QUANTITATIVE STRUCTURE-ACTIVITY RELATIONSHIP: PREDICTION OF ANAEROBIC TRANSFORMATION OF CHLOROACETANILIDE HERBICIDES

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Nomenclature and List of Symbols

C=O	Carbonyl-carbon
CESV	Connolly excluded solvent volume
CMA	Connolly molecular area
K_H	Henry's Constant
K_{ow}	Octanol-water partition coefficient
MR	Molar Refractivity
MW	Molecular weight

Chapter 1

Introduction

For thousands of years, humans have been in search of undiscovered areas for further plant and animal development to support the booming population growth. In the process of exploration, nature's biological entities warranted the need for mitigating the impedimental behavior of same plantae, fungi and animalia on the enrichment of the earth's resources. Particularly, weeds, plants and grasses erect barriers for optimal production and utilization of primary croplands. To combat these hindrances, one course humans have delved into is the design, manufacturing and implementation of pesticides. Though these chemical advances help alleviate the arduous labor in crop cultivation, initially scientists and engineers unknowingly set in place the beginning of harmful environmental and public health obstacles.

The dominant backlash of uncontrolled applications of pesticides on croplands, including those in the chloroacetanilide herbicide family (alachlor, acetochlor, propachlor, butachlor and metolachlor), is the contamination of groundwater. Moreover, these pollutants pose potential adverse animal and human health effects by way of contaminated groundwater consumption. Approximately 23 million people in the United States use untreated groundwater as their main source of drinking water while the remainder of drinking water is treated with expensive technology or inadequate

treatment methods for the removal of pesticides [1]. Hence, research has turned to understanding the pesticides' fate and modifications in chemical structure (*i.e.* transformation) when undergoing water treatment and while moving through the subsurface.

1.1 Motivation

The fate and transport of the chlorinated acetanilide pesticides is chiefly governed by the type of transport medium (hydrogeology) where they are acted upon by physical, chemical and biological processes. Although some research has been performed on the transformations occurring in surface waters and in subsurface layers under methanogenic and sulfate-reducing conditions, there are only limited investigations into quantifying the chemical and biological fate of these pesticides under other anaerobic transformations such as those with bisulfide and nitrate-reducing cultures. Previous research for example, has indicated that abiotic reactions occur between these herbicides and bisulfide, possibly leading to dechlorination.

As previously stated, only limited research has been performed correlating the chemical and biological degradation rate of chloroacetanilides to the alterations in their chemical structures under anaerobic conditions in the presence of denitrifying bacteria or bisulfide. However, the science of quantitative structure-activity relationships (QSARs) can be used as a tool to not only correlate transformation rates to compounds' structures or properties but to predict transformation rates of other similar compounds. Specifically, QSAR is a mathematical model used to relate known activity of a congeneric series of compounds to their structure or properties to predict other compounds' unknown activity. QSARs have not been extensively investigated for chloroacetanilide herbicides participating in abiotic and biotic reactions under anaerobic conditions, though there is a considerable amount of toxicological QSAR

research.

Initially, QSARs were developed to predict the activity and properties of pharmaceuticals and pesticides, principally for conception and design purposes [2]. However, as the need for cost-effective bioremediation techniques increased due to escalations in environmental contamination, the original objective of QSARs expanded to encompass the prediction of organic chemical properties and activities such as solubility, Henry's constant, and bioconcentration. Additionally, this technique can provide insight into the causation and mechanism of physical, biological and chemical transformations of contaminants. From such analyses can evolve effective bioremediation technology and ultimately enhance the quality of the environment and public health.

Resources aiding the effort of establishing QSARs include chemical and biochemical software programs. For this thesis work, ChemDraw and Chem3D Pro 10.0® and Gaussian 03® were used to measure structural characteristics and properties for chloroacetanilides. ChemDraw 10.0® allows the user to build any compound in two dimensions. From this program, constructed compounds can be imported into Chem3D Pro 10.0® where the compounds are drawn in three dimensions and property/structural characteristics can be calculated. Gaussian 03® is a similar program to Chem3D Pro 10.0® , and comparisons of properties computed in Chem3D Pro 10.0® and Gaussian 03® can be made. These programs allow for timely property/structural calculations for compounds that otherwise would require inexpedient experiments to determine the unavailable data of these compounds. The use of such programs brings scientists and engineers closer to developing full-scale QSARs and to understanding the remediation technology needed for the betterment of the environment.

1.2 Contribution

Chloroacetanilide herbicides demonstrate desirable pre- and post-emergence regulation of weeds and grasses in an assortment of corn, cotton and soybean crops [3]. Additionally,

over the last two decades, more than 50 million kg of these herbicides have been used annually in the United States for a variety of crops including soybeans, peanuts, corn, wheat, broadleaf weeds, [4]. More importantly, studies have detected chloroacetanilides and their metabolites in groundwater and surface waters ([5], [6]). Their presence in these water sources raises concern since according to United States Environmental Protection Agency, alachlor, acetochlor, metolachlor, and propachlor are considered to be carcinogenic ([7], [8], [9], [10]).

Because of their ubiquitous presence in groundwater, persistence and potential adverse effects, five commonly used chloroacetanilide herbicides - alachlor, acetochlor, propachlor, butachlor and metolachlor - were chosen for a research project to further investigate their environmental fates under anaerobic conditions through use of quantitative structure-activity relationships. Among the main objectives of this research were the following:

1. To estimate solubility (S), Henry's constant (K_H), and octanol-water partition coefficient (K_{ow}) of selected herbicides - alachlor, acetochlor, propachlor, butachlor and metolachlor - using ChemDraw Pro 10.0® and Chem3D Pro 10.0®,

2. To correlate the kinetic data of abiotic reactions between bisulfide and chloroacetanilide herbicides to their computed chemical structural and properties using Chem3D Pro 10.0® and Gaussian 03®, and

3. To correlate the kinetic data of biotic reactions in nitrate-reducing cultures and chloroacetanilide herbicides to their computed chemical structural descriptors and properties using Chem3D Pro 10.0® and Gaussian 03®.

The overall goal of this thesis was to perform preliminary analyses that can be used in a full-scale QSAR investigation for quantifying the abiotic and biotic degradation rates chloroacetanilide herbicides under anaerobic conditions. Furthermore, these results can be used as an initial reference point for similar research efforts on other pesticides.

1.3 Thesis Outline

Contained hereafter, Chapter 2 includes a discussion on the current literature. In particular, this chapter prefaces the work performed by Walker [11] and Qin [12] regarding the transformation and degradation kinetics of chloroacetanilides in nitrate-reducing and bisulfide environments, respectively, which provide the original data used in this analysis. Chapter 3 describes the methodology, materials and modeling tools adopted in these analyses. The results and discussion of the research are presented in Chapter 4. Chapter 5 includes the conclusions made from this work and recommendations for further research. Lastly, Appendix A contains all collected data and constructed plots used in this work.

Chapter 2

Literature Review

2.1 Introduction

Over the past 20 years, a substantial amount of research has been performed in order to gain a better understanding about the transformations of xenobiotic pesticides under various conditions in groundwater and surface water. This research comprises a diverse group of investigations from a multitude of scientific and engineering disciplines. Thus, research analyses have entailed the following non-exhaustive list: water contamination surveys (sources and prevalence), bioremediation investigations (treatment and removal), and fate and transport studies (lab and in-situ).

This review of the literature will briefly summarize the significance of several research accomplishments related to the QSAR work characterized in this thesis. Firstly, this review discusses the persistence of pesticides in groundwater and their non-point and point sources. Consecutively, a brief description of the health and environmental implications of groundwater contamination will be presented, followed by a thorough delineation of the research performed over the transformations of pesticides including descriptions of earlier work performed by Qin [12] and Walker [11] that provide the original data for this thesis research. Subsequent reviews include details of the selected chloroacetanilide herbicides for this thesis research: alachlor, acetochlor, propachlor, butachlor and metolachlor. Fur-

thermore, this review describes the quantitative-structure activity relationship techniques and the relation to degradation of chloroacetanilides. Finally, this section concludes with a summary of this work's literature assessment.

2.2 Pesticide Contaminated Groundwater

As previously stated, the blanketed applications of pesticides pose a potential threat to the environment and public health as supported by various studies that have detailed numerous cases of groundwater contamination by these chemicals. In 2000, the United States utilized pesticides on over 900,000 farms and in 70 million homes of which the majority of pesticides were herbicides [13]. This use resulted in the urban residences of northern United States treating their lawns at an equivalent rate to that of farmers in the food production industry. Consequently, the phenomenal use and persistence of pesticides in the northern United States have led to approximately 75% of municipal wells and 70% of private wells containing pesticides and their metabolites [14]. On a national basis, out of approximately 1500 domestic and public supply well samples taken throughout the U.S. between 1992 and 1996, about 40% were contaminated with pesticides [15]. According to the United States Geological Survey, more than 50% of the wells tested contained pesticides in the water in areas of agricultural and urban groundwater and more than 50% of the agricultural areas contained herbicides between 1991 and 1997 [16].

Some of the most widely used chloroacetanilide herbicides in the United States include alachlor, acetochlor, propachlor, butachlor and metolachlor [17]. In 1996, 1.15, 1.67, and 0.647 million pounds of alachlor, metolachlor and acetochlor were applied to corn crops in Wisconsin, respectively. In the vicinity of these cropland areas, 70% of private wells contained concentrations of an alachlor metabolite between 1.1 and 27 μg/L , and 7% contained the parent compound alachlor [14]. In the southern United States during the early nineties, over 50 and 15 tons of metolachlor and alachlor, respectively, were transported into the Gulf of Mexico, via surface waters, completely contaminating the Mississippi River navigable reach. Chloroacetanilides are also the dominant herbicides used for the corn crops

in Iowa accounting for 38% of herbicides used in this state. Thus, surveys have shown that 75% of municipal wells were contaminated with metolachlor, alachlor, and acetochlor metabolites with a median value of summed concentrations of 1.2 μg/L . Additionally, in surface waters, the parent compounds had a median value of 6.4 μg/L [18]. Another study in Iowa measured up to 16 μg/L of alachlor in groundwater [19]. There are many other studies that have shown groundwater contamination by chloroacetanilides and correspondingly instigated various risk assessment studies.

According to the Environmental Protection Agency [20], these herbicides can promote unsafe conditions for the ecological environment and human health. For example, acetochlor, butachlor, and alachlor can result in tumors in the nasal olfactory epithelium and thyroid. Though the EPA has concluded that metolachlor and propachlor do not cause such tumors, the National Resource Defense Council (NRDC), World Wildlife Fund (WWF), Consumers Union (CU), and Institute for Environment and Agriculture (IEA) have presented evidence that both chloroacetanilides display similar mechanisms. Therefore, all five chloroacetanilides can induce oncogenic effects.

Currently, the EPA has set the drinking water Maximum Contaminant Level (MCL) for alachlor at 2 μg/L or 2 ppb [21]. Acetochlor, butachlor, propachlor, and metolachlor await further investigation to establish their MCLs but presently have unregulated monitoring programs in place.

As evidenced by the information presented, chloroacetanilides currently pose potential hazards to the environment and human health especially due to their unpredictable frequency of contamination in groundwater and surface waters. Hence, an intermediate step to elucidate the fate and transport of these chemicals begins with the understanding of their sources.

2.3 Pesticide Contaminated Groundwater Sources

Herbicide contamination of groundwater stems from point-source or non-point-source pollution. Point-sources primarily encompass the facilities categorized in the commercial industry

and sometimes include those on a smaller scale such as accidental spills, back siphoning, and storage leaking with on-farm usage. Non-point sources embody a much larger area, predominantly where broadcast applications are made to crops or soil [19]. Historically, the handling and use of herbicides were not monitored cautiously, resulting in multiple non-point sources to be labeled point sources. The lack of discernment between the types of sources inhibits the ability to curtail future problems with herbicide contamination. Though distinguishing between point sources and non-point-sources may be ambiguous at times, both forms of release into the environment can conclusively initiate the transport of pollutants into the topsoil, subsurfaces and groundwater.

Upon application, the fate and transport of herbicides are mostly dependent upon their sorption and persistence. These processes occur along side other phenomena such as volatilization, advection, chemical decomposition, biological degradation, photolysis, and groundwater interactions [22]. In order to research methods to remediate herbicide-contaminated soils and groundwater, the exploitation of these fate processes by scientists and engineers is essential.

2.4 Transport of Xenobiotic Chemicals

As previously mentioned, xenobiotic chemicals can enter the ground in various ways. In doing so, they can enter the environment as a pure compound or as a solute, where they can infiltrate through the topsoil, subsurfaces, and ultimately the groundwater. This behavior is dictated by several processes such as solubilization, volatilization, sorption, advection, and chemical/biological transformation. The following sections include brief descriptions of each process.

2.4.1 Dissolution and Solubility

Upon contact with subsurface waters, xenobiotic compounds can be fully or partially dissolved into the water. The extent of dissolution is dependent upon the compound's aqueous solubility (*i.e.* hydrophilicity) - the amount of solute that dissolves in a known amount of

water at a specific temperature. For example, if a substance has a low solubility (*i.e.* is highly hydrophobic), that chemical can be a separate liquid or solid, relative to the solvent, or remain as a gas. Hence, the fate and transport of the substance will be primarily governed by its relative density with respect to water and its volatilization potential and its tendency to convert into a gas. In contrast, a compound with a high solubility is largely regulated by its polarity and molecular size. Other factors affecting solubility include, but not limited to, are nature/number/location of functional groups, pH, co-solutes, and temperature [2].

For pesticides, those with solubility greater than 30 mg/L in groundwater are considered potentially unsafe. Characteristically en masse, chloroacetanilides have solubility greater than 30 mg/L [23]. Alachlor, acetochlor, butachlor, and metolachlor have solubility values of 240, 223, 530, and 580 mg/L respectively [3]. Correspondingly, high solubility increases the ability of the pollutant to move within the site or off-site via runoff or leaching. Furthermore, dissolution in natural organic matter is the main activity underlying the process of sorption. Therefore, an understanding of solubility assists in the comprehension and exploitation of sorption properties of xenobiotic chemicals.

2.4.2 Volatilization

Corresponding to dissolution in understanding the fate and removal of organic contaminants is the process of volatilization. Along with solubility, this concept is also described through Henry's constant, K_H, which can be "thought of as a partition coefficient between water and the atmosphere" [2]. This constant controls the accumulation tendency at equilibrium. In particular, at a low K_H, contaminants tend to accumulate in the aqueous phase in contrast to those with a high K_H partitioning more into the gaseous phase [24]. Upon establishing the dominant phase of the contaminant, its fate thereafter depends on other chemical properties. Henry's constant has a strong dependency on temperature such that at a lower temperature, compounds have lower volatility. This property is important in considering the widespread application of herbicides and their exposure to humans. Hence, an understanding of this phenomenon is needed for the remediation of herbicide contaminants to protect human health.

2.4.3 Sorption

Another pertinent process contributing to the fate and transport and biological activity of xenobiotic substances in the soil environment is sorption [25]. Sorption refers to absorption and adsorption - the incorporation or uptake of an element by a cell or organism and physical adhesion onto the surface of another liquid or solid [26]. In conjunction with sorption is the process of desorption - the removal of substance from the surface of another. Sorption is measured by the soil adsorption coefficient (K_{oc}), particularly, the higher the K_{oc}, the stronger its adsorption to soil organic matter and the lesser its capability to leach into the groundwater. These phenomena can be rate-limiting factors affecting biodegradability, bioavailability, subsurface transport, and bioremediation. Specifically, they influence the amount of contaminant in the aqueous phase, on aquifer solids, and retardation/attenuation in groundwater ([25], [27]).

The sorption potential of a substance is dependent on the chemical/physical properties of the sorbate and sorbent. Such properties can include hydrophobicity, molecular size, and fraction of organic matter in soils and aquifer solids. Thus, the effects of sorption are not only described by the K_{oc} but also by the octanol-water partition coefficient (K_{ow}) ([25], [27]). Generally, the K_{ow} behaves similarly to the K_{oc} whereby a high K_{ow} value results in low solubility.

Another potential facet of sorption is its effect on biodegradation. The solid phase (aquifer sediment) contains the bulk of the bacteria capable of degrading xenobiotic chemicals. Therefore, an increase in localized sorption increases the degradation ability of the bacteria or can limit the available substrate for promoting the degradation of xenobiotic chemicals [28].

2.4.4 Advection and Hydrodynamic Dispersion

Regarding a chemical's dissolution in water is its transport into other subsurface areas through advection and hydrodynamic dispersion. Advection is the transport of contaminants by the flow of water. Hydrodynamic dispersion is another form chemical migration

encompassing mechanical dispersion and molecular diffusion. Molecular diffusion is facilitated by concentration gradients whereas mechanical dispersion is largely due to the varying groundwater velocities through tortuous pathways creating a mixing environment [29]. Possible dilution of chemical concentration can occur. However, as the contaminants move throughout the subsurface, they may encounter a hydrogeologic environment not conducive to biodegradation as described in the previous section. Thus, advection and dispersion play important-intrinsic roles in the remediation of xenobiotic chemicals in groundwater.

2.5 Transformation of Xenobiotic Chemicals

Upon entering soil and water subsurfaces by way of the aforementioned processes, xenobiotic chemicals can be further disturbed (degraded or transformed) by other biological and/or chemical mechanisms. The remainder of this literature review focuses on the chemical influence of bisulfide and the biological manipulation of nitrate-reducing bacterial cultures on chloroacetanilide herbicides in regards to their biodegradation rates. The following sections provide a review of the biological and chemical processes related to these herbicides via an introduction to the work performed by Walker [11] and Qin [12].

2.5.1 Biotransformation

Biotransformation of a chemical is due to microorganisms (aerobic, anaerobic, or facultative). Such transformation can be mediated through the following various mechanisms for xenobiotic compounds [2]:

1. Contaminant functions as the primary substrate - inorganic or organic electron donor providing a main energy source for microorganisms,

2. Contaminant functions as the electron acceptor to produce energy for the system,

3. Contaminant serves as a secondary substrate - substrate coexisting with a primary substrate in order to provide a net energy for growth, or

4. Contaminant is a cometabolic microorganism which does not use organics as primary or secondary substrates but is able to fortuitously transform the organic.

Substrate-enzymatic reactions include hydrolysis (nucleophilic substitution), oxidation, and reduction processes. Factors influencing these mechanisms include pH, temperature, nutrient availability, electron acceptor/donor conditions, chemical reactivity, and type of bacterial culture [30]. Various studies have shown that under anaerobic conditions, halogenated aromatic compounds are more prone to reduction rather than oxidation and, in general, lead to less toxicity and bioaccumulation. Ultimately, anaerobic dehalogenation reactions effectively degrade parent compounds and increase the degradation ability of their metabolites ([31], [32]). However, although these studies have shown that the parent compounds can be degraded effectively, the potential environmental and public health effects have not been identified for their metabolites. Typically, degradation of organics by way of hydrolysis lead to detoxifying the contaminant, but for reduction reactions, the products are usually more toxic [33]. Furthermore, there exist numerous factors dictating the success of biotic processes, as previously stated, such that when one is limiting, the potential for microbial-activity inhibition increases.

Though research has shown that chloroacetanilides can degrade under anaerobic biotic conditions, degradation under anaerobic abiotic conditions has begun to receive more attention in the last decade. Many studies have focused on the dechlorination of halogenated aromatics, combined with nitrate reduction, employing a variety of denitrifying bacteria and electron acceptor conditions.

2.5.2 Chemical Transformation

Similar to biotransformations, abiotic (chemical) reactions also involve oxidation-reduction and hydrolysis processes where they are a function of pH, temperature, moisture content, organic content, and chemical concentration. However, chemical transformations do not depend on nutrient availability or microbial concentration. Generally, abiotic reactions are slower than biotic ones and according to Bouwer and McCarty [34], can work with one

another - “abiotic processes originate with biotic transformations which with existence of reductants, oxidants, acids and bases around their living environments, microorganisms can obtain energy for cell growth and maintenance through a series of oxidation-reduction reactions, by utilizing or producing these reactants, which may results in environmental changes of the system, pH and electrochemical potential. Such environmental changes can finally result in abiotic degradation reactions such as hydrolysis and/or chemical oxidation or reduction of compounds.” Of particular interest are the reactions between hydrogen sulfide (reduction product of sulfate reduction) and haloaliphatics establishing hydrogen sulfide as “one of the most common, abundant and reactive nucleophiles in hypoxic [anaerobic] aqueous environments” [35]. Furthermore, studies have shown that the products between aliphatic compounds and hydrogen sulfide exist widely in the environment [35]. To date, little research has been conducted on the reactions between chloroacetanilide herbicides and bisulfide, but studies have suggested that chloroacetanilides undergo abiotic transformation [36].

2.6 Anaerobic Transformation: Abiotic and Biotic Reactions of Halogenated Compounds

Transformation of chlorinated aromatics under anaerobic conditions has received considerable attention due to their prevalence in groundwater in such environment. Both biotic and abiotic species exist in such environments. Biotic reactions refer to “all processes involving the participation of metabolically active microorganisms abiotic reactions encompass a host of processes mediated by compounds generally associated with biological activity, but not necessarily directly involving active microorganisms” [37].

The following sections present the literature available for anaerobic biotic and abiotic reactions in groundwater with respect to halogenated aromatic compounds.

2.6.1 Impact of Bisulfide

Associated with anaerobic conditions is bisulfide which results from the microbial reduction of sulfate. Its parent compound, hydrogen sulfide, is well known for its colorless appearance and potent odor of "rotten eggs" [38]. Despite the heavy industrial use of hydrogen sulfide, it is also a natural product from the degradation of organic matter. Consequently, this chemical nuisance finds its way into drinking water via groundwater. Total sulfide concentrations have been reported to reach 10^{-3}M ([39], [40], [41]). Lemley and others [42] state that at a concentration as low as 0.5 mg/L of hydrogen sulfide can add an offensive taste and foul odor, and Pomeroy and Cruse [43] found these effects at concentrations as low as 0.0001 mg/L .

In water at 25°C with a pH range from 6 to 9 (*i.e.* natural water pH range), hydrogen sulfide's primary ionic species is bisulfide [35]. The prevailing existence of hypoxic (anaerobic) environments in saturated subsurfaces (pristine and contaminated) fosters this most abundant and reactive nucleophile ([44], [39]). Furthermore, with sulfate as the terminal electron acceptor, reductive dechlorination seems to occur under these conditions [45]. Therefore, one of the primary focuses of this research is to study the effects of this process towards chloroacetanilide degradation as well as its relationship to the herbicides' structures and properties. Contained hereafter are the research data that have been collected regarding the elucidation of the reaction mechanism and reactivity of halogenated aromatic compounds in the presence of bisulfide.

HS^- Studies

According to Schwarzenbach *et al.* [40], Barbash and Reinhard [35], the abiotic reaction between organic contaminants and sulfide species is environmentally beneficial inasmuch the proven studies of haloaliphatics abiotic transformation by bisulfide. For instance, reaction products of bisulfide and aliphatic compounds have been detected in numerous groundwater samples. Wilber and Garrett [46] suggested that aryl herbicides may undergo similar transformations abiotically in groundwater.

One of the major reductive processes in hydrogeologic subsurface systems is the dehalo-

genation of haloaryl compounds where the dominant abiotic electron donors in anaerobic systems are reduced iron and sulfur groups [12]. The reduction of nitroaromatic compounds is also a frequent occurring reaction. In a study conducted by Schwarzenbach and coworkers [47], nitrobenzene was reduced by the iron porphyrin and quinine facilitation of electron transfer from sulfide to the contaminant. Similarly, Yu and Bailey [48] observed the reduction of nitrobenzene in solution with sulfide species.

Generally, reductive dechlorination occurs via nucleophilic or free radical substitution [37]. Substantial research investigating the nucleophilic substitution of haloaliphatic compounds has been widely reported by various scientists ([40], [49], [50]). Barbash and Reinhard [35] reported that nucleophilic substitution controlled the reaction under hypoxic conditions in the dehalogenation of 1,2-dichlorethane and 1,2-dibromoethane. Consequently, bisulfide was considered a soft nucleophile due to its loosely held and more polarizable electron cloud's availability for nucleophilic attack. A soft nucleophile is typically a species that is large, highly polarizable and has low energy highest occupied molecular orbitals while a soft electrophile has similar characteristics causing a substitution reaction between the soft nucleophile and electrophile.

Studies on the substitution of haloacyl-sustituted anilines with sulfide species are limited. The limited available research on chloroacetanilide and bisulfide will be discussed hereafter. However, Wolfe and Macalady [33] recommended determining the role of each functional group and their relative transformation potential in combination by analyzing the factors affecting the transformation kinetics. In doing so, structural descriptors of organics must also be inspected. Thus, one of the main objectives of this thesis research was to correlate structural characteristics of chloroacetanilides to their degradative activity through the use and practice of quantitative structure-activity relationships.

2.6.2 Impact of Nitrate Reduction

Under anaerobic nitrate-reducing conditions, many early studies revealed much difficultly in the enrichment and isolation of the responsible microorganisms for halogenated aryl compounds. Thereby, numerous halogenated aromatic compounds have been labeled re-

calcitrant under anaerobic denitrifying conditions. However, Bouwer and Cobb [51] found that the addition of an electron to an in situ bioremediation scheme promotes the rapid utilization of oxygen, resulting in anoxic conditions. Ergo, biotransformation under nitrate reducing and in anoxic environments has become a notable area of research.

Some studies have been able to elucidate the enrichment culture able to degrade halogenated aromatics in a denitrifying environment [52]. Most nitrate-respiring microorganisms are found in environments such as lakes, rivers, soils, and oceans in anoxic conditions ([53], [54], [55]). Due to their prevailing existence, "Anaerobic processes are beneficial for eliminating pollutants from contaminated sites, in which oxygen is often unavailable due to its quick depletion with easily utilizable substrates, low solubility in water and low rate of transportation in saturated porous matrices such as soils and sediments. Denitrifying bacteria, which are basically categorized as aerobes, have received attention because they could be active under anoxic conditions. Their facultative trait allows them to have a more extensive range of habitats with different oxygen concentrations than other microbial groups" [52]. Therefore, there have been reports of the potential of such bacteria to degrade haloaryl contaminants in hydrogeological subsurfaces and attempts to elucidate these mechanisms. These studies will be discussed below.

Nitrate Reduction Studies

Because of their activity under anoxic conditions, denitrifying bacteria have received considerable attention concerning their role in abiotic reactions with halogenated aromatic and alkyl compounds. More specifically, the halogen (primarily chlorine) was attached directly to the benzene ring, and the halogenated contaminant acted as alternate electron acceptors under anaerobic conditions.

Sanford and coworkers [56] conducted a study on myxobacteria able to dechlorinate 2-chlorophenol testing different electron donors - acetate, pyruvate, diatomic hydrogen, succinate, formate, and lactate. They concluded that dechlorination and nitrate reduction occurred in the same culture, with acetate being the best electron donor. However, 2-chlorophenol was fully degraded. When continuously adding 2-chlorophenol, nitrate re-

duction was inhibited. Thus, nitrate was not the preferred electron acceptor and inhibited dechlorination at concentrations greater than 5mM. Picardal and others [57] also concluded that at concentrations greater than 3mM of nitrate, dechlorination was inhibited. In contrast, Bae and others [52] reported that at 5mM nitrate concentration, degradation of 2-chlorophenol occurred, though not involving reductive dechlorination and most likely was due to the different denitrifying culture used in both experiments. Consistent with this study, 3-chlorobenzoate and 4-chlorobenzoate were degraded under denitrifying conditions, but there were no metabolites detected. Therefore, it was not conclusive whether reductive chlorination was the initial step in the degradation of the chlorobenzoates [58].

Though as evidenced by these studies that the secondary substrate utilization capabilities of denitrifying cultures are not consistent throughout the environment, biological degradation of xenobiotic substances remains significant. To date, very little literature exists in reference to the effects of nitrate-reducing bacteria on the degradation of chloroacetanilides and moreover, research into other haloacyl-substituted anilines awaits investigation. Studies particular to each chloroacetanilide will be discussed later in this chapter.

2.7 Pesticide Analysis and Transformation Kinetics

Analyzing and quantifying the rate of biotransformation is another key aspect that must be established to fully understand the fate of contaminants in certain environments. Thus, kinetic experiments were performed by Qin [12] and Walker [11] to determine the rate constants for each chloroacetanilide compound under bisulfide and nitrate-reducing anaerobic conditions, respectively. These results will be used in the QSAR investigation of this thesis.

In determining the rate constants of halogenated aliphatic and aryl compounds, the type of kinetics and experiment was thoroughly examined. Numerous studies have successfully implemented a second-order model in aqueous environments to describe a nucleophilic substitution with a nucleophile or a reductive reaction with a reductant ([59], [35], [49], [50],

[47]). Therefore, despite the various methods and kinetic expressions available to determine the rate constants, the use of small-volume batch reactors and a pseudo-first-order decay model were employed in determining the decay rates of each chloroacetanilide based on previous research ([12], [60]).

In general, many of the kinetic expressions used in quantifying the biodegradation rates of xenobiotic chemicals were derived from Monod and Michaelis-Menten equations ([61], [62]). As previously stated, after extensive investigation into the order of the reaction, a pseudo first-order model was used in Qin [12] and Walker's [11] research to express the disappearance of herbicides under conditions resembling groundwater environments as closely as possible. To follow is a brief description of the methods employed by Qin [12] and Walker [11] in analyzing and quantifying the rate of chemical and biological transformation, respectively.

2.7.1 Experimental Systems with Bisulfide

Work perfomed by Qin [12] focused on evaluating the abiotic reaction of chloroacetanilide herbicides with bisulfide in anaeraobic environments. In brief, this section describes the analytical methods and batch reactors studies used to determine the abiotic transformation rates of selected herbicides.

For the batch reactor studies, a solution containing 50mM of phosphate buffer was stripped of oxygen in a nitrogen environment and dosed with known concentrations of bisulfide and herbicide. After complete mixing, the solution was transferred to a series of batch reactors and sealed to mitigate volatilization of the hydrogen sulfide. To limit temperature fluctuation, the reactors were incubated in the dark (temperature range of 5°C to 50°C). Samples were collected periodically for herbicide and sulfide analysis. These analyses will be briefly described below.

Solid phase extraction techniques employed by Qin [12] were taken from those described by Thurman and coworkers [63]. PrepSep C-18 cartridges were used as the extraction columns where 50 mL samples from batch reactors were passed through these columns. Following air drying, the cartridges were eluted with 2 mL of ethyl acetate, and extracts

were stored in a dark room at 4°C before gas chromatography (GC) analysis.

For pesticide analysis, GC with an electron capture detector (ECD) was used where extracted samples and herbicide standards were injected onto a silica capillary column. To quantify concentrations, the comparison of relative areas was recorded by an integrator. Five calibration standards for each experiment were used to calibrate the GC, and duplicate runs were performed for each sample and standard. The average of each measurement was computed.

For sulfide analysis, the Iodometric Method was employed where an aliquot of 0.025N standard iodine solution, 2 mL of 6N HCl and 50 mL of sample containing sulfide were added sequentially in a flask. The unreacted iodine in solution was back-titrated with 0.025N $Na_2S_2O_3$ solution.

Investigations into the order of the reaction supported the assumption that a second-order model could be used to describe the following reaction:

$$\frac{d[C]}{dt} = -k_{HS^-}[HS^-][C] \tag{2.1}$$

where k_{HS^-} is the second-order rate constant for the reaction between bisulfide and the herbicide, $[HS^-]$ is the concentration of bisulfide and $[C]$ is the concentration of the herbicide. During periods of constant bisulfide concentration, equation 2.1 can be approximated by a pseudo-first-order decay as follows:

$$\frac{d[C]}{dt} = -k_{obs}[C] \tag{2.2}$$

where the pseudo-first-order rate constant, k_{obs}, is given by:

$$k_{obs} = k_{HS^-}[HS^-] \tag{2.3}$$

From these equations, the plot of $ln[C]$ vs. $time$ yields k_{obs}. The quotient of k_{obs} and the measured bisulfide concentration yields k_{HS^-}. From rates of transformation of each herbicide in the presence of various concentrations of bisulfide, second-order rate constants

were developed.

2.7.2 Experimental Systems with Denitrifying Bacterial Culture

Data for the rate of acetanilide biotransformation under nitrate-reducing conditions was obtained from an unpublished study performed by Walker [11]. What follows is a brief description of how these experiments were performed.

A solution of inorganic salts containing trace minerals and a phosphate buffer served as the aqueous medium for the cultures. Included was sodium nitrate ($NaNO_3$) at an initial nitrate concentration of 200 mg/L NO_3^-–N. Earlier work [64] had shown acetanilide biotransformation to be primarily cometabolic, meaning that a readily degradable organic substrate was required to support the maintenance of the microbial culture. As such, acetate (sodium acetate) was added to provide an initial acetate concentration of 90 mg/L . This ratio of acetate to nitrate ensured that the cultures would be carbon-limited. That is, the amount of nitrate exceeds the amount required by stoichiometry for the metabolism of acetate under nitrate-reducing conditions. Though nitrate-reduction under these conditions is an alkalinity-producing reaction, the phosphate buffers included in the aqueous medium were adequate to ensure a pH of 6.8 - 7.2.

The aqueous solution was next stripped of oxygen under a nitrogen environment, such that dissolved oxygen was measured to be no more than 0.5 mg/L . The solution was then seeded with a small aliquot of effluent from the biotower reactors at the Stillwater (OK) municipal wastewater treatment plant. The biotower at this plant was known to contain anoxic zones in which nitrate-reduction occurred, and as such, its effluent was certain to contain facultative nitrate-reducing bacteria. Within 48 hours, the culture was shown to be actively reducing nitrate. Following a five-day period in which the nitrate-reducing biomass was allowed to grow, the culture was then well mixed and distributed among a series of 1 L reactors (three replicates each for each of the five acetanilide herbicides under investigation). These reactors were immediately dosed with an aqueous stock solution of

one of the herbicides, resulting in an initial concentration of approximately 100 μg/L . After thorough mixing, a sample was immediately taken to determine the initial concentration. In addition, a set of three abiotic control reactors was established. These reactors were identical to the biological reactors, but were not seeded with the microbial culture. All reactors were kept sealed, in the dark, at room temperature (23°C) over the experimental period.

The biological and control reactors were then monitored for herbicide concentration over time. The herbicide concentration was tested on a 50 mL sample taken from each reactor. The sample was analyzed by the solid-phase extraction method described by Qin [12]). At the end of the experimental period (approximately 20 days), the reactors were analyzed for volatile suspended solids (VSS) as an estimate of bacterial solids concentration. The data was then plotted assuming the cometabolic biotransformation reaction could be described as a second order reaction, as seen in the following equation:

$$\frac{d[C]}{dt} = -k_{bio}[X][C] \tag{2.4}$$

where C is the herbicide concentration (μg/L), X is the microbial solids concentration (mg VSS/L), and k_{bio} is the biotransformation rate under nitrate-reducing conditions. Over the relatively brief time of the experiment, the biomass concentration could be treated as constant, and so the above equation can be treated like a pseudo-first order reaction. Hence, a plot of the natural log of the herbicide concentration versus time yielded a line whose slope was equal the value ($k_{bio}[X]$). The value of k_{bio} could then be estimated by dividing the slope by the biomass concentration X. These values are the average of the three replicates, which in all cases were within 10% of each other. It should also be noted that the abiotic control reactors exhibited minimal loss of herbicide over the time of the experiment, as expected. This indicates that the pesticide loss seen in the other reactors was primarily due to biological transformation reactions.

2.7.3 Bisulfide and Nitrate-reducing Rate of Transformation Constants

Rationale for the use of the pseudo-first-order rate model can be found in the graduate work of Wilber [60] and Qin [12]. Below are the second-order rate constants for each herbicide under these conditions collected from Walker [11] and Qin [12]:

Table 2.1: Bisulfide and biological rate reaction constants for chloroacetanilide herbicides.

	k_{HS^-} [a] $(\frac{L}{mg\,HS^- \cdot hr})$	k_{bio} [b] $(\frac{L}{mg\,VSS \cdot hr})$
Alachlor	0.00160	0.00026
Acetochlor	0.00112	0.00051
Butachlor	0.00083	0.00052
Metolachlor	0.00037	0.00027
Propachlor	0.00255	0.00028

[a] Source: Qin [12]

[b] Source: Walker [11]

As shown in Table 2.1, Walker's qualitative observation concluded that more complex molecules are transformed faster than those with less complicated substituents does not hold entirely. Furthermore, he concluded that for biological transformation, access to the chlorine molecule is less likely to be the dominant structural parameter controlling the rate of reaction; instead, factors influencing the microorganisms' ability to attack substituted branches would be more significant [11]. In contrast, the data in Table 2.1 upholds the notion that the most simplistic, substituted structure (propachlor) reacts the fastest while the most heavily, complicated substituted structure (metolachlor) reacts the slowest. Additionally, Qin [12] qualitatively described the trend among these rates as consistent with the notion that the least and most simply, substituted structure (propachlor) reacts fastest while the most heavily substituted (metolachlor) reacts slowest. Likewise, the two herbicides with the most similar structures (alachlor and acetochlor) also had the closest rates of reaction. In a similar investigation performed by Beestman and Deming [65] and Zimdahl and Clark [66], degradation rates of four chloroacetanilides were as follows in decreasing order: propachlor,

alachlor, butachlor and metolachlor.

2.8 Quantitative Structure-Activity Relationships

Due to the demand for safer chemicals in medical and agricultural disciplines, scientists and engineers have been working over the last 20 years to design substances based on mitigating toxic effects on the ecological and human environment. A principle component of achieving this goal has involved rational molecular design strategies ([67], [68], [69]). These methodologies were first implemented in pharmaceutical and drug design, but in the last decade, they have emerged in areas of bioremediation and engineering risk assessment applications. An integral piece of this research includes the science of quantitative structure-activity relationships (QSARs). For simplicity's sake, structure-function relationships include studies of quantitative-structure activity relationships (QSAR), quantitative structure-property relationships (QSPR), and quantitative structure-toxicity relationships (QSTR) and will be referred to as general QSARs in this work.

QSARs are largely exploited by industries to expeditiously predict the biological/chemical activity and reactivity of organic compounds in the environment and engineered systems based on structural-congeneric compounds of known activity and reactivity. These algorithms assist in elucidating the reaction mechanisms and pathways of organic contaminants in the environment and, accordingly, metabolites can be identified. Thus, the purpose of this section is to describe the nature and benefits of QSARs for understanding and predicting the behavior of xenobiotic chemicals.

2.8.1 Underlying Principles of QSARs

QSARs predict the functions of a congeneric series of compounds by attempting to statistically correlate its functions to structural molecular characteristics and properties (*i.e.* descriptors). For purposes of this discussion, structure refers to the molecular characteristics, activity to chemical or biological effects (substitution, toxicity, biotransformation), and property refers to environmental fate characteristics such as solubility, volatility, Henry's

constant, etc. [2]. The main assumptions in the QSAR approach when used in predicting biological fate is that "the factors governing the events in a biological system are represented by the descriptors characterizing the compounds, whose biological activity is expressed via the same mechanism" and "all physical, chemical, and biological properties of a chemical substance can be computed from its molecular structure, encoded in a numerical form with the aid of various descriptors" [70]. Similar assumptions are made regarding behavior in abiotic chemical reactions.

2.8.2 QSAR Model

QSAR algorithms are multivariate mathematical relationships between a set of descriptors (properties or structural), x_{ij}, and a chemical or biological activity, y_i. For compound i, the linear relationship relating descriptors, x_{i1}, x_{i2} to activity, y_i, is as follows:

$$y_i = x_{i1}m_1 + x_{i2}m_2 + \ + x_{in}b_n + e_i \tag{2.5}$$

where m is the linear slope expressing the correlation between property x_{ij} with activity y_i of compound i, and e_i is a constant [70]. Typically, the slopes and e_i are found through regression analyses such as simple linear regression (SLR), multiple linear regression (MLR), variant MLR (stepwise MLR), partial least squares, and principal component analysis (PCA) [70].

2.8.3 QSAR Model Validation

The validity of the QSAR model chosen is dependent on several criteria. The following list summarizes these requirements:

- Biological or chemical activity must relate to physicochemical properties
- Chemical activities must be based on same mechanism
- Congeneric chemicals should be used in analyses

These guidelines assist in the selection of the appropriate chemical sets. As stated previously, the series of compounds must exhibit a specific activity through a common mechanism that can be modeled by a QSAR equation.

Chloroacetanilide QSAR Validation

For chloroacetanilides, the USEPA established that alachlor, acetochlor and butachlor should be grouped together based on a common end-point and known toxicity for this end-point - nasal turbinate tumors in rats [20]. Their assessment details the chemical and biological common group mechanisms. Although, the USEPA did not incorporate metolachlor and propachlor into this assessment. There have been disputes over exclusion of these two chemicals put forth by the NRDC, WWF, IEA, and CU as stated earlier in this report. Therefore, the activity based on the same mechanism has been established for this group of five chloroacetanilides. Furthermore, these chemicals display notable consubstantial structural arrangements, completing the criteria for QSAR validation.

2.8.4 QSAR Descriptors

The selection process for descriptors has typically included those of this science's origin, Hammet parameters, which are electronic parameters relating the electronic influence of a substituent to the difference between the log of the acid dissociation constant of a substituted and unsubstitued benzoic acid. However, these values typify the influence of substituents directly attached to a benzene parent compound. Thus, this electronic descriptor is not integrated into this report's analysis of QSARs for chloroacetanilides. Over the history of QSARs, the variety and diversity of descriptors have come to encompass topological, geometrical, quantum chemical indices, and properties such as molecular size, shape, symmetry, complexity, branching, cyclicity, stereoelectronic character, K_{ow}, Henry's constant, K_{oc}, and solubility. In developing a QSAR, a selection of descriptors can cause collinearity and overdetermination. Hence "one needs to extract distinct and orthogonal or uncorrelated structural information from the collection of diverse predictors in order to develop useful QSAR/QSPR models" [71].

The rationale for the descriptors chosen for chloroacetanilide herbicides was briefly discussed with the transport of xenobiotic chemicals. The selection of descriptors was based on environmental fate parameters - Henry's constant, solubility, and octanol-water partition coefficient - that are believed to strongly influence the degradation of herbicides. Each parameter and its descriptors are discussed below.

Henry's Constant Descriptors

Predictive methods for the Henry's constant are essential in understanding the behavior of contaminants in the environment and can also be used corroboratively with solubility and vapor pressure data. Although measurements of polyaromatic pesticides with low volatility are difficult to generate reliable data Nagamany *et al.* have presented a new approach in estimating K_H, using group and bond contribution factors [24]. Their method revealed a strong correlation between Henry's constant of a solute and its molecular structural characteristics involving the connectivity indices and polarizability. Thus, potential descriptors to correlate Henry's constant to degradation rates of chloroacetamide herbicides were molar volume, dipole moment, and total connectivity index. The higher the dipole moment, the more the chemical can react with water and ultimately, the more concentrated it is in the aqueous phase. The larger the molar volume of a contaminant the greater the difficulty of the chemical to remain in solution because it requires a larger solvent cavity. Other descriptors that were considered were temperature and vapor pressure. The higher the temperature results in a higher the tendency of a chemical to exist in the gas phase. Likewise, the higher the vapor pressure, the more it will evaporate. Furthermore, molar refractivity was chosen as a descriptor to characterize the size of the compound since it is dependent on temperature and index of refraction.

Solubility Descriptors

Solubility is primarily a function of molecular size and polarity. Thus, the descriptors for Henry's constant are very similar for solubility. However, Nagamany and Speece [72] have shown a different aspect of polarizability used in predicting solubility, one that is

dependent on the number of carbon, chlorine and hydrogen atoms of the contaminant and topological diameter. Potential descriptors for the QSAR analysis include topological diameter, Connolly molecular area, Connolly excluded solvent volume, molecular topological index, and Wiener index.

2.8.5 Octanol-water Partition Coefficient Descriptors

The octanol-water partition coefficient K_{ow} is a measurement of differential solubility of a compound between water and n-octanol. This value measures the hydrophobicity and hydrophilicity of a substance. Additionally, the prediction and modeling of the migration of dissolved hydrophobic organic substances in soil and groundwater is characterized by this parameter. Potential descriptors for this parameter were based on previous work ([73], [74], [75]) and include molar volume, molecular surface area, and molecular weight.

2.8.6 QSAR and Chloroacetanilide Degradation

The importance of QSARs has increased over the past 20 years. Scientists and engineers are perpetually researching the fate and transport and remediation technology of organic contaminants. With the use of this science, cost-effective and rapid predictions of chemical and biological activity of herbicides can be made while simultaneously contributing to the ceaseless efforts of bioremediation.

The following section describes the chloroacetanilide herbicide family and the research conducted on their transformation and relevant QSAR descriptors.

2.9 QSAR and Chloroacetanilide Transformation

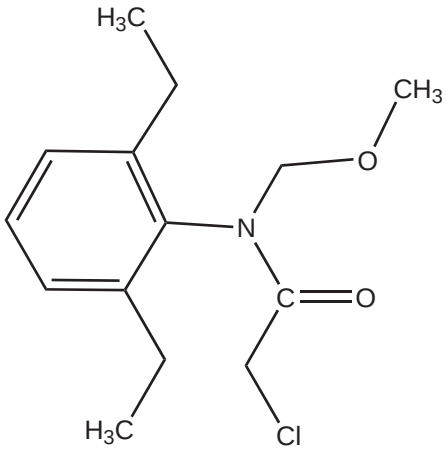
In regards to the chemical and biological degradation of the acetanilide compounds of interest in this thesis, a vast amount of research has been conducted into the elucidation of their reactions in specific hydrogeological mediums. However, to date, no laboratory or field studies have been performed on acetanilide herbicides for full scale quantitative structure-activity relationships with respect to predicting their degradation as a function of their

structure, activity, and properties. Therefore, the following review describes the current literature concerning the properties and transformations of alachlor, acetochlor, butachlor, metolachlor, and propachlor on a collective and individual basis.

2.9.1 Chloroacetanilides

The herbicide structures of alachlor, acetochlor, butachlor, metolachlor, and propachlor are shown in Figures 2.1, 2.2, 2.3, 2.4, and 2.5, respectively.

Alachlor

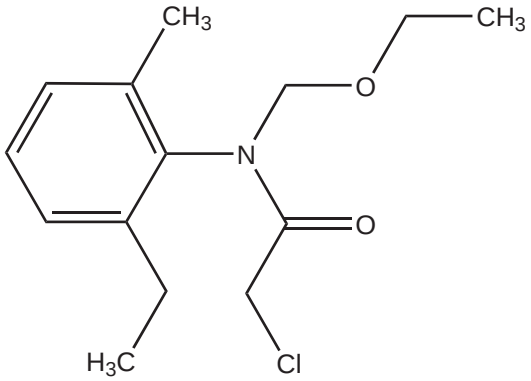


Properties

CAS Number[a]	15972-60-8
Chemical Formula[a]	$C_{14}H_{20}ClNO_2$
Boiling Point[a]	100ºC at 0.02 mm Hg
Density[a]	1.133 g/mL at 25/15.6ºC
Organic Carbon Partition Coefficient[b]	2.279
Melting Point[a]	39.5 ºC to 40.5ºC
Molecular Weight[a]	269.8
Physical State[a]	White, odorless, crystalline solid
Water Solubility[a]	240 mg/L at 25ºC
Vapor Pressure[a]	2.2 x 10^{-5} mm Hg at 25 ºC

Figure 2.1: Alachlor structure and properties.
[a]Source: Chem3D Pro
[a]Source: Weed Science Society of America [3]
[b]Source: CRWR [76]

Acetochlor

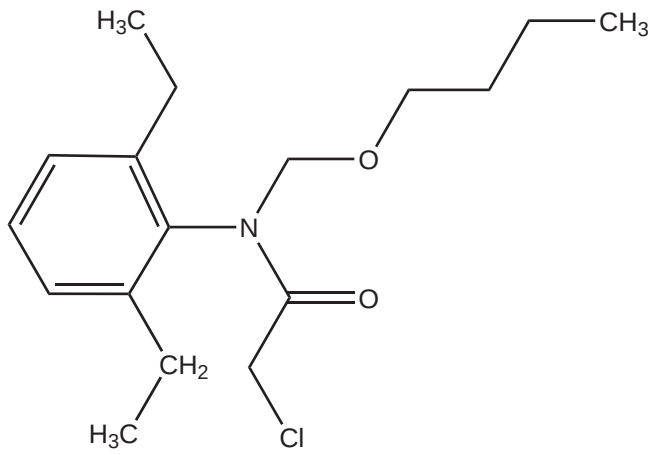


Properties

CAS Number[a]	34256-82-1
Chemical Formula[a]	$C_{14}H_{20}ClNO_2$
Density[a]	1.136 g/mL at 20ºC
Organic Carbon Partition Coefficient[b]	2.642
Molecular Weight[a]	269.8
Physical State[a]	Aromatic colorless thick liquid
Water Solubility[a]	223 mg/L at 25ºC
Vapor Pressure[a]	3.4 x 10^{-8} mm Hg at 25ºC

Figure 2.2: Acetochlor structure and properties.
[a]Source: Chem3D Pro
[a]Source: Weed Science Society of America [3]
[b]Source: CRWR [76]

Butachlor

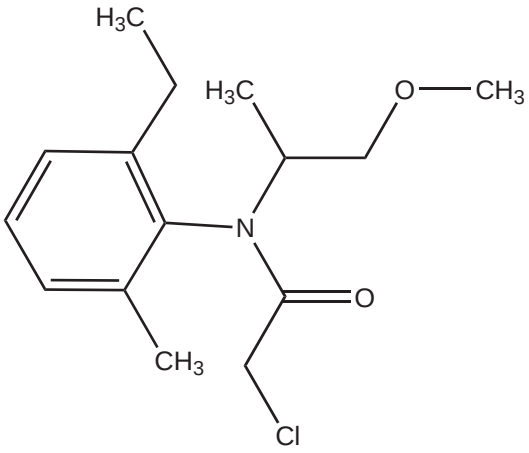


Properties

CAS Number[a]	23184-66-9
Chemical Formula[a]	$C_{17}H_{26}ClNO_2$
Boiling Point[a]	156ºC at 0.5 mm Hg
Density[a]	1.070 g/mL at 25ºC
Organic Carbon Partition Coefficient[b]	4.114
Melting Point[a]	< -10ºC
Molecular Weight[a]	311.9
Physical State[a]	Amber-colored liquid
Water Solubility[a]	23 mg/L at 24ºC
Vapor Pressure[a]	4.5 x 10^{-6} mm Hg at 25ºC

Figure 2.3: Butachlor structure and properties.
[a]Source: Chem3D Pro
[a]Source: Weed Science Society of America [3]
[b]Source: CRWR [76]

Metolachlor



Properties

CAS Number[a]	51218-45-2
Chemical Formula[a]	$C_{15}H_{22}ClNO_2$
Boiling Point[a]	100ºC at 0.001 mm Hg
Density[a]	1.085±0.005 g/mL at 20ºC
Organic Carbon Partition Coefficient[b]	2.513
Molecular Weight[a]	283.8
Physical State[a]	Off-white to colorless, odorless liquid
Water Solubility[a]	530 mg/L at 20ºC
Vapor Pressure[a]	1.3 x 10^{-5} mm Hg at 20ºC

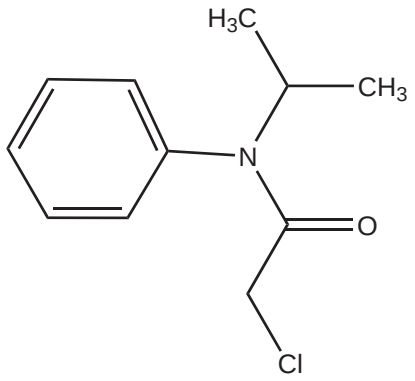
Figure 2.4: Metolachlor structure and properties.

[a]Source: Chem3D Pro

[a]Source: Weed Science Society of America [3]

[b]Source: CRWR [76]

Propachlor



Properties

CAS Number[a]	1918-16-7
Chemical Formula[a]	$C_{11}H_{14}ClNO$
Boiling Point[a]	110ºC at 0.03 mm Hg
Density[a]	1.134 g/mL at 25ºC
Organic Carbon Partition Coefficient[b]	1.793
Melting Point[a]	77 ºC
Molecular Weight[a]	211.7
Physical State[a]	Light tan solid
Water Solubility[a]	580 mg/L at 20ºC
Vapor Pressure[a]	2.3 x 10^{-4} mm Hg at 25ºC

Figure 2.5: Propachlor structure and properties.

[a]Source: Chem3D Pro

[a]Source: Weed Science Society of America [3]

[b]Source: CRWR [76]

As shown by Figures 2.1-2.5, these compounds are aniline derivatives where the nitrogen is a tertiary amine forming an acetanilide base compound. On the alpha carbon of the amide functional group is a chlorine substituent. Because of this halogen, chloroacetanilides are considered halogenated alkyl compounds. Each herbicide differs in that their amide side chain substituents to the benzene ring are of different length and type (*i.e.* alkyl and ether groups). Because of their structural similarities, these herbicides are expected to possess comparable chemical/physical properties, transformation mechanisms, modes of actions, and selectivity. Some modes of action for this group of herbicides have been established. Sharp [77] proposed the mode of action for chloroacetanilides is the inhibition of protein synthesis in target plants, and the Environmental Protection Agency [20] found their toxicological mode of action to be the production of tumors of the nasal olfactory epithelium in rats. Thus, their degradation rates based on structure, activity, and properties can be expected to be similar.

The main use of these chemicals is for pre-emergence and post-emergence control of grasses and broad-leaved weeds in primarily corn, rice, and soybean crops. Their widespread use can be seen in Iowa where approximately 7 million kg of metolachlor, alachlor, and acetochlor was applied to farmland in 1995 [18]. In 1996, 1.15 million lb of alachlor was applied over 692,000 acres of farmland in Wisconsin [14]. They are strongly recalcitrant to chemically breaking down by radiant light and do not volatilize easily. However, they have shown to degrade chemically and biologically in soil subsurfaces. Various studies have shown that alachlor, acetochlor, butachlor, metolachlor, and propachlor have been detected in groundwater samples ([78], [14], [79]). Tsumura [80] has confirmed the existence of butachlor in tap water.

The subsequent section of this literature review is devoted to characterizing the physical and chemical properties of each chloroacetanilide of interest and to detail the contemporary explorations on the transformation of each herbicide.

Alachlor

Of the chloroacetanilide herbicides examined in this thesis, alachlor [(2-chloro-N-(2,6-diethylphenyl)-N-(methoxymethyl)acetamide] was the second herbicide to be registered with the United States Environmental Protection Agency. The EPA registered alachlor in 1969 as a selective herbicide for control of broadleaf weeds and grasses on corn, soybeans, peanuts, sorghum, and beans. Furthermore, alachlor is used a pre-emergent and post-emergent control chemical for corn/soybeans and peanuts, respectively [7]. Nationally, 25.6 million lb/yr of alachlor was applied to over 14 million acres/yr of field crops in 1991-1995 [81]. Some of the common names for alachlor include Partner®, Bronco®, Lariat®, and Lasso® [3].

As mentioned, alachlor has been detected in a number of surface and groundwater samples nationwide. The USEPA promulgated its maximum contaminant level at 2 μg/L and is classified as oncogenic [7]. In the USGS 1992-2001 groundwater report, 15% of the agricultural streams tested revealed alachlor concentrations exceeding the benchmark [82]. A study of 76 U.S. Midwestern streams in 1997 detected a pre-emergence concentration of alachlor of 18.3 μg/L and a post-emergence concentration of 7.8 μg/L . Moreover, the metabolites of alachlor, ethane sulfonic acid (ESA) and oxanillic acid (OXA), were also detected, some at much higher levels. The pre and post-emergence detections for alachlor ESA were 93.4 and 3.02 μg/L while the both detections for alachlor OXA were 1.06 and 4.3 μg/L , respectively [6]. One of the most comprehensive studies on alachlor concentrations in finished surface drinking water was conducted by the Acetochlor Registration Partnership in 1995 and 1996 [83]. Samples were taken from 173 monitoring wells located in eastern half of the United States. Results from this research showed the maximum alachlor concentration detected was 4 μg/L . From 1989 to 1992, the University of North Carolina-Asheville Environmental Quality Institute detected concentrations of alachlor up to 68 μg/L [83].

Previous studies have shown alachlor to degrade chemically and biologically in the environment. Alachlor transformation has been shown to be influenced by its volatility. Chesters et al. reported the spraying application of alachlor may cause it to volatilize in environments with moist soils and higher temperatures [84]. Glotfelty and coworkers [85] also reported the

rapid volatilization of alachlor in moist soils and increased soil heating and atmospheric turbulence. The transformation of alachlor not only occurs through volatilization but through microbial degradation as well.

Biotransformation is thought to be one of the major pathways for degrading alachlor. Numerous studies have reported the degradation of alachlor and other chloroacetanilides to be a cometabolic process ([86], [87], [88]). In a study conducted by Kaufman and Blake [89], additional carbon sources may promote the growth of a soil fungus Rhizoctonia and the degradation of alachlor. Pothuluri *et al.* [90] reported that the half-life of alachlor was decreased by the addition of available carbon sources for surface and aquifer samples. This decrease suggested nutrient limitation and cometabolic transformation was occurring. Alachlor degradation has also been shown to occur under anaerobic nitrate-reducing conditions. A variety of studies reported the transformation of alachlor in anaerobic environments. However, degradation studies under nitrate-reducing conditions are limited.

Chemical degradation of alachlor has been shown in many research efforts. Li and coworkers [91] reported the degradation of alachlor by small amounts of Fe(II) and Mn(II), but alachlor degradates were not identified. Eykholt and Davenport [92] also showed that iron can degrade alachlor and metolachlor. They found prominent ions suggesting the dechlorination alachlor and metolachlor. Anaerobic abiotic transformation of alachlor has also been researched. Novak *et al.* [93] observed rapid decay of alachlor in the presence of sulfide. Furthermore, Stamper and coworkers [94] observed degradation of alachlor, propachlor and metolachlor at elevated sulfide levels. Reported by Gan *et al.* [95], alachlor, acetochlor, metolachlor and propachlor may undergo nucleophilic substitution at the acetyl carbon in the presence of thiosulfate. Additionally, another study showed that soil microorganisms producing bisulfide degraded chloroacetanilides [96].

2.9.2 Acetochlor

Replacing alachlor in the 1990s, acetochlor [2-chloro-N-(ethoxymethyl)-N-(2-ethyl-6-methylphenyl)acetamide] was registered in 1994 also as pre-emergent for the control of weeds for field corn, sorghum, wheat, tobacco, and popcorn [8]. Trade names for acetochlor include

Acenit®, Guardian®, Harness®, Trophy®, and Winner® ([3], [97], [98]). In 1991-1995, 2 lb/treated acre per year of acetochlor were applied to field crops [81]. Acetochlor was conditionally registered for its application on corn crops with the intentions of reducing alachlor, and by 1997, acetochlor had effectively evolved into the new alachlor whereby becoming one of the most predominantly used herbicides in the United States [82]. Additionally, the United States Department of Agriculture [99] reported a 32% in alachlor use between 1993 and 1994.

Despite the increasing use of acetochlor over the past decade and its particularly close structure with respect to alachlor, there is no MCL established for this chemical. Moreover, in 1997, concentrations as high as 21.3 μg/L were detected in runoff samples wherefore the concentration of its metabolites, acetochlor ethane sulfonic acid (ESA) and oxanillic acid (OXA), were reported as high as 5.01 and 4.27 μg/L , respectively [6]. Other studies have shown acetochlor concentrations to reach 1.2 μg/L in wells and surface water ([99], [14]). The USEPA has not established an MCL for acetochlor, but it has been classified as "likely to be carcinogenic to humans" [8].

Work was conducted to investigate the degradation and detoxification of acetochlor in soils by inorganic amendments. Though minimal microbial activity was detected, sodium thiosulfate enhanced the degradation of alachlor resulting in dechlorination of acetochlor [100]. Microbial degradation has also been examined Xu and others [101] where they isolated and characterized *Pseudomonas oleovorans* to be a pure bacterial culture capable of degrading acetochlor. They proposed a degradative pathway including processes of dechlorination, N- and C-dealkylation, hydroxylation and dehydrogenation occurring at the acetyl carbon. Results from a study conducted by Feng [102] also suggested that the dechlorination of acetochlor occurs as the acetyl carbon; however, in his work, microbial conjugation at this carbon is due to glutathione.

2.9.3 Butachlor

As with acetochlor and alachlor, butachlor [N-butoxymethyl-2-chloro-2',6'-diethylacetanilide] is also used as a pre-emergence control of annual grasses and broadleaf weeds. Butachlor

is mostly used in Asian, South American, and African countries to control aquatic weeds and seeded rice. Due to the prevalent use of butachlor in other countries, research into its fate and transport in the environment are exceptional in the United States. Although, Yu and coworkers [103] identified a bacterial culture in wheat rhizosphere soil able to degrade butachlor. This herbicide was also found to degrade in both soil and water microbially, and upon addition of decomposed cow manure, microbial activity spontaneously increased enhancing butachlor degradation [104].

2.9.4 Metolachlor

Metolachlor [2-chloro-N-(2-ethyl-6-methylphenyl)-N-(2-methoxy-1-methylethyl)acetamide] was first registered 1976 in the United States. Some of the common names for metolachlor include Bicep®, Dual®, Pennant®, and Milocep® [3]. The use of this herbicide covers a broad spectrum of applications such as corn, soybeans, and sorghum crops pre and post-emergence control of weeds; lawns and turf weed control; ornamental plants, shrubs, trees, vines and fence/hedgerows; and right-of-way weed control [9]. The USGS [81] reported 57.9 million lb/yr of metolachlor treating 31.3 million acres/yr of field and vegetable crops in 1991-1995 and 0.8 million lb/yr to applied to turf, fence/hedgerows and landscaping settings in 1987, 1989 and 1990.

In agricultural areas, metolachlor was one of the two most detected herbicides in both streams and groundwater, exceeding the detection frequency of alachlor [82]. Similar to acetochlor and alachlor, metolachlor's metabolites were also detected. The range of concentrations in 1997 for metolachlor were from 12.3 μg/L to 124.3 μg/L in the Midwestern United States. For metolachlor ESA, concentrations were from 6.36 μg/L to 12.4 μg/L and for metolachlor OXA, 3.83 to 6.37 μg/L [81]. Another study reported 57 μg/L of metolachlor in a Louisiana pond right after a fish kill [105]. Currently, there is no MCL established for metolachlor. However, metolachlor is considered a Group C possible human carcinogen [9].

Metolachlor has been found to degrade chemically and biologically. Satapanajaru and others [106] found zerovalent iron to degrade metolachlor via dechlorination and enhanced

the degradation rates upon the addition of Al, Fe(II) or Fe(III) salts. Furthermore, microbial dechlorination of metolachlor has been detected to a varying extent though no mineralization occurred [90]. Similar to alachlor, cometabolism has been observed for metolachlor in many studies ([107], [108], [109]).

2.9.5 Propachlor

Propachlor [2-chloro-N-(1-methylethyl)-N-phenylacetamide] was registered by the USEPA in 1964 as a pesticide [110]. Some of propachlor's trade names include Ramrod®, Bexton®, Prolex®, Kartex®, and Satecid® [111]. Propachlor is registered to be used as a pre-emergence herbicide on corn, soybeans, grain sorghum, cotton, and peas.

According to the World Health Organization (WHO) [112], the volatility of propachlor mostly occurs on moist soil surfaces with windy conditions. However, the primary mechanism of acylanilide dissipation is microbial degradation [113]. Similar to other chloroacetanilides, propachlor has been shown to degrade in the presence of sulfur species. Zheng and others [114] concluded thiourea is well capable of dechlorinating propachlor via nucleophilic substitution.

2.10 Literature Values of Chloroacetanilide Properties

Literature values for the octanol-water partition coefficient were used in this work to validate the use of the software programs employed. The partition coefficients for alachlor, acetochlor, butachlor, metolachlor, and propachlor were found to be 2.64, 2.48, 3.71, 3.28 and 2.31, respectively ([115], [116], [117], [10]). Furthermore, as will be discussed hereafter, the software program used to compute the descriptor and property values was not able to calculate the solubility for these herbicides. Thus, solubility values from literature were used in the correlation computations. The solubility for alachlor, acetochlor, butachlor, metolachlor, and propachlor were found to be 240, 223, 23, 520, and 580 mg/L [3].

2.11 Summary

Shown previously, this review of part of the current literature in the environmental fields of groundwater quality, subsurface phenomena, chemical and biological transformation, fate and transport processes, and chemical and biological transformation kinetics is evidence of the substantial progress made to help understand the transformation and fate chemicals such as alachlor, acetochlor, butachlor, metolachlor, and propachlor in aerobic environments. Notwithstanding these research efforts, limited literature has focused on elucidating the various biotic and abiotic degradation pathways occurring between these herbicides and bisulfide and nitrate-reducing cultures in anaerobic environments. Furthermore, quantitative structure-activity relationships have not been established for this series of chloroacetanilides in predicting and understanding their chemical and biotransformation under these conditions. Hence, the current literature provides the basis for the objective of this study where a number of significant questions may be answered. Among these are the following: What correlations exist between abiotic/biotic rate constants and structure/property descriptors? What are the most probable degradation products of these chloroacetanilides? What are the best descriptors to be used in developing an algorithm predicting the rate of chemical or biological transformation?

The research described herein can be used a preliminary analysis for a full scale QSAR analysis leading to a stronger understanding of the biotic and abiotic transformations of these herbicides in anaerobic environments. With this information and further research achievements, possible bioremediation techniques can be developed to improve the groundwater quality and moreover, exploit the science of quantitative structure-activity relationships in the field of environmental science and engineering.

Chapter 3

Methods and Materials

3.1 Introduction

Following a brief history of the research project that led to the current effort, this chapter describes the experimental methods employed for this thesis. For more information into the analytical techniques, equipment, and materials used in the earlier work, refer to the literature review of this report.

This study focused on evaluating potential descriptors for quantifying the chemical and biological degradation rates of five chloroacetanilides - alachlor, acetochlor, butachlor, metolachlor, and propachlor - in an anaerobic environment. Previous research endeavors by Qin [12] and Walker [11] reported degradation rates of this herbicide family with bisulfide and under denitrifying conditions, respectively. The effects of molecular structure and property characteristics were investigated via the science of quantitative structure-activity relationships. Such characteristics included Henry's constant, octanol-water partition coefficient, and solubility. In addition to these parameters, other various descriptors were chosen and simple linear regression correlations were established. Descriptors, structure and property, included in the analysis were carbon-chlorine (C-Cl) bond length, molecular weight (MW), carbonyl-carbon (C=O carbon) atomic charge, dipole moment, molar refractivity (MR), Connolly molecular area (CMA), Connolly excluded solvent volume ($CESV$),

carbon-chlorine (C-Cl) bond energy, octanol-water partition coefficient ($\log(K_{ow})$), solubility, and Henry's constant (K_H). The goal of this thesis was to provide a database of descriptors for future research to ultimately perform a full-scale QSAR for chloroacetanilide degradation. The following information gives a brief description and use of the software packages in accomplishing this goal.

3.2 CambridgeSoft® Software: ChemOffice 2006® Experimental Tools

ChemOffice 2006® (CambridgeSoft® , Cambridge, MA) is a robust software suite comprising of two programs, Chemdraw and Chem3D Pro 10.0®. Chemdraw Pro 10.0 was used to construct the 2-dimensional herbicide structures. Chem3D Pro 10.0® was used to import drawings from Chemdraw Pro 10.0® in order to find the most appropriate conformation in three-dimensions. Thermodynamic properties such as Henry's law constant, $\log(K_{ow})$, molar refractivity, and solubility were computed using this tool. Other computed properties included those of electronic and steric influence such as Connolly molecular area, Connolly excluded solvent volume, atomic charge, and molecular weight. The use of these tools in the attempt to elucidate the mechanism of degradation and the rate of degradation is described below.

3.2.1 ChemDraw Pro 10.0®

As mentioned previously, ChemDraw Pro 10.0/tr was used to construct the 2-D structures of alachlor, acetochlor, butachlor, metolachlor, and propachlor. Figures 2.1, 2.2, 2.3, 2.4, and 2.5 in the literature review of this report present these images.

3.2.2 Chem3D Pro 10.0®

The 2-D structure of each herbicide was imported into Chem3D Pro 10.0® where the stereochemical information included in ChemDraw Pro 10.0® structures was manually checked in

the 3-D model. Molecular mechanics were then computed for each chemical in order to optimize the geometry of the compound and find its lowest energy conformation (*i.e.* identify a set of low-energy conformers that are in equilibrium with each other). By performing this calculation, each 3-D drawing was then altered to its most stable configuration[1]. Chem3D Pro 10.0® uses the Eigenvector Following (EF) routine as the default geometry optimization routine for minimization calculations. Each herbicide was run under MM2 calculations.

Computational Properties

After MM2 calculations, thermodynamic, steric, electronic, and hydrophobic parameters were computed for each herbicide. Thermodynamic properties included the following:

- Henry's law constant, K_H (unitless)
- Log(K_{ow})
- Molar refractivity, MR (cm^3/mol)
- Solubility, S (mg/L)

Steric, electronic, atomic and hydrophobic parameters included the following:

- Connolly molecular area, CMA ($Å^2$)
- Connolly excluded solvent volume, $CESV$ ($Å^3$)
- Molecular weight, MW (g/mol)
- Carbonyl carbon charge (Mulliken)

Another property measured in this software program included the carbonyl-carbon atomic charge. All the above values were based on classical mechanics.

After many unsuccessful attempts of computing the solubility for each herbicide in Chem3D Pro 10.0®, an email was sent to the CambridgeSoft inquiring about the zero value output for all chemicals analyzed by the program. Technical support explained that the

[1]ChemOffice Desktop 2006 Manual

reason behind the zero values for solubility was due to a "bug" in the software program. Thus, the correlation of this property to the degradation rates was not possible. However, literature values [3] were used in this part of the analysis, considering all other calculated parameters were close to available literature values, as will be discussed in Chapter 4.

3.3 Gaussian 03® Software Tool

For comparison, Gaussian 03® (Gaussian, Inc., Wallingford, CT) software was used to calculate the bond length, bond energy, and dipole moment using quantum mechanics. Energy calculations and geometry optimization calculations were performed using Gaussian 03®. Specifically, this software is a hybrid density functional method that includes Becke's 3-parameter nonlocal-exchange functional with the correlation functional of Lee-Yang-Parr, B3LYP. The 6-31G all-electron split-valence basis set includes the polarization d-function on non-hydrogen atoms is employed for all calculations. Frequency calculations confirm that the stable geometries have real vibrational frequencies.

3.4 Literature Comparison of Software Computed Properties

Property computations for the octanol-water partition coefficient from Chem3D Pro 10.0® were compared to literature values to test the accuracy of the program. In exception of the solubility as previously discussed, the program was concluded to be accurate. Percent differences will be compared and discussed in Chapter 4.

3.5 Microsoft Excel® Statistical Analysis

After all computations were run in Chem3D Pro 10.0®, the environmental properties were linearly correlated to the k_{HS^-} and k_{bio} rate constants found in previous experiments ([12], [11]) Microsoft Excel®. Descriptors (properties and values calculated by the software) were

then chosen for each fate parameter and were correlated to the degradation rates as well. The following correlations were made:

- k_{HS^-} vs. Solubility, $\ln(k_{HS^-})$ vs. Solubility
- k_{HS^-} vs. Henry's constant, $\ln(k_{HS^-})$ vs. Henry's constant
- k_{HS^-} vs. $\log(K_{ow})$, $\ln(k_{HS^-})$ vs. $\log(K_{ow})$
- k_{bio} vs. Solubility, $\ln(k_{bio})$ vs. Solubility
- k_{bio} vs. Henry's Constant, $\ln(k_{bio})$ vs. Henry's Constant
- k_{bio} vs. $\log(K_{ow})$, $\ln(k_{bio})$ vs. $\log(K_{ow})$

Based on these correlations, additional descriptors were chosen, and the output values for each descriptor for all five chloroacetanilides were correlated to the degradation rates. These plots are as follows:

- k_{HS^-} vs. Molar Refractivity, $\ln(k_{HS^-})$ vs. Molar Refractivity
- k_{HS^-} vs. Connolly Molecular Area, $\ln(k_{HS^-})$ vs. Connolly Molecular Area
- k_{HS^-} vs. Connolly Excluded Solvent volume, $\ln(k_{HS^-})$ vs. Connolly Excluded Solvent Volume
- k_{HS^-} vs. Molecular Weight, $\ln(k_{HS^-})$ vs. Molecular Weight
- k_{HS^-} vs. C-Cl Bond Length, $\ln(k_{HS^-})$ vs. C-Cl Bond Length
- k_{HS^-} vs. C-Cl Bond Energy, $\ln(k_{HS^-})$ vs. C-Cl Bond Energy
- k_{HS^-} vs. C=O Carbon Charge, $\ln(k_{HS^-})$ vs. C=O Carbon Charge
- k_{HS^-} vs. Dipole Moment, $\ln(k_{HS^-})$ vs. Dipole Moment

Each plot for k_{HS^-} and $\ln(k_{HS^-})$ was also constructed for k_{bio} and $\ln(k_{bio})$ with respect to individual descriptors. From these plots, simple linear regression lines were calculated using Microsoft Excel®. Conclusions based on the correlation coefficient, r^2, are discussed

in Chapter 5 as well as the significance of each descriptor in the development of QSARs relating to the transformation rate constants.

Chapter 4

Results and Discussion

4.1 Introduction

This chapter presents and discusses the experimental data results from Qin [12] and Walker [11], along with the descriptor computations from Chem3DPro 10.0® . Moreover, the correlations between the rate constants and descriptors were assessed statistically, followed by a qualitative discussion of the steric, atomic, and electronic effects of the herbicide structures on the relative reactivities of the abiotic and biotic reaction of five chloroacetanilide herbicides (alachlor, acetochlor, butachlor, metolachlor, and propachlor) under anaerobic conditions.

4.2 Bisulfide and Nitrate Reduction Degradation Analyses

As described in the literature review, separate series of experiments were performed previous to the current research to investigate the reaction of a series of chloroacetanilide herbicides with bisulfide [12] and with nitrate-reducing bacteria [11]. Data collected from these reaction studies are shown in the table below (Table 2.1 in the literature review).

As stated previously, the trend among these rates is generally consistent with the notion

Table 4.1: Bisulfide and biological rate reaction constants for chloroacetanilide herbicides.

	k_{HS^-} [a] $(\frac{L}{mg\,HS^- \cdot hr})$	k_{bio} [b] $(\frac{L}{mg\,VSS \cdot hr})$
Alachlor	0.00160	0.00026
Acetochlor	0.00112	0.00051
Butachlor	0.00083	0.00052
Metolachlor	0.00037	0.00027
Propachlor	0.00255	0.00028

[a] Source: Qin [12]

[b] Source: Walker [11]

that the least and most simply, substituted structure (propachlor) reacts fastest while the most heavily substituted (metolachlor) reacts most slowly [12]. Additionally, this qualitative observation that more complex molecules are transformed faster than those with less complicated substituents does not hold entirely. Other chemical structure properties, especially those that affect chemical/enzyme interaction, may also play a role. For biotransformation, the likelihood that access to the chlorine molecule is the dominant structural parameter that controls the reaction rate is low. Therefore, other factors related to the ability of the microorganisms to attack the substituents will likely be more important.

4.3 Chem3D Pro 10.0® Computation Results

This section presents the results computed in Chem3D Pro 10.0® and Gaussian 03®. The results include the property calculations of thermodynamic, electronic, atomic, and steric parameters as well as the more structural parameters, such as bond lengths and atomic charges of specific atoms in the herbicide structures.

4.3.1 Molecular Mechanics

After importing each herbicide into Chem3D Pro 10.0® from ChemDraw 10.0®, molecular mechanics 2 (MM2) calculations were run to minimize the energy of the compound and determine its most stable conformation through calculating molecular energies. Factors

the software consider include bond bending, van der Waals interactions, bond stretching/compression, torsional strain, and electrostatic interactions. The minimum energy configuration was determined with water as the assumed solvent. As an example of the minimum energy configuration calculation, Figure 4.1 depicts the difference of butachlor before molecular mechanics were applied to this herbicide.

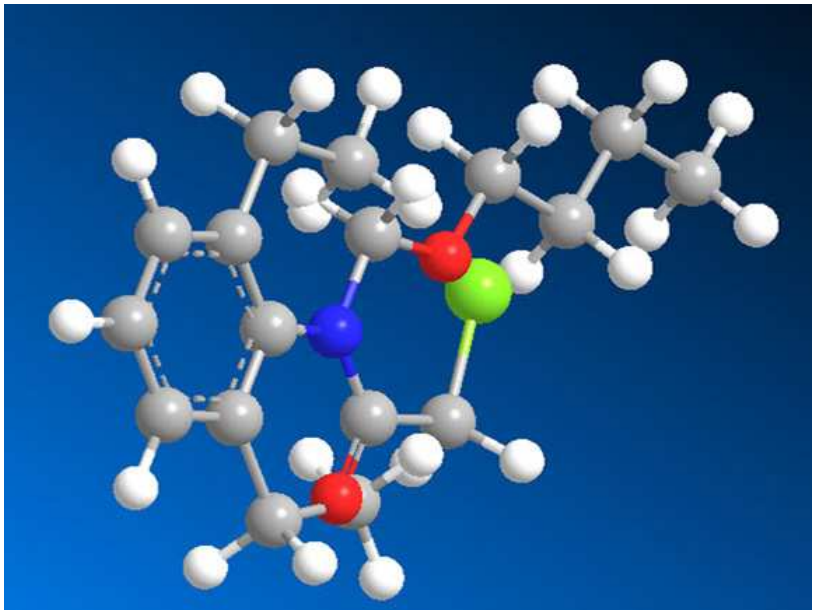


Figure 4.1: Butachlor before energy minimization.

In Figure 4.1 above, the carbonyl oxygen binds to an alkyl carbon in the ortho position, and the hydrogen atoms from the ortho alkyl substituents bond to the hydrogen atom of an ether carbon before minimization. This configuration renders the structure inaccurate.

Figure 4.2 shows butachlor after energy minimization via molecular mechanics calculations. After MM2, the carbonyl oxygen and hydrogen atoms are no longer bonded to other atoms in the molecule, and its structure is now a 3-dimensional representation of the butachlor molecule.

In Figures 4.1 and 4.2, the chlorine atom is lime green, oxygen atoms are red, lone pair of electrons are pink, the nitrogen atom is blue, carbon atoms are gray, and hydrogen atoms are white.

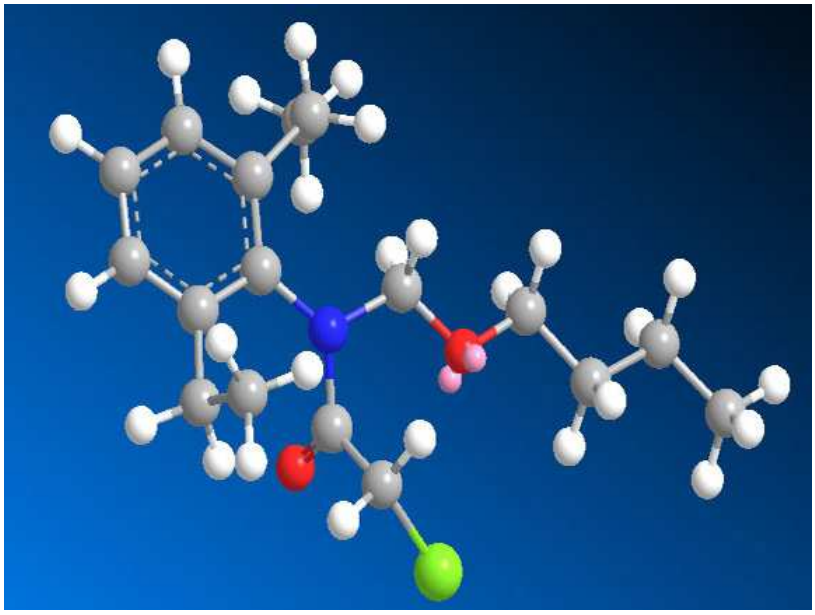


Figure 4.2: Butachlor after energy minimization.

4.3.2 Property Computations

Properties computed in Chem3D Pro 10.0® include $\log(K_{ow})$, K_H, molar refractivity, Connolly molecular area (CMA), Connolly excluded solvent volume ($CESV$), molecular weight (MW), and carbonyl-carbon atomic charge. Properties computed in Gaussian 03® include dipole moment, bond length, and bond energy.

The validity of the program was checked based upon its comparison of its calculated value to literature values of the octanol-water partition coefficient. The percent differences are shown in Table 4.2. Because the percent difference is low, the calculations for all other properties are assumed to be accurate and valid. Table 4.3 shows the thermodynamic properties computed in Chem3D Pro 10.0® along with the literature values for solubility. Tables 4.4 and 4.5 show the electronic, steric, and atomic properties of each chloroacetanilide.

Table 4.2: Percent difference between literature value and Chem3DPro 10.0® computations for K_{ow}.

	Alachor	Acetochlor	Butachlor	Metolachlor	Propachlor
K_{ow}	2.35	2.27	3.63	3.31	2.49
Literature value	2.64[a]	2.48[b]	3.7[c]	3.28[a]	2.3[d]
Percent Difference	11.6%	8.8%	1.9%	0.9%	7.9%

[a]Source: AWRA [115]
[b]Source: Walker [116]
[c]Source: Lu *et al.* [117]
[d]Source: EPA [10]

Table 4.3: Thermodynamic properties of chloroacetanilide herbicides.

	$\log(K_{ow})^a$	${K_H}^a$	Solubility[b]	MR[a]
units	*unitless*	*unitless*	(mg/L)	$(cm^3/mole)$
Alachlor	2.35	8.308	240	73.93
Acetochlor	2.27	8.308	223	74.07
Butachlor	3.63	7.939	23	87.8
Metolachlor	3.31	8.185	520	78.94
Propachlor	2.49	6.462	580	57.96

[a]Source: Chem3D Pro 10.0®
[b]Source: WSSA [3]

Table 4.4: Chem3D Pro 10.0® steric and atomic properties of chloroacetanilide herbicides.

	CMA	CESV	MW	C=O Carbon Charge
	$(\mathring{A}^2)$	$(\mathring{A}^3)$	$(g/mole)$	$(Mulliken)$
Alachlor	223.5	194.539	269.7671	0.532
Acetochlor	227.156	195.461	269.7671	0.494
Butachlor	263.768	229.504	311.84684	0.483
Metolachlor	229.513	212.621	283.79368	0.464
Propachlor	179.665	152.858	211.68796	0.542

Table 4.5: Gaussian 03® electronic and steric properties of chloroacetanilide herbicides.

	Dipole Moment ($Debye$)	C-Cl Bond Length ($\mathring{A}$)	C-Cl Bond Energy ($kJ/mole$)
Alachlor	2.9785	1.8956	300.39
Acetochlor	3.7384	1.89396	345.52
Butachlor	5.371	1.89496	355.51
Metolachlor	4.9036	1.89584	355.78
Propachlor	4.8607	1.89838	399.09

4.4 Statistical Analysis Discussion

Shown in Table 4.6 are the correlation values between the thermodynamic descriptors and bisulfide and nitrate reducing rate constants and the natural log of those values. Table 4.7 shows the correlations between steric, electronic and atomic descriptors and the rate constants.

Table 4.6: Correlations values between thermodynamic properties and rate constants.

	K_H	Solubility	$\log(K_{ow})$	Molar Refractivity
$units$	$unitless$	(mg/L)	$unitless$	$(cm^3/mole)$
k_{HS}-	0.61	0.12	0.39	0.74
$\ln(k_{HS}$-)	0.37	0.00	0.47	0.52
k_{bio}	0.08	0.56	0.05	0.27
$\ln(k_{bio})$	0.07	0.54	0.05	0.26

Table 4.7: Correlations values between atomic, electronic, and steric descriptors and rate constants.

	C-Cl Bond Length	C-Cl Bond Energy	C=O Carbon Charge	Dipole Moment	CESV	CMA	MW
$units$	$\mathring{A}$	$(kJ/mole)$	$(Mulliken)$	$(Debye)$	$(\mathring{A}^3)$	$(\mathring{A}^2)$	$(g/mole)$
k_{HS}-	0.50	0.11	0.91	0.04	0.81	0.64	0.75
$\ln(k_{HS}$-)	0.22	0.02	0.91	0.12	0.60	0.39	0.50
k_{bio}	0.46	0.00	0.16	0.05	0.23	0.38	0.27
$\ln(k_{bio})$	0.44	0.00	0.16	0.05	0.22	0.36	0.25

Correlation coefficients, r^2, measure the degree of linearity between two variables. If there is a perfect linear relationship, r^2 will be equal to $\pm$ 1. For this study, r^2 values ≤ 0.40 were considered to be of 'low significance' correlations, and r^2 values > 0.40 were considered to be of 'high significance' correlations. Found in Appendix A are the computed descriptor values for each herbicide and the correlation coefficients determined between each pair of variables (rate constants vs. descriptors).

4.4.1 Thermodynamic Property-Kinetics Correlations

From the calculations performed in both software programs, Table 4.6 shows the correlation values for the k_{bio} and k_{HS^-} rate constants and the thermodynamic properties.

From Table 4.6, correlations for the biological rate constants and thermodynamic properties were poor. These low correlations are likely be due to the degradation independency of these parameters. More specifically, the substrate-specificity of microorganisms could be the dominating factor in the degradation of these herbicides, and the thermodynamic properties will likely not reflect those effects. Furthermore, $\ln(k_{bio})$ was used to make energetic correlations as well. Noticeably, the change in correlations between k_{bio} and $\ln(k_{bio})$ regressions were very minimal. Therefore, there must be other descriptors relating to the degradation rate constants based upon substrate-enzymatic biochemical properties of the herbicide compounds rather than on its thermodynamic and energy properties. Another possibility for these small differences is the minimal change in rates such that ln(1-x) is approximately - ln(x). Furthermore, the reaction mechanism has not been established for this denitrifying bacterial culture which limits the examination of the environmental effects on the rate of intermediate formation and breakdown. The absence of bacterial identity also limits the ability to understand the type of reaction catalyzed. Thermodynamic properties could be more useful if metabolites and their rates in this study were identified and measured. Another possible reason for the low correlations could be that there are relatively small differences in the biological rate constants leaving minimal variation to occur for the independent variable (*i.e.* the structural parameter). This, too, would indicate that the substrate-enzyme interaction is relatively unaffected by these parameters.

In contrast, the correlations for the bisulfide reaction were higher, and the behavior of the correlations for the biological reactions can assist in understanding this reaction. Each of the key calculated parameters will be discussed below in terms of its correlation with k_{HS^-}.

Solubility Correlation

The correlations for the abiotic rate constants were higher than those of the nitrate-reduction rates, albeit the solubility correlation was much lower. Possible reasons behind this low correlation could be due to the descriptors for solubility - size and polarity. If one descriptor is not correlated to the rate constants, the overall correlation for solubility with respect to the degradation rate constant will also be low. These descriptors will be discussed in the electronic, steric, and atomic correlation analysis.

In regards to the biological rate constant, the solubility correlation is much greater than the correlation with the bisulfide rate constant. The r^2 value between k_{bio} and solubility was 0.56, and the r^2 value between k_{HS^-} and solubility was zero.

Henry's Constant Correlation

Similar to the solubility correlation, the Henry's constant did not relate to the biological rate constants well. A possible reason could be that despite the amount of herbicide in the aqueous phase, substrate specificity depends on the steric and topological properties of the herbicides. Stated previously, Henry's constant and solubility are highly related. Hence, suggestions made regarding the understanding of the biological rate constant and solubility correlations can also be applied to the Henry's constant.

For the bisulfide reaction, the correlation to Henry's constant was surprisingly higher than its correlation to solubility. Again, Henry's constant is dependent on the vapor pressure of the pure compound and solubility of the herbicide. Thus, since the solubility correlation for k_{HS^-} was low, the correlation with Henry's constant was expected to behave similarly.

Log(K_{ow}) Correlation

The correlation of log(K_{ow}) with the biological rate constants was approximately zero. This relationship indicates that the rate of biodegradation is not strongly dependent on the ratio of the hydrophobicity to hydropholicity of the herbicides. This relationship was expected since partition coefficients for this group of congeneric compounds vary more than the miniscule changes in the biodegradation rates. Similar to Henry's constant, the octanol-water partition coefficient is dependent on the solubility. Thus, one of the descriptors for the partition coefficient could correlate poorly to the rate constant, as well as a descriptor, for the solubility relating poorly to the rate constant. Consequently, the overall effect creates a very unsuccessful correlation.

Similar reasons can be applied to the low correlation for the bisulfide rate constant. However, the correlation might imply that solubility could be a possible descriptor, though not a heavily weighted one, for the degradation rate.

Molar Refractivity

The correlation made between the molar refractivity and the biological rate constant was low with an r^2 of 0.27. Since molar refractivity represents the size and polarity of a compound, the denitrifying enzymes may not be able to fit around the substrate due to steric hindrance of substrate geometry which may explain the low r^2 value. Thus, k_{bio} may not be a function of size and/or polarity.

In regards to the bisulfide rate constant, the correlation was much higher. Figure 4.6 shows the linear regression for this descriptor. This relationship may imply that the size and polarity play an important role in the reaction of these herbicides with bisulfide in anaerobic environments. Furthermore, by inspection, the degradation rate increases as the molar refractivity decreases. Studies described in the literature review have shown that these herbicides dechlorinate under anaerobic conditions with bisulfide. Therefore, this trend may suggest that as the chlorine becomes less hindered by alkyl substituents (size descreases), the degradation rate increases.

4.4.2 Steric-, Electronic- and Atomic-Kinetics Correlations

From the calculations performed in both software programs, Table 4.7 shows the correlation values (r^2) between the rate constant (k_{HS^-} and k_{bio}) and the electronic, atomic, and steric properties.

As shown in Table 4.7, the electronic, atomic, and steric correlation coefficients for the biological rate constants were considerable low, similar to the thermodynamics correlation coefficients found in Table 4.6. In addition to the lack of biochemical information on the denitrifying bacterial culture, substrate-specificity, and reaction mechanisms, the relation of these electronic, atomic, and steric effects is difficult to delineate. Furthermore, the metabolites under these conditions were not identified over the duration of this research, nor in other literature. Therefore, suggestions about the rate constant-descriptor relationship would be speculation. There is not compelling evidence demonstrating the dependency of the biological rate constants on these descriptors. Again, the relatively small difference between all the k_{bio} values indicates some major mechanism that may be uninfluenced by these parameters.

These electronic, atomic and steric descriptors were chosen on the basis of other research efforts proposing a mechanism between bisulfide and these chloroacetanilides. Resultantly, the discussion hereafter primarily pertains to the correlations determined in this thesis work for the bisulfide reaction.

Steric Descriptors

Topological/steric descriptors include the Connolly excluded solvent volume (ESV), the Connolly molecular area (MA), and molecular weight (MW). Their linear correlation coefficients with k_{HS^-} were 0.81, 0.64, and 0.75, respectively. Figures 4.3, 4.4, and 4.5 show the plots and regression correlations for each topological/steric descriptors.

Since the solvent excluded volume is computed from the molecular area, the behavior of these plots (CESV and CMA) was expected to be similar. The correlation coefficients,

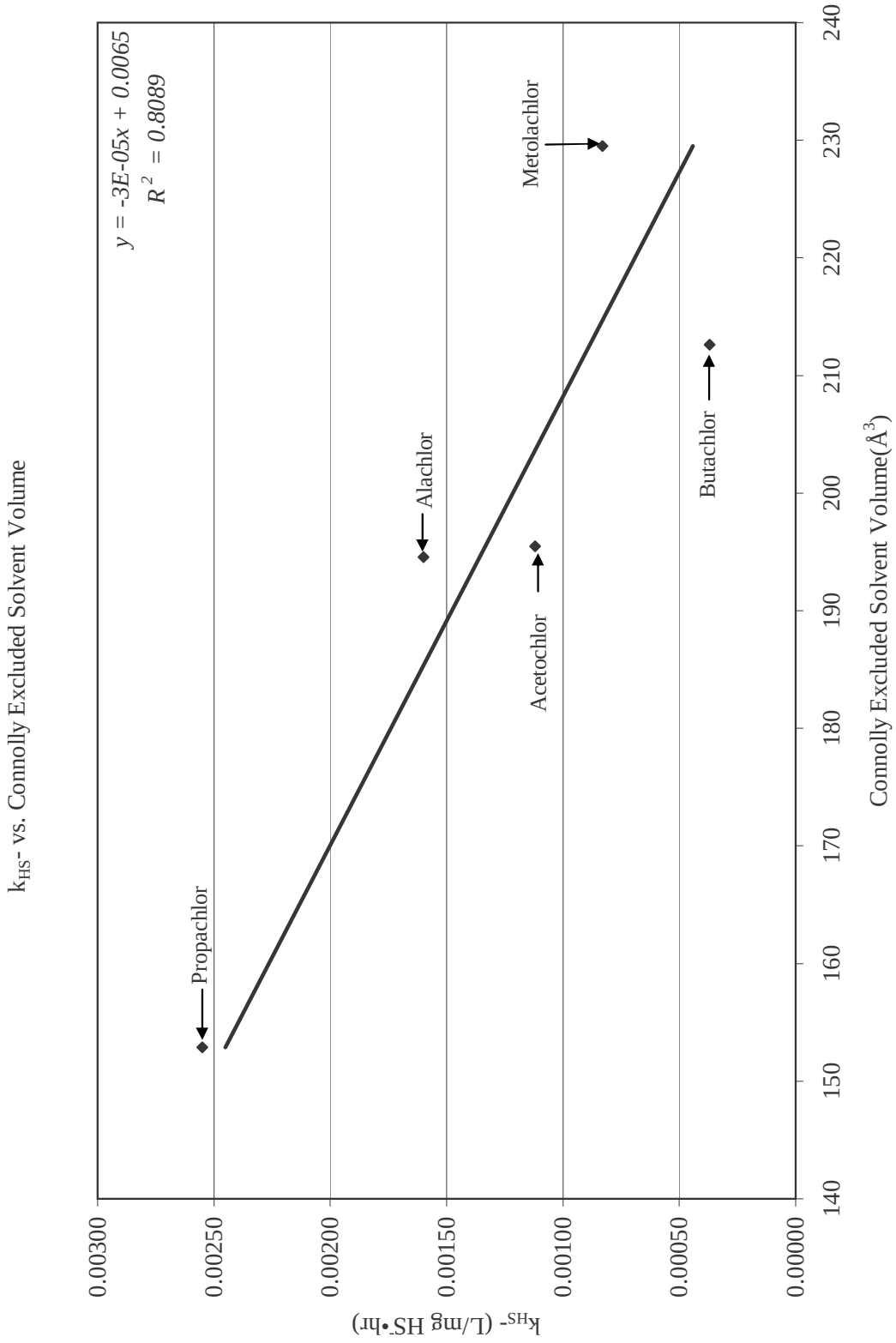


Figure 4.3: k_{HS^-} and Connolly excluded solvent volume correlation plot.

Figure 4.4: k_{HS^-} and Connolly molecular area correlation plot.

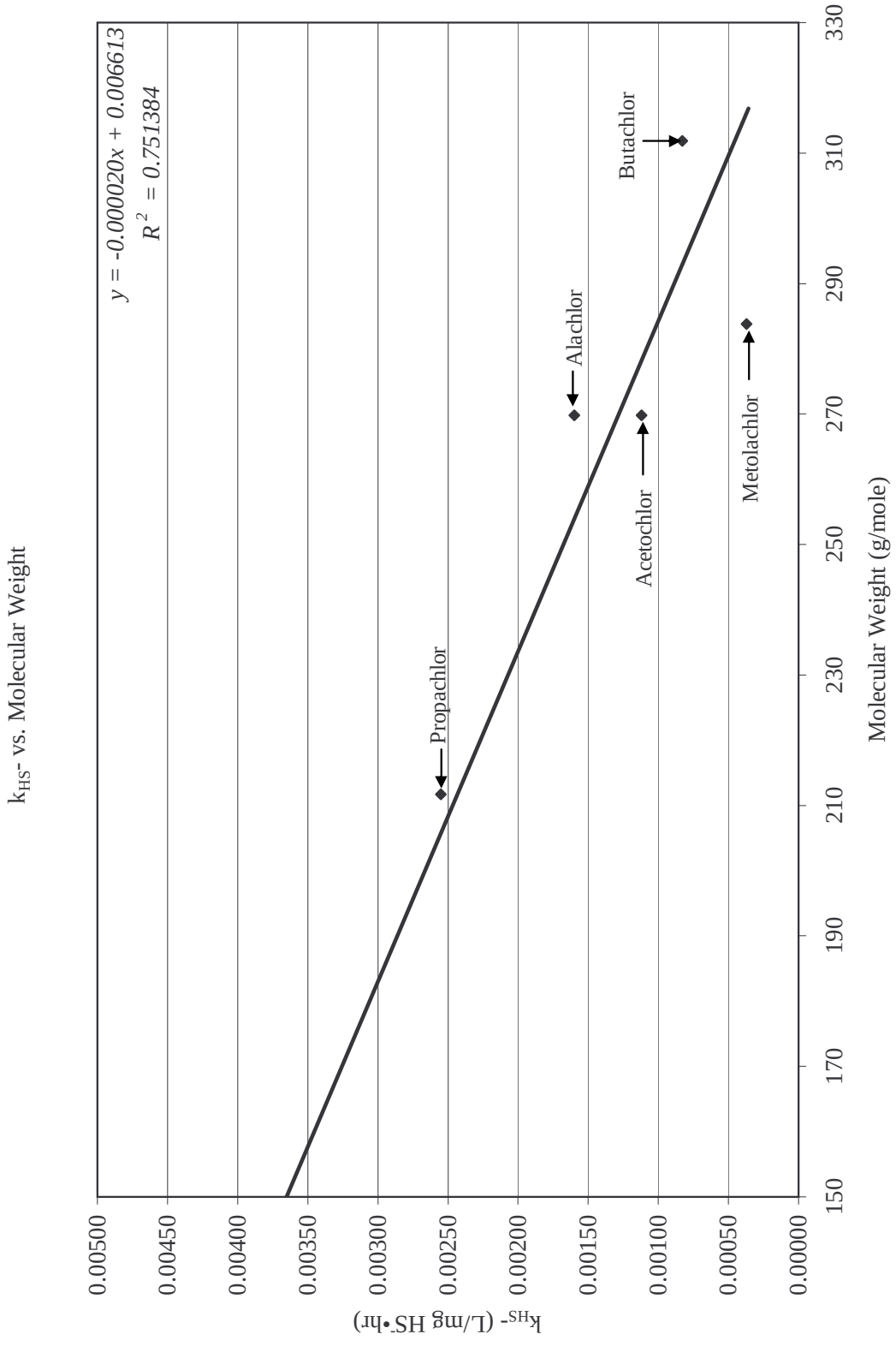


Figure 4.5: k_{HS^-} and molecular weight correlation plot.

however, were not as comparable as expected. This difference could be the result of the long alkyl butyl side chain on butachlor from the ether carbon, increasing the surface area in comparison to other herbicides whose ether alkyl chain is comprised of a methyl or ethyl group. Though there is more surface area for the butachlor side chain, the relative volume added, because of this increase in area, did not tremendously affect the solvent excluded volume correlation. These correlations from Figures 4.3, 4.4, and 4.5 suggest that the degradation rate is dependent upon the size and shape of the compound as an entity and the shape near the chlorine substituent. This latter variable will be discussed in the next section.

Atomic and Electronic Descriptors

As shown in Table 4.7, the bond energy and bond length of the C-Cl bond did not correlate well with k_{HS^-}. This low relationship shows that the dechlorination of these herbicides is not exceptionally dependent on the strength or energy of the bond, but more likely the carbonyl carbon. In particular, if these herbicides undergo an S_N2 nucleophilic substitution, the carbonyl carbon charge would more likely influence the rate at which these herbicides dechlorinate since it would delocalize the negative charge of the carbon center during the transition state [118]. Therefore, the carbonyl charge was computed and correlated to the degradation rate.

From Table 4.7, the linear correlation shows that the carbonyl carbon charge is strongly related to the rate of degradation with bisulfide. As the oxygen atom inductively pulls the electrons away from the carbon, the electron density decreases about the carbonyl carbon. This decrease in electron density causes the carbonyl carbon to act more as an electrophile. Thus, the nucleophile, bisulfide, could undergo an S_N2 substitution at the carbon.

The last electronic, steric and atomic descriptor tested was the dipole moment to describe the polarizability of each herbicide. Recall, the solubility, Henry's constant, and octanol-water partition-coefficient were dependent on the polarizability. However, the correlation of this descriptor to the bisulfide rate constant was approximately zero. This value could suggest that the environmental fate parameters would be heavily dependent on size and shape instead of the polarizability of the compound.

4.5 Summary of QSAR Descriptor Statistical Analysis

All properties computed in Chem3D Pro 10.0® for alachlor, acetochlor, butachlor, metolachlor, and propachlor are shown in Tables 4.3 and 4.4. Properties computed in Gaussian 03® can be found in Table 4.5. Furthermore, correlation values computed in Microsoft Excel® are shown in Tables 4.6 and 4.7.

Described by Qin [12], the second order rate constants for alachlor, acetochlor, butachlor, metolachlor, and propachlor were 0.00160, 0.00112, 0.00083, 0.00037, and 0.00255 ($\frac{L}{mg\,HS^{-}\cdot hr}$), respectively. Thus, in order of decreasing rate transformation for abiotic degradation for the five chloroacetanilide herbicides with bisulfide is as follows: propachlor > alachlor > acetochlor > butachlor > metolachlor. The order of these abiotic rate constants may be attributed to the differences in molecular structure. The rate constant for alachlor is less than that of propachlor possibly due to the steric hindrances of the alkyl substituents attached to alachlor's benzene ring. Such hindrance may inhibit a nucleophilic attack on the acetyl carbon or the carbonyl carbon. Though this same assessment does not hold for the remaining herbicides, the size and shape of these herbicides is believed to contribute to the order of the abiotic rate constants, as will be discussed below.

For the nitrate-reducing second order rate constants, Walker [11] described that the most heavily substituted herbicide reacts faster than those with less complex substituents. He concluded that for biological transformation, access to the chlorine molecule is less likely to be the dominant structural parameter controlling the rate of reaction. Instead, factors influencing the microorganisms' ability to attack substituted branches would be more significant.

Thermodynamic properties computed in Chem3D Pro 10.0® include solubility, Henry's constant, octanol-water partition coefficient, and molar refractivity. Correlations between these properties and k_{bio} were low ($r^2 \leq 0.40$) except the correlation with solubility. The correlation value of k_{bio} and solubility was determined to be 0.56, which was considered a high correlation ($r^2 > 0.40$). The correlations between k_{bio} and C-Cl bond energy, C=O carbon charge, dipole moment, Connolly excluded solvent volume, Connolly molecular area, and molecular weight were also considered low correlations. The r^2 for the C-Cl bond length was higher with a value of 0.44. The most strongly correlated descriptor was the solubility descriptor with an r^2 value of 0.56.

The opposite results occured with the correlations between k_{HS^-} and the thermodynamic properties. These r^2 values were higher than those with k_{bio} except the correlation with solubility, which yielded an r^2 value < 0.20. For the atomic, electronic and steric

properties, high correlations with k_{HS^-} occurred with the C-Cl bond length, C=O carbon charge, Connolly excluded solvent volume, Connolly molecular area, and molecular weight. Low correlations for the k_{HS^-} included the C-Cl bond energy and dipole moment. The most strongly correlated descriptor was the C=O charge with an r^2 value of 0.91.

Overall, this study has revealed potential descriptors to be used in a full-scale QSAR analysis. More research is needed along with this effort to establish the degradation products and pathways of the bisulfide and nitrate-reduction reactions to better understand the influence of descriptors on the activity of these herbicides.

Chapter 5

Conclusions and Future Work

5.1 Conclusions

The research study described herein provided a preliminary analysis that can be used for a full scale quantitative structure-activity relationships study for chloroacetanilides - alachlor, acetochlor, butachor, metolachlor, and propachlor. The main focus of this research effort included the following:

1. Correlations between thermodynamic properties (Henry's constant, solubility, molar refractivity, and octanol-water partition coefficient) and chemical/biological anaerobic transformation rate constants

2. Correlations between steric/electronic/atomic properties (Connolly molecular area, Connolly excluded solvent volume, dipole moment, carbon-chlorine bond length and energy, molecular weight, and carbonyl carbon atomic charge) and chemical/biological anaerobic transformation rate constants

After a review of the results of this research, a number of conclusions can be drawn. These are listed below.

- Structure of the series of chloroacetanilide herbicides can be drawn in ChemDraw Pro 10.0® and imported into Chem3D Pro 10.0®, which successfully predicted their octanol-water partition coefficients ($\log(K_{ow})$ with respect to published values.

- For the reaction with bisulfide, the following descriptors have predictive value for the transformation rate:
  - Carbon-chlorine bond length, $r^2 = 0.50$
  - Carbonyl carbon charge, $r^2 = 0.91$
  - Connolly excluded solvent volume, $r^2 = 0.81$
  - Connolly molecular area, $r^2 = 0.64$
  - Molecular weight, $r^2 = 0.75$
  - Molar refractivity, $r^2 = 0.74$
  - Henry's constant, $r^2 = 0.61$
  - Octanol-water partition coefficient, $r^2 = 0.47$
- The most strongly correlated descriptor for the bisulfide reaction was the carbonyl carbon charge, which suggests a nucleophilic attack on the carbonyl carbon could be one of the major degradation pathways.
- For the nitrate-reducing biodegradation rates, the descriptors were less correlated than those in comparison to the bisulfide rate constants.
- The descriptos with the most predictive value for the nitrate-reducting rate constants include the following:
  - Solubiliy, $r^2 = 0.56$
  - Carbon-chlorine bond length, $r^2 = 0.46$
- The most strongly correlated descriptor was the carbon-chlorine bond length, which suggests dechlorination could be one of the major degradation pathways using this group of denitrifiers.

5.2 Recommendations for Further Research

Based on the current literature for quantitative structure-activity relationships on chloroacetanilides as well as the results obtained from this study, the following partial list of recommended studies should be addrssed.

- Additional chloroacetanilides should be tested and included for full-scale QSAR analysis. Other herbicides from this family include, but are not limited to, butenachlor, delachlor, diethatyl, dimethachlor, metazachlor, pretilachlor, propisochlor, prynachlor, terbuchlor, thenylchlor, and xylachlor.

- Laboratory experiments to measure solubility, Henry's constant, octanol-water partition coefficient, and other measurable descriptors should be performed to compare results computed from software programs and to validate the developed QSAR algorithm.

- Statistical analyses should be performed on fragments of each herbicide especially to better understand enzyme-substrate interactions and abiotic mechanisms.

- Gas chromatograph - mass spectrometry methods should be employed to identify degradates for a specific pathway and determine rate of reactions for these pathways.

- Denitrifying bacterial culture should be isolated and characterized to exploit enzyme-substrate binding and kinetics.

- Other QSARs should be tested. These relationships include structure-property, property-property and property-activity.

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Appendix A

Rate Constants, Descriptor Values, Correlation Plots

A.1 Bisulfide and Nitrate-reducing Rate Constants

The following data include the abiotic and biotic rate constants ([12] and [11]).

Table A.1: Bisulfide and biological rate reaction constants for chloroacetanilide herbicides

	k_{HS^-} [a] $(\frac{L}{mg\,HS^- \cdot hr})$	k_{bio} [b] $(\frac{L}{mg\,VSS \cdot hr})$	$\ln(k_{HS^-})$ $(\frac{L}{mg\,HS^- \cdot hr})$	$\ln(k_{bio})$ $(\frac{L}{mg\,VSS \cdot hr})$
Alachlor	0.00160	0.00026	-6.43	-8.25
Acetochlor	0.00112	0.00051	-6.79	-7.58
Butachlor	0.00083	0.00052	-7.09	-7.56
Metolachlor	0.00037	0.00027	-7.90	-8.22
Propachlor	0.00255	0.00028	-5.97	-8.18

[a] Source: Qin [12]

[b] Source: Walker [11]

A.2 Descriptor Values

The following section includes descriptor values for the environmental fate parameters, thermodynamic, atomic, steric, and electronic properties selected for the preliminary QSAR analysis.

Table A.2: Descriptor Values

	C-Cl Bond Energy[a] $(kJ/mole)$	C=O Carbon Charge[b] $(Mulliken)$	$\log(K_{ow})$[b] $unitless$	${K_H}^b$ $unitless$	Solubility (mg/L)
Alachlor	300.39	0.532	2.35	8.308	240
Acetochlor	345.52	0.494	2.27	8.308	223
Butachlor	355.51	0.483	3.63	7.939	23
Metolachlor	355.78	0.464	3.31	8.185	520
Propachlor	399.09	0.542	2.49	6.462	580

[a]Gaussian 03®

[b]Chem3D Pro 10.0®

Table A.3: Descriptor Values

	CMA[a] $(\mathring{A}^2)$	CESV[a] $(\mathring{A}^3)$	Molecular Weight[a] $(g/mole)$	Dipole Moment[b] $(Debye)$	C-Cl Bond Length[b] (Å)
Alachlor	223.5	194.539	269.7671	2.9785	1.8956
Acetochlor	227.156	195.461	269.7671	3.7384	1.89396
Butachlor	263.768	229.504	311.84684	5.371	1.89496
Metolachlor	229.513	212.621	283.79368	4.9036	1.89584
Propachlor	179.665	152.858	211.68796	4.8607	1.89838

[a]Gaussian 03®

[b]Chem3D Pro 10.0®

A.3 Correlation Values between Descriptors and Rate Constants

The tables below present the correlation values, R^2, for the biotic/abiotic rate constants and descriptors.

Table A.4: Correlations values between thermodynamic properties and rate constants

	K_H	Solubility	$\log(K_{ow})$	Molar Refractivity
units	*unitless*	(mg/L)	*unitless*	$(cm^3/mole)$
k_{HS}-	0.61	0.12	0.39	0.74
$\ln(k_{HS}$-)	0.37	0.00	0.47	0.52
k_{bio}	0.08	0.56	0.05	0.27
$\ln(k_{bio})$	0.07	0.54	0.05	0.26

Table A.5: Correlations values between atomic, electronic and steric descriptors and rate constants

	C-Cl Bond Length	C-Cl Bond Energy	C=O Carbon Charge	Dipole Moment	CESV	CMA	MW
units	$\mathring{A}$	$(kJ/mole)$	$(Mulliken)$	$(Debye)$	$(\mathring{A}^3)$	$(\mathring{A}^2)$	$(g/mole)$
k_{HS}-	0.50	0.11	0.91	0.04	0.81	0.64	0.75
$\ln(k_{HS}$-)	0.22	0.02	0.91	0.12	0.60	0.39	0.50
k_{bio}	0.46	0.00	0.16	0.05	0.23	0.38	0.27
$\ln(k_{bio})$	0.44	0.00	0.16	0.05	0.22	0.36	0.25

A.4 Correlation Plots

Figure A.1: k_{HS^-} and $\ln(K_{ow})$ correlation plot.

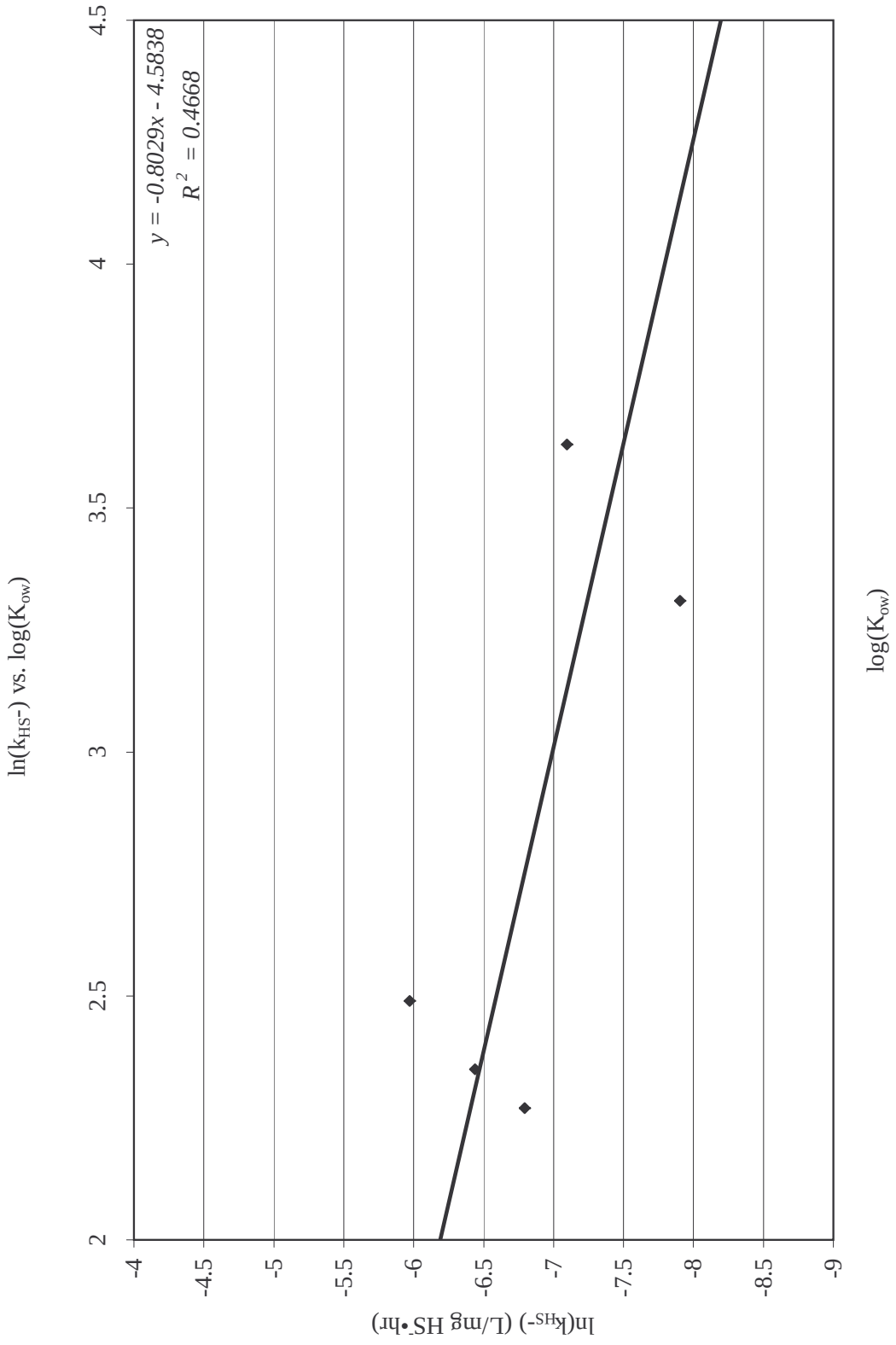

Figure A.2: $\ln(k_{HS^-})$ and $\ln(K_{ow})$ correlation plot.

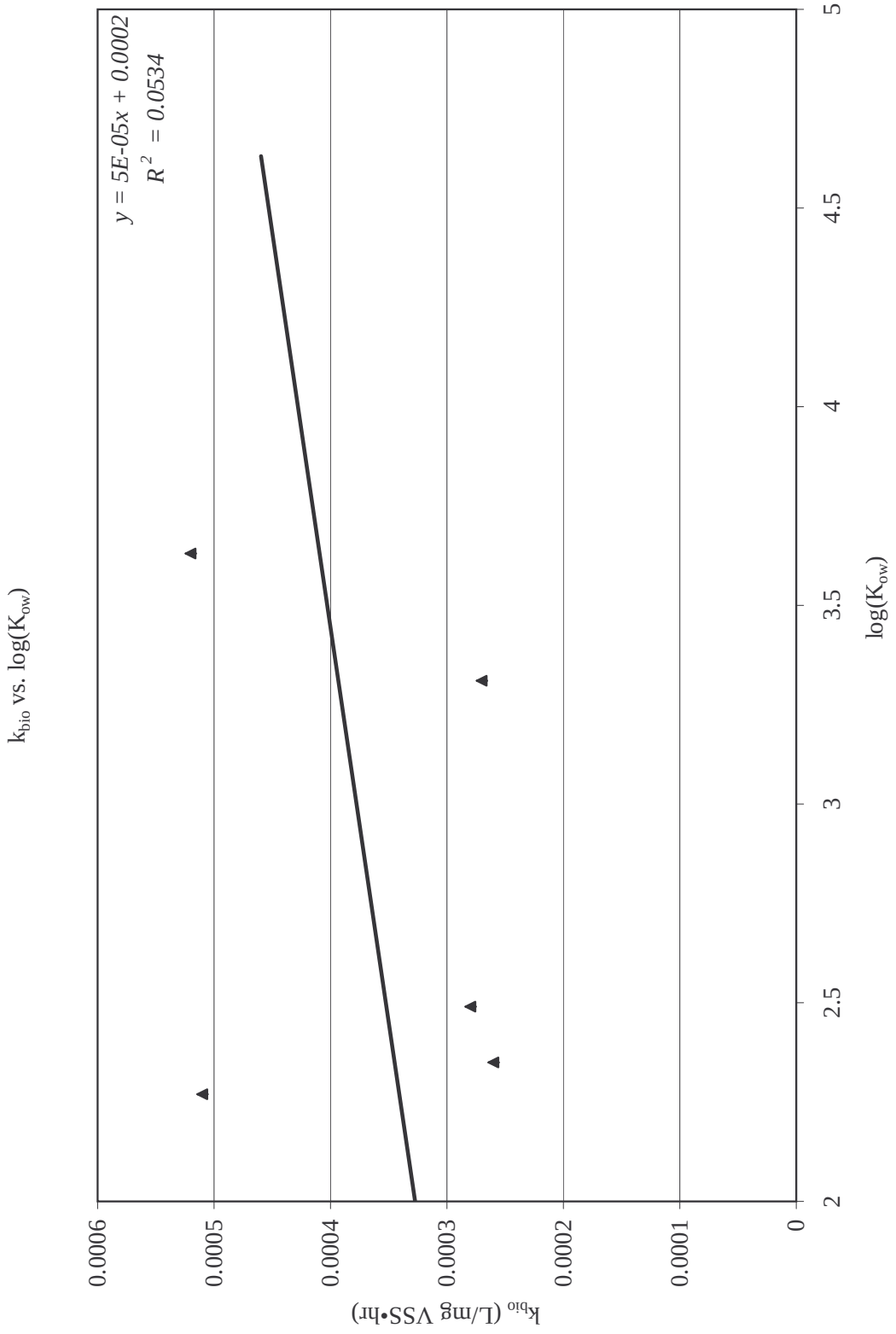

Figure A.3: k_{bio} and $\ln(K_{ow})$ correlation plot.

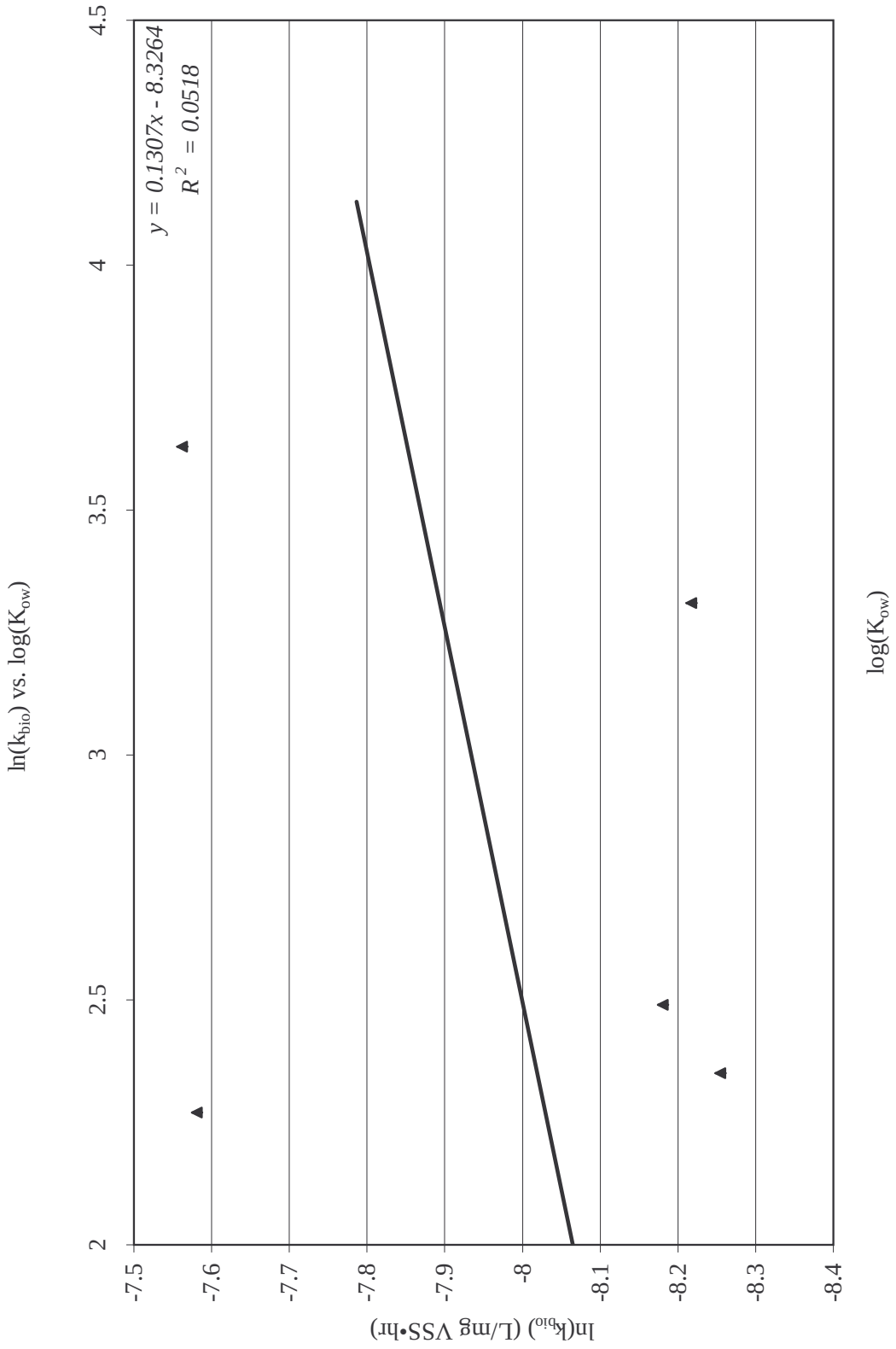

Figure A.4: $\ln(k_{bio})$ and $\ln(K_{ow})$ correlation plot.

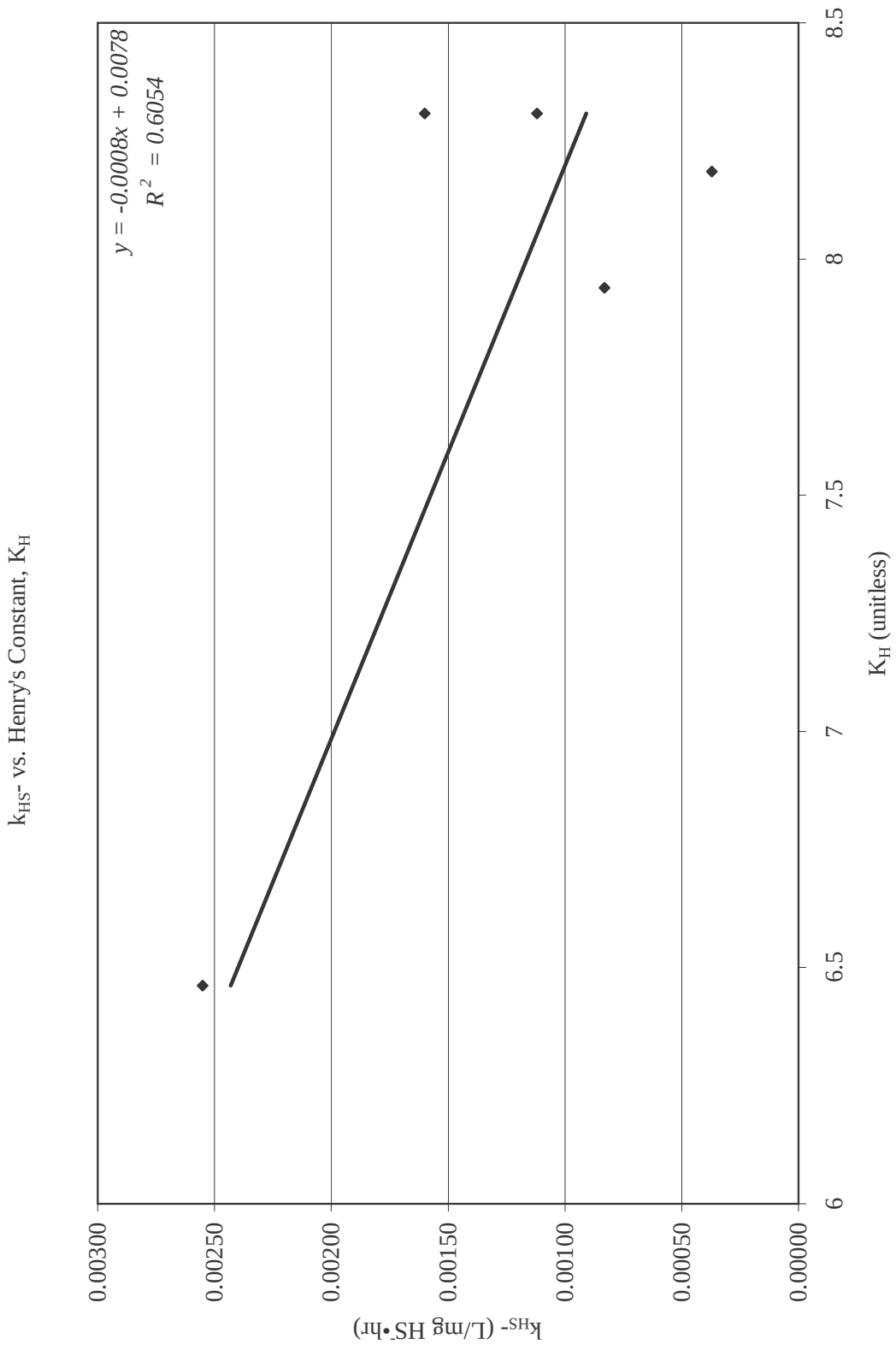

Figure A.5: k_{HS^-} and K_H correlation plot.

Figure A.6: $\ln(k_{HS^-})$ and K_H correlation plot.

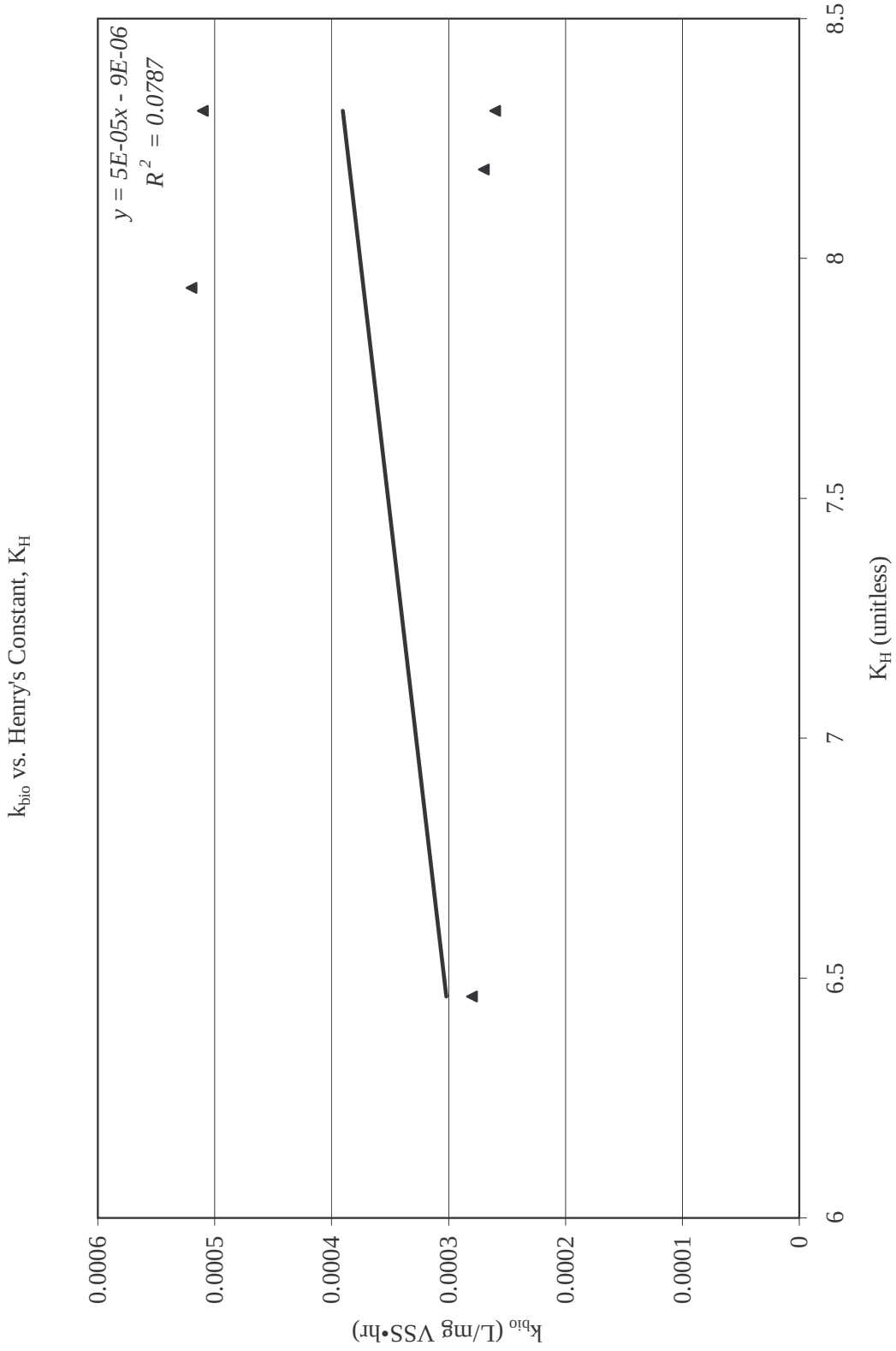

Figure A.7: k_{bio} and K_H correlation plot.

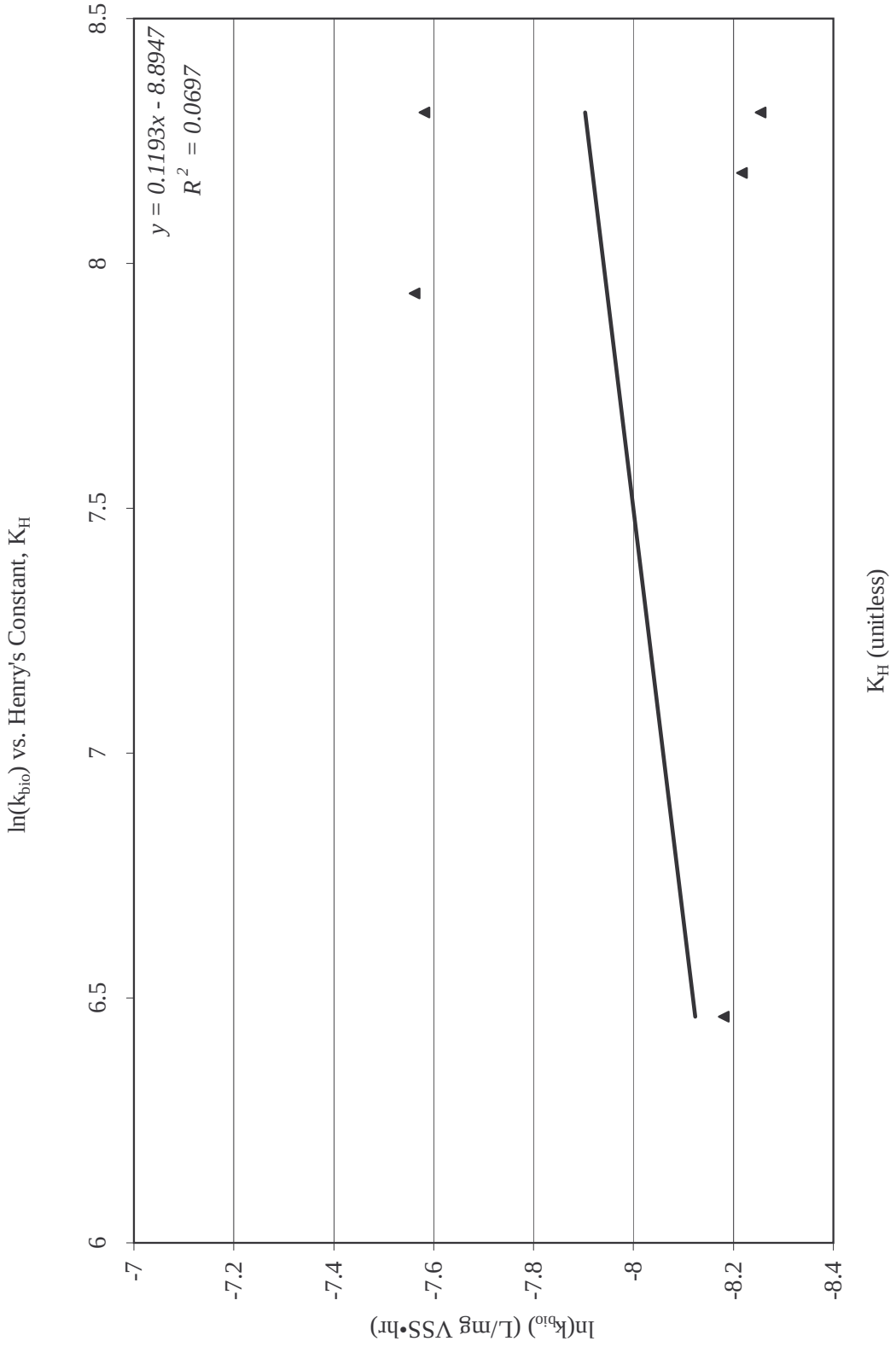

Figure A.8: $\ln(k_{bio})$ and K_H correlation plot.

Figure A.9: k_{HS^-} and solubility correlation plot.

Figure A.10: $\ln(k_{HS^-})$ and solubility correlation plot.

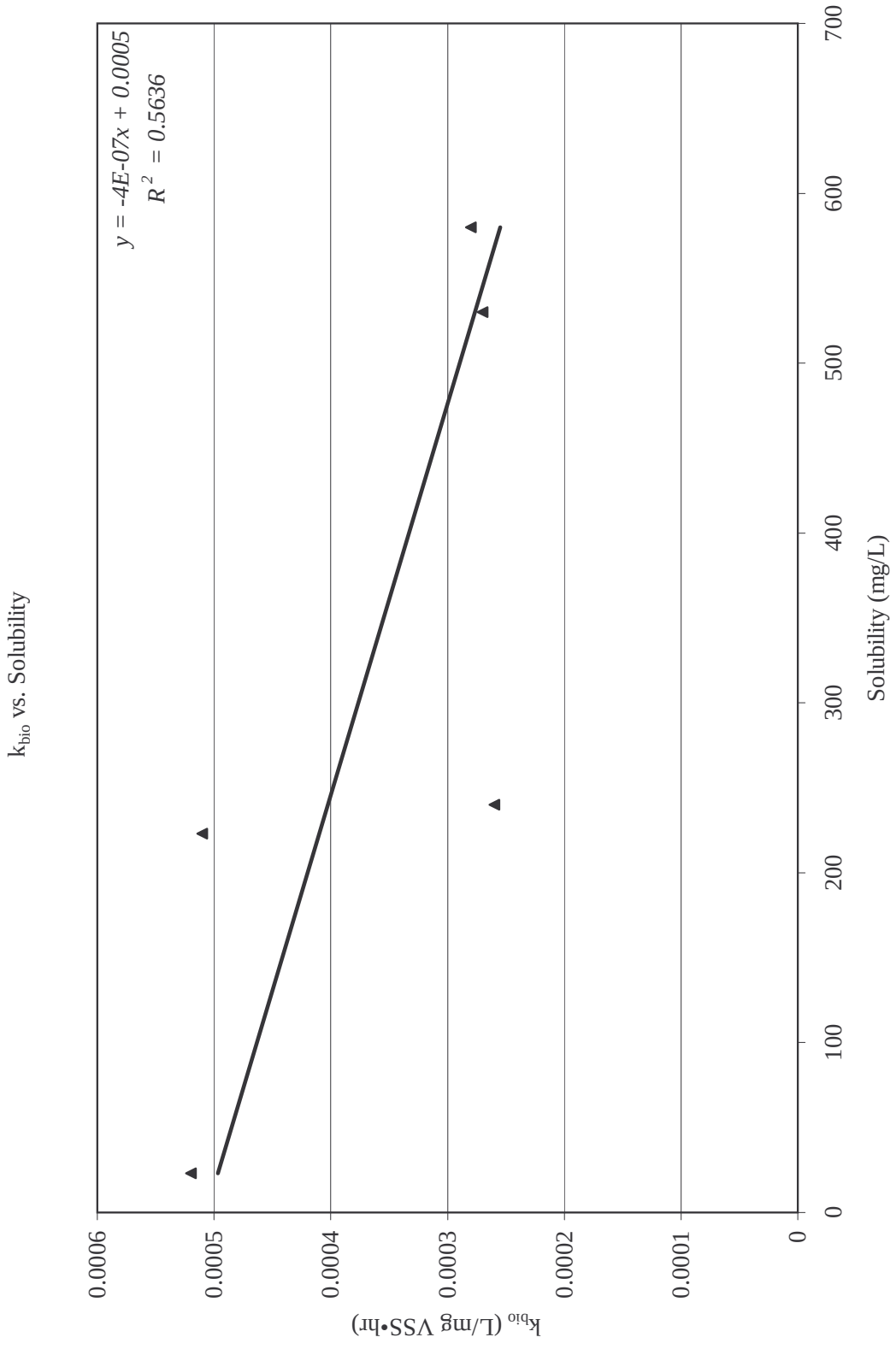

Figure A.11: k_{bio} and solubility correlation plot.

Figure A.12: $\ln(k_{bio})$ and solubility correlation plot.

Figure A.13: k_{HS^-} and molar refractivity correlation plot.

Figure A.14: $\ln(k_{HS^-})$ and solubility correlation plot.

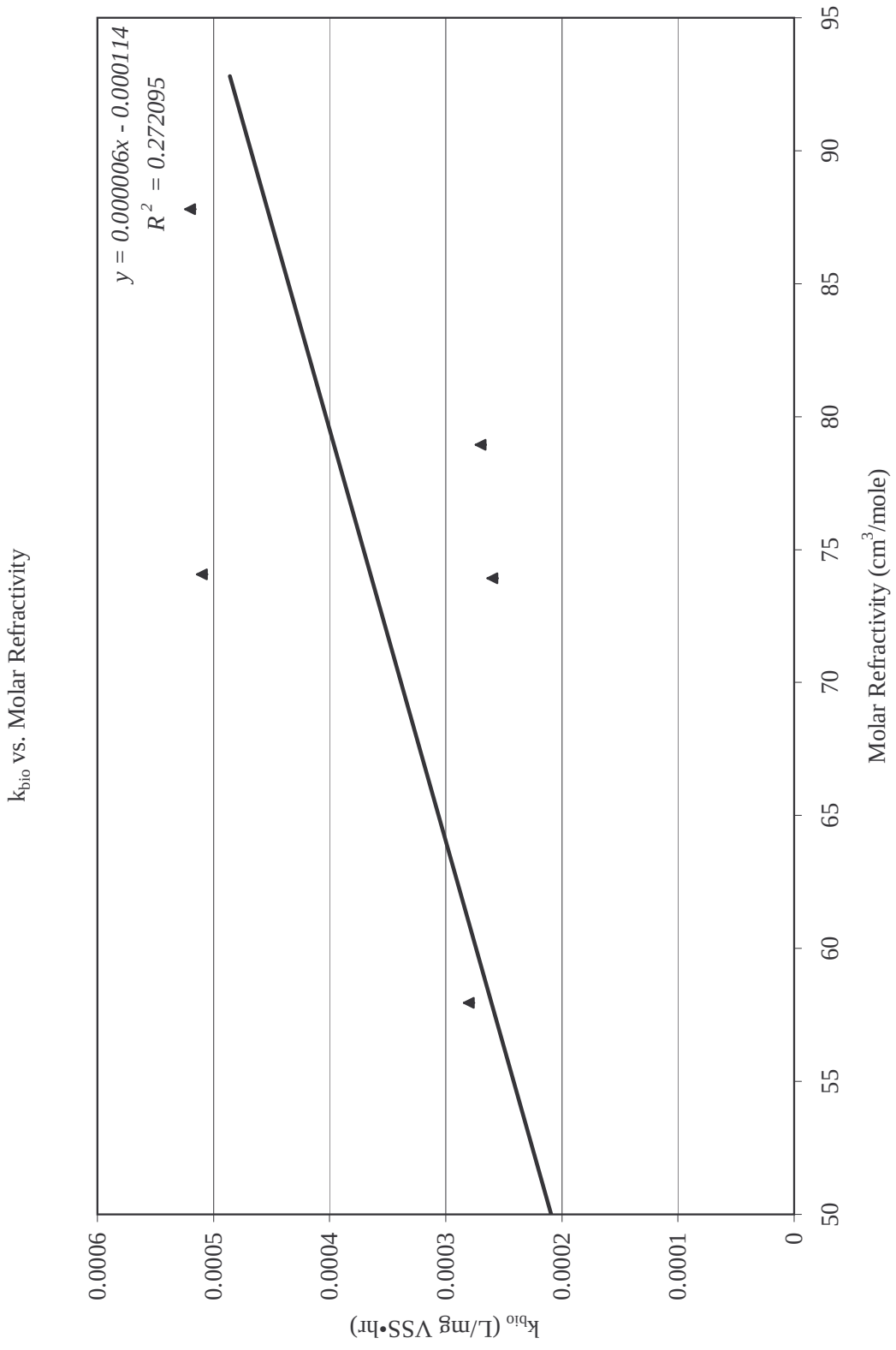

Figure A.15: k_{bio} and molar refractivity correlation plot.

Figure A.16: $\ln(k_{bio})$ and molar refractivity correlation plot.

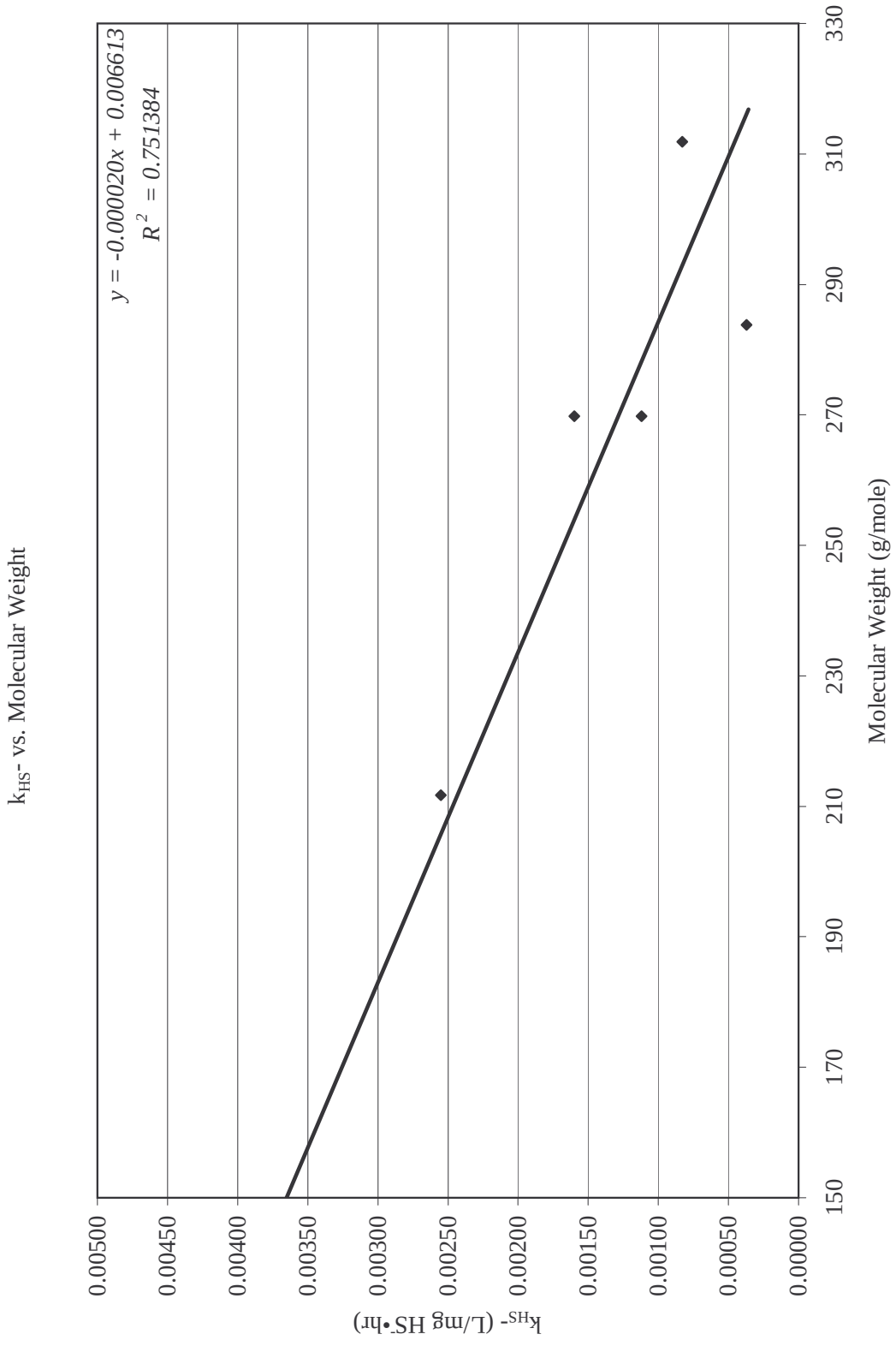

Figure A.17: k_{HS^-} and molecular weight correlation plot.

Figure A.18: $\ln(k_{HS^-})$ and solubility correlation plot.

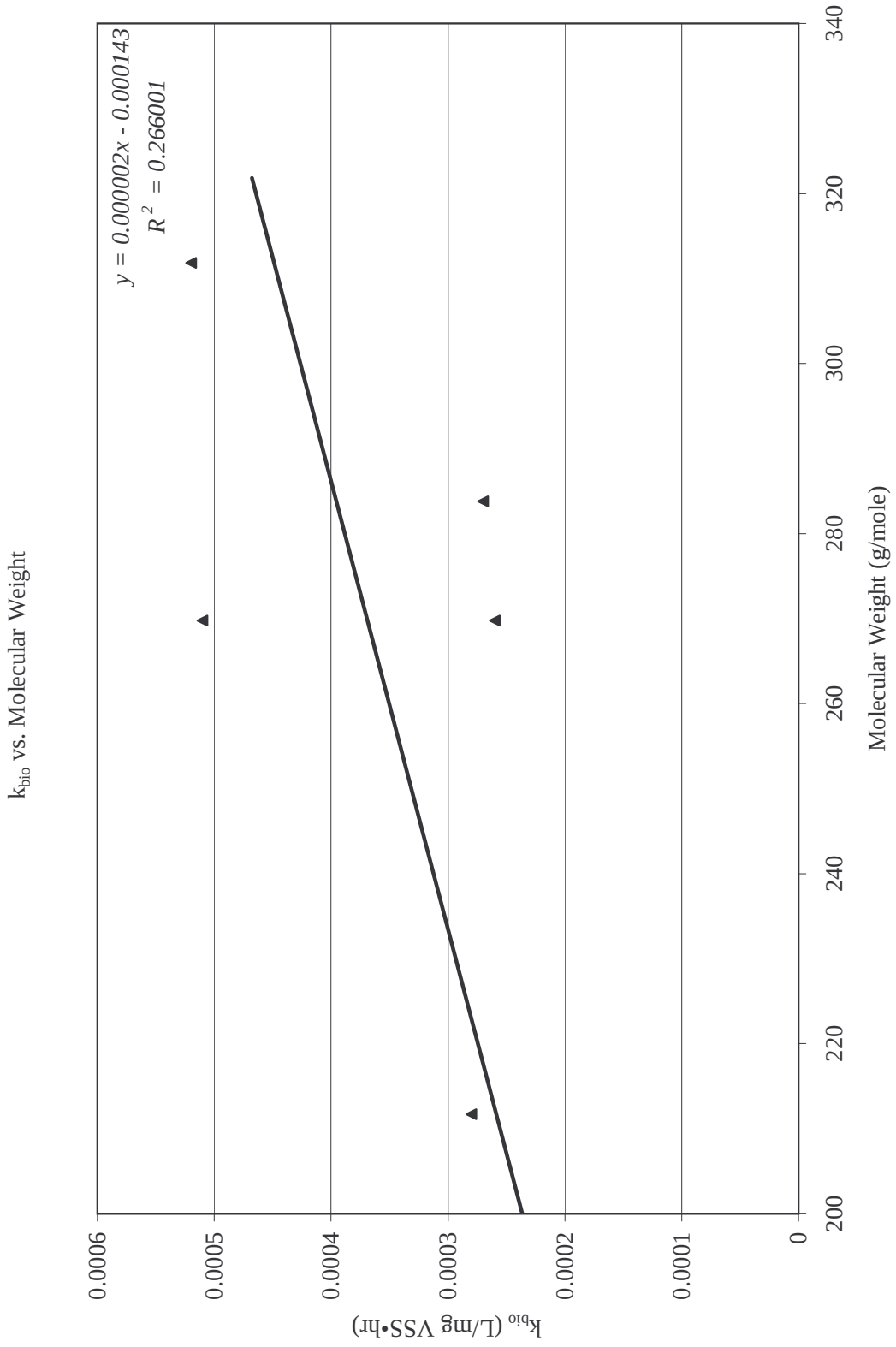

Figure A.19: k_{bio} and molecular weight correlation plot.

Figure A.20: $\ln(k_{bio})$ and molecular weight correlation plot.

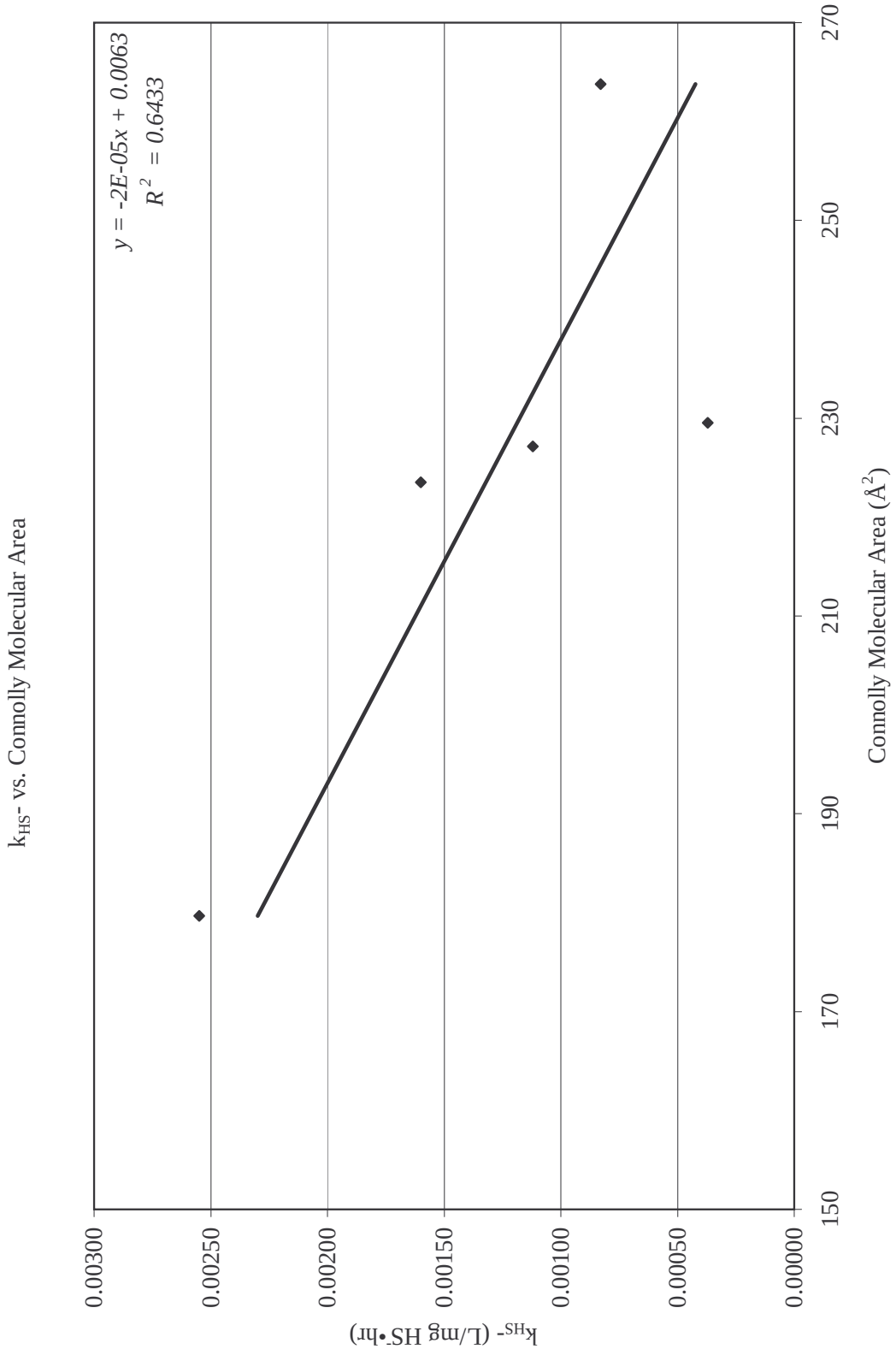

Figure A.21: k_{HS^-} and Connolly molecular area correlation plot.

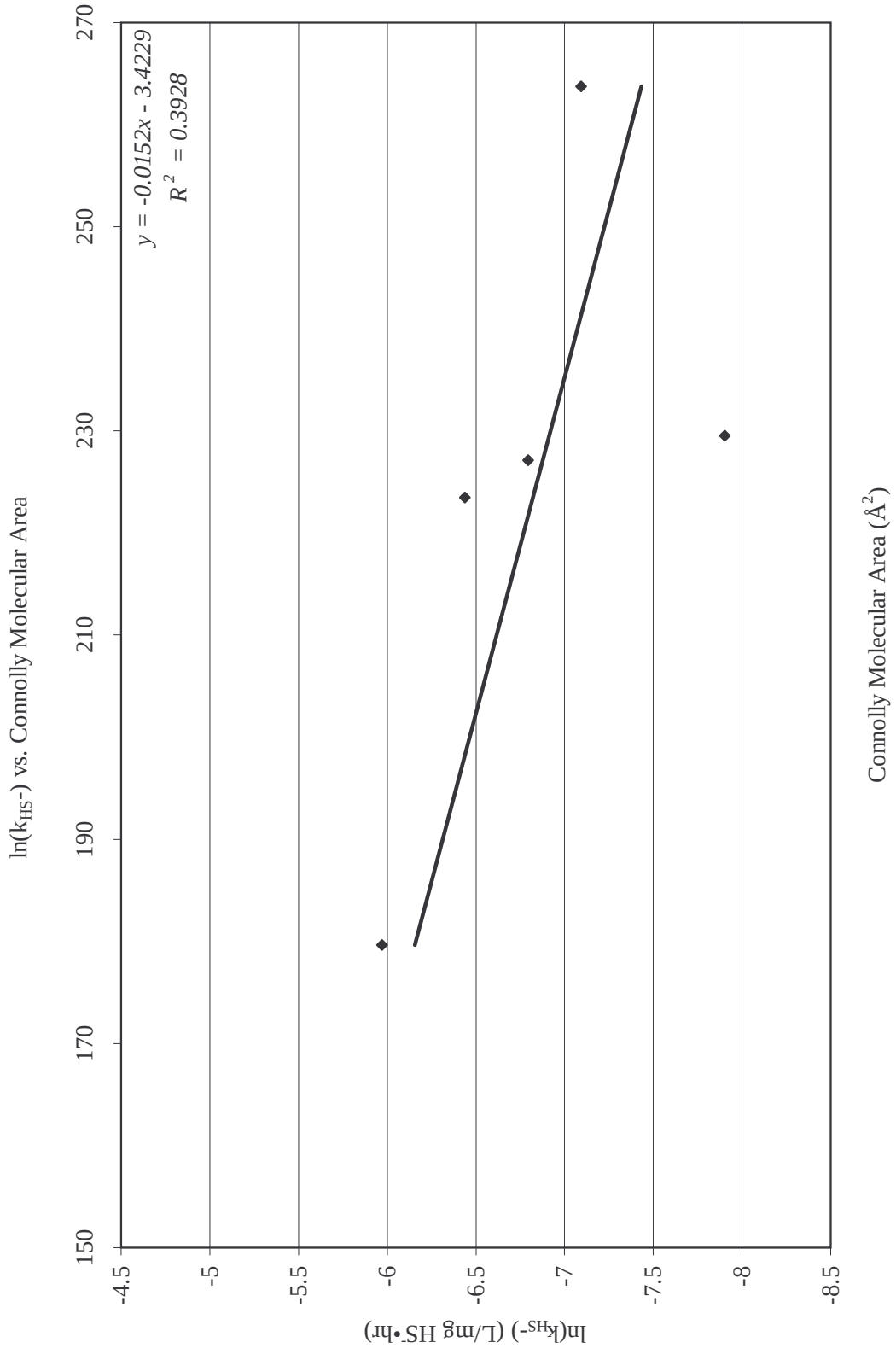

Figure A.22: $\ln(k_{HS^-})$ and Connolly molecular area correlation plot.

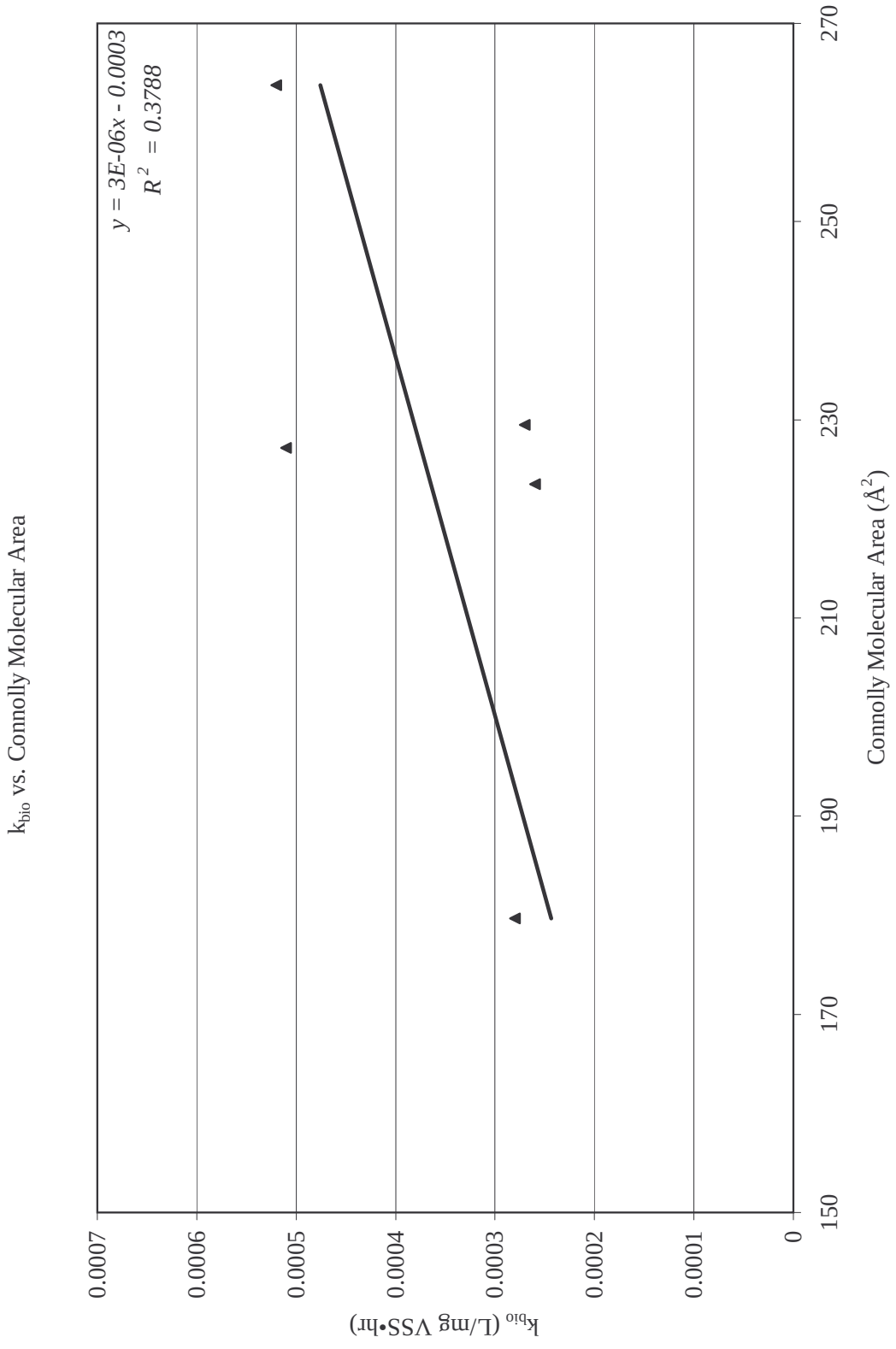

Figure A.23: k_{bio} and Connolly molecular area correlation plot.

Figure A.24: $\ln(k_{bio})$ and Connolly molecular area correlation plot.

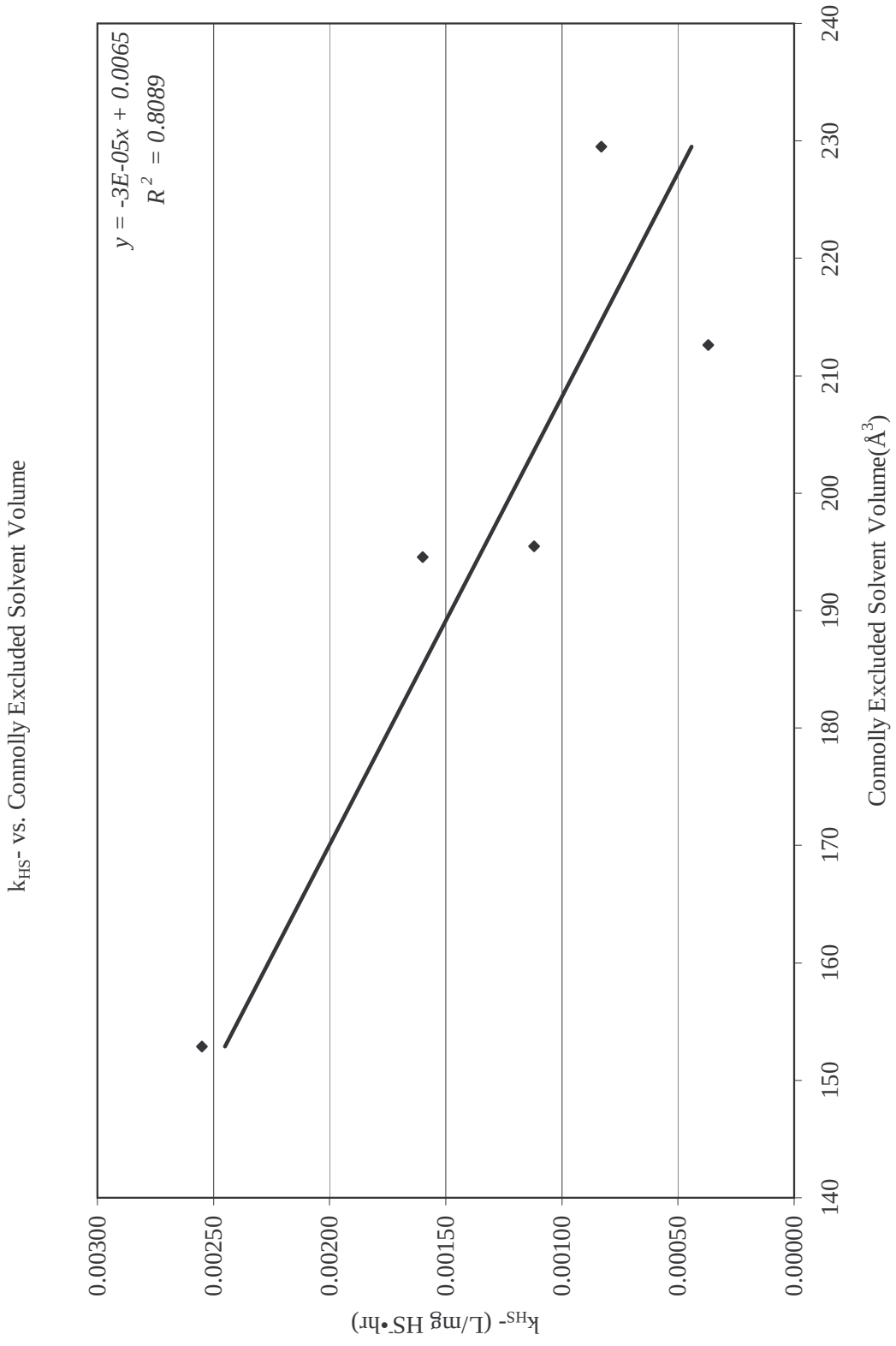

Figure A.25: k_{HS^-} and Connolly excluded solvent volume correlation plot.

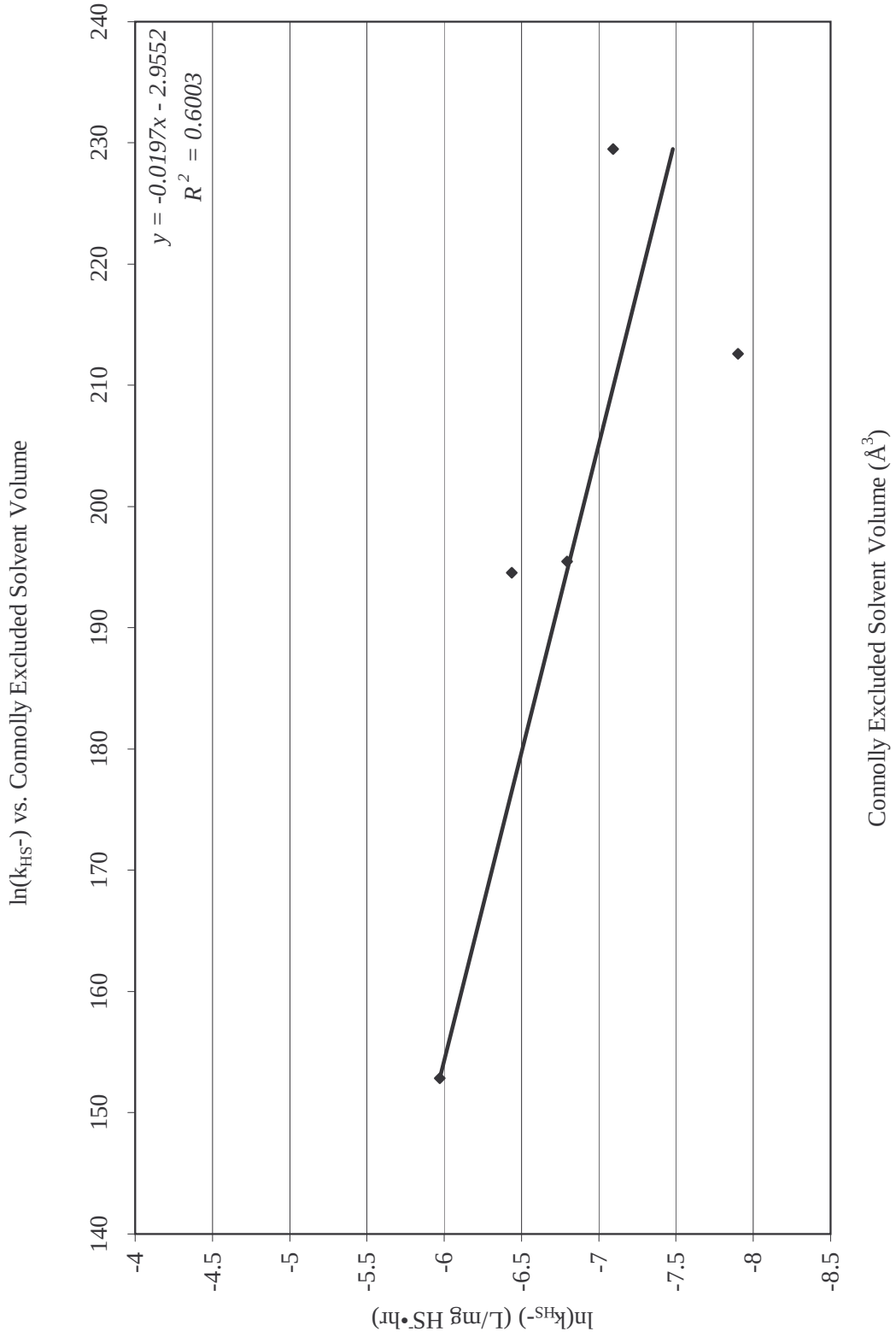

Figure A.26: $\ln(k_{HS^-})$ and Connolly excluded solvent volume correlation plot.

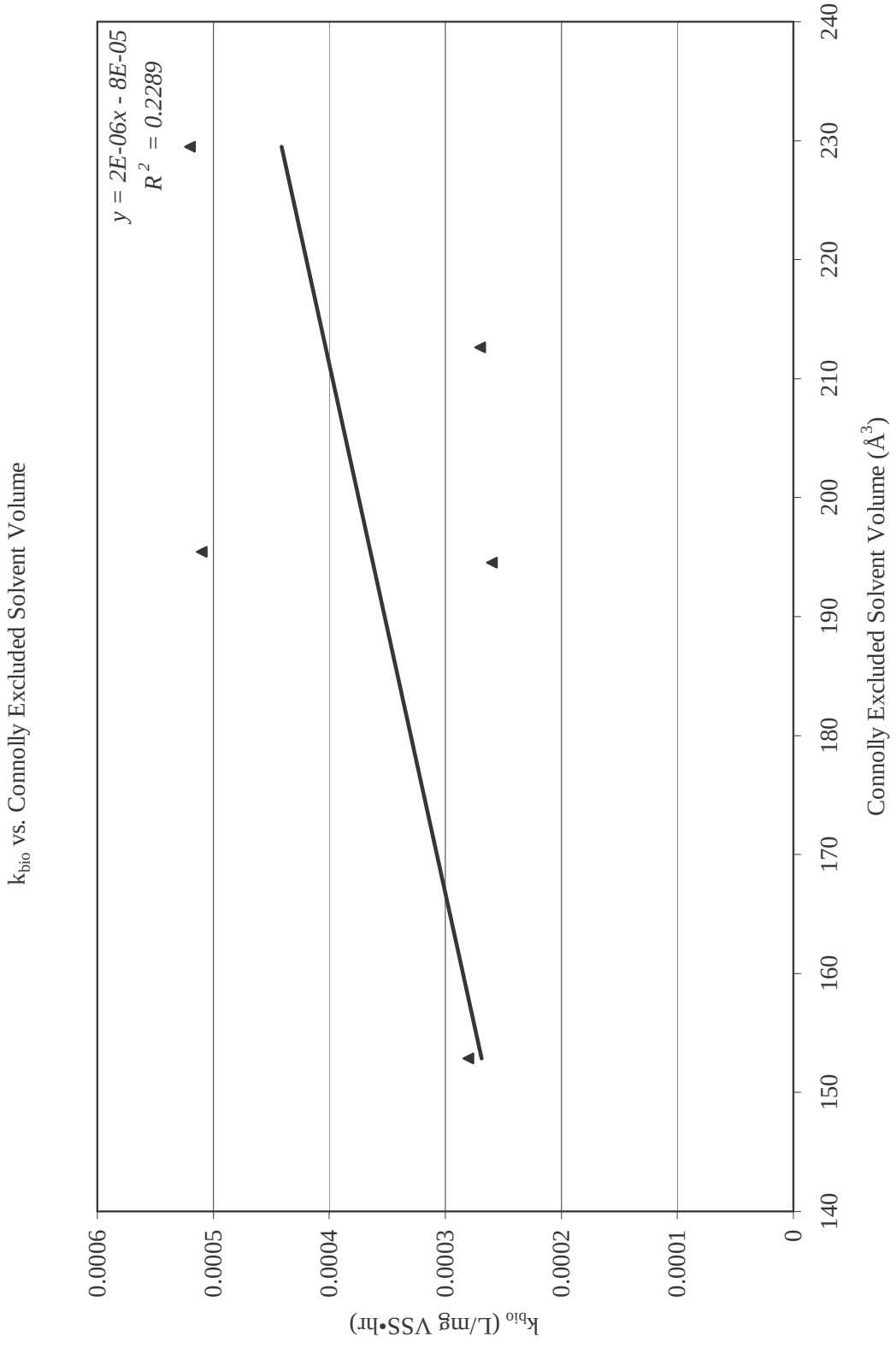

Figure A.27: k_{bio} and Connolly excluded solvent volume correlation plot.

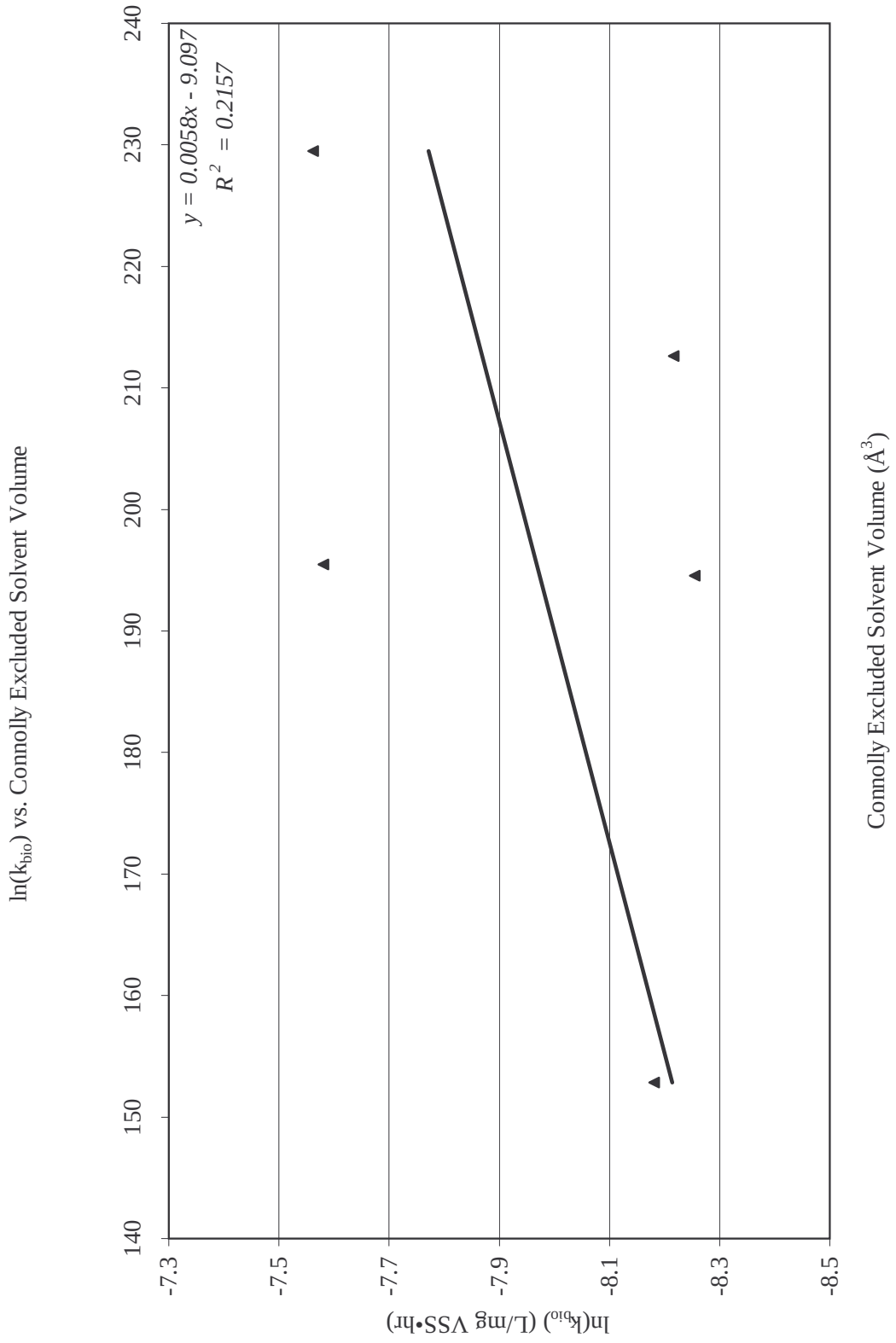

Figure A.28: $\ln(k_{bio})$ and Connolly excluded solvent volume correlation plot.

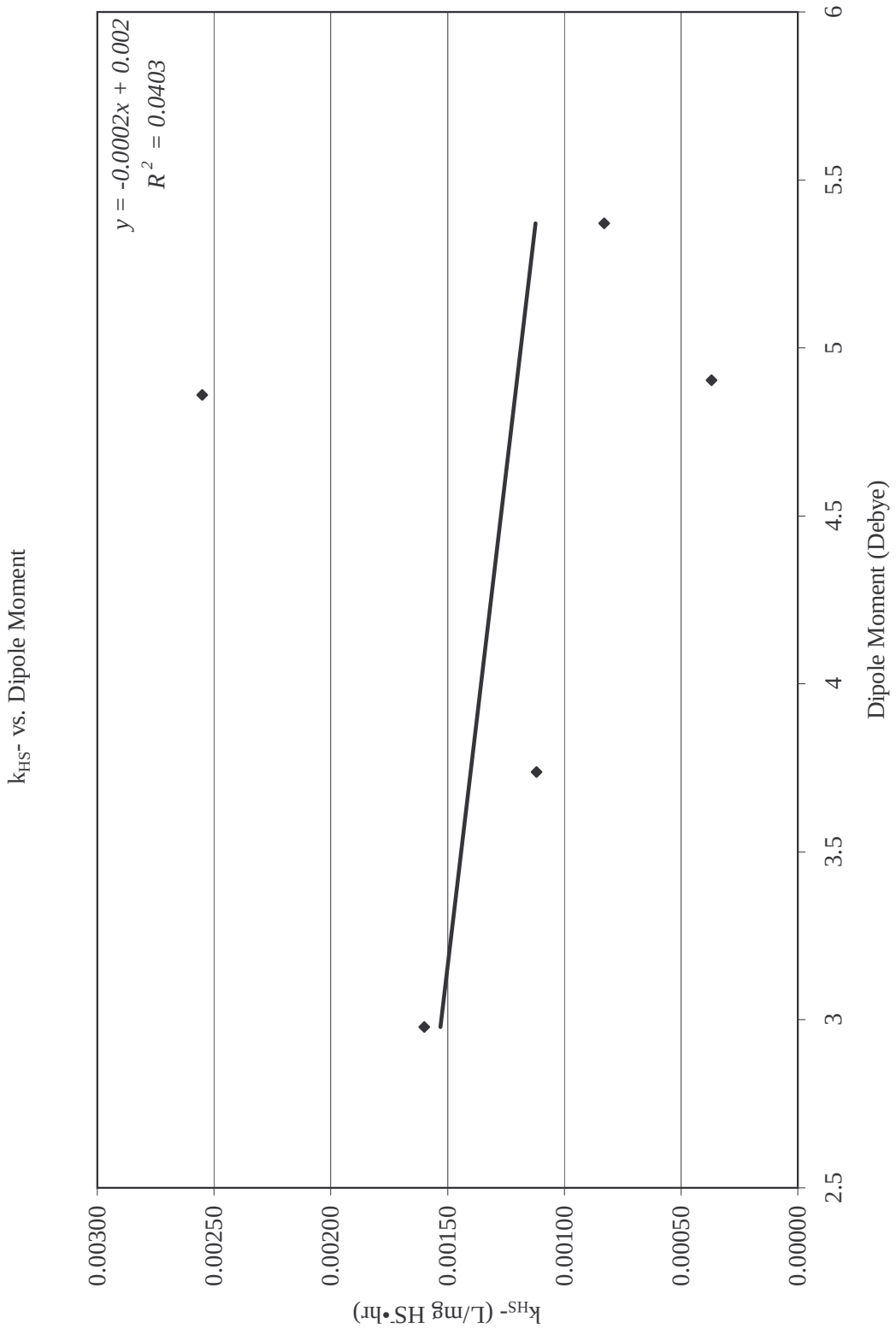

Figure A.29: k_{HS^-} and dipole moment correlation plot.

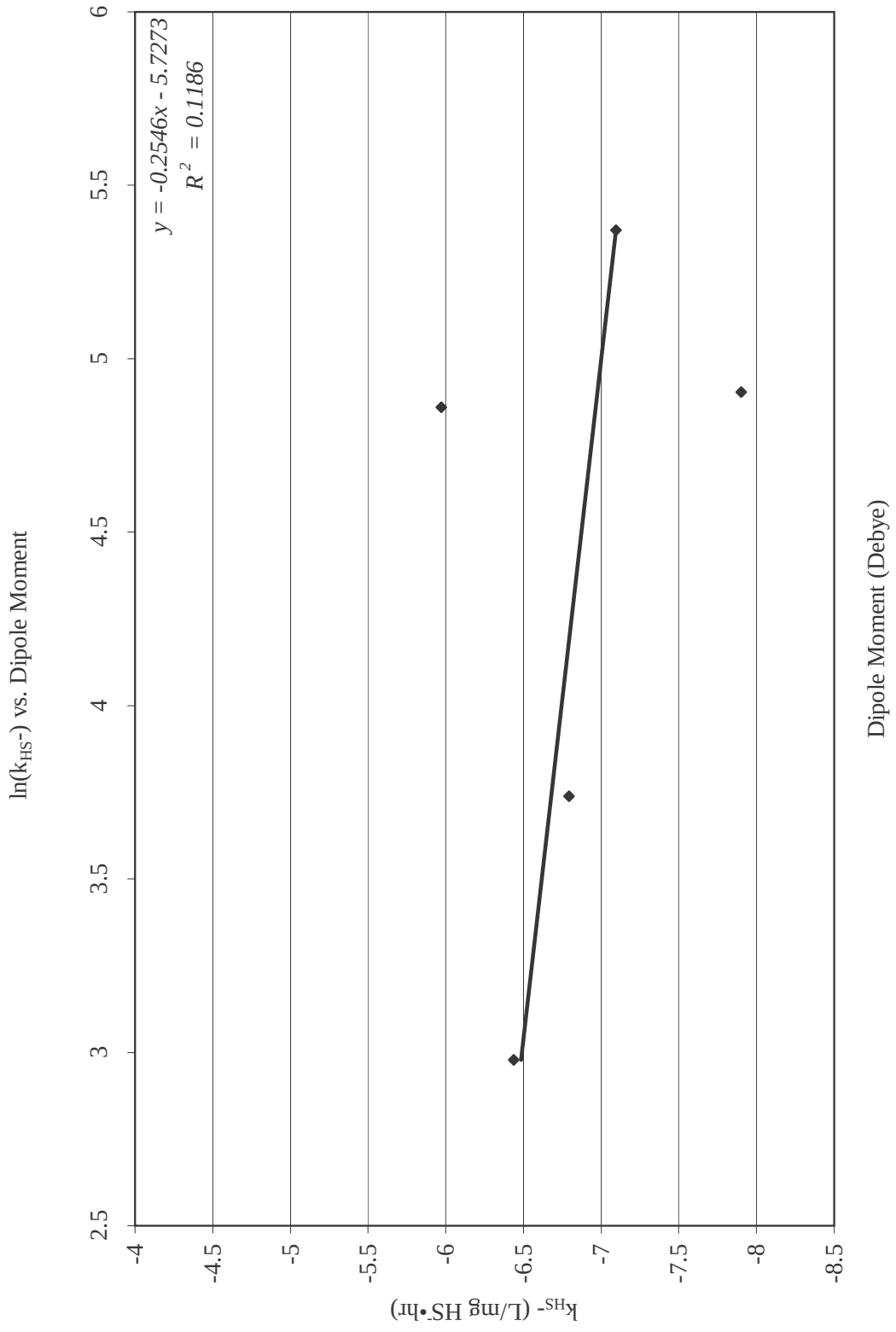

Figure A.30: $\ln(k_{HS^-})$ and dipole moment correlation plot.

Figure A.31: k_{bio} and dipole moment correlation plot.

Figure A.32: $\ln(k_{bio})$ and dipole moment correlation plot.

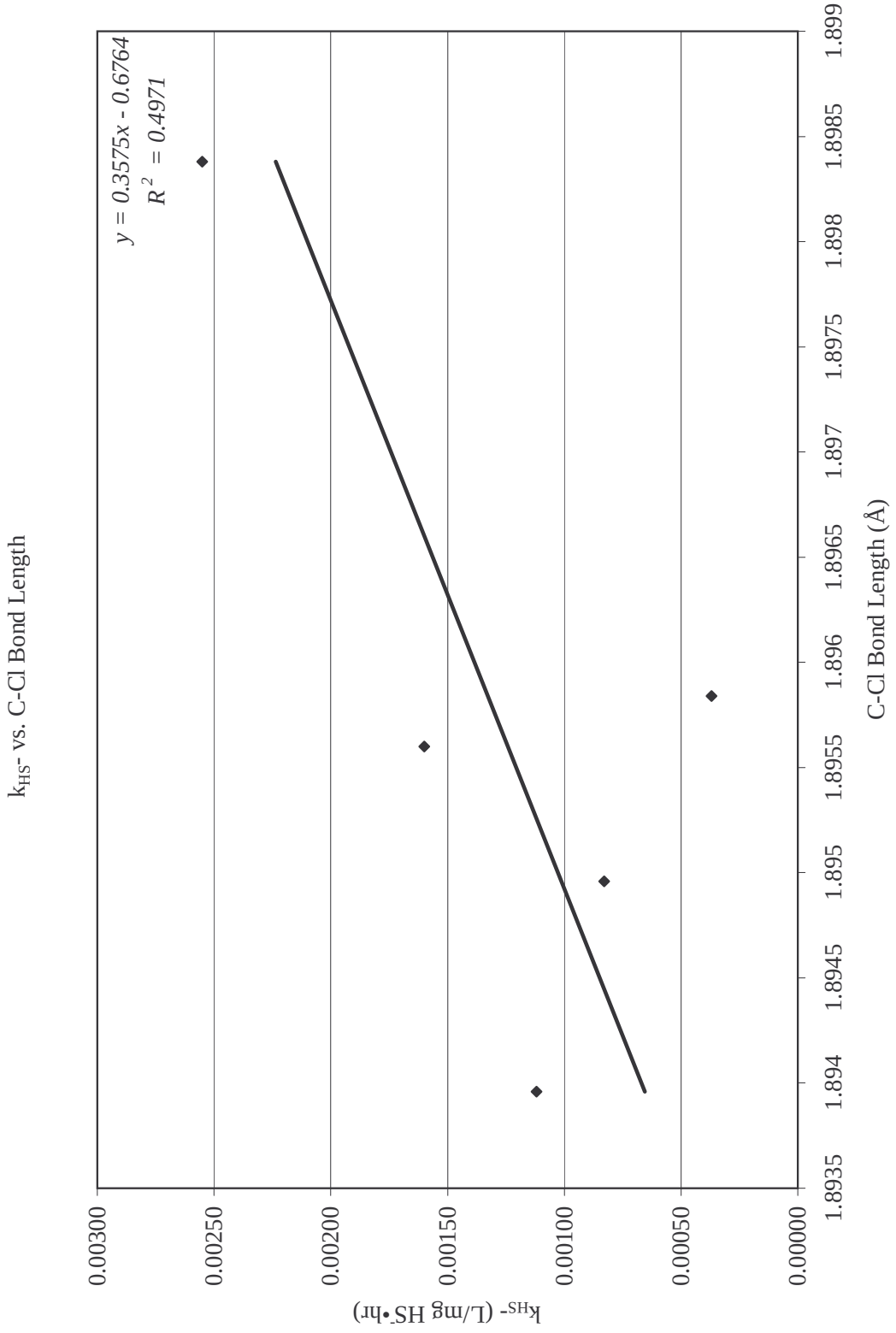

Figure A.33: k_{HS^-} and C-Cl bond length correlation plot.

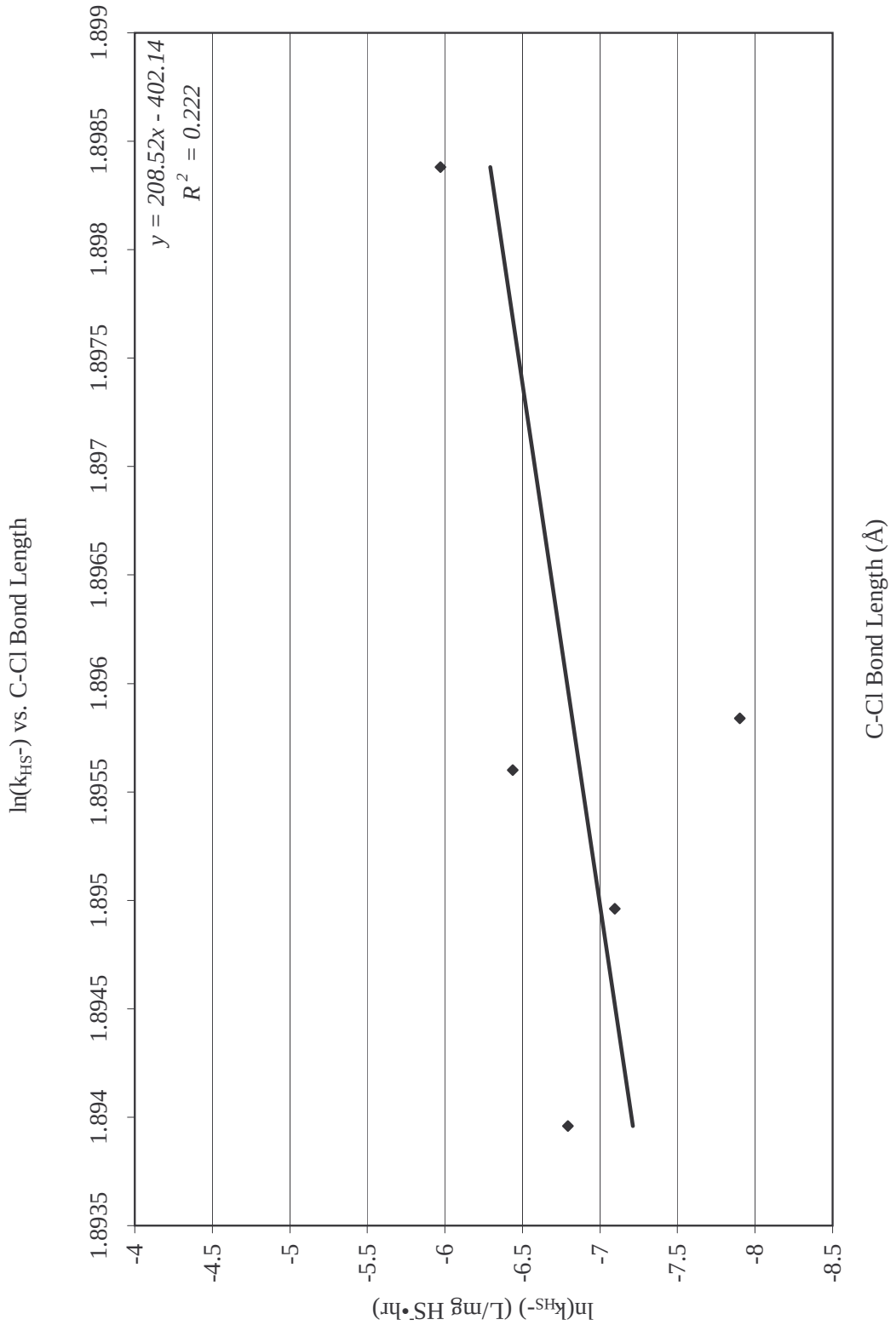

Figure A.34: $\ln(k_{HS^-})$ and C-Cl bond length correlation plot.

Figure A.35: k_{bio} and C-Cl bond length correlation plot.

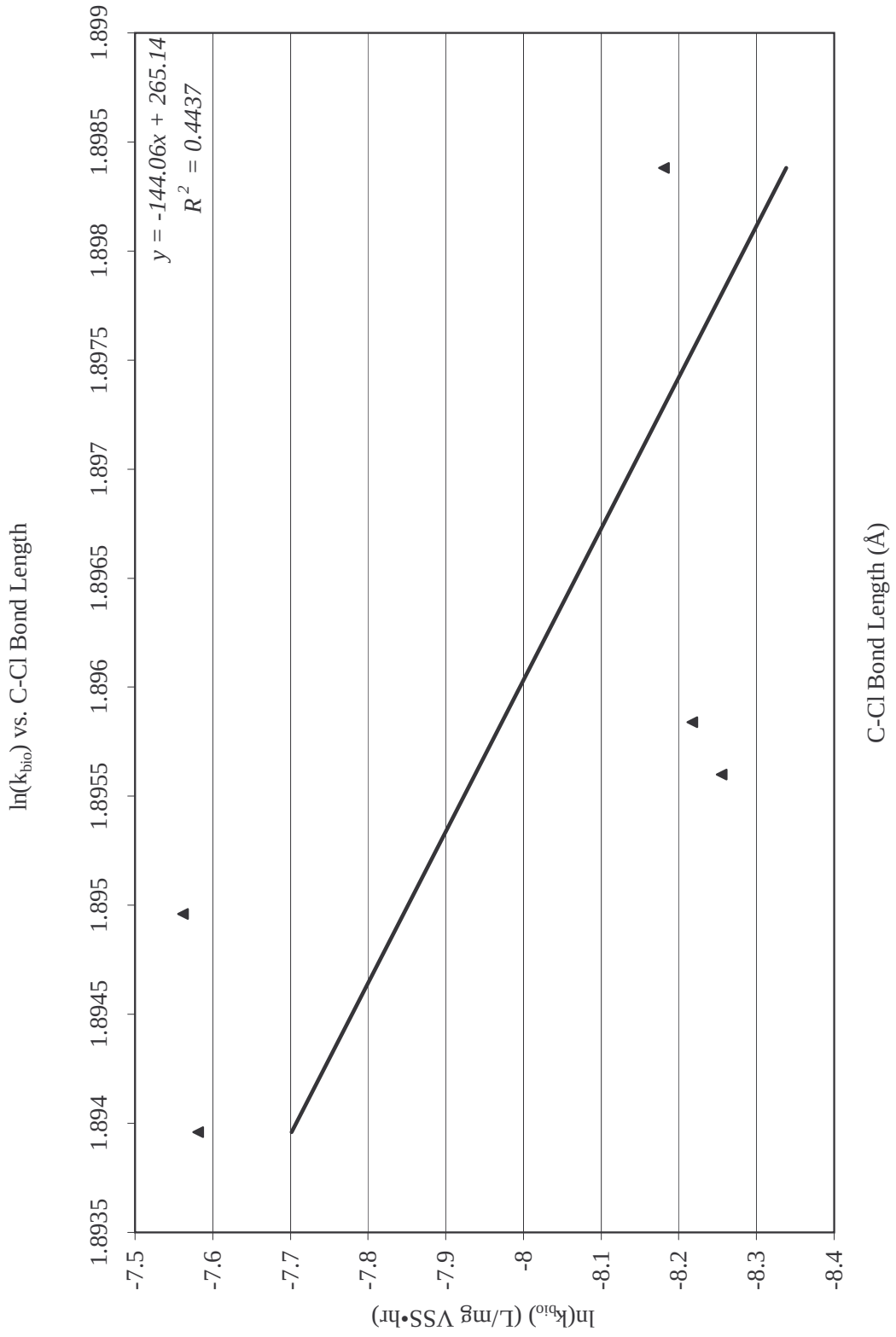

Figure A.36: $\ln(k_{bio})$ and C-Cl bond length correlation plot.

Figure A.37: k_{HS^-} and C-Cl bond energy correlation plot.

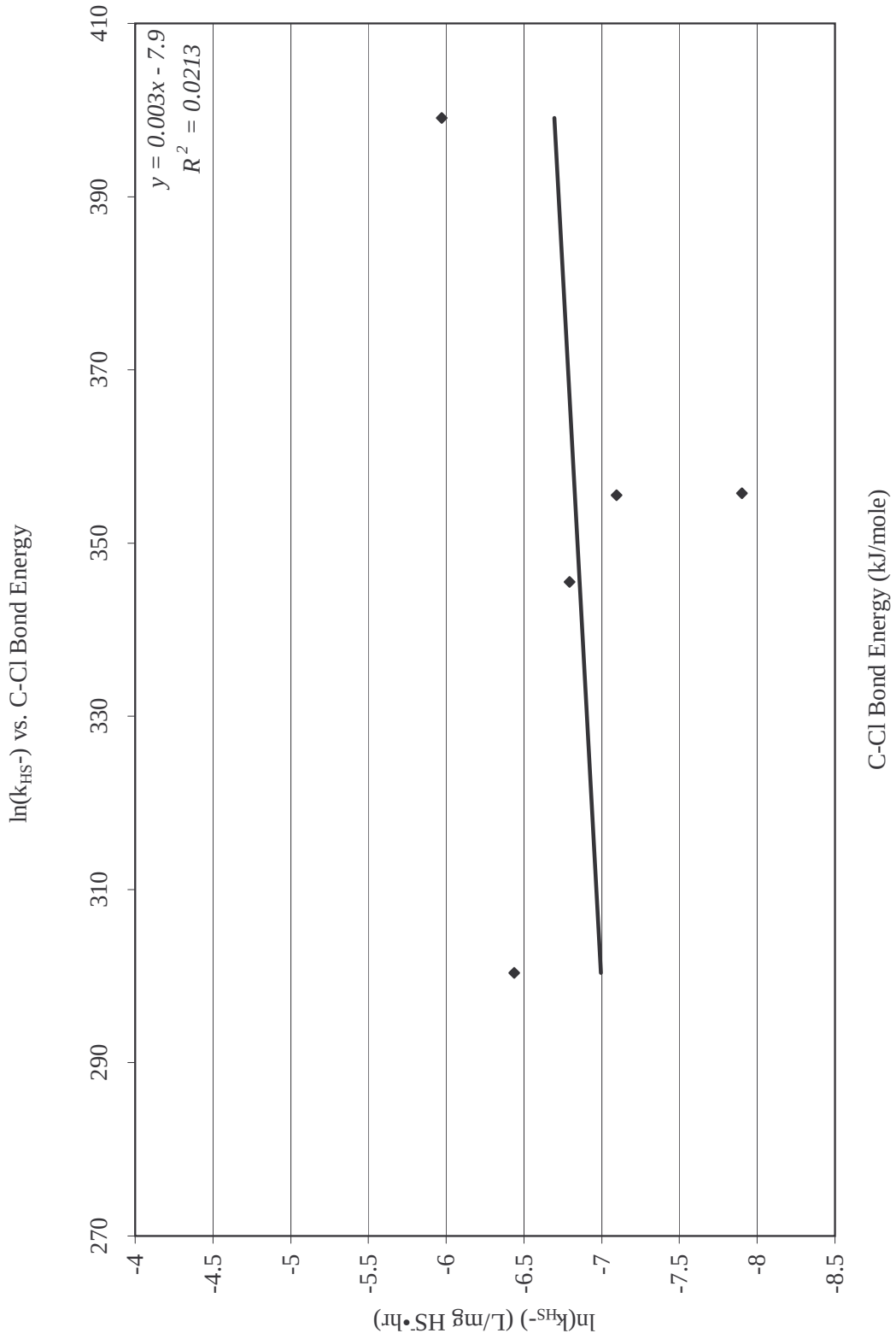

Figure A.38: $\ln(k_{HS^-})$ and C-Cl bond energy correlation plot.

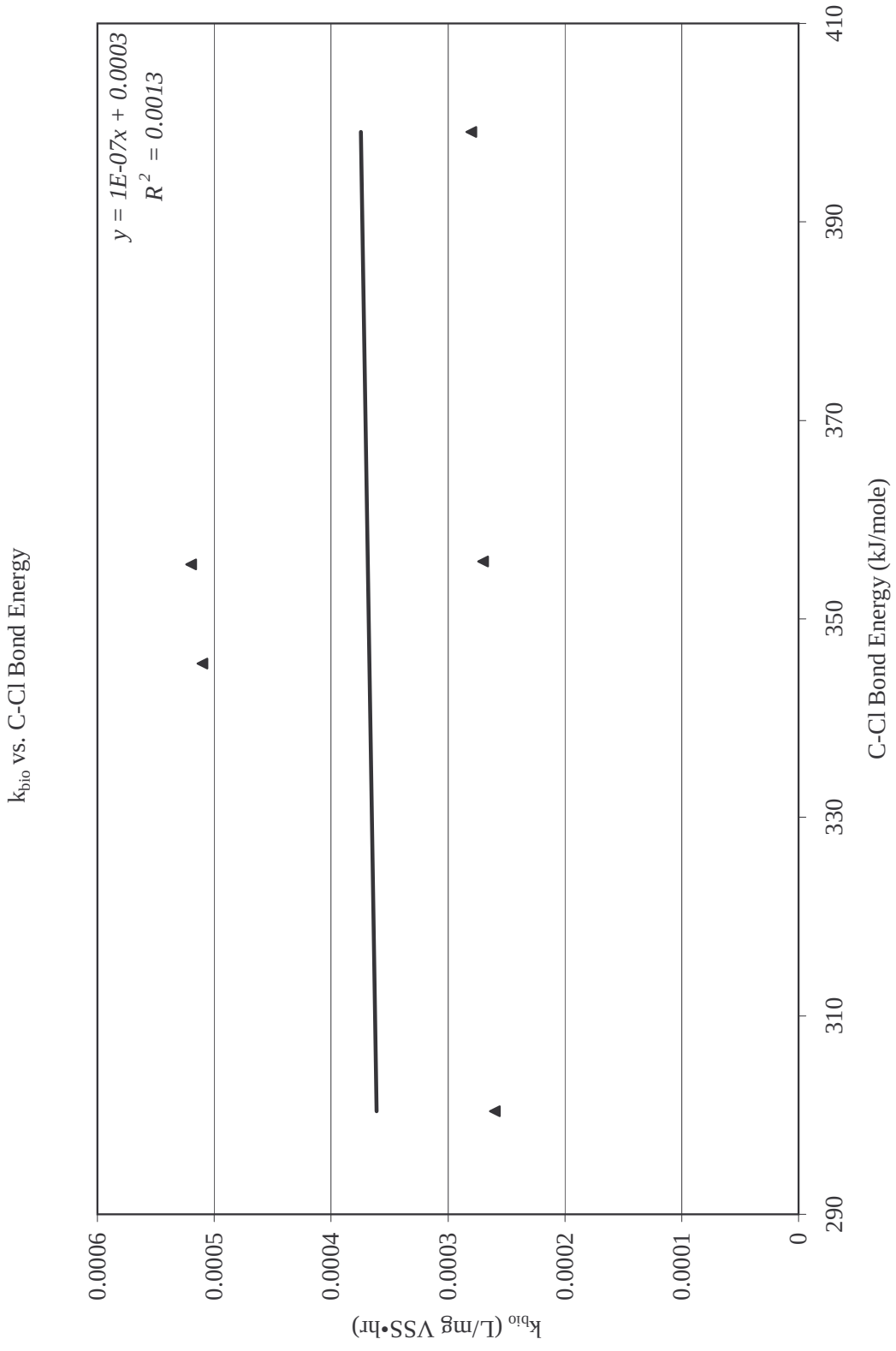

Figure A.39: k_{bio} and C-Cl bond energy correlation plot.

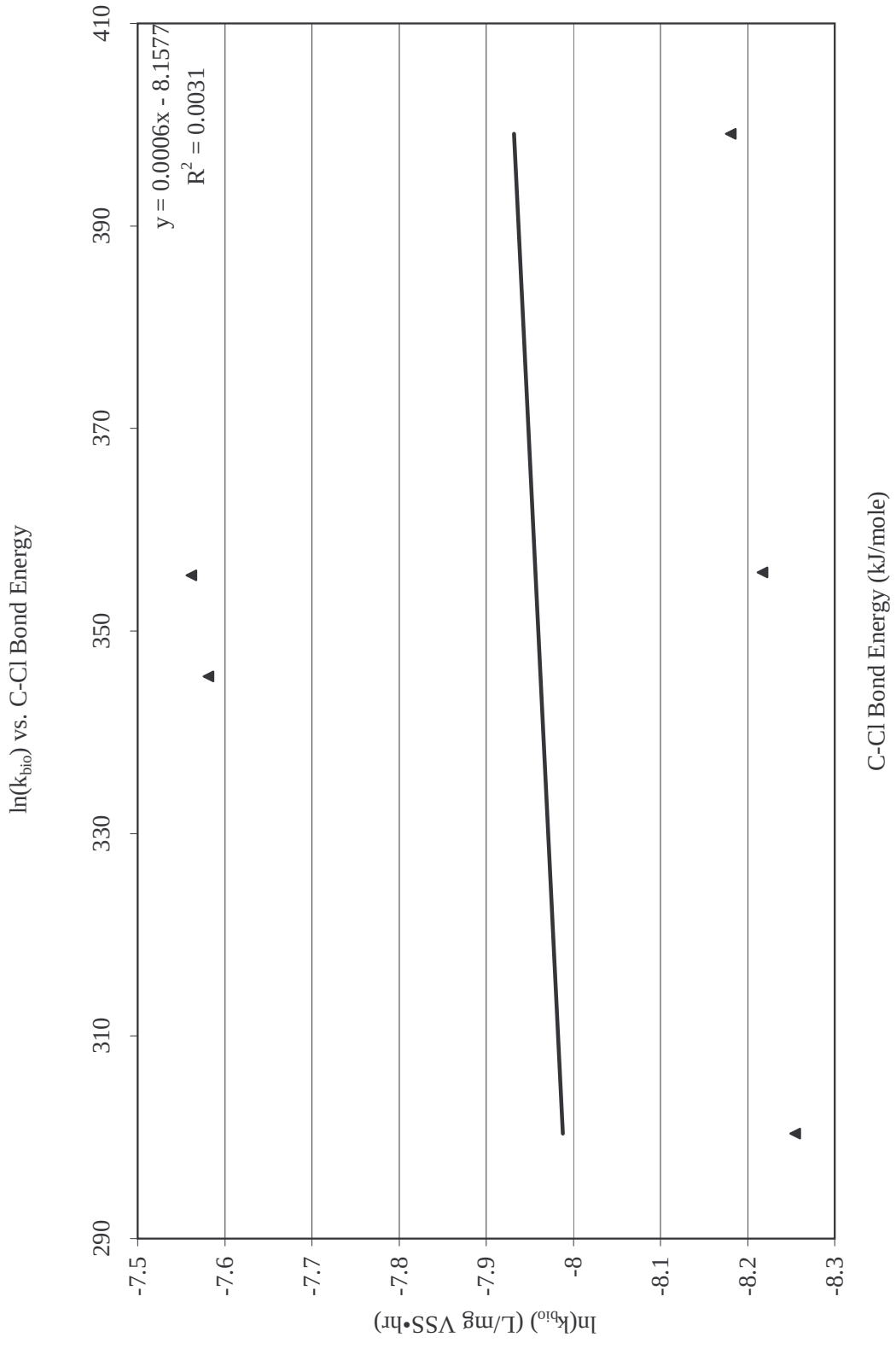

Figure A.40: $\ln(k_{bio})$ and C-Cl bond energy correlation plot.

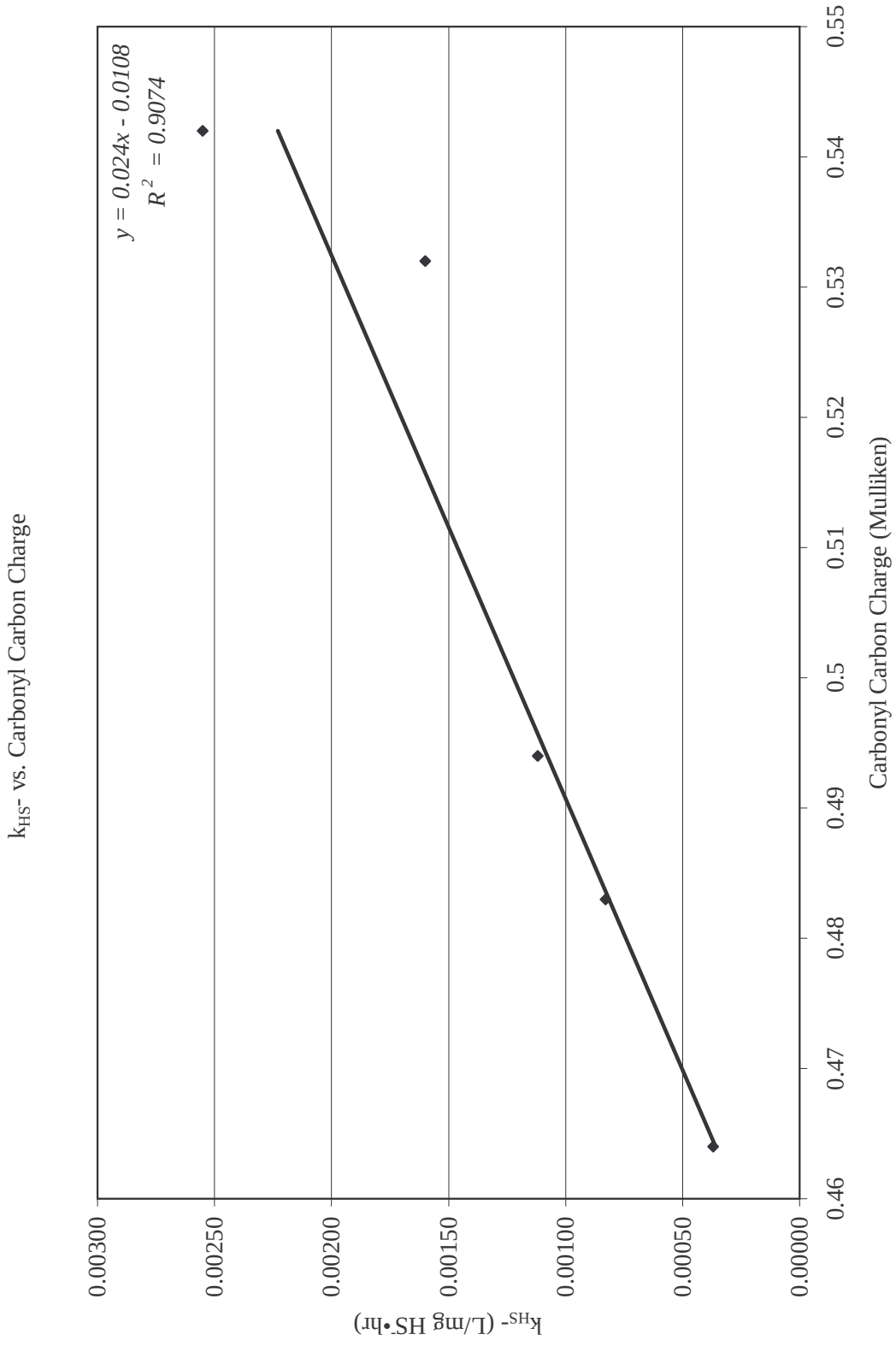

Figure A.41: k_{HS^-} and C=O carbon charge correlation plot.

Figure A.42: $\ln(k_{HS^-})$ and C=O carbon charge correlation plot.

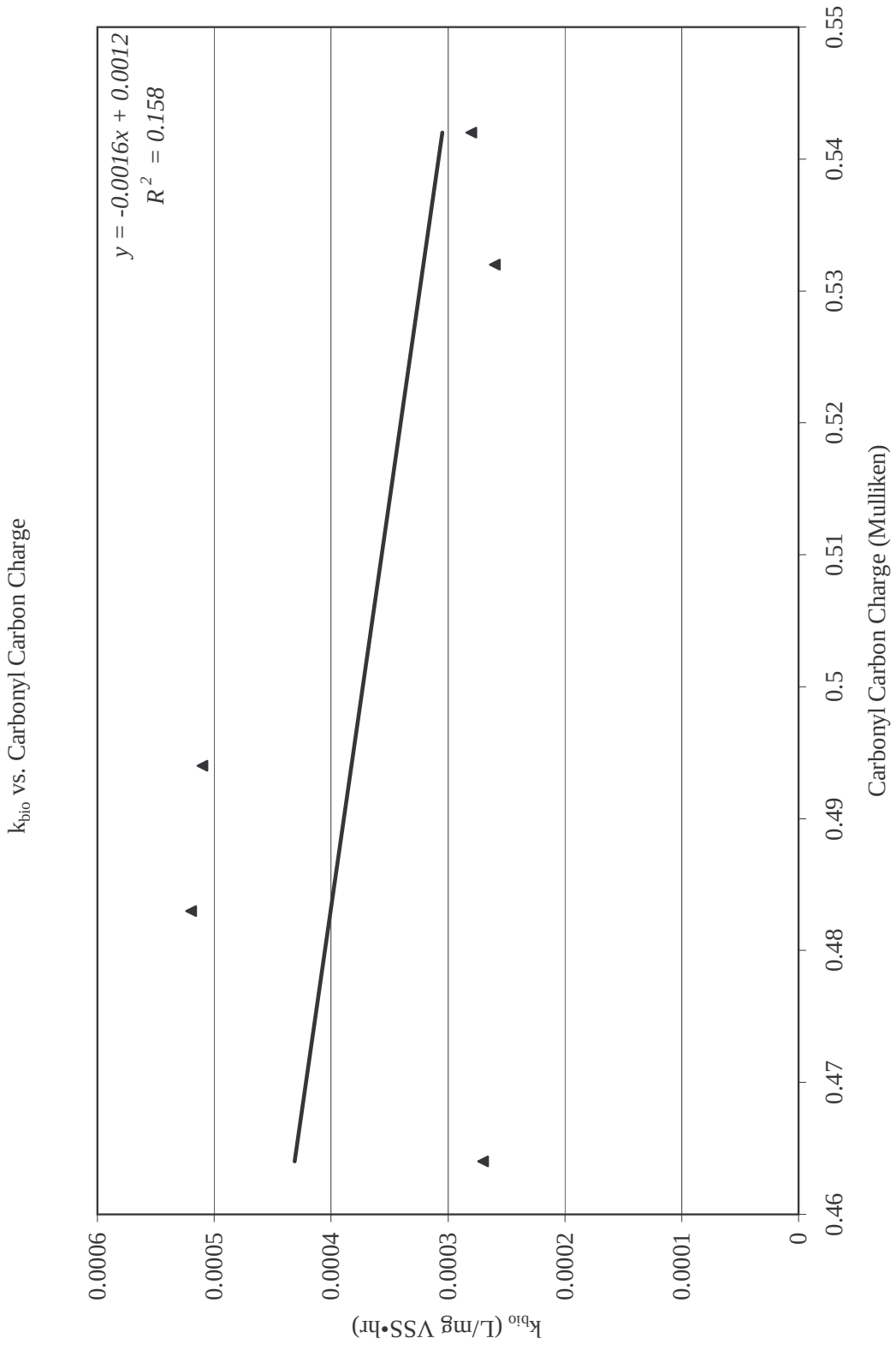

Figure A.43: k_{bio} and C=O carbon charge correlation plot.

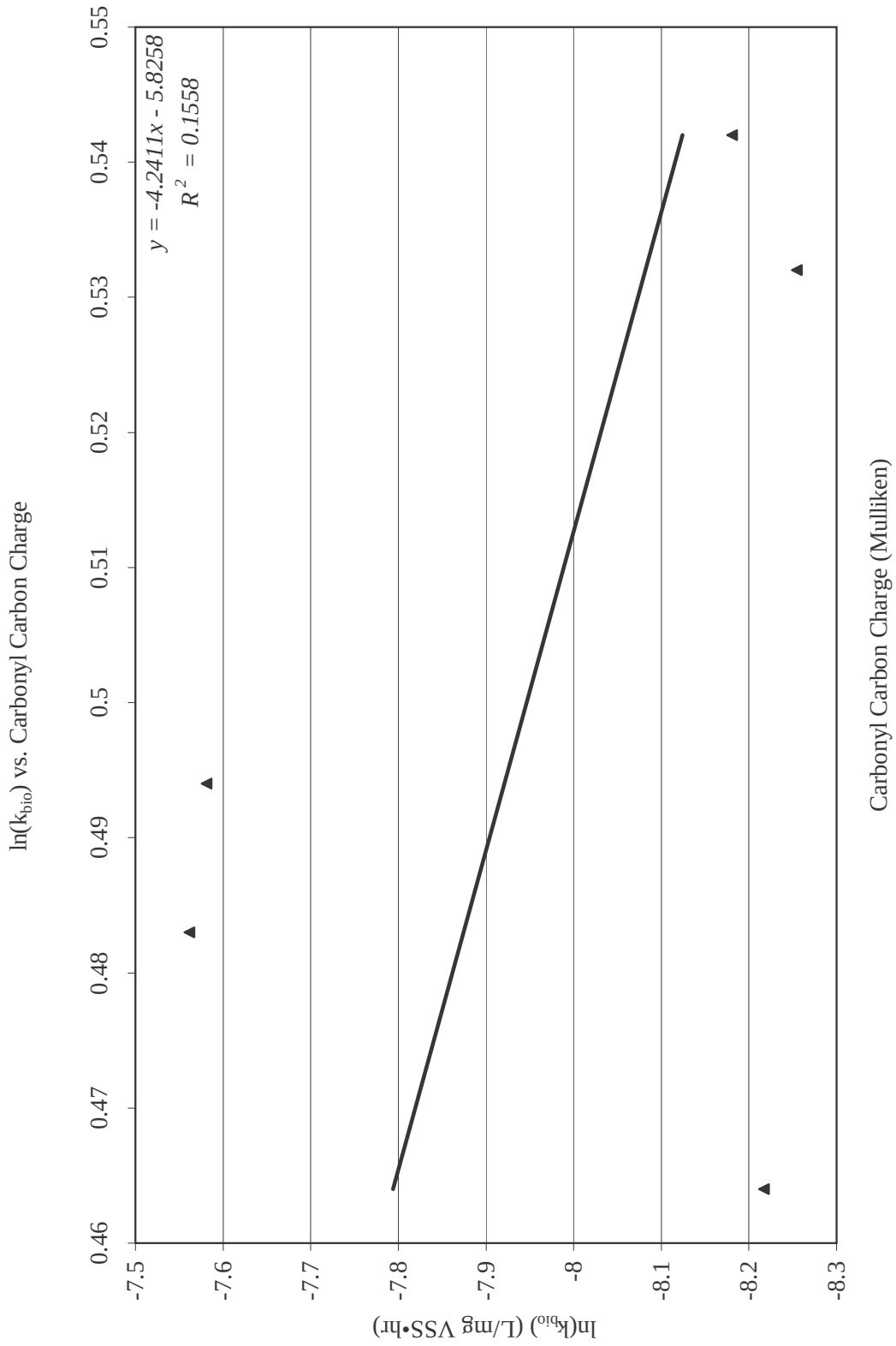

Figure A.44: ln(k_{bio}) and C=O carbon charge correlation plot.

A.5 Molecular Mechanics Figures

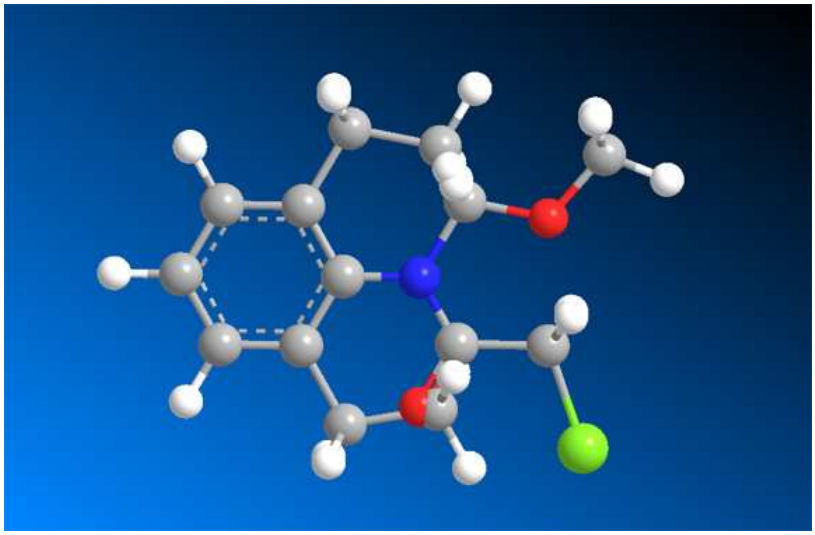

Figure A.45: Alachlor before energy minimization.

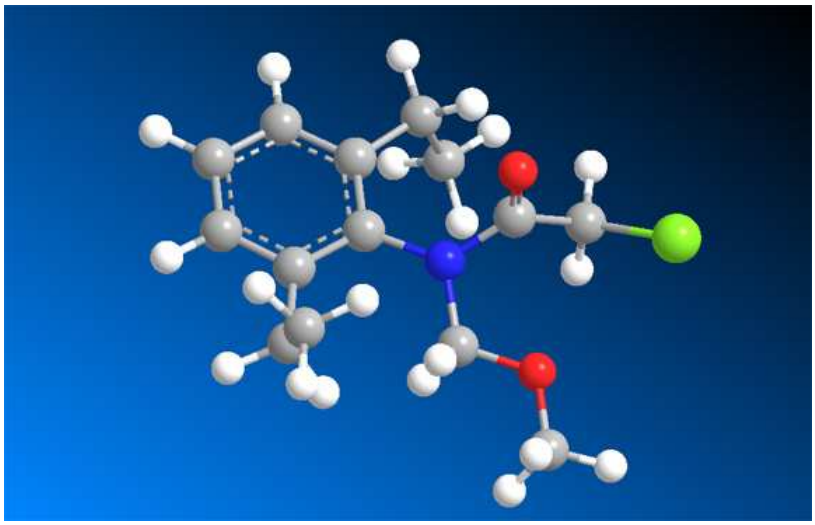

Figure A.46: Alachlor after energy minimization.

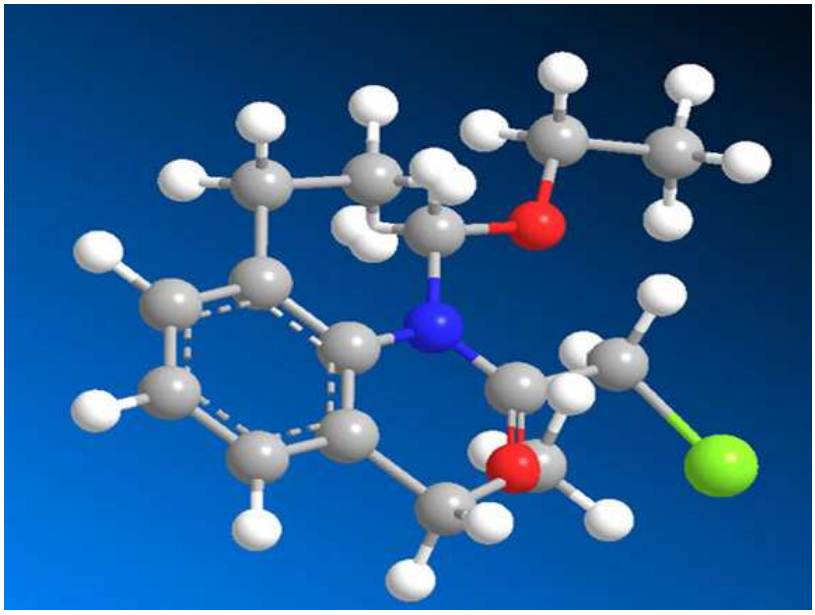

Figure A.47: Acetochlor before energy minimization.

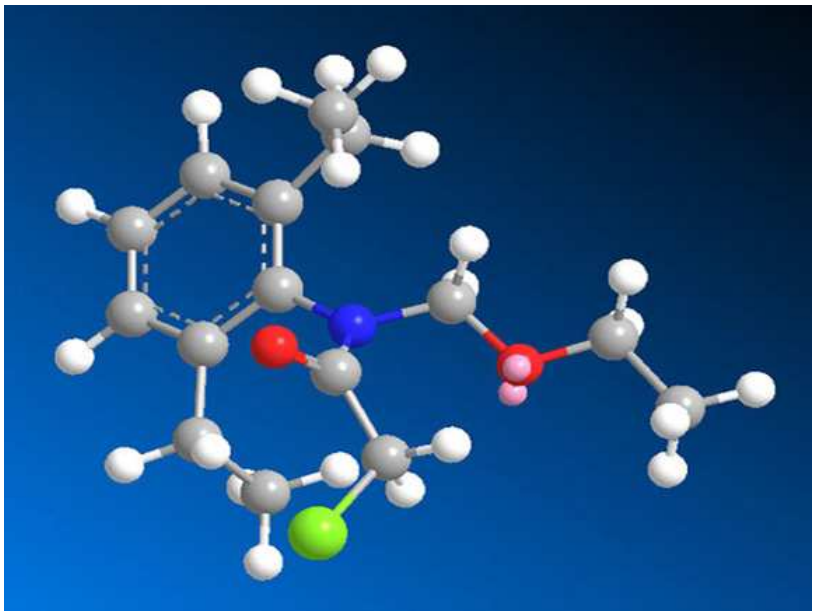

Figure A.48: Acetochlor after energy minimization.

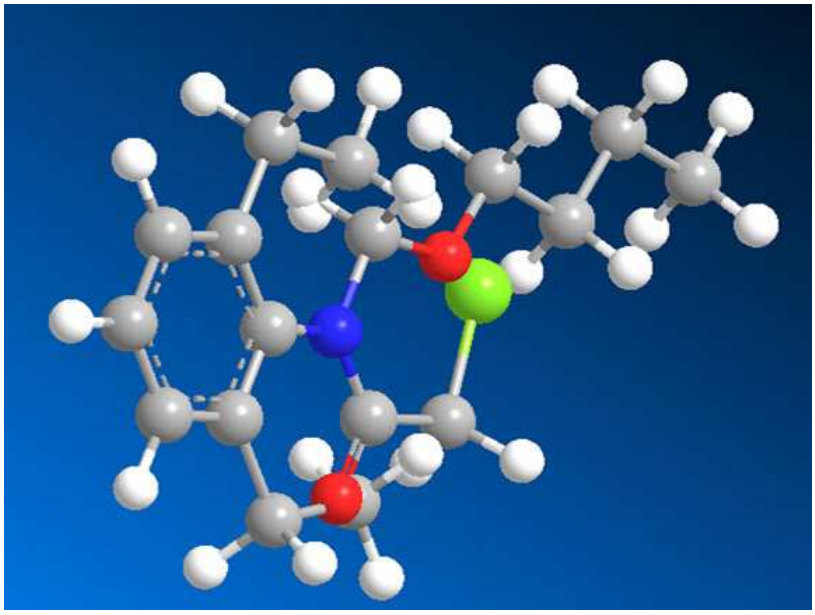

Figure A.49: Butachlor before energy minimization.

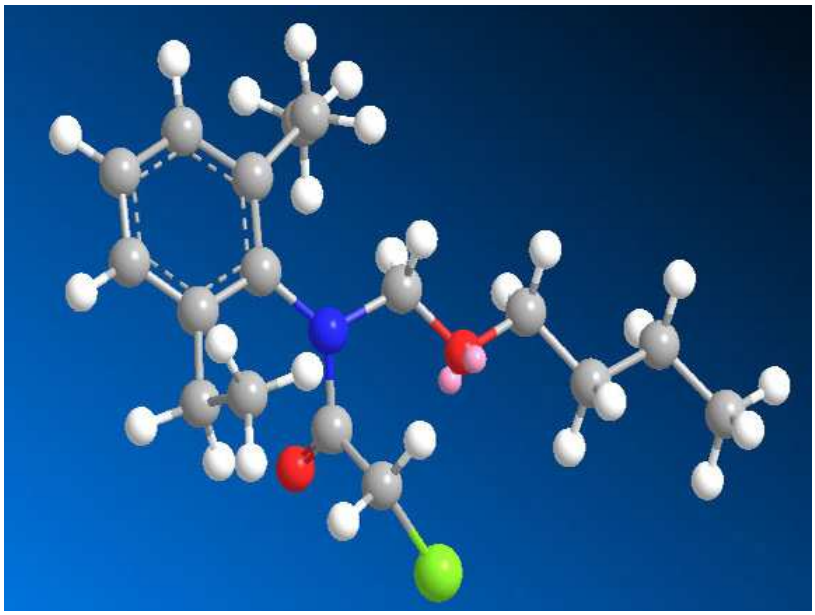

Figure A.50: Butachlor after energy minimization.

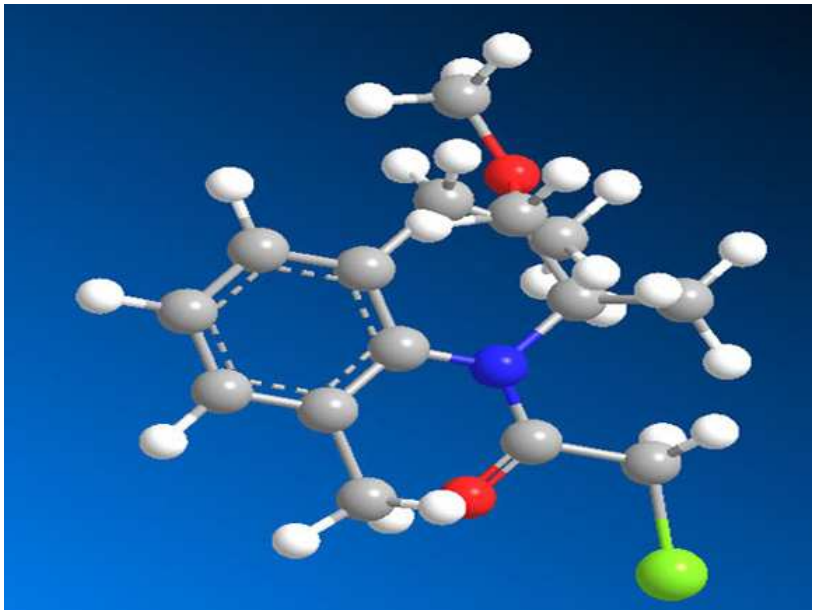

Figure A.51: Metolachlor before energy minimization.

Figure A.52: Metolachlor after energy minimization.

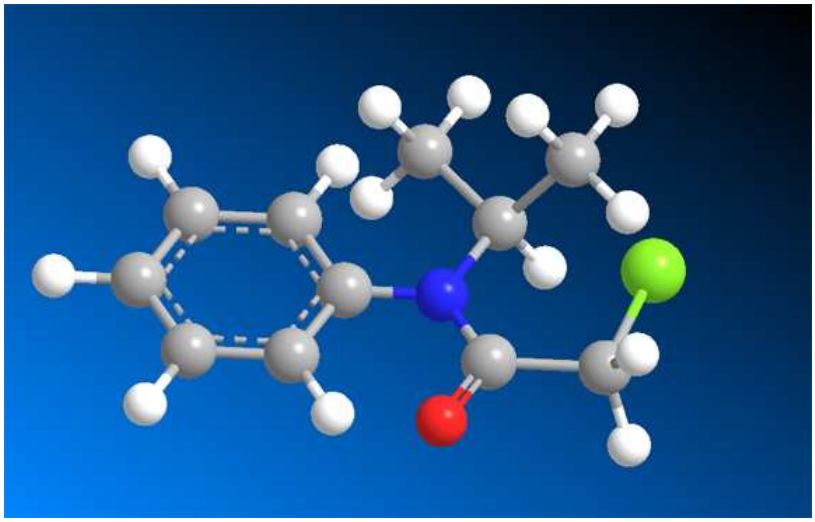

Figure A.53: Propachlor before energy minimization.

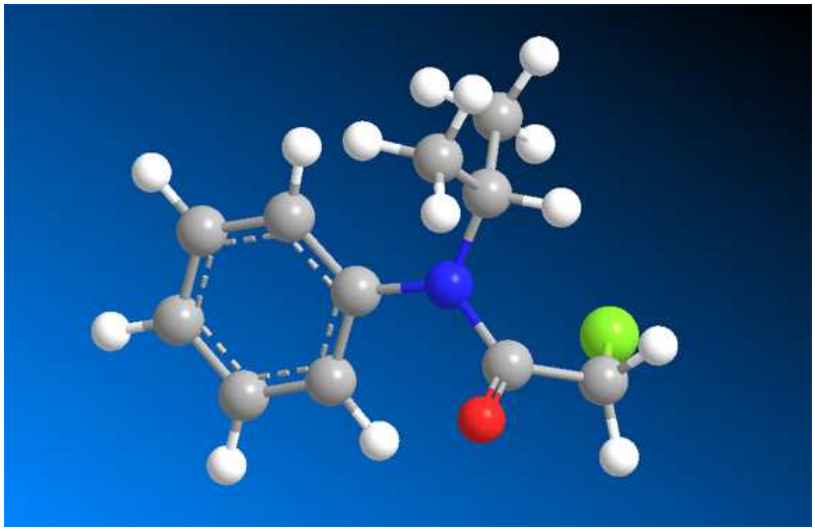

Figure A.54: Propachlor after energy minimization.

VITA

Angela Robyn Kana

Candidate for the Degree of

Master of Science

Thesis: QUANTITATIVE STRUCTURE-ACTIVITY RELATIONSHIP: PREDICTION OF ANAEROBIC TRANSFORMATION OF CHLOROACETANILIDE HERBICIDES

Major Field: Environmental Engineering

Biographical:

Personal Data: Born in Daegu, South Korea on September 13, 1982, the daughter of John and Ginger Kana.

Education: Received Bachelor of Science degree in Civil and Environmental Engineering from Oklahoma State University, Stillwater, Oklahoma in May 2005. Completed the requirements for the Master of Science degree with a major in Environmental Engineering at Oklahoma State University in July 2007.

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Professional Memberships: Chi Epsilon, Tau Beta Pi, Phi Kappa Phi, Phi Eta Sigma, ASCE

Name: Angela Kana Date of Degree: July, 2007

Institution: Oklahoma State University Location: Stillwater, Oklahoma

Title of Study: QUANTITATIVE STRUCTURE-ACTIVITY RELATIONSHIP: PREDICTION OF ANAEROBIC TRANSFORMATION OF CHLOROACETANILIDE HERBICIDES

Pages in Study: 133 Candidate for the Degree of Master of Science

Major Field: Environmental Engineering

Scope and Method of Study: The purpose of this work is to perform a preliminary analysis for future use in a full-scale quantitative structure-activity relationship (QSAR) analysis to ultimately predict the anaerobic transformation rates of chloroacetanilide herbicides with bisulfide and denitrifying bacteria and to elucidate degradation mechanisms and pathways. Chem Draw 10.0®, Chem3D Pro 10.0®, and Gaussian 03®were chemical software programs used to compute the data used in the statistical analysis of this study.

Findings and Conclusions: A preliminary analysis of quantitative structure-activity relationships was conducted over five selected chloroacetanilide herbicides: alachor, acetochlor, butachlor, metolachlor, and propachlor. Multiple structural, thermodynamic, atomic, electronic and steric descriptors were examined in regards to their correlations with nitrate-reducing rate constants and bisulfide reaction rate constants. Experimental results suggested there are various potential descriptors to predict the activity of these herbicides with bisulfide. Most notably, the descriptors carbonyl-carbon charge, Connolly excluded solvent volume, and molecular weight displayed the highest correlations for the bisulfide reaction. A limited number of descriptors were found to correlate well with the nitrate-reducing rate constants. Future areas of research might include further laboratory testing of descriptors, additional chloroacetanilides to examine, and testing to isolate denitrifying bacteria capable of degrading these herbicides.

Advisor's Approval: _____

Dr. Gregory Wilber