially for municipalities that are using surface water supplies, there is a definite need for ongoing monitoring of organic chemicals. Public water supply systems that pump directly out of rivers or streams into their treatment plants are periodically subject to the stormwater runoff from streets and industries and to non-point source pollution from agricultural runoff. Systems that use the water from natural lakes or manmade reservoirs may be less subject to the concerns of non-point source pollution from runoff. However, they are more susceptible to organic chemical input from groundwater that was contaminated from past practices of waste disposal. In addition, reservoirs and lakes are affected by temperature stratification and mixing at different seasons of the year, which, when combined with the input mixture that each individual system has received, determine the final organic chemical content of the raw water coming into any municipality's treatment plant.

There is also a need for monitoring the chlorinated compounds that result from the treatment plant's own operations in producing the finished water. These compounds will vary in composition and concentration depending on both the raw water conditions and the unit operations at each individual treatment plant.
Background discussion of TCP and TCE

TCP is a chemical component in paint and varnish removers, and is used as a solvent and degreasing agent. It also has shown up in groundwater pollution studies as an impurity resulting from the manufacture of an agricultural fumigant that was used from the early 1940's through the late 1970's. One main area of use was by the pineapple growers in the Hawaiian Islands. A product of the Shell Chemical Company named DD (a combination of dichloropropane and dichloropropene) was found to range in TCP content from 0.4% to 7%, and was used alone as a soil fumigant or in combination with other chemicals such as ethylenedibromide and dibromochloropropane. It is now believed that products contaminated with TCP were applied liberally for a thirty year period in Oahu by Dole, Libby, and Del Monte (11).

TCP has also been found more recently in the groundwaters of California (11) and in several other sites in North America (77). TCP is known to be a severe health risk to humans and is listed as carcinogenic and mutagenic on its material data sheet. The compound is distinguished by the fact that it is readily absorbed through the skin and targets organs such as the liver, kidney, and pancreas. The EPA is in the process of promulgating a maximum contaminant level (MCL) for drinking water supplies, which is expected to be released after 1995. The MCL is anticipated to be in the 50-100 part per trillion range (64). Physicochemical characteristics of TCP are listed in Table 1.

TCE is a chlorinated aliphatic compound that has been used widely as an industrial degreasing agent for many years. It has occurred frequently in
groundwater contamination situations and is one of the most commonly detected xenobiotic compounds in all types of water (5). The occurrence of TCE has been a direct result of industrial pollution and as a byproduct of the breakdown of another chemical such as tetrachloroethylene (PCE). Studies have shown no reactivity by algae toward TCE (5). Although one study has investigated the biosorption of TCE by green algae (5), the vast majority of studies to date have concentrated on the sorption of pesticides and PCB's (26, 25, 24, 23, 22, 19, 17, 12, 10). Physicochemical characteristics for TCE are listed in Table 1.

Background discussion of *Thalassiosira pseudonana*

*Thalassiosira pseudonana* (Hasle and Heimdal, 1970) is a centric marine diatom with an average diameter of 5-10 μm (53) (see Figure 1). The silicon content of *T. pseudonana* has been measured at 0.6 -1.5x10^{-12} g Si/cell (79). Studies of the lipid composition of diatoms have concentrated on the fatty acid content. Almost 80% of the fatty acids of *Thalassiosira* are saturated or have only one double bond (77% are C_{16}, 8% are C_{14}, and 9% are C_{20}) (53). There are no consistent differences noted in any studies in the literature between the lipid (fatty acid) composition of freshwater and saltwater diatoms. Werner notes that differences as great as those between species are found among studies from different laboratories.
Summary of Objectives

In summary, the objectives of this study were:

-Determination of the potential for sorption of TCP and TCE on a specific diatom, *Thalassiosira pseudonana*, by developing isotherms and bioconcentration regression relationships from laboratory data, and

-Development of a simple and efficient methodology for the identification and quantification of chlorinated organics in raw and finished water using capillary column gas chromatography technology and a mass balance approach.
<table>
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<th>MW</th>
<th>$K_{ow}$</th>
<th>H</th>
<th>Mp</th>
<th>Bp</th>
<th>SG</th>
<th>Pvap</th>
<th>Sol.</th>
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<tr>
<td></td>
<td>°C</td>
<td>°C</td>
<td></td>
<td>°C</td>
<td>°C</td>
<td></td>
<td>(atm)</td>
<td>mg/L</td>
<td></td>
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<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>1,2,3-Trichloropropane</td>
<td>147.4</td>
<td>102</td>
<td>0.009</td>
<td>-14.7</td>
<td>156</td>
<td>1.39</td>
<td>0.0045</td>
<td>1900</td>
<td>CH$_2$ClCICICH$_2$Cl</td>
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<td></td>
<td>(3)</td>
<td>(72)</td>
<td>(8)</td>
<td>(80)</td>
<td>(11)</td>
<td>(11)</td>
<td>(11)</td>
<td>(3)</td>
<td></td>
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<tr>
<td>Trichloroethylene</td>
<td>131.4</td>
<td>204</td>
<td>0.3</td>
<td>-85</td>
<td>88</td>
<td>1.46</td>
<td>0.0704</td>
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<td>(4)</td>
<td>(4)</td>
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<td>(4)</td>
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(A)= Aldrich Chemicals, H= Dimensionless Henry’s Law Constant, Mp= Melting point, Bp= Boiling Point, SG= Specific Gravity, Pvap= Vapor Pressure, Sol.= Solubility, (3)= Reference Sited, MW= Molecular Weight, $K_{ow}$= Octanol Water Partition Coefficient
E. Paasche, 1973

Lipid Content: 25%, Werner

Weight: $0.5 \times 10^{-11}$ g/cell, Berdanier

Diameter: 5-10 um, Paasche

Figure 1

**THALASSIOSIRA PSEUDONANA**
CHAPTER II
LITERATURE REVIEW

Relationship to Work of Other Investigators

Basic Concepts

A solute's bioconcentration factor (BCF) is defined as the ratio of its equilibrium biological concentration \( C_{\text{bio}} \) to its equilibrium aqueous concentration \( C_{\text{aq}} \) as follows:

\[
BCF = \frac{C_{\text{bio}}(\text{equilibrium})}{C_{\text{aq}}(\text{equilibrium})} = \frac{(\text{Mass Solute Sorbed/ Mass of Cells})}{(\text{Mass Aqueous Solute/ Volume of Water})}
\] (1)

where: Mass Aqueous Solute = (Mass Aqueous Solute Initial - Mass Solute Sorbed)

Different investigators have used various combinations of units for the BCF, which makes it impossible to compare regression results without first verifying the system of units used to develop the BCF. For the remainder of this dissertation the units used for the BCF will be the following:

\[
\frac{\text{ug, Mass Solute Sorbed}}{\text{g, Mass of Cells}} = \frac{L}{g}
\] (2)
Results from other studies presented in this report have been converted to this system of units.

Baughman and Paris (27) discussed that the bioaccumulation coefficient was simply the ratio of the equilibrium concentration in the cell to the equilibrium concentration in the medium. If the metabolism of the solute was negligible, this simple concept provided the mathematical definition of bioaccumulation and should be measurable in sorption experiments:

\[
BCF = \frac{\left[ P \right]_{\text{cell}}}{\left[ P \right]_{\text{aq}}} = \frac{Q}{C} \tag{3}
\]

The Freundlich isotherm has also been used in considerable research over the years especially in microbial accumulation studies:

\[
Q = K_f C^n \tag{4}
\]

When the value of \( n \) becomes unity, then \( K_f = BCF \). The value of \( n \) may be related to our current inability in these experiments to distinguish between adsorption and absorption. A simple manner of performing a linear regression on the isotherm data by plotting the log value of the equilibrium sorption (\( Q \)) versus the log value of the equilibrium aqueous concentration (\( C \)) is provided by the Freundlich isotherm. The BCF regression equation is also easily derived from the resultant Freundlich isotherm equation since \( \log BCF = \log Q - \log C \). The method outlined above has been used to develop the regression relationship for the BCF during this study.
Throughout this report, the concept of biosorption will be discussed as the disappearance of the subject chemical from the aqueous phase. The studies that have been done to date, including the results of this laboratory investigation, have not been able to distinguish between the different transfer mechanisms that may be occurring. The two major mechanisms to be considered are transport into the cell and adsorption onto the cell surface. A cell surface is a lipid bilayer, and the lipid content of the cell is hypothesized to play a major role in determining the partitioning of hydrophobic organic chemicals between the aqueous phase and the biomass.

Lipids in the cell envelope behave like a lipid micelle with a hydrophilic exterior and a hydrophobic interior (61). A small organic molecule with a high enough octanol-water partition coefficient ($K_{ow}$, indicative of the chemical's solubility in lipids) could penetrate the cell membrane and partition into the lipids. Amy (61) also hypothesizes that uncharged organic compounds with an apparent molecular weight (AMW) less than 600 g could be transported through the cell membrane into the cell regardless of the compound's $K_{ow}$. This hypothesis is also supported by additional studies which have indicated that the level of biosorption is comparable for live or dead biomass, suggesting that passive diffusion into the cell is more important than an active transport mechanism. Amy's study used an ultrafiltration membrane to separate compounds from a kraft mill effluent with an AMW less than 1000 g. A sorption study that was run comparing the sorption of the separated compounds to the sorption potential of the total effluent produced isotherms that showed that lower AMW compounds more effectively partitioned into the cell lipids.
Amy's sorption isotherms were based on a measure of total organic halides (TOX) in a control sample as compared to a filtered sample. No indication is given that any consideration was given to the sorption occurring on the system glassware or on the filters (which may have been very hydrophobic) during the experiment.

**Isotherm and Regression Studies**

Smets and Rittman (5) conducted biosorption studies for 1,1,2-trichloroethylene on three different green algae types (*Scenedesmus quadricauda, Selenastrum capricornutum, and Chlorella vulgaris*) from an algal culture collection at the University of Texas at Austin, and the TCE was obtained as a uniformly $^{14}$C-labeled compound. The aqueous phase equilibrium concentration of TCE was determined in a scintillation counter. The dry weight of the biomass was determined by filtering and drying the glass filter at 104°C overnight. Total solids concentration of the biomass ranged from 0.1 g/L to 0.63 g/L in this study.

The samples were incubated in a reciprocal shaker for two hours and then incubated in an upright position for an additional two hours allowing the biomass to settle before analysis of the aqueous phase. This permitted the laboratory personnel to sample the supernatant without disturbing the settled biomass and precluded the need for centrifugation of the samples since Smets and Rittman did not intend to try to measure the TCE sorbed on the pellet. Control samples of TCE in distilled water also were analyzed to correct for volatilization and non-biological adsorption on the vial walls.
Sorption was determined to follow non-linear Freundlich isotherms by regressing the data on a log-log plot of equilibrium sorption versus equilibrium supernatant concentration. The resultant BCF's from this analysis range from 1.4 (TCE equilibrium concentration of 1 µg/L) to 5.37 (TCE equilibrium concentration of 1 mg/L). Resultant BCF regression equations are presented in Table 2). The BCF's determined were about an order of magnitude greater than those normally predicted for sediment-soil systems (68, 69, 70, 71, 72, 73, 74, 75) or bacteria dominated systems (27, 76). The range of BCF's did include the values which would have been calculated for TCE based on the analyses by Casserly et al. (BCF= 2.6) (6). However, the value predicted by Mailhot's study (BCF= 0.84) (26) did not fit into the data range. The greater sorption was attributed to the greater lipid content of the algae and/or to a different distribution of lipid containing molecules in the algae.

The advantages of Smets and Rittman's experimental method were:

- it allowed for quantification of the equilibrium sorption while requiring only two measurements (that of the supernatant equilibrium concentration and a control concentration),

- it did not invoke the technologically complicated methods and high monetary expenses involved in use of gas chromatographic analyses,

- it provided for indirect quantification of the extent of sorption onto the system components, and

- its simplicity (only two measurements required, and no separations or extractions) limited the sample handling time and therefore reduced the losses due to volatilization.
**Table 2**

**COMPARISON OF RESULTS OF RECORD**

<table>
<thead>
<tr>
<th>ALGAL TYPE</th>
<th>REGRESSION EQUATION</th>
<th>PREDICTED BCF</th>
<th>REFERENCE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Selenastrum</td>
<td>$\text{Log BCF} = 0.46 \text{Log } K_{ow} - 0.64$</td>
<td>TCP=1.53</td>
<td>TCE=2.06</td>
</tr>
<tr>
<td></td>
<td>$R^2=0.83$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Selenastrum</td>
<td>$\text{Log BCF} = 0.36 \text{Log } K_{ow} - 0.90$</td>
<td>TCP=0.67</td>
<td>TCE=0.85</td>
</tr>
<tr>
<td></td>
<td>$R^2=0.91$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chlorella</td>
<td>$\text{Log BCF} = 0.145 + 0.195\text{LogC}$</td>
<td>$1.4&lt;\text{BCF}&lt;5.37^*$</td>
<td>TCE</td>
</tr>
<tr>
<td></td>
<td>$R^2=0.98$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Scenedesmus</td>
<td>$\text{Log BCF} = 0.177 + 0.151\text{LogC}$</td>
<td>$1.51&lt;\text{BCF}&lt;4.27^*$</td>
<td>TCE</td>
</tr>
<tr>
<td></td>
<td>$R^2=0.96$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Selenastrum</td>
<td>$\text{Log BCF} = 0.344 + 0.064\text{LogC}$</td>
<td>$1.5&lt;\text{BCF}&lt;3.44^*$</td>
<td>TCE</td>
</tr>
<tr>
<td></td>
<td>$R^2=0.79$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thalassiosira</td>
<td>$\text{Log BCF} = -0.041 + 0.094\text{LogC}$</td>
<td>$0.91&lt;\text{BCF}&lt;1.74^*$</td>
<td>TCE</td>
</tr>
<tr>
<td></td>
<td>$R^2=0.88$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thalassiosira</td>
<td>$\text{Log BCF} = -0.418 + 0.056\text{LogC}$</td>
<td>$0.38&lt;\text{BCF}&lt;0.56^*$</td>
<td>TCP</td>
</tr>
<tr>
<td></td>
<td>$R^2=0.98$</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*BCEF range represents $1\text{ug/L}<\text{C}<1\text{mg/L}$*
The disadvantages of Smets and Rittman's experimental method were:
- there was no actual measurement made of the equilibrium sorption concentration, or the system sorption concentration, and
- concentration was only measured indirectly through the measurement of radioactivity.

Baughman and Paris (27) have stated that the measurement of radiation is not specific for the compound under study. Also, labeled compounds are not noted for high purity, and therefore even a small amount of impurity with a significant difference in the partition coefficient from the compound of interest could cause a great deal of difference in the experimental results.

Casserly et al. (6) performed a study of the sorption of eight different organic compounds (benzene, toluene, chlorobenzene, 1,2-dichlorobenzene, naphthalene, phenanthrene, di-n-butylphthalate, and pyrene) on the green algae *Selenastrum capricornutum* to determine what was the level of sorption of organics on algae, and also to determine if there was a relationship between sorption and octanol-water partition coefficients. All of the organics chosen by Casserly et al. were benzene ring compounds varying from one to four rings with various functional groups attached. The sorption experiments were conducted for a duration of 24 hours and were performed with each compound individually as well as with all compounds dosed simultaneously. A linear experimental design was devised that allowed the investigators to get duplicate samples for control and total organic concentration at 0-hour and at 24-hour.

The extraction procedure was a variation of the method developed by Henderson et al. (67) using 2 mL of methylene chloride for each 120 mL sample bottle as the solvent for the extraction. This method provided a total extraction of the system (including glassware) for each extraction made. Another sample
was taken at the 24-hour time and centrifuged to separate the supernatant and the pellet. Extractions were then made of the supernatant and the pellet after centrifugation to determine the equilibrium concentrations. Sufficient data were not given in the report to know if the investigators took into account that their extraction procedure would also provide a total system extraction of the supernatant and pellet samples including glassware.

Analyses were performed on a Perkin-Elmer gas chromatograph using a flame ionization detector and a 6 ft. x 1/8 in. stainless steel column with Carbowax 1500 on 80/100 mesh. Sample size was 1 µL; injector temperature was 140°C; detector temperature was 160°C; a column temperature program varying between 60°C and 160°C was used; and the carrier gas was helium at 30 mL/min.

A BCF was calculated for each organic compound, and the log BCF value was plotted against the Log Kow value for each compound. A linear regression was performed through the resultant data points. (Regression equation presented in Table 2.) The results indicated a linear relationship between the base 10 log value of the bioconcentration factor and the base 10 log value of the octanol-water partition coefficient for the organic chemicals used in the study. Also, the base 10 log value of the bioconcentration factors for this experiment on S. capricornutum fell between 3.34 and 4.29 for the compounds dosed individually. These bioconcentration factors are 10 to 100 times those which have been estimated for the bioaccumulation of organics by fish using the same organic chemicals.

The advantages of this experimental method were:
- they actually attempted to measure directly the equilibrium concentrations for the solid and liquid phases,
-they attempted to take enough measurements to enable them to perform a mass balance on the experimental system, and
-their extraction method (at least theoretically) provided a means for quantifying the sorption onto the system glassware.
The disadvantages of this experimental method were:
-the procedure was time consuming. The number of samples needed to produce the data required that a set of samples run at the 24-hour time period are actually extended over a large duration of time depending on the time for the organic peak to elute on the gas chromatograph. This large duration of time is a problem especially for volatile chemical analyses and for artifacts such as additional sorption onto the system, and bacterial metabolization that may be occurring,
-the gas chromatograph system was technologically complex in and of itself to operate,
-the extraction procedure used provided a complete extraction of the system including the glassware, and
-the extraction procedure probably did not provide accurate results for the pellet extraction if for no other reason than the fact that the organic chemical was concentrated on/in the pellet, and none of the organic chemical was in the liquid that the pellet was resuspended in.
Baughman and Paris (27) observed that the general procedure of centrifuging the cells after they have obtained equilibrium concentration and then discarding the supernatant and resuspending the cells in an organic chemical free medium before performing extraction has often been followed to evaluate the potential for desorption or the strength of the sorption. The fallacy in using this procedure to determine the desorption or to try to extract the sorbed
chemical has been that even if there are no kinetic limitations, detectable desorption cannot occur unless a concentration change greater than the experimental error can occur for the biomass and sorbed chemical in question because the fraction of solute in each phase is a function only of the ratio of the masses of the two phases.

Miyazaki and Thorsteinson (24) extracted their equilibrated biomass and liquid mixture three times before filtering, and then extracted the biomass three times. They were still not able to recover the total radioactivity from their labeled DDT, and concluded that some of the DDT or its metabolite(s) were bound intracellularly, or were otherwise not extractable by the solvent system used.

Mailhot (26) investigated several different regression relationships for the algal bioaccumulation of nine different organic compounds based on ten different physicochemical properties of the compounds. Again, as in several of the other reported experiments, Selenastrum capricornutum was the alga of choice. Values were determined from the literature for boiling point, density, melting point, molecular weight, solubility, and octanol/water partition coefficient for each of the compounds studied. Values were calculated for molar volume, parachor, capacity ratio, and connectivity index. The molar volume is the volume occupied by one mole and is numerically equal to the the molecular weight divided by the density. The parachor is a molecular size parameter which corrects the molar volume for surface tension. The capacity ratio of a chromatographic column is the ratio of the difference in the retention time of the solute and the mobile phase to the retention time of the mobile phase. The connectivity index is a measure of the degree of structural branching of the chemical.
The nine compounds studied by Mailhot were benzoic acid, anthracene, atrazine, DDT, benzene hexachloride, chloroform, hexachlorobenzene, hexachlorobiphenyl, and tetrachlorobenzene. These compounds were chosen because all of them could be obtained in a radioactive labeled form, and because they represented a wide range of physicochemical properties and structural types.

Mailhot’s article does not report the duration of the sorption experiments or at what point she considered that equilibrium had been reached except for benzoic acid, chloroform, atrazine, and benzene hexachloride, which were the most hydrophilic of the compounds tested. The uptake times reported for these compounds were very extensive, and the actual uptake measured was very near the detection limit for the scintillation counter procedure. Mailhot reported spiking the algae with these compounds and returning the cultures to the incubator for extended periods of time (atrazine - 28 hours, benzene hexachloride - 44 hours, benzoic acid and chloroform - 6 days). She then filtered four replicate samples of the culture and one of a control sample without algae and used the difference of the average of the replicates and the control as the sorption.

The remainder of the compounds were analyzed by performing a count of total radioactivity and then centrifuging the sample. A count of the supernatant was taken, and then the pellet was resuspended in the supernatant and another count was taken. A count was also taken from a control sample without algae. Pellet activity was calculated by correcting the pellet sample for the activity of the supernatant. Mailhot does not report the concentration of the organic compounds used in the experiments. The biomass concentration was approximately 100,000 cells/mL.
A BCF was calculated for each compound based on the experimental results, and these values were regressed individually against the various physicochemical properties of the compounds. The BCF was best predicted by the octanol-water partition coefficient and the capacity factor (the regression equation for the BCF and the octanol-water partition coefficient for the hydrocarbons only from this study is presented in Table 2). Disregarding the values of BCF for benzoic acid and atrazine which are extremely low, the BCF's calculated in this study ranged from 0.69 for chloroform to 37 for hexachlorobiphenyl.

Mailhot's experiment had the same advantages and disadvantages as the experiment performed by Smets and Rittman (5) for the most part. However, there were some differences. Mailhot attempted to measure, at least indirectly, the activity of the pellet by centrifuging the sample, and then measuring the activity of the supernatant. The pellet was then resuspended by vortexing with the supernatant, and another activity measurement was made to correct for the activity of the supernatant. This was one step further than Smets and Rittman went by measuring only the control and supernatant activities and calculating the pellet activity. This procedure should have been an improvement over that of Smets and Rittman. Although Mailhot measured the control activity, she made no comment on whether she considered or tried to correct for sorption onto the system glassware. Also, Mailhot didn't address losses due to volatilization although she appeared to be operating her samples with a large headspace.

Mailhot's experiment pointed out the difficulties encountered in pursuing this type of analysis with hydrophilic compounds. Very extensive periods of time (up to 6 days) were required to get sorption of some of the chemicals used, and
still the results for benzoic acid and atrazine appeared to be so low as to be suspect for validity. Also, there seemed to be an experimental problem with using live cultures of algae and allowing them to incubate up to six days while performing a sorption experiment. There would be a growth potential for the algal population which Mailhot does not address in her article.

Mailhot's study incorrectly identified the regression equation developed by Baughman and Paris (27) as resulting from a study of *S. capricornutum*, while Smets and Rittman (5) correctly identified it as a regression equation based on combining several studies of microbial cells. Baughman and Paris combined the data from seven different published studies of bacterial sorption, algal sorption, and sorption on other organic materials to develop a regression equation relating increasing bioconcentration (K_B) with increasing octanol/water coefficient (K_{OW}) value. No information concerning the data from the experiments used was presented. Although none of the details of the methods used is presented, it was interesting to note that the regression equation for Log BCF versus Log K_{OW} produces an R^2 = 0.954. The value of the BCF for TCE for this regression would be 0.11, while the value of the BCF for TCP would be 0.03. This regression equation was not included in Table 2 since it was based on data from several different sources and sorption on different types of materials.

**Difficulties Encountered in Past Analyses**

Some of the most serious shortcomings in experimentation to date have involved the washing of cells and the filtration of biomass (27). Due to the com-
position of most membrane filters, and their hydrophobic nature, they may have sorbed large quantities of solute resulting in serious overestimation of BCF's. Radiolabeled solutes have also been used by several experimenters (5,26) to overcome some of the problems with filtering or centrifugation. However, measurement of radiation was not specific for the compound in question, and labeled compounds have been known to have impurities which could affect the observed value of the BCF if they partitioned differently than the study compound. Above discussions have shown how previous experimenters have attempted to overcome the problems with centrifugation, cell washing, and filtration by gravity settling and using indirect measurements such as radioactivity. Most experimenters have resorted to not completing the mass balance by not measuring one or more specific equilibrium concentration.

Another problematic area of this type of analysis is to get an accurate measurement of the equilibrium sorption concentration on and/or in the algae. This problem has been alluded to previously in this discussion and discussed briefly by Miyazaki and Thornsteinson (24). The amount of solute remaining in the biomass after n extractions can be calculated by the following equation (27):

\[
X_n = X_o \left[ \frac{m}{V + \frac{m}{BCF}} \right]^n = X_o \left[ \frac{1}{\frac{V}{mBCF} + 1} \right]^n
\]

(5)

\(X_n = \) amount of solute remaining in the biomass after n extractions
\(X_o = \) initial amount of solute in the biomass
\[ V = \text{mass of the aqueous phase} \]
\[ \text{BCF} = \text{bioconcentration factor} \]
\[ m = \text{mass of the biomass} \]
\[ n = \text{number of extractions} \]

Once the laboratory study has been sufficiently completed to determine a Freundlich isotherm and therefore to derive a regression equation for the BCF, there should be sufficient data to calculate, through an iterative procedure, the number of extractions that need to be performed to quantify the amount of solute in the biomass. However, for a study such as the one performed herein using a modification of the extraction method as presented by Henderson et al. (67), the multiple extraction procedure would become very complicated and would lead to additional losses through volatilization. Also, the additional extractions would require more bottles which would lead to further losses due to sorption on the glassware. Additionally, a method would have to be found to remove the extra solvent after each extraction. Further research and thought is required to look into these complications.

I performed one extraction on the resuspended pellet for each set of experiments. As would be predicted by the forgoing calculations and discussion, the resultant concentration from one extraction was considerably lower than was expected from the values being recorded for total concentration, control concentration, and supernatant concentration. Therefore, the equilibrium sorption concentration for the pellet was calculated by using a mass balance approach to each experiment as explained in the methods section of this report.
Cox (10) conducted a survey of bioconcentration of DDT in marine phytoplankton collected from Monterey Bay in California. The phytoplankton had been collected over a period of time from 1955 to 1969 and preserved. Cox hypothesized that, since the uptake of DDT was rapid and irreversible, he could quantify the increase or decrease in DDT bioconcentration by the phytoplankton which were one of the first links in the food chain.

The samples were filtered onto glass-fiber filters after a rough filtration to remove larger zooplankton. The filters and samples were ground together in three rinses of hexane, which were pooled, concentrated, and chromatographed on silica gel microcolumns. Eluates from the columns were concentrated under nitrogen and analyzed using gas chromatography. The analyses were performed on a Beckman GC-4 gas chromatograph with dual columns and two electron capture detectors according to standard methods published by the USDA in the Pesticide Analytical Manual at that time.

Cox found that the concentration of DDT residues in the phytoplankton had increased over the years. He performed an analysis of the relationship of the size of the standing crop of algae to the concentration of the DDT in the biomass. From the theoretical curve produced, he concluded that a fixed amount of pesticide residue had become incorporated in the algal material present in a given volume of water, regardless of the density of the standing crop. However, the density of the standing crop affected the final concentration of acquired residues according to the relationship that the partition coefficient of DDT residues for phytoplankton and similar materials declined as the density of phytoplankton increased.
Cox's conclusion held implications for the experimental results that have been discussed so far in this review and for the laboratory investigation that I performed at Ohio State. Since the range of the potential values for the BCF of the diatoms and the chemicals that were used in the study were unknown, the experimentally determined BCF's could be a result of the choice of the biomass concentration.

Pore and Sorenson (17) immobilized the algae, *Prototheca zopfii* in agar beads and placed them in a fixed bed column which they termed a bioaccumulating column (BAC). Comparative columns of powdered activated carbon (also immobilized in agar), and columns of agar beads without algae or carbon were run parallel to the algal columns. *Prototheca zopfii* were chosen because *Prototheca sp.* were typical of the living sedimental algae that make up part of fresh water sediments, especially those with organic pollution. Pesticides such as Chlordecone, a halogenated, polycyclic aromatic compound, have been frequently found to be concentrated in the humic layer of aquatic sediments.

The algal column, the powdered activated column, and the control column were all fed a total of one liter of the Chlordecone (1 mg/L) over a 2-hour period. The column effluent was extracted three times with chloroform, and the chloroform extracts were pooled and evaporated to 1 mL. The GC analysis was performed on a Packard 421 with a 6 foot glass column containing 3% QF1 on 100-120 mesh chromasorb Q. The analysis was carried out isothermally with the injector at 280°C, the column at 230°C, and the FID detector at 280°C.

The experimental results for the Chlordecone analysis indicated nearly identical removal of Chlordecone for the algal and activated carbon BAC's
(approximately 100%). The pure agar bead control column results indicated that approximately 70% removal of the Chlordecone occurred on the glass and beads of the system alone without the benefit of the algae or of the activated carbon.

A second set of experimental columns was run with five different columns. One column was an algal BAC prepared in the same manner as the first experiment. There were also three other algal BAC's: one with half the algae as the first; one with one-fourth the algae as the first; one with half the algae as the first, and the algae were formalin-killed before making the agar beads; and one control column of agar beads alone. The authors found for algal concentrations at 50% of the original columns that the algal BAC removed 98% of the Chlordecone; the one-fourth strength algal column removed 93% of the Chlordecone; and the formalin-killed algal BAC resulted in 82% removal. This indicated that the formalin-killed algae were providing removal of 12% of the Chlordecone in addition to the 70% removal due to adsorption onto the system agar and glassware.

An additional column experiment was run using another very hydrophobic chemical termed aflatoxin G1 by the authors (no other details concerning this chemical were given in the report). The results of this study indicated that the agar beads alone removed 20% of the chemical, whereas the algae removed only an additional 5% of the aflatoxin. The formalin-killed algae actually were found to remove less than the agar beads alone, and this result was attributed to experimental error. Therefore, the authors concluded that the algae neither bioconcentrated or metabolized the aflatoxin.

Since the formalin-killed algae removed considerably less Chlordecone than the live immobilized algae, the authors concluded that the removal of
Chlordecone was dependent on active metabolism of the algae. Also, since the aflatoxin was not accumulated by the algae (and was reported to be highly hydrophobic), the authors questioned the validity of passive lipid partitioning as the removal mechanism.

The results presented by Pore and Sorenson were counter to the rest of the literature results presented so far. Also, the experimental design used by these authors was quite different from the studies presented by the other authors. The Chlordecone was mainly being removed by adsorption onto the agar and glassware of the system (reported as 70% removal), and the fact that the algae and activated carbon were immobilized in the agar makes the accuracy of the presented results suspect. Also, the authors reported that they did not feel that the carry-over of chemical adsorbed on the system glassware from one experiment to the next was necessary to be taken into account due to the concentration level of the experiment. Again, the discounting of the effects of this sorption activity may have been detrimental to the study's results.

Vance and Drummond (19) studied the accumulation of Dieldrin, DDT, Aldrin, and Endrin by the green algal species, *Scenedesmus* and *Oedogonium*, and blue green algal species, *Microcystis* and *Anabaena*. The algae were allowed to grow in the presence of the chlorinated hydrocarbons for a period of seven days. The algae then were filtered from the media and soxhlet extracted with hexane. Further sample cleanup was accomplished with florisil columns and methylene chloride.

GC analysis was performed on a dual-column gas chromatograph equipped with electron capture detectors and a five foot by 1/8 in. glass column packed with SE-30. The column temperature was 185°C; the detector temperature was 185°C; and the injector temperature was 190°C. The carrier
gas was nitrogen at 50 ml/min, and the injected sample size was 2 μL.

The pesticide accumulation studies were run at three different concentrations (0.02 mg/L, 0.5 mg/L, and 1.0 mg/L) for each pesticide and for each type of algae. In addition, standard Warburg respiration analyses were run to determine if the metabolism of the algae was affected by the presence of the pesticides over the seven-day period. No impact was seen in the algae's respiration over the seven day period and no unusual breakdown product from any of the pesticides was found during the experiments that would have indicated that they were being metabolized by the algae. The experimenters concluded that although the pesticides appeared to act as metabolic inhibitors in certain plant species based on cited literature, no inhibition was seen at the 1 mg/L level or lower over a seven-day period in the algae tested. There appeared to be little or no degradation of the pesticides by the algae.

It is difficult to conclude what the bioconcentration factor would be for Vance and Drummond's experiment based on the data presented in the literature. Concentrations of the pesticides on or in the algae were reported for the experiments run at the mg/L range for initial aqueous concentration. However, the equilibrium aqueous concentrations for the pesticides are not presented in the article. The researchers claimed that the pesticides were concentrated over 100 times the original concentrations by the algae. The bioconcentration factors based on the data presented with the initial aqueous concentration ranged from 0.1 to 0.3 to be on a consistent units basis with the data that has been presented in this review so far.

Werner and Morschel (22) performed a comparative experiment of the adsorption of Dieldrin by four strains of diatoms (three of Nitschia and one of Cyclotella) and ten different species of two different genera of green algae
(Chlamydomonas and Scenedesmus). The algae were taken from various collections and cultured for use in the experiments. The uptake measurements were performed by using $^{14}$C-Dieldrin and a liquid scintillation counter with the Dieldrin concentration set at $0.135 \mu$g/mL. The uptake by the cultured algae was also compared to the uptake of dead diatoms which had been stored in the freezer and were thawed out for these experiments.

The dead cells of the diatoms were found to leave only slightly more of the Dieldrin behind than the living cells of the same diatom species over the same time period. Also, the shells of the diatoms were found to adsorb some of the Dieldrin, although much less than the living or dead cells of the the diatoms. The authors concluded that the organic material of the shells (binding proteins, pectic substances, chitan fibers, etc.) may be involved in the adsorptive properties of the diatom strains.

The authors concluded in general that the diatoms tested were more effective in removal of Dieldrin than the strains of the green algae tested. However, they specifically stated that they could not make a generalized statement that diatoms were overall better than green algae which were better than flagellates for adsorption of organic chemicals based on their results.

Werner and Morschel's results were presented in plots of "% Dieldrin Remaining" versus "time". The plots were interesting in that the diatoms overall reached equilibrium within about 1 to 2 hours, while the Scenedesmus appeared to require most of a 24-hour period to reach equilibrium after which the aqueous Dieldrin concentration appeared to start rising possibly as a result of desorption. The Chlamydomonas plots showed a decreasing rate of removal.
of the Dieldrin over a 30 hour period and did not appear to have reached equilibrium within that time.

Another interesting note is that the Dieldrin was not toxic to the growth of the algae during the experiments. The *Chlamydomonas* and the live *Nitschia* (diatoms) species had reported increases of from slightly over $1.5 \times 10^6$ cells/mL to slightly over $4 \times 10^6$ cells/mL during the 27 hours. However, even with the continued increase of the biomass for the live diatoms and that reported for the *Chlamydomonas* during the experiment, the cells of the dead diatoms (which obviously weren't increasing during the experimental period) were just as effective as the live diatoms and more effective than the green algae tested in removing the Dieldrin based on percent remaining at any time period.

These experimental observations seemed to indicate that there may have been excessive sorption of the chemical on the experimental system which wasn't completely taken into account. Also, the problems with using an indirect measure such as $^{14}$C labeling to measure radioactivity instead of the concentration of the chemical of interest have been discussed before in this review.

Miyazaki, and Thorsteinson (24) isolated 10 different diatom species from a sample of ditch water near Manitoba, Canada. The pure cultures were allowed to grow for two weeks and then transferred to 125 mL Erlenmeyer flasks along with 50 mL of growth medium inoculated with 0.71 mg/L $^{14}$C-DDT. The inoculated cultures were incubated in a gyratory water bath shaker for a two week period before analysis for sorption. The total mixture was extracted three times with n-hexane-ether before filtering the suspension medium off.
After the filtering, the cells also were extracted three times with acetone. The extracts from both procedures were combined before analysis. The radioactivity was monitored to determine the total amount of DDT that could be recovered, and thin layer chromatography was utilized to determine if DDT was reduced to any of its metabolites by the diatoms.

The investigators found that some of the DDT was degraded to DDE, and that different diatoms produced different rates of degradation. The *Nitzschia* analysis results indicated that 0.4% of the DDT was converted to DDE, and approximately 63% of the DDT was recovered from the combined extractions. An unidentified diatom species, called *Species A* was also evaluated, and the results indicated approximately 1% of the DDT was converted to DDE, while approximately 60% of the original DDT was recovered. The total DDT and metabolites recovered in the form of radioactivity being considerably less than the initial inoculation led the investigators to conclude that some of the DDT or its metabolites were bound intracellularly or were for some other reason not extractable by the solvent system used.

Again, not enough data was presented to determine what portion of the DDT or metabolites may have been sorbed onto the experimental system, or whether the investigators took this possibility into consideration. Also, the problems discussed earlier with using radioactivity rather than direct measurement of the chemical of interest to determine sorption exist. However, the interest in this study was that it was the only literature reference found that indicated that metabolism of the chlorinated hydrocarbon by the diatom *Nitzschia* and by the unidentified *Species A* was occurring as a possible sink for the chemical in addition to sorption processes.
Clayton et al. (12) studied the bioaccumulation of Polychlorinated Biphenyls (PCB) in marine zooplankton in Puget Sound. Samples were collected from Puget Sound, and chlorinated hydrocarbons and lipids were extracted through a rather complicated scenario of four successive homogenations with hexane-acetone in a teflon tissue grinder. Further sample cleanup was accomplished using florisil column chromatography and ethanol-KOH saponification. A Tracor gas chromatograph, Model MT-220 equipped with two $^{63}$Ni Electron Capture Detectors and a 2 mm x 180 cm glass column with an SP 2240/SP 2401 stationary phase was used to identify the PCB's, which were detected along with pesticides in all samples. GC-MS was used to confirm several of the analyses and to provide quantification.

The chlorinated biphenyl residues were normalized to four separate biomass parameters: fresh weight, lipid weight, dry weight, and carbon weight. Analyses were performed in triplicate and produced analytical uncertainties as follows: lipid fraction, 10%; dry fraction, 1%; and carbon fraction, 4%.

The authors first calculated the equilibrium sorption concentration of the PCB's based on the fresh weight of the zooplankton. A multiple stepwise correlation analysis was then performed for the fresh weight equilibrium value correlated with the following items: lipid, dry, and carbon fractions of the biota; the equilibrium concentration of the PCB's in the water; the standing stock of biomass per cubic meter of water; chlorophyll-a; salinity; and temperature. These correlations were performed for PCB's with 4,5, and 6 chlorinated functional groups (due to their being the most statistically abundant of the PCB's
in the samples). The results showed the the fraction of lipid content was always the first chosen regression variable and accounted for approximately 75% of the variation. No other parameter was found to contribute more than 8% variation. Based on the results of this correlational analysis, Clayton felt that it was appropriate to use the equilibrium sorption concentration based on the lipid fraction as the most meaningful normalization parameter. Clayton's definition of the BCF was based on this normalization.

Clayton et al. then looked at the difference in the BCF across different zooplankton species to determine if there was a variation. The results indicated that the BCF values were independent of the faunal differences within the uncertainty of the data. It is reasonable to conclude that the PCB concentration in the water acted as a virtually infinite reservoir of PCB for uptake by the lipids in the zooplankton. Clayton et al. concluded that if a lipid-water partitioning process were applicable to any species (i.e. fish, mammals, plankton), comparable to that observed in the zooplankton, that all biota in a given ecosystem should have similar PCB residue levels normalized to lipid content if an infinite reservoir of concentration were available to them.

The study concluded that the level of the organic chemicals in the ambient water and in the zooplankton normalized to the lipid content controlled the degree of bioaccumulation especially when the lipid content was over 2% of the fresh weight. The lipid-water concentration ratio was found to be independent of the type of fauna used in the experiment. Also, the results indicated that once equilibrium was established the zooplankton possessed surface characteristics favoring establishment of rapid exchange between the internal lipid pools and the aqueous medium.

A major conclusion made by authors' study was that the biocon-
centration that was occurring in the zooplankton was a resultant of the passive lipid partitioning process rather than as a result of food chain biomagnification.

Veber et al. (23) conducted a pilot-scale outdoor experiment to determine the uptake of PCB's by two different strains of algae, *Scenedesmus acutus* and *Chlamydomonas geitleri* from a wastewater which was composed of municipal wastewater and piggery wastewater. The average influent PCB concentration was 40.5 µg/L. The algal cultivation surface was a series of angled glass slides which the pumped wastewater was allowed to trickle down over. The wastewater supply tank was 150 L. The dilution rate was varied between 0.1 - 0.5 per day. The volume of the suspension that determined the dilution rate was withdrawn once a day, and the volume was replaced with fresh wastewater. The evaporation loss was replaced with tap water. Apparently recirculation from the storage tank and across the slides occurred only at night.

PCB removal efficiency varied between 45 - 100 % (dilution rate 0.5 day⁻¹ and 0.1 day⁻¹, respectively). No discussion was included in the report as to the bacterial content of the wastewater, and the authors comment that the rate of PCB elimination increased with increasing proportion of wastewater in the medium.

The algae were extracted and a GC analysis was run using an ECD detector. A 1.2 m column with 80/100 mesh Chromasorb W was used for the separation. No further details were given concerning the GC analysis or extraction procedures used. Evaluation of the sorption capacity of the individual algal species was presented as the PCB content in dry algal biomass for each species (213 µg/g for *S. acutus* and 157 µg/g for *C. geitleri*) at the maximum dilution rate.
The authors stated that the influent PCB concentration was 40.5 μg/L on the average and the PCB removal was stated as 45% at the maximum dilution rate. Therefore, the aqueous PCB equilibrium concentration was approximately 22.3 μg/L at 0.5 day⁻¹. This equilibrium concentration would produce a quite high bioconcentration factor of 7 L/g for C. geitleri to 10 L/g for S. acutus. The problem with the study was that the authors didn’t separate out the affect of the bacterial removal of the PCB’s. The authors’ own analysis indicated that increasing removal rate was observed with increasing proportion of wastewater in the system, indicating at least qualitatively, the effect of the bacteria on removal of the PCB’s. Also, no discussion or consideration was given to the impact of sorption on the treatment/cultivation system along with the sorption onto the jars that the samples were collected and stored in.

Keil et al. (25) had conducted literature reviews indicating that the marine diatom Cylindrotheca closterium was capable of absorbing, concentrating, and metabolizing DDT and were interested if the diatom was capable of similar performance with PCB’s.

A two-week incubation period of the diatoms with PCB’s was conducted before cell counts were made. The PCB concentrations were run at 0.1, 0.01, and 0.001 ppm, and a control sample was run. After the two-week period, the diatoms were separated by centrifugation. The dry diatom pellets were extracted with 1 mL of acetone, and GC analysis was conducted on a 6 foot by 1/4 in. glass column packed with SE 30 (injector: 235°C, column: 200°C, detector: 350°C, carrier gas flow 60 mL/min). The detector type and carrier gas type were not identified in the literature.
The investigators found the extractable PCB concentrations from the diatom pellets to be the following concentrations: 109 ppm at the 0.1 ppm initial aqueous concentration, 4.7 ppm at the 0.01 ppm initial concentration, and 0.0 ppm at both the 0.001 ppm and control concentrations. No discussion was offered as to the total percent of the original PCB's recovered, or whether there was consideration given to possible sorption on the system components. There was no discussion regarding the laboratory procedures for transfers, separations, and extractions. Also, authors did not discuss whether any metabolization of the PCB's occurred during the experiment as was one of their original goals.

**Color Removal During Sorption Studies**

Lee et al. (15) studied the decolorization of bleached kraft mill effluent (BKME) by different populations of pure and mixed algae and by a mixed population of bacteria. The bacterial population in the absence of algae was found to not remove color. The pure and mixed algal populations were found to remove from 50-70% of the color. Color and ultraviolet light absorbance of the algal populations before and after removal of the BKME color was very similar, and the researchers concluded that since the light absorbance of the algae wasn't changed during the removal of color from the BKME, color removal was not accomplished by an adsorption mechanism.

The total organic content in solution remained constant during the experiments causing the investigators to conclude that the reduction in absorbance was not caused by removal or destruction of the organic molecules. If the chromophores were being assimilated by the algae for production of algal
biomass, CO₂, and H₂O, there should have been a change in the organic content of the aqueous solution. Lee concluded that the results suggested that the algae caused some type of metabolic transformation of the colored molecules to non-colored molecules (possibly through some enzymatic system) without any assimilation, adsorption, or degradation.

Lee et al. stated that the experiments were controlled, and that there was no microbial growth in the pure algal experiments. Also, bacterial experiments were incubated in the dark with the absence of algae. The pure bacterial experiments showed no removal of color in the aqueous solution. The pure bacterial experiments provided further proof that any color removal found in the other experiments was due to the algae.

There is no indication in the literature that any attempt was made to distinguish between the original lignin-related TOC and the final TOC, a large percentage of which could be hypothesized to be from product formation due to algal and/or microbial degradation activities. Lee et al., however, concluded that since the TOC remained constant during the growth experiments, the reduction of absorbance at 280 nm in the aqueous solution could not be the result of removal of the organic molecules. Therefore the change in absorbance had to be due to some change or shift in the characteristics of the molecules which was responsible for the absorbance rather than in the elimination of the molecules themselves.

Since the authors' experiment relied on two indirect measures, light absorbance at 280 nm and TOC of the aqueous solution, to reach its conclusions, it encountered some difficulties. It cannot be concluded for certain that some of the original lignin-related TOC was not transformed by the algae. Also, there was no discussion or quantification of sorption effects on the system
containers and glassware used during the experiment. However, the results of the experiment provided an interesting hypothesis for future research, that some of the characteristics of a molecule could be changed or transformed by the algae while the molecule and many of its other characteristics (TOC for example) remained intact.
CHAPTER III
EXPERIMENTAL DESIGN AND OPERATIONAL METHODS

Materials

Diatoms

Cultured, preserved, concentrated diatoms that are representative of the diatoms found in an oligotrophic lake or reservoir were purchased from the Coast Seafoods Company in Quilcene, Washington. The diatoms were shipped preserved in 50% Propylene glycol and concentrated to 10 billion cells/mL. Coast Seafoods cultured the diatoms and has marketed them to be used as a food source in raising other marine fauna (i.e. oysters, shrimp, etc.) for commercial consumption. Research laboratories have also been using the diatoms as a food source for marine fauna in ongoing environmental research projects (i.e. zebra mussels) (81). The concentrated, preserved diatoms were stored in a laboratory refrigerator at 5°C throughout the study.

It was decided that this procedure would be better than collecting and culturing samples for the proposed laboratory experiments due to the time and space requirements necessary to culture diatoms in the laboratory and for better control of the experimental results. It has been noted (27) that pure axenic cultures should be used when it is desirous to make a comparison between
different types of organisms. When system properties are to be assessed, mixed cultures should be preferred.

The diatoms were analyzed by standard Gooch crucible non-filterable solids analysis (50) to provide an approximate idea of the unit cell weight based on the figure of 10 billion cells/mL provided by the manufacturer. An average cell weight of approximately $0.5 \times 10^{-11}$ g was determined through this procedure.

Centrifuge

A Beckman J2-21 refrigerated centrifuge (Beckman Instruments, Palo Alto, California) was used for separation of the biomass phase from the aqueous phase. Different speeds were experimented with to determine the minimum time and speed combination necessary to get good separation between the phases. Complete separation was defined operationally and qualitatively as the minimum speed and time necessary to acquire complete visual separation of the liquid and solid phases. Additionally, the residue of the propylene glycol on the surface of the diatom pellet had to rinse easily from the pellet without dislodging the solids. Also, the solids had to be able to be removed from the centrifuge tube with relative ease. It was felt that if all of these three requirements were met, the propylene glycol would be removed, and the cells of the diatoms would not be damaged.

Speeds ranging from 10,000 rpm to 3,000 rpm and time periods ranging from 5 minutes to 10 minutes were analyzed over a two week period to determine an operationally optimum set of conditions. At all speeds 5 minutes
appeared to be sufficient to produce complete visual separation. At 10,000 rpm the propylene glycol was easily poured off and rinsed from the surface of the diatoms without disturbing the pellet. It was very difficult to remove the pellet from the centrifuge tube, and repeated rinsings and scrapings were required. The consistency of the pellet was similar to that of dry mud. At 5,000 rpm, the diatom pellet was easily rinsed without disturbing the surface, and the pellet was considerably more easy to remove from the centrifuge tube. At 3,000 rpm, it was difficult to rinse the propylene glycol from the surface of the pellet without losing a considerable mass of the cells in the rinsing operation. The pellet appeared to be rather loosely packed in the centrifuge tube although it was not fluid enough to be poured out.

Based on the optimization runs made in the centrifuge, it was determined to use a speed of 4,000 rpm for a time period of five minutes for the purpose of spinning off the propylene glycol from the diatoms. Approximately 6-7 mL of the concentrated diatoms as shipped were diluted with carbonate free deionized water to a volume of 38 mL in the centrifugation tube before centrifugation. The average weight of the tube containing the diluted diatoms was 47.7 g.

The centrifuge was set for a refrigeration temperature of 10°C +/- 2°C to counteract the heat buildup in the rotor during the spinning operation. With the size of the rotor being used (model no. JA-14) and the speed (4,000 rpm), the compensation was set at zero. The brake was set at a setting of 2, which required about 10 minutes to stop the centrifuge once the initial five minute centrifugation operation was complete. The brake setting of two was determined to be necessary to not disturb the surface area of the pellet resulting in resuspension of part of the solid phase into the aqueous phase.

The propylene glycol was poured off, and the pellet was rinsed visually
clean from any residue after completion of the centrifugation run. The pellet was then placed into a 1 L volumetric flask and diluted to 1 L volume with carbonate free deionized water. A magnetic stirrer was used to keep the solids suspended in the aqueous phase. Total solids, volatile solids, and non-filterable solids (50) were determined for the 1 L suspension that was made up for each set of experiments. The 1 L biomass solution was stored in a laboratory refrigerator at 5°C until the experimental run that it was being used in was complete to prevent any possibility of biological breakdown of the biomass. During the first phase of the study the volatile solids concentration generally ranged from 0.5 - 0.6 g/L (see Table 3), and during the second phase of the study the volatile solids concentration was maintained from 0.8 - 0.9 g/L.

**Glassware**

50 mL borosilicate glass serum bottles with sleeve type rubber stoppers (13x18 mm) (Thomas Scientific, Swedesboro, NJ) were purchased to be used for the contact and extraction phase of the experiment. The retractable sleeve stopper had a continuous 1 mm thick membrane over the hollow plug portion of the stopper which permitted syringe needle puncture and was self-closing to reseal the bottle. The bottles were filled with the prepared diatom fluid or with the control fluid, carbonate free deionized water, and then sealed with the stoppers.
Table 3

SUMMARY OF SOLIDS CONCENTRATIONS

<table>
<thead>
<tr>
<th>DATE</th>
<th>VOLATILE SOLIDS, g/L</th>
<th>TOTAL SOLIDS, g/L</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Phase 1</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4/22</td>
<td>0.51</td>
<td>0.78</td>
</tr>
<tr>
<td>4/29</td>
<td>0.57</td>
<td>0.84</td>
</tr>
<tr>
<td>5/5</td>
<td>0.55</td>
<td>0.81</td>
</tr>
<tr>
<td>5/13</td>
<td>0.64</td>
<td>0.91</td>
</tr>
<tr>
<td><strong>Phase 2</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5/20</td>
<td>0.53</td>
<td>0.80</td>
</tr>
<tr>
<td>6/3</td>
<td>0.59</td>
<td>0.83</td>
</tr>
<tr>
<td>6/10</td>
<td>0.59</td>
<td>0.83</td>
</tr>
<tr>
<td>6/17</td>
<td>0.65</td>
<td>0.95</td>
</tr>
<tr>
<td>6/18</td>
<td>0.92</td>
<td>1.34</td>
</tr>
<tr>
<td>6/22</td>
<td>0.82</td>
<td>1.19</td>
</tr>
<tr>
<td>6/25</td>
<td>0.78</td>
<td>1.09</td>
</tr>
</tbody>
</table>
Micro-Syringes

Gas tight micro-syringes of various sizes (Hamilton Company, Reno, Nevada) were used to inject additional fluid (diatom, control, or chlorinated compound) and to evacuate air to eliminate any headspace in the bottles. After each use during the experimental phase, the syringes were cleaned by twice rinsing with hexane and then three times rinsing with carbonate free deionized water.

The entire extraction process was completed with 1 mL syringes during the first phase of the isotherm experiments. During the second phase of the isotherm experiments, the extraction process was completed using 2,500 µL syringes with Luer-Loc tips. Disposable 21 gauge, 1 1/2 in. long steel tips were used with these syringes. Details of the extraction process and reasons for the changes in the syringes are included later in the discussion.

Centrifuge Bottles

Centrifuge bottles were 50 mL polycarbonate bottles with a Teflon insert, buna-n rubber o-ring, and screw cap assembly (Beckman Instruments, Palo Alto, CA). In the first phase of the isotherm experiments, these bottles were used during the contact phase. However, it was very difficult to complete the extraction process in these bottles. It was found during the first phase of the experiments that it was nearly impossible to get a representative sample of the sorption of the chlorinated compound on the surface of the polycarbonate centrifuge bottles. In the second phase of the isotherm experiments, the contact phase of the process was not completed in the centrifuge bottles. The contact
phase for all samples was completed in the glass serum bottles, and one sample was transferred to the polycarbonate centrifugation bottles to be centrifuged after the contact phase.

**Chemicals**

High-grade purity TCP (99%) and TCE (98%) (Aldrich Chemical Company, Milwaukee, Wisconsin) were acquired for the experiments. Suitable stock solutions were made for the inoculations for each run of the experiment. The TCP and TCE were diluted to a 1000 ppm concentration in methanol in a sealed serum bottle. The stock solution was stirred for several hours on a magnetic stirrer before being used to inoculate a series of bottles for an isotherm analysis. All stock solutions were stored in a refrigerator at a constant temperature of 5°C.

**Gas Chromatograph**

The gas chromatograph used in the isotherm analyses was a Varian Model 3700 GC equipped with a Model 8000 Autosampler (Varian, Walnut Creek, CA). Various settings for the temperature of the components and the attenuation were tried throughout the first phase of the isotherm experiments as presented in Table 4. The model 3700 had two universal detector bases and a large enough oven space to allow two columns to be run in parallel with the same or different detectors. Expansion interfaces on the model 3700 allowed
<table>
<thead>
<tr>
<th>PHASE 1</th>
<th>PHASE 2</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>DETECTOR:</strong> 63 Ni ECD 200°C, 220°C</td>
<td><strong>DETECTOR:</strong> FID 180°C, 200°C</td>
</tr>
<tr>
<td><strong>COLUMN:</strong> DB-1 105°C, 110°C, 120°C, 135°C, 160°C</td>
<td><strong>COLUMN:</strong> DB-624 100°C, 105°C</td>
</tr>
<tr>
<td><strong>INJECTOR:</strong> 100°C</td>
<td><strong>INJECTOR:</strong> 100°C, 0°C (63°C)</td>
</tr>
<tr>
<td><strong>ELECTROMETER:</strong> ECD = 10 Output = - Attenuation = 128, 1024</td>
<td><strong>ELECTROMETER:</strong> Mode: Ion Detect Output = + Attenuation = 128 Range = 10⁻¹⁰</td>
</tr>
<tr>
<td><strong>GASES:</strong> H₂ = 8 mL/min +/- (80 cm/sec) H₂ + N₂ = 40 mL/min</td>
<td><strong>GASES:</strong> N₂ Carrier = 6 mL/min N₂ Makeup = 25 mL/min H₂ = 25 mL/min Air = 300 mL/min</td>
</tr>
<tr>
<td><strong>INJECTION SIZE:</strong> 1 μL</td>
<td><strong>INJECTION SIZE:</strong> 0.5 μL</td>
</tr>
</tbody>
</table>
the addition of an autosampler and the use of various data recorders or integrators to provide a hard copy of the results of the chemical analyses.

A "hot on-column" direct injection liner was purchased from Varian and inserted into the standard packed column injector body of the 3700 GC. A liner was also installed in the detector fitting in the oven. The installation of the two liners allowed the use of a megabore 0.53 mm inside diameter capillary column to elute the chlorinated compounds.

The "hot on-column" injector was completed by inserting the capillary column to form a seal with the liner just below the septum seal of the injector needle. A 26 gauge needle will just barely fit inside of the 0.53 mm megabore column allowing the injections to be made directly into the column. The advantages of using the on-column injection technique were:
- that it limited the decomposition of the sample to a minimum when the analyses were being done at a low injector temperature such as was the case in this study, and
- it minimized any syringe discrimination, especially when accompanied by the use of the autosampler.

The disadvantages of this on-column injection technique were:
- the sample size was usually limited to 0.5 μL, larger samples resulted in distorted peak shapes,
- there was a greater chance for wide peaks,
- there was a greater chance of a build up of non-volatile residues in the column, especially if a retention gap was not used to retain the non-volatile compounds, and
- there was a possibility of damaging the inside of the column by phase stripping due to the needle scraping the inside of the column.
Detectors

Two detectors were used in this analyses, one for each of the two phases of the research. The first phase was performed using a $^{63}$Ni Electron Capture Detector (ECD) (Varian Corp), a specific detector for electron-absorbing compounds such as chlorinated hydrocarbons in gas chromatography. The detector was set at 200°C or 220°C throughout the phase one analysis. The operator's manual for the 3700 GC (82) stated that the ECD must be operated a minimum of 50°C above the column temperature.

The ECD is not only a specific detector for chlorinated hydrocarbons, but also allows detection of chlorinated hydrocarbons down to the low µg/L range. This detection range is a mandatory prerequisite for the development of the proposed methodology to have widespread application in the water treatment industry and for field studies involving contamination of groundwater or surface-waters.

The second phase of the work was performed using a Flame Ionization Detector (FID), a general detector which operated based on the organic compound burning in a combination of air and hydrogen to generate positive and negative ions. The positive ions were collected producing an electrical current proportional to the amount of the organic compound which was burned. The current was amplified by the 3700 GC's electrometer and recorded by the integrator or recorder interfaced to the GC.

The FID proved to be a very stable and robust detector for use in the measurement of chlorinated hydrocarbons. However, the FID is limited in that its lower detection limit is approximately 50 µg/L. This limitation restricts the FID
from having general applicability in field studies involving low levels of organic contamination. However, the FID is very applicable for laboratory studies operation in higher concentration ranges.

The operator’s manual (82) recommended the FID be operated at gas flow rates of 300 mL/min of air and 30 mL/min of hydrogen and carrier gas. Both phases of this investigation were conducted using a combination of hydrogen and nitrogen gasses in the 3700 GC. The second phase also required high purity air for the FID. The phase one ECD investigation was conducted using hydrogen gas as the carrier gas at approximately 7 mL/min along with nitrogen gas as the makeup gas at approximately 37 mL/min. The actual determination of the gas setting was made by evaluating the velocity of a nonretained compound such as hexane at 80 cm/sec through the column system. The pneumatics of the autosampler system were also operated with the nitrogen gas. The nitrogen gas that was used for makeup was dried in a heated on-line filter before entering the GC system to remove moisture.

In the second phase using the FID detector, the nitrogen gas was used as the carrier gas at approximately 6 mL/min. The air was run at 300 mL/min, and the hydrogen and nitrogen makeup gas were both operated at approximately 25 mL/min. The rates used in this analysis were again based on meeting a non-retained flow rate of approximately 80 cm/sec through the capillary column.

The nitrogen gas was filtered through an in-line gas purifier (Model 2-3800, Carrier Gas Purifier, Supelco, Bellefonte, Pennsylvania) before entering the GC and capillary column systems.
**Gas Flow Meter**

A digital electronic flow meter (Varian Corp., Intelligent Flow Meter) was used throughout all of the experiments on an ongoing basis to keep the 3700 GC pneumatics controls and all of the three individual gas supply tank pressure gauges calibrated. The flow rates of the gases were monitored on the pneumatics control panel and on the individual pressure gauges on each tank. Any change in either the control panel readout or the gas tank pressure reading was followed up by checking the gas flow rates through the detector with the digital flow meter.

**Integrator**

An SP 4270 Computing Integrator (Spectra Physics, San Jose, California) was interfaced to the 3700 GC. The SP4270 provided a report summary printout for each chromatogram that was run (typical chromatograms and partial summary report are presented in Figure 2 and Figure 3). The report provided correlated information for each peak consisting of the following: peak position number, the particular peak’s area percentage of the total area under all of the peaks in that run, the run time that the peak occurred, the area under that peak, and the baseline correction method chosen by the integrator to integrate the area under the curve.
Figure 2

TYPICAL CHROMATOGRAM AND SUMMARY REPORT, TCE
Figure 3

TYPICAL CHROMATOGRAM AND SUMMARY REPORT, TCP
Capillary Columns

I chose capillary columns for these experiments due to the advantages that they offered over the packed column design in the analysis of chlorinated hydrocarbons. The design of the capillary column provides higher resolution than a packed column due to the length of the column being longer than a typical packed column, and the number of theoretical plates per unit length is also increased. A typical packed column may be 6 ft in length whereas the capillary columns I used were 30 m in length. Both of the columns used in this experiment provided over 1000 theoretical plates per meter.

The capillary column also provides a shorter analysis time than a packed column due to the fact that the capillary column has much less stationary phase than the packed column. In these experiments the columns chosen had either a 5 μm or 3 μm stationary internal liquid phase which was very nonpolar to be compatible with the elution of the nonpolar chlorinated hydrocarbons.

The capillary columns also provide greater sensitivity than a typical packed column. The sensitivity is increased by optimizing the carrier gas used in the analysis, and the increased column length tends to diminish the effects of fluctuation in the gas flow rate that might be more obvious in a shorter column. Also, the fact that there is less stationary phase present tends to reduce the effects of bleeding of the stationary phase that is observed in packed columns. The capillary columns, however, are limited in the sample size that can be used due to the decreased volume of the stationary phase.

The capillary column used in the first phase of the isotherm experiments (ECD) was a DB-1, 30 meters in length, 0.53 mm inside diameter, with a 5 um internal fixed phase coating (J & W Scientific, Folsom, California). The DB-1
coating is a cross-linked, surface bonded dimethylpolysiloxane compound. The cross-linking and surface bonding processes gave the polymer increased chemical stability. Therefore, the stationary phase was not significantly affected by larger sample injections and processes such as direct aqueous injections, on-column injections, and solvent rinsing of the column. The dimethylpolysiloxane coating was the most nonpolar siloxane stationary phase available for GC work, and was equivalent to AT-1 or SE-30 coatings (Alltech, San Jose, California) which were mentioned quite frequently in the literature. The 5 μm film thickness was chosen because the on-column solvent injection method chosen required increased stability of the column internal phase. The thicker stationary phase also permitted the solvent used in the extractions to elute quickly and completely before the chlorinated compounds used in the analysis eluted. The 30 m length was chosen to provide higher resolution at a lower speed than a shorter column would have provided. The reason for the use of the 0.53 mm inside diameter of the column was discussed earlier in the information about the autosampler and the injector body inserts.

The capillary column used in the second phase of the work (FID) was a DB-624, 30 meters in length, 0.53 mm inside diameter, with a 3 μm internal fixed phase coating (J & W Scientific). Most of the explanations for the column parameter selections for this column remained the same as for the column used in the first phase of the experimentation. The DB-624 column stationary phase was still very nonpolar, but it had been slightly modified from the chemical composition of the DB-1 stationary phase to be more selective for halogenated compounds. The DB-624 had a 6% substituted phenylmethylpolysiloxane stationary phase.
An apolar, extremely inert, column with a thicker coating than those normally employed was required to permit the isothermal analysis that was being performed in this study (1). The procedure required the ability to elute the solvent used in the extraction procedure quickly at a column temperature of approximately 100°C before the chlorinated hydrocarbons being studied were eluted.

**Cleaning Protocol**

All glassware used in this study were cleaned with a laboratory cleaning detergent (Alconox) and rinsed with deionized water. The glassware was dried at 170°C for at least one hour in accordance with Standard Methods (50). GC sample vials were washed and rinsed as the rest of the glassware, and then rinsed with a nitric acid solution before drying in a muffle furnace at 400°C for at least one hour. Muffled vials were stored in an aluminum container covered with aluminum foil until they were used.

As a matter of GC system maintenance, the system was taken down after each complete set of sampling run, and a solvent cleaning system was used to clean out any nonvolatile contaminants that had collected in the injector system along with any solvents or chlorinated hydrocarbons that may have contaminated the injectors due to column backflash. The procedure was initiated by completely shutting the GC system down. Once the oven had cooled to room temperature, the column was removed and stored. The injector body inserts
were removed and washed in nitric acid solution and muffled at 550°C for one hour.

A solvent wash system was built which consisted of using a 43 mL glass sampling bottle with a screw-on plastic orifice lid with a hard rubber insert seal such as is used in EPA approved field sampling programs for water quality parameters. Two holes were punched in the rubber insert and a plastic tube was inserted into each hole. Gas tight ferrules and fittings were attached to the plastic tubing. The carrier gas supply to the injector bodies was disconnected inside the GC, and one of the tubes was connected to the supply side of the carrier gas system while the other tube was connected to the injector.

The solvent wash system was run by using the carrier gas at a very low flow rate (approximately 10 psig) to push the solvents through the injector system. Three different solvents were used for each cleanup procedure in the following order: methanol, methylene chloride, and hexane. Approximately 40 mL of each solvent was used at the end of each run to clean the injector system.

During the experimental runs the standard operation of the GC was to inject one sample into the column for separation followed by one injection of hexane through the autosampler system to purge any of the previous sample from the autosampler system before injecting the next sample. The septum which sealed the system between the autosampler syringe and the injector body of the GC was replaced after each complete run of a set of samples.
Background for Experimental Method Development

Grob and Habich (1) performed an evaluation of the direct aqueous injection (DAI) of halogenated hydrocarbons into capillary columns using ECD detectors. One major problem with the ECD was that it was very slow to recover from the influence of water if the system retained water releasing it from the system slowly over a prolonged period of time. The elution of water had to come to a quick finite end to limit the effect of water on the ECD. Therefore, the report recommended that for using the DAI process in capillary columns with ECD the column must be made out of very inert material to keep the column from retaining water.

Secondly, the injection port needed to be efficient, minimizing dead space. Vaporizing injectors tended to have a lot of dead space which retained water and released it over a prolonged period of time. Therefore, the report recommended on-column injection techniques should be used in place of the vaporizing injector port. The main problem with using on-column injection techniques with DAI was that nonvolatile components eventually built up in the inlet end of the column requiring periodic cutting of a piece of the inlet end. In-lieu of cutting a piece of the column, guard column sections could be purchased through most capillary column manufacturers to be used to protect the column from nonvolatile components.

Grob and Habich investigated the use of extremely apolar columns to elute the water as quickly as possible. The two stationary phases chosen were SE-30 (a pure methyl silicone phase) and SE-54 (a slightly more polar silicone
phase containing phenyl). Five halogenated compounds (methylene chloride, chloroform, 1, 1, 1-trichloroethane, trichloroethylene, and tetrachloroethylene) were run on the columns at three different temperatures (70°C, 95°C, and 105°C). Three different column lengths (17.5 m, 16.5 m, and 39 m) and three different stationary phase thicknesses (3.3 μm, 4.8 μm, and 5.0 μm) were also evaluated to determine the relative effect of the column length and stationary film thickness on control of compound retention and to evaluate if the thicker stationary phases would perform better for a longer period of time using DAI. Hydrogen gas at a velocity of 30 cm/sec was used as the carrier gas, and Nitrogen gas at 50 mL/min was used as the makeup gas.

The best results in terms of controlling the water peak widths and the tailing effect of the large water solvent peak while the other compounds were eluding, occurred at the higher temperatures using the thicker stationary phase. The study recommended using a column temperature of from 100°C to 105°C with a stationary phase thickness of 5 μm. The column length necessary depended on the column length needed to separate the lightest hydrocarbon in the ongoing study. They also found that it may be necessary to increase the carrier gas velocity to 50 cm/sec to 70 cm/sec to assure ideal peak shape. The results also indicated that if column temperatures were much above 100°C, and if a sample size above 1μL was used, that secondary cooling should be used to control backflow of sample into the injector body.

Grob and Zhangwan (59) indicated that it would be attractive to be able to devise a more-or-less direct injection technique that would allow the future development of a fully automatic on-line analysis of certain types of samples
such as drinking water. They attempted to develop a precolumn which could be attached to the ahead of the injection end of the apolar separation column to create a retention gap effect. The precolumn was supposed to create a space which was wettable by the solvent (in this case water). As the solvent created a film which was evaporated by the carrier gas, the sample would be pushed into the separation column. The precolumn material, therefore, needed to have two main attributes:

- it needed to be wettable by water, and
- it needed to have poor retention or activity to allow the sample to pass into the separation column.

Grob and Zhangwan's study evaluated the following different types of glass material for the precolumn: raw fused glass, raw soft glass, soft glass etched with KOH, and raw Duran glass. Each type of glass was also evaluated for different coatings such as carbowax to deactivate the surface. The results of the study were disappointing in that no material was found which was sufficiently wettable and deactivated at the same time. If the material could be sufficiently deactivated, it lost its wettability. Therefore, the findings indicated that it was not only unnecessary, but also pointless to install a precolumn for a DAI process when the flooding into the separation column could not be prevented by a deactivated column material.

**Extraction Methodology Background**

Henderson et al. (67) developed a simple liquid-liquid extraction procedure for separating halomethanes from water. The procedure that they developed used pentane for the extracting solvent, and the chemicals that they
were extracting from water were chloroform, carbon tetrachloride, dichlorobromomethane, chlorodibromomethane, and bromoform. Their samples were identified using a Tracor 560 gas chromatograph with a $^{63}$Ni ECD. The column was a 6 ft x 2 mm glass packed column with 10% squalane on Chromasorb. The process was run isothermally at 67°C with argon/methane as the carrier gas at a flow rate of 20 mL/min. The makeup gas was also argon/methane at a flow rate of 60 mL/min. A Varian Model 435 digital integrator was used to record the peaks and calculate the area under the peaks.

The sample bottles were 120 mL serum bottles which were sealed with a Teflon coated rubber septum with an aluminum retainer ring crimped over the rim of the bottles. The bottles were completely filled and evacuated so there was no headspace which would have allowed volatilization. Two 10 cc syringes with 22 gauge needles were inserted through the septum of the sample bottle simultaneously. One of the syringes contained 3 to 5 mL of the pentane solvent, while the other syringe was empty. The syringes needed to be positioned in the sample bottle so that the pentane could be injected into the sample while the empty syringe was used to simultaneously withdraw an equal amount of the sample.

The authors then brought the partitioning process to equilibrium by shaking the bottles for 15 minutes at 500 rpm on a gyratory platform shaker. The bottles were then allowed to sit until a sufficient sample of the organic phase could be withdrawn for injection into the gas chromatograph. The process as proposed by Henderson et al. has been modified and used by other experimenters (5, 6) as discussed earlier in the literature review section.
The importance of a process such as developed by Henderson et al. was in the advantages of time and simplicity it could bring to the gas chromatographic process over the conventional techniques of purge and trap for volatile organics analysis in water. The study conducted in this investigation took a modification of the Henderson et al. method for extraction and combine it with an on column injection technique which was projected to produce results similar to the DAI technique proposed by Grob and Habich (1). I have also combined the use of the ECD or FID with a capillary column for separation and identification of the organic compounds. The technique proposed and used in this study eliminated the need for retention gap procedures such as discussed by Grob and Zhangwan (59). The results of this study hold a great deal of promise for the development of future on-line or simplified GC techniques that could be used in any municipal water treatment plant for the identification of organic chemicals in the drinking water supply with little technical training or expertise on the part of the personnel doing the analysis.

**Phase I-ECD Methods**

Experiments were conducted in a range of concentrations from 5 μg/L to 100 mg/L. The lower part of the study range reflects field concentrations that have been observed in many groundwater and surface water contamination situations (11, 19,22, 54, 64, 77, 78), while the higher concentrations allowed comparison with existing literature data (5, 6, 17, 18, 27) as a check on the experimental procedures.
The experimental procedure was divided into two main sections. The first phase of the experiments was conducted using a $^{63}$Ni ECD and the DB-1 capillary column. During the first phase of experimentation, bottles containing the biomass and solute along with parallel control bottles of water and solute were placed in a temperature controlled shaker bath (Model 50 Shaker Bath, Precision Scientific Co., Chicago, Illinois) for 24 hours. The twenty-four hour period should have been sufficient for biosorption potential to be reached based on literature reviews (5,26,27,61). Incubations were conducted over a range of different times during the second phase of the experiments to verify that equilibrium had indeed been reached. These time variations are discussed further in the text concerning the second phase. Temperature was controlled at 25°C during the experiments.

For each sampling run the following bottles were set up: control at $t_0$, control at $t_f$, total at $t_f$, centrifuge at $t_f$ (see Figure 4). The control at $t_0$ bottle was immediately extracted using a modification of the Henderson et al. method (67) and a sample taken and injected into the GC. The control at time final samples and the total samples were incubated in sealed glass serum bottles, and the centrifuge sample was incubated in a sealed polycarbonate centrifuge bottle. At the end of the twenty-four hours, the sample bottles were removed from the water shaker bath and prepared for centrifugation or extraction. The control at $t_f$ and the total at $t_f$ samples were extracted and injected into the GC for analysis.

The centrifuge at $t_f$ sample was put into the centrifuge for five minutes at 4,000 rpm with the other control settings as indicated in the discussion on methods. The supernatant was poured into a glass serum bottle which was
SCHEMATIC OF STUDY SAMPLING PROCEDURE
tapped off with carbonate free deionized water and sealed with a sleeve type rubber stopper. Any headspace was immediately removed using a gas tight microsyringe and additional deionized water. The pellet was resuspended with deionized water and poured into a glass serum bottle. The bottle was topped off with deionized water, and the same procedure was used to seal the bottle and evacuate any headspace.

The centrifuge tube was then filled with deionized water, and hexane which was the solvent being used for the extraction procedure, was injected under the water surface before reinserting the Teflon and o-ring seal and recapping the tube. The tube was then shaken for three to five minutes on a wrist action shaker (Model BB Wrist Action Shaker, Burrell Corp., Pittsburgh, Pa.). The tube was then allowed to sit while the phases separated. The screw cap, and Teflon seal had to be removed to sample the organic phase before injection into the GC. This extraction procedure using the centrifuge tube proved to be both difficult to accomplish, and inadequate to produce accurate results. Therefore, this process was modified during the second phase of the study as discussed later.

The extraction process as proposed by Henderson et al. (67) was modified in this phase of the study. The 60 mL glass serum bottle filled with the sample was injected with 1 mL of hexane while 1 mL of the sample was simultaneously withdrawn from the bottle. This method was easy to use once I developed some experience and dexterity with the procedure. By keeping the injecting needle at a location near the bottom of the rubber sleeve (top of the sample bottle), the withdrawing syringe could be positioned near the middle of the sample bottle (basically inserted as far as the syringe needle will allow). The pressure differential created by injecting the hexane near the top of the
bottle automatically caused the receiving gas tight micro-syringe to fill with the sample. The serum bottle was then placed on the wrist action shaker for a period of three to five minutes to provide the chlorinated compound with uniform time and mixing to partition into the hexane.

The serum bottle was allowed to sit for approximately three to five minutes while the phases separated. Then a sample was taken from the serum bottle with a gas tight micro-syringe and injected into a GC sample vial with a screw-on cap and Teflon coated rubber septum. Typically 200uL to 300 µL could be recovered without getting any water into the hexane that was being withdrawn from the serum bottle. Again, my ability to withdraw the separated hexane from the bottle improved with experience in the method. The GC sample vial was set into the auto sampler which injected a 1 µL sample on column into the DB-1 capillary column.

During the phase one experiments, different column temperatures were tried in an effort to determine if the optimum temperature for running the experiments at an isothermal temperature was near 100°C or higher. The DB-1 column and the GC system including the 63Ni ECD experienced considerable difficulties and instabilities at the higher column temperatures. The major difficulty in running the column at temperatures over 100°C with a 1 µL sample was the tendency for the sample to backflash into the injector body. The sample backflash then contaminated the injector system for future sample injections causing erroneous results. The range of temperatures used for the column, injector, and detector during phase one are shown in Table 4.
Phase 2 - FID Methods

During the second phase of the experiments several changes were made affecting different aspects of the experimentation based on the experiences of the first phase of the study. The DB-1 column was switched to a DB-624 column which still had a very nonpolar stationary phase, but was designed to be selective to separate nearly all halogenated organic compounds. Approximately 6% of the functional groups in its chemical matrix were substituted. The detector system was switched from the ECD to a flame ionization detector (FID). The column temperature was held between 100°C and 105°C throughout the second phase. The injector temperature control was left at 0°C to reduce the chances of backflash of the sample into the injector body. However, monitoring indicated that the injector temperature was around 64°C throughout the second phase due to heat transfer from the column (oven) into the injector body. The detector temperature ranged from 180°C to 200°C. The sample injection size on the autosampler was reduced from 1 μL to 0.5 μL to reduce the chance of flooding the detector.

The sample preparation and extraction procedures were also changed during the second phase of the experimentation to improve the accuracy of the results. The amount of hexane used for extraction was increased from 1 mL to 2 mL. The gas tight microsyringes used for the extraction procedure were switched from the 1 mL syringes in the first phase to 2.5 mL syringes. The switch in the procedure made it easier to obtain approximately 500 μL of
organic solvent after the extraction procedure with less chance of getting water in the sample.

The centrifuge tubes were not used during the incubation period. All of the samples were incubated in the sealed glass serum bottles. After the contact period, the total sample at $t_f$ and the control sample at $t_f$ were extracted in a similar manner as in the first phase with the exception of the change in the volume of the hexane used. The centrifuge sample was poured from the glass serum bottle into a polycarbonate centrifuge tube, sealed, and centrifuged as in the first phase. The glass serum bottle that had been emptied into the centrifuge tube was filled with deionized water, sealed with a rubber sleeve stopper, and extracted to determine the sorption on the glass.

The supernatant from the centrifuge tube was poured into a glass serum bottle, topped off with deionized water, sealed with a rubber sleeve stopper, and extracted for injection into the GC. The pellet in the centrifuge tube was resuspended with deionized water, poured into a glass serum bottle, sealed with the rubber sleeve stopper, and extracted for injection into the GC. This method proved much more successful and reproducible than the method which attempted to extract a sample in the centrifuge tube during the first phase of the experimentation.

The gasses were changed for the FID as indicated in the earlier methods and materials section and Table 4. The alternating process of injecting a sample and then rinsing the autosampler system with hexane, which was initiated in the first phase, was continued. The procedure of taking the system down and solvent washing of the injector system after the completion of a sampling run was also continued.
The contact time was varied during the second phase of the experiments for the samples with an initial aqueous concentration of 10 mg/L. The incubation times were run at 2, 4, 6, 8, 10, and 12 hours rather than running all of the samples for a 24 hour contact time. The isotherms developed for both TCP and TCE showed agreement with previous literature studies (27), which indicated that the sorption process was fairly rapid with equilibrium probably being achieved in a matter of a few hours. There was no discernable pattern of increased sorption found after a two to four hour incubation period. Within the experimental error of the procedures the value for the calculated sorption were found to be constant up to the 12-hour incubation time. The isotherms are discussed in more detail in Chapter 4 and are shown in Figure 5 and Figure 6.

The constant temperature shaker bath was eliminated from the procedure during the second phase. The shaker bath was difficult to use during the first phase, as it allowed the diatoms to settle out to the bottom of the serum bottles, so they had to be turned on a regular basis to keep resuspending the diatoms. In the second phase a rotational mixer (Model 811 Fisher-Kendall Mixer, Fisher Scientific) was converted with adjustable clamps to be able to hold the serum bottles for a sample run. This mixer rotated at a slow constant rate on a horizontal axis keeping the solids in suspension during the contact time. Also, the room temperature was kept within a range of 25°C to 29°C. Since the study involved the use of preserved rather than live biomass, the room temperature control was satisfactory.
CHAPTER IV
FINDINGS AND RESULTS

Phase I- ECD

Gas Chromatograph

During the first phase of experimentation, the detector and injector temperatures were left fairly constant as indicated in Table 4. However, the column temperature was varied through five different temperature settings to find an optimum isothermal temperature at which to run the separation analysis. As discussed earlier in the section on goals and objectives, one of the main purposes of this laboratory study was to develop a simplified laboratory analysis that could be used at small municipality water-quality laboratories utilizing personnel with minimal technical training. Hence, the development of an isothermal temperature procedure would greatly simplify the type of GC oven required and the knowledge base of the operator required.

The literature (52) indicated that running the oven at too high a temperature at the time of the injection could cause problems with sample backflash into the injector body since an on-column hot body injector liner was being used. The results of varying the column temperature in this phase verified the general comment of the literature. It was found that temperatures of
120°C and over could cause sample backflash into the injector body system. The situation caused by a sample backflash into the injector body system of the GC could have been very detrimental to the accuracy of the study's results. Once the injector system became contaminated, especially with non-volatile components, it could lead to contamination of the column. This situation eventually did occur in the first phase of this study.

Once the chemical compounds have backflashed into the injector body from the column, they may condense inside the injector body due to the lower temperatures in the injector system. Also any non-volatile compounds which were in the sample could line the surface of the injector body. During subsequent sample injections part of the formerly backflashed compounds may get carried into the column causing erroneous or confusing peaks to occur on the printout of the chromatogram.

Capillary Column and Autosampler

In this study, the capillary column became contaminated with compounds from the high temperature backflash after the oven temperature was run at temperatures higher than 120°C during the initial phase of experimentation. The procedure to correct the resultant contamination was to take the column off-line and to cut approximately one meter of the column off the influent end and dispose of it. Some of the contamination could be seen in the lead end of the column by looking at it under a phase contrast microscope. Cutting a meter of length off the lead end of a 30 meter column does not drastically change the time through the column and therefore the velocity of a non-retained compound.
Since the DB-1 column was a cross-linked and surface bonded dimethyl polysiloxane stationary phase, it was also possible to solvent rinse the column to remove contamination without damaging the stationary phase. The solvent rinse procedure was also used on the column to remove contamination in addition to removing part of the influent end of the column.

Once the column had been cleaned, the solvent rinse system had to be used on a larger scale on the injector system of the GC. The solvent rinse system is described in detail in the methods and materials section of this study.

Another source of difficulty in the first phase of the experimentation was the sample injection size. The 1 μL size was chosen as a starting point to give a reasonable representative sample for the GC separation, although the literature (1, 52) indicated that this sample size was probably at the upper limit of volume that could be used for the GC separation using a capillary column and ECD. As stated before, the intent of this study was to develop a comparatively easy method to provide reproducible results for laboratory personnel that are not GC experts. The 1 μL sample injection size was tried to provide a more workable volume of injection for the analyst to use in this method. The 1 μL volume tended to flood the detector in a fairly short period of time after only five or six sample injections. The flooding conditions that occurred were even worse if the extraction process was not performed effectively and allowed water to be injected along with the hexane solvent. Once the detector had been flooded, it took up to 12 hours to get the detector to recover (dry out) so that it could be properly used for analysis again.
Sample Handling

During the first phase the samples that were to be centrifuged were incubated in the polycarbonate centrifuge bottle during the contact period. This procedure caused some difficulties to the results of the first phase. From a theoretical point of view, the fact that a polycarbonate bottle rather than a borosilicate glass bottle was used for this portion of the sorption experiment could cause an artifact in the results due to possible different sorption potentials of the two different glass material matrices. By far the more realistic problem that was experienced was the extreme difficulty and almost impossibility in using the Henderson et al. extraction method to get any results that were indicative of the sorption of the chlorinated compounds onto the walls of the centrifuge bottle. It was impossible to perform the simultaneous sample extraction/solvent injection into the polycarbonate bottles that were being used since they didn’t have a resealable rubber septum sleeve like the serum bottles did. Not only did this make the separation technique accuracy suspect, but also using the Henderson et al. method with these bottles allowed a much greater potential for volatilization from the system during the extraction process. In addition to these potential problems, the hexane and water phases in the centrifuge bottles did not separate well following the procedure of shaking the bottles and allowing them to sit as was followed for the serum bottles. This problem made it difficult to even extract 100 μL of solvent without getting water in the extraction.

The water shaker bath that was used during the first phase of the experiment did a good job of keeping the serum bottles at a constant temperature. However, the motion of the shaker bath could not keep the
diatoms in suspension. Therefore, the bottles had to be inverted every hour during the contact period to resuspend the diatoms. The diatoms actually settled rather rapidly in the serum bottles and would have had to be inverted every five minutes or so to keep the diatoms completely in suspension. This problem with keeping the diatoms mixed in suspension could have some bearing on the experimental results and could cause an artifact in the results due to the experimental methods of the first phase.

**External Standard**

During the first phase of the experimentation an external standard of known concentration was purchased for both the TCE and TCP from Varian Corporation to be used as a calibration for the process. Quite a few of the contamination problems during the first phase of the experimentation were caused by using these standards along with the Henderson et al. extraction method, and the high column temperatures. The external standard method was chosen to provide quantification of the results and to provide a simpler procedure than using an internal standard throughout the procedure. Although the external standard method was workable, the standards chosen were “off-the-shelf” standards which were very high in concentration (2 mg/mL) and contained approximately 30 chlorinated compounds each. The specific standards chosen coupled with the methods used caused some problems for the study due to contamination of the GC system. A better set of standards needed to be used.
Detector

The ECD was an excellent and very sensitive detector to use for this procedure. The only problems that were encountered with its use were due to the methods used for the sample injection and column operation. However, there could be some technical difficulties in owning, storing, and using an ECD due to the radioactive source used in its function. Proper safety training, licensing, and reporting procedures need to be followed on an annual basis to use and maintain such a detector. A simpler detector would be better for a small municipality to use in their laboratory procedures.

Phase 2 - FID

Gas Chromatograph

During the second phase, the results of the first phase experimentation were used to develop constant settings for all aspects of the experimentation so that consistent data could be developed for biosorption isotherms. The detector temperature was consistently run at 180°C or 200°C in accordance with the operator’s manual recommendations. The column or oven temperature was kept at 100°C or 105°C throughout the second phase in response to the results from the first phase which indicated sample backflash was still occurring at 120°C. I decided that 100°C was the lowest temperature that the procedure should be run at isothermally, and that it was better to run the method at 105°C
because there was always the possibility that some water would be in the extraction of the sample depending on how experienced the analyst was with the extraction method. Running the oven at 105°C allowed any water that was in the sample to be volatilized and seemed to do a good job of not causing sample backflash into the injector body. The injector temperature was turned off during the second phase of the experiment. By setting the injector at 0°C, the heat from the oven kept the injector at approximately 63°C. This heat transfer actually caused a fortuitous situation by forming a region of lower temperature right at the inlet end of the capillary column. This in turn provided a solvent effect for this analysis without going to the additional expense and control hardware to install secondary cooling or some venting method. The solvent effect limited the chance of any part of the sample volatilizing in the injector before the sample was in the column or of any sample backflash into the injector body.

Capillary Column and Autosampler

The solvent rinse procedure that was developed in the first phase of the experimentation and discussed in the materials and methods section was used as a standard operating procedure for the injector system during the second phase of the study. After each complete sampling run the column was taken off-line and stored while the injector system was cleaned with the solvent rinse system. This method worked extremely well as a precautionary method during the second phase, and the DB-624 column did not become contaminated at any time during this phase of the experiment. Therefore, no part of the column had
to be sacrificed, and no rinsing and reconditioning of the column was necessary
during the second phase.

The solvent rinse system that was used for the injector system cleanup
was fairly easily constructed from spare parts that were in the laboratory as
described in the methods and materials section. In addition, manufactured
solvent rinse systems can be purchased from companies that manufacture GC
systems and appurtenances. However, for use in smaller municipality
laboratory, it would be better for an on-line solvent rinse system to be developed
that would allow the operator to set valves and switches to run the system. The
system used in this study had all of the components necessary to complete the
solvent rinsing but required the operator to disconnect the carrier gas supply to
the injector system within the GC pneumatics system. Also, the operator had to
be fairly dexterous at handling hazardous solvents as detailed in the materials
and methods section.

Based on the results of the first phase of the experimentation, the
decision was made to set the autosampler volume to 0.5 μL for the second
phase of the procedure to prevent flooding of the detector and to allow more
continuous analysis without having to wait for the detector to recover. The
initial belief in the first phase of the experiment was that a 1 μL sample would be
easier to accomplish for a more reproducible method. However, the 1 μL
sample was too large to be used consistently for this method, and should be
replaced by using the autosampler set at 0.5 μL. Even experienced GC
analysts should seriously consider using an autosampler for this procedure to
prevent phase stripping on the megabore column due to the injector needle
scraping the inside of the column.
Sample Handling

In response to the fact that the solvent extraction procedure in the first phase was allowing some water to be included in the injected sample, I decided to increase the solvent extraction volume from 1 mL to 2 mL for the second phase of the study in hope of making the extraction procedure easier and more effective. The gas tight micro syringes were increased in size from 1 mL in the first phase to 2.5 mL in the second phase. The syringe tips were also changed from a 26 gauge permanent needle in the first phase to a 21 gauge disposable needle and luer lock fitting in the second phase. The syringes in the second phase were easier to handle and use accurately, and the needles were less prone to bending or breaking at the interface with the glass syringe. Also, there was less chance of carry-over contamination from the needles since the needles were disposed of after each sample separation.

The method of using the centrifuge bottle during the contact phase of the experiment was eliminated during the second phase since it was almost impossible to get a representative sample on an ongoing basis using Henderson’s extraction method with the centrifuge bottle. There was no way to pressurize the centrifuge bottle to simultaneously remove a portion of the sample from the bottle while injecting the hexane. This caused an inaccurate withdrawal and injection, sample overflow from the bottle, and allowed for increased sample volatilization.

The procedure was modified by incubating all sample in the borosilicate glass serum bottles sealed with the rubber stopper sleeves. At the end of the contact period, the rubber sleeve stopper was removed and the sample was poured into the centrifuge bottle which was sealed and centrifuged as before.
The serum bottle was then filled with deionized water and resealed. The bottle then was extracted by the standard method used throughout the experiment and analyzed on the GC to provide a value of the chlorinate compound which was sorbed onto the glass. The centrifuged sample was separated, and the supernatant and pellet were analyzed as in phase one.

The shaker bath which was initiated in phase one was used to control temperature and to keep the diatoms suspended. The method did not do a good job of keeping the diatoms suspended as they settled out every 15 minutes or so. The procedure of inverting the bottles every hour during the first phase partially addressed this issue, but may have introduced an artifact into the procedure by limiting the actual contact time some of the diatoms were experiencing. I partially addressed this problem, however, by allowing a 24-hour contact time during the first phase. As the second phase of the experiment was looking at shorter contact times to achieve equilibrium, the means of keeping the diatoms suspended had to be addressed by a different means.

A Fisher-Kendall mixer was modified as described in the methods and materials section to be able to hold up to six serum bottles with attached bottle clamps. This mixer was allowed to rotate at a slow constant speed on an axis turning parallel to the floor or table during the contact period. This procedure kept the diatoms in suspension by constantly turning the bottles end-over-end during the contact period. The constant temperature control was lost by using this method. However, I decided since the biomass was not alive, and the room was maintained in a temperature range of 26°C to 29°C (depending on whether the GC oven was on or not), that the constant temperature bath was not as important to the process as the mixing aspect. If an experiment were being
conducted with live biomass, the experimenters would want to consider using a mixing method such as the one used in phase 2 of this experiment within the confines of a constant temperature room.

The contact time during the second phase of the experiment was varied to see if it was necessary to allow a 24 hour contact time for these particular compounds and for this specific diatom to reach equilibrium conditions. The contact times were reduced to 2, 4, 6, 8, 10, and 12 hour periods. The results of the reduced contact time are discussed in the next section.

Several of the samples were lost during the second phase of the experiment due to a malfunction of the autosampler system. The adjustment of one of the cams that controlled the insertion depth of the autosampler syringe needle into the GC sample bottle was sticking causing the cylinder not to fully extend. The result of this mechanical malfunction was that the sample bottle was not properly pressurized, and therefore the autosampler was not withdrawing a liquid sample from the sample vial. The chromatograph printouts of these samples provided an inaccurate and much reduced response representative of whatever concentration the gas tight syringe was able to withdraw from the headspace of the sample bottle. Again, a problem such as this pointed out the fact that the operator of the GC system had to be well enough versed in the operation of its components to monitor the operation of the system and to realize when certain components were malfunctioning and needed to be adjusted or repaired.
External Standard

The external standards used in the first phase as purchased from Varian were not continued in the second phase due to the possibility of contamination of the system and confusion of the results by purposely introducing chlorinated compounds into the analysis which were not part of this specific study. The use of the external standards should still be considered by the municipalities using this procedure to develop a reference printout for a range of chlorinated compounds depending on the purposes for which they are using the analysis method (i.e. raw water analysis or treated water analysis). However, for the purpose of this experiment the stock solutions developed for the TCP or TCE were used as the external standard throughout the second phase of the work to limit contamination of the system.

Detector

The ECD was replaced with the FID in the second phase of the analysis. The FID is discussed in the methods and materials section of the text. The ECD worked well in the first phase analysis, however, it was a more sensitive and delicate instrument to deal with. Also, the operator had to have a greater base of knowledge and skill to deal with the requirements of running the detector using a radioactive source. The FID was not as sensitive as the ECD and could not provide the results that the ECD would in the 1 ppb area. However, it was rather simple to use and performed well in the 50 μg/L to 100 μg/L range and at higher concentrations for laboratory analyses.
For this particular analysis, the FID functioned well until one point in the experimentation where the detector flame went out and the GC flameout device would not relight the detector. Initially it was presumed that the sample could have flooded the detector (although this was puzzling since the sample size had been reduced 50%). When continued attempts to restart the FID failed, the FID was disassembled, and it was discovered that the ferrule that sealed the hydrogen supply to the flame had broken. The cracked ferrule caused the FID to get an improper mixture of air and hydrogen, and the flame wouldn't stay lit. This situation pointed out that even though attempts were made to simplify a process such as this GC separation, there were certain aspects which a laboratory technician, who did not understand the function of the particular GC being used, would not be able to remedy without the assistance of a manufacturer's representative.

**General Analysis**

**Data**

The six values for the area under the curve from each GC sampling run (see Figures 2 and 3) were compared to the value for the known concentrations of the external standards which were run to determine the concentration of the chlorinated compounds for the following samples:

1. deionized water at t₀,
2. deionized water at tᵣ,
3. total sample including the concentration on the bottle at tᵣ,
4. bottle at tᵣ,
5. supernatant or aqueous equilibrium at $t_f$, and
6. pellet at $t_f$.

The values for 1 and 2 were used as a check for losses due to volatilization during the contact time. The total sample concentration for 3 was used as the starting concentration. The supernatant concentration for 5 was used as the aqueous equilibrium concentration. The equilibrium concentration on or in the pellet was calculated by subtracting the supernatant and bottle concentrations from the total concentration. This value was compared to the measured pellet concentration, and the higher number of the two values was used as the equilibrium sorption concentration for the experiment at that contact time to be conservative. (See Table 5 for the data values.)

I adopted this analysis method for the reasons mentioned in the literature review (24, 27). It was not possible to resuspend the pellet in deionized water (which had no concentration of the chlorinated compound in question) to be extracted successfully by a once-through method such as was used in this study without performing a series of extractions to remove all of the sorbed compound from the solid. Since this experiment was only set up for one extraction, the chlorinated compounds were most likely not completely extracted from on or in the solid. The calculated value of the pellet concentration provided the most conservative value of the sorption potential of this diatom for TCP and TCE.
<table>
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<tr>
<th>SUPERNATE C&lt;sub&gt;equilibrium&lt;/sub&gt;</th>
<th>BOTTLE TOTAL</th>
<th>TOTAL SOLIDS</th>
<th>CALCULATED SORPTION CONC.</th>
<th>CALCULATED Q&lt;sub&gt;equilibrium&lt;/sub&gt;</th>
<th>LOGQ</th>
<th>LOGC</th>
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(Table Continues)
Table 5 (Continued)

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<th>TOTAL SOLIDS</th>
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<th>CALCULATED SORPTION CONC.</th>
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<th>LOGC</th>
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<td>(g/L)</td>
<td>(ug/L)</td>
<td>(ug/g)</td>
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Isotherm Development

A simple linear regression procedure was performed on the data for TCP and for TCE using the base 10 logarithm of the equilibrium sorption value (Log Q) versus the base 10 logarithm of the aqueous concentration equilibrium value (Log C) (see Table 6 and Table 7). The result of the regression procedure is called the Freundlich Isotherm which is defined mathematically as follows:

\[ Q = KC^n \]  \hspace{1cm} (6)

\[ \log Q = \log K + n \log C \]  \hspace{1cm} (7)

- \( Q \) = equilibrium sorption density of the chemical compound on the biomass, (\( \mu \)g substance/g cell mass)
- \( C \) = aqueous phase equilibrium concentration (\( \mu \)g substance/ L \( H_2O \))
- \( K \) = Freundlich Constant
- \( n \) = Freundlich exponent

Graphical representations of the Freundlich Isotherm for TCE and TCP are shown in Figures 5 and 6, respectively.
Table 6

STATISTICAL ANALYSIS FOR TCE ISOTHERM

DEP VAR: LOGQ  N: 9  MULTIPLE R: 0.946  SQUARED MULTIPLE R: 0.896
ADJUSTED SQUARED MULTIPLE R: 0.881  STANDARD ERROR OF ESTIMATE: 0.195

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<tr>
<th>VARIABLE</th>
<th>COEFFICIENT</th>
<th>STD ERROR</th>
<th>STD COEF TOLERANCE</th>
<th>T</th>
<th>P(2 TAIL)</th>
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ANALYSIS OF VARIANCE

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<th>MEAN-SQUARE</th>
<th>F-RATIO</th>
<th>P</th>
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Resultant Equations:

\[ \log Q = 1.094 \log C - 0.041 \]
\[ \log BCF = 0.094 \log C - 0.041 \]

Typical Values of BCF:

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<th>BCF (L/g)</th>
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Table 7
STATISTICAL ANALYSIS FOR TCP ISOTHERM

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<th>STD COEF TOLERANCE</th>
<th>T</th>
<th>P (2 TAIL)</th>
</tr>
</thead>
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ANALYSIS OF VARIANCE

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<th>SOURCE</th>
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<th>MEAN-SQUARE</th>
<th>F-RATIO</th>
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Resultant Equations:

\[
\log Q = 1.056 \log C - 0.418 \\
\log BCF = 0.056 \log C - 0.418
\]

Typical Values of BCF:

<table>
<thead>
<tr>
<th>C equilibrium (μg/L)</th>
<th>BCF (L/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.38</td>
</tr>
<tr>
<td>1,000</td>
<td>0.56</td>
</tr>
<tr>
<td>10,000</td>
<td>0.64</td>
</tr>
<tr>
<td>100,000</td>
<td>0.73</td>
</tr>
</tbody>
</table>
Figure 5

**TCE ISOTHERM**

Log $Q = 1.094 \log C - 0.041$

Based on Control tf

Log $Q$ vs. Log $C$ graph with data points for Control tf, Total tf, and Control to.
$\log Q = 1.056 \log C - 0.418$

Based on Control tf

Figure 6

TCP ISOTHERM
**BCF Regression Relationships**

Once the statistical analysis was completed, and the regression equation for the Freundlich isotherm was calculated, the bioconcentration factor as described in the literature review section was obtained as follows:

\[
\text{BCF} = \frac{Q}{C} \ (L \ H_2O / g \ cell \ mass) \quad (8)
\]

so, \( \text{BCF} = KC^{n-1} \) \quad (9)

and, \( \log \text{BCF} = \log K + (n-1) \log C \) \quad (10)

The mathematical expression for the calculated BCF and the values of the BCF at various aqueous equilibrium concentrations are presented in Table 6 and Table 7.

The comparison of the BCF expressions for TCE and TCP which were determined in this laboratory analysis were also presented in Table 2 in comparison with the regression equations determined by Smets and Rittman (5) for the sorption of TCE on three types of green algae. Figure 7 shows the graphical representation of all three of Rittman’s regression equations for the BCF of TCE on green algae plotted along with the two resulting BCF regression equations for TCE and TCP on *Thalassiosira pseudonana* from this study.
Figure 7

BCF REGRESSIONS
BCF and Octanol Water Partition Coefficient

The two regression equations (5, 26) for the relationship between BCF and $K_{ow}$ presented a simple relationship which could be useful if it were actually applicable to a wide range of situations. Figure 8 shows the graphical representation of these regression equations which evaluated the relationship between Log BCF and Log $K_{ow}$. However, the relationship presented in these two regressions oversimplified the interactions that took place in the sorption process and were useful as a predictive model only for the specific type of algal biomass and the specific type of organic compound involved in the study. One could also go further and say that the relationships derived apply only at the approximate biomass and chemical compound concentrations used in the study.

A regression relationship between BCF and $K_{ow}$ could still have value as an operational and monitoring tool to a water supply manager of a specific facility even though it wouldn’t have wide application to other facilities. Field samples could be taken of a raw water supply (river) or storage (reservoir) facility. Biomass density determinations would have to be made in the laboratory from the field samples. Aqueous chemical concentrations could be determined using the Henderson et al. extraction method and the GC separation method developed in this study. Sorbed chemical concentrations on the biomass could be determined using the same methods as presented in this study. A regression relationship could be determined for that particular supply or storage facility for the consortium of algae that were present in the water. The relationship would provide the BCF for each chemical compound on or in the biomass present versus the $K_{ow}$ of the individual compounds. The relationship
Figure 8
LOG BCF VS LOG Kow
would also give some insight into the interactive or competitive effects of the various compounds present. The regression would be useful for evaluating the supply or storage facility water quality and for consideration of necessary treatment processes in the plant or pretreatment processes before the plant depending on the biomass density and chemical sorption on that biomass.

The Freundlich isotherm relationship that led to the Log BCF versus Log C relationship held more promise for the development of a model of understanding of the relationships which occurred to produce the biosorption potential that I was measuring.
CHAPTER V
SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

Summary and Conclusions

Sorption Results

Sorption experiments using preserved, concentrated diatoms to evaluate the sorption potential of TCP and TCE on the diatoms were successfully conducted in the Civil Engineering laboratories at The Ohio State University. The resultant data from these experiments led to the development of Freundlich isotherms which in turn led to the development of regression relationships for the BCF of these two compounds on the diatom *T. pseudonana*. The Freundlich isotherms determined were:

\[
\begin{align*}
\text{TCE: } \log Q &= 1.094 \log C - 0.041 \quad (11) \\
\text{TCP: } \log Q &= 1.056 \log C - 0.418 \quad (12)
\end{align*}
\]

The BCF regressions developed from these isotherms were:

\[
\begin{align*}
\text{TCE: } \log \text{BCF} &= 0.094 \log C - 0.041 \quad (13) \\
\text{TCP: } \log \text{BCF} &= 0.056 \log C - 0.418 \quad (14)
\end{align*}
\]

The regression equations developed indicated significantly lower BCF's for these two compounds on this diatom in an aqueous phase equilibrium concentration range of 1 \(\mu\)g/L to 1 mg/L than had been predicted using the regression equations developed by Smets and Rittman (5) in a study of TCE and green algae. Casserly et al.'s (6) regression relationship between BCF
and $K_{ow}$, which was based on data from an isotherm experiment using green algae and other organic chemicals besides TCE and TCP, also predicted higher BCF's for TCP and TCE than were found in this study. Still another study by Mailhot (26), using green algae and other organic compounds besides TCE and TCP, produced a regression relationship which predicted a higher value for the BCF of TCP and a lower value for the BCF of TCE than was predicted in this study.

**Past Studies**

Several obvious problems existed in comparing the sparse literature with the current study. The studies of record, except for Rittman's study (5), did not involve the use of TCE or TCP. Most of the past studies involved indirect measurement of the sorption potential by measuring the distribution of radioactive labeling rather than an actual compound concentration. Some of the past studies did not take into account the sorption potential for the chemical compounds on the system glassware, etc. Also, in addition to the different chemical compounds used, none of the studies of record which developed regression relationships used diatoms as the study biomass.

It was apparent from the past literature review and from the study just completed that the regression relationship between BCF and $K_{ow}$ was very specific for a certain combination of chemical compounds and biomass, and almost certainly for a specific source water and all of its specific chemical parameters. A regression relationship between BCF and $K_{ow}$ for a specific reservoir would probably be of use to the facility operators as an operation tool. Beyond such a specific use, one would have to be very careful and selective
about using a relationship developed in one source water situation to be
applied in another. Specifically, the source water chemical parameters,
biomass type and distribution between types, and organic compounds of
interest would have to be evaluated before attempting to use such a
relationship in a different location from the one in which it was developed.

Methodology

The current study was successful in developing a new, simplified pro­
cedural method which could be applied to the analysis and evaluation of raw or
treated water supplies for the identification and quantification of chlorinated
hydrocarbons. The method developed in this study resulted from modifying an
extraction method developed in another study (67), and combining the results
of the extraction process with a modification of a packed column GC to take
advantage of the efficiencies in separation technology now offered by “off-the­
shelf” capillary column products. The method developed could be adopted and
successfully run by water treatment personnel at a municipal treatment
plant with a small amount of technical training. Also, the method could be
developed into a packaged “off-the-shelf” system either to be used by
municipalities for continuous on-line monitoring of water quality or to be used by
researchers and consultants for field studies of groundwater or surface-water
chemical quality.

The problems encountered in the first phase of the study related to
determining the proper sample size for the column and detector of choice, and
the GC oven and injector temperature settings for the analysis. Also, the
problems encountered in the extraction procedure related to the volume of the
solvent used in the extraction process, and to the type of glassware and type of mixer used during the contact period to keep the diatoms suspended. These operational parameters were all optimized during the first phase of the analysis, and the system worked very well during the second phase of the study.

The problems encountered in the second phase of the study all related to mechanical malfunctions of the instrumentation. A cam that controlled the needle injection in the sample vial and, therefore, proper pressurizing of the sample bottle malfunctioned through some of the analysis causing only headspace gases to be injected into the column, and in essence causing the loss of the sample to the study. A problem with the restart mechanism for the FID flame was actually caused by a cracked ferrule which led to an improper mixture of air and hydrogen to keep the flame lit.

These problems pointed out the need for ongoing maintenance of a laboratory instrument such as a GC which was used in this separation technique. Most municipalities which would undertake the evaluation of chlorinated chemicals in their water supplies through this type of technique should definitely enter into a maintenance contract with the GC company which supplied the instrumentation.

The problems of actually measuring the components of the mass balance in this study which were encountered in the few past studies that had been done were not completely overcome. The type of glassware and sealed extraction system used in this experiment would not allow multiple extractions to be performed accurately when volatile chemicals were the compound of interest. Also, it would have been very difficult to accurately remove the solvent from one extraction and then to apply new solvent for another extraction of the same sample. The procedure used probably resulted in an underestimation of
the amount of the compound which was sorbed in or on the pellet. A procedure for estimating the number of extractions required to completely extract the compound from the resuspended pellet was presented earlier in the discussion. The amount of the compound sorbed in or on the pellet was calculated by the values determined for the other samples in the procedure.

Determining the magnitude of the compound sorbed to the biomass in this manner should have erred on the side of overestimating the actual bioconcentration potential since the mass balance was balanced by attributing all of the compound that was in the supernatant or on the glass to being sorbed on the pellet. Having made this comment, it is even more interesting that the BCF's determined through this study were lower than those that might have been expected based on the previous literature concerning sorption on green algae.

Lipid Content

Finally, some comment needs to be made concerning the lipid content of the diatoms used in the study. As discussed in the literature review, the few studies that have been completed to try to quantify the sorption of organic compounds on algal biomass have speculated that the increased sorption potential or BCF that they have observed (greater than would be expected for soil or other sediments of similar organic content) may be due to the lipid content of algae. Perhaps the organic compound was being absorbed or partitioning into the lipids inside the algal cell was occurring in addition to the adsorption that was occurring on the outside of the algal cell.
Because the lipid content of the green algae *Selanastrum* was on the order of 6%, this study hypothesized that a coastal diatom such as *T. pseudonana* with 20% or greater lipid content would exhibit an even greater BCF than that which had been experienced in the previous studies with green algae. Since diatoms in general have a greater lipid content than the green algae at any given time during the year, there appeared to be potential for greater sorptive capacity that could be beneficial environmentally if properly understood and enhanced in contaminated source water systems. The resultant BCF regressions were actually quite disappointing in comparison to previous studies which were completed concerning the green algae and chlorinated compounds.

**Surface Adsorption Phenomenon**

Although sufficient data were not developed by this study to conclusively detail why the diatom sorption potential proved to be lower, some hypotheses can be made. Some of the problems with previous studies have been discussed earlier in the text, and it has been stated that the potential measured and reported in previous experiments may have overestimated the actual bioconcentration potential in green algae. However, the case made for lipid absorbance or linear partitioning in the green algae causing a higher sorption potential than would be expected in other forms of matter with similar organic content may be true. The fact that the diatoms with higher lipid content do not exhibit similar bioconcentration potential as would be expected based solely on the lipid content may also be true.
The cell wall of the diatom is composed primarily of glass (SiO₂) (29). The silicon content of the diatom shell may actually control the sorption process and cause the isotherm of organic compound sorption to be more dependent on competition for surface adsorption sites rather than on partitioning into the lipids contained inside the diatom cell even though the lipid content is considerably higher than that of green algae. The organic content of SiO₂ is very low (f_{oc} < 0.0001 (72)) leading one to believe that linear partitioning would also be very low or at least inhibited by the glass cell wall. However, the surface area for Lichrosorb 60 (Merck), a diatomaceous earth material was very high (500 m²/g) in Schwarzenbach and Westall’s study (72) using porous silica particles with an average particle diameter of 30 μm. This large surface area could be indicative of a major surface adsorption phenomenon.

A linear isotherm would be indicative of simple partitioning of the organic compound out of the water into the organic phase. A nonlinear isotherm (log-log for example) would indicate a surface adsorption phenomenon such as competition for adsorption sites was occurring instead of or in addition to sorption or partitioning into the cell lipids. It was not possible to distinguish between the different rates of sorption or the different sorption processes that were occurring by the methods employed in this study.

As has been previously discussed, if partitioning of the organic compound into the cell lipid were the only mechanism contributing to biosorption in algae, the BCF would equal the linear partition coefficient (K_p). By definition:

\[ \log K_p = \log K_{OC} + \log f_{OC} \]  

(15)
Schwarzenbach and Westall (72) have shown that:

\[ \log K_{OC} = 0.72 \log K_{OW} + 0.49 \quad (R^2 = 0.95) \quad (16) \]

for a series of nonpolar organic compounds with \( K_{OW} \) ranging from 1.59 to 6.72. Therefore, by substituting their equation into the definition for \( K_p \), the following relationship can be derived:

\[ \log K_p = 0.72 \log K_{OW} + 0.49 + \log f_{OC} \quad (17) \]

From the results of this study and the previous study (5) performed with green algae, the BCF was found not to be equal to \( K_p \). The \( K_p \) predicted by the above equation for TCE in or on S. capricornatum assuming an organic content of approximately 6% was 8.5 which is considerably higher than the range of 1.4 to 5.4 for the BCF found by Rittman (5) for TCE over an aqueous equilibrium concentration range of 1 \( \mu \)g/L to 1 mg/L. If this equation were considered for a coastal diatom with an \( f_{OC} \) of 0.25, \( K_p \) values of 35.6 and 21.6 for TCE and TCP, respectively, would be predicted. Obviously the sorption in diatoms is controlled by some other process in addition to the linear partitioning process which reduces the sorption potential that would be predicted by the linear partition theory. Although sufficient data was not generated by this study to conclusively say what the other process was that reduced the linear partition potential, surface adsorption to the SiO\(_2\) cell wall of the diatoms, along with the fact that the cell wall may reduce the diffusion potential to the lipids in the cell interior seem to be plausible reasons which could lead to a formulation of the BCF equation as follows:

\[ \text{BCF} = \log K_{OC} + n \log f_{OC} \quad (18) \]

Where, \( n \) represents the diffusional resistance factor based on the cell wall
material coupled with the surface adsorption phenomenon that is occurring for the specific algal-organic compound pairing in question.

**Recommendations for Future Research**

**Product Development**

The recommendations for future research to follow-up on this study can be divided into the two main areas of development of practical applications and further basic research. This study successfully showed that a modified extraction technique could be combined with an on-column injection into a GC capillary column run at an isothermal temperature to determine and quantify the presence of chlorinated organics in water from a raw water or treated water source. Further work could be done to develop a working prototype of a GC machine that could be used by municipal laboratory technicians to test their water supplies on a daily basis in-lieu of more complicated purge and trap techniques, using temperature programmed GC-MS and sophisticated separation and concentration techniques which require a specialized degree of training, high equipment costs, and a great deal of time to complete.

Specifically it is suggested further work be undertaken to design an on-line solvent rinse system which could be used for ongoing maintenance of the injector system to reduce contamination from sample backflash. The results of this study suggested that the isothermal temperature needs to be set at 100°C to 105°C to make sure that any water that gets into the extraction is volatilized. If a way of improving the extraction technique to reduce water interference
without making it too complicated could be developed, the isothermal temperature could be reduced, thereby reducing the chance of backflash and injector contamination. If the chance of contamination could be reduced, the time of the cycle between solvent rinses could be increased.

**Laboratory Methodology**

Further work should be done running the ECD with all of the procedural modifications that were adopted in the second phase of the study. It is believed that the modifications that led to the success of the FID process would also improve the results and stability of the ECD process. The ECD was a much more sensitive detector than the FID and may be more to the liking of some analysts for its increased sensitivity. However, the results of the FID were found to be very satisfactory, and it was found to be a more robust and forgiving mechanism to the untrained operator. For most field studies or treatment process monitoring, the ECD would need to be used due to the low organic concentrations typically experience.

Additional work could be done to evaluate, modify, and optimize the portion of the procedure that resuspends and extracts the organic compound from the pellet. The method used in this study did not provide a complete extraction of the organic compound from the pellet, and in most cases underestimated the concentration of the organic compound on the pellet. Also, additional evaluation of the glassware used in the centrifugation process, could lead to a change in the type of cap used. If a cap with a septum which would allow injections from a gas tight syringe could be used during the centrifugation, the procedure could be modified and some of the sample transfers and
additional glassware could be eliminated. This would probably be better for the overall procedure as it would eliminate losses due to volatilization and possible additional sorption factors on system glassware.

**Additional Field and Laboratory Study Topics**

In addition to the chlorinated compounds that are being evaluated in this study and have been evaluated in past studies, it is suggested that further practical research be undertaken to evaluate the sorption potential for soluble organic chemicals (SOC) representative of the agricultural pesticides being used that affect the raw water sources of many municipal water utilities in the Midwest. These studies should look at sorption potential for a consortium of green algae and diatoms representative of the raw water source being evaluated. There is also great potential for using the methods developed in this study for evaluation of insitu conditions in raw water supplies for chlorinated organics or for SOC. For example, samples could be taken in the field at the upground reservoir supplying a town which were representative of the algae concentration in the reservoir. These samples could be extracted and GC separation could be undertaken on-site in the field with a portable GC, or samples could be sealed and refrigerated and taken back to the lab as long as samples could be analyzed in a fairly short time period. Consideration would have to be given to a possible shift in the equilibrium conditions between phases if too long of a contact period were allowed before laboratory analysis.

Also from a practical viewpoint, consideration should be given to combining the effects of volatilization, mixing, and sorption in a laboratory
analysis to be able to quantify the magnitudes of the different phenomena in a raw water supply that is being used by a municipality.

There are many topics that could be considered from a basic research perspective to continue this work. The results of this study indicated a considerably lower sorption potential for diatoms than had been predicted by green algae studies or than would have been predicted by linear partitioning theories. Other studies have indicated that the sorption potential in algae is approximately the same whether the algae are live or dead. Future studies are needed to evaluate whether in fact the sorption potential for live diatoms is greater than that of dead diatoms. It should be reasonable that the magnitude of the uptake of the organic compound into the live cell at equilibrium would be equivalent to the maximum specific uptake of the cell as predicted by Powell's equation (84). This value could be a bigger percentage of the value predicted by linear partitioning theory than that experienced in this study with the dead diatoms which was probably controlled by the surface adsorption phenomenon due to the cell wall structure.

Along with future research using living cells, evaluation of the change in the biomass concentration during the contact period due to population growth factors should be considered as this has not been evaluated by any previous investigators using live cultures, and contact periods as long as 24 hours are being used to reach equilibrium. Also, experimentation should be done in constant temperature rooms to evaluate the effect of different temperature ranges on the sorption potential of live algae.

Additional theoretical and experimental work needs to be done to separate and quantify the surface adsorption and diffusion-linear partitioning phenomena that have been suggested by this study and to some extent by
other researchers. In addition to separating these phenomenon, more thought and experimentation should be put into evaluating Cox's (10) theory that the standing crop density controlled the magnitude of the observed sorption phenomenon. Obviously if linear partitioning theory controlled the situation, Cox's statement would be incorrect. However, the surface adsorption phenomenon may control the observed BCF at least in diatoms. This could be checked by running a series of experiments over a range of biomass solids concentration for each initial aqueous concentration of the organic compound.

There is also a need for further laboratory analysis of the competitive factors that could be going on in a raw water source. These studies should look at evaluating the sorption of a consortium of algae for single and multiple organic compounds. Competitive factors also need to be evaluated for a mixture of compounds with axenic cultures of algae.

TCP Treatability

Beyond the basic and applied research needed for the topic of biosorption in algae, and the additional work needed in the development of the extraction and separation technique presented in this study, further work is needed in the evaluation of TCP. The data presented in the Risk Reduction Engineering Laboratory (RREL) Treatability Database (80) for TCP was very sparse, and some of the data was incorrect based on the information found in the literature search for this study. Research has indicated that the occurrence of TCP in North America has become more widely recognized, and the USEPA should be ready to promulgate a maximum contaminate level for this compound in drinking water supplies. The only current treatment process that has been
evaluated for the removal of TCP is granular activated carbon adsorption. It is recommended that further research on treatability studies for TCP in general be conducted, and specifically evaluation of reverse osmosis for TCP removal is suggested.
CHAPTER VI

BIBLIOGRAPHY


Bibliography (Cont.)


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Bibliography (Cont.)


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