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Acid dissociation of chloroguaiacols and pH dependent sorption to estuarine sediments

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Acid Dissociation of Chloroguaiacols and pH Dependent Sorption to Estuarine Sediments

A Dissertation

Presented to

The Faculty of the School of Marine Science
Virginia Institute of Marine Science
The College of William and Mary in Virginia

In Partial Fulfillment
Of the Requirements for the Degree of
Doctor of Philosophy

by

Jennifer Lisa Gundersen
1995

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APPROVAL SHEET

This dissertation is submitted in partial fulfilment of
the requirements for the degree of

Doctor of Philosophy

Jennifer Lisa Gundersen

Approved, April, 1995

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DEDICATION

In loving memory of my mother

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I would like to extend sincere thanks to all those who made this work possible. Recognition must first go to my committee members. Dr. Robert C. Hale took me under his wing way back in '88. He provided technical, financial and moral support for lo these many years, taught me the right way to do analytical chemistry and gave me the freedom to pursue new interests, no matter how strange. I will be forever in his debt. Thanks to Dr. William G. MacIntyre for his always intriguing perspectives and our subsequent debates on life, the universe and everything, for showing me that P-Chem can be fun and for teaching me that since we don't understand the structure of water, none of it matters anyway. Thanks to Dr. Robert J. Huggett, who provided me with invaluable opportunities to work with researchers worldwide and made sure I had the supplies I needed when I was actually at VIMS. Thanks also to Drs. Howard I. Kator and Richard T. Di Giulio for their reviews and critiques of my prospectus and this manuscript (I will forever know the difference between regression and correlation).

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The Chesapeake Bay Yacht Clubs Association supplied the funding that enabled me to purchase chloroguaiacol standards and other equipment and is gratefully acknowledged.

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ABSTRACT

Chloroguaiacols are common components of bleached kraft pulp mill effluent known to bioaccumulate in aquatic organisms, and sorb onto particulate material and sediments. Ionization properties of the chloroguaiacols must be known in order to explain their sorptive behavior in aquatic systems. To obtain this information, thermodynamic acid dissociation constants (pK_a) of guaiacol (*o*-methoxyphenol) and nine chloroguaiacols have been determined by spectrophotometric methods. These experimental pK_a values have been compared with literature values, values predicted by the Hammett equation and predictions by quantum chemical calculations using MOPAC 6.0 with AM1 and MNDO Hamiltonian operators. Experimental and predicted acidities were linearly related, and the correlation and regression equations obtained have been used to calculate pK_a values for those chloroguaiacols that were unavailable for experimental measurement.

Sorption of chloroguaiacols onto sediments may result in reduced bioavailabilty to aquatic species, increased degradation rates or rapid burial. Conversely, it may result in increased exposure to deposit feeding species with subsequent transfer to higher trophic levels. The sorption of four chloroguaiacols (5-chloroguaiacol, 4,6-dichloroguaiacol, 4,5,6-trichloroguaiacol and tetrachloroguaiacol) with pK_a values ranging from 9.06 to 6.06 was investigated on four estuarine sediments. The effect on sorption of aqueous phase pH, sediment organic carbon content and degree humification was investigated. Sorption coefficients, K_d, were determined at two pH values (6.6 and 7.8). Linear relationships were observed between K_d and both %total organic carbon(TOC) and %humic acids at each pH. K_{oc} and K_{humic}, sorption coefficients normalized to %TOC and %humic acids, were calculated. In addition to the amount of TOC present, the type of organic matter (ie. humic acids) is an important factor in sorption.

Aqueous phase pH governs the ionization of chloroguaiacols. Sorption of the ionized and molecular species are different due, in part, to the different solubilities of the species and was reflected in the different K_d values determined at each pH.

ACID DISSOCIATION OF CHLOROGUAIACOLS AND pH DEPENDENT SORPTION TO ESTUARINE SEDIMENTS.

Chapter I

General Introduction

The first writing paper, produced from mulberry bark, was made in China by Ts'ai Lun in the year 105. For centuries after that paper was made by the sheet, by hand, from bamboo and old rags. In 1798, a machine was developed to make a continuous sheet of paper, revolutionizing its production. As larger quantities of paper were produced, old rags became more scarce and a new source of fiber was necessary. Wood became, and remains, the primary fiber source for the paper industry (Leokum, 1965).

Three basic wood pulping processes are in general use today. These include: kraft (or sulfate), sulfite and mechanical pulping (McLeay, 1987). The kraft process is, by far, the most widely employed. In this process, wood chips are digested under pressure, at 160-180°C. Sodium hydroxide and sodium sulfide dissolve most of the lignin and other soluble compounds, known collectively as extractives. This leaves only the insoluble cellulose fibers, or pulp, along with some residual lignin which imparts a characteristic brown color. Unbleached pulp is suitable for the production of items such as paper grocery bags. Bleaching processes are used to brighten the pulp for white paper products. There are two main types of pulp bleaching: those that complete the delignification process such as chlorination, and those that chemically oxidize or reduce the chromophoric groups (Minor, 1982).

Chlorination is by far the most commonly used bleaching method. The process of pulping and bleaching is a multi-stage operation that can vary from mill to mill, although some steps are common to all. The most common stages include: chlorination (abbreviated as C), caustic extraction (E) and treatments with hypochlorite(H), chlorine dioxide (D), a mixture of chlorine and chlorine dioxide (C/D), oxygen (O), peroxide (P) and ozone (Z). Typical pulping and bleaching sequences are represented as CEDED or (C/D)EHDED. Oxygen is now being used by many mills as a prebleaching step, in sequences such as OCEDED. In response to increasing regulatory pressures to reduce chlorinated organic byproducts, new processes involving total chlorine free (TCF) and elemental (as in Cl_2) chlorine free (ECF) bleaching are being developed.

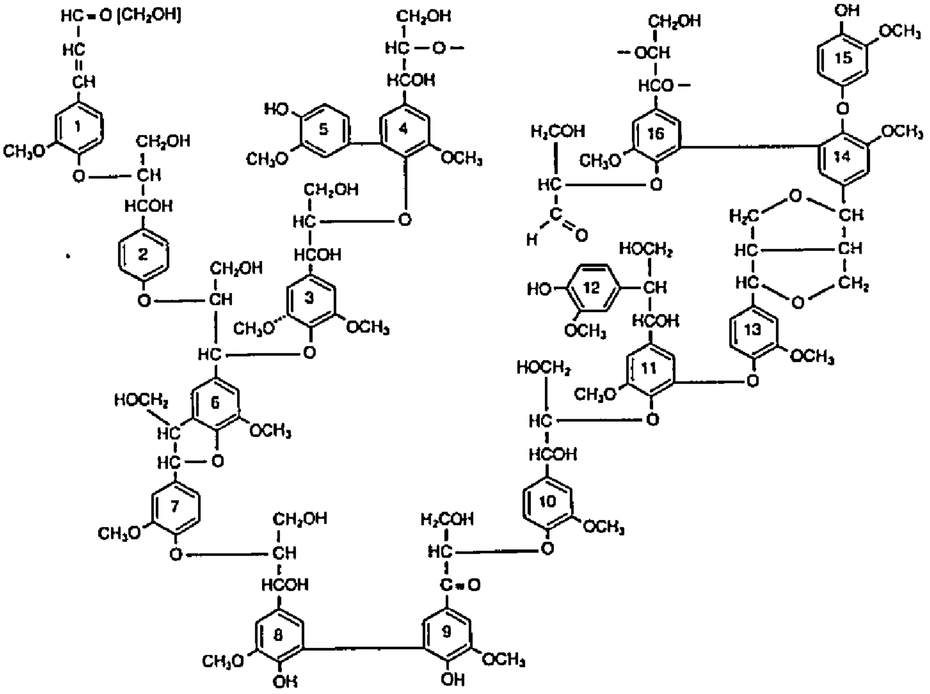
The pulp and paper industry in the U.S. and worldwide has recently become subject to close environmental scrutiny. Recent reviews (Suntio *et al.*, 1988; McLeay, 1987; Voss *et al.*, 1981; Owens, 1991) have shown that hundreds of chlorinated organic compounds, by-products of the pulping and bleaching processes, are present in pulp mill effluents at levels ranging from parts per million to parts per trillion. These compounds are considered to be a significant source of toxicity to organisms in the waters surrounding the mills and are now the target of regulatory action. (Stanley, 1995.)

Lignin (Figure 1) is a highly complex, aromatic polymer. Its breakdown during pulping and bleaching spawns a multitude of byproducts, many of which

Figure 1.

Primary Structures of Lignin

(from Kringstad *et al.*, 1984)



HC=O (CH₂OH)
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
CH₂OH
HOCH₂
H₂COH
HCOH
OCH₃
CH₃O
HO
OH
C=O

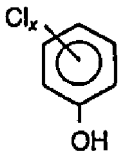
remain uncharacterised (Owens, 1991). Known components of pulp mill effluents include several classes of compounds including resin acids, fatty acids, aromatic and polynuclear aromatic compounds. Chlorinated compounds including phenols, guaiacols (*o*-methoxyphenols), catechols (*o*-dihydroxybenzenes), veratroles (dimethoxybenzenes) and syringols (dimethoxyphenols) have been detected in the effluents (Figure 2). In this dissertation physical properties affecting the fate of chloroguaiacols in aquatic systems are examined and methods to determine them in aquatic systems are evaluated.

The amount and types of organochlorine produced is a function of the overall process, as well as the type of wood used. Hall *et al.* (1989) examined wastewater, at various points throughout the pulping and bleaching processes, from four different Canadian paper mills. All of the mills used slightly different bleaching procedures and all but one used softwoods, such as pine or spruce. The amount of total organochlorines discharged from each plant varied greatly. Among the softwood plants, the study showed that both oxygen prebleaching and an increased substitution of ClO_2 for Cl_2 greatly reduced the amount of total organochlorines discharged from pulp mills. The hardwood plant, using oxygen prebleaching, produced the lowest concentration of total organochlorines. This pattern has been supported by a number of other researchers (Axegård *et al.*, 1988, Heimberger *et al.*, 1988, Gergov *et al.*, 1988, Germgård *et al.*, 1985, Owens *et al.*, 1994). Activated sludge treatment of the wastewater prior to discharge has also been found to significantly reduce total organochlorine concentrations in effluents (Gergov *et al.*, 1988).

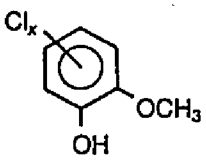
Figure 2.

Phenolic compounds commonly identified in BKPPME

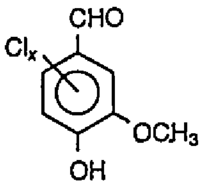
(from Kringstad *et al.*, 1984)



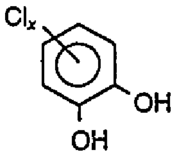
Chlorinated
phenols



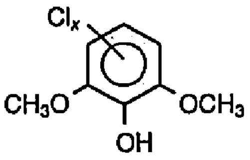
Chlorinated
guaiacols



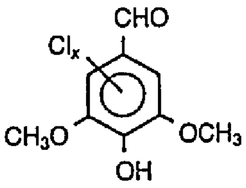
Chlorinated
vanillins



Chlorinated
catechols



Chlorinated
syringols



Chlorinated
syringaldehydes

Normal bleached kraft pulp mills generate 1 to 8 kg of AOX (adsorbable organic halogen) per ton of pulp depending on the ratios of Cl to ClO_2 used (Hall *et al.*, 1989). At a pilot plant in Finland AOX, loads have been cut to 0.1kg/ton pulp by using TCF and ECF bleaching (MacLeod, 1994). AOX is a measure of the amount of total halogens bound to organic matter in a water sample (EPA, 1993.) While these results are promising, the cost of redesigning existing plants to produce TCF or ECF bleached pulp is prohibitive. One industry estimate predicts it will cost $11.5 billion ($7.5 billion more than EPA estimates) to meet currently proposed EPA standards under the Clean Air and Clean Water Acts. Considering this, plants will undoubtably be discharging significant amounts of organochlorines for many years to come (Stanley, 1995).

In the bleached kraft pulp process up to 55% of the original weight of the wood is dissolved in the pulp liquors (Axegård, 1988; McLeay, 1987; Kringstad *et al.*, 1984; Minor, 1982). A typical bleached kraft pulp mill that produces 750 tons of pulp per day must treat and discharge between 20 and 45 million gallons of effluent daily (Walden, 1976). Given the present state of bleaching technology, a typical mill releases between 750 to 6,000 kg of AOX daily. Even if AOX can be reduced to 0.1 kg/ton of pulp produced, 75 kg of halogenated compounds would be released into receiving waters.

A recent review of the environmental effects of bleached kraft pulp and paper mill effluent (BKPPME) in the aquatic environment cites organochlorines as one of the major classes of compounds of concern because of their persistence in the environment, resistance to degradation in traditional wastewater treatment processes and their propensity for bioconcentration and biomagnification (Owens, 1991). Organochlorines in BKPPM effluents are often reported in the literature simply as TOCl (total organic chlorine) or AOX, the latter being the more commonly used test. To date about 70% of the AOX with molecular weights under 1,000 amu have been identified. These low molecular weight compounds are of greatest concern because most toxic and mutagenic effects are attributed to this fraction. Between 50 and 70% of all AOX have molecular weights over 1,000 amu and consist primarily of chlorinated lignins. Some effluent regulations now include limits on TOCl or AOX, along with requirements for total effluent toxicity testing. To date, there are few regulations designed specifically for individual compounds characteristic of pulp and paper mills. In 1993, EPA proposed new regulations to monitor individual compounds from pulp mills being discharged to the air and water (EPA, 1993). This proposal has still not been promulgated and it appears that it may be changed to be based on treatment conditions rather than the discharge of specific compounds (Stanley, 1995). Similar regulatory methods are being considered in Canada, the U.K., and several European countries (Johnson, 1991).

Most past toxicological work regarding pulp and paper mills has centered on the effects of whole effluent on aquatic species. For example, retarded growth rates have been noted in barnacles and mussels near a sulfite pulp mill outfall in British Colombia (Wu *et al.*, 1980). Physiological and biochemical changes were noted in perch sampled between 2 and 10 km downstream from a Swedish bleached kraft mill's outfall. Effects cited include: reduced gonad growth, enlarged liver, induction of some cytochrome P-450-dependent enzymes such as 7-ethoxyresorufin *O*-deethylase (EROD) activity, and hematological alterations. EROD activity is a measure of enzyme activity associated with the metabolism of xenobiotic compounds such as polynuclear aromatic hydrocarbons (Andersson *et al.*, 1988). A significant increase in EROD activity was noted in rainbow trout that were caged downstream from a Finnish bleached kraft pulp mill (Lindström-Seppä *et al.*, 1990). Lehtinen *et al.* (1990) noted that effluents from traditional chlorine bleaching processes in Scandinavia had a much stronger physiological effect on immature rainbow trout than effluents from plants that employed oxygen prebleaching, or high percentages of ClO_2 in lieu of elemental chlorine.

Since there are hundreds of potential toxic compounds in BKPPME, it is hard to attribute toxic effects to specific compounds. Due to the small amount of toxicity information available for chlorinated phenolics on aquatic species, toxicity equivalency factors (TEF) have been derived for these compounds relative to pentachlorophenol. TEF increase with increasing chlorination. It has been suggested that the overall toxicity of a multi-component effluent such as BKPPME can be estimated by summing the TEF of the constituents (Kovacs *et al.*, 1993).

Toxicity data for individual guaiacols on aquatic species are limited. LC_{50} (96hr) values have been reported for guaiacol, 4,5-dichloroguaiacol (4,5-CG), 3,4,5-trichloroguaiacol (3,4,5-CG), and tetrachloroguaiacol on rainbow trout (*Salmo gairdneri*)(Voss *et al.*, 1980). Values ranged from 44mg/l for guaiacol to 0.32 mg/l for tetrachloroguaiacol. The pH appeared to have an effect on the toxicity of tetrachloroguaiacol, with LC_{50} values decreasing with decreasing pH. Increased toxicity was observed in sea urchin, *Strongylocentrotus purpuratus*, sperm with increasing degrees of chloroguaiacol chlorination (Cherr *et al.*, 1987).

A 16-month mesocosm experiment studied the fate and chronic effects of 4,5,6-trichloroguaiacol (4,5,6-CG) (Rosemarin *et al.*, 1990). Organisms examined ranged from algae to invertebrates and fish. Impaired reproduction was noted in both benthic and planktonic invertebrates. The mean weight of the fish decreased with increasing 4,5,6-CG concentration. Other sublethal measurements, such as histological or biochemical changes, were not made. The study indicated that bioaccumulation increased with trophic level.

Metabolites of several components of the effluents, including chlorinated guaiacols, veratroles and resin acids have also been detected in the bile of fish in Finland and the former Soviet Union (Oikari, 1986: Oikari *et al.*, 1988). An extensive, longterm monitoring program on a Canadian river system near a bleached kraft pulp mill analyzed effluent, receiving water, sediments, insects and fish. Concentrations of several chloroguaiacols in the bile of mountain whitefish (*Prosopium williamsoni*) and longnose sucker (*Catostomus catostomus*) were at the mg/l level while levels of the same compounds in water were at μg/l levels or below detection limits ($\approx$ 50ng/l). Numerous chlorophenolics were also detected, in the ppm range, in *Hydropsyche* caddisflys (larval stage filter feeders) 140km downstream of the mill. Levels of chlorophenolics generally dropped as the bleaching process at the plant was modified from 25% to 100% ClO_2 substitution (Owens *et al.*, 1994). Cytochrome P4501A induction was noted in both species of fish examined in this study (Klopper-Sams and Benton, 1994, Klopper-Sams *et al.*, 1994). Other adverse effects on the population of either species was not noted (Swanson *et al.*, 1994)

Chlorinated organic compounds, including chloroguaiacols, have been reported in waters receiving pulp mill effluents (Carey *et al.*, 1988a & 1988b; McKague *et al.*, 1988; Paasivirta *et al.*, 1988b; Voss, 1983; Xie *et al.*, 1986) and sediments in the vicinity of the mills (Paasivirta *et al.*, 1988a, Eder *et al.*, 1980 and de Sousa *et al.*, 1988). Most research has taken place in Scandinavia and Canada, where pulp and paper processing is a major industry. A recent, comprehensive survey of chlorophenolic (CP) distribution near a bleached kraft pulp mill examined variations in CP concentrations in the water column and suspended sediments related to seasonal riverflow and mill process modifications (Owens *et al.*, 1994). Chlorophenolic concentrations in the water and sediment were highest when the mill used a high percentage of Cl_2 in bleaching and decreased with increasing ClO_2 substitution. Results for the biota sampled concurrently mirrored these results as discussed above.

The fate of chlorinated compounds in BKPPME depends on their molecular weight and structure. The lower molecular weight compounds, such as chloroform, are volatile and do not generally accumulate in receiving waters or sediments (Voss, 1983). It has also been suggested that chloroform is relatively resistant to sorption by humic substances (Xie *et al.*, 1986). According to Abrahamsson *et al.*, (1989) the fate and transport of chloroform and other low molecular weight compounds depend primarily on volatilization and dilution. The higher molecular weight and lipophilicity of chlorinated phenolics, as well as the chemical composition of the water, are important factors in determining their fate. Along with volatilization and dilution, chlorinated phenolics are also subject to microbial degradation, particle sorption and subsequent sedimentation (Abrahamsson *et al.*, 1989.).

Biotransformation of chlorinated phenolics has been reported under both aerobic and anaerobic conditions. Studies have been conducted in wastewater treatment systems, as well as receiving waters and sediments. Highly chlorinated phenolics were reductively dechlorinated by anaerobic bacteria during wastewater treatment. Demethylation was also observed. Chloroveratroles were converted first to chloroguaiacols, then to chlorocatechols, prior to dechlorination. Complete dechlorination, however, was not observed under these conditions (Woods *et al.*, 1989). Allard *et al.* (1988) examined the fate of chloroguaiacols and chlorocatechols in sediments under both aerobic and anaerobic conditions. *O*-methylation of chloroguaiacols to form chloroveratroles occurred under aerobic conditions; while demethylation of both chloroguaiacols and chloroveratroles, resulting in chlorocatechol formation, was observed under anaerobic conditions. It was suggested that this was the reason chlorocatechols predominated in sediments, while chloroveratroles were found primarily in biota. A guaiacol cycle has been proposed for the transformation of chlorinated phenolics in contaminated environments (Remberger *et al.*, 1986). A number of researchers (Nielson *et al.*, 1987; Eriksson *et al.*, 1985 and Abrahamsson *et al.*, 1989) have also reported on microbial transformations of chlorinated phenolics and chlorolignins. These transformations generally involved methylation or demethylation but not complete dechlorination. In the presence of guaiacol, the dechlorination and demethylation of 4-CG, 5-CG and 4,5-CG by an *Acinetobacter junii* strain has been reported. Chloroguaiacols with substitution at the 6-position were not metabolized by this bacterium. (González *et al.*, 1993)

Recent advances in pulp and paper technology have reduced the amounts of chloroguaiacols being generated, but because they have not been eliminated their presence remains an environmental concern. It is, therefore, important to understand the factors affecting the environmental fate of chloroguaiacols in aquatic systems. These factors include the ionization properties and sorptive reactions of chloroguaiacols and are examined in this dissertation.

Ionization properties of chloroguaiacols must be known in order to explain their sorptive behavior in aquatic systems. In the first section of this dissertation, thermodynamic dissociation constants (pK_a, where pK_a = -log K_a) of guaiacol and 9 chloroguaicols were determined by spectrophotometric methods, representing the first comprehensive determination of aqueous phase pK_a values for chloroguaiacols that have been identified in BKPPME. The hypothesis that experimentally determined thermodynamic pK_a values are linearly related to values predicted by the Hammett equation and to gas phase acidities made by quantum chemical calculations using MOPAC 6.0 was tested.

In the second section, the pH dependent sorption of four chloroguaicols, with pK_a values ranging from 9.06 to 6.06, was investigated on four estuarine sediments. Three hypotheses were tested: 1) pH affects chloroguaiacol sorption to sediments, 2) the amount of total organic carbon in a sediment affects chloroguaiacol sorption and 3) the amount of humic acids in a sediment affects chloroguaiacol sorption. Sorption of a chloroguaiacol onto sediments may result in its reduced availability to certain aquatic species, increased degradation rates or rapid burial. On the other hand, it may lead to increased exposure to deposit feeding benthic species with subsequent transfer to higher trophic levels.

Effective methods of chloroguaiacol determination are necessary in order to monitor BKPPME for regulatory purposes as well as to evaluate process changes that may reduce chlorinated organics in effluents. The National Council of the Pulp and Paper Industry for Air and Stream Improvement (NCASI) has developed an analytical method to determine chlorinated phenolics in BKPPME. This method is becoming an industry standard in monitoring effluents for chlorophenols. In the third section, the NCASI method is compared to one developed specifically for chloroguaiacols by the author.

Chapter II

Dissociation Constants of Chloroguaiacols in Water: A Comparison of Measured and Predicted Values

Introduction

The Brønsted-Lowry definition of an acid is a substance that can donate a positively charged hydrogen ion (H^+). In water a strong acid will completely ionize while a weak acid will do so only partially. Chloroguaiacols and other phenolics are examples of weak acids. Their degree of dissociation is represented by an acidity constant,

$$K_a = \{H^+\}\{G^-\}/\{HG\}$$

where {} represent activities of the ionized, G^-, and molecular, HG, guaiacol species. Acidity, or dissociation, constants are often referred by the term pK_a, where $-\log K_a = pK_a$.

Acid dissociation of chlorinated phenolics is a determinant of their sorptive properties and hence their environmental transport and fate. Lee *et al.* (1990) measured effects of pH and sediment organic content on the sorption of ^{14}C pentachlorophenol (PCP, $pK_a' = 4.75$) by several natural soils and sediments with organic carbon contents ranging from 0.2 to 57.5%. The study indicated that, at $pH<7$, PCP is predominantly neutral and is hydrophobically sorbed. Sorption of the neutral species increased with increasing organic carbon content of the sorbent, as well as decreasing pH. At $pH > 7$, sorption of the pentachlorophenolate ion (PCP^-) and formation and sorption of neutral metal-PCP^- pairs becomes important.

PCP^- is far more soluble in water than PCP. It follows that PCP will sorb more readily than its ionized form. As pH decreases, overall sorption increases. Another factor adding to the increase in sorption is that as pH decreases, sediment surfaces become more protonated and may present positively charged sites that will attract PCP^-. Several other studies (Schellenberg, 1984; Lagas, 1988; Boyd, 1982 & 1989, Fisher, 1990, Issacson and Frink, 1984) have been conducted regarding the sorption of chlorophenols by sediments, soils and aquifer materials with similar results.

The investigation reported in this dissertation was undertaken to determine the acid dissociation constants of nine chloroguaiacols and to assess the accuracy to which these results can be predicted by empirical and quantum chemical methods. As discussed above, knowledge of these dissociation constants is required because ionization properties determine the interaction between phenolic compounds and surfaces of sediments, soils, aquifer materials and living cells, and thus the environmental fate and toxicity of these compounds.

Measurements of acidities of some chloroguaiacols have been made. Gas chromatography was used to simultaneously determine the pK_a values and octanol-water partition coefficients of 4,5-CG, 3,4,5-CG, 4,5,6-CG and 3,4,5,6-CG (Xie *et al.* (1984). HPLC was used to determine the pK_a of 5-CG, 6-CG, 4,5-CG, 4,6-CG, 3,4,5-CG, 3,4,6-CG, 4,5,6-CG and 3,4,5,6-CG (Li *et al.* (1991). Methanol concentration was observed to have a large effect on the apparent pK_a. The determinations by Li *et al.* were made in 60/40 methanol/water solutions, so the results are inapplicable to aqueous solutions and are not used in later data comparisons.

In the present work, acid dissociation constants of guaiacol and nine chloroguaiacols (4-, 5-, 6-, 4,5-, 4,6-, 3,4,5-, 3,4,6-, 4,5,6- and 3,4,5,6-CG) have been determined through spectrophotometric methods. There are six other possible chlorinated guaiacols, but these were not commercially available at the time of the experiment, and, to date, have not been identified in BKPPME. This indicates either that steric and kinetic limitations may occur in their production under the conditions of pulp mill operation or that the previous lack of available standards prevented positive identification of these compounds.

Predictive methods for pK_a determination for these compounds are a way to obtain the data necessary for the estimation of their environmental behavior. With the advent of more powerful computing capabilities, quantum chemical calculation is now a viable method for predicting physical properties of environmentally relevant compounds and has been recently applied to dioxins (Thompson, 1994) and PCBs (Greaves *et al.*, 1994).

Materials and Methods

Spectrophotometric pK_a determination

Chlorinated guaiacol standards (all with purities of 99+% with the exception of 4-CG which was 90-95%) were obtained from Helix Biotech, Richmond, B.C., Canada. Guaiacol (98%) and chlorophenols (99+%) were purchased from EPA Reference Standards, Research Triangle Park, N.C. Stock solutions of these standards were prepared in methanol (HPLC grade, Burdick and Jackson) with nominal concentrations of 1mg/ml. Phosphate, borate, carbonate and HCl/KCl buffers were prepared according to Dien and Lentner (1970) using degassed, deionized water. Ionic strengths of the buffers were in the range of 0.05 to 0.16eq/l.

The dissociation constants, reported as pK_a, for each chloroguaiacol were determined by the *uv*-vis spectrophotometric method described in Albert and Sergeant (1984). Although more time consuming than potentiometric titration, this method offers the advantage that the pK_a of compounds with low aqueous solubilities, such as chloroguaiacols, can be determined with greater accuracy.

In the spectrophotometric method, the dissociation constant is measured by directly determining the ratio of ionized and molecular species concentrations in a series of solutions of known pH, all being near the expected pK_a. In order to do this the approximate dissociation constant must be known. In the case of CG, the pK_a can be estimated using the Hammett equation (discussed below).

The first step is to compare the spectra of the molecular and ionized species and determine the optimal analytical wavelength, the wavelength at which the greatest difference in absorbances exist. Representative absorption spectral scans of 3,4,5-CG from 200 to 400nm at pH 1.6, 12 and 7.19 (Figure 3) show the molecular, ionized and mixed species responses, respectively, with the analytical wavelength, λ, identified. The absorbance of the mixed species is then read at this wavelength. Assuming Beer's Law holds true, the observed absorbance, A, of the mixed species is the sum of the absorbances of the ion (A_i) and molecule (A_m):

$$A = A_i + A_m.$$

Absorbance of either component is related to its molar concentration, c, by:

$$A = \varepsilon l c$$

where ε is the molar absorption coefficient and l is the optical length of the cell. In the mixture, the concentration of the ionized and molecular species are $F_i c$ and $F_m c$, respectively. A_i and A_m are then $\varepsilon_i F_i c l$ and $\varepsilon_m F_m c l$, respectively. F_i and F_m, the ionized and molecular fractions, F_i and F_m, are:

$$F_i = [A^-]/([A^-]+[HA]) \text{ and } F_m = [HA]/([A^-]+[HA]).$$

Figure 3.

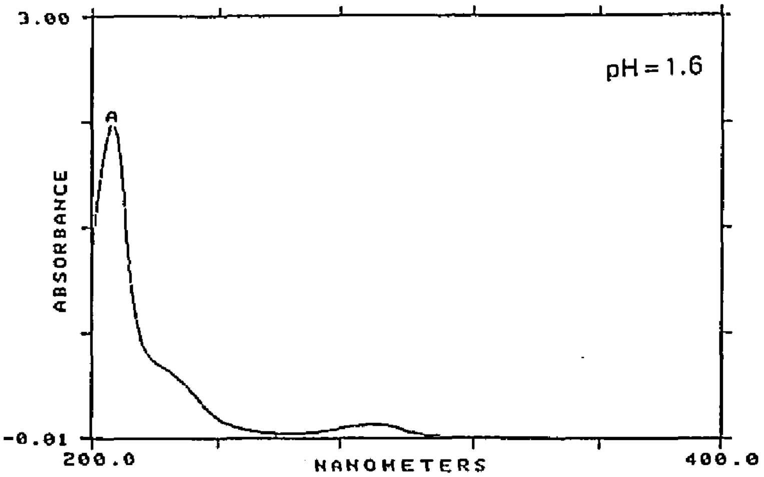
Absorbance spectra of 3,4,5-chloroguaiacol species:

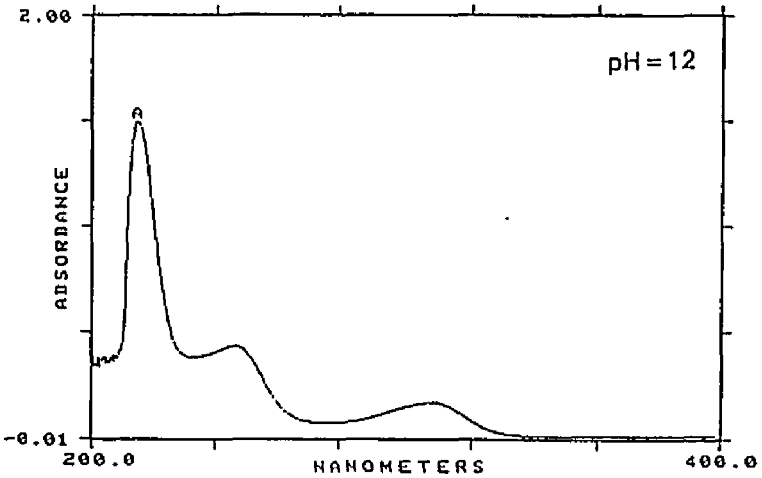
pH=1.6: molecular species

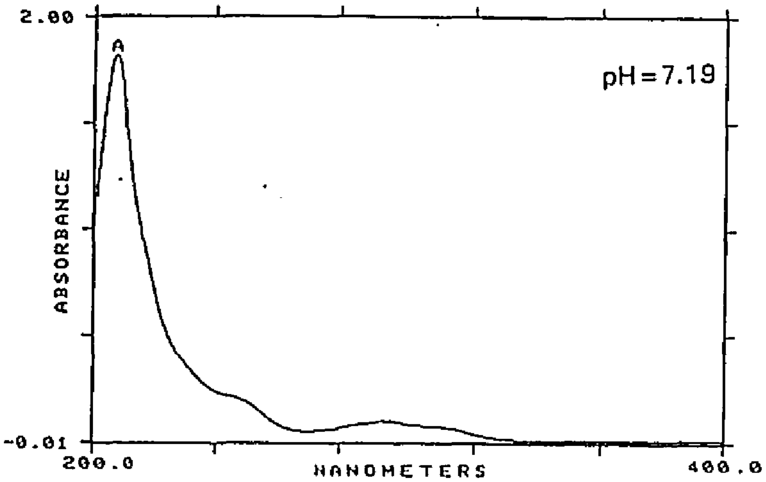
pH=12: ionized species

pH=7.19: mixed species

analytical wavelength (λ)=308nm


3.00
-0.01
200.0
400.0
ABSORBANCE
NANOMETERS
A
pH = 1.6


2.00
-0.01
200.0
400.0
ABSORBANCE
NANOMETERS
A
pH = 12


2.00
-0.01
200.0
400.0
ABSORBANCE
NANOMETERS
A
pH = 7.19

Replacing [HA] with $[H^+][A^-]/K_a$ gives

$$F_i = K_a/([H^+] + K_a) \text{ and } F_m = [H^+]/([H^+]+K_a).$$

If l is constant,

$$A = (\varepsilon_i F_i + \varepsilon_m F_m)c$$

and since $\varepsilon = A/c$ with F_i and F_m defined above,

$$\varepsilon = \{\varepsilon_i K_a/([H^+] + K_a)\} + \{\varepsilon_m [H^+]/([H^+] + K_a)\}.$$

If the total concentration is held constant, absorbance, A, can replace ε and

$$pK_a = pH + \log[(A_i - A)/(A - A_m)].$$

For analysis, 0.10ml of the stock solution was dissolved in 10.0ml of buffer solution resulting in chloroguaiacol concentrations of ca. 10^{-5}M and a negligible methanol concentration of 1%. The pH of the solution was not affected by the methanol. Analytical wavelengths for the chloroguaiacols were between 248 and 308nm. At 250nm pure methanol has an absorbance of 0.02, decreasing to 0.005 at 300nm, corresponding to absorbances between 0.002 and 0.0005 at analytical concentrations. Addition of 1% methanol would not be expected to change the dielectric constant of the solution and would therefore not affect pK_a determinations.

Five potential analytical wavelengths were read using a Gilford Response *uv*-visible spectrophotometer (Ciba Corning Diagnostics Corp., Oberlin, Ohio). pH was measured with an Orion model 290A pH meter with a model 91-57BN Triode electrode (Orion Corp. Boston, Mass.). The dissociation of phenolics is highly temperature dependent. Phenol exhibits a change of 0.012 pK_a units per degree (°C). For this reason, the cell and cell holder were thermostated at 25±0.5°C. The most sensitive analytical wavelength was selected for dissociation constant determination.

Dissociation constants determined by this method are apparent values, written as K_a'. K_a' is an apparent constant because the pH electrode measures hydrogen ion activity, while the absorbance ratios represent concentration ratios of the guaiacolate ion, G^-, and neutral guaiacol, HG. The thermodynamic K_a is:

$$K_a = \{H^+\}\{G^-\}/\{HG\} = \{H^+\}[G^-]\gamma_{G^-}/[HG]\gamma_{HG} = K_a' \gamma_{G^-}/\gamma_{HG}$$

where { } represent activities, [ ] represent concentrations, and γ is the molar activity coefficient, which is defined to be unity at infinite dilution in water. Since γ_{HG} is near unity in the experimental solutions, $K_a \approx K_a'\gamma_{G^-}$. The ionic strength, I, resulting mainly from the concentration of the buffer ions present in the experimental solutions causes γ_{G^-} to be considerably less than one. In reasonably dilute solutions, γ_{G^-} can be calculated from the Debye-Huckel limiting law for the G^- species in a solution of ionic strength:

$$\gamma_{G^-} = 0.5\sqrt{I}$$

I equals $\frac{1}{2}\sum_i C_i z_i^2$ where C_i is the concentration of each ionized species and z_i is the charge of each species in solution. In this work, the Debye-Huckel γ_{G^-} is applied to calculate thermodynamic K_a from K_a' values. The resulting pK_a values are used in comparisons with pK_a values predicted by the Hammett equation and relative acidities predicted by quantum chemical models.

In principle, pK_a values could be obtained by determining pK_a' values in a series of buffers of decreasing ionic strength and extrapolating to infinite dilution. However, measurements at lower ionic strengths were experimentally impractical due to reduced buffer capacity and consequent errors in pH determination.

Prediction of dissociation constants by quantum chemical calculation.

Quantum chemical calculation of relative acidities of the chlorinated guaiacols was executed using MOPAC 6.0, a semi-empirical molecular orbital modelling package obtained from Quantum Chemistry Program Exchange, Dept. of Chemistry, Indiana University, Bloomington, Indiana. The MNDO and AM1 Hamiltonian operators in MOPAC were used to calculate heats of formation for all the chlorinated guaiacol molecules, and their corresponding deprotonated guaiacolate, singly charged anions. MNDO and AM1 methods are in common use, and are described by Hirst

(1990). AM1 is a modification of the MNDO calculation involving a new parameterization. Both methods were used to examine the effects of the new parameterization. *Ab initio* methods were not used because the computation time needed for acidity calculations on all chloroguaiacols was prohibitive.

Relative gas phase acidities of the chloroguaiacols with respect to guaiacol may be defined by the heat of reaction ($\delta\Delta H^0_{(g)}$) for (Fujio *et al.*, 1981; Radom, 1974):

$$\text{chloroguaiacolate} + \text{guaiacol} \rightarrow \text{guaiacolate} + \text{chloroguaiacol}$$

$\delta\Delta H^0_{(g)}$ was calculated from the MOPAC determined heats of formation of each species in this reaction.

In addition to calculation heats of formation, MOPAC determines other molecular properties and optimizes molecular geometry. To run MOPAC, a matrix representing the approximate geometry of the molecule is constructed. Keywords specify which calculations are to be done and the matrix is input into the program to run. A portion of typical outputs for both the ionized and molecular forms of tetrachloroguaiacol are presents in Figures 4 and 5.

Aqueous acidities differ from gas phase acidities, primarily due to hydration effects. However these acidities will be linearly related if hydration energies are similar for the dissociation reaction of all the chloroguaicols investigated. Fujio *et al.* (1981) demonstrated that this relation held for substituted phenols.

Figure 4.

MOPAC 6.0 output for tetrachloroguaiacol

```
                 SUMMARY OF   AM1   CALCULATION
                                                       VERSION  6.00
C7H4O2Cl4

AM1
3,4,5,6-tetrachloroguaiacol

 GRADIENT TEST NOT PASSED, BUT FURTHER WORK NOT JUSTIFIED
 SCF FIELD WAS ACHIEVED

    HEAT OF FORMATION        =       -75.315064 KCAL
    ELECTRONIC ENERGY        =    -13489.973477 EV
    CORE-CORE REPULSION      =     10403.315119 EV
    GRADIENT NORM            =         5.129298
    DIPOLE                   =         2.61518 DEBYE
    NO. OF FILLED LEVELS     =        36
    IONIZATION POTENTIAL     =         9.419241 EV
    MOLECULAR WEIGHT         =       261.919
    SCF CALCULATIONS         =        79
    COMPUTATION TIME =  7 MINUTES AND  50.870 SECONDS

                 FINAL GEOMETRY OBTAINED
AM1
3,4,5,6-tetrachloroguaiacol

# Atom Bond lgth  Bond angle Twist angle  Neighboring    Charge
                                            Atoms (#)
1   C  0.0000000    0.000000    0.000000  0    0    0    0.1026
2   C  1.4062602    0.000000    0.000000  1    0    0   -0.0843
3   C  1.4051663  119.074139    0.000000  2    1    0   -0.0336
4   C  1.4045319  120.606309    0.566708  3    2    1   -0.0836
5   C  1.4062846  120.419038   -0.868824  4    3    2   -0.0444
6   C  1.3992717  119.149158   -0.396067  5    4    3   -0.0169
7   O  1.3697861  121.170537  177.320893  1    6    5   -0.2255
8  Cl  1.6919236  120.568472 -178.885252  2    1    6    0.0499
9  Cl  1.6930940  119.563294 -179.634975  3    2    1    0.0463
10 Cl  1.6945810  119.833196  179.352567  4    3    2    0.0395
11 Cl  1.6935581  120.318955  179.674905  5    4    3    0.0372
12  O  1.3838163  123.599844  175.605186  6    5    4   -0.2261
13  C  1.4334135  115.150399   68.892374 12    6    5   -0.0779
14  H  1.1143537  112.582539    7.830308 13   12    6    0.0890
15  H  1.1185037  105.246718  128.453860 13   12    6    0.0893
16  H  1.1173442  106.824781 -113.758083 13   12    6    0.0935
17  H  0.9733910   78.096037  177.432200  7    6    1    0.2453
```

Figure 5.

MOPAC 6.0 output for tetrachloroguaiacolate anion

```
                    SUMMARY OF    AM1    CALCULATION
                                                         VERSION   6.00
C7H3O2Cl4

AM1 CHARGE=-1
3,4,5,6-tetrachloroguaiacol

 GRADIENT TEST NOT PASSED, BUT FURTHER WORK NOT JUSTIFIED
 SCF FIELD WAS ACHIEVED

    HEAT OF FORMATION       =      -117.222588 KCAL
    ELECTRONIC ENERGY       =    -13173.191281 EV
    CORE-CORE REPULSION     =     10098.371417 EV
    GRADIENT NORM           =         6.924967
    DIPOLE                  =         4.05748 DEBYE
    NO. OF FILLED LEVELS    =        36
    CHARGE ON SYSTEM        =        -1
    IONIZATION POTENTIAL    =         3.883719 EV
    MOLECULAR WEIGHT        =       260.912
    SCF CALCULATIONS        =        30
    COMPUTATION TIME =  2 MINUTES AND  50.050 SECONDS

                      FINAL GEOMETRY OBTAINED
 AM1 CHARGE=-1
 3,4,5,6-tetrachloroguaiacol

#Atom   Bond lgth   Bond Angle Twist angle  Neighboring     Charge
                                             Atoms (#)
1    C  0.0000000    0.000000     0.000000   0     0     0    0.2902
2    C  1.4454558    0.000000     0.000000   1     0     0   -0.2547
3    C  1.3868090  121.977643     0.000000   2     1     0    0.0413
4    C  1.3992749  120.962601    -0.071060   3     2     1   -0.2271
5    C  1.4092688  119.830720     0.378738   4     3     2    0.0220
6    C  1.3830402  119.694678     0.701895   5     4     3   -0.1320
7    O  1.2574780  121.252194  -176.835794   1     6     5   -0.4438
8   Cl  1.7094501  118.010178   178.409490   2     1     6   -0.0556
9   Cl  1.7131941  119.928212   179.973385   3     2     1   -0.0541
10  Cl  1.7085522  120.261009  -179.766578   4     3     2   -0.0578
11  Cl  1.7110273  119.251027   179.903892   5     4     3   -0.0500
12   O  1.3915217  119.130339   173.563568   6     5     4   -0.2137
13   C  1.4248869  114.069410   123.903320  12     6     5   -0.0583
14   H  1.1146538  112.892505     0.496660  13    12     6    0.0863
15   H  1.1183679  106.505592   120.808753  13    12     6    0.0678
16   H  1.1194350  106.636095  -121.013037  13    12     6    0.0394
```

In the present work, MOPAC predicted pK_a values were calculated from the linear regression of thermodynamic pK_a versus $\delta\Delta H^0_{(g)}$(kcal/mole) using both MNDO and AM1 methods. Departures from perfect correlation between experimental aqueous acidities and computed gas phase acidities may have several sources including experimental error, effects of neglect of hydration energies in computations, simplifying assumptions made in the MOPAC calculations, and computational errors.

Prediction of dissociation constants by the Hammett equation.

Hammett predictions of thermodynamic dissociation constants in water (McDaniel and Brown, 1957, Hammett, 1970) were made according to the equation:

$$pK_{a,\mathrm{Hammett}} = pK_a^0 - \rho(\Sigma\sigma) \tag{1}$$

where pK_a^0 is the pK_a of the unsubstituted aromatic acid, ρ is a constant determined for a specific reaction, and σ is the Hammett constant for a particular substituent on the ring.

The empirically determined Hammett constants were initially based on linear free energy relationships (LFER) of meta- and para- substituents on benzoic acid, and later extended to include ortho- position substitutions. The substituent constant is defined as:

$$\sigma = \log K_i - \log K_0$$

where K_0 and K_i are the acidity constants of benzoic acid and the substituted benzoic acid, respectively (Hammett, 1970). The constant, ρ (standardized as 1 for the ionization of benzoic acids), relates to a specific ionization reactions. It is determined as the slope of the line generated by plotting $\log(K_i/K_0)$ for benzoic acid versus the aromatic acid in question (Schwarzenbach *et al.*, 1993). Phenol was used as the reference acid, with pK_a^0, σ and ρ values taken from Perrin *et al.* (1981).

Results and Discussion

Experimental Results.

Experimental, predicted and literature pK_a values and relative acidities ($\delta\Delta H^0_{(g)}$) from MOPAC are presented in Table 1. The pK_a' values were corrected to pK_a values as described above.

Three separate pK_a' determinations were made on 4,6-CG to test the precision of the method, giving 7.84±0.02, 7.76±0.03 and 7.77±0.05 with n=7 in all cases. To verify the accuracy of the method, pK_a values were determined for two chlorophenols (3,4-CP and 2,4-CP) with published dissociation constants that are analogs of 4,5-CG and 4,6-CG. The pK_a values determined for 3,4-CP (8.70±0.05, n=7) and 2,4-CP (8.07±0.04, n=7) were in reasonable agreement with literature values (Table 2). The pK_a values for their chloroguaiacol analogs (4,5-CG and 4,6-CG) are 8.53±0.04 and 8.03±0.04, respectively. This indicates that the *ortho*-methoxy group does have an effect on the acid dissociation. Literature values in Table 2 for chlorophenols are not thermodynamic, so exact agreement with the values reported here is not expected.

Comparison of MOPAC Predicted and Experimental pK_a Values.

Experimental thermodynamic pK_a values were linearly related to $\delta\Delta H^0_{(g)}$(kcal/mole) from MNDO calculation (Figure 6) and AM1 calculation (Figure 7). MOPAC predicted pK_a values were calculated from a simple linear regression (Zar, 1984) of $\delta\Delta H^0_{(g)}$ versus experimental pK_a.

Table 1. pK_a data from Hammett equation, experimental results and relative acidity, as $\delta\Delta H^0_{(g)}$, kcal/mole, from MOPAC data.

Compound	pK_a (s.d.) (n=7)†	pK_a' Lit.‡	Hammett pK_a	MNDO $\delta\Delta H^0_{(g)}$	MNDO pK_a	AM1 $\delta\Delta H^0_{(g)}$	AM1 pK_a
3,4,5,6-CG	6.06 (0.02)	6.25[a]	6.22	28.44	6.17	19.99	6.23
3,5,6-CG	n.m.	n.a.	6.75	21.93	7.12	15.78	7.12
3,4,6-CG	6.71 (0.03)	n.a.	7.04	23.11	6.95	16.14	7.04
4,5,6-CG	7.19 (0.02)	7.20[a]	7.04	22.95	6.97	16.20	7.03
5,6-CG	n.m.	n.a.	7.58	15.76	8.02	14.35	7.42
3,6-CG	n.m.	n.a.	7.58	15.81	8.01	10.90	8.16
3,4,5-CG	7.55 (0.03)	7.56[a]	7.73	20.70	7.30	14.69	7.35
4,6-CG	8.03 (0.04)	n.a.	7.87	16.72	7.88	12.11	7.90
3,5-CG	n.m.	n.a.	8.27	14.17	8.25	10.22	8.30
6-CG	8.49 (0.04)	n.a.	8.40	8.77	9.05	8.39	8.69
4,5-CG	8.53 (0.04)	8.52[a]	8.56	14.76	8.17	12.17	7.89
3,4-CG	n.m.	n.a.	8.56	14.38	8.22	9.77	8.40
3-CG	n.m.	n.a.	9.09	6.64	9.36	4.20	9.58
5-CG	9.06 (0.04)	n.a.	9.09	7.17	9.28	5.35	9.34
4-CG	9.50 (0.05)	n.a.	9.38	8.02	9.16	5.26	9.35
guaiacol	10.13 (0.04)	9.98[b] 9.85[b]	9.92	0	10.33	0	10.47

† n.m. indicates pK_a values were not measured

‡ n.a. indicates pK_a values were not available

[a] Data from Xie *et al.* 1984

[b] Data from Serjeant and Dempsey, 1979

Table 2. Reported pK_a's chlorophenols (CP)

Compound	pK_a	Method**
2,3,4,5-CP	5.92[a]	1
	6.61[b]	2
	6.96[c]	x
	5.64[c]	x
3,4,5-CP	7.87[a]	1
	7.81[b]	2
	7.83[d]	3
2,3,4-CP	7.34[a]	1
	6.97[a]	x
	7.66[a]	x
3,4-CP	8.87[a]	1
	8.68[b]	2
	8.58[d]	3
2,4-CP	8.51[a]	1
	8.09[b]	2
	7.70[d]	3
	7.89[d]	3
4-CP	9.70[a]	1
	9.10[d]	5
	9.42[d]	3
	9.38[d]	4
2-CP	9.13[a]	1
	8.55[d]	3
	8.48[d]	4
	8.25[d]	3

[a] Data from Li *et al.* 1991, [b] Data from Xie *et al.* 1984, [c] Data reported in Suntio *et al.* 1988
[d] Data reported in Dempsey and Serjeant, 1979

** Methods used are as follows:

1: High performance liquid chromatography
2: Simultaneous determination of partition coefficients and pK^a by gas chromatography
3: Absorbance spectrophotometry
4: Calorimetric methods
5: pH titration
x: method not reported.

Figure 6.

$\delta\Delta H^0_{(g)}$ vs. thermodynamic pK_a from MNDO calculations

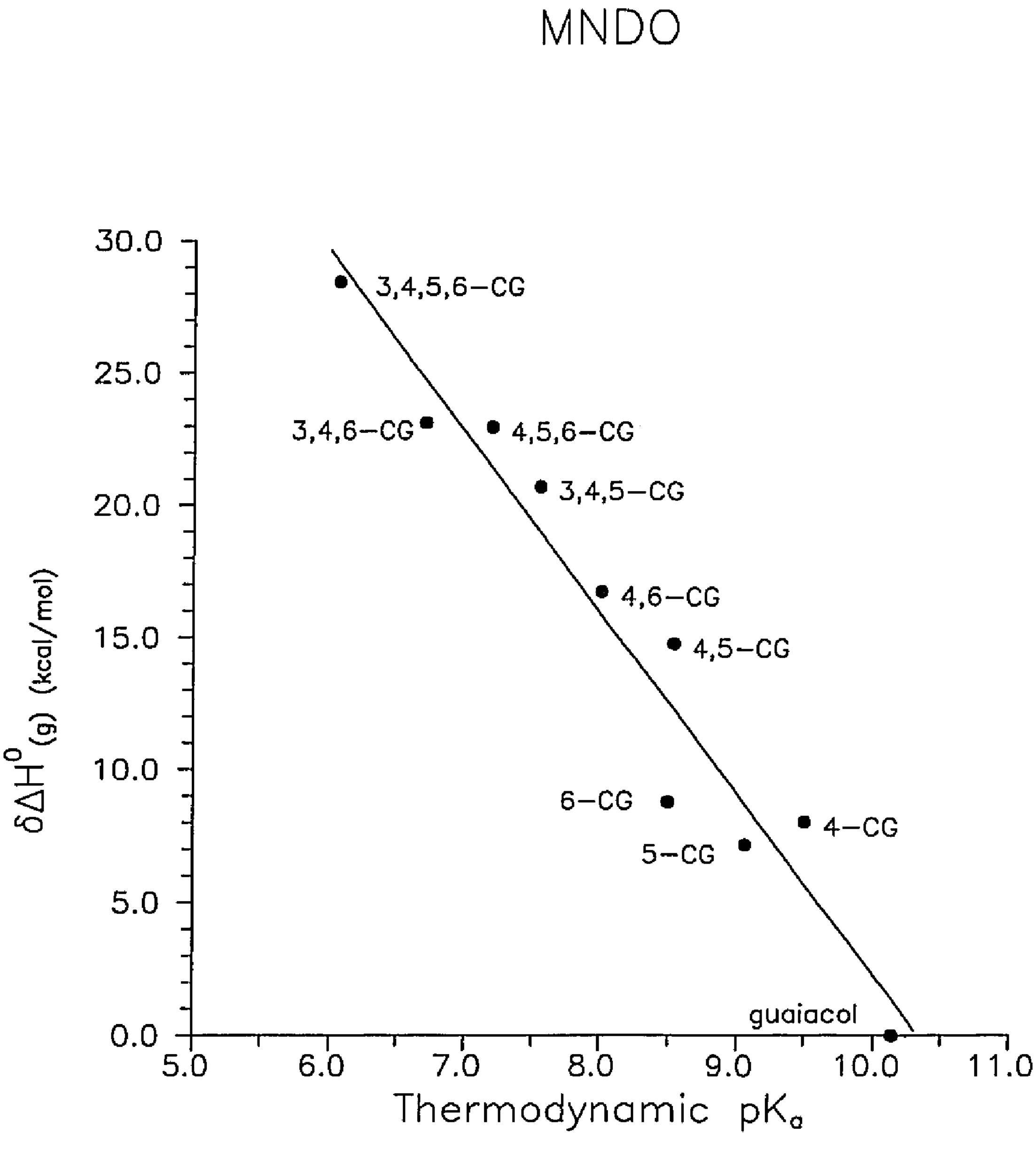
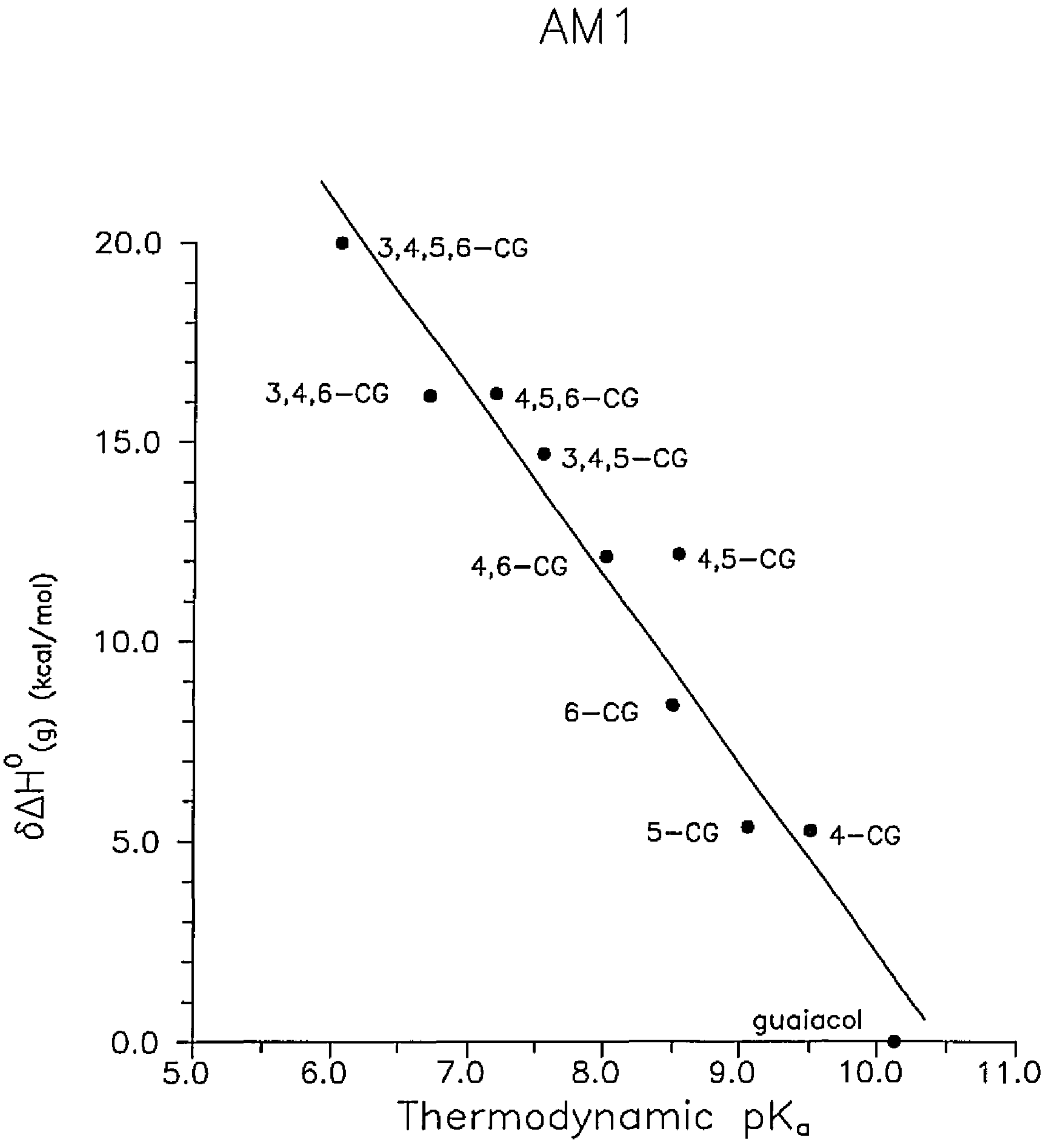

MNDO
δΔH⁰(g) (kcal/mol)
30.0
25.0
20.0
15.0
10.0
5.0
0.0
5.0
6.0
7.0
8.0
9.0
10.0
11.0
Thermodynamic pKa
3,4,5,6-CG
3,4,6-CG
4,5,6-CG
3,4,5-CG
4,6-CG
4,5-CG
6-CG
4-CG
5-CG
guaiacol

Figure 7.

$\delta\Delta H^0_{(g)}$ vs. thermodynamic pK_a from AM1 calculations


AM1
δΔH°(g) (kcal/mol)
20.0
15.0
10.0
5.0
0.0
5.0
6.0
7.0
8.0
9.0
10.0
11.0
Thermodynamic pKa
3,4,5,6–CG
3,4,6–CG
4,5,6–CG
3,4,5–CG
4,6–CG
4,5–CG
6–CG
5–CG
4–CG
guaiacol

For $\delta\Delta H^0_{(g)}$ from AM1:

$$\delta\Delta H^0_{(g)}(\text{kcal/mole}) = -4.713(\mathrm{p}K_a) + 49.326,\ r^2 = 0.943$$

For $\delta\Delta H^0_{(g)}$ from MNDO:

$$\delta\Delta H^0_{(g)}(\text{kcal/mole}) = -6.831(\mathrm{p}K_a) + 70.55,\ r^2 = 0.945$$

Both AM1 and MNDO calculations predicted a similar pK_a for 3,4,6-CG and 4,5,6-CG, although thermodynamic values differed greatly. Differences between the MOPAC and thermodynamic values for particular chloroguaiacols may, in part, be due to solvation effects which are not included in the MOPAC model. The new parameterization incorporated in the AM1 Hamiltonian did not enhance the linearity of the relationship.

Factoring in the effects and interactions with water would likely improve this relationship. Efforts have been made to modify MOPAC-type calculations to include interactions with water (Cramer and Truhlar, 1992). However, all available models treat water as a continuous medium, which dictates that local effects of the water structure cannot be sufficiently defined to permit accurate calculation of aqueous acidities of the chloroguaiacols from the vapor phase acidities. Given the acceptable r^2 obtained here using gas phase acidities, only modest benefit might be gained from current models that include hydration.

Comparison of Hammett Predicted and Experimental pK_a Values.

The pK_a values predicted empirically by the Hammett equation were correlated (p=0.05) to the experimental thermodynamic values (Figure 8):

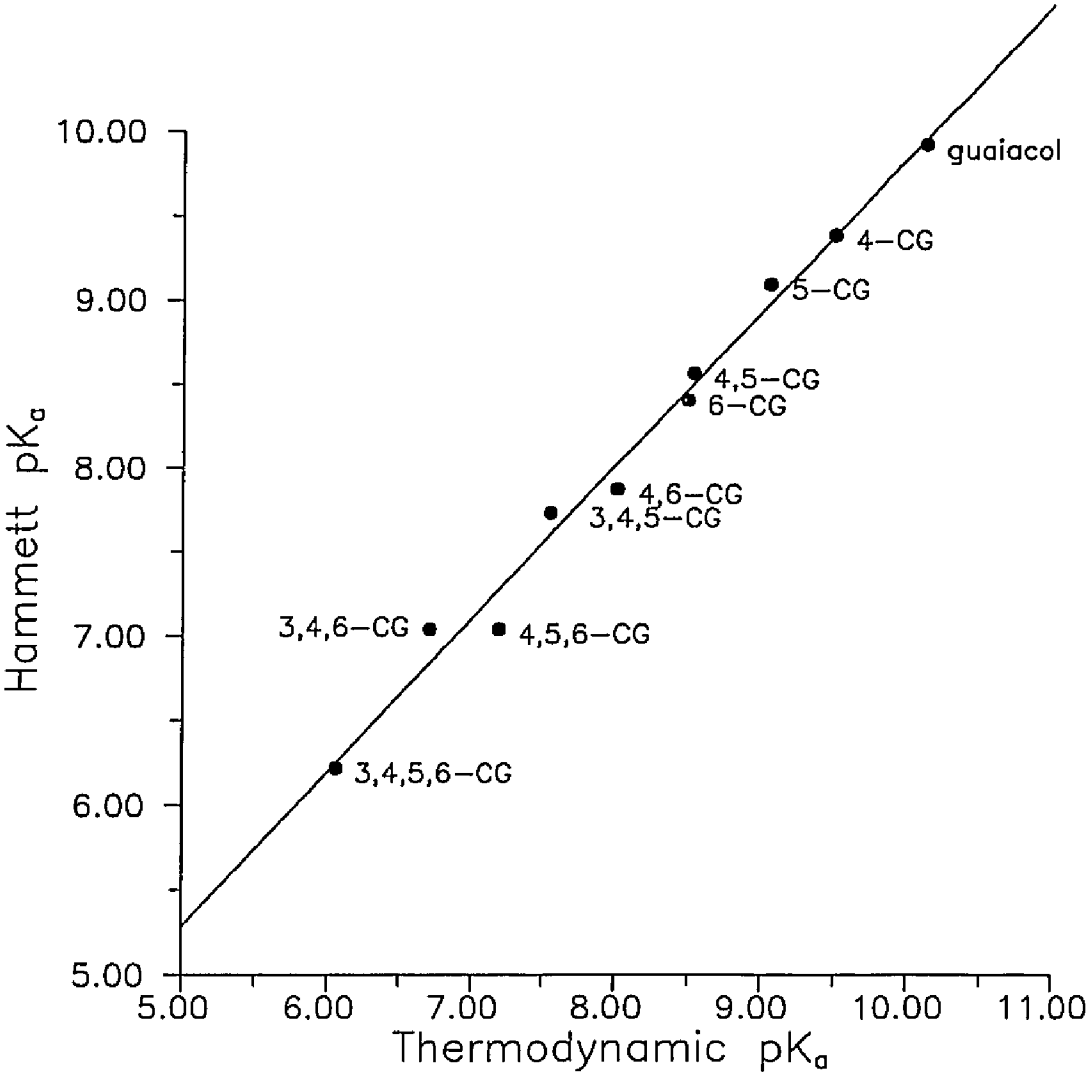
$$pK_{a,\mathrm{Hammett}} = 0.909\ pK_{a,\mathrm{thermo}} + 0.7398, \quad r = 0.993$$

Hammett predicted and experimental values compared favorably in all cases indicating that values calculated for the commercially unavailable CG's should be accurate. Monosubstituted CG's generally exhibited the least difference between predicted and thermodynamic pK_a values. This behavior may be due to the manner in which the Hammett method was established.

The largest deviations were noted for 3,4,6-CG and 3,4,5-CG suggesting that substitution at the 3- position, adjacent to the methoxy group, has a significant effect. Hammett predicted (Perrin *et al.*, 1981) pK_a values for chloroguaiacols are identical to analogous chlorophenols. The Hammett predicted pK_a for a substituted phenolic compound is based on σ values determined for the substituent's position relative to the hydroxyl group. It was developed for monosubstituted *meta-* and *para-* substituents and later extended to the *ortho-* substituents (Hammett, 1970, Perrin, 1981). The σ value for an *ortho-* methoxy group is 0.0, so the only substituent effect for chloroguaiacols is from chlorine. Due to differences in molecular symmetry, some chlorophenols have more than one analogous chloroguaiacol.

Figure 8.

Hammett predicted pK_a vs. thermodynamic pK_a



10.00
9.00
8.00
7.00
6.00
5.00
Hammett pK$_a$
5.00
6.00
7.00
8.00
9.00
10.00
11.00
Thermodynamic pK$_a$
guaiacol
4–CG
5–CG
4,5–CG
6–CG
4,6–CG
3,4,5–CG
3,4,6–CG
4,5,6–CG
3,4,5,6–CG

Based on these rules, 3,4,6-CG and 4,5,6-CG have the same Hammett pK_a (7.04). The structural difference between these molecules is the position of the *meta*- chlorine with respect to the methoxy group. Thermodynamic values are 6.71 and 7.19, respectively, indicating effects of this structural difference.

Comparison of Experimental and Literature Acidity Values.

The thermodynamic values reported here compare favorably with those reported in Xie *et al.* (1984) which were also done in aqueous media. This method involved partitioning, extraction and derivatization of the compounds prior to analysis and pK_a' determination. While these values are good approximations, thermodynamic values were not computed, and the method forced the assumption that γ_{G^-} was equal to one and that all molecular CG was transferred to the octanol phase. Nonetheless, ionic strengths in the Xie *et al.* (1984) experiment were on the order of 0.05 eq/l so the reported values are probably close to the thermodynamic values.

The pK_a' determinations by Li *et al*, (1991) were carried out in 60% methanol and are not suitable for comparison with results reported here. The change in pK_a with methanol concentration is not linear, so the results cannot be extrapolated to aqueous phase values. The difference in pK_a with methanol concentration is caused by the different dielectric constants of water and methanol (78.5 and 31.5, respectively). Dielectric constants measure how well a solvent can separate oppositely charged species, in this case the guaiacol anion and H^+. The higher value for water indicates that species are more easily dissociated, leading to higher K_a (or lower pK_a) values.

Comparison of Hammett and MOPAC methods

Hammett values are calculated from experimental data for other molecules in aqueous solutions, and thus hydration effects are included in Hammett predictions. As noted above, relative acidities calculated by MOPAC are quantum chemical calculations of properties of the isolated molecule in the gas phase at 0 K and thus do not include hydration effects. The correlation between predicted and experimental pK_a values by MOPAC may be weaker than that for Hammett due, in part, to the absence of these hydration effects. Computer capabilities may evolve to permit quantum mechanical calculation of a system including a chloroguaiacol and many water molecules. This would include solvation energies and would potentially provide a better correlation with experimental values.

The greatest differences between experimental thermodynamic values and MOPAC predictions were for the mono- and dichloroguaiacols, while the Hammett predictions for these compounds showed the least deviation. Since the Hammett σ values were calculated from acidity determinations of monosubstituted benzoic acids, this would be expected. Substituent interactions for polychloroguaiacols would not be contained in Hammett calculations. Since MOPAC calculations were done for each individual compound, the combined effect of all substituents is included. The hydration shells and energies may be different for lower chlorinated guaiacols, resulting in the differences in correlation.

This work demonstrates the utility of predictive methods for acid dissociation constants and provides a consistent set of pK_a values for chloroguaiacols that have been found in the environment. Knowledge of these dissociation constants is essential when assessing the potential environmental fate and effect of chlorinated guaiacols in aquatic systems.

Conclusions

This work represents the first comprehensive determination of thermodynamic pK_a values for chloroguaiacols that have been identified in BKPPME. Thermodynamic pK_a determination is necessary to be able to effectively compare chloroguaiacol dissociation constant values because it corrects for ionic strength differences between the experimental solutions.

MOPAC-based gas phase acidity calculations were linearly related to experimental thermodynamic pK_a values with r^2 values of 0.94 for both the AM1 and MNDO Hamiltonian operators. Hammett predicted pK_a values were strongly correlated to experimental thermodynamic pK_a values with $r^2 = 0.987$. MOPAC calculations appeared to be somewhat better at predicting pK_a values for higher substituted chloroguaiacols than the Hammett equation, possibly because the Hammett σ values are derived from monosubstituted benzoic acids and do not include effects of interaction between the substituents. Hammett calculations, however, do incorporate solvation effects and are slightly more effective at predicting the dissociation constants of mono- and di-chloroguaiacols. These methods for pK_a prediction are useful tools for determining dissociation constants to within tenths of pK units.

Chapter III

pH Dependent Sorption of Chloroguaiacols to Estuarine Sediments

Introduction

Sorptive reactions significantly influence the fate and effect of organic compounds in the environment. Sorbent, sorbate and solvent properties all contribute to the observed sorption of an organic compound in an aqueous system.

The hydrophobic or hydrophilic nature of a sorbate describes whether its dissolution in water is energetically favorable. Dissolution of a molecule in a solvent involves changes in the free energy of the solute/solvent system. There are both enthalpic and entropic contributions to the free energy change. Enthalpic contributions represent the solvent:solvent, solute:solute and solvent:solute interactions, ie. the energies involved in making or breaking such associations. Entropic contributions include the entropies of cavity and ice formation along with enhanced randomness. In dissolving hydrophobic organic compounds (HOC) in water, several factors must be considered. The solute molecule must be isolated from other solute molecules. Hydrogen bonds between water molecules must be broken in order to create a cavity for the solute and new associations must form between the solute and solvent (Schwarzenbach *et al.*, 1993).

Large amounts of energy are required to dissolve HOC in water. It is therefore energetically favorable to leave the aqueous phase and either dissolve into organic substances associated with the sorbent or adhere to a mineral surface by weak intermolecular (van der Waals or dipole-dipole) forces. On the other hand, hydrophilic characteristics such as high polarity may lead to higher aqueous solubility, resulting in a diminished tendency to sorb (Schwarzenbach *et al.*, 1993, Karickoff, 1984).

When the total organic carbon (TOC) content of a sorbent is greater than 0.1%, TOC is considered to be the dominant factor in the partitioning of hydrophobic organic compounds including aromatic and polynuclear aromatic hydrocarbons, PCB and pesticides (Karickoff *et al.*, 1979, Chiou, 1979, Means *et al.*, 1980). More recently, the composition of the organic matter, especially the amount and type of humic acids present, has also been concluded to be significant (Dousset *et al.*, 1994, Payá-Pérez *et al.*, 1992, Garbarini *et al.*, 1986, Rutherford *et al.*, 1992, Carter *et al.*, 1982, Gauthier *et al.*, 1987, Chiou *et al.*, 1983).

For low (<0.1%) TOC sorbents, such as aquifer materials, other regulating characteristics must be considered along with TOC. These include: particle size (and therefore surface area), metal oxide coatings of the sorbent particles (Barber, 1994, Libelo, 1995), the age of organic matter and amount and type of clays present (Banerjee *et al.*, 1985, Karickoff, 1984).

For neutral HOC, aqueous phase composition generally plays a minor role in sorption. If the sorbate is ionized, the composition of the natural waters becomes important. In some cases, changes in ionic strength (by orders of magnitude) have been reported to have a small effect on sorption of pentachlorophenol (Lee *et al.*, 1990) and DDT (Carter *et al.*, 1982). In contrast, other workers have reported that such changes had no observable effect on the sorption of PCB, PAH, and other aromatic compounds (Karickoff *et al.*, 1979), and organic acids including several substituted phenols (Jafvert, 1990).

For ionizable species, such as guaiacols, the solution pH plays an important role in sorption. As mentioned above, hydrophobicity and solubility of the sorbate affect its tendency to sorb. In general, ionized species are much more soluble than their neutral counterparts. It follows that sorption of the ionized and nonionized species will be different, and that the neutral species will tend to sorb more readily. When the pH of the aqueous phase is within about 1 pK unit of the pK_a, both the ionized and molecular species of a compound are present in substantial amounts in solution and sorption of both must be considered.

Lee *et al.* (1990) summarized the theory of acid dissociation and partitioning. For weak organic acids, eg. phenols and guaiacols, the dissociation of the acid, HA, is represented by the following:

$$HA \rightleftharpoons A^- + H^+$$

$$K_a = [A^-][H^+]/[HA]$$

where K_a is the acid dissociation constant, $[A^-]$ is the concentration of the anion, $[H^+]$ is the hydrogen ion concentration and [HA] is the concentration of the undissociated molecule. For this case, activity coefficients are assumed to be near unity. Given the pH and pK_a of the compound (where $pK_a = -\log K_a$ and $pH = -\log[H^+]$), the fraction of neutral phenolic, ϕ_n, can then be calculated as follows:

$$\phi_n = [HA]/([HA] + [A^-])$$

substituting for $[HA] = [H^+][A^-]/K_a$,

$$\phi_n = 1/\{1+(K_a/H^+)\}.$$

Since $K_a = 10^{pKa}$ and $[H^+] = 10^{pH}$,

$$\phi_n = 1/(1 + 10^{pH-pKa}).$$

The distribution of the neutral and ionic species between sediment and aqueous phases can be defined as:

$$K = ([HA]_s + [A^-]_s)/([HA]_w + [A^-]_w)$$

where the subscripts s and w refer to sediment and water, respectively. This K value is actually a combination of sorption, or partition coefficients for two distinct species and is the value measured in most partitioning experiments. K values for the neutral and ionized species can be expressed as:

$$K_n = [HA]_s/[HA]_w \quad \text{and} \quad K_i = [A^-]_s/[A^-]_w,$$

where the subscripts n and i refer to neutral and ionized species.

If it is assumed that only the neutral species is sorbed onto sediments or soils, and that the organic carbon content (OC) of the sorbent determines the degree of sorption, the sorption coefficient with respect to organic carbon content is:

$$K_{oc,p} = K_{oc,n}\phi_n \tag{1}$$

where $K_{oc,n} = (K_n/OC)$ and p refers to the predicted value. This equation is applicable to most hydrophobic, unionizable organic compounds; but will underestimate sorption if there is a significant sorption of the ionized species.

When considering the binding of the ionic species to a sorbent, ion exchange is not believed to be the primary method of sorption. Instead, Lee *et al.* (1990) has proposed that a neutral ion pair forms and binds to the sorbent; or that the hydrophobic end of the molecule orients towards the sorbent, while the more polar end associates with the aqueous phase. In either case the sorption would still be governed by the organic carbon content of the sorbent. The overall sorption can then be described as:

$$K_{oc,p} = K_{oc,n}\phi_n + K_{oc,i}(1-\phi_n) \tag{2}$$

where $K_{oc,i} = (K_i/OC)$. Due to the difference in their hydrophobicities, the sorption of the ionized species will be less than that of the neutral species. If the anionic species does not significantly contribute to the total sorption, (i.e., $K_{oc,n} >> K_{oc,i}$) Equation 2 can be reduced to Equation 1. The validity of this model will be addressed further in the discussion section of this chapter.

For heterogenous sorbents, sorption coefficients can also be further subdivided to consider the various components of the sorbent. For an estuarine sediment made up of various ratios and types of sand, silt and clay, sorption of an organic compound will vary on each size fraction and the different materials composing each fraction will also have distinct sorption coefficients. In this way, the sorption coefficient measured on a natural material is in fact a summation of coefficients for the various components of the material.

Sorption coefficients are determined by plotting the equilibrium concentration in solution versus the amount sorbed. The resulting line is usually fit to the Freundlich isotherm:

$$C_s = KC^n_w$$

where C_s and C_w are the concentrations sorbed (s) and in solution (w), K is referred to as the Freundlich constant and n is the measure of nonlinearity. If $n<1$, it becomes harder to sorb molecules as the concentration in solution increases. This can occur when concentrations reach the point where most of the available binding sites have been filled. When $n=1$, the isotherm is linear, and the affinity of the sorbate for the sorbent remains constant at all concentrations. In situations where $n> 1$, sorption increases with increasing concentration. This may occur when the sorbed molecule actually changes some characteristic of the sorbent altering its sorbing properties or when multilayer sorption occurs. In most cases, all three types of isotherms exhibit linearity at low concentrations. When linear, the K value can be written as $K = C_s/C_w$ and is determined by the slope of the line (Schwarzenbach *et al.*, 1993).

It should be noted here that sorption or partition coefficients are reported in several ways. When not corrected or normalized to TOC, they are generally reported as K_d, K_{ads}, or K_p. The value is determined by the slope of the line generated by plotting concentration in solution (in ug/ml, g/l or mole/l) versus the amount sorbed (in g/kg sorbent or mole/kg sorbent). Values are usually reported in l/kg or ml/g sorbent. K_{oc} is determined as above.

The sorption and partitioning of chlorophenols, especially pentachlorophenol, has been investigated extensively. The influence of pH on octanol/water (Westall *et al.*, 1985, Lee *et al.*, 1990) and sediment/water (Schellenberg *et al.*, 1984, Lee *et al.*, 1990, Fisher, 1990) partitioning has been reported. The octanol/water partition coefficient, K_{ow}, is often used to estimate or predict K_{oc}, because K_{oc} values are generally more difficult and costly to determine. K_{ow} is also used in predicting the lipophilicity and bioaccumulation potential of a compound and is correlated to the compounds aqueous solubility. (Chiou, 1979, 1983).

Lee *et al.* (1990) examined the sorption of pentachlorophenol (PCP, $pK_a=4.75$) on a series of sediments of varying organic carbon content. The study showed that at $pH<7$, hydrophobic sorption of the non-ionized form predominates. Sorption of the neutral species increased with increasing organic carbon content of the sorbent, as well as with decreasing pH. At $pH>7$, sorption of the pentachlorophenolate ion (PCP^-) and formation and sorption of neutral metal-PCP^- pairs was significant.

In a comparable study, up to 90% of phenol, 2-chlorophenol and 2,4-dichlorophenol irreversibly sorbed onto the sediment (Issacson and Frink, 1984). Several other studies have been conducted regarding the sorption of various chlorophenols by sediments, soils and aquifer materials with similar results (Schellenberg *et al.*, 1984; Lagas, 1988; Boyd, 1982 & Boyd *et al.*, 1989). Fisher (1990) reported comparable results and also observed that PCP accumulation in biota increased as pH decreased.

Few studies of a similar nature have been reported for chloroguaiacols. Seip *et al.* (1986) determined K_{oc} values for several chlorinated phenolics including tetrachloroguaiacol and 4,5,6-trichloroguaiacol on three different Norwegian soils. A good correlation between aqueous solubility and K_{oc} was observed for the chlorophenols studied. Water solubility data for chloroguaiacols do not exist, so similar correlations between K_{oc} and solubility could not be made for chloroguaiacols. The effect of solution pH on sorption was not examined in this study, although soil pH was reported. Results of this study are summarized in Table 3 .

In a mesocosm study designed to examine the fate and effects of 4,5,6-CG on aquatic species from several trophic levels, 99% of the parent chloroguaiacol recovered at the end of the experiment was associated with the sediment, some in the form of catechols and lower chlorinated CG. Actual measurements of partition coefficients were not made, but the affinity of the compound for the sediment is apparent (Rosemarin *et al.*, 1990).

As part of a study on the biotransformation of chloroguaiacols, K values of 1.3 and 1.4 (ml/kg of organic carbon, note: units are different from most K values) for 4,5,6-CG and tetrachloroguaiacol have been reported. The pH under which these values were obtained was not described. The aqueous medium was VV 2, an aqueous bacterial growth medium containing trace minerals (Remberger *et al.*, 1986).

It is often difficult to compare reported K_d values between sediments because they are dependent on sediment TOC. It is for this reason that the term K_{oc} is often used. For phenolic compounds, comparison of K_{oc} values is also troublesome because the K_{oc} values are also pH dependent. By assuming insignificant sorption of the phenolate anion, and calculating ϕ_n for the pH at which each isotherm was determined, Schellenberg *et al.* (1984) calculated K_{oc} values for 8 different chlorophenols as nonionzed species on three different sediments. The values are summarized in Table 4 and show the general trend that the more chlorinated, thus more hydrophobic, phenols have a greater tendency to sorb than the more hydrophilic, less chlorinated phenols. This study used lake and river sediments with organic carbon contents of 9 and 3% respectively, and aquifer materials with organic carbon contents of 0.8% and 0.03%. Batch isotherm experiments were carried out in stoppered test tubes to which 0.5-4 g dry weight sorbent, water of fixed pH and ionic strength and the compound of interest were added. Samples were allowed to equilibrate for 15-20 hours at constant temperature, although equilibrium was generally reached within 8 hours. The concentration of phenol remaining in the aqueous phase was determined by HPLC.

Lee *et al.* (1990) used a variety of sorbents, with organic carbon contents ranging from 0.2 to 57.5%, to examine the pH dependent partitioning of ^{14}C labelled pentachlorophenol. The batch equilibrium method, similar to the procedure discussed above, was used. The concentration of pentachlorophenol remaining in the aqueous phase was determined by liquid scintillation. Zachara *et al.* (1986) also employed a similar method to look at the role of pH, soil type and temperature on quinoline sorption (reported pK_a=4.94).

Table 3. Reported K_{oc} values for chloroguaiacols (CG) and pentachlorophenol in column and batch sorption experiments from Seip *et al.* (1986).

Compound	K_{oc} column	K_{oc} batch	Soil pH	TOC%
4,5,6-CG	972	1050	5.6	0.2
	>475	550	7.4	2.2
	>475	5054	4.2	3.7
Tetra-CG	1192	1250	5.6	0.2
	238	1182	7.4	2.2
	>475	9946	4.2	3.7
PCP	922	1550	5.6	0.2
	293	386	7.4	2.2
	>475	8405	4.2	3.7
2,4,6-CP	564	360	5.6	0.2
	91.2	109	7.4	2.2
	>475	1946	4.2	3.7

Table 4. K_{oc} values for nonionized chlorophenols on various sorbents. Reported in Schellenberg *et al.* (1984).

Compound	K_{oc} Sed. 1*	Sed. 2*	Sed. 3*	Avg K_{oc}
2,3-CP	223	461	595	426
2,4-CP	266	654	715	545
2,4,6-CP	830	1310	1070	1070
2,4,6-CP	1760	2850	2380	2330
3,4,5-CP	2940	4420	3690	3680
2,3,4,6-CP	6040	7690	6190	6640
2,3,4,5-CP	13200	13900	12500	13200
PCP	39000	35800	23800	32900

*f_{oc} in g_{oc}/g

Sediment 1: 0.094 (lake sediment)
Sediment 2: 0.026 (river sediment)
Sediment 3: 0.0084 (aquifer material)

Fisher (1990) also used ^{14}C-labelled PCP in 5 liter microcosms with sediments containing either 0 or 3% organic carbon and pH's of 4, 6 or 8. Three trophic levels of organisms were introduced and the system was monitored for seven days. At the end of the experiment, biota, sediment and water were analyzed.

The batch method is the most commonly used technique in determining sorption coefficients. Although not flawless, the use of a common method allows for the relatively simple comparison of results with those of other researchers. Other methods have been employed to study sorption processes. Column-type experiments have been used to more closely emulate processes involving surface water infiltration and groundwater flow and are useful in looking at rates of sorption. The results of such experiments compare favorably with batch isotherms for heavy metals (Burgisser *et al.*, 1993) and organics (Lee *et al.*, 1988). However, interferences from sorption to experimental equipment can be more difficult to assess than in batch experiments (Lion *et al.*, 1990). Seip and co-workers (1986) conducted both column and batch isotherms with chloroguaiacols and chlorophenols (Table 3).

In the present study, the effect of aqueous phase pH, along with the amount and type of organic matter present in the sorbent, on the sediment/water partitioning of four chlorinated guaiacols with pK_a values ranging from 6.06 to 9.06 was examined. Four different estuarine sediments were collected from the Catlett Island National Estuarine Research Reserve Site (NERRS) on the York River in Virginia. TOC values ranged from 0.1 to 3.5%, representing the range of organic carbon concentrations commonly encountered in estuarine environments. Two different pH values, 6.6 and 7.8 are also representative of natural waters (Snoeyink *et al.*, 1980). The pK_a values reported in Chapter II of this work were used to determine the fraction of neutral and ionized CG present at the experimental pH values and the sorption of the two species was evaluated. The results can be used to used to predict the fate and effects of common BKPPME components in receiving waters.

Materials and methods

Sediment collection, characterization, processing

Sediments were collected from the Catlett Islands National Estuarine Research Reserve, on the York River in Virginia. Sites were selected to represent various types of estuarine sediments ranging from a well scoured, sandy point to a protected tidal mudflat. Sediments were air-dried, passed through a 2mm sieve and stored in sealed glass jars at 5°C until use.

BET surface areas were determined, in triplicate, from single point isotherms on a Micromeritics (Norcross, GA) Flowsorb 2300 with a thermal conductivity detector. Gas flow was 30% N_2/70% He at 15 cm^3/min.

Total organic carbon was determined on acidified sediments with a Carlo Erba NA 1500 Carbon/Nitrogen Analyzer ($n \geq 3$).

Grain size analysis was conducted, in triplicate, by the wet sieving method to determine sand. Silt and clay were determined by sedimentation (McManus, 1988).

Percent humic acids were determined gravimetrically in triplicate. A standardized method of humic acid of extraction does not exist. The method employed here is based on recommendations by the International Humic Substances Society (Aiken *et al.*, 1985, MacCarthy, P., 1995, personal communication). Sediment and NaOH (0.5N, 1/10:w/v) were placed in test tubes with teflon lined caps, sparged with N_2, sealed and extracted by agitating on an orbital shaker table at

150rpm overnight. The samples were centrifuged at 3000rpm for 30 minutes and the supernatant containing humic and fulvic acids was siphoned into a clean tube and acidified to $pH < 2$ with 12N HCl. The precipitated humic acids were centrifuged at 3000rpm for 30 minutes and the supernatant containing fulvic acids, Na^+ and Cl^- was removed. Deionized water was added to the humics to remove additional Na^+ and Cl^- that could adulterate the mass of the humics. The mixture was acidified to prevent dissolution of humics and centrifuged as above. The supernatant was removed and the humics rinsed into a tared crucible for drying overnight at 75°C. Sequential extractions were not done because the possibility of adulteration of the precipitated humics by sand or clay particles would be greater as lower amounts of humic acids were extracted.

Sediment characteristics are compiled in Table 5. A 2-tailed Student's *t*-test (Zar, 1984) showed no significant difference ($p=0.05$) between average %TOC or %humic acid values for CY and YR1. The p-values for a significant difference between these sediments was 0.13 for %TOC and 0.069 for %humic acids.

Preparation of spiking solutions

Chloroguaiacol standards (purity 99+%) were purchased from Helix Biotech, Richmond, B.C. Canada. Phosphate buffers (1/15M) were prepared according to Dien and Lentner (1970) using degassed, deionized water. Stock solutions of each CG were prepared with nominal concentrations of 1-2mg/ml in methanol (reagent grade, Burdick and Jackson). To prepare the spiking solution, stock solution was added to the buffer or the CG was dissolved directly to achieve desired CG concentrations. Methanol concentrations were 1.5% or less in the final spiking solutions.

Equilibrium Sorption Isotherms

The batch equilibrium method was used to measure equilibrium sorption isotherms at room temperature (23±3°C). For each isotherm, 0.3 to 5.0 grams of sediment were weighed into methanol rinsed scintillation vials (20ml) with foil lined caps. Ten ml of spiked buffer solution (containing 1 to 4 chloroguaiacols) were added to each vial and the vials sealed. It is accepted that several sorbates in the same batch do not affect sorption at concentrations far from saturation (Schellenberg *et al.*, 1984). Preliminary kinetic experiments indicated that equilibrium was reached within 12 hrs. To ensure equilibrium, samples were shaken for 24 hours at 125 rpm on an orbital platform shaker (Junior Orbit Shaker, Lab Line Institute, Melrose Park, Ill). Five concentrations were prepared for each isotherm. Initial sorbate concentrations ranged from 0.75 to 100μg/ml. For each isotherm, each concentration was run in triplicate. The concentration ranges used for each CG was limited by detection limits and solubility of compound. When possible, sorbent weight was selected to achieve 50% sorption (based on preliminary isotherms).

Table 5. Sediment characteristics

Sediment*	% Total Organic Carbon (sd) n≥3	% sand (sd) n=3	% silt (sd) n=3	% clay (sd) n=3	% Humic (sd) n≥3	Surface area, m^2/g (sd) n≥3
CM	3.52 (0.26)	5.45 (0.21)	51.03 (2.87)	43.51 (2.84)	1.20 (0.13)	13.56 (0.186)
CY	0.56 (0.10)	76.63 (2.18)	8.57 (2.62)	14.80 (0.45)	0.077 (0.032)	7.85 (0.103)
YR1	0.68 (0.07)	85.34 (3.97)	7.45 (3.93)	7.21 (0.22)	0.16 (0.042)	2.50 (0.123)
YR2	0.11 (0.02)	98.15 (.43)	0.66 (.071)	1.19 (0.43)	0.026 (0.018)	0.701 (0.0125)

* CM=Catlett muck
CY=Catlett@York R.
YR1=York R. 1
YR2=York R. 2

After equilibration, the samples were centrifuged at 3500rpm for 30min. One ml of the supernatant was transferred to autosampler vials and acidified with 3μl glacial acetic acid for HPLC analysis.

Sorbent-free blanks, also prepared in triplicate for each concentration, were run with each isotherm, to determine the extent of sorption onto the vial. Sorbate-free isotherms were run for each sediment/pH combination to ensure that the sediments were free of HPLC interferences at the wavelengths and retention times of interest. Two to four isotherms were conducted for each compound at each treatment combination.

HPLC Analysis

Samples were acidified with glacial acetic acid to prevent a change in pH of the mobile phase upon introduction of the pH buffered sample to the column, significantly improving chromatography. Ten to 100μl of sample were analyzed on a Waters HPLC equipped with a model 510 pump, 717plus autosampler and 486 detector with a Whatman ODS-2 10μ reverse-phase C_{18} column and C_{18} reverse-phase guard column cartridge. Operating conditions were: isocratic with 79/20/1 MeOH/H20/glacial acetic acid with a flow rate of 1ml/min. The detector was set at 240nm. Five point calibration curves were run at the beginning and end of each analysis and continuing calibration standards were analyzed after every 5 to 10 samples. Average run times for each sample were between 10 and 20 minutes

Determining *K*

The distribution coefficient, K_d, for the linear portion of the isotherm, was determined by plotting the equilibrium concentration in solution (μg/ml) versus the amount sorbed (μg/g sediment) (Figure 9), the slope of the resulting line being K_d. The linear portion of the curve was determined visually. The concentration in solution was determined by HPLC as above and the amount sorbed was determined by the difference between the initial aqueous concentration (corrected for sorption to the reaction vial) and the amount remaining in solution.

To account for systematic errors associated with each isotherm run, eg. temperature fluctuations in the room, the results of all isotherms for each treatment were combined and plotted together to calculate a single K_d, by simple linear regression, for each treatment. A representative plot of the combined isotherms is given in Figure 10. K_{oc} and K_{humic}, sorption coefficients normalized to the percent organic carbon and humic acid, respectively were calculated by dividing K_d by the fraction of organic carbon (f_{oc} = %TOC/100) or humic acid (f_{humic} = %humics/100).

Figure 9.

Typical sorption isotherm.
Sample shown: YR1 at pH 6.6 with tetrachloroguaiacol.
Slope of the line through first four concentrations
represents K_d.

York R. 1: pH 6.6 tetrachloroguaiacol

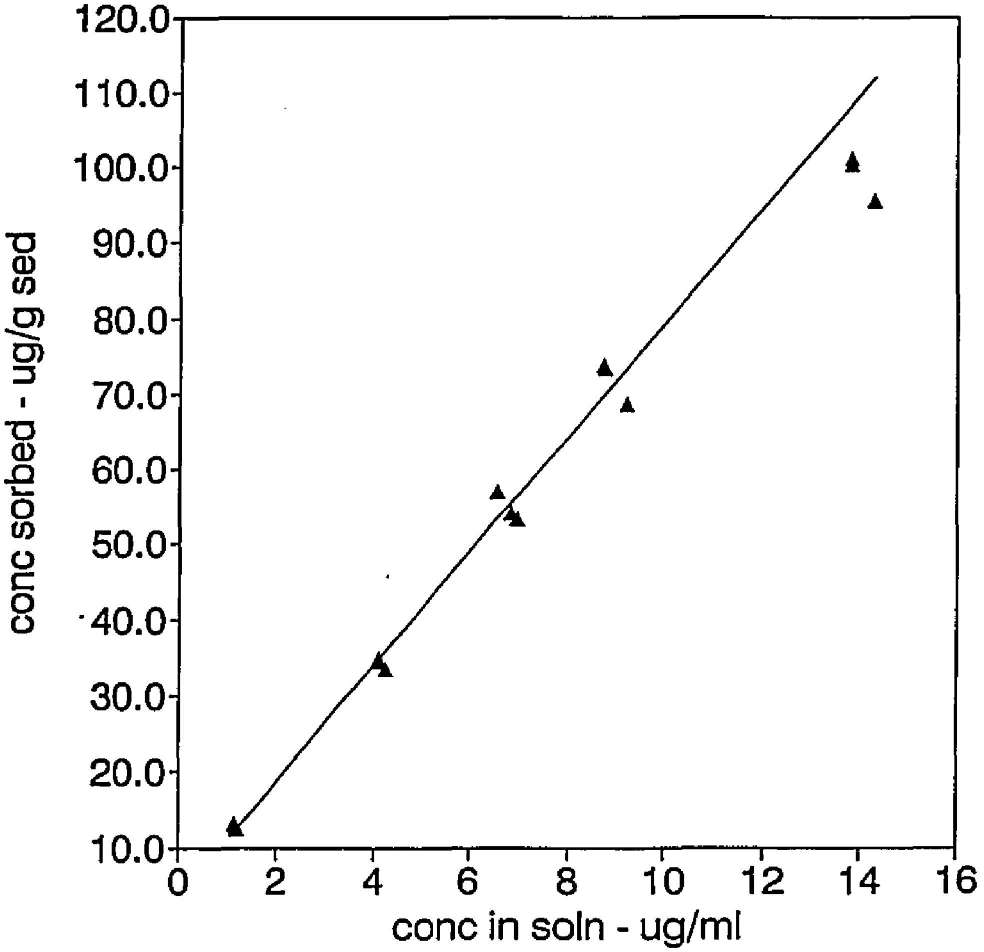
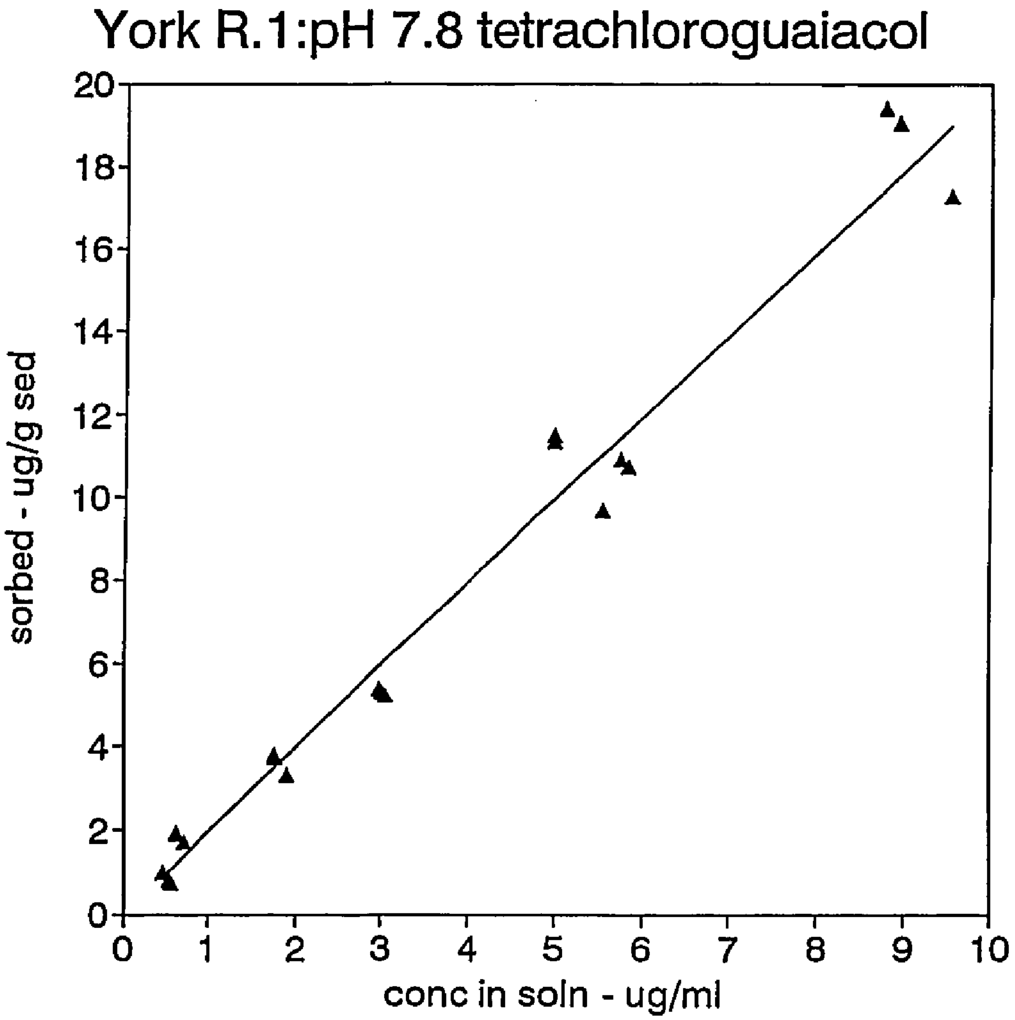


Figure 10.

Plot of results of combined isotherm runs.
Sample shown: YR1 at pH 7.8 with tetrachloroguaiacol.
Slope of the line represents K_d.



York R.1:pH 7.8 tetrachloroguaiacol
sorbed - ug/g sed
conc in soln - ug/ml
0
2
4
6
8
10
12
14
16
18
20
0
1
2
3
4
5
6
7
8
9
10

RESULTS AND DISCUSSION

Experimentally determined and derived *K* values for 5-, 4,6-, 4,5,6- and tetrachloroguaiacol (CG) are presented in Table 5. Sediment characteristics are compiled in Table 5. In the figures that follow, the effects of pH and sediment characteristics on sorption are demonstrated. Error bars are not included on the graphs because standard errors of the K_d values computed from the isotherm data are very small (see Table 6) and would not be discernible.

pH effects

Figures 11 through 14 depict the range in K_d values for each CG with each sediment and the effect of pH on K_d. All calculated K_d values (slopes) were significant at p=0.05. For 4,6-dichloroguaiacol, 4,5,6-trichloroguaiacol and tetrachloroguaiacol, the K_d values at pH 6.6 and 7.8 were significantly different (p=0.05) for each sediment. Little difference in the K_d values with pH was observed for 5-chloroguaiacol for each sediment, because at both pH values, 5-chloroguaiacol was almost entirely nonionized (see Table 6). A 2-tailed *t*-test for the homogeneity of slopes (Zar, 1984) showed no significant difference (p=0.05) between the K_d values, at pH 6.6 and 7.8, for 5-chloroguaiacol on each sediment.

The difference in sorption coefficients of the other chloroguaiacols with pH on each sediment is evident and is primarily the result of the differences in the neutral fraction present at each pH. Sorption of organic anions is often considered insignificant. At pH 7.8, 98% of the tetrachloroguaiacol is ionized, yet the K_d values, on a given sediment, are similar to those of 4,5,6-CG which is only 80% ionized. Although these two compounds are predominantly ionized at pH 7.8, their sorption coefficients are almost twice that of the lower chlorinated 5-CG and 4,6-CG which are predominantly neutral at the same pH. For higher chlorinated CG, the sorption of the chloroguaiacol anion cannot be disregarded.

Table 6. Experimental *K* values for chloroguaiacols

Compound (pK_a)	Sediment	pH	K_d (std error)	r^2	n=	K_{oc} (K_d/f_{oc})	K_{humic} (K_d/f_{hum})
5-G (9.06) $\phi_{n,\ 6.6}$= 0.99 $\phi_{n,\ 7.8}$= 0.95	CM*	6.6	3.61 (0.15)	.965	24	101	301
		7.8	3.79 (0.19)	.953	21	107	315
	CY*	6.6	0.48 (0.03)	.923	24	85	621
		7.8	0.38 (0.05)	.785	21	68	491
	YR1*	6.6	0.80 (0.03)	.976	24	117	497
		7.8	0.73 (0.04)	.943	24	107	454
	YR2*	6.6	0.12 (.003)	.986	24	106	447
		7.8	0.13 (0.01)	.856	21	106	447
4,6-G (8.03) $\phi_{n,\ 6.6}$= 0.96 $\phi_{n,\ 7.8}$= 0.63	CM	6.6	12.23 (0.38)	.980	24	346	1019
		7.8	6.43 (0.07)	.997	27	182	536
	CY	6.6	1.39 (0.04)	.982	27	249	1812
		7.8	0.90 (0.05)	.953	21	160	1164
	YR1	6.6	2.72 (0.11)	.972	21	400	1701
		7.8	1.31 (.04)	.981	24	193	822
	YR2	6.6	0.37 (0.005)	.997	21	335	1415
		7.8	0.21 (0.007)	.979	24	190	804

Compound (pK_a)	Sediment	pH	K_d (std error)	r^2	n=	K_{oc} (K_d/f_{oc})	K_{hum} (K_d/f_{hum})
4,5,6-G (7.19) $\phi_{n,\ 6.6}=$ 0.80 $\phi_{n,\ 7.8}=$ 0.20	CM	6.6	46.66 (1.38)	.961	48	1318	3888
		7.8	12.35 (0.31)	.986	24	349	1029
	CY	6.6	4.72 (0.30)	.856	45	842	6124
		7.8	1.73 (0.16)	.859	21	309	2244
	YR1	6.6	8.91 (0.27)	.963	42	1310	5568
		7.8	2.71 (0.17)	.931	21	398	1693
	YR2	6.6	1.16 (0.06)	.893	45	1058	4476
		7.8	0.35 (0.03)	.875	24	318	1345
tetra-G (6.06) $\phi_{n,\ 6.6}=$ 0.22 $\phi_{n,\ 7.8}=$ 0.02	CM	6.6	46.03 (1.36)	.984	21	1301	3836
		7.8	11.65 (1.03)	.888	18	329	971
	CY	6.6	3.96 (0.18)	.956	24	708	5145
		7.8	0.822 (0.07)	.898	18	147	1068
	YR1	6.6	7.80 (0.26)	.980	21	1147	4876
		7.8	1.99 (0.08)	.973	21	293	1246
	YR2	6.6	1.21 (0.04)	.976	21	1098	4646
		7.8	0.239 (0.03)	.810	21	217	918

*: Indicates no significant difference in K_d values for sediment between pH 6.6 and 7.8 at a 0.05 probability level.

Figure 11.

K_d values for Catlett Muck sediment (CM) for four chloroguaiacols at pH 6.6 and 7.8

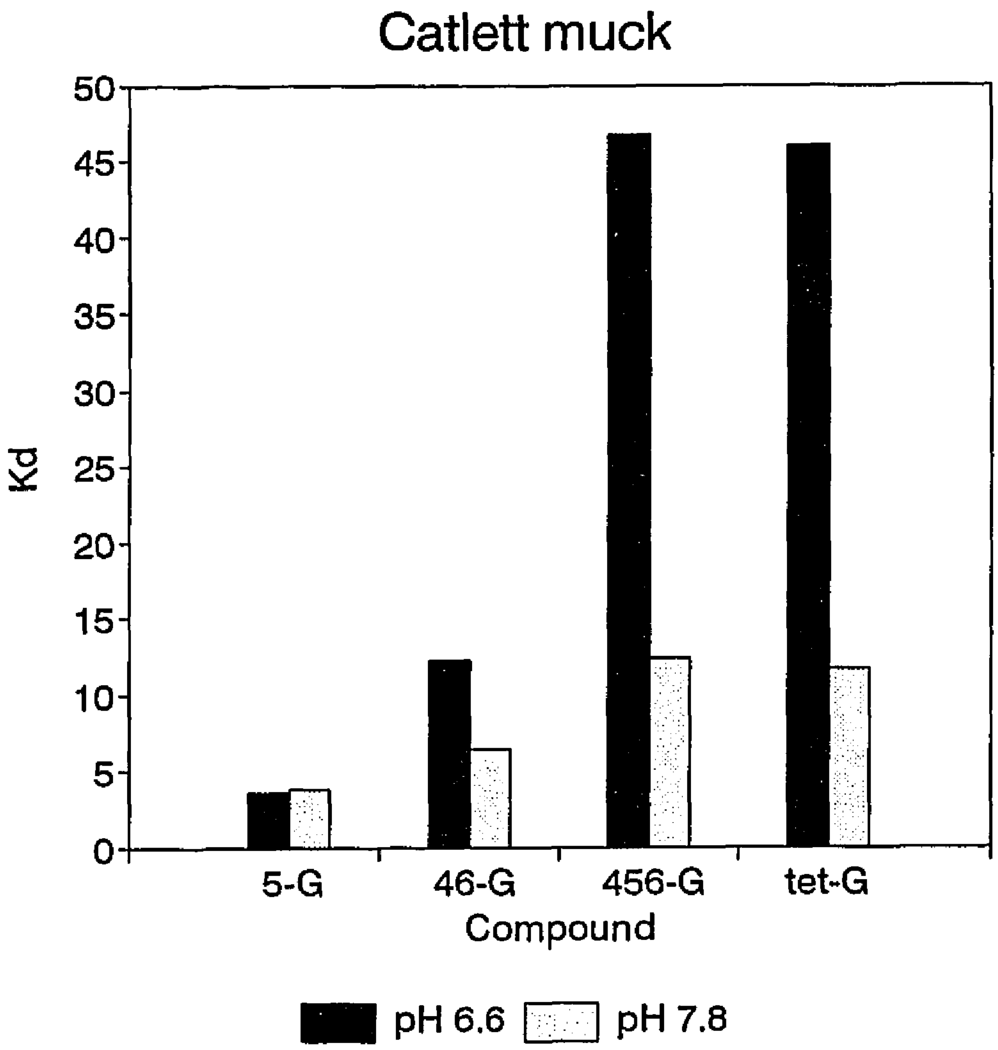

Catlett muck
Kd
50
45
40
35
30
25
20
15
10
5
0
5-G
46-G
456-G
tet-G
Compound
pH 6.6
pH 7.8

Figure 12.

K_d values for Catlett@York sediment (CY) for four chloroguaiacols at pH 6.6 and 7.8

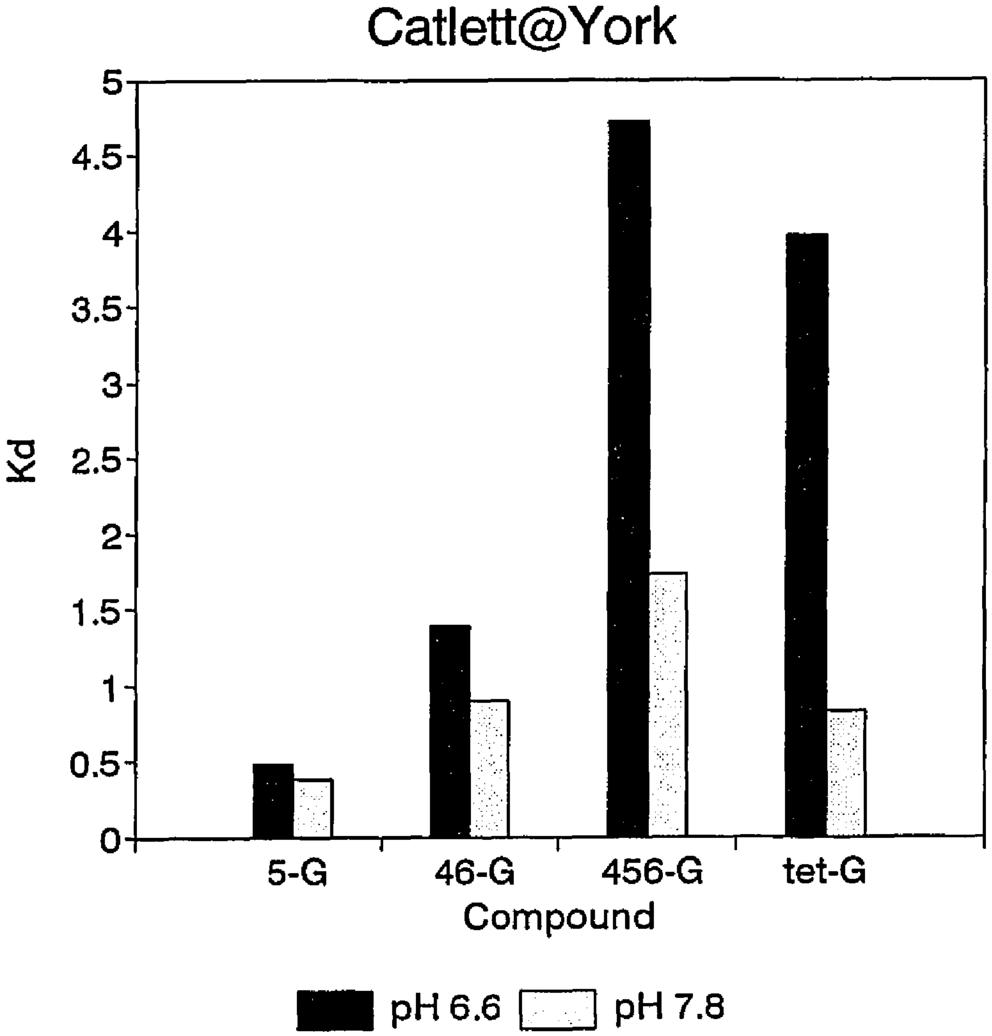
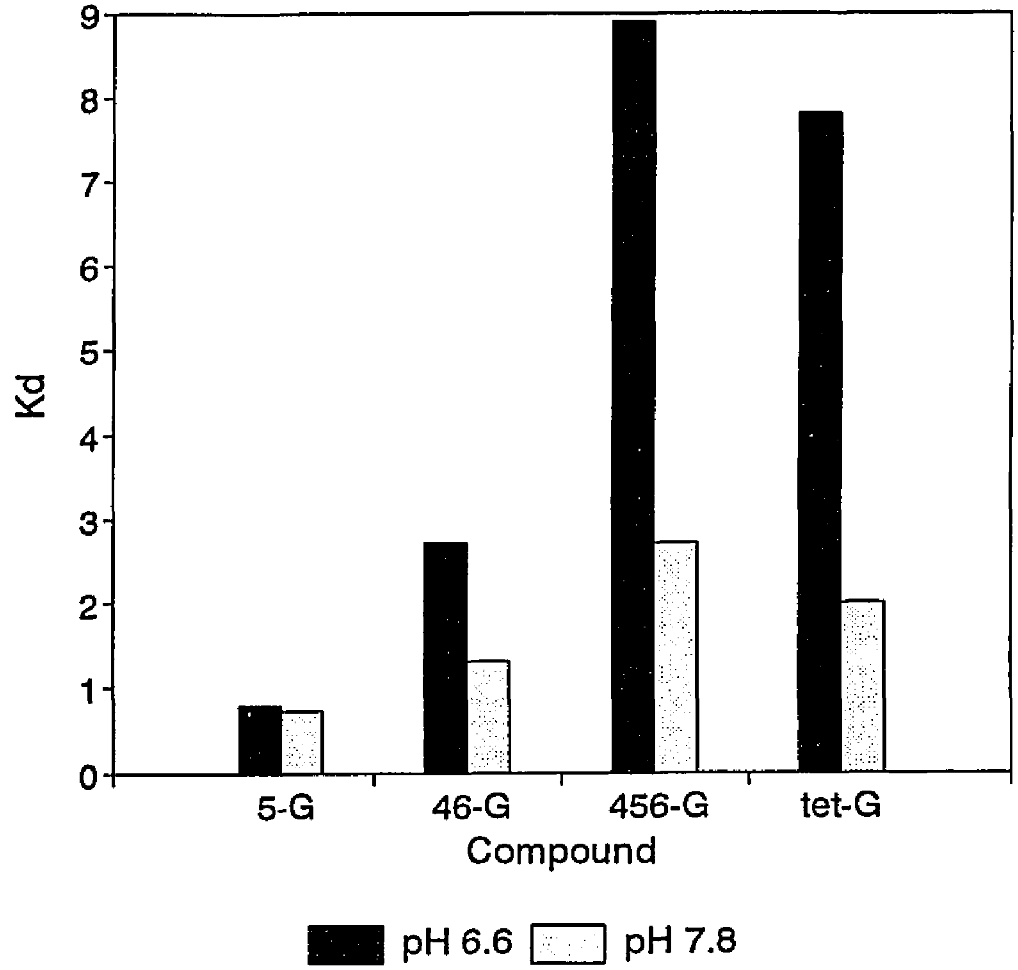

Catlett@York
Kd
5
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
5-G
46-G
456-G
tet-G
Compound
pH 6.6
pH 7.8

Figure 13.

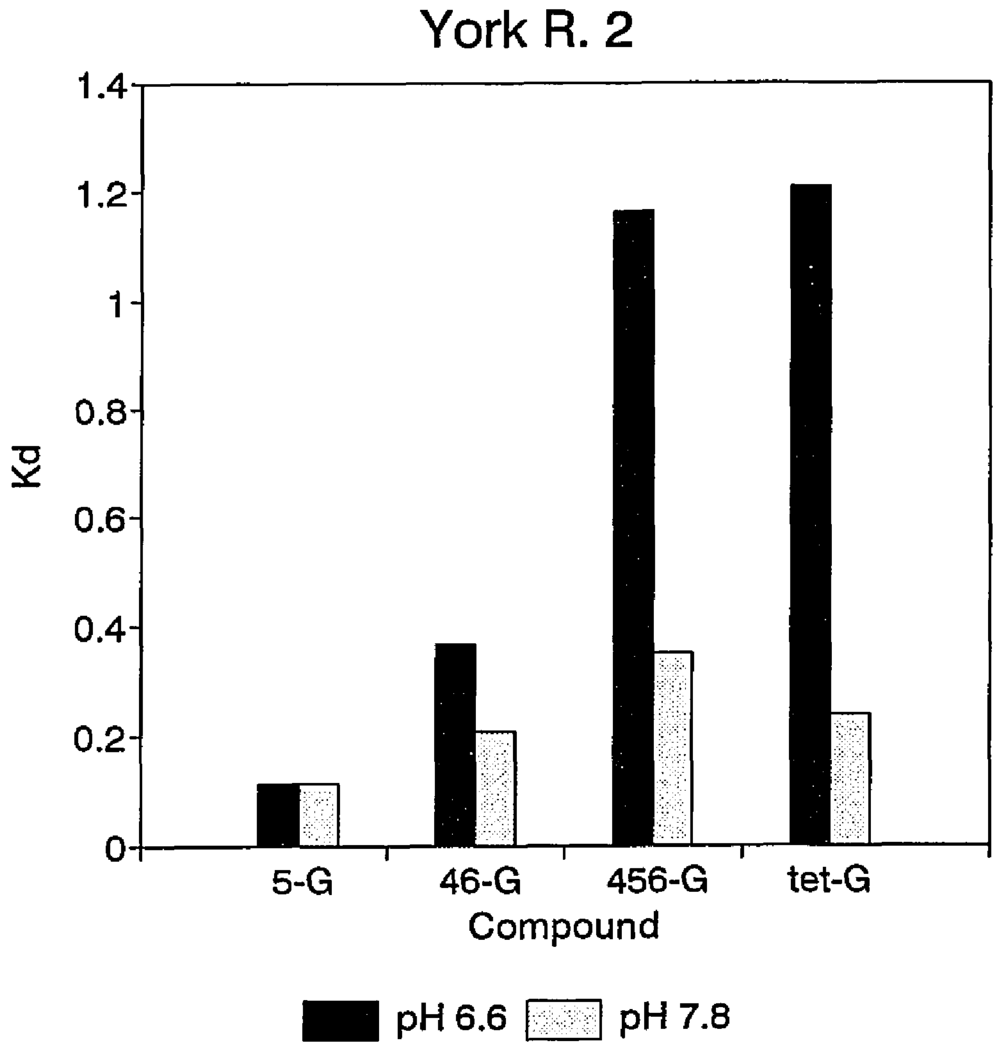
K_d values for York R. 1 sediment (YR1) for four chloroguaiacols at pH 6.6 and 7.8



York R. 1

Figure 14.

K_d values for York R. 2 sediment (YR2) for four chloroguaiacols at pH 6.6 and 7.8


York R. 2
Kd
1.4
1.2
1
0.8
0.6
0.4
0.2
0
5-G
46-G
456-G
tet-G
Compound
pH 6.6
pH 7.8

Organic carbon effects

In Figures 15 through 18, the extent to which calculation of K_{oc} can normalize partition coefficients is demonstrated. For CM, YR1 and YR2, the K_{oc} values are quite similar for a given treatment (pH/CG combination) but CY values are generally lower. As discussed earlier, it is not only the amount, but also the type of organic carbon present, that is an important factor in determining sorption. YR1 and CY have essentially the same %TOC yet their respective K_d and K_{oc} values are very different. The reason may lie in the amount or contribution of humic acids present in the two sediments. YR1, the better sorbing substrate has about two times more humic acids by weight than CY.

K_{humic} is not a commonly used term but was calculated and the values for each compound were plotted against each sediment in the same manner as K_{oc} (Figures 19-22). The values for CY and YR1 converged, indicating that the humic portion of the organic carbon is an important factor in partitioning.

The K_{humic} values for Catlett muck are generally lower than the other three sediments. This may result from the higher overall TOC content, but humic acids are a larger percent (calculated as [%humic/%TOC]100) of TOC for CM than for any other sediment. It would seem, then, that the K_{humic} values for CM should actually be higher than the other sediments. It is possible that the humic acids present in CM are in some way different from those in the other sediments. Analysis of C, H, N, O, and S along with a measure of the degree of aromaticity would be necessary to further characterize the material. Determination of humic acids is a relatively simple

Figure 15.

K_{oc} values for 5-chloroguaiacol on each sediment at pH 6.6 and 7.8.

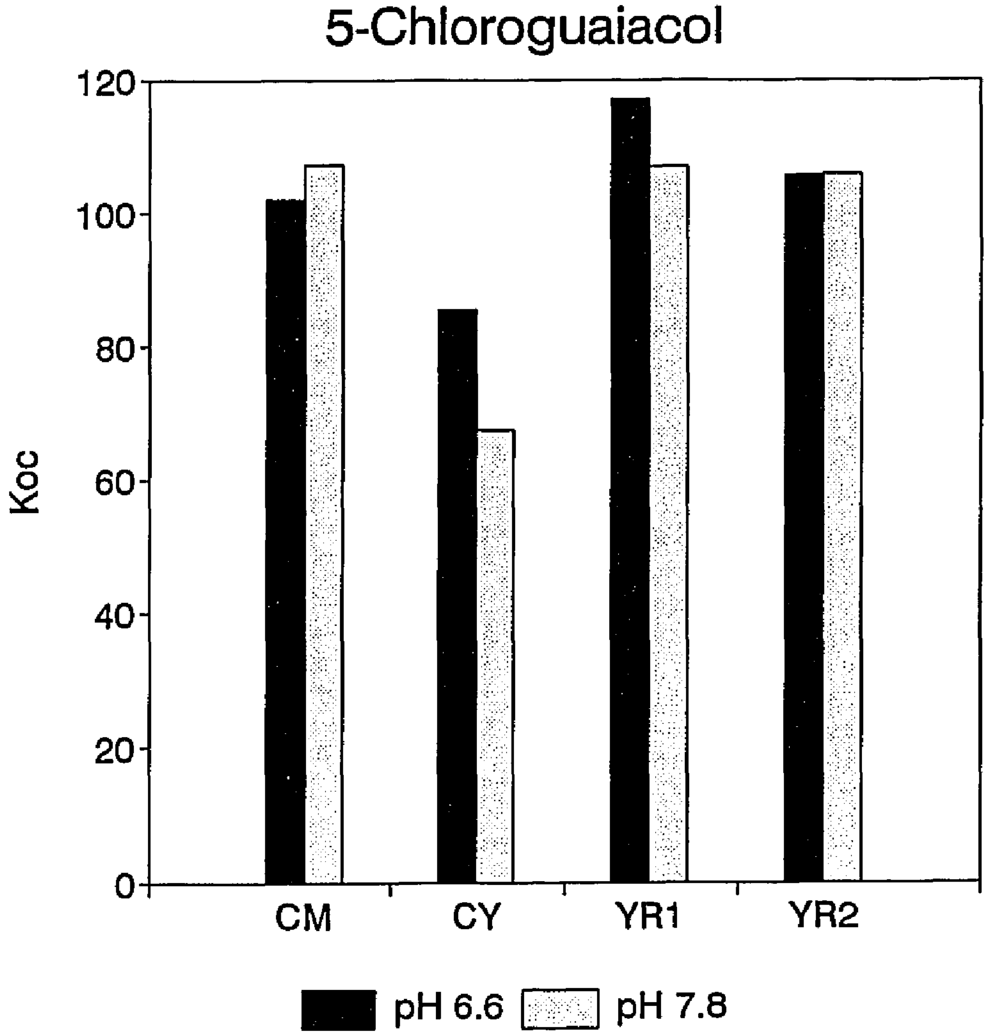
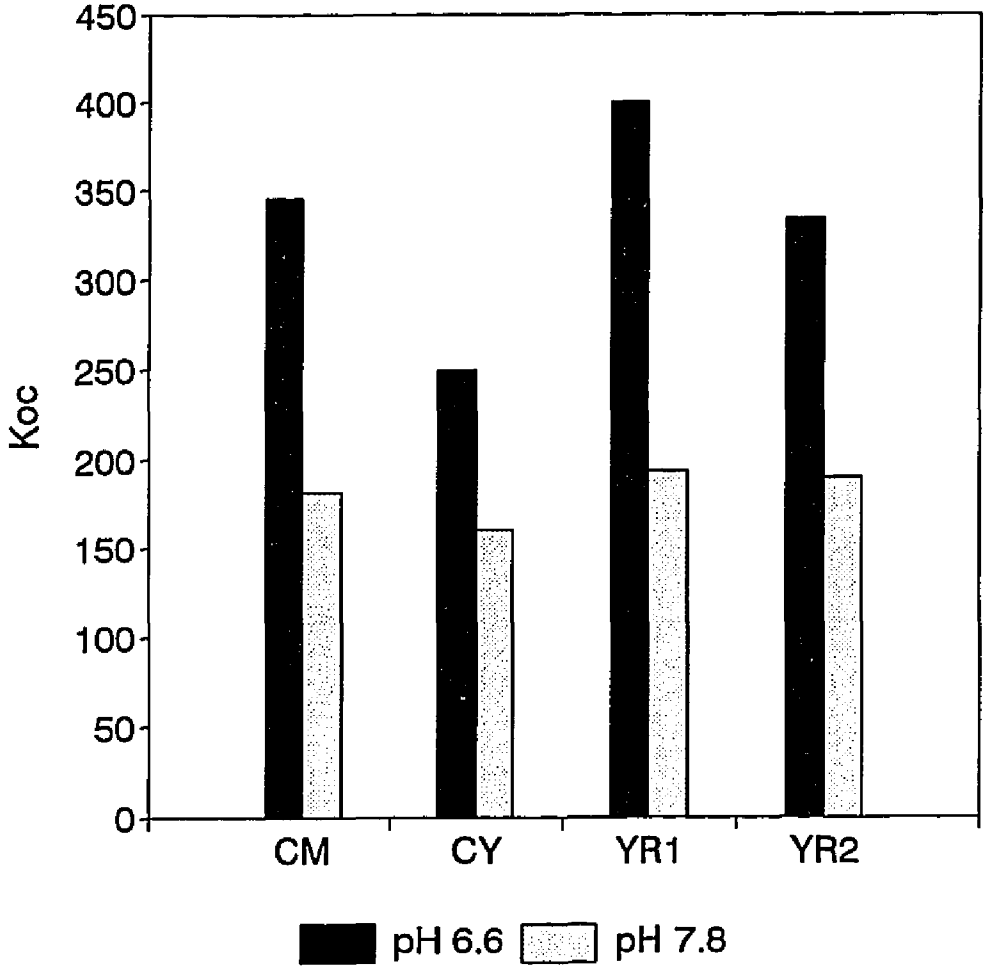

5-Chloroguaiacol
Koc
0
20
40
60
80
100
120
CM
CY
YR1
YR2
pH 6.6
pH 7.8

Figure 16.

K_{oc} values for 4,6-dichloroguaiacol on each sediment at pH 6.6 and 7.8.



4,6-Dichloroguaiacol

Figure 17.

K_{oc} values for 4,5,6-trichloroguaiacol on each sediment at pH 6.6 and 7.8.

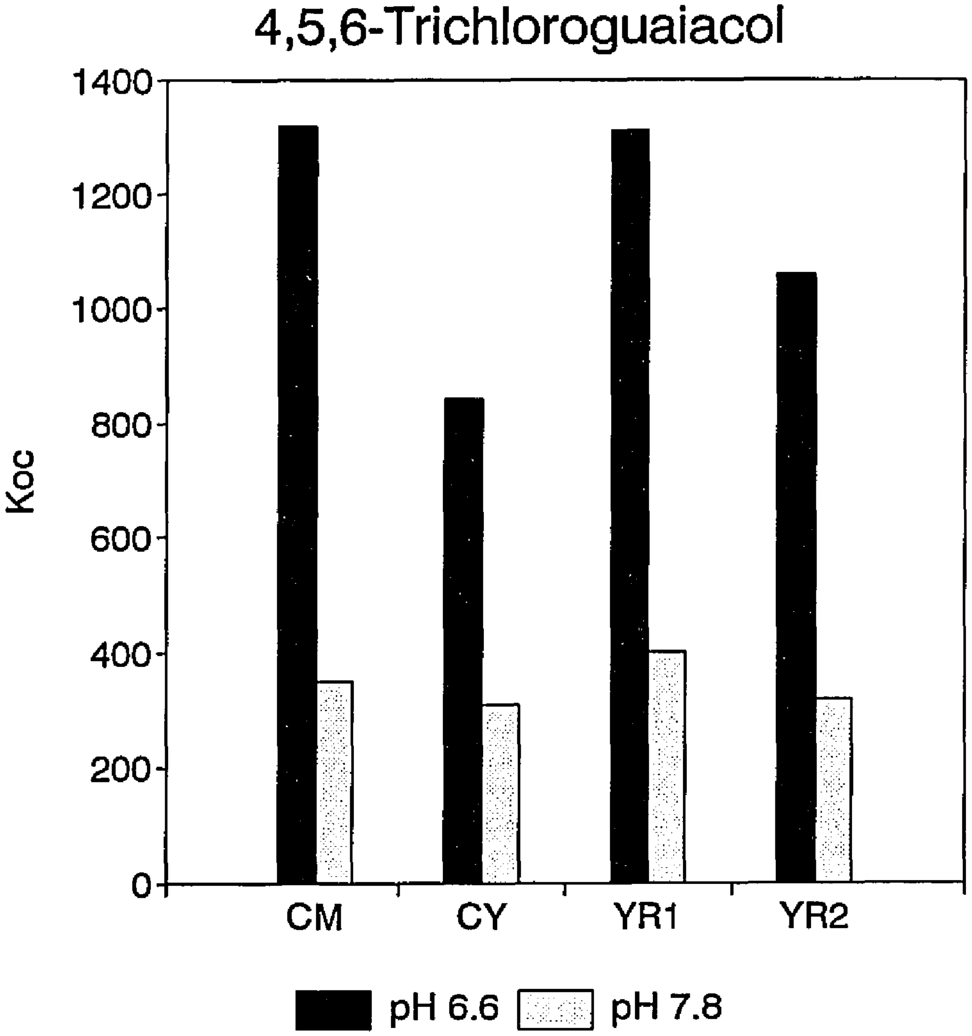

4,5,6-Trichloroguaiacol
Koc
1400
1200
1000
800
600
400
200
0
CM
CY
YR1
YR2
pH 6.6
pH 7.8

Figure 18.

K_{oc} values for tetrachloroguaiacol on each sediment at pH 6.6 and 7.8.

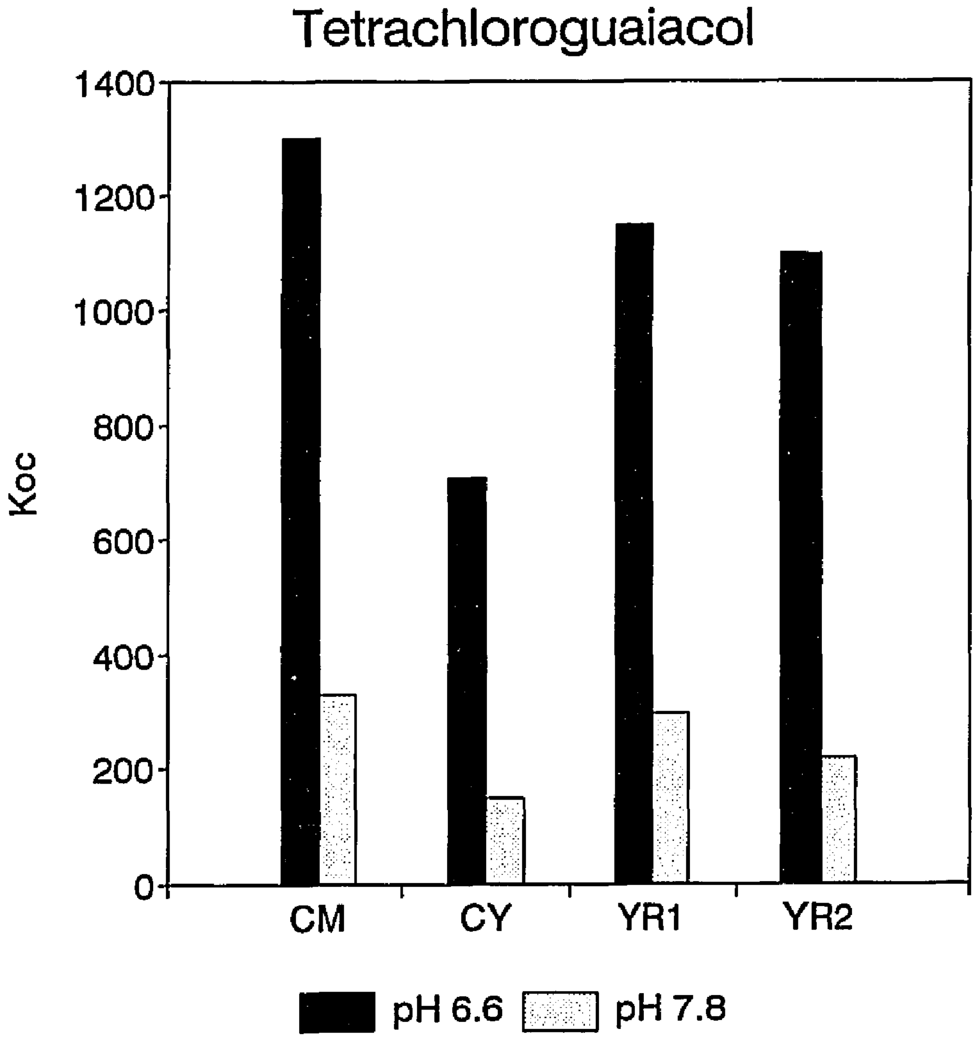

Tetrachloroguaiacol
Koc
1400
1200
1000
800
600
400
200
0
CM
CY
YR1
YR2
pH 6.6
pH 7.8

Figure 19.

K_{humic} values for 5-chloroguaiacol on each sediment at pH 6.6 and 7.8.

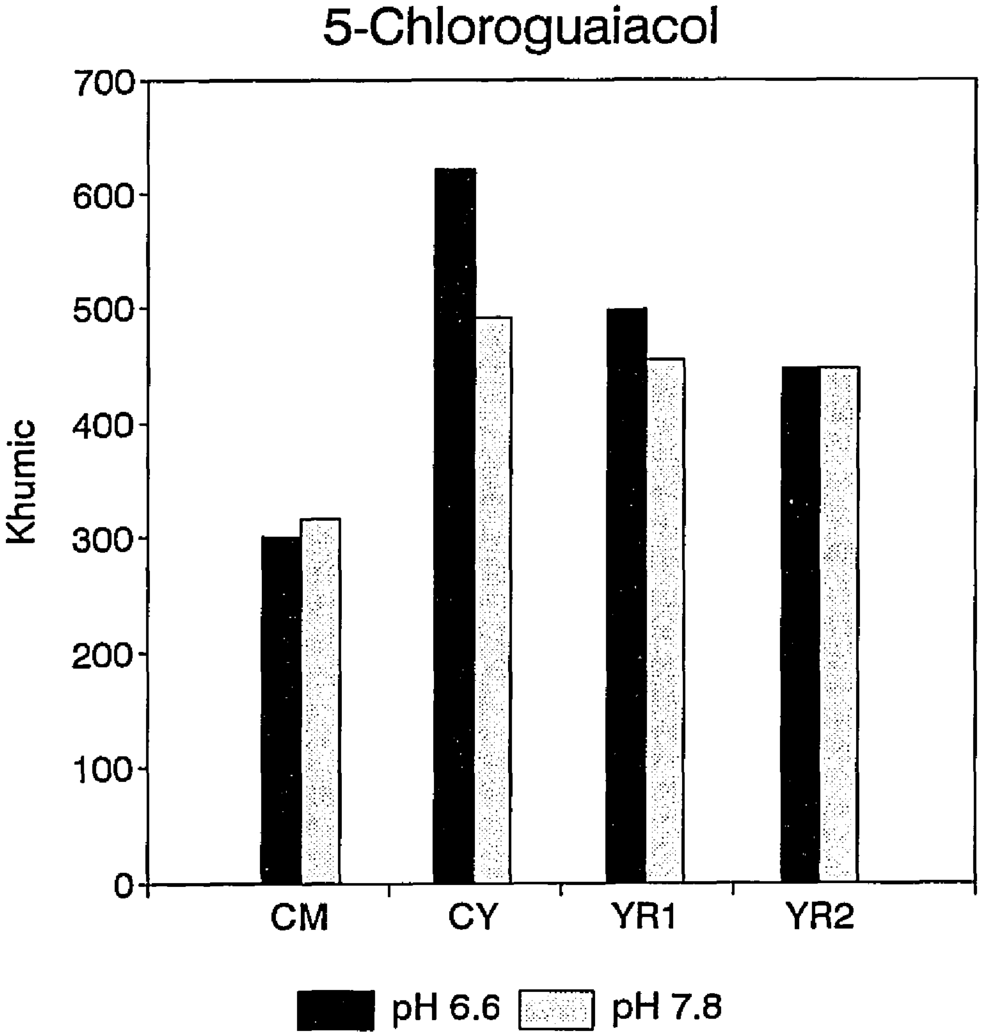

5-Chloroguaiacol
Khumic
700
600
500
400
300
200
100
0
CM
CY
YR1
YR2
pH 6.6
pH 7.8

Figure 20.

K_{humic} values for 4,6-dichloroguaiacol on each sediment at pH 6.6 and 7.8.

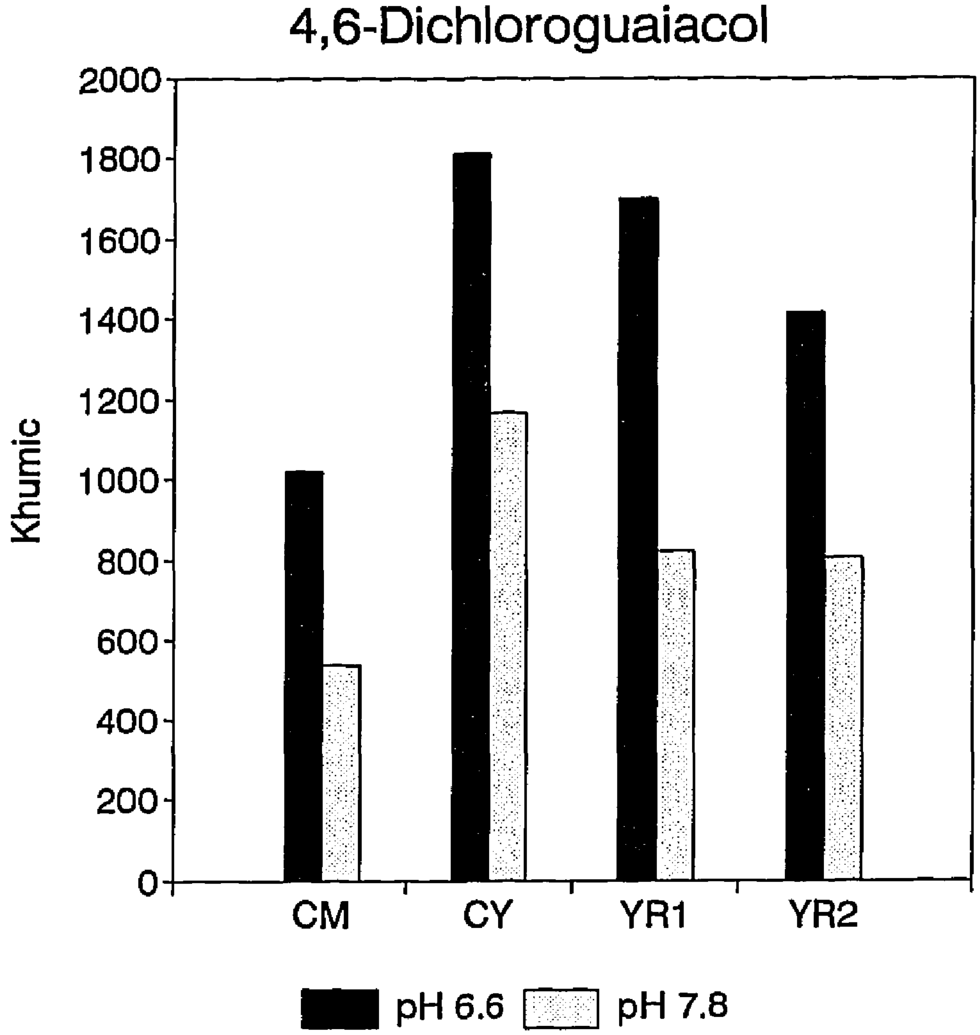

4,6-Dichloroguaiacol
Khumic
2000
1800
1600
1400
1200
1000
800
600
400
200
0
CM
CY
YR1
YR2
pH 6.6
pH 7.8

Figure 21.

K_{humic} values for 4,5,6-trichloroguaiacol on each sediment at pH 6.6 and 7.8.

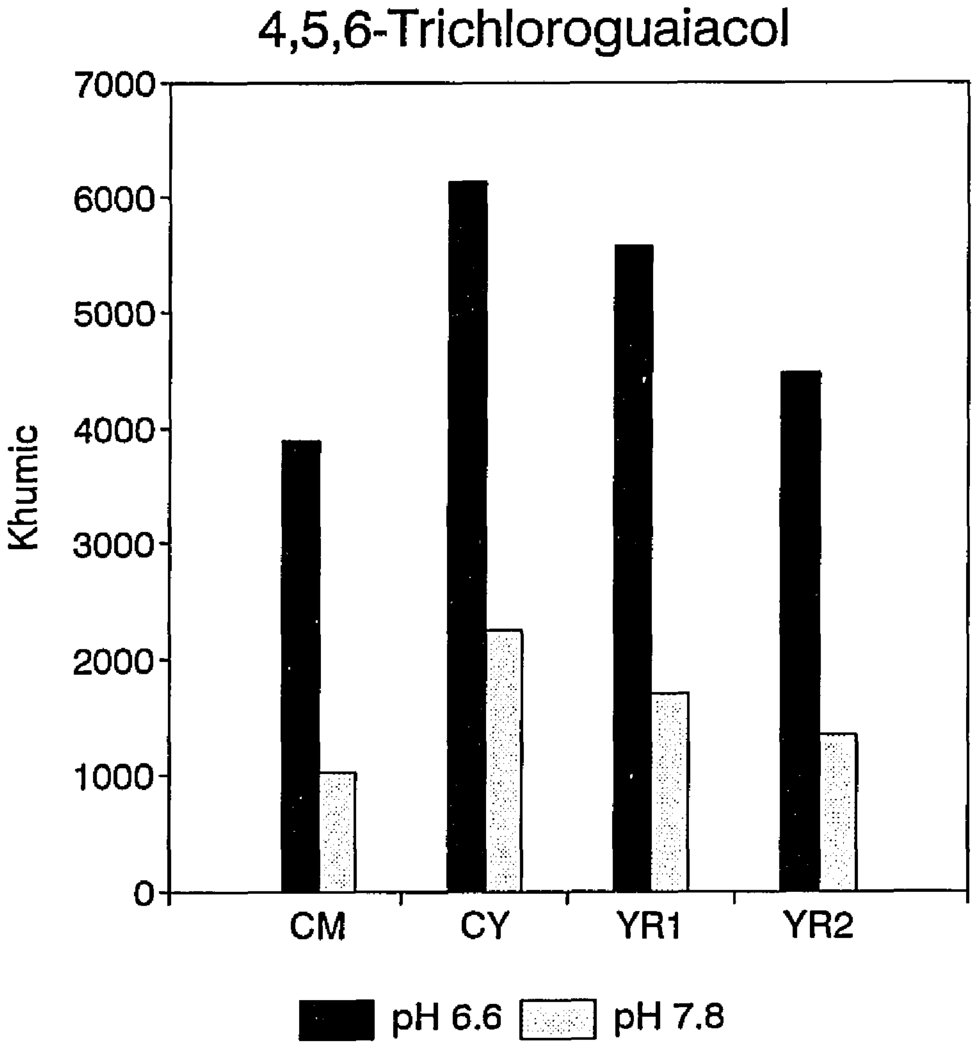
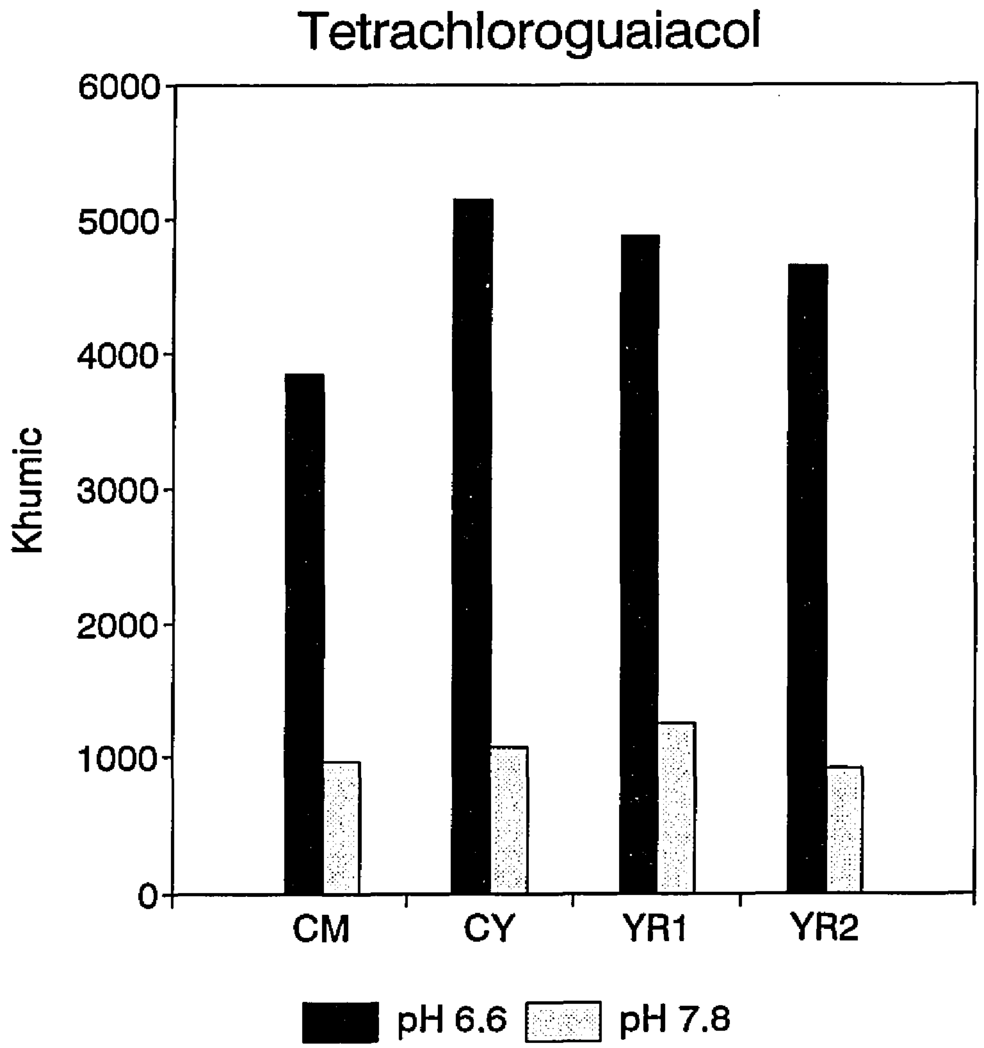

4,5,6-Trichloroguaiacol
Khumic
7000
6000
5000
4000
3000
2000
1000
0
CM
CY
YR1
YR2
pH 6.6
pH 7.8

Figure 22.

K_{humic} values for tetrachloroguaiacol on each sediment at pH 6.6 and 7.8.


Tetrachloroguaiacol
Khumic
6000
5000
4000
3000
2000
1000
0
CM
CY
YR1
YR2
pH 6.6
pH 7.8

procedure as done here and is a useful parameter in assessing sorption. K_{humic} can be used in conjunction with K_{oc} in describing and understanding sorptive reactions.

Another possibility is that because of its high degree of humification, more humic acids from CM could have dissolved into the aqueous phase compared to other sediments. The presence of colloidal material, such as dissolved humics, in solution can result in an apparent increase in water solubility and consequently a decrease in sorption of HOC. This phenomenon has been observed for PCB in natural waters (Baker *et al.*, 1986) and other pollutants (Chiou *et al.*, 1986). Carlberg *et al,* (1986) suggested that humic substances in natural waters can reduce the bioavailability of pollutants including PCB, pesticides and phenolics.

The effect of colloidal material on the determination of sorption coefficients has been reported. Early work on the determination of sorption coefficients noted a "solids concentration effect" where *K* values appear to decrease with an increase in the experimental solid/water ratio (Gschwend and Wu, 1985, O'Connor and Connolly, 1980). Further investigation into this phenomenon indicated that as the solid concentration increased, the concentration of nonsettling or nonfilterable macromolecules also increased. This colloidal material increased the apparent aqueous solubility, reducing sorption.

It is possible that relative to the other sediments, there was a higher concentration of dissolved humic acids in CM samples resulting in the relatively lower K_{humic} values. In future studies, measurements of dissolved organic carbon could address this question. For most isotherms, the sediment weights used were: 0.3gCM, 1.0gCY, 1.0gYR1 and 5.0gYR2. Multiplying the sediment weight by f_{humic}, the amounts of humic acid in each sample were 3.60, 0.77, 1.60, 0.26mg, respectively.

Plots of K_d versus both %TOC (Figures 23-26) and %humic acids (Figures 27-30) exhibit linear relationships as evidenced by the regression equations for each line (see figure captions). All regression lines based on the four points plotted (representing the four sediments) were significant at a 0.05 probability level. For the three point regressions that excluded the CM sediment, only three regression lines were significant: 4,6-dichloroguaiacol at pH 6.6 with %humic acids and tetrachloroguaiacol at pH 6.6 and 7.8 with %humic acids. Since humic acids are a portion of the TOC, a multiple regression of the two factors could not be made.

Attempts to derive K_i and K_n from K_d

In the introduction to this chapter, K_d was suggested to be the sum of the sorption coefficients for the ionized, $K_{d,i}$, and neutral, $K_{d,n}$, chloroguaiacols based on the fractions of each species present at a given pH and that K_d could be resolved not its components. In order to calculate these two values, K_d at two pH values must be determined and the system of equations:

at pH 6.6: $$K_{d,\,6.6} = K_{d,n}\phi_{n,\,6.6} + K_{d,i}(1-\phi_{n,\,6.6})$$

and

at pH 7.8: $$K_{d,\,7.8} = K_{d,n}\phi_{n,\,7.8} + K_{d,i}(1-\phi_{n,\,7.8}),$$

solved. When actual experimental values are used to solve this system, negative values for $K_{d,i}$ are obtained. The reason for this is that the model assumes that the sorbent characteristics were the same for each determination of K_d. Organic matter, especially humic acids, behave very differently at various pH values and thus their sorbent capacities change.

Humic acid is a general term for a category of refractory, naturally occurring, biogenic, heterogenous organic substances with high molecular weight (Aiken *et al.*, 1985). Humic substances contain many different acidic functional groups, including carboxyl and phenolic groups. The pK_a values for most carboxylic acid groups is between 2 and 6. For phenolic groups, the pK_a range is between 8 and 12. At a given pH, certain groups will, therefore, be protonated while others will be ionized. The ionized groups will be more hydrophilic and the protonated groups more hydrophobic (Perdue *et al.*, 1984). The effect of this is that at lower pH values, the humic acid, as a whole, will be more hydrophobic in nature and tend to coil, or bunch up, presenting less surface area to the water and a favorable atmosphere for sorbing. As pH increases, more hydrophilic groups will be present and the molecule will uncoil. Ionic strength also has an effect on humic coiling, with high ionic strength causing the humic to coil (Murphy *et al.*, 1994).

Figure 23.

K_d versus %TOC for 5-chloroguaiacol.

Regression equations

For all four points on line:

pH 6.6: $Y = 1.02x + 0.01$, $r^2 = 0.99$,

pH 7.8: $Y = 1.09x - 0.08$, $r^2 = 0.99$

For first three points (representing YR2, YR1, CY):

pH 6.6: $Y = 1.09x - 0.03$, $r^2 = 0.92$,

pH 7.8: $Y = 0.94x - 0.01$, $r^2 = 0.85$

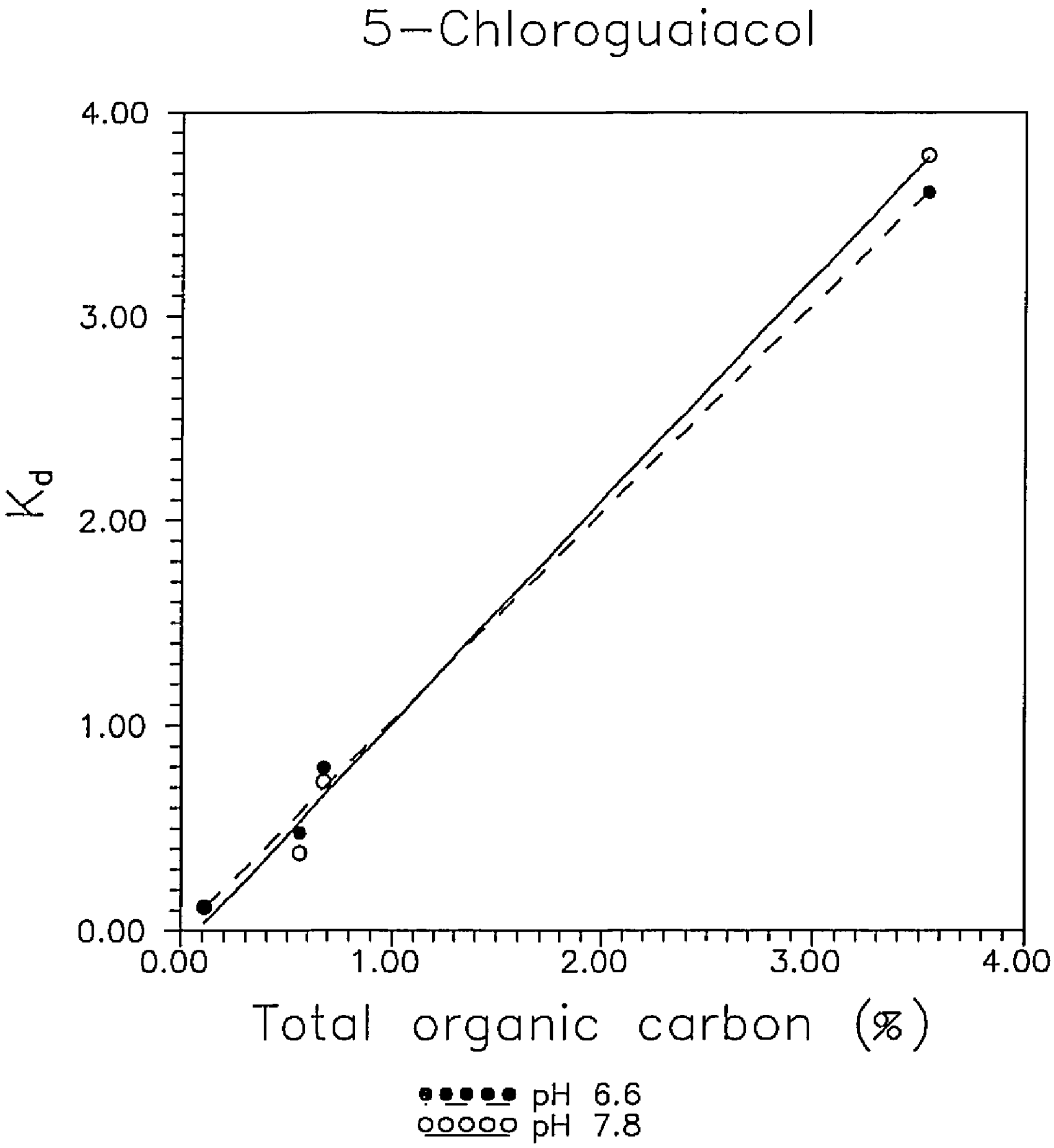

5–Chloroguaiacol
4.00
3.00
2.00
1.00
0.00
K_d
0.00
1.00
2.00
3.00
4.00
Total organic carbon (%)
●●●●● pH 6.6
ooooo pH 7.8

Figure 24.

K_d versus %TOC for 4,6-dichloroguaiacol.

Regression equations

For all four points on line:

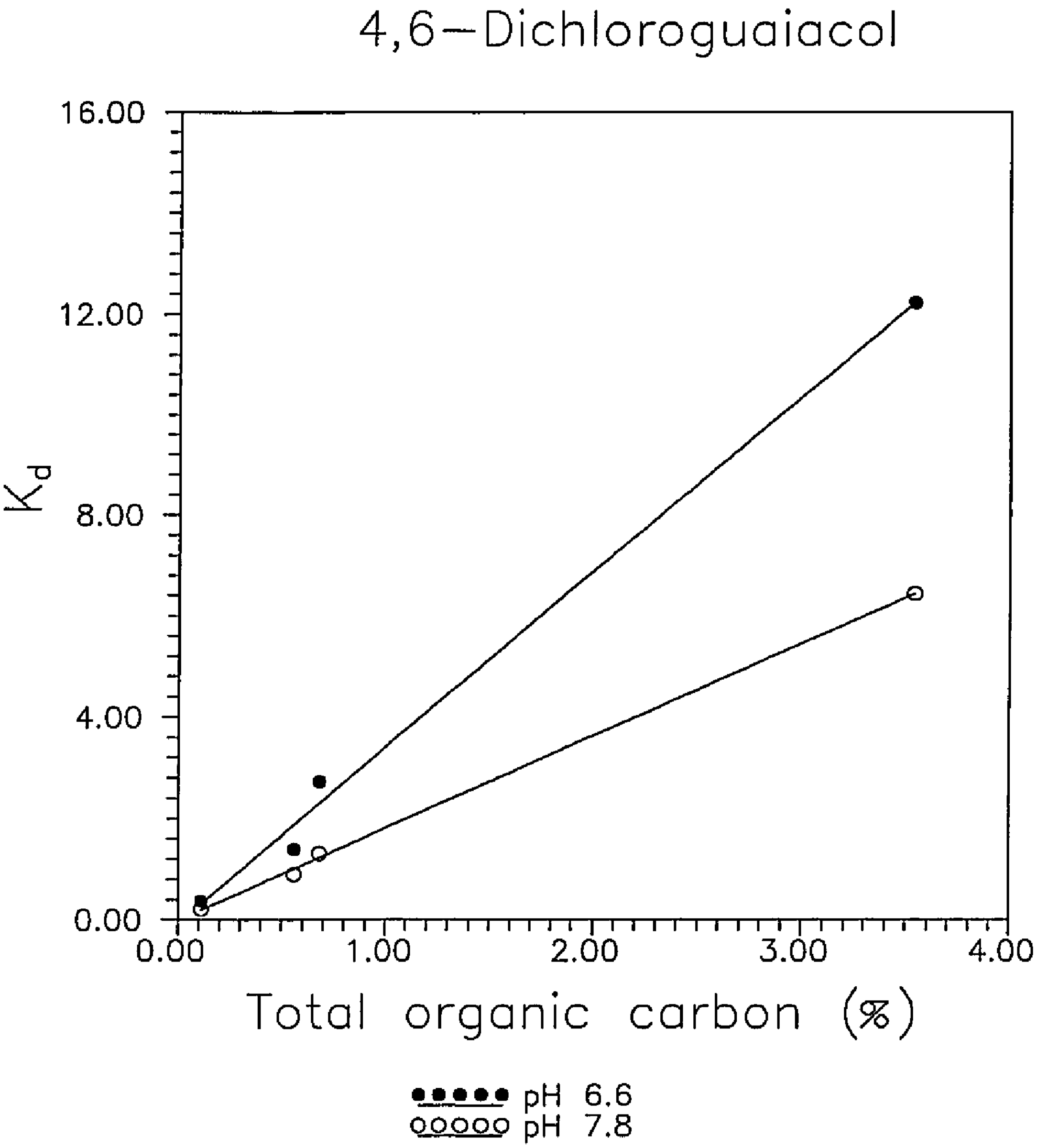
pH 6.6: $Y = 3.47x - 0.07$, $r^2 = 0.99$,

pH 7.8: $Y = 1.82x + 0.01$, $r^2 = 0.99$

For first three points (representing YR2, YR1, CY):

pH 6.6: $Y = 3.62x - 0.13$ $r^2 = 0.85$,

pH 7.8: $Y = 1.83x - 0.02$, $r^2 = 0.97$



4,6–Dichloroguaiacol

Figure 25.

K_d versus %TOC for 4,5,6-trichloroguaiacol.

Regression equations

For all four points on line:

pH 6.6: $Y = 13.46x - 1.10$, $r^2 = 0.99$,

pH 7.8: $Y = 3.49x + 0.02$, $r^2 = 0.99$

For first three points (representing YR2, YR1, CY):

pH 6.6: $Y = 12.03x - 0.48$, $r^2 = 0.89$,

pH 7.8: $Y = 3.84x + 0.02$, $r^2 = 0.95$

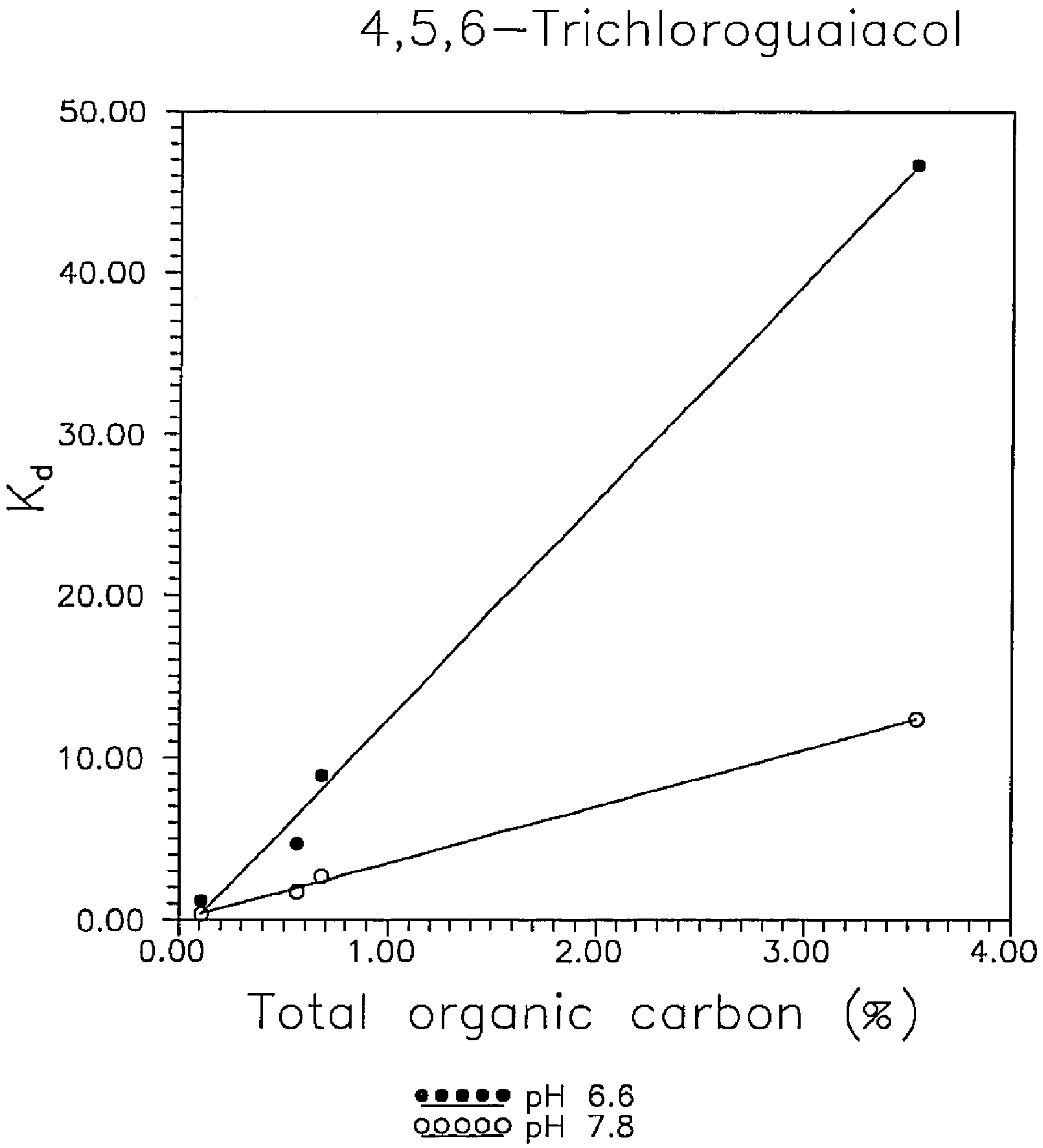

4,5,6-Trichloroguaiacol
K_d
50.00
40.00
30.00
20.00
10.00
0.00
0.00
1.00
2.00
3.00
4.00
Total organic carbon (%)
●●●●● pH 6.6
○○○○○ pH 7.8

Figure 26.

K_d versus %TOC for tetrachloroguaiacol.

Regression equations

For all four points on line:

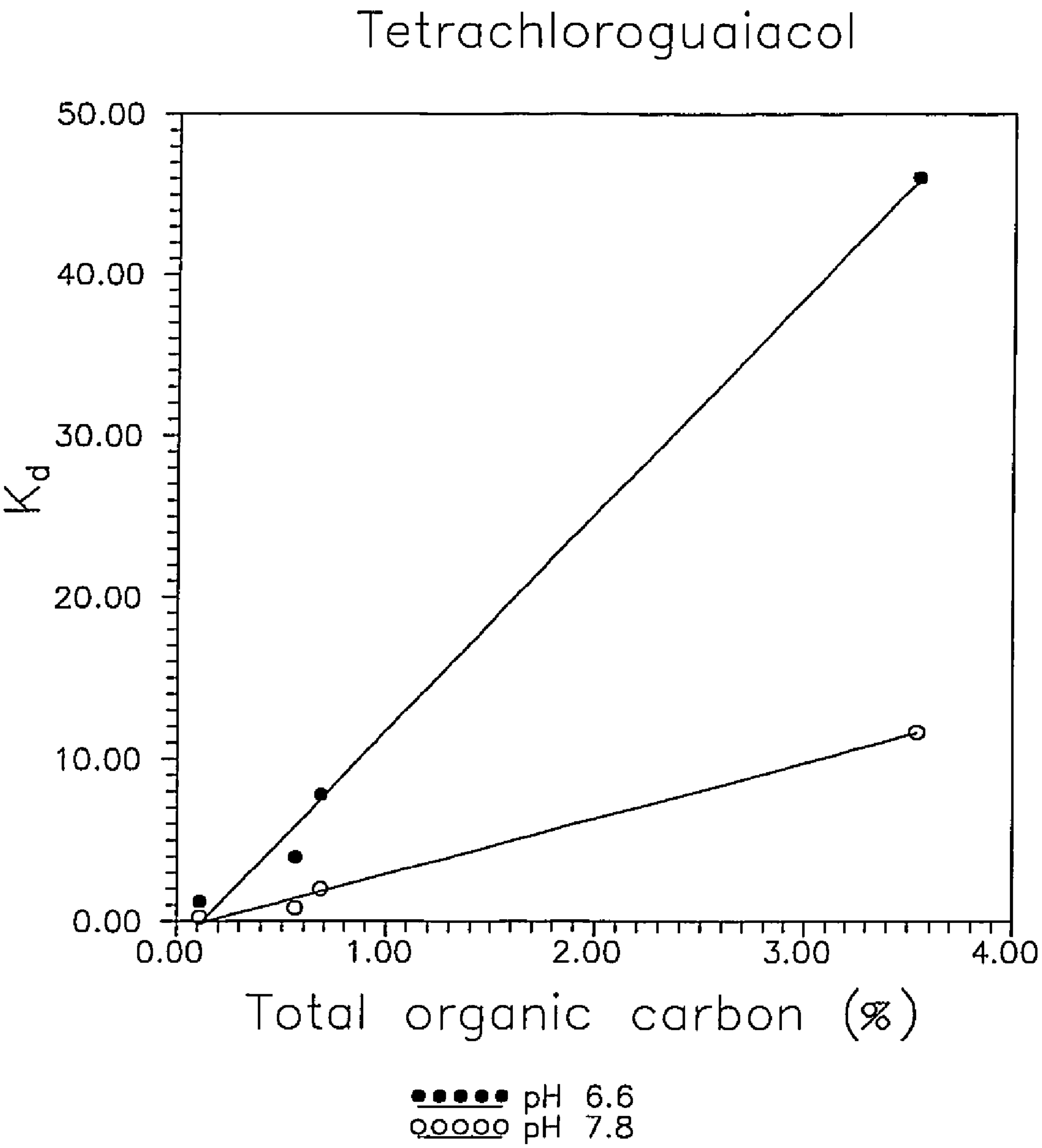
pH 6.6: $Y = 13.41x - 1.65$, $r^2 = 0.99$,

pH 7.8: $Y = 3.42x + 0.50$, $r^2 = 0.99$

For first three points (representing YR2, YR1, CY):

pH 6.6: $Y = 10.08x - 0.21$, $r^2 = 0.84$,

pH 7.8: $Y = 2.59x + 0.15$, $r^2 = 0.76$



Tetrachloroguaiacol
50.00
40.00
30.00
20.00
10.00
0.00
K_d
0.00
1.00
2.00
3.00
4.00
Total organic carbon (%)
●●●●● pH 6.6
○○○○○ pH 7.8

Figure 27.

K_d versus %humic acids for 5-chloroguaiacol.

Regression equations

For all four points on line:

pH 6.6: $Y = 2.85x + .021$, $r^2 = 0.99$,

pH 7.8: $Y = 3.05x + 0.13$, $r^2 = 0.99$

For first three points (representing YR2, YR1, CY):

pH 6.6: $Y = 4.95x + 0.03$, $r^2 = 0.97$,

pH 7.8: $Y = 4.52x + 0.01$, $r^2 = 0.99$

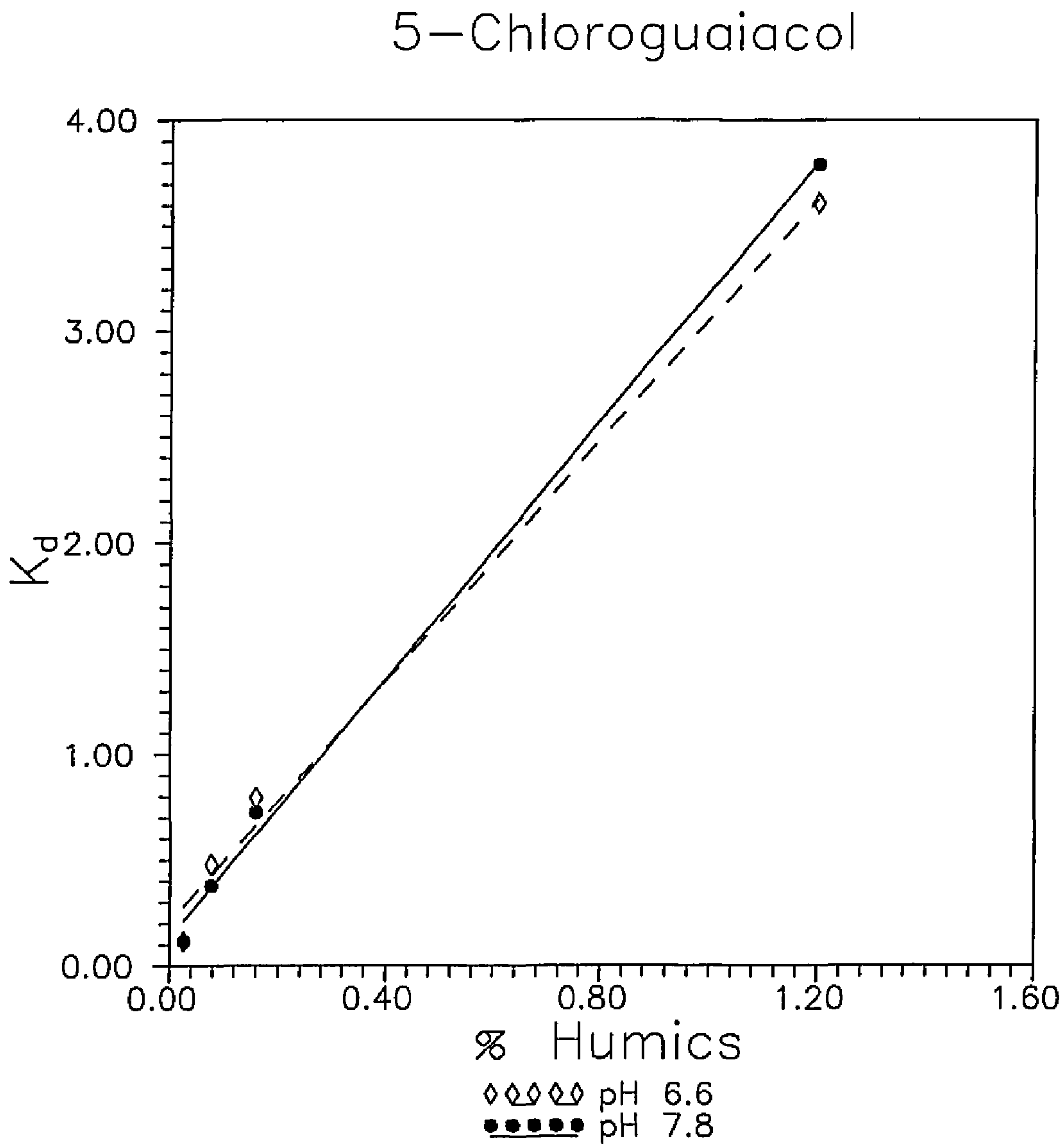

5−Chloroguaiacol
4.00
3.00
2.00
1.00
0.00
K_d
0.00
0.40
0.80
1.20
1.60
% Humics
◊◊◊◊◊ pH 6.6
●●●●● pH 7.8

Figure 28.

K_d versus %humic acids for 4,6-dichloroguaiacol.

Regression equations

For all four points on line:

pH 6.6: $Y = 9.73x + 0.62$, $r^2 = 0.99$,

pH 7.8: $Y = 5.08x + 0.35$, $r^2 = 0.99$

For first three points (representing YR2, YR1, CY):

pH 6.6: $Y = 17.41x - 0.03$, $r^2 = 0.99$,

pH 7.8: $Y = 7.94x + 0.11$, $r^2 = 0.93$

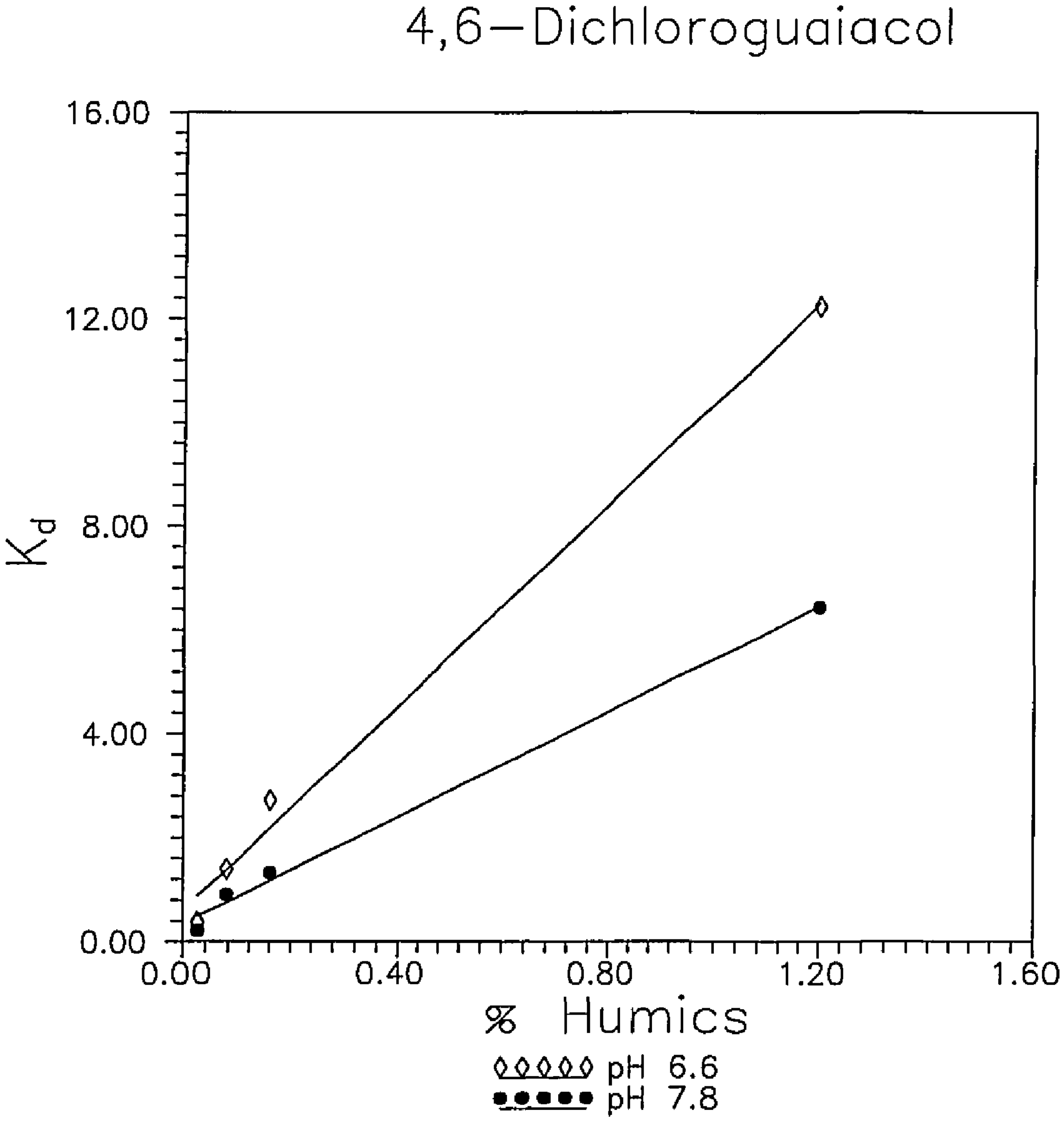

4,6−Dichloroguaiacol
K_d
16.00
12.00
8.00
4.00
0.00
0.00
0.40
0.80
1.20
1.60
% Humics
◊◊◊◊◊ pH 6.6
●●●●● pH 7.8

Figure 29.

K_d versus %humic acids for 4,5,6-trichloroguaiacol.

Regression equations

For all four points on line:

pH 6.6: $Y = 37.70x + 1.57$, $r^2 = 0.99$,

pH 7.8: $Y = 9.74x + 0.72$, $r^2 = 0.99$

For first three points (representing YR2, YR1, CY):

pH 6.6: $Y = 57.10x - 0.08$ $r^2 = 0.99$,

pH 7.8: $Y = 17.04x + 0.10$, $r^2 = 0.95$

4,5,6-Trichloroguaiacol

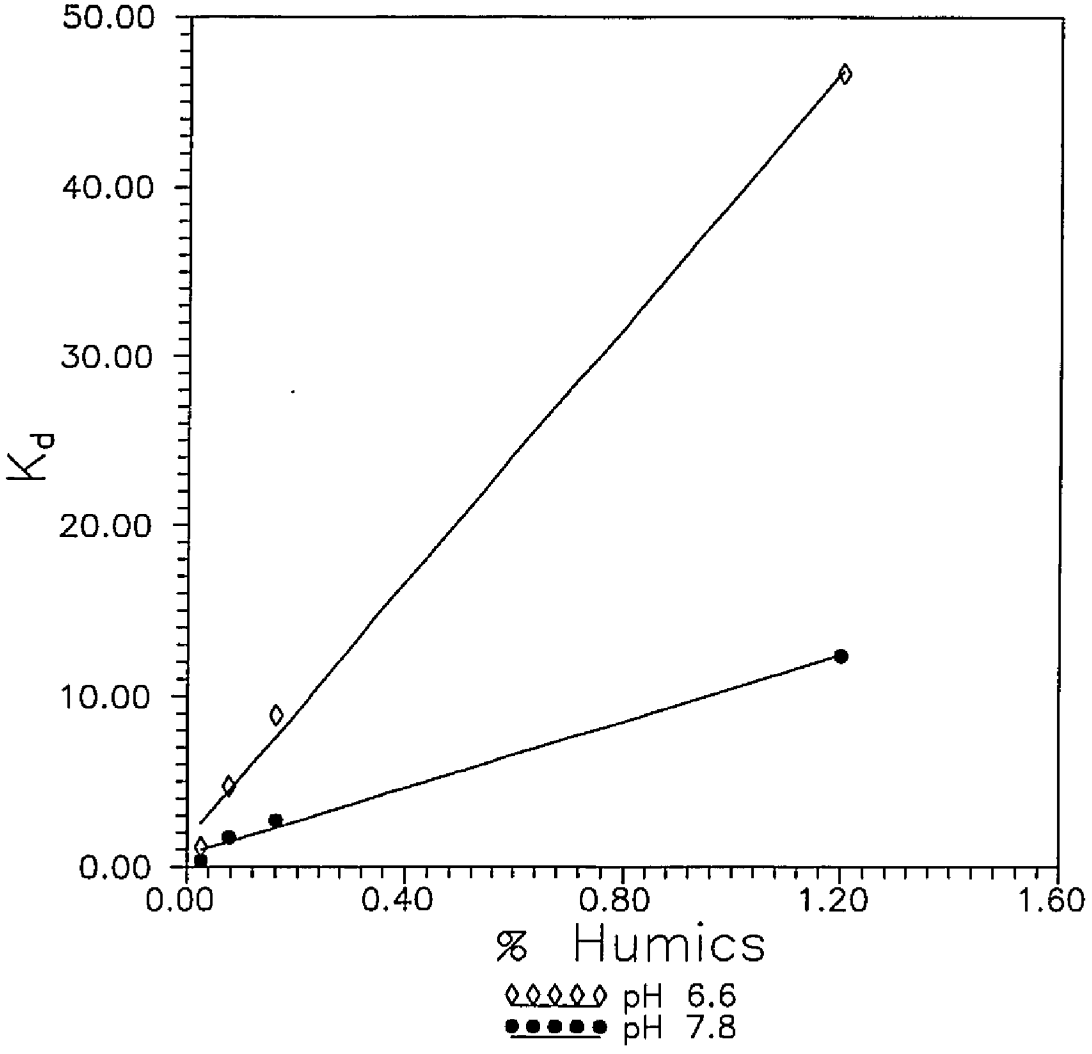


Figure 30.

K_d versus %humic acids for tetrachloroguaiacol.

Regression equations

For all four points on line:

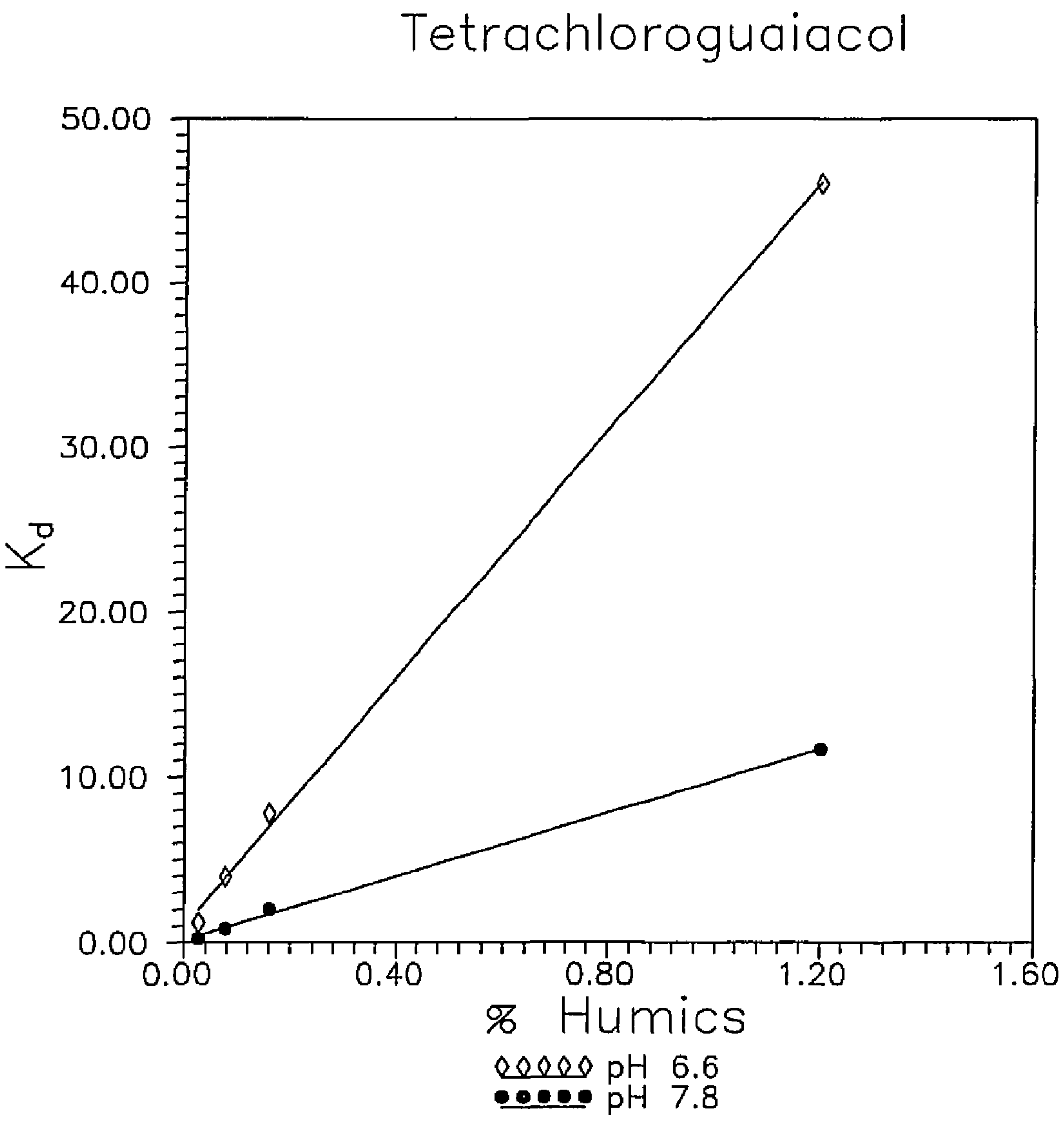
pH 6.6: $Y = 37.61x + 0.99$, $r^2 = 0.99$,

pH 7.8: $Y = 9.59x + 0.17$, $r^2 = 0.99$

For first three points (representing YR2, YR1, CY):

pH 6.6: $Y = 48.92x + 0.03$, $r^2 = 0.99$,

pH 7.8: $Y = 13.20x - 0.14$, $r^2 = 0.99$


Tetrachloroguaiacol
50.00
40.00
30.00
20.00
10.00
0.00
K_d
0.00
0.40
0.80
1.20
1.60
% Humics
◊◊◊◊◊ pH 6.6
●●●●● pH 7.8

Comparison with published data

Seip *et al.* (1986) reported K_{oc} values for 4,5,6-CG and tetra-CG on three different soils. If the soil pH is taken as an approximation of the aqueous phase pH experienced during both the column and batch experiments, some comparisons can be made with data presented here. Results from the soil with a pH of 7.4 and TOC of 2.2% can be compared with those of Catlett muck. The soil pH lies between the two used in this experiment and the TOC is comparable to CM. Most significant is the fact that K_{oc} values of 4,5,6-CG and tetra-CG for the batch runs conducted by Seip differ by about a factor of two, tetra-CG being the higher value, while comparable values obtained in the isotherms conducted by the author were quite similar. In fact, in all isotherms determined here, K values for the two compounds are almost the same.

The data from Schellenberg *et al.* (1984) in Table 4 indicate that, for nonionized phenolics, more highly chlorinated hydrophobic compounds will sorb to a greater degree than lower chlorinated compounds. This data does not consider the effect of pH on sorption. Some comparisons can, nonetheless, be made. For the dichlorophenols, K_{oc} values ranged from 200 to 600 and trichlorophenols were between 800 and 4400. At pH 6.6, 96% of 4,6-CG and 80% of 4,5,6-CG are in the nonionized form and K_{oc} values lie between 100 and 200 for 4,6-CG and 350 and 700 for 4,5,6-CG. Given the extrapolation made to determine the K_{oc} for the phenols and the degree of ionization of the CG, the values seem reasonably similar.

Effect of sediment on aqueous phase characteristics

For all isotherms, phosphate buffers were prepared at 1/15M. The ionic strengths of the pH 6.6 and 7.8 buffers were 0.096 and 0.18, respectively. Several experimental factors may combine to alter this ionic strength. Salts from interstitial water (salinity = 14, during sampling), that precipitated during sediment drying, would be redissolved upon introduction of spiked water, increasing the ionic strength of the experimental solution. In addition, carbonate shell fragments in the sediment may have dissolved during the agitation, further buffering the solution.

These effects were evident when, at the end of each isotherm, the pH of individual vials was recorded. While the pH of the buffer added to each at the beginning of each isotherm was identical, the pH values of the individual solutions at the end of the run varied by up to ±0.25 pH units from the value of the original buffer. This would seem to be a major component of the experimental variation. A change in pH changes the value of ϕ_n, thus altering the overall K_d.

Unfortunately, attempts to alter the sediment to minimize these effects will change the sediment characteristics and the results will not reflect the sediment tested. Increasing the ionic strength of the buffer would help maintain the desired pH, but would also have the effect of "salting out" organic ions. If the intent is to model interactions with marine sediments where the ionic strength of seawater is ≈ 0.7, this would be appropriate or even necessary. However, in examining freshwater or estuarine systems (the more common environment for pulp mill outfalls), such ionic strengths would be excessive.

Conclusions

The sorption, represented by K_d, of four different chloroguaiacols onto four different estuarine sediments was shown to increase with increasing chlorine substitution of the molecule, %total organic carbon content of the sediment and %humic acids in the sediment. For the di-, tri- and tetrachloroguaiacols, sorption decreased as aqueous phase pH increased due to the increase in the fraction of the chloroguaiacol in the more hydrophilic ionized form. The monochloroguaiacol was almost entirely in the nonionized form at both pH values and no significant difference in K_d was observed.

Sorption coefficients were linearly related to both %TOC and %humic acids content of the sediments. It is generally accepted that sediment %TOC is a controlling factor in sorption of organic compounds to sediments where %TOC > 0.1. Humic acid content has recently been suggested to also be a controlling factor in sorption. Calculation of sorption coefficients normalized to sediment %TOC (K_{oc}) and %humic acids (K_{humic}) indicated that humic acids, along with total organic carbon control the sorption of chloroguaiacols to estuarine sediments.

Chapter IV

Evaluation of Analytical Methods to Determine Chloroguaiacols in Water

Introduction

Regulatory overview

In the mid-1970's the United States Environmental Protection Agency (EPA) began regulating pulp and paper mill effluents. The parameters monitored were classified as conventional pollutants: BOD (biochemical oxygen demand), TSS (total suspended solids) and pH. In 1979, EPA developed a list of 126 "Priority Pollutants" to be regulated under the Clean Water Act. Pentachlorophenol and several trichlorophenols, compounds commonly found in BKPPME, were included on the list and became subject to regulation. As the complexity of these effluents was realized, nonconventional, chlorinated pollutants present in BKPPME, but not on the Priority Pollutant list, were found to be of biological significance. In lieu of regulating all of these individual compounds, the parameters AOX (adsorbable organic halogens) and COD (chemical oxygen demand), were added to the list of conventional pollutants already being monitored. These are the parameters being regulated today (USEPA, 1993).

In October, 1993, EPA published the "Development Document for Proposed Effluent Limitations Guidelines and Standards for the Pulp, Paper and Paperboard Industry". In this document, the regulation of compounds specific to the paper industry was proposed. In developing the proposal, EPA looked at 443 organic compounds commonly reported in effluents from different types of mills throughout the United States. If, among other criteria, the compound was detected at concentrations considered to be of biological concern in the effluent at more than one of the mills involved in the pilot study, it was included on the list. Of the 28 chlorinated phenolics on the initial list, 12 were included on the final list of compounds for regulation, including four chloroguaiacols: tetra-CG, 3,4,5-CG, 3,4,6-CG and 4,5,6-CG. This document also included a description of the method to be used by regulators to determine chlorophenolics in effluents. The protocol is based on one developed by the National Council of the Pulp and Paper Industry for Air and Stream Improvement (NCASI), now referred to as Method 1653.

As mentioned in Chapter I, a ruling on what BKPPME parameters will be regulated has not been finalized. If specific compounds are to be monitored, the method used to analyze them must be reliable, selective and sensitive. In this chapter analytical methods for chlorinated phenolics, including Method 1653, are reviewed and discussed.

A preliminary study was conducted by the author to assess the feasibility of using trifluoroacetic anhydride derivatization and GC with the halogen specific, electrolytic conductivity detector (ELCD) as an alternative to the *in situ* acetylation method currently in use. This method is described and evaluated below.

Review of analytical methods

In addition to Method 1653, there are several methods in current use to determine chlorinated phenolics in aqueous media, with no method predominant. At environmental concentrations, the determination of phenolic compounds is often done by gas chromatography (GC).

The hydroxyl group on phenolic compounds makes them slightly polar. On most commonly used GC columns this causes chromatographic problems such as peak tailing, resulting in inaccurate integration of peak areas. For this reason, phenolic compounds are often derivatized at the hydroxyl group to mask its polarity.

In a recent review of analytical methods for alcohols and phenols, gas chromatographic methods for both derivatized and underivatized phenols are described (Smith and Johnson, 1993). Derivatization is done for several purposes. In addition to masking polarity, it can increase volatility, improve stability of the analyte, aid in compound separation and enhance the analytes response on specific detectors. The major disadvantages of derivatization are: possible incompleteness of the derivatization reaction, difficulty in removing the derivatizing agent from the sample, additional time involved in the analysis, losses of analyte at each additional step involved, and unwanted chemical alteration of the sample caused by derivatizing conditions (ie. temperature or acidity) (Watson, 1993). In some cases, derivatized compounds are less stable than their underivatized counterparts (Baugh, 1993).

Derivatization of phenols is not generally done for quality control purposes when single analytes at high concentrations are being evaluated by GC, but when peak resolution is crucial to the interpretation of results for complex mixtures of analytes, derivatization is often necessary.

Common derivatizing reactions for phenols include acylation, alkylation and silylation (Knapp, 1979). Alkylation involves the replacement of the hydroxyl hydrogen with a nonpolar group such as a methyl, heptyl or pentafluorobenzyl. The derivatizing agents used include diazomethane, methyl iodide/potassium carbonate or pentafluorobenzyl bromide. Acid anhydrides, acid chlorides or reactive amides are often used to prepare acyl derivatives. Trimethylsilylation is a commonly used silylation reaction for phenols.

Within the pulp and paper industry, acylation is, by far, the most commonly reported derivatization method for phenolics. To determine chlorophenolics in BKPPME, several methods use organic solvent extraction of acidified samples, followed by derivatization of the components in the separated organic phase. Acetic anhydride is the most frequently used derivatizing agent (Rosemarin *et al.*, 1990, Owens *et al.*, 1994, Morales *et al.*, 1992). Pentafluorobenzyl derivatives have also been prepared following dichloromethane extraction (Lee, H-B., 1984). Extraction of phenolics on an Amberlite XAD-2 column, followed by elution and derivatization of the extract with heptafluorobutyric anhydride has also been reported (McKague, 1981). Another column extraction method used C_{18} to isolate the phenolics, which were then derivatized with acetic anhydride (Renberg and Lindstrom, 1981). GC with an electron capture detector (ECD) and/or mass spectrometer (MS) were used in all of the above analyses.

Acetylation of phenolics prior to extraction from the aqueous phase has been reported by several researchers (Stark *et al.*, 1985, Xie *et al.*, 1986, Voss *et al.*, 1981, Paasivitrta *et al.*, 1992a, Lee, H-B. *et al.*, 1989). In these techniques, the sample pH is fixed to pH 7, acetic anhydride added and the derivatized phenolics extracted with an organic solvent. These methods employ also ECD or MS based methods.

The method developed by NCASI for the EPA (Method 1653) uses the *in situ* acetylation approach (NCASI, 1986). In this method, the pH of a 1 liter water sample is adjusted to 7 with sodium hydroxide (6N) or sulfuric acid (6N), internal standards are added and the pH is raised to between 9.5 and 11 with potassium carbonate. *In-situ* acetylation of the phenolic compounds is accomplished by adding 25 ml of acetic anhydride to the sample in a covered beaker and stirring for 3-5 minutes. The derivatized compounds are sequentially extracted with three hexane aliquots (200 ml each) by either separatory funnel or stir bar extraction. The extracts are combined and the volume is reduced to 0.5ml with a gentle stream of nitrogen. Analysis is by GC/MS with electron impact (EI) ionization on a DB-5 GC column. The method has minimum detection limits for chloroguaiacols ranging from 0.1 to 1 μg/l and minimum quantification limits from 1 to 5 μg/l (USEPA, 1993, LaFleur, 1992, personal communication).

Calibration standards for Method 1653 are prepared by adding the stock calibration solution to 1 liter of deionized water and carrying out *in situ* acetylation and extraction as described above.

Materials and Methods

Prior to the analysis of an effluent sample, samples of filtered (0.45μm) York River water spiked with chloroguaiacols and a deionized water blank were hexane extracted as described below to assess the effectiveness of the solvent. Underivatized extracts were analyzed by GC-ELCD. Samples were not derivatized so that % recoveries of analytes from the extraction step would not be combined with effects of derivatization. Due to the poor peak shape, analysis was based on peak height, rather than area. Triplicate extractions were done. Averaged % recoveries of four chloroguaiacols tested (4,6-CG, 3,4,5-CG, 4,5,6-CG and 3,4,5,6-CG) were between 132 and 139%. The blank was free of interferences.

Initial derivatization tests were made by preparing chloroguaiacol solutions directly in hexane and derivatizing as described below. In this way extraction effects would not be included in analyses. Based on comparison of retention times of the underivatized compounds, GC-ELCD analysis indicated that the compounds were essentially completely derivatized. When the derivatization time was shortened, peaks representing the underivatized chloroguaiacols were apparent along with the derivatized compound.

Once extraction and derivatization methods were confirmed, a wastewater sample was obtained from Chesapeake Corporation, West Point, Virginia, a bleached kraft pulp and paper mill. The sample was a 24-hour composite of treated wastewater, collected immediately prior to discharge. At the time of sample collection, the mill employed chlorine (Cl_2) bleaching with secondary wastewater treatment processes. The sample was analyzed according to the trifluoroacetic anhydride method described below.

Trifluoroacetic anhydride (TFAA) was purchased from Pierce Chemicals, Rockford, IL. and was used without further purification. To minimize hydrolysis to trifluoroacetic acid, the TFAA was stored under nitrogen in a desiccator. To further limit atmospheric exposure of the entire supply of TFAA, several 1ml aliquots of TFAA were placed in 2ml amber glass vials with teflon lined screw cap lids, sealed under nitrogen and stored in a desiccator. These individual aliquots were then used in subsequent derivatization procedures. Chloroguaiacol standards were obtained from Helix BioTech, Vancouver, B.C. Phenol standards were purchased from Aldrich Chemicals, Milwaukee, WI. All solvents used were HPLC grade (Burdick and Jackson, Muskegon, MI).

In the TFAA method used here, the pH of a 1 liter aqueous sample, previously spiked with internal standards (2,6-dichloro-4-fluorophenol (DCFP) and 2,4,6-tribromophenol), was adjusted to ≤ 2 with 12N HCl and extracted three times with hexane (150, 100 and 100ml) in a 2000ml separatory funnel. The volume of the combined hexane extract was reduced by rotary evaporation and transferred to a 10ml test tube with a teflon lined screw cap and evaporated under a stream of nitrogen to a volume of approximately 1ml. 400μl of 0.1M pyridine in hexane was added, followed by 25μl of TFAA. The tube was capped, shaken and allowed to react for 1 hour at room temperature. The extract was washed twice with 0.5ml aliquots of DI

H_2O and once with 0.5ml of 0.1M phosphate buffer (pH=7) to remove trifluoroacetic acid generated by the reaction. The hexane layer was siphoned off the top, run through a Pasteur pipet packed with Na_2SO_4 to remove residual water and analyzed by GC with an ELCD, or Hall, detector (OI Corp, College Sta., TX) on a 30m x 0.32mm i.d DB-5 column with 0.25μ film thickness. The original column temperature program was 90°C for 4 minutes, then increased by 4°/minute to 310°C and held for 10 minutes (90/4/4/310/10). Several temperature program modifications were tested in an attempt to improve separation of early eluting compounds with the final program being 50/5/4/310/10. Rates of 1 or 2°C/min did not improve separation of coeluting compounds.

Compound identification and confirmation was by gas chromatography with negative chemical ionization mass spectrometry (GC-NCIMS) on an Extrel ELQ 400-2 quadrupole mass spectrometer.

Calibration standards were prepared by adding the desired volume of chloroguaiacol stock solution (nominal concentration 1mg/ml in acetone) to 1.5 to 2ml of hexane. Acetone was removed with a gentle stream of nitrogen, the volume was reduced to about 1ml and derivatized as above.

Results and Discussion

Experimental Results

Figure 31 presents chromatograms of TFAA derivatized and underivatized chloroguaiacol standards and tribromophenol, analyzed on the Hall detector. A definite improvement in chromatography is evident in the derivatized sample. Retention times of the underivatized compounds appear to be shorter than the derivatized because different temperature programs were employed (see methods section).

Chromatograms of the extracted, derivatized wastewater sample (Figure 32), analyzed by ELCD, ECD and flame ionization detection (FID), demonstrate the specificity of ELCD over ECD as well as the improved response of ELCD over FID.

Several chloroguaiacols were detected by GC-ELCD (Figure 33) in this sample, based on retention time comparison with standards and confirmed by GC with negative chemical ionization mass spectrometry (GC-NCIMS).

Concentrations of guaiacols detected are as follows:

Compound	Concentration (μg/l)
4,6-CG	0.58
4,5-CG	0.55
3,4,5-CG	1.35
4,5,6-CG	0.34
tetra-CG	1.45

Standards for other chloroguaiacols were not available at the time of analysis.

Monochloroguaiacol isomers were tentatively identified, based on retention time, by GC-ELCD, but could not confirmed by GC-NCIMS. Like ELCD, NCIMS is also specific to halogenated compounds. However, observed responses on NCIMS to chloroguaiacol standards tended to be much lower than the ELCD. It is possible that the response of the monochloroguaiacols was insufficient for measurement.

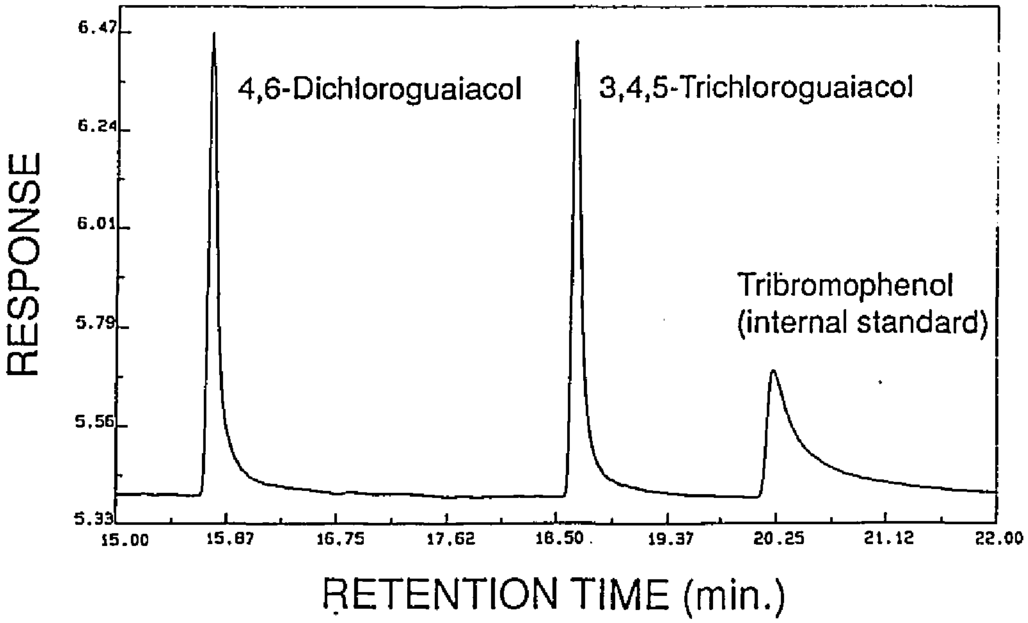
Figure 31.

ELCD chromatogram of underivatized (top) and TFAA derivatized (bottom)

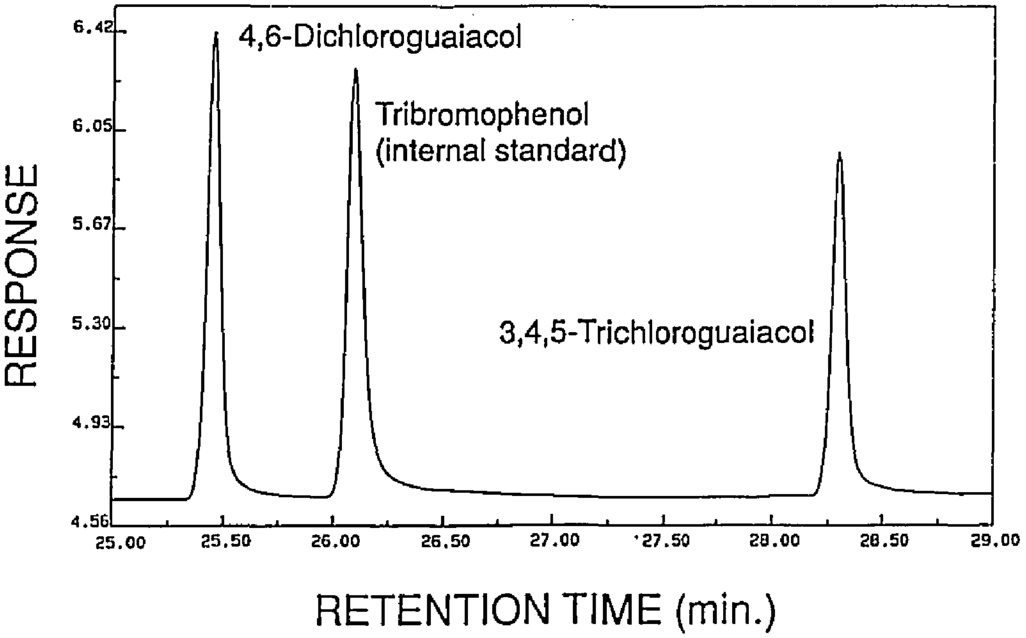
chloroguaiacol standards and tribromophenol.

(Note: Retention times for underivatized compounds are

shorter due to different temperature programs)



RESPONSE
6.47
6.24
6.01
5.79
5.56
5.33
4,6-Dichloroguaiacol
3,4,5-Trichloroguaiacol
Tribromophenol
(internal standard)
15.00
15.87
16.75
17.62
18.50
19.37
20.25
21.12
22.00
RETENTION TIME (min.)



RESPONSE
6.42
6.05
5.67
5.30
4.93
4.56
4,6-Dichloroguaiacol
Tribromophenol
(internal standard)
3,4,5-Trichloroguaiacol
25.00
25.50
26.00
26.50
27.00
27.50
28.00
28.50
29.00
RETENTION TIME (min.)

Figure 32.

Gas chromatograms of treated BKPPME sample,

analyzed by ECD, ELCD and FID.

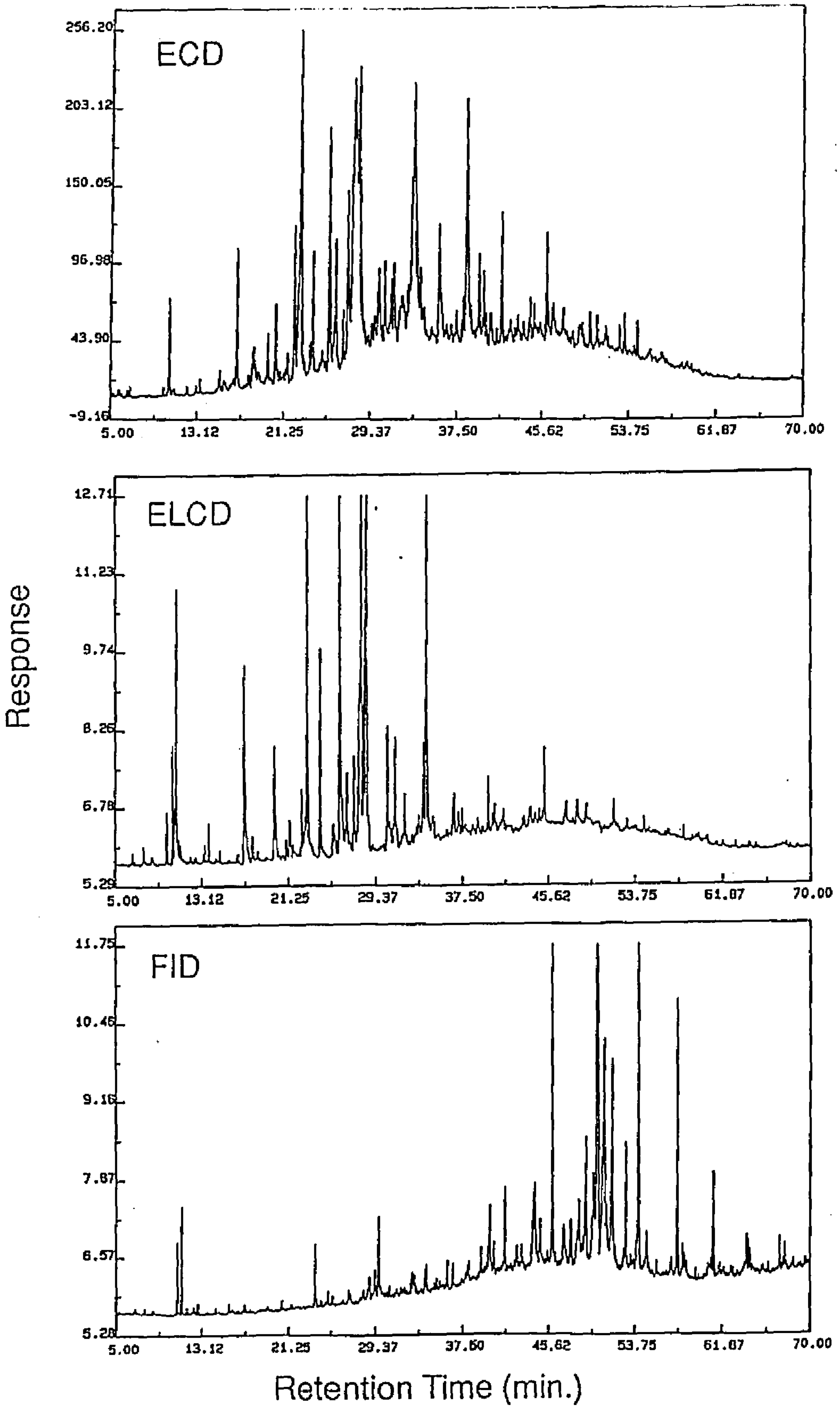
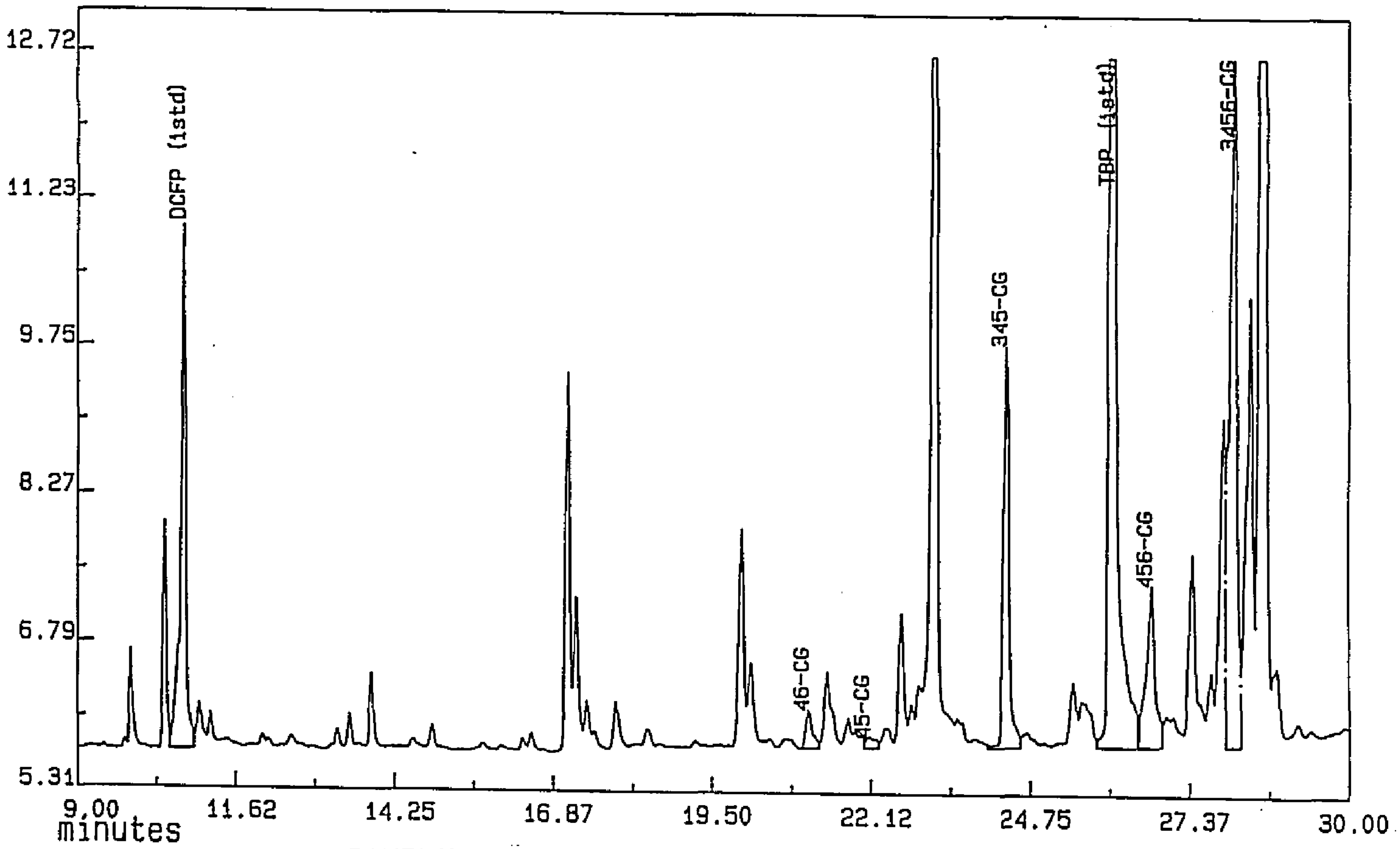

ECD
256.20
203.12
150.05
96.98
43.90
-9.16
5.00
13.12
21.25
29.37
37.50
45.62
53.75
61.87
70.00
ELCD
12.71
11.23
9.74
8.26
6.78
5.29
5.00
13.12
21.25
29.37
37.50
45.62
53.75
61.87
70.00
FID
11.75
10.46
9.16
7.87
6.57
5.28
5.00
13.12
21.25
29.37
37.50
45.62
53.75
61.87
70.00
Response
Retention Time (min.)

Figure 33.

GC-ELCD chromatogram of treated effluent sample with chloroguaiacol peaks marked.



SAMPLE: 24hr comp hex extr rederiv 1ul .2fv

Discussion

Within the pulp and paper industry, the method of determining chlorinated phenolics in aqueous samples has changed little since Voss *et al.* first published the *in situ* acetylation method for GC analysis in 1981. Two potential advantages of the TFAA method were considered worthy of further investigation. First, the ELCD can operate in a halogen-specific mode. This would eliminate a large number of potentially interfering non-halogenated BKPPME components. The ECD is responsive not only to halogens, but also to other electronegative atoms such as oxygen, nitrogen, sulfur and phosphorous. Compounds containing these constituents will be detected by an ECD, generating much more complex chromatograms than ELCD (Figure 33) and difficulty in resolving individual peaks (Baugh, 1993). Second, the incorporation of three additional halogens onto the phenolic compound would generate a greater response over that of an acetyl derivative. This factor could counterbalance the fact that ECD is somewhat more sensitive than ELCD. ECD detection limits are on the order of 0.050 picograms ($pg = 10^{-12}g$) of halogen on-column (Baugh, 1993). For the ELCD, tenths of picograms of halogen can be detected in the halogen mode (OI Corp, 1988).

Fluorinating a phenolic compound tends to increase its volatility over that of the underivatized phenol. This is often a desired effect in chromatography. In the case of mono- and dichloroguaiacols and the internal standard DCFP, the increased volatility lead to losses during sample transfer and blowdown. In standard mixes blown down to less than 0.1ml, DCFP and monochloroguaiacols exhibited little or no response, indicated they had evaporated during blowdown. At the slowest temperature program rate used here, monochloroguaiacol isomers in standard mixes could not be resolved. Temperature programs with 1 or 2°C/minute increases increased run times without improving resolution of monochlorinated isomers.

The use of a solvent with a lower boiling point, such as pentane (bp=35°C), would allow for a lower initial column temperature and possibly better separation of early eluting compounds. Conversely, maintaining the low volume of extract necessary for GC analysis (0.2ml) would be more difficult with such a volatile solvent.

During method development, the ELCD system tended to malfunction (giving no response or poor chromatography) after approximately 10-20 samples were run, with repair time often being on the order of days or weeks. An ELCD is notorious for frequent breakdowns, and thus, artifacts of the analytical method were not originally envisioned to be the cause of the problems. The Pierce derivatization method suggests using 1.0M phosphate buffer (pH=6.0) to quench the reaction and take up excess trifluoroacetic acid generated by the derivatization. During the initial development of this procedure, a 0.1M buffer (pH=7) was inadvertently used. When the recommended buffer was used, failure of the instrument occurred after only three or four sample injections.

Consultation with Pierce representatives at this stage suggested that artifacts of the derivatization procedure could lead to problems with ELCD systems that would not interfere with the recommended ECD or FID systems (E.Conklin, personal communication, 1994). It became apparent that residual acids or phosphate buffer in the sample extract could damage the Ni reaction tube of the ELCD. At the suggestion of Pierce technical services representatives, sequential water washes were added to the procedure. (The pH of the aqueous phase after water and buffer washes was between 5 and 7.) However, loss of ELCD response still occurred after only a few injections. Each new attempt at modification of the procedure resulted in significant amounts of downtime of the ELCD system. Due to the large number of users of this system, access to the equipment was limited and further attempts to modify the method were not possible.

Other complications related to this derivatization have been encountered, making it a less practical alternative to AA derivatization methods. TFAA is highly corrosive and moisture sensitive, much more so than AA. It requires storage under nitrogen and can easily corrode nonglass parts of storage containers. In the presence of water, it readily forms trifluoroacetic acid (pK_a =-0.26), a much stronger acid than acetic acid (pK_a = 4.7) which forms less easily by the hydrolysis of AA (Rochester, 1970). TFAA must be handled with great care because of the caustic nature of the compound.

TFAA derivatization must be done in an organic solvent, requiring more steps than the *in situ* AA derivatization. Attempts to remove by-products will create more experimental steps in an already complicated procedure, potentially producing losses of analytes with each additional step. Other attempts to remove damaging byproducts were difficult to do because other users of the ELCD could not afford additional delays in analysis.

Conclusions

For a newly developed method to become a viable alternative to existing methods, it must demonstrate certain advantages over the other methods. Among other things, it should be easier to perform, more accurate and reproducible, have lower detection limits or require fewer safety precautions. As it stands the TFAA method does not meet these criteria. This is not to say that the Method 1653 is the best method to use in regulation. One conspicuous potential problem lies in the manner in which calibration standards are derivatized. These standards are dissolved in water and acetylated *in situ*, then extracted in the same procedure as samples. The procedure involves several steps with the potential for losses of analytes at each step. Low recovery of calibration standards would result in inaccurate quantitation of samples. Another consideration is the fact that acetic anhydride is readily hydrolysed in water thus, the derivatization reaction competes with the hydrolysis of the anhydride. Incomplete derivatization could result.

A method that incorporates derivatization of analytes that have already been concentrated in an organic extract may afford a more complete derivatization, provided interferences from the derivatization are eliminated. Column or solid phase extraction methods may reduce the amount of solvents necessary in the extraction stage. However, the high amount of suspended solids present in BKPPME may affect flow and efficiency.

Minimal manipulation of calibration standards should be done in order to limit losses of the standards. By derivatizing standards directly in an organic phase, extraction inefficiencies would be eliminated.

Chapter V

Conclusions

This work represents the first comprehensive determination of thermodynamic pK_a values of the chloroguaiacols that have been identified in BKPPME. Thermodynamic pK_a values are required for intercomparison of chloroguaiacol dissociation constants and comparison with predictive models because corrections are made to compensate for differences between the experimental solutions.

MOPAC-based gas phase acidity calculations were linearly related ($p=0.05$) to experimental thermodynamic pK_a values, with r^2 values of 0.94 for both the AM1 and MNDO Hamiltonian operators. Hammett predicted pK_a values were significantly correlated ($p=0.05$) to experimental thermodynamic pK_a values, with $r=0.993$ ($r^2 = 0.987$). MOPAC calculations appeared to be somewhat better at predicting pK_a values for higher substituted chloroguaiacols than the Hammett equation, possibly because the Hammett σ values are derived from monosubstituted benzoic acids and do not include effects of interaction between the substituents. Hammett calculations, however, do incorporate solvation effects and may be slightly more effective at predicting the dissociation constants of mono- and di-chloroguaiacols. These methods for pK_a prediction are useful tools for determining dissociation constants to within tenths of pK units, and should be useful for other determinations of acid dissociation of other phenolic compounds in BKPPME and natural waters.

Sorption of an organic compound onto a sediment may result in reduced bioavailability to aquatic species, increased degradation rates or burial. Conversely, it may result in increased exposure to deposit feeding species and subsequent transfer to higher trophic levels. In this work, the sorption, represented by K_d, of four chloroguaiacols onto four estuarine sediments was shown to increase with increasing chlorine substitution of the molecule, %total organic carbon content of the sediment and %humic acids in the sediment. For the di-, tri- and tetrachloroguaiacols, sorption decreased as aqueous phase pH increased due to the increased ionized fraction of the chloroguaiacol. The monochloroguaiacol was almost entirely in the nonionized form at both pH values and no significant difference in K_d was observed, indicating that ionized and nonionized species sorb differently.

Sorption coefficients for each chloroguaiacol were linearly related to both %TOC and %humic acids content of the sediments. It is generally accepted that sediment %TOC is the controlling factor in sorption of organic compounds to sediments with %TOC>0.1. It has recently been suggested that humic acid content is also a factor affecting sorption in sediments with both high and low %TOC. Calculation of sorption coefficients normalized to %TOC (K_{oc}) and %humic acids (K_{humic}) for the sediments used here indicated that humic acid content and total organic carbon together control the sorption of chloroguaiacols to estuarine sediments.

Chloroguaiacol concentrations in BKPPME may soon be regulated by the EPA. If they are to be monitored and regulated, the methods used to analyze them must be reliable, selective and sensitive. A preliminary study was conducted to assess the feasibility of a new method involving trifluoroacetic anhydride (TFAA) derivatization as an alternative to methods currently in use.

For a newly developed method to become a viable alternative to existing methods, it must demonstrate certain advantages over the other methods. Among other things, it should be easier to perform, more accurate and reproducible, have lower detection limits or require fewer safety precautions. At present, the TFAA method does not meet these criteria. This does not imply that EPA Method 1653 is the best method to use in regulation. One conspicuous potential problem lies in the manner in which calibration standards are derivatized. These standards are dissolved in water and acetylated *in situ*, then extracted in the same procedure as samples. The procedure involves several steps with the potential for losses of analytes at each step. Low recovery of calibration standards would result in inaccurate quantitation of samples. Another consideration is the fact that acetic anhydride is readily hydrolysed in water thus, the derivatization reaction competes with the hydrolysis of the anhydride. Incomplete derivatization could result.

Minimal manipulation of calibration standards should be done in order to limit losses of the standards. By derivatizing standards directly in an organic phase, extraction inefficiencies would be eliminated.

Thoughts for Future Research

Thermodynamic dissociation constants were determined on 9 of the 15 possible chloroguaiacols. The remaining six chloroguaiacols were commercially unavailable at the time of the study, but have recently become available. Experimental determination of pK_a values for these compounds would further test the predictive abilities of MOPAC-based gas phase acidity calculations and the Hammett equation. As computer computational power increases, aqueous phase acidity calculations using MOPAC-type or *ab initio* quantum chemical calculations may become practical and should be attempted.

The examination of the sorptive reactions of ionizable and nonionizable organic compounds should be pursued along several avenues. The effect of varying amounts and types of humic acids on sorption will not only illustrate the role humic acids play in sorption, but may also give more insight into the composition of the humics. Spectroscopic and elemental analysis of the humic acids extracted from the sediments used in this experiment may explain the difference in K_{humic} values for CM and the other sediments. Different extraction efficiencies may also be a factor in the different observed K_{humic} values. A standardized extraction method for humic acids from sediments could possibly minimize observed variations. The effect of pH and ionic strength on the sorptive abilities of humics acids should be examined. This would be most easily done with nonionizable organic compounds and could later be extended to ionizable compounds. The different mechanisms of sorbing or binding ionized and nonionized species to a sorbent should be investigated.

This investigation treated four compounds on four sediments with total organic carbon contents of 0.1, 0.56, 0.68 and 3.5% at two pH values. Examining the sorption onto natural sediments with %TOC between 0.68 and 3.5% would give a better understanding of the relationship between K_d, K_{oc} and K_{humic}. Isotherms run at other pH values would allow for an further investigation of the effect of pH on sorption where potential linear relationships between K_d and pH could be explored. Sorption of chloroguaiacols from solutions with concentrations closer to environmental concentrations (μg/L range) should be examined. This would be most practical with radio-labelled compounds, which are currently prohibitively expensive in the quantities required for batch isotherm.

In conducting preliminary experiments to determine sorption equilibrium, isotherms were run on CM and CY with tetrachloroguaiacol at pH 6.6 and 7.8. Samples were equilibrated for 1, 12 and 24 hours. Samples examined from the 1 hour equilibration displayed higher sorption coefficients than the 12 and 24 hour samples, which were the same. This phenomenon has been observed by other researchers (Libelo, personal communication, 1994) and may be attributed to a "wetting effect" when dried sediments are used in batch isotherm experiments. Kinetic sorption experiments comparing sorption onto fresh (ie. never dried), pre-wet and dried sediments may elucidate this phenomenon. Sediment weights on fresh sediments could be determined after the isotherm is completed by decanting the supernatant, drying and weighing the material. Salts precipitated from the overlying buffer should have a minimal effect on sediment weight if sufficient supernatant is decanted prior to drying. By using fresh sediments, microbial effects could also be examined.

Most batch equilibrium isotherms are conducted using buffer solutions made from deionized water and inorganic salts. Such solutions do not include the colloidal material usually present in natural waters. Sorption experiments using natural water or aqueous phases containing known quantities of dissolved organic matter should be conducted to assess the role dissolved organic matter on sorption.

Although the TFAA method does not appear to be useful as reported, possible modifications may improve the method. Solid-phase extraction may be employed to either expedite the initial extraction or as a cleanup method after derivatization. However, the high amount of suspended solids present in BKPPME may affect flow and efficiency. Other separation techniques may aid in the cleanup of the sample, making it less complex and therefore more amenable to GC-ECD analysis. Further work is needed to determine the artifacts causing the degradation of the GC-ELCD system.

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Born in Point Pleasant, N.J., April 13, 1963. Graduated from St. Peter Grammar School, Point Pleasant, N.J., in 1977. Graduated from St. Rose High School, Belmar, N.J. in 1981. Earned a B.S. in Chemistry from the College of William and Mary, Williamsburg, VA in 1985. Earned an M.A. in Chemistry from the College of William and Mary, Williamsburg, VA in 1986. Was employed as a Site Investigator/Chemist by the Virginia Department of Waste Management - Superfund Program from 1986 to 1988. Entered the doctoral program in Marine Science at the College of William and Mary in 1988 so that all diplomas would match. Accepted a position as a Chemist with the USEPA Research Laboratory in Narragansett R.I.