DEVELOPMENT AND COMPARISON OF 17β-ESTRADIOL SORPTION ISOTHERMS FOR THREE AGRICULTURALLY PRODUCTIVE SOILS FROM DIFFERENT PHYSIOGRAPHIC REGIONS IN VIRGINIA

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Abstract

Natural steroid estrogens such as 17β-estradiol in low nanogram per liter concentrations can adversely affect the reproductive health of aquatic organisms. The overall goal of this research was to quantify the sorption of 17β-estradiol to three soils considered to be agriculturally productive from different physiographic regions in Virginia to aid in modeling the concentration of estrogens available for transport in runoff from agricultural fields.

Batch equilibrium experiments were conducted with various concentrations of 17β-estradiol (E2) in a background solution of 5 mM calcium chloride and 100 mg/L sodium azide added to four separate soil samples representative of productive agricultural soils from three different physiographic regions of Virginia. Groseclose loam, Myatt sandy loam and Cecil loam were supplied by the Crop and Soil Environmental Sciences Department at Virginia Tech. All soils were collected from the plow layer (0 to 15 cm) except for an additional Cecil soil sample from the Bt horizon. The concentration of E2 in the liquid phase was measured by gas chromatography/mass spectrometry (GC/MS) and was used to find the time to reach equilibrium and to develop sorption isotherms for each soil.

The time required to reach equilibrium for all soils was less than 24 hours. A linear isotherm provided the best fit to model the sorption of E2 to Cecil and Myatt soils (R^2 = 0.94 and 0.96, respectively). For Groseclose soil, the general form of the Freundlich isotherm fit best (R^2 = 0.98), although the linear isotherm also provided a good fit (R^2 = 0.93). The sorption of E2 to agricultural soil appears to be related to the organic carbon content of each soil (Pearson coefficient, 0.82). Attempts to analyze and create isotherms for conjugated E2 by deconjugating with metholysis were unsuccessful.

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Chapter 1: Introduction

Investigations of agricultural nonpoint source (NPS) pollution typically focus on a relatively narrow range of targeted toxic and nutrient compounds. However, regular application of pesticides, irrigation water, soil amendments, and fertilizers, including animal manure, may result in the transport of numerous other organic chemicals into surface waters via runoff and potentially into ground water via leaching. One group of organic chemicals that has garnered attention in recent years is commonly called endocrine disruptors. These compounds can be any of a number of chemicals that interact with the endocrine, or hormonal, system to mimic or block receptor binding, or alter the rate of hormonal synthesis or metabolism (Myers et al., 2001). US EPA (1998a) defines endocrine disrupting chemicals (EDCs) as "exogenous agents that interfere with the production, release, transport, metabolism, binding, action or elimination of the natural hormones in the body responsible for the maintenance of homeostasis and the regulation of developmental processes."

The group of recognized EDCs includes pesticides, polychlorinated biphenyls (PCBs), dioxins, and steroid hormones. Steroid hormones of interest include estrogens such as estrone and estradiol, progesterone, testosterone, and cortisol. Estrogens in the environment are of particular concern because they are bioactive at low concentrations. Estrogenic compounds in surface water can have a number of effects on aquatic species, including inducing vitellogenin (VTG) synthesis in male fish. Vitellogenin is an egg yolk precursor protein associated with female fish. Intersexuality during sexual differentiation has also been reported (Tyler and Routledge, 1998). Although estrogens are naturally present in the environment, increased estrogen concentrations have been measured in surface water and ground water from wastewater treatment effluent and runoff from agricultural fields (Peterson et al., 2000; Kolpin et al., 2002).

Research has focused on estrogens released from point sources, specifically wastewater treatment plants, into surface water. Limited research has been done on the environmental fate and persistence of these compounds in agricultural soils where manure was land applied (Nichols et al., 1997; Finlay Moore et al., 2000). The amount of estrogen in runoff is a function not only of the amount applied but also of the rate of degradation and sorption of the compound to soil. A few studies have been done on a limited range of soils to examine the sorption of free estrogen to

soil. The sorption of free estrogens to soils and sediments appears to be highly dependent on the soil's characteristics. The conjugated forms of estrogens, glucuronides and sulfates, have been largely ignored when sorption was analyzed.

Conjugated forms have also been ignored when looking at the stability of estrogens in agricultural soil, although a few studies (e.g., Colucci et al., 2000) have been done on the stability and sorption of estrogens in agricultural soil. While the rate of degradation of the conjugated forms of estrogen under typical manure handling procedures has not been determined, it can be predicted that conjugated forms will degrade to the free and bioactive form (Layton et al., 2000). Studies on conjugated estrogens in wastewater treatment plants found a decrease in conjugated estrogen concentration in the effluent when compared to the influent (Gentili et al., 2002). Although the deconjugation reactions will most likely proceed quickly, the conjugated form of estrogen is not expected to sorb to soil to the extent that the free (unconjugated) form does. This increases the risk of conjugated estrogens reaching surface water. Conjugated estrogens could then be deconjugated to the bioactive free form.

Predicting the environmental fate of estrogens from land applied manure or grazing operations is complicated because of the degradation of estrogens in the presence of microorganisms. More studies on sorption and degradation need to be done to fully understand which factors are most important to predict concentrations in runoff from agricultural fields. One factor that needs to be explored is the difference in behavior in the soil environment between conjugated and free forms of estrogen.

1.1. Objectives

The overall goal of this research was to further evaluate the environmental fate of steroid estrogens in agricultural waste applications. Specifically, the objectives were to:

1. Investigate methods used to measure estrogens and develop a method using GC/MS to measure free 17β-estradiol and conjugated 17β-estradiol concentrations in an aqueous soil matrix; and
2. Develop isotherm equations to describe the sorption of estrogen to soil in the absence of biodegradation.

1.2. Research Hypotheses

1. A measurable amount of estrogen sorbs to each soil.
2. The aqueous concentration of estrogen is related to the sorbed concentration of estrogen and can be described by an isotherm equation.
3. The sorption of conjugated estrogens to soil is significantly less than the sorption of free estrogens.
4. The sorption of estrogens is positively correlated to the organic carbon content of soil.

Chapter 2: Literature Review

This chapter examines the concentrations of estrogens that have been found in surface waters, wastewater treatment plant effluent, and livestock wastes. It also summarizes previous studies on the environmental fate of estrogens including the sorption of estrogens to soil. Methods that can be used to quantify estrogens are discussed in this review; focus is placed on laboratory techniques used to evaluate estrogens with gas chromatography-mass spectrometry (GC/MS). These techniques include extraction, deconjugation of conjugated estrogens, and derivatization.

2.1. Endocrine Disrupting Chemicals

Endocrine disruptors are a group of compounds that interact with the endocrine system to mimic or block receptor binding, or alter the rate of hormone synthesis or metabolism (Myers et al., 2001). Compounds with reported endocrine disrupting properties include organtin compounds (pesticides), polychlorinated and polybrominated compounds, alkylphenolic compounds, bisphenolic compounds, phthalate esters, and steroid hormones. Natural steroid hormones such as estrone (E1) and 17β-estradiol (E2) can have dangerous endocrine disrupting effects to humans and wildlife at concentrations as low as picograms or nanograms per liter of water (Colborn et al., 1993).

Reproductive and developmental effects in fish have been found to result from exposure to ambient concentrations of endocrine disrupting chemicals present in typical British rivers (Jobling et al., 1998). Increased steroid estrogen concentrations in surface water have resulted in a high incidence of intersexuality in wild populations of riverine fish (roach; *Rutilus rutilus*) and widespread sexual disruption in wild populations (Jobling et al., 1998). Estrogenic substances can increase the induction of the yolk precursor protein, vitellogenin (Vtg), in male fish. Vtg concentrations in male fish, such as the zebrafish (*Danio rerio*), are now being used as a biomarker to indicate estrogen contamination in waters (Rose et al., 2002).

When analyzing the estrogenic activity of various compounds, it is typical to compare the estrogenic activity of a compound to that of 17β-estradiol, the most estrogenic natural steroid estrogen. The estrogenic activity of estrone, E1, is 0.21 times that of E2. The estrogenic activities of 17α-estradiol and estriol are 0.01 and 1.3×10^{-3}, respectively. Conjugated estrogens

are much less bioactive than free forms with relative estrogenic activity ratios compared to E2 ranging from 5.9 x 10^{-7} to 5.3 x 10^{-5} (Matsui et al., 2000). By comparison, two estrogenic industrial chemicals, nonylphenol and bisphenol A, have estrogenic activity ratios of 1.0 x 10^{-3} and 2.7 x 10^{-4}, respectively (Matsui et al., 2000). These ratios were calculated based on the yeast estrogen screen (YES) assay described later in this review.

2.2. Chemistry of Estrogens

The molecular structures of natural steroid estrogens including 17β-estradiol (E2 or estradiol), estrone (E1), and estriol (E3) all contain an aromatic ring (Figure 2-1). The structures of these three estrogens differ in the functional group attached in the C-16 and C-17 positions. Estriol has hydroxyl groups at both the C-16 and C-17 positions. Estrone has a carbonyl group at the C-17 position and estradiol has a hydroxyl group at the C-17 position.

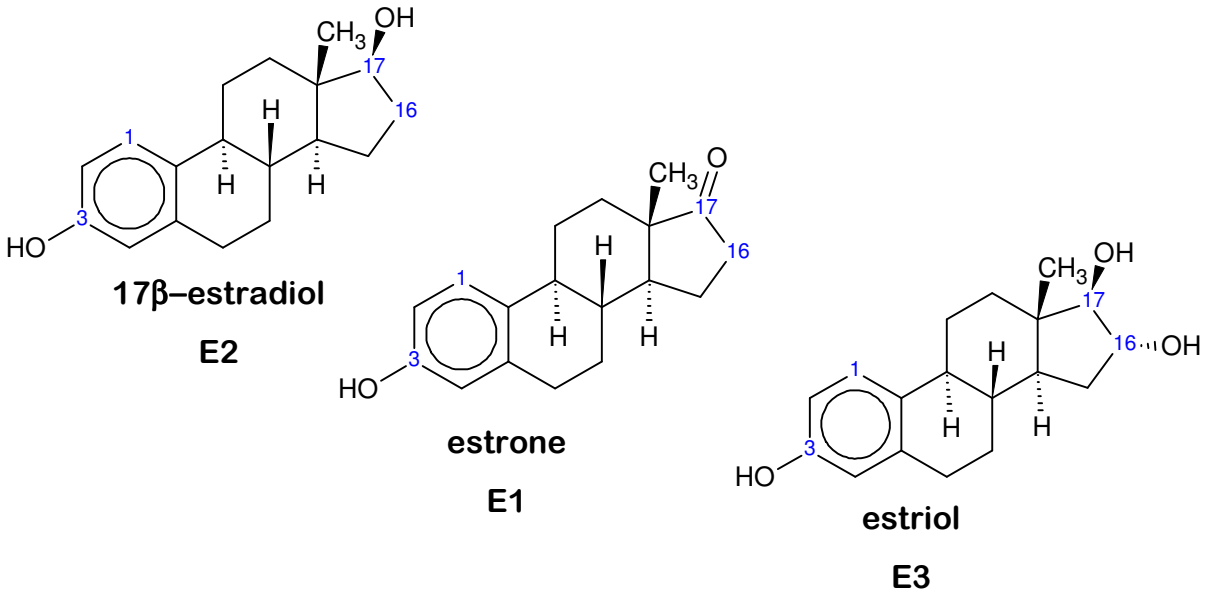

Figure 2-1. Molecular structures of 17β-estradiol (E2), estrone (E1), and estriol (E3).

The β configuration of estradiol occurs when the hydroxyl group at position C-17 of the molecular structure of estradiol points downward from the molecule. Estradiol has an alternate steriochemical arrangement where the hydroxyl group at C-17 points upward from the molecule in the α configuration (Figure 2-2).

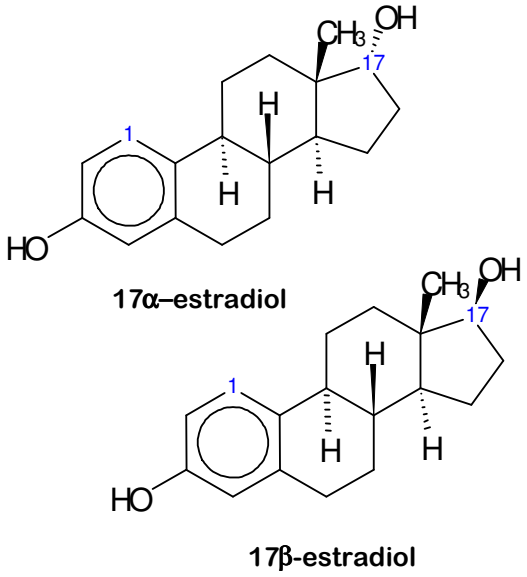

Figure 2-2. Molecular structures of sterioisomer forms of estradiol.

Conjugated estrogens have the same molecular structure as the free form with the exception of a sulfate and/or a glucuronide group substituted at the C-3 and/or the C-17 position. Two conjugated forms of E2 are shown in Figure 2-3; these are not the only conjugated forms of E2.

In general, free estrogens are non-volatile, have low solubility in water, and are moderately hydrophobic weak acids (Hanselman et al., 2003). Physiochemical properties of estradiol, estrone, and estriol are given in Table 2-1. Physiochemical data for conjugated estrogens were not found in the literature, but conjugated estrogens are expected to have a greater aqueous solubility due to their polar functional groups (Hanselman et al., 2003).

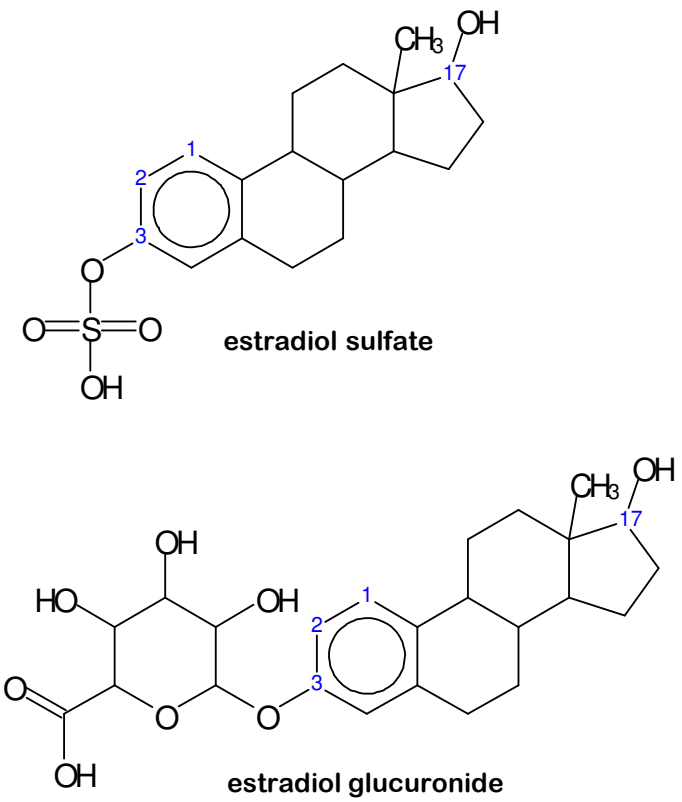

Figure 2-3. Molecular structures of two conjugated forms of estradiol.

Table 2-1. Selected physiochemical properties of estrogens (Hanselman et al., 2003)*

Property	Estradiol $C_{18}H_{24}O_2$	Estrone $C_{18}H_{22}O_2$	Estriol $C_{18}H_{22}O_3$	Reference
Molecular weight (g/mol)	272.4	270.4	288.4	(Lai et al., 2000) (Tabak et al., 1981)
Solubility in water (mg/L)	3.9-13.3	0.8-12.4	3.2-13.3	(Hurwitz and Liu, 1977) (Batra, 1975)
Vapor pressure (Pa)	3×10^{-8}	3×10^{-8}	9×10^{-13}	(Lai et al., 2000) (Lai et al., 2002) (Holthaus et al., 2002)
Log K_{ow}	3.1-4.0	3.1-3.4	2.6-2.8	(Lai et al., 2000) (Lai et al., 2002)
pK_a	10.5-10.7	10.3-10.8	10.4	(Hurwitz and Liu, 1977) (Lewis and Archer, 1979)

K_{ow} – octanol-water partition coefficient, K_a-acid ionization constant.
*Reprinted with permission from (Hanselman et al., 2003). Copyright (2003) American Chemical Society.

2.3. Laboratory Assays for Identifying Estrogens

Immunoassays and gas chromatography/mass spectrometry (GC/MS) are two of the most common methods used to measure estrogen concentrations in the laboratory in recent studies. Immunoassays are usually less expensive than GC/MS methods, but are susceptible to cross reaction and false positives. Mass spectrometry methods can detect low concentrations of estrogens and are more selective than immunoassay methods. This section summarizes the various methods that have been used to analyze estrogens.

2.3.1 Enzyme Immunoassays

Commercially available enzyme immunoassays (EIA) specific for the quantification of 17β–estradiol were used in recent work measuring estrogen contamination in runoff from applied animal manure (Nichols et al., 1997; Finlay Moore et al., 2000; Raman et al., 2004). The specificity, sensitivity, low cost, and speed of EIA made it a popular assay for estrogen analysis (Nichols et al., 1997; Finlay Moore et al., 2000). Estrogen immunoassays were initially developed for quantification of 17β–estradiol in tissue culture media, human saliva, urine, and serum (Raman et al., 2001). The application of these assays to animal wastes and other organic samples may be subject to matrix effects associated with protein binding, humic substances, and endogenous enzymes (Hanselman et al., 2004). In addition, EIA may be subject to cross–reactivity with 17α–estradiol, estrone, and estriol. Also, variations occur in kit quality between manufacturers (Raman et al., 2001; Hanselman et al., 2004). Although EIA has been used successfully in recent studies, this method may present difficulties when used with complicated matrices such as soil.

2.3.2 Gas Chromatography

Gas chromatography-mass spectrometry (GC/MS) has been used in a number of studies despite its cost and time requirements (Lai et al., 2000; Raman et al., 2001). Raman et al. (2001) analyzed estrogenic compounds using a GC/MS method in which the sample was derivatized before using GC/MS. A similar method, which also required derivatization, was proposed by Kuch and Ballschmitter (2001) using high resolution GC with negative chemical ionization MS detection (HRGC-(NCI)-MS). High sensitivity and selectivity are achieved by these methods for free estrogens, but these methods are not applicable to conjugated forms of estrogens (Isobe et

al., 2003). In order to test for conjugated estrogens using GC/MS, the estrogen must first be deconjugated using a method such as methanolysis or enzymatic hydrolysis (Tang and Crone, 1989; Hoffmann et al., 1997). Unlike EIA, GC/MS methods allow for detection of more than one target compound in each sample. This is useful when analyzing compounds that might degrade, such as estrogens.

Estrogens need to be derivatized before they can be analyzed with GC/MS. Derivatization deactivates the hydroxyl and keto functional groups on estrogenic steroids, making the compounds more volatile. Two basic derivatization procedures for free estrogens involve silylation and use of pentafluorobenzyl compounds. A list of chemicals that have been used for derivatization of estrogens for GC/MS analysis is in Table 2-2.

Table 2-2. List of chemicals used for derivatization of estrogens for analysis by GC/MS.

Abbreviation	Chemical Name	Reference
MSTFA	N-methyl-N-(trimethylsilyl)-trifluoroacetamide	(Ternes et al., 1999; Lai et al., 2000; Spengler et al., 2001; Andersen et al., 2003; Ding and Chiang, 2003; Quintana et al., 2004)
BSTFA	O-Bis(trimethysilyl)trifluoracetamide	(Desbrow et al., 1998; Raman et al., 2001; Ding and Chiang, 2003; Raman et al., 2004)
TMSI	Trimethylsilylimidazole	(Ternes et al., 1999; Lai et al., 2000; Nakamura et al., 2001)
TMCS	Trimethylchlorosilane	(Desbrow et al., 1998; Ding and Chiang, 2003)
DTE	Dithioerythritol	(Ternes et al., 1999)
PFBBr	Pentafluorobenzyl bromide	(Cathum and Sabik, 2001; Nakamura et al., 2001; Fine et al., 2003)
TEA	Trietylamine	(Xiao et al., 2001)
SIL A	Dimethyldichlorosilane in toluene	(Belfroid et al., 1999; Croley et al., 2000; Huang and Sedlak, 2001)

The silylation derivatization method forms trimethylsilyl (TMS) ethers of the hydroxyl and keto functional groups (Kolpin et al., 2002). Various mixtures of two silylating agents, MSTFA and BSTFA, have been used for estrogen derivatization procedures. A mixture of MSTFA/TMSI/DTE at a 1000:2:2 volume to volume to weight ratio was used in a number of studies (Ternes et al., 1999; Lai et al., 2000; Andersen et al., 2003). However, Quintana et al. (2004) determined TMSI was unnecessary. Ding and Chiang (2003) evaluated the use of various

silylating agents and recommended the use of BSTFA with 1% TMCS for derivatization of estrogenic steroids over the use of MSTFA. With this derivatization mixture, secondary derivatives were reported to make up only 3% or less of the derivatized sample (Ding and Chiang, 2003). Raman et al. (2001; 2004) also used BSTFA, while Hoffmann et al. (1997) and Desbrow (1998) used a mixture BSTFA and TMCS to derivatize. The time required for derivatization procedures using silylation varied from 30 to 100 minutes and the temperature varied from 60 to 85 ^{o}C.

Pentafluorobenzyl (PFB) compounds were used in other studies as derivatizing agents. Cathum and Sabek (2001) used PFB bromide, 5% 18-crown-6 in acetone, while Xiao et al. (2001) used a mixture of 5% TEA in acetonitrile and PFB chloride. Nakamura et al. (2001) and Fine et al. (2003) used a two-step derivatization with 10% aqueous potassium carbonate and acetone solution of 5% PFB bromide followed by drying, washing, and extracting and adding TMSI.

There are other methods used to derivatize estrogens for GC/MS analysis. Among the other compounds that have been used are heptafluorobutyric anhydride, pentafluorpropionic acid anhydride, and SIL A reagent (Belfroid et al., 1999; Croley et al., 2000; Huang and Sedlak, 2001).

2.3.3 Other Methods to Quantify Estrogens

Other methods to measure estrogen concentration in various matrices include liquid chromatography, radioimmunoassay (RIA), fluorimetry, and colorimetry. Both high performance liquid chromatography (HPLC) and liquid chromatography tandem mass spectrometry (LC-MS/MS) have been used successfully to measure estrogens (Belfroid et al., 1999; Isobe et al., 2003; Lee et al., 2003; Tashiro et al., 2003; Lagana et al., 2004). The use of LC-MS/MS for evaluation of both conjugated and free estrogens eliminates the need for the deconjugation and derivatization steps required for GC/MS (Isobe et al., 2003). Although immunoassays such as ELISA (enzyme linked immunoassay) and RIA (radioimmunoassay) offer relatively simple protocols and high sensitivity, neither method is able to analyze conjugated estrogens. GC/MS procedures, while sensitive and selective, require deconjugation and derivatization before analyzing conjugated forms of estrogens. It is unclear whether methods used to deconjugate were successful because of the low concentrations of conjugated estrogens

in the samples (Belfroid et al., 1999; Finlay Moore et al., 2000). Belfroid et al. (1999) attempted to analyze glucuronide conjugates with GC-MS/MS after enzymatic deconjugation, but found estrogen glucuronides above the detection limit in only one sample. In studies where conjugated estrogens are expected to be present, LC-MS/MS methods allow direct measurement of conjugated hormones.

Older studies of estrogens used colorimetric techniques that lacked sensitivity and selectivity for estrogens (Cohen, 1969). These methods were replaced by highly sensitive RIA and EIA. However, both methods can suffer from cross reactivity (Hanselman et al., 2003).

All of the methods listed above have drawbacks and advantages, but have produced comparable results. Vos (1996) compared ELISA (a commercially available EIA) and RIA and found satisfactory specificity, accuracy, precision, and sensitivity with ELISA. ELISA also compared well to GC/MS methods for the measurement of free estrogens (Raman et al., 2004). The deciding factor for which method to use is often the availability of equipment. RIA requires special laboratory procedures to work with radiation and EIA requires a plate reader. GC/MS and LC/MS-MS equipment can be prohibitively expensive for laboratories that are not already equipped.

2.3.4 Recombinant Yeast Estrogen Screen (YES) Assay

In cases where a measurement of the total estrogenic activity of a sample is desired, the recombinant yeast estrogen screen (YES) can be used. The YES assay utilizes an estrogen-inducible expression system in yeast cells *Saccharmyces cerevisae* (Routledge and Sumpter, 1996). The yeast cells also contain expression plasmids carrying the reporter gene *lac-Z,* which is used to measure the receptors' activity. When the cells are incubated in the presence of estrogenic compounds, the *lac-Z* product, β-galactosidase, is secreted into the medium and causes the chromatogenic substrate, chlorophenol red –β-galactopyranoside (CPRG), to turn red (Holbrook et al., 2002). This color change can be quantified by measuring absorbance. This assay does not differentiate between estrogenic compounds, but is useful to measure the total estrogenic activity.

2.4. Sources of Estrogens in the Environment

Although estrogens are released into the environment from a variety of natural sources, negative environmental effects attributed to relatively high estrogen concentrations have been documented downstream of wastewater treatment facilities. This section discusses briefly the concentrations of estrogens released from wastewater treatment plants. It is harder to attribute estrogen pollution to nonpoint sources (NPS) such as land applied manure and animal grazing operations. The concentrations of estrogens in various forms of animal waste are discussed here.

2.4.1 Estrogens from Wastewater Treatment Plants

While the fate of estrogenic compounds in wastewater treatment systems remains an area of debate, surveys of wastewater treatment plant effluent have provided evidence that some natural steroid estrogens survive the treatment process. These concentrations ranged from <0.15 to 64 ng/L (Table 2-3). The large range of concentrations is most likely due to differences in treatment processes, influent concentrations, and sampling time of year between studies. Because estradiol is bioactive at concentrations < 1 ng/L, relatively small concentrations can cause endocrine disrupting effects when released steadily into surface water (Colborn et al., 1993).

Table 2-3. Ranges and median concentrations of estradiol (E2) and estrone (E1) in wastewater effluents.

	Concentration range (median) ng/L		
Location	E2	E1	Reference
UK	2.7-12 (5.9)	1.4-76 (9.9)	(Desbrow et al., 1998)
Germany/Canada	<1-64 (6, <1)[a]	--	(Ternes et al., 1999)
Netherlands	<0.4-12 (0.7)	<0.4-47 (4.5)	(Belfroid et al., 1999)
USA	<0.01-3.7 (0.7)	--	(Snyder et al., 1999)
USA	1.6-7.4	--	(Huang and Sedlak, 2001)
UK	1.6-7.4 (2.6)	6.4-29 (9.8)	(Xiao et al., 2001)
Italy	0.44-3.3 (1.0)	0.43-18 (1.3)	(Baronti et al., 2000)
Germany	<0.15-5.2 (0.4)	<0.1-18 (1.5)	(Kuch and Ballschmiter, 2000)

[a] Median values calculated separately for data from German and Canadian WWTP

2.4.2 Estrogens from Livestock

All species, sexes, and classes of livestock excrete steroids in both urine and feces. The steroid hormones in feces exist primarily in the free or unconjugated form, while the steroid hormones in urine are primarily in the conjugated form (Shore and Shemesh, 2003). In manure storage, as in wastewater treatment facilities, conjugated estrogens are expected to be rapidly deconjugated by microorganisms in feces (Layton et al., 2000).

Different species of farm animals can excrete different forms of estrogens by different routes. Cattle excrete mostly 17α-estradiol, 17β-estradiol, and estrone, while swine and poultry excrete 17β-estradiol and estrone but rarely excrete 17α-estradiol (Lange et al., 2002). Poultry and swine primarily excrete estrogens in urine, while much of the estrogen excreted by cattle occurs in feces. Estimated rates of urinary estrogen excretion per 1000 kg LAM (live animal mass) for dairy cattle, sows, and chickens are shown in Table 2-4. The values in Table 2-4 do not discriminate among various forms of estrogen. Very large ranges were found for pregnant animals because estrogen excretion can vary widely during different stages of pregnancy.

Table 2-4. Estimated rates of estrogen excretion. (Adapted from Hanselman et al., 2003)*

	Urinary excretion rate µg/day	Feces excretion rate µg/day
Pregnant Dairy Cattle	640-18300	300-12600
Nonpregnant Dairy Cattle	460-540	390-800
Pregnant Sows	0-214000	320-1680
Nonpregnant Sows	0-1100	30-900
Laying Hen Chickens	1350-3930	--
Nonlaying Hen Chickens	380-1950	--

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Chicken manure has much higher concentrations of estrogenic hormones than other livestock manures because birds have higher concentrations of hormones than mammals (Shore et al., 1995). The estrogen content varies with the life-cycle stage of the chickens. Shore et al. (1993; 1995) found total estrogen content in poultry litter ranging from 65 ± 7 µg/kg dry weight for female immature broilers (7 weeks) to 533 ± 40 µg/kg dry weight for 5-month old layers and from 14 ± 4 µg/kg dry weight for male immature broilers to 93 ± 13 µg/kg dry weight for 5-month old roosters. Finlay-Moore et al. (2000) and Nichols et al. (1997; 1998) reported concentrations in broiler litter of 17β-estradiol ranging from 33 to 904 µg/kg.

In order to estimate the probability of estrogens in runoff and ground water from land applied manure, the concentrations of both free and conjugated estrogens in livestock waste prior to land application need to be known, taking into account the degradation of estrogens in storage, handling, and treatment practices. Raman et al. (2004) surveyed dairy and swine waste storage facilities to determine free estrogen concentrations under different manure handling procedures. The results of this study (Table 2-5) indicate that the amount of estrogen in manure is largely dependent on manure handling procedures.

Table 2-5. Concentration of estrone and estradiol in various swine and dairy manure storage facilities (Adapted from Raman et al., 2004).* All concentrations were reported as ppb.

	Range of Mean Concentrations (ppb)	
Sample type	Estrone	Estradiol
Swine finishing lagoon	5.9-11	1.8-3.3
Swine finishing hoop structure	30-78	32-49
Swine farrowing lagoon	5.9	3.9
Swine farrowing pit	31-150	12-29
Dairy dry-stack semisolid	13-80	12-27
Dairy dry-stack solid	12-37	5.8-25
Dairy holding pond	2.5-5.6	0.8-1.9

*Reprinted with permission from (Raman et al., 2004). Copyright (2004) American Chemical Society

Data from the cited studies are limited in scope. The concentration and form of estrogen in manure depend on the species, sex, life cycle stage, and treatment of the manure. In order to gain a reliable estimate of estrogen concentration in various land applied manures, more extensive studies are needed that include a wide range of waste treatment and handling practices.

Studies indicate that estrogens can be transported to surface water via runoff after manure has been land applied. Finlay-Moore et al. (2000) found sizable edge-of-field losses of estradiol (20-2530 ng/L) from grasslands amended with broiler litter. Nichols et al. (1997) illustrated that runoff of E2 was significantly influenced by the rate of applied poultry litter and Shore et al. (1995) found increased estrogen content in streams containing runoff from fields where chicken litter with 28 μg/kg total estrogen was applied.

Two studies examined ways to minimize estrogen concentrations in runoff. Nichols et al. (1997) treated broiler litter with alum and reduced E2 concentrations in runoff by 42%. Nichols et al. (1998) found that grass filter strips of 6-m (20 ft), 12.2-m (40 ft), and 18.3-m (60 ft) width

reduced transport of E2 by 79%, 90%, and 98%, respectively, from fields. This indicates that the use of some best management practices (BMPs) designed to minimize the transport of nutrients, such as filter strips, can also be effective in minimizing the transport of estrogens.

2.5. Estrogens in the Environment

This section details the concentrations of estrogens that have been found in surface and ground water and summarizes studies of the environmental fate of steroid estrogens. Focus is placed on two aspects of the environmental fate of estrogen, degradation and the sorption of estrogen to soils and sediments.

2.5.1 Estrogens in Surface and Ground Water

Current research indicates the presence of bioactive concentrations of estrogens in surface water. Three naturally occurring estrogens, estradiol, estrone and estriol, were included in a survey conducted by the USGS of sites susceptible to contamination from human, industrial, and agricultural wastewater (Kolpin et al., 2002). The concentrations reported by Kolpin et al. (2002) are up to two orders of magnitude higher than the concentrations reported by other studies (Table 2-6). Despite the discrepancy, the concentrations reported are within the concentration range that can cause physiological effects (Shore and Shemesh, 2003). Endocrine disruption has been reported in fish at concentrations as low as approximately 1 ng/L (Routledge et al., 1998). Table 2-6 summarizes the concentrations of E1 and E2 reported in various countries.

Table 2-6. Summary of concentrations of estrone and estradiol found in surface water.

Location	Sample type	Concentration Range (ng/L) (median)		Reference
		Estrone	Estradiol	
USA	streams	<LOD-112 (27)	<LOD-93 (9)	Kolpin et al. (2002)
USA	Sacramento River Delta	NM	0.08 ± 0.02	Huang and Sedlak (2001)
USA	Colorado River Delta	NM	0.8 ± 0.24	Huang and Sedlak (2001)
Canada	St. Lawrence River	<2-6	<2-38	Cathum and Sabik (2001)
UK	Thames	0.2-10 (6.6)	0-7.1 (1.9)	Xiao et al. (2001)
Germany	river	0.10-4.1 (0.40)	0.15-3.6 (0.3)	Kuch and Ballschmiter (2001)
Italy	Tiber River	5-12 (8)	2-6 (4)	Lagana et al. (2004)
Italy	Tiber River	1.5	0.11	Baronti et al. (2000)
The Netherlands	coastal/estuarine and rivers	<0.3-5.5 (<LOD)	<0.1-3.4 (0.3)	Belfroid et al. (1999)
Japan	109 major rivers	NM	<LOD-27 (2.1)[ab] <LOD-24 (1.8)[ac]	Tabata et al. (2001)
Japan	Manko tidal flat	2.2-53.0 (8.6)	<LOD-8.7 (<LOD)	Tashiro et al. (2003)
Japan	Tamagawa River	3.4-6.6 (3.8)	0.6-1.0 (0.8)	Isobe et al. (2003)
Japan	Lake Kasumigaura	0.2-0.8 (0.7)	<LOD	Isobe et al. (2003)

[a]Arithmatic mean (± standard deviation in paratheses), [b]Summer sampling, [c]Autumn sampling
LOD—limit of detection, NM—not measured

Most of the studies listed in Table 2-6 included only the free forms of 17β-estradiol and estrone. Belfroid et al. (1999) included the conjugated glucuronide form of estrogen but found no estrogen glucuronides above the limit of detection downstream of the effluent from five wastewater treatment plants. Isobe et al. (2003) also included conjugated forms; while they found no detectable concentrations of estrogen glucuronides, they found concentrations of estrone sulfate of 0.3-0.8 ng/L and estradiol sulfate of <LOD-0.4 ng/L in surface water. Estrogen sulfates are expected to be more persistent in the environment than glucuronides (Vos, 1996).

Only the survey by the USGS reported 17α-estradiol concentrations in surface water. 17α-estradiol was found in 5.7 % of the streams sampled, while 17β- estradiol was found in 10-10.6% of the streams depending on the E2 quantification method used (Koplin et al., 2002). The presence of 17α-estradiol can be interpreted as an indicator of contamination by agricultural sources, specifically from cattle as most other animals excrete estradiol in the 17β configuration (Lange et al., 2002).

Estrogens have also been found in lake water that supplies drinking water to Israel. Estrogen concentrations in the Sea of Galilee reached 20-22 ng/L in drought conditions and dropped to 4 ng/L in non-drought conditions (Shore et al., 1995). After filtration and chlorination, drinking water contained 11-19 ng/L in the drought conditions but dropped to < 0.5 ng/L in the normal year (Shore et al., 1995). This indicates the possibility that excess estrogens released into water supplies are not always removed by drinking water treatment.

Studies that surveyed E2 levels in ground water illustrate that E2 can also be found in ground water especially in karst areas. A survey of five springs in a mantled karst landscape in Northwest Arkansas found levels of E2 ranging from 6 to 66 ng/L in ground water suspected to be contaminated by animal waste (Peterson et al., 2000). Wicks et al. (2004) found levels of E2 ranging from 13 to 80 ng/L in a survey of eight springs in the Ozark Plateau Aquifer (OPA).

2.5.2 Estrogen Degradation in the Environment

The degradation of free estrogens, in particular 17β-estradiol, has been found to be rapid in sediment and water. E2 is oxidized to E1, which can be further converted to estriol (E3) (Ying et al., 2002). Microorganisms from English rivers degraded E2 to E1 and E1 to E3 with half lives ranging from 0.2-9 days when incubated at 20 ^{o}C (Jurgens et al., 2002). The degradation of conjugated estrogens in soil and water is not well studied. Although conjugated estrogens are not bioactive compared to free estrogens, they can be converted to free estrogens by bacteria in the environment (Ternes et al., 1999; Baronti et al., 2000).

Colucci et al. (2001) studied the decrease in extractable/bioavailable concentrations and mineralization of ^{14}C-17β-estradiol in loam, sandy loam, and silt loam soils from Canada. The biological activity of estrogens was determined by a yeast (YES) assay and was found to rapidly dissipate in all soils. Colucci et al. (2001) found that 17β-estradiol was rapidly converted to less bioactive estrone. Because autoclaving did not prevent the oxidation of estradiol to estrone, the

degradation process was assumed abiotic. In addition to the degradation of estradiol in this experiment, the decrease in extractable concentrations by mineralization (cleavage of the phenolic ring) was measured and no consistent effects of soil pH, organic matter, or texture on the mineralization process were found. Although no studies were found that evaluate the stability of conjugated estrogens in soil, the rate of deconjugation of estrone glucuronide was found to occur very rapidly (~30 minutes) in a fecal environment (Vos, 1996). Estrone sulfate was found to be much more stable and was not deconjugated over this time period.

2.5.3 Sorption of Estrogens to Soil and Sediment

Sorption to soil is an important component of the environmental fate of estrogens because it can slow the transport to surface water, allowing the estrogen to be degraded to non-bioactive compounds. Studies, such as Yu et al. (2004) and Lai et al. (2000), indicate that free estrogens are strongly sorbed to soil. This is supported by column experiments conducted by Casey et al. (2003) that indicated that no estrogen was leached through the soil column for five different soils. However, research indicates that free estrogens present in chicken litter have enough mobility to be transported in runoff from fields (Finlay Moore et al., 2000; Shore and Shemesh, 2003). No studies were found in the literature that evaluated the sorption of conjugated estrogens.

Lee et al. (2003) suggested that hydrophobic partitioning into organic carbon is the dominant mechanism for E2 sorption to soils. Hydrophobic, or linear, partitioning refers to the separation of nonpolar organic molecules out of the polar soil solution and into the nonpolar organic matter of the soil (Bohn et al., 2001). This mechanism is synonymous with the partitioning of nonpolar compounds into a nonpolar phase such as octanol. Polar compounds are more strongly attracted by hydrogen bonding to the O_2 on silicated surfaces, edge hydroxyls and carboxyl, hydroxyl and amino groups of organic matter.

If hydrophobic partitioning is the dominant sorption mechanism, the sorption of E2 to soils is expected to be highly correlated to organic matter or organic carbon content. Casey et al. (2003) and Lai et al. (2000) both reported correlations between the sorption of E2 and organic matter and total organic carbon (TOC) content of soils and sediment, respectively (correlation coefficients ranging from 0.79-0.94). However, estradiol sorption to iron oxide and clay minerals in the absence of organic carbon indicates that hydrophobic partitioning cannot be the

only sorption mechanism (Lai et al., 2000; Van Emmerik et al., 2003). Lai et al. (2000) reported 40% of the estrogen added to iron oxide (no organic carbon) was sorbed.

An adsorption isotherm is a graph of the equilibrium amount of a compound adsorbed plotted against the equilibrium solution concentration of the compound at a fixed temperature (isotherm), pressure, and solution chemistry (pH and ionic strength) (Essington, 2004). When the exact mechanism of partitioning is not known, adsorption is referred to as sorption. Isotherms can be used to model the equilibrium partitioning between sorbed and aqueous phases to estimate the amount of contaminant that will be available for transport in surface runoff.

A common method for developing soil sorption isotherms is described by Essington (2004). Sorption isotherms can be developed using batch equilibrium experiments where a mass of soil is in contact with a volume of solution that contains an initial concentration (C_i) of the compound of interest (adsorbate). The ionic strength is selected to mimic that of the soil solution and is normally controlled by a salt common to the soil environment, such as calcium chloride ($CaCl_2$). Usually the solution pH is allowed to be controlled by the soil. The experiment is repeated using several different reaction vessels with different initial concentrations of the compound of interest that are equilibrated with a mass of soil while agitated under constant temperature. After reaching equilibrium, the solution is separated from the soil via centrifugation or filtration and the solution is analyzed for the compound of interest. The sorbed concentration can be determined using a mass balance.

The three most common isotherms used to model sorption to soils are Freundlich, linear, and Langmuir. The Freundlich isotherm is strictly an empirical relationship. The Freundlich isotherm is (Essington, 2004):

$$q = K_d c_{eq}^{\ n} \qquad \textbf{2.1}$$

Where q = sorbed concentration (mg/kg),
K_d = the Freundlich distribution coefficient,
c_{eq} = equilibrium concentration (mg/L), and
n = parameter between 0 and 1.

The constants in Equation 2.1 can be found by taking the log of both sides and then plotting the log of the equilibrium aqueous concentration by the log of the sorbed concentration.

A special case of the Freundlich isotherm occurs when n = 1. As n approaches 1, surface site homogeneity increases (Essington, 2004). The equation then becomes the linear sorption isotherm. The parameters for the linear sorption isotherm can be determined by fitting a linear regression to a plot of equilibrium concentration by sorbed concentration.

A linear isotherm is often used if hydrophobic partitioning is assumed to be the dominant sorption mechanism. If a linear isotherm can be used to describe sorption, the solubility of E2 and the octanol-water coefficient (K_{ow}) can be related to the organic carbon-normalized partition coefficient (K_{oc}) shown in Equation 2.2 (Essington, 2004):

$$K_p = K_{oc} f_{oc} \qquad \textbf{2.2}$$

Where K_p = the partitioning coefficient, and
f_{oc} = the fractional organic carbon content of the soil.

Equation 2.2 can be useful in estimating the partitioning of hydrophobic compounds such as E2 into the organic carbon of soil.

The Langmuir isotherm was originally developed as a theoretical equation describing the adsorption of gas to a solid. There are a number of assumptions associated with this equation (adapted from Essington (2004)):

- Adsorption occurs at specific sites on a surface;
- The surface is homogeneous;
- There is an adsorption maxima as the monolayer of adsorbed molecules on the surface becomes filled by adsorbate;
- The heat or energy of adsorption is constant over the entire surface and independent of temperature;
- Adsorbed species do not interact;
- The volume of the monolayer is independent of temperature; and
- Equilibrium is attained.

The Langmuir isotherm equation is (Essington, 2004):

$$q = \frac{bK_L c_{eq}}{(1 + K_L c_{eq})} \qquad \textbf{2.3}$$

Where q = sorbed concentration (mg/kg),
b = the adsorption maxima (mg/kg),
K_L = adjustable parameter (L/kg), and
c_{eq} = equilibrium concentration (mg/L).

The adsorption constant K_L can be described as a measure of the intensity of the adsorption isotherm, where K_L times b is the slope of the isotherm as c_{eq} approaches zero.

All E2 sorption studies reported in the literature have used the Freundlich isotherm to describe the sorption of E2 to soil with the exception of Casey et al. (2005). The published Freundlich parameters vary widely among different soils and sediment. It is unclear whether the wide variation in sorption isotherm parameters is due to soil properties (Figure 2-4; Table 2-7) or differences in experimental analysis. There are some discrepancies in the reported or assumed time to equilibrium. There are also differences related to degradation of E2 to E1. Many studies did not attempt to sterilize soil and the calculated sorbed concentration could include loss of E2 due to degradation (Lai et al., 2000; Casey et al., 2003; Lee et al., 2003; Brion et al., 2004).

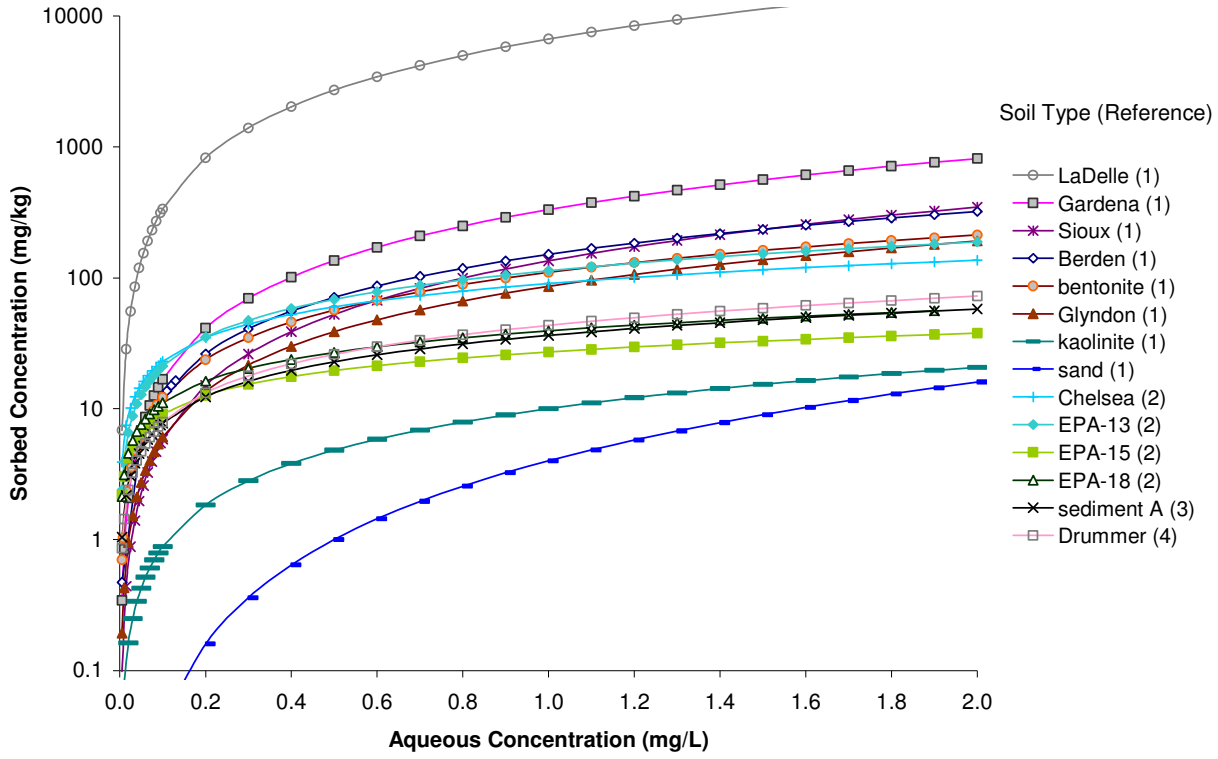


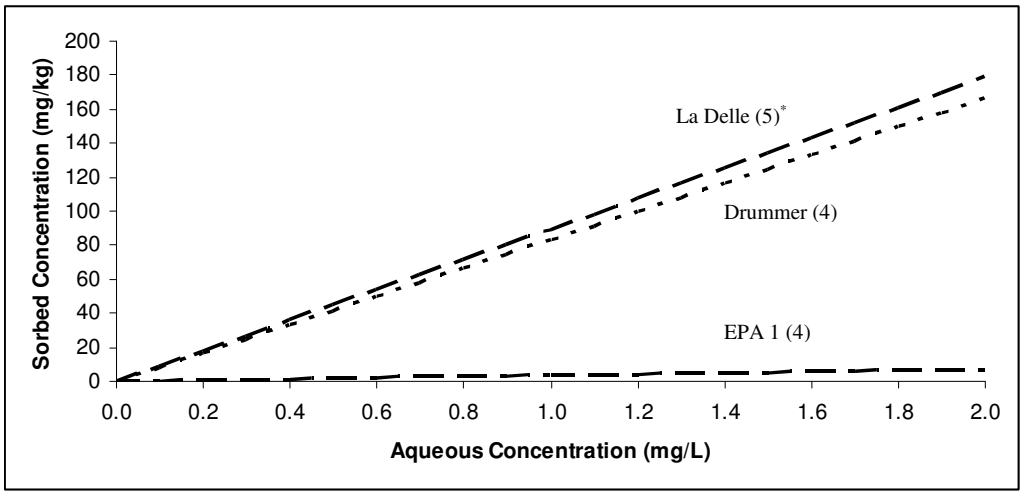
Figure 2-4. Comparison of Freundlich isotherms for E2. (1) Casey et al. (2003); (2) Yu et al. (2004); (3) Lai et al. (2000); (4) Lee et al. (2003). Soil characteristics are included in Table 2-6.

Table 2-7. Description of soils and sediments used in estradiol sorption experiments.

Soil	Description	OC (%)	SSA (m^2/g)	pH	Reference
Bearden-silty clay loam	fine-silty, mixed, superactive, frigid Aeric Calciaquolls	7.5	175	6.4	(1), (5)
Gardena-clay loam	coarse-silty, mixed, superactive, frigid Pachic Hapludolls	5.3	154	6.4	(1), (5)
Glyndon-sandy clay loam	coarse-silty, mixed, superactive, frigid Aeric Calciaquolls	3.3	123	NR	(1), (5)
LaDelle-silt loam	fine-silty, mixed, superactive, frigid Cumulic Hapludolls	9.2	151	7.9	(1), (5)
Sioux-loam	sandy-skeletal, mixed frigid Entic Hapludolls	7.5	106	7.8	(1), (5)
bentonite		0.0	654	NR	(1)
kaolinite		0.0	43	NR	(1)
Sand		0.0	NR		(1)
Topsoil	Chelsea, MI	5.45[a]	3.92	6.8[b]	(2)
EPA-13	sediment	4.5[a]	12.5	6.8[b]	(2)
EPA-15	sediment (Ohio River, IN)	0.95[a]	15.2	6.8[b]	(2)
EPA-18	sediment (Mississippi River, KY)	0.66[a]	12.6	6.8[b]	(2)
Sediment A	Blackwater Estuary sand:silt:clay 1.6:74:25	1.1[a]	NR	NR	(3)
EPA1	sand:silt:clay 94:0:6	0.22	NR	7.3	(4)
Drummer	sand:silt:clay 66:14:21	2.91	NR	7.2	(4)

[a]measured as TOC (wt%); [b]pH adjusted to 6.8; NR-not reported
(1) Casey et al. (2003); (2) Yu et al. (2004); (3) Lai et al. (2000); (4) Lee et al. (2003); (5) Casey et al. (2005)

Although the sorption of E2 to soils and sediments is described as hydrophobic partitioning, only two studies (Lee et al., 2003 and Casey et al., 2005) developed linear isotherms to describe the sorption of E2 to soils and sediment (Figure 2-5). The descriptions of these soils and sediment are included in Table 2-7. The soil with the greatest organic carbon content has the greatest partitioning coefficient and vice versa.



* K from 24-hr sampling time.

Figure 2-5. Linear isotherms developed for sorption of E2 to soils and sediments. (4) Lee et al. (2003); (5) Casey et al. (2003). Soil and sediment characteristics are included in Table 2-7.

It is difficult to attribute the reported decrease in E2 concentration in studies that only measure the estrogen content in the aqueous phase because a decrease in the aqueous phase due to degradation of estradiol can be mistaken for sorption. Colucci et al. (2001) noted the possibility of abiotic degradation when they found oxidation of estradiol in autoclaved soils. Lee et al. (2003) also warned that clay and oxide surfaces may catalyze abiotic degradation reactions of estrogens resulting in an overestimation of sorption. Casey et al. (2003) also reported a correlation between estradiol sorption and particle size, and between estradiol sorption and silt content. Although Yu et al. (2004) developed sorption isotherms, they did not identify any trends with relation to soil properties.

There are some discrepancies regarding the rate of estrogen sorption to soil. Lai et al. (2000) reported time to equilibrium on the order of one hour, while Casey et al. (2003) and Lee et al. (2003) reported time to equilibrium of 48 and 72 hours, respectively. Yu et al. (2004) reported apparent equilibrium within seven to ten days. These studies involved the sorption of estrogens to river sediments and soils with a wide range of properties. Because of this difference in sorption rates, all future studies should record data for a number of days to ensure that equilibrium has been reached.

2.6. Summary and Conclusions of Literature Review

Increased estrogen concentration in surface water can cause endocrine disrupting effects in aquatic species. Natural hormones are much more bioactive (have greater estrogenic activity) than synthetic estrogenic endocrine disrupting chemicals such as pesticides and plasticizers. Therefore, a much smaller concentration can have the same effect.

Estrogens have been found in surface water downstream of point sources such as wastewater treatment plants (Kolpin et al., 2002). While a few studies (e.g. Finlay-Moore, 2000) have found that estrogens from land applied manure are transported in runoff, it is more difficult to quantify the amount of estrogen from nonpoint sources such as land applied manure. After application, estrogens in manure can degrade, become sorbed to soil, or be transported in runoff or water leaching to ground water.

The environmental fate of estrogen that has been land applied is a subject of recent debate. Degradation of estrogens in soil has been found to be rapid, but the mechanisms of degradation are not well-understood (Colucci et al., 2001). Some research has been done on sorption of estrogens to soils, but sorption is largely dependent on the soil and there are discrepancies in reported times to equilibrium. No research has been done on sorption of conjugated estrogens to soils. Because conjugated forms are more polar, they are expected to be more mobile. The ability to quantify the sorption of free and conjugated estrogens to different soils is necessary to predict the estrogen content in runoff from grazing systems and land applied manure. In order to simplify the complex natural systems and get an accurate measure of the sorption of estrogen, experiments need to be carried out in the absence of microbial degradation. Based on a review of previous studies that quantify estrogens in various matrices, the most effective method to measure both conjugated and free estrogens would be LC-MS/MS. However, GC/MS combined with deconjugation and derivatization should provide an acceptable alternative.

Chapter 3: Methods

A series of batch equilibrium sorption experiments was conducted to determine partitioning of 17β-estradiol (E2) between soil and aqueous phases. All sorption experiments were set up as continuously shaken glass bottles with Teflon lined caps. Each bottle contained a weight of soil as the sorbent and a concentration of E2 in a background solution as the sorbate. In order to eliminate as many sources of E2 degradation as possible, the soil-water systems in this experiment were chemically sterilized to minimize biodegradation and covered to minimize photolysis. Temperature was measured at every sampling time and was kept at 22 ± 2 ^{o}C. The pH was measured at various sampling times. All sample containers were glass and all lids used had Teflon liners to minimize sorption to bottles, lids, and intermediate sample containers such as vials, test tubes, and pipettes.

3.1. Chemicals and Solutions

Stock solutions of E2 at concentrations of 100 mg/L and 500 mg/L were made by dissolving E2 in high-performance liquid chromatography (HPLC)-grade methanol. All stock solutions were stored at 4^{o}C in glass bottles with Teflon-lined caps. The bottles were wrapped in foil to minimize photolysis. Appropriate volumes of the stock solutions were added to a background solution to obtain the final solutions for each experiment. The background solution consisted of an electrolyte, calcium chloride ($CaCl_2$), at a level of 5 mM and a biological degradation inhibitor, sodium azide (NaN_3), at a concentration of 100 mg/L. The volumetric fraction of methanol in each solution was <0.5% (v/v), a concentration level at which no measurable effect of the cosolvent, methanol, was found for sorption of organic pollutants (Yu et al., 2004). The chemicals used for the E2 solutions as well as other chemicals used in these experiments for liquid/liquid extraction and derivatization procedures are listed in Table 3-1.

Table 3-1. Summary of chemicals used in sorption experiments.

Chemical	Supplier
Dichloromethane (DCM)	Fisher Chemical (Fairlawn, NJ)
Calcium Chloride ($CaCl_2$)	Fisher Chemical (Fairlawn, NJ)
Sodium Azide (NaN_3)	Fisher Chemical (Fairlawn, NJ)
Hexanes	Fisher Chemical (Fairlawn, NJ)
Methanol	EMD Chemicals, Inc. (Gibbstown, NJ)
17β-estradiol (E2)	Sigma Aldrich (St. Louis, MO)
O- bis(trimethylsilyl)trifluoracetamide (BFTSA)	ACROS Organics (Morris Plains, NJ)
Trimethylsilylimidazole (TMSI)	ACROS Organics (Morris Plains, NJ)

3.2. Soils

Samples representative of productive agricultural soils from three different physiographic regions of Virginia were supplied by the Crop and Soil Environmental Sciences Department at Virginia Tech (Figures 3-1 and 3-2). The soils had been collected from fields in the Appalachian Ridge and Valley, Piedmont, and Atlantic Coastal Plain regions (Lawrence, 2000). The Ridge and Valley soil was a Groseclose loam from Montgomery County, classified as a clayey, mixed mesic Typic Hapludult. The Coastal Plain soil was a Myatt fine sandy loam from Isle of Wight County, classified as a fine-loamy, siliceous, thermic Typic Ochraquult. The Piedmont soil was a Cecil loam from Amelia County, classified as a fine, kaolinitic, thermic Typic Kanhapludult. All soils were collected from the plow layer (0 to 15 cm). An additional Cecil soil sample was collected from the Bt horizon (Zelazny, 2005).

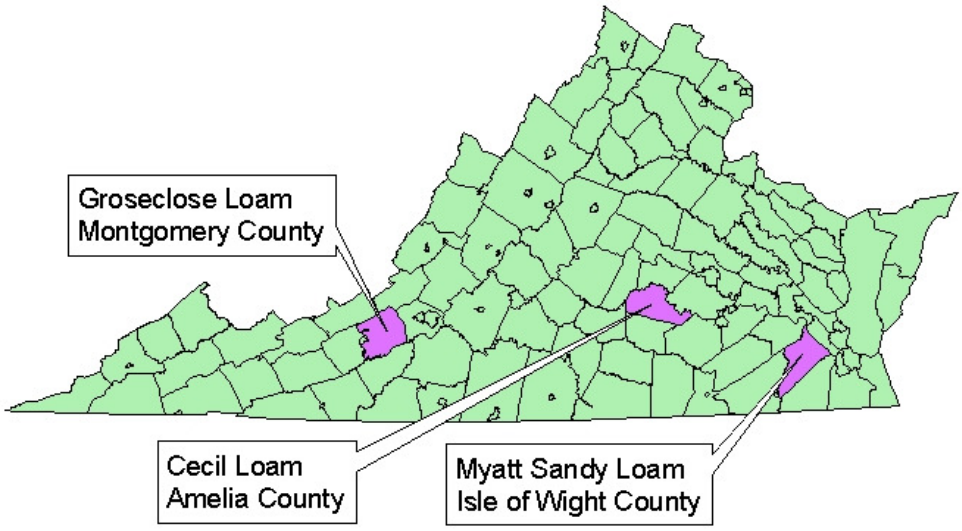


Figure 3-1. Map of Virginia showing counties where agriculturally productive soils were collected from different physigraphic regions.

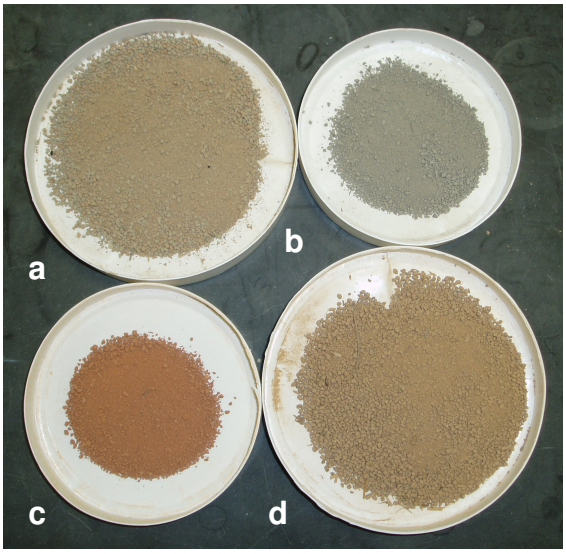


Figure 3-2. Soils used in sorption experiments. a.) Groseclose, b.) Myatt, c.) Cecil Bt horizon, d.) Cecil.

Following collection, all soils were air-dried and clods were broken up by hand using a mortar and pestle, sifted through a 2-mm sieve, and thoroughly homogenized by mixing in a portable electric cement mixer (Lawrence, 2000). Previously, the Virginia Cooperative Extension Soil Testing Laboratory at Virginia Tech determined pH (1:1 ratio of soil to water) and percent organic matter (Lawrence, 2000). The organic matter content of each soil was converted to organic carbon content by dividing by the Van Bemmelen factor of 1.724 (Richardson and Vepraskas, 2001). It should be noted that although this is a common conversion, it is very general and is not always accurate for all soils. This conversion was used in order to compare the organic content of the soils used in this study to the organic content of soils in published studies. Cation exchange capacity was determined by the Soil Survey Laboratory at Virginia Tech. Characteristics of each soil are listed in Table 3-2. Before weighing for sorption experiments, the soil samples were dried in a 100°C oven then mixed by hand.

Table 3-2. Soil characterization: pH, organic matter, organic carbon, and cation exchange capacity (Lawrence, 2000).

Soil Type	pH	Organic Matter (%)	Organic Carbon[a] (%)	CEC ($cmol_{(+)}/kg$)
Groseclose	6.7	3.8	2.2	5.45
Cecil	6.6	3.1	1.8	4.61
Cecil Bt Horizon	ND	0.2	0.1	ND
Myatt	6.1	2.3	1.3	3.76

ND- not determined

[a] % organic carbon determined by dividing by 1.724 (Richardson and Vepraskas, 2001)

3.3. Soil-Water System Setup

Each experiment was set up as a collection of soil-water systems. With the exception of the preliminary concentration experiment, each system consisted of 150 mL of E2 solution with a mass of soil, determined by a preliminary experiment, in a 250-mL glass bottle with a Teflon-lined cap. This volume, 150 mL, was chosen to ensure that there was excess solution when each system was sampled five times (15 mL per sample). For the preliminary experiment, only 50 mL of E2 solution was added to each bottle because each system was only sampled once. The methods used to sample and analyze each sample are described in the following sections. The

bottles were acid washed, rinsed, and autoclaved prior to each use. The soil-water system bottles were placed randomly on a shaker and covered with foil.

3.4. Estradiol Determination

In order to determine the concentration of E2 in the aqueous phase from the batch experiments containing soil and E2 solution, each sample was prepared using the series of steps shown in Figure 3-3. Samples were taken from well-mixed bottles in order to retain the soil to solution ratio. The soil was removed from each sample by centrifugation and the E2 in the aqueous phase was extracted using liquid/liquid extraction. The extracted E2 was then derivatized and analyzed by gas chromatography/mass spectrometry (GC/MS).

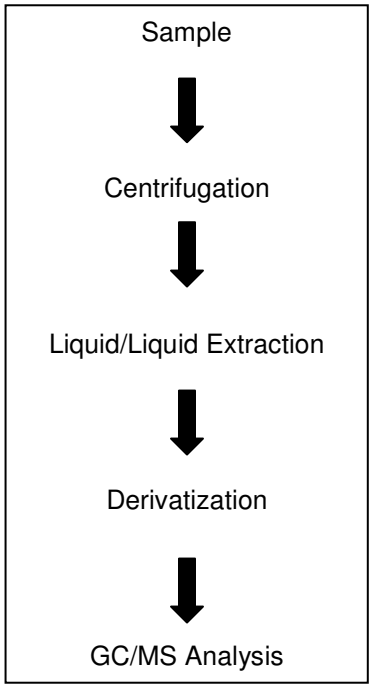


Figure 3-3. Major steps in E2 sample preparation for GC/MS analysis.

3.4.1 Sample Preparation

Samples (15 mL) of the soil-water solution were taken with glass pipettes from well-mixed systems containing E2 solution and soil. The samples were then centrifuged in 15-mL glass centrifuge tubes at 750 x *g* for 30 minutes at 22 ^{o}C. The supernatant was decanted and

saved to be analyzed for E2 concentration. Liquid/liquid extraction with dichloromethane (DCM) was used to extract E2 from the aqueous phase. Ten mL of sample was placed in a 25-mL vial. One mL of DCM was added to each sample and each sample was shaken vigorously for 30 seconds (Lai et al., 2000). The phases were then allowed to separate into a top aqueous layer and a bottom organic DCM layer. Being careful not to remove the aqueous layer, 0.5 mL was removed from the DCM layer and was placed in a 10-mL test tube. An additional 1 mL of DCM was added to the 25-mL vial containing the sample. The vial was shaken for 30 seconds and the phases were allowed to separate. Another 0.5 mL aliquot was removed from the DCM layer and added to the same 10-mL test tube for a total of one mL in the tube. The DCM in the tube was evaporated to dryness using nitrogen, leaving behind dried E2 residue.

3.4.2 Derivatization

In order to derivatize the samples for GC/MS analysis, a mixture of O-bis(trimethylsilyl)trifluoracetamide (BFTSA) and 1% (v/v) trimethylsilylimidazole (TMSI) was used. The derivatization mixture used in this study was slightly different than the mixture suggested by Ding and Chiang (2003), who suggested the use of BFTSA with 1% (v/v) trimethylchlorosilane (TMCS). Derivatization of estrogens is required for GC/S analysis to increase analyte volatility and improve chromatographic separation (Ding and Chiang, 2003). The samples were derivatized by resuspending the dried extraction residue with BFTSA with 1% (v/v) TMSI. The resuspended samples were vortex mixed and heated at 70^{o}C for 30 minutes. The samples were then dried at 70^{o}C under nitrogen.

3.4.3 GC/MS Analysis

The derivatized and dried samples were resuspended in hexane with 0.5 mg/L mirex and transferred to 2-mL, amber GC/MS vials. Mirex was used as an internal standard for GC/MS analysis. The GC/MS specifications used in this study are shown in Table 3-3.

Table 3-3. Gas chromatography-mass spectrometry parameters.

GC column	J & W Scientific DB-5MS + DG capillary column 30 m; film thickness 0.25µm; i.d. 0.25 mm
Inlet temp	260°C
Carrier gas	helium at 1mL/min flow
GC temp	100°C 2 min; 20°C/min until 260°C; 10°C/min to 290°C
GC	Agilent 6980 Series
MS	Agilent 5973 MSD (mass selective detector)

A calibration curve using standard solutions of 0.10, 0.50, 1.0 and 2.0 mg/L E2 solutions was constructed to relate the measured response, or area under the peak calculated by the GC/MS, to concentration. Mirex and derivatized E2 were identified by retention time and confirmed by a peak identity with a secondary ion. Because E2 has the potential to degrade to estrone (E1), output from the GC/MS was checked for E1 responses by confirmation with a characteristic mass spectral fragment. The average retention time for mirex was 17.3 minutes. The retention times for derivatized E1 and E2 were 18.3 and 18.7 minutes, respectively.

After all experiments were completed, a set of standards containing 0.1, 0.5, 1.0, and 2.0 mg/L E1 and E2 were analyzed on the GC/MS to obtain relative peak responses for E1 and E2. This was done to attempt to quantify the amount of E2 that degraded to E1 in each system.

3.4.4 Deconjugation

Two conjugated estrogens, 17β-estradiol-3- sulfate and 17β-estradiol-3- (β-D-glucuronide), were analyzed using the method described earlier (Figure 3-3) with the addition of a deconjugation step (Figure 3-4). The deconjugation method used was described by Tang and Crone (1989). Anhydrous methanolic HCl (1M, 1 mL) was added to the dried residue from liquid/liquid extraction with dichloromethane. The vial was capped and incubated at 60°C for five minutes. The solvent was then evaporated at 60°C with nitrogen, leaving dried E2 residue that was then derivatized with BFTSA with 1% TMSI and analyzed using GC/MS.

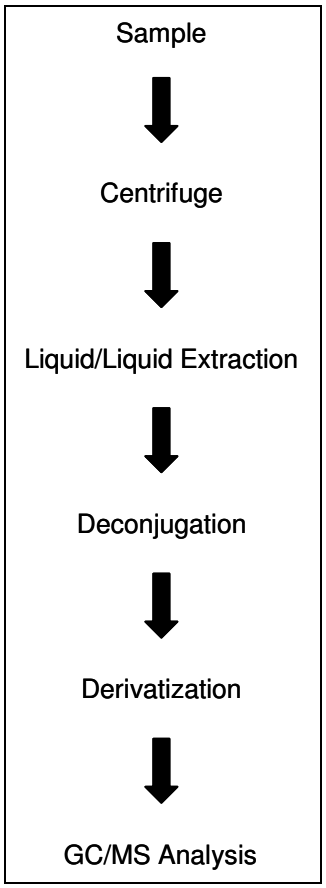


Figure 3-4. Major steps in conjugated E2 sample preparation for GC/MS analysis.

3.5. Percent Recovery

Because the derivatization mixture used was slightly different than those described in the literature, an experiment was conducted to determine the percent recovery for the E2 measurement methods. It was not possible to separate extraction and derivatization efficiency, so the experiment was designed to measure the total percent recovery of E2 in a soil matrix.

A mixture of Groseclose soil and the background solution of $CaCl_2$ and sodium azide with an unknown E2 concentration was centrifuged and the aqueous portion was saved. This Groseclose soil matrix was supplemented with three different spikes. All spikes and the unknown solution were analyzed for E2 concentration in triplicate. The experimental procedure for this experiment is shown in Table 3-4. This experiment was only conducted with Groseclose soil; it is possible that the recovery could vary among soil matrices.

Table 3-4. Experimental design to determine percent recovery of E2 from a Groseclose soil solution.

E2 Concentration	No. of replications for each solution
Unknown (no spike)	3
Unknown + 0.5 mg/L spike	3
Unknown + 1.0 mg/L spike	3
Unknown + 2.0 mg/L spike	3

In order to prepare the spiked solutions, 40 mL of the unknown solution was added to a 50-mL flask, a known concentration of 100 mg/L stock solution was added, and deionized water was added to bring the solution up to 50 mL. This resulted in a 20% dilution of the unknown solution. The percent recovery for each spiked sample was calculated by:

$$\%Recovery = \frac{\left(C_t - 0.8 \times C_u\right)}{C_s} \times 100 \qquad \textbf{3.1}$$

where C_t = the total concentration measured by GC/MS,
0.8 = dilution factor,
C_s = the concentration of the spike, and
C_u = the unknown concentration measured by GC/MS.

3.6. Method Detection Level

The method detection level (MDL) is the constituent concentration that when processed through the complete method produces a signal with a 99% probability that it is different from the blank (APHA, 1998). In order to prepare a matrix to determine the MDL, Groseclose soil was mixed with the background solution of $CaCl_2$ and sodium azide. E2 was added to this matrix at a concentration of 0.05 mg/L. The concentration was chosen to be within the range of one to five times the calculated MDL. Seven portions of this solution were analyzed using the GC/MS procedure described previously. All sample processing steps were included in the determination. The standard deviation of the measured E2 concentration was computed. From a table of the one-sided *t* distribution, the value of *t* for 7-1 = 6 degrees of freedom at the 99% level is 3.14 (Ott and Longnecker, 2001). The MDL is 3.14 times the standard deviation.

3.7. Preliminary Soil to Solution Ratio Experiment

A preliminary experiment was conducted to determine a sorbent to solution ratio for each experiment that would yield a 30-70% reduction in aqueous–phase E2 concentration at equilibrium conditions as suggested by Yu et al. (2004). This experiment was conducted for the Groseclose and Cecil soils with two concentrations of E2 solution and three soil to solution ratios for each concentration (Table 3-5). Fifty mL of each concentration of E2 in the background solution was added to three dry weights of soil.

Table 3-5. Experimental design for preliminary concentration experiments with Cecil and Groseclose soils.

Mass of soil (sorbent)	No. of replications for each solution	
	Solution A (0.1 mg/L)	Solution B (1.0 mg/L)
0.0 (control)	2	2
1.0 g	2	2
3.0 g	2	2
5.0 g	2	2

3.8. Time to Equilibrium Experiment

After an appropriate range of sorbent to E2 solution ratios had been determined with the preliminary concentration experiment, an experiment was conducted to determine time to equilibrium using the highest and lowest E2 concentrations used in the final sorption experiment. In this experiment, 6.0 g of soil and 150 mL of E2 solution were added to each bottle. Samples were taken following the procedure described in Section 3.4 between one day and ten days to determine the approximate time to equilibrium. Sample times for the final experiments were determined based on the results from the preliminary tests. These preliminary tests were necessary because there is some discrepancy in the literature as to time to equilibrium for E2 sorption. Lai et al. (2000) reported equilibrium was reached in less than five hours, while Yu et al. (2004) reported that equilibrium was not reached until seven to ten days. Table 3-6 illustrates the experimental design for preliminary time to equilibrium experiments for Cecil, Cecil Bt horizon, and Myatt soils. For these soils, samples were taken for the lowest and highest

concentration at 1, 3, 5, 7, and 9 days. For Grosclose soil, additional samples were taken for three other concentrations at 1, 2, 4, 7, and 10 days (Table 3-7).

Table 3-6. Experimental design for preliminary time to equilibrium experiments for Cecil, Cecil Bt, and Myatt soils.

	No. of replications for each day of sampling				
	Day 1	Day 3	Day 5	Day 7	Day 9
Lowest concentration (0.05 mg/L)	3	3	3	3	3
Highest concentration (2.0 mg/L)	3	3	3	3	3
Control (no soil) (0.05 mg/L)	3	3	3	3	3
Control (no soil) (2.0 mg/L)	3	3	3	3	3

Table 3-7. Experimental design for preliminary time to equilibrium experiments for Groseclose soil.

	No. of replications for each day of sampling				
	Day 1	Day 2	Day 4	Day 7	Day 10
Lowest concentration (0.5 mg/L)	3	3	3	3	3
Highest concentration (2.0 mg/L)	3	3	3	3	3
0.1 mg/L	3	3	3	3	3
0.5 mg/L	3	3	3	3	3
1.0 mg/L	3	3	3	3	3
Control (no soil) (0.05 mg/L)	3	3	3	3	3
Control (no soil) (2.0 mg/L)	3	3	3	3	3

3.9. Experimental Setup

The final experiment was designed to measure the concentration of 17β-estradiol in the aqueous phase after the time determined in a preliminary experiment to be sufficient to reach equilibrium sorption to soil. For each soil, there were 21 total bottles. These bottles consisted of five concentrations determined by the preliminary experiment, one control at the lowest concentration, and one control at the highest concentration. All concentrations and controls were in triplicate. The experimental design for this experiment is shown in Table 3-8.

Table 3-8. Experimental design for sorption isotherm experiments.

	No. of Replications for each E2 concentration				
	0.05 mg/L	0.10 mg/L	0.50 mg/L	1.0 mg/L	2.0 mg/L
Groseclose	3	3	3	3	3
Myatt	3	3	3	3	3
Cecil	3	3	3	3	3
Cecil B horizon	3	3	3	3	3
Control (no soil)	3	-	-	-	3

3.10. Determination of Isotherms

The equilibrium aqueous concentrations were used to create sorption isotherms for each soil. E2 concentration was measured in the aqueous phase. To develop a sorption isotherm, both aqueous and sorbed concentrations are required. The concentration of E2 sorbed to soil was determined by the mass balance approach:

$$q = \frac{(c_i - c_{eq})V}{M} \qquad \textbf{3.2}$$

where q = sorbed concentration (mg/kg),
c_i = initial solution concentration (mg/L),
c_{eq} = measured equilibrium concentration (mg/L),
V = Volume of solution added (L), and
M = mass of soil added (kg).

The initial concentration in this equation is the concentration of the solution made from standard stock solutions in the laboratory. The mass balance approach is based on two assumptions: E2 was only sorbed onto soil and not onto laboratory equipment, and E2 did not degrade. The first assumption was verified by including controls at the highest and lowest concentration in the experimental setup to account for sorption of E2 to laboratory equipment. The second assumption was evaluated by checking each sample for degradation indicated by the presence of E1.

In order to develop an isotherm for the compounds used in this experiment, the data were fitted to Freundlich (eq. 2.1), linear, and Langmuir isotherms (eq. 2.3). Linear regression of the linearized forms of the Freundlich and Langmuir isotherms was done in SAS (SAS, 2003). The linear isotherm was developed using linear regression with an intercept equal to zero using SAS. The SAS code can be seen in Appendix A.

Chapter 4: Results and Discussion

The experimental data from the experiments described in Chapter 3 are presented in this chapter. Difficulties with the deconjugation method as well as developed isotherms and correlations between soil characteristics and sorption of E2 are discussed.

4.1. Experimental Conditions

Experimental conditions were monitored throughout the experiments. A high, a low, and, a current temperature were taken at every sampling time. The temperature was maintained at 23 ± 2 ^{o}C. The temperature data are shown in Table B-1 in Appendix B. The pH of the soil-water systems was also measured at various sampling times (Table B-2). The measured pH for Cecil soil systems ranged from 5.7 to 6.2 with mean pH of 6.0. Cecil Bt horizon soil systems had mean pH of 4.4 and a range of 4.3 to 4.7. Groseclose soil systems had a mean pH of 5.8 with a range of 5.6 to 6.2. Myatt soil systems had a range of 5.2 to 5.6 with a mean of 5.4. The pH was not buffered in these experiments because the pH was not expected to influence sorption of E2.

4.2. GC/MS Analysis

Based on a mean percent recovery of E2 of 88%, GC/MS was an acceptable method for measuring free E2 in aqueous soil solution when combined with liquid/liquid extraction with dichloromethane and derivatization with BFTSA with 1% TMSI for samples with E2 concentrations greater than the MDL of 0.03 mg/L E2. However, this procedure was very time consuming, requiring a minimum of 8 hours to prepare 24 samples for GC/MS analysis. Because the laboratory equipment used to dry the estrogen samples could only be used for 12 samples at a time, the required laboratory preparation time increased by about 8 hours for each set of 24 samples. However, the use of GC/MS had a major advantage over methods such as EIA in the ability to directly analyze for degradation products such as estrone (E1).

The mass spectra used to identify mirex and derivatized E1 and E2 are shown in Figure 4-1. The characteristic mass spectral fragments were m/z 272 for mirex, m/z 416 for E2, and m/z 342 for E1.

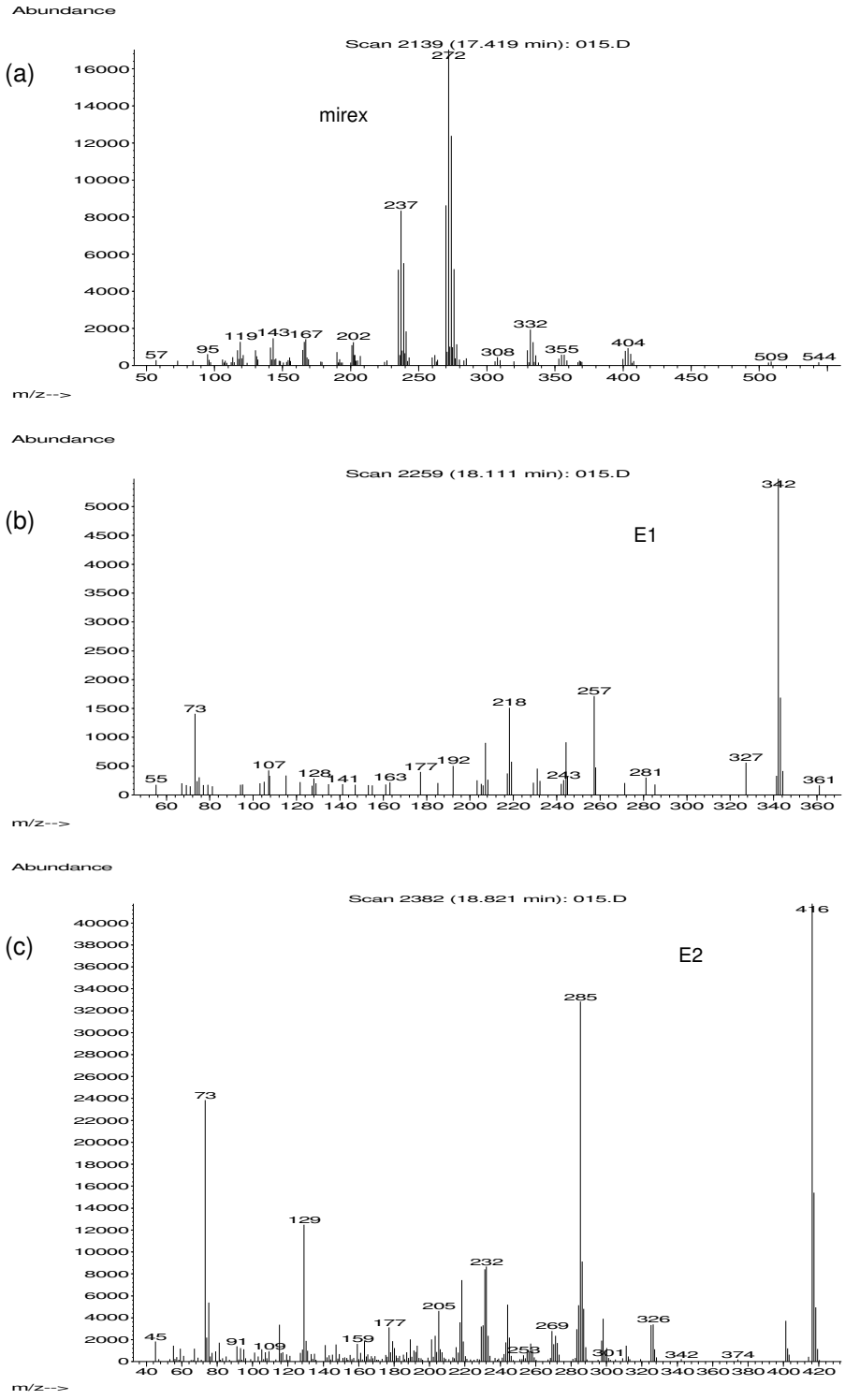


Figure 4-1. Mass spectra and characteristic mass spectral fragment of (a) mirex (272), (b) TMS derivative of estrone (342), and (c) TMS derivative of estradiol (416).

The average retention time for mirex for calibration analyses run after the column was changed on 02/28/05 was 17.3 minutes. The retention times for derivatized E1 and E2 were 18.3 and 18.7 minutes, respectively. A chromatogram from 03/21/05 showing mirex and derivatized E1 and E2 peaks is shown in Figure 4-2.

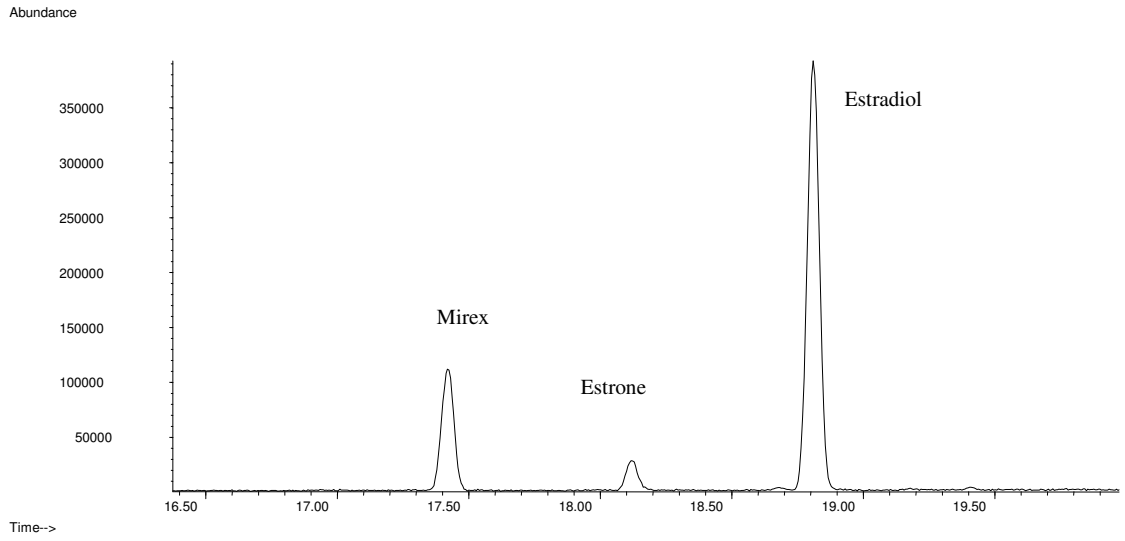


Figure 4-2. Example of chromatogram showing mirex and derivatized estradiol and estrone peaks.

One of the major issues with the procedure for analyzing E2 described in Chapter 3 is the appearance of double peaks at the retention time for derivatized estradiol (Figure 4-3). The mass spectra for these peaks could not be identified using known spectra, but the spectra contained many of the mass spectral fragments common to estrogens. It is hypothesized that this peak is underivatized E2. However, because it is not possible to analyze these samples accurately for E2 concentration, samples with double peaks were discarded. This did not affect the creation of sorption isotherms because data sets were chosen from the sample dates after time to equilibrium that had the most complete sample set after double peaks were discarded. However, a calibration curve had to be discarded. This is discussed in the section on calibration curves.

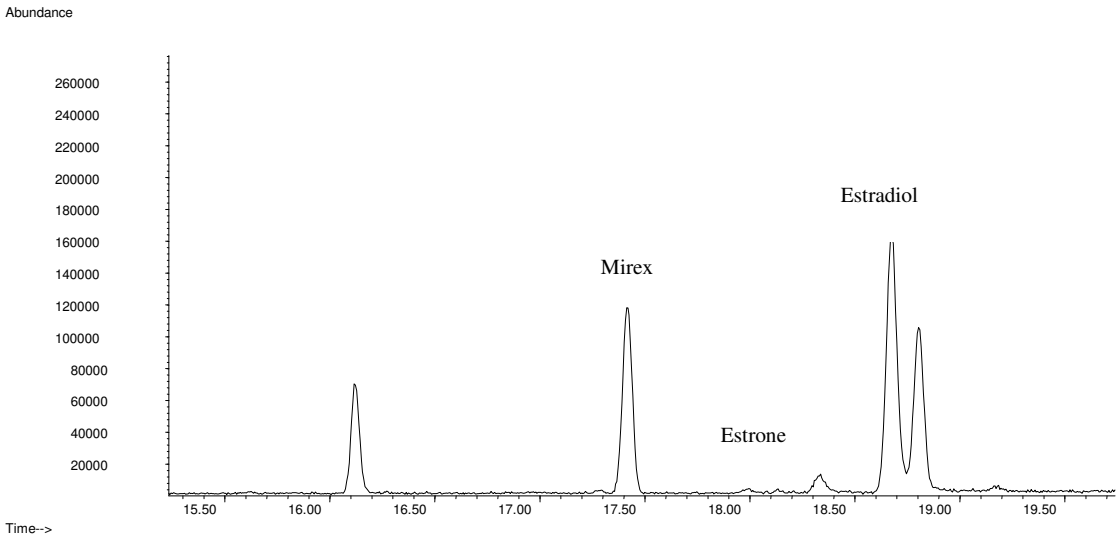


Figure 4-3. Example of chromatogram showing derivatized estradiol and estrone, and mirex peaks. This chromatogram illustrates the two peaks that appeared at the retention time for derivatized estradiol.

4.2.1 Conjugated Estrogens

The method described in Chapter 3 for analyzing the conjugated estrogens, 17β-estradiol-3- sulfate and 17β-estradiol-3- (β-D-glucuronide), was not successful in this study. During preliminary work using the methanolysis deconjugation method described by Tang and Crone (1989), E2 could not be recovered consistently when analyzed by GC/MS (data not included). Thus, the methanolysis method was considered unsuitable for conjugated estrogen analysis and only free E2 was used in the sorption experiments. Based on a study by Finlay-Moore (2000), this method of deconjugation was expected to work because it successfully cleaved E2-sulfate in E2-D-glucuronide pure solutions. However, Finlay-Moore (2000) concluded that the methanolysis deconjugation procedure of Tang and Crone (1989) was not suitable for measuring conjugated estrogens in a study measuring runoff of estrogen content in poultry litter impacted runoff. Measured values of free E2 increased (≤150%) in some runoff samples and decreased (≤63%) in other samples after methanolysis. However, it was not specified if there was an actual conjugated estrogen content of the poultry litter. It was not within the scope of this research to evaluate other methods for deconjugation of estrogens, but future studies should examine the use of other deconjugation procedures such as enzymatic hydrolysis. Enzymatic hydrolysis using β-

glucuronidase from *E. coli* or β-glucuronidase/ arylsulphatase has successfully deconjugated estrogen glucuronides but may not always be successful with the more stable estrogen sulfates (Vos, 1996; Hoffmann et al., 1997; Belfroid et al., 1999; Raman et al., 2001). The issue of deconjugating estrogen sulfates can be avoided entirely by using a LC-MS/MS method for analyzing conjugated estrogens, as proposed by Isobe et al. (2003). However, LC-MS/MS instruments remain expensive and there was not one available for this research.

4.2.2 Calibration Curves

Calibration curves were created as a linear regression of the ratio of the concentration of the compound of interest (E2) to the concentration of the internal standard (mirex) and the ratio of the response factor of the E2 to the response factor of the mirex (Figure 4-4). There was some variation between the slopes and intercepts found by linear regression on different sample days. Table 4-1 lists the slope and y-intercept for each linear regression of the calibration data. Raw data values used to develop calibration curves can be found in Table C-1 in Appendix C.

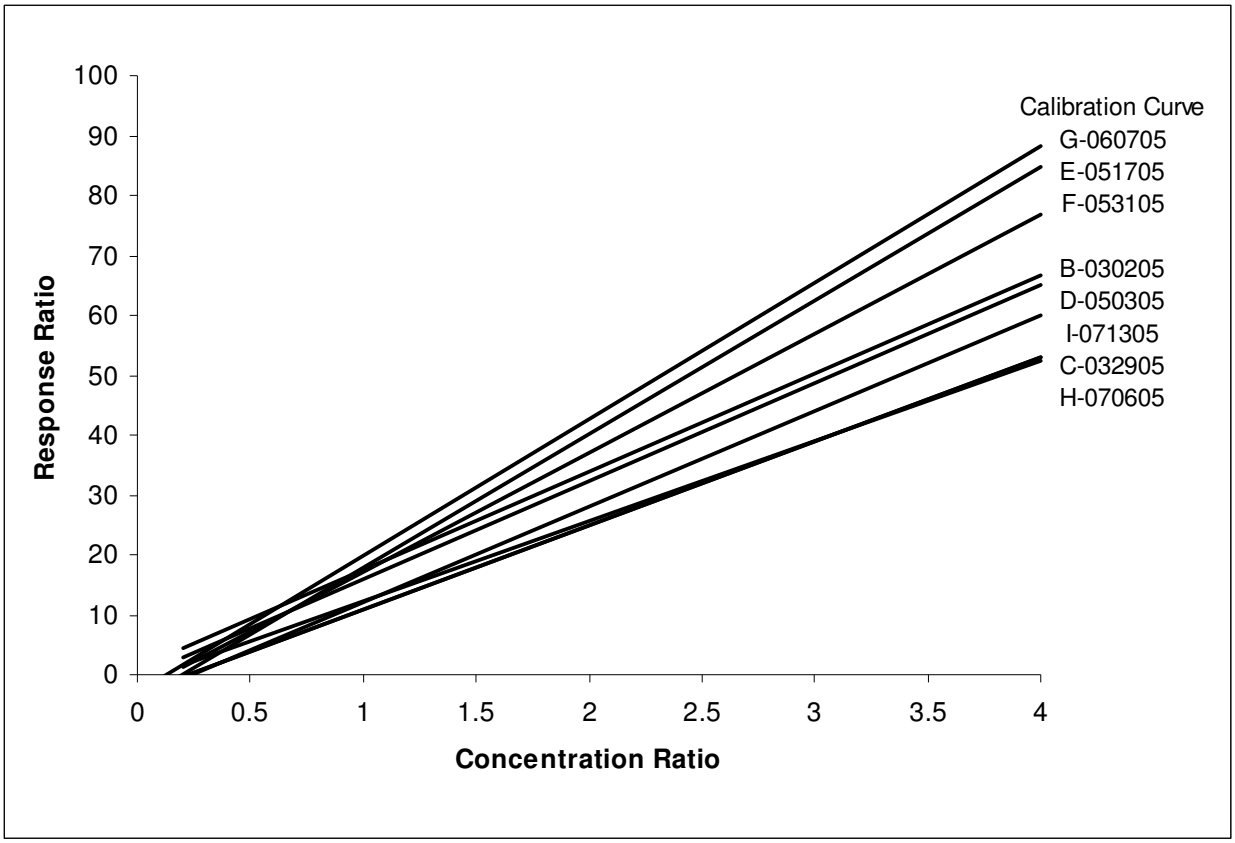


Figure 4-4. Calibration curves used to calculate E2 concentrations from GC/MS responses. The response ratio is the ratio of the E2 response to the internal standard, mirex, response. The concentration ratio is the ratio of the E2 standard concentration to the concentration of the internal standard. The slope and intercept for each curve are shown in Table 4-1.

Table 4-1. Calibration curve slope and intercept.

Calibration Curve	Date	Slope	y-intercept	R^2
A	01/29/05	1.07	-0.093	0.998
B	03/02/05	16.18	-0.48	0.994
C	03/29/05	13.86	-4.34	0.909
D	05/03/05	16.00	-1.36	0.915
E	05/17/05	22.43	-4.42	0.996
F	05/31/05	19.91	-2.86	0.999
G	06/07/05	22.81	-2.98	0.996
H	07/06/05	14.58	-2.03	0.980
I	07/13/05	15.96	-3.89	0.992

Each set of samples was analyzed with a calibration curve created on the same GC/MS analysis day, except for samples from the time to equilibrium experiment for Cecil, Cecil Bt horizon, and Myatt soils analyzed on 04/19/05 and 04/26/05. No calibration curve was

developed for 04/19/05 and the standard curve for 04/26/05 had underivatized E2 (two peaks) and was rejected. The samples were then analyzed using a calibration curve developed from standards analyzed on 05/03/05. The 05/03/05 date was chosen because the mirex responses for 04/19/05 and 04/26/05 were similar to the mirex response on 05/03/05. The mean mirex responses for these dates were 32850, 34011, and 32311, respectively. Also,samples from the preliminary experiment run on 01/14/05 were analyzed using a calibration curve created on 01/20/05 because no curve was created for 01/14/05. The mean mirex responses were 305016 for samples analyzed on 01/14/05 and 334517 for the standards and samples analyzed on 01/20/05.

4.2.3 Internal Standard

An internal standard, mirex, was used to correct for GC/MS variation. The measured mirex responses varied among GC/MS analysis dates. The mirex responses for the samples from 01/20/05 were much higher than for the following GC/MS analysis dates (Figure 4-5). The average mirex response for 01/20/05 was approximately 443000, while the average responses for other analysis dates ranged from approximately 3200 to 8600. While the reason for this difference is unknown, it was hypothesized that the mirex concentration in the 0.5 mg/L secondary stock solution was degrading. Beginning on 03/02/05 a new 0.5 mg/L solution was made for each sample analysis date by adding 25 μL of 1000 mg/L stock solution to a 50-mL volumetric flask. The mirex responses remained relatively stable (see Figure 4-5). It is suspected that the two orders of magnitude difference was most likely due to use of a different stock solution to prepare the 0.5 mg/L solution of mirex rather than the degradation of mirex in the stock solution. After March 2005 care was taken to ensure all samples were prepared using the same mirex stock solution that was prepared fresh for every sample analysis date.

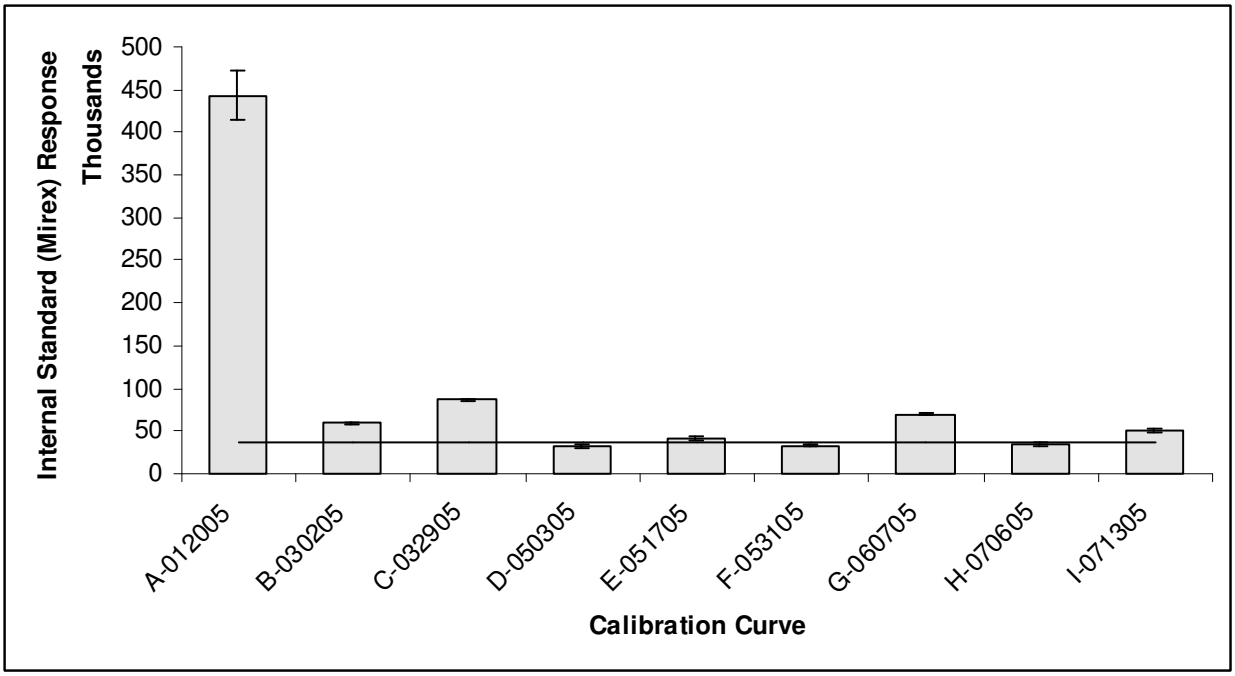


Figure 4-5. Mean internal standard (mirex) response by calibration curve date. Error bars indicate standard deviation. The horizontal line is the mean of the internal standard responses for all dates excluding A-012005.

4.2.4 Degradation of Estradiol to Estrone

Although the systems were sterilized with sodium azide, each sample was checked for the primary degradation product of E2, estrone (E1). Derivatized E1 was identified using the characteristic mass spectral fragment at m/z 342 (Ding and Chiang, 2003). Many samples had a small amount of estrone. The samples were checked after the experiment for E1 to determine if E2 in the experiments had degraded. Because the samples were checked after the fact for estrone, no estrone calibration curves were made. The presence of estrone indicates there was E2 degradation despite the addition of sodium azide. Either the sodium azide did not keep the system entirely sterile, or E2 was degrading by abiotic means such as photolysis or iron oxide catalysis. The presence of E1 in the sample complicates the mass balance used to develop the sorption isotherms. Because only the E2 in the aqueous phase was measured and because the sorbed E2 concentration was calculated by a mass balance, any E2 that had degraded was mistakenly assumed to be sorbed to the soil.

In order to approximate the concentration of E1 relative to the final E2 concentration, a series of standards of both E1 and E2 were run on the GC/MS to get a ratio of the peak response

of E1 to the peak response of E2. Figure 4-6 shows the calibration curves obtained from this analysis. The lines are approximately parallel and therefore it was decided that a single ratio could be used to estimate the concentration of E1. The ratio of the E1 response to the E2 response for the same standard concentrations was almost constant (Figure 4-7). Because the data from the lower concentrations appear to be outliers, the median ratio value was chosen rather than the mean ratio. This value was 0.88.

To estimate the amount of E1 in each sample, the ratio of the E1 to the E2 response for each sample was multiplied by the calculated E2 concentration and divided by 0.88. This should not be taken as an exact measure of the E1 present in each sample, but as an estimate used to evaluate the degradation of E1 in the sterile soil water systems.

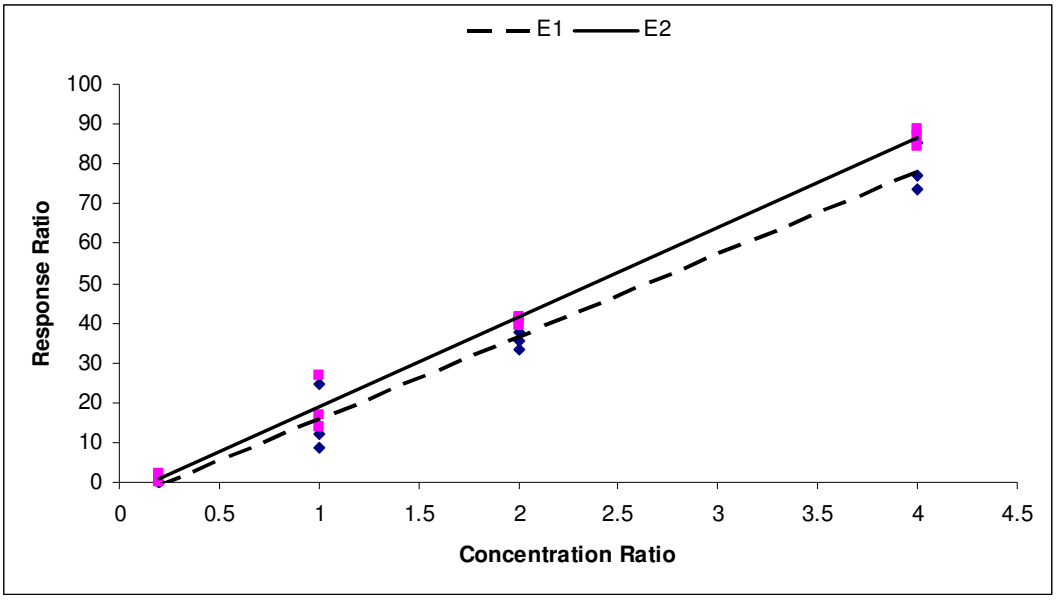


Figure 4-6. Comparison of calibration cuves for E2 and E1. The concentration ratio is the ratio of the compound of interest (E1 or E2) to the concentration of the internal standard, mirex. The response ratio is the ratio of the peak response of the compound of interest to the response of mirex.

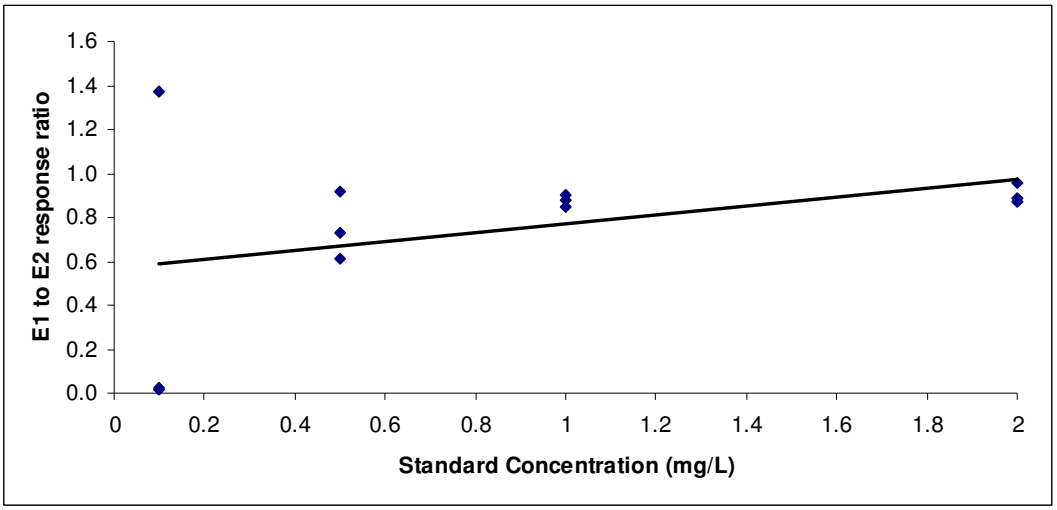


Figure 4-7. Ratio of E1 to E2 peak response area from GC/MS versus standard concentration. The median value of the ratios is 0.88.

The resulting estimated percentages of degradation of E2 to E1 for each soil are shown in Table 4-2. The Cecil soil from the Bt horizon had the least E2 degradation. For Myatt, and Cecil soils, the lower initial concentrations had a greater ratio of E1 to E2 than for the higher initial concentrations. This would indicate that sorption was overestimated, especially at the lower initial concentrations.

Table 4-2. Mean degradation of E2 to E1. Expressed as a %.

	Mean Degradation of E2 to E1 (%)			
Initial E2 concentration	**Myatt**	**Cecil Bt**	**Cecil**	**Groseclose**
0.1 mg/L	21	0.0	36	0
0.5 mg/L	3	0.0	16	2
1.0 mg/L	2	0.1	4	17
2.0 mg/L	0	0.1	3	14

It was suggested by Lee et al. (2003) that the abiotic degradation of estradiol could be catalyzed by iron oxide in the soils. Although no data were available regarding the iron oxide content of these soils, the Cecil Bt horizon soil was expected to have the highest iron oxide content (Zelazny, 2005). Because the Cecil Bt horizon soil had the least mean degradation of E2 to E1, it appears that the degradation of estradiol in these experiments is the result of a mechanism other than iron oxide catalysis.

These results reinforce the notion that the metabolites (E1) of E2 need to be monitored when conducting any environmental fate study. These results were not used to correct the equilibrium concentration values of E2 or the sorbed concentration, because they are a very rough estimate of the amount of E1 that was formed in the systems. Because E1 is much less bioactive than E2, the degradation of E2 is a further mechanism to remove E2 from the soil solution. It is noted that some E2 did degrade and may be mistakenly attributed to the sorbed concentration.

4.2.5 Method Detection Level

The method detection level for the sample processing including extraction, derivatization, and GC/MS analysis from samples from a spiked Groseclose soil matrix was determined to be 0.03 mg/L. All data points below this level were considered invalid and removed from the data set. Unfortunately, samples taken from bottles spiked at the lowest initial concentration level of 0.05 mg/L E2, commonly fell below this level. Therefore, data analysis was conducted with only four concentration levels instead of five. The assumption that this MDL is valid for all soil matrices was tested using the first seven 0.05 control data points from the time to equilibrium experiment for Groseclose soil. Using these data, a MDL of 0.02 mg/L was calculated for the control with no soil.

A similar experiment by Lai et al. (2000) reported a GC/MS detection limit (DL) of 0.005 mg/L. The MDL for this study was an order of magnitude higher than the detection limit for the Lai et al. (2000) study, however, that limit was reported as a detection limit rather than a method detection limit. The detection limit does not take into account all processing steps before the use of the GC/MS. The difference between the MDL and the DL is an indication of the variability introduced by the number of sample processing steps required to analyze E2 samples with GC/MS. The samples in this experiment were concentrated by 20; for future experiments the method could be revised to include a greater concentration step which would measure smaller values. Sample sizes in these experiments were 10 mL. If the concentration factor was increased by ten, then the sample size would have to be 100 mL. This would greatly limit the number of samples that could be taken. A higher concentration factor could also introduce greater error due to the concentration steps.

4.2.6 Percent Recovery of Estradiol

The percent recovery of E2 is the amount of E2 recovered from samples spiked with a known amount minus the measured unknown E2 concentration. It was not possible to separate the efficiency of E2 extraction by dichloromethane and the efficiency of the derivatization procedure; therefore, the reported E2 recovery is the total recovery of all sample processing steps. The percent recovery of E2 was determined because a slightly different derivatization mixture was used in this procedure than in other studies that have used GC/MS for E2 analysis and to check that the procedure was working properly. The derivization mixture in this experiment was a mixture of BFTSA with 1% (v/v) TMSI, while Ding and Chiang (2003) recommended a mixture of BFTSA with 1% (v/v) TMCS. The results of this experiment are shown in Table 4-3. An average recovery of 88 ± 13 % of E2 was found in the Groseclose soil matrix with a 95% confidence interval. The raw data for this experiment are in Table C-3 in Appendix C. The mean and confidence interval were calculated using SAS and the code and output can be seen in Tables A.2 and D.2, respectively. This percent recovery is for method validation purposes only. This recovery percent was not used in data analysis because all standards used for calibration were made up in water and were subjected to all sample processing steps and, therefore, subjected to the efficiencies of extraction and derivatization.

Table 4-3. Results of percent recovery experiment.

Sample	C_t (mg/L)	C_t-0.8C_u	% recovery	Avg. Recovery
Unknown	0.58			
Unknown	0.45			
Unknown	0.34*			
Unknown + 0.5 spike	0.77	0.36	71.6	71.6
Unknown + 0.5 spike	0.60*	--	--	
Unknown + 0.5 spike	0.39*	--	--	
Unknown + 1.0 spike	1.27	0.86	85.8	81.1
Unknown + 1.0 spike	1.26	0.85	84.8	
Unknown + 1.0 spike	1.14	0.73	72.8	
Unknown + 2.0 spike	2.57	2.16	107.9	105.2
Unknown + 2.0 spike	2.42	2.01	100.4	
Unknown + 2.0 spike	2.56	2.15	107.4	

*double peaks, discarded

C_t = final E2 concentration, C_u = measured unknown E2 concentration

The percent recovery increased with increasing spike concentration. There are two explanations for this result. One is that with a greater spike, or aqueous, concentration there is better recovery. Second, the calibration curve overestimated the final concentration for the 2.0 mg/L spikes. The calibration curve used to calculate the concentration only extended to 2.0 mg/L. The final concentration of the 2.0 mg/L spike and the unknown concentration was approximately 2.4 mg/L. It is possible that the calibration curve overestimated the recovered concentration at this level.

4.3. Determining Concentration Range

The E2 concentrations and the mass of soil used in the final isotherm experiments were determined by using a preliminary concentration experiment. The six soil mass, solution volume, and E2 concentration scenarios (three ratios of solution volume to soil mass mixed with E2 concentrations of 0.1 and 1.0 mg/L) from the preliminary concentration experiment yielded percent reductions of aqueous E2 for Groseclose and Cecil soils ranging from 16% to 70% (Table 4-4). The raw data for this experiment are in Table C-4 in Appendix C.

Table 4-4. Percent sorbed to Groseclose and Cecil soil.

Soil	Solution to soil ratio	Initial E2 Concentration	
		0.1 mg/L	1.0 mg/L
Groseclose	100 mL E2 solution: g soil	21	16
Groseclose	50 mL E2 solution : g soil	51	34
Groseclose	16.7 mLE2 solution : g soil	65	67
Cecil	100 mL E2 solution: g soil	28	33
Cecil	50 mL E2 solution : g soil	33	48
Cecil	16.7 mLE2 solution : g soil	62	70

A solution volume to soil mass ratio was chosen to yield a reduction in aqueous E2 concentration within the range of 30-70% for initial E2 concentrations of 0.1 mg/L and 1.0 mg/L. For both soils and both concentrations, the solution to soil ratios of 50 mL/g soil and 16.7 mL/g soil yielded a percent reduction in aqueous E2 concentration between 30-70%. The final ratio chosen was between these two values at 25 mL/g soil.

4.4. Determining Time to Equilibrium

In order to develop a sorption isotherm, the system being studied is assumed to be at equilibrium. For previous sorption studies of E2 to soil and sediment, the published time to reach equilibrium varied from less than 5 hours to 7-10 days (Lai et al., 2000; Yu et al., 2004). For this study, the smallest time step was 24 hours and samples were taken up until 9-10 days to make sure equilibrium was reached.

For all soils tested, it appeared that equilibrium was reached within 24 hours (Figures 4-8 and 4-9). While there was some variation between sampling days, the overall trend in aqueous concentration had a very sharp decrease in concentration within the first 24 hours. The initial concentration was estimated as the average of the control concentrations shown on each graph.

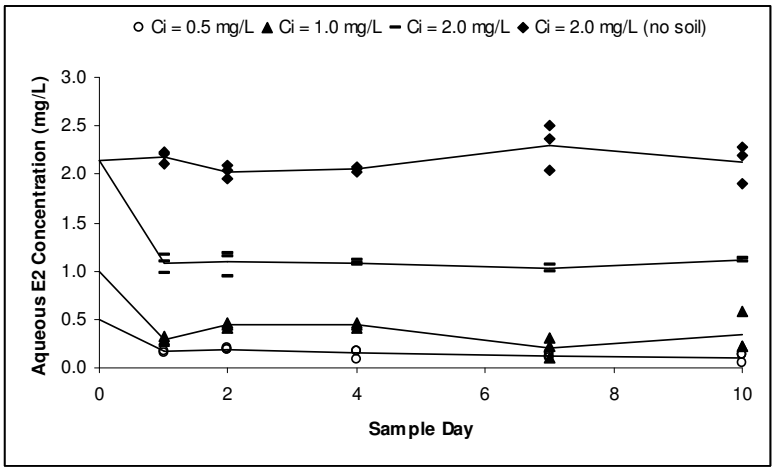


Figure 4-8. Aqueous E2 concentration by sample day for batch experiments using Groseclose soil and a control (no soil) with three different initial concentrations.

The time to equilibrium experiment for Groseclose soil was carried out at five initial concentrations. The results from initial concentrations of 2.0 mg/L, 1.0 mg/L, and 0.5 mg/L are shown in Figure 4-8. For all concentrations, equilibrium was reached within the first 24 hours.

The time to equilibrium experiments for Cecil, Cecil Bt horizon, and Myatt soils were conducted at the same time with the same initial solutions and controls. After the first 24 hours, the sample concentrations trended upward for all three soils and the control (Figure 4-9). Because the control also showed this trend it was assumed that it was due to variation in measurements and that equilibrium was again reached within 24 hours. Although this experiment is lacking data points at times less than 24 hours, the results indicate an agreement with studies that found time to equilibrium within the order of one day (Lai et al., 2000; Casey et al., 2003) rather than 7 to 10 days as suggested by Yu et al. (2004).

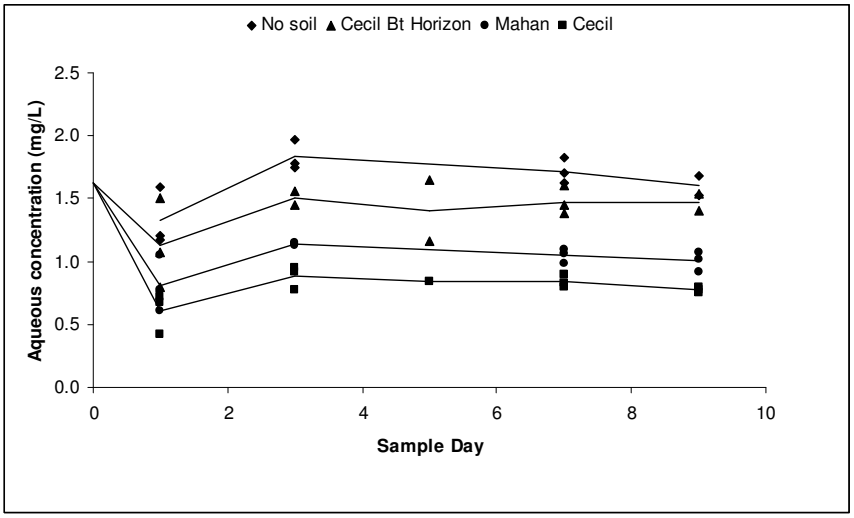


Figure 4-9. Aqueous E2 concentration by sample day for batch experiments using Cecil, Cecil Bt horizon, and Myatt soils. The initial concentration was 2.0 mg/L. Data were analyzed using a calibration curve from May 3, 2005.

4.5. Control Analysis

Controls were included with each experiment to ensure that E2 was sorbed to the added soil and not the glassware used in the experiment. The raw data from these controls can be seen in Appendix C. For this analysis, all controls were used except for those from the time to equilibrium experiments for Cecil and Cecil Bt horizons. These data were excluded because the calibration curve used to analyze the data was from a different analysis date and resulted in control concentrations that did not match those of initial concentrations. Excluding these data, the control for 0.05 mg/L averaged 115% of the initial concentration and the control for 2.0 mg/L averaged 99% of the initial concentration. Based on these results, it was concluded that the sorption to glassware was negligible.

4.6. Sorption Isotherms

Isotherm equations were used to model the equilibrium partitioning between sorbed E2 and aqueous E2. Data taken after 24 hours (time to reach equilibrium) for each soil were fitted to Freundlich, linear, and Langmuir isotherms. The sampling date after 24 hours that had the most complete data set was chosen to develop sorption isotherms for Cecil, Myatt and Groseclose soils. The same data set was used for all three isotherms for each soil. The raw data used to develop these isotherms can be seen in Appendix C in Tables C-7, C-8, C-9, and C-10.

4.6.1 Groseclose Sorption Isotherms

Data from one sample date and four initial concentrations were used to develop isotherms for Groseclose soil (Table 4-5). The data from the lowest initial concentration of 0.05 mg/L was discarded because it fell below the MDL for the procedures used.

Table 4-5. Data used to create sorption isotherms for Groseclose soil.

Initial E2 Concentration (mg/L)	Final E2 Concentration (mg/L)	Mean Final E2 Concentration (mg/L)	E2 sorbed* (mg/kg)	Mean E2 sorbed* (mg/kg)	% E2 sorbed
0.1	0.03	0.03	1.8	1.8	73
0.1	0.03		1.8		
0.5	0.19	0.19	7.8	7.8	62
0.5	0.19		7.8		
0.5	0.20		7.5		
1.0	0.42	0.45	14.5	13.8	55
1.0	0.45		13.8		
1.0	0.47		13.3		
2.0	0.95	1.10	26.3	22.6	45
2.0	1.15		21.3		
2.0	1.19		20.3		

*Determined by mass balance assuming all E2 did not degrade.

Of the three isotherm equations fit to the sorption of E2 to Groseclose soil data, the Freundlich sorption isotherm had the highest R^2 value (0.98). However, all isotherms had relatively high R^2 values (Figure 4-10). The linear isotherm resulted in an R^2 value of 0.93 and the Langmuir sorption isotherm produced an R^2 value of 0.88.

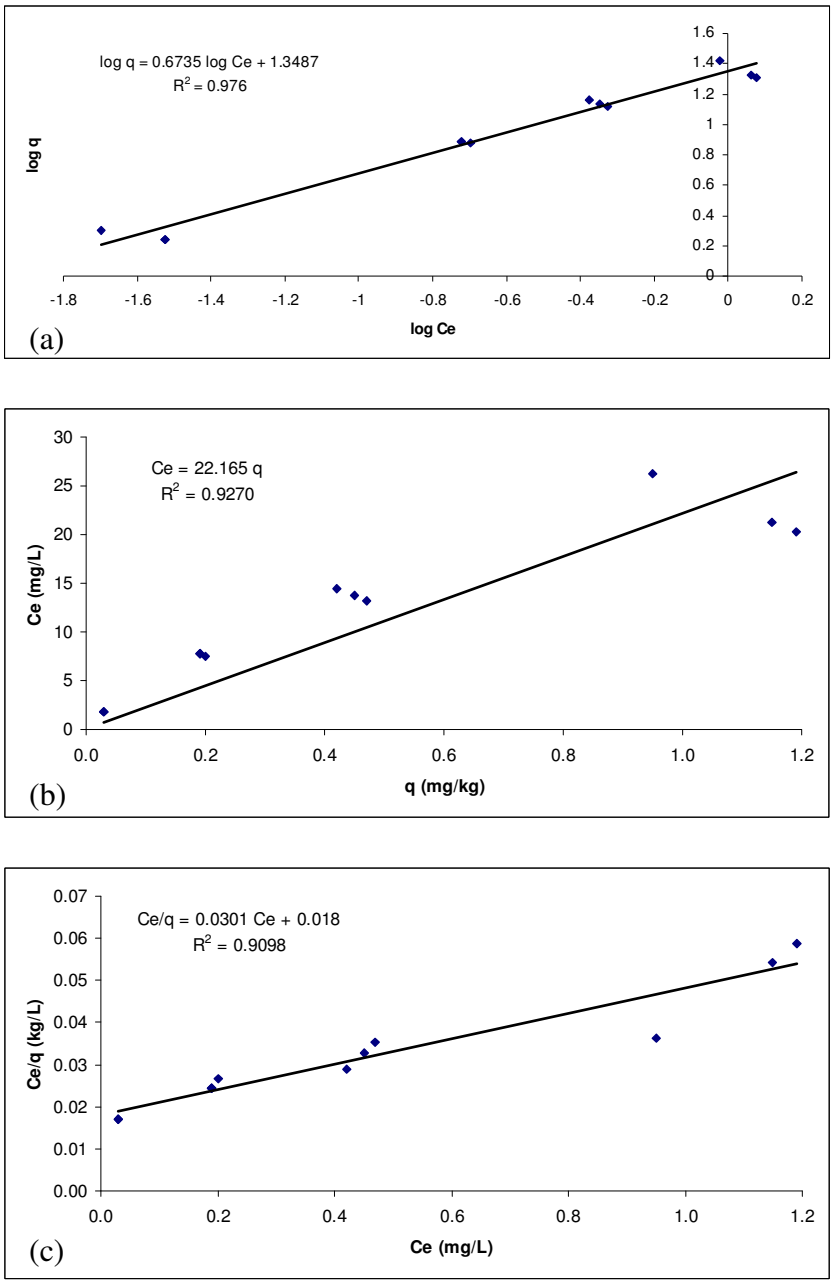


Figure 4-10. Isotherms for sorption of E2 to Groseclose soil. (a) Freundlich (b) Linear (c) Langmuir. Ce is the equilibrium aqueous E2 concentration (mg/L) and q is the equilibrium E2 sorbed concentration (mg/kg).

4.6.2 Myatt Sorption Isotherms

Similar to Groseclose soil, isotherms were created for the sorption of E2 to Myatt soil using the data shown in Table 4-6. Data from the lowest initial concentration of 0.05 mg/L were not detected above the MDL.

Table 4-6. Data used to create sorption isotherms for Myatt soil.

Initial E2 Concentration (mg/L)	Final E2 Concentration (mg/L)	Mean Final E2 Concentration (mg/L)	E2 sorbed* (mg/kg)	Mean E2 sorbed* (mg/kg)	% E2 sorbed
0.1	0.08	0.09	0.5	0.3	13
0.1	0.08		0.5		
0.5	0.24	0.27	6.5	5.9	47
0.5	0.29		5.3		
1.0	0.51	0.46	12.3	13.4	54
1.0	0.48		13.0		
1.0	0.40		15.0		
2.0	1.10	1.11	22.5	22.3	45
2.0	1.12		22.0		

*Determined by mass balance assuming all initial E2 did not degrade.

The Freundlich isotherm developed for the sorption of E2 to Myatt soil from Isle of Wight County fit the data reasonably well ($R^2 = 0.91$; Figure 4-11). However, the value of n for the Freundlich isotherm should lie between 0 and 1 (Essington, 2004). In this regression, n is 1.48. For these sorption data, the linear isotherm had a higher R^2 (0.96) and described the data better than the Freundlich isotherm. The Langmuir isotherm did not fit the data ($R^2 = 0.26$).

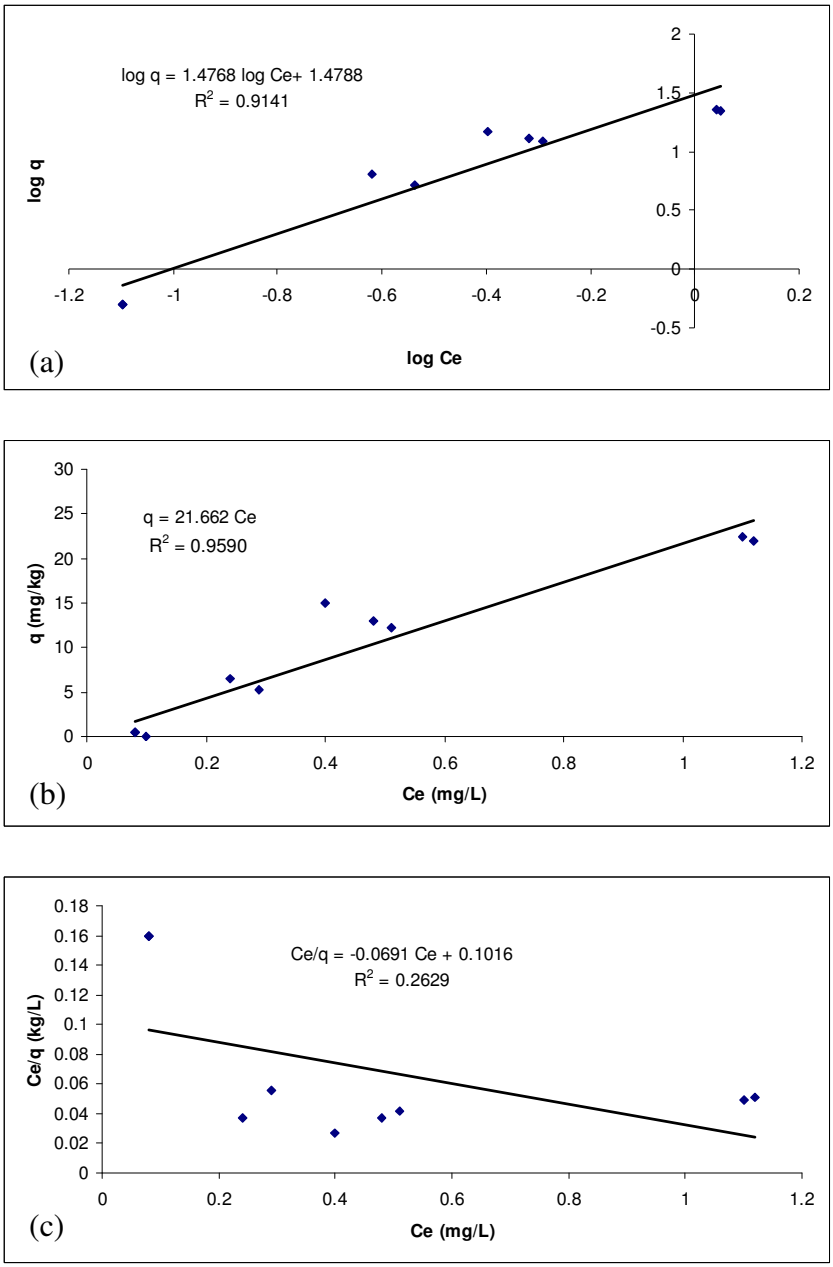


Figure 4-11. Isotherms for sorption of E2 to Myatt soil. (a) Freundlich (b) Linear (c) Langmuir. Ce is the equilibrium aqueous E2 concentration (mg/L) and q is the equilibrium E2 sorbed concentration (mg/kg).

4.6.3 Cecil Sorption Isotherms

Sorption isotherms were developed using data from five initial concentrations. The data for the lowest initial concentration (0.05 mg/L) were included in this analysis because they were at or above the MDL for the procedures used to measure E2 concentration (Table 4-7).

Table 4-7. Data used to create sorption isotherms for Cecil soil.

Initial E2 Concentration (mg/L)	Final E2 Concentration (mg/L)	Mean Final E2 Concentration (mg/L)	E2 sorbed* (mg/kg)	Mean E2 sorbed* (mg/kg)	% E2 sorbed
0.05	0.03	0.04	0.5	0.38	30
0.05	0.04		0.3		
0.1	0.05	0.05	1.3	1.17	47
0.1	0.05		1.3		
0.1	0.06		1.0		
0.5	0.42	0.42	2.0	2.08	17
0.5	0.39		2.8		
0.5	0.44		1.5		
1.0	0.60	0.59	10.0	10.33	41
1.0	0.64		9.0		
1.0	0.52		12.0		
2.0	1.15	1.19	21.3	20.17	40
2.0	1.19		20.3		
2.0	1.24		19.0		

*Determined by mass balance assuming all initial E2 did not degrade.

The Freundlich sorption isotherm for sorption of E2 to Cecil soil shown in Figure 4-12 fits the general trend of the data ($R^2 = 0.81$) but did not describe the data as well as the isotherm created for Groseclose soil. Like Myatt soil, the linear form of the Freundlich isotherm described the data better ($R^2 = 0.94$) than the general form. The Langmuir isotherm did not appear to be applicable to Cecil soil ($R^2 = 0.02$).

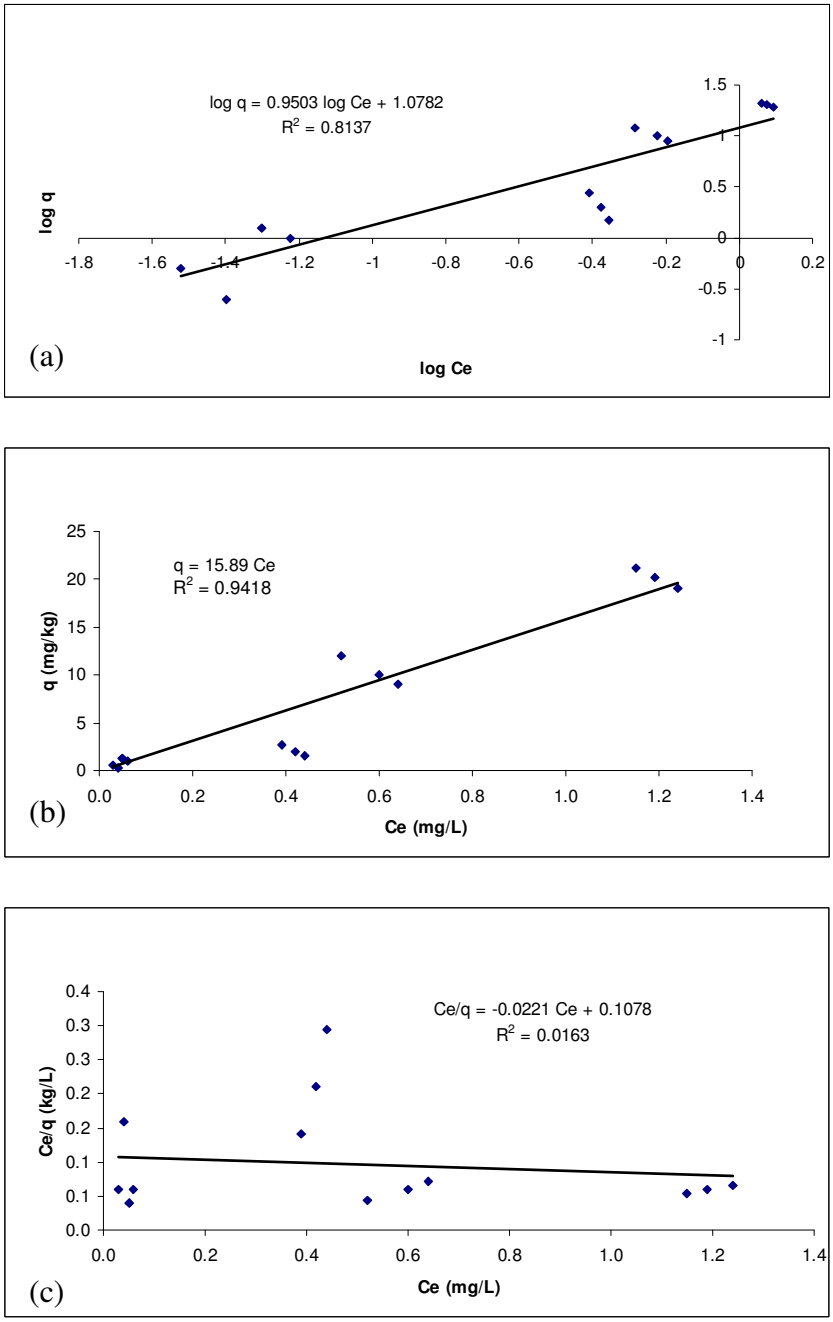


Figure 4-12. Isotherms for sorption of E2 to Cecil soil. (a) Freundlich, (b) Linear (c) Langmuir. Ce is the equilibrium aqueous E2 concentration (mg/L) and q is the equilibrium E2 sorbed concentration (mg/kg).

4.6.4 Cecil Bt Horizon Sorption Isotherms

To create an isotherm for the sorption of E2 to Cecil Bt horizon, E2 equilibrium concentrations from four initial E2 concentrations were used. Cecil Bt was included in this study because it has much lower organic carbon content than the other soils used. The data used to develop isotherms are presented in Table 4-8.

Table 4-8. Data used to create sorption isotherm for Cecil Bt horizon soils.

Initial E2 Concentration (mg/L)	Final E2 Concentration (mg/L)	Mean Final E2 Concentration (mg/L)	E2 sorbed* (mg/kg)	Mean E2 sorbed* (mg/kg)	% E2 sorbed
0.1	0.08	0.09	0.5	0.2	7
0.1	0.09		0.3		
0.5	0.21	0.21	7.3	7.4	59
0.5	0.20		7.5		
0.5	0.29		5.3		
1.0	0.53	0.52	11.8	11.9	48
1.0	0.49		12.8		
1.0	0.55		11.3		
2.0	1.57	1.50	10.8	12.4	25
2.0	1.32		17.0		
2.0	1.62		9.5		

*Determined by mass balance assuming all initial E2 did not degrade.

None of the isotherms developed to model the sorption of E2 to Cecil Bt horizon soil described the data very well (Figure 4-13). The highest R^2 value was again for the linear form ($R^2 = 0.73$). However, visually, this regression did not follow the trend of the data. The Langmuir isotherm did not describe E2 sorption to Cecil Bt horizon soils.

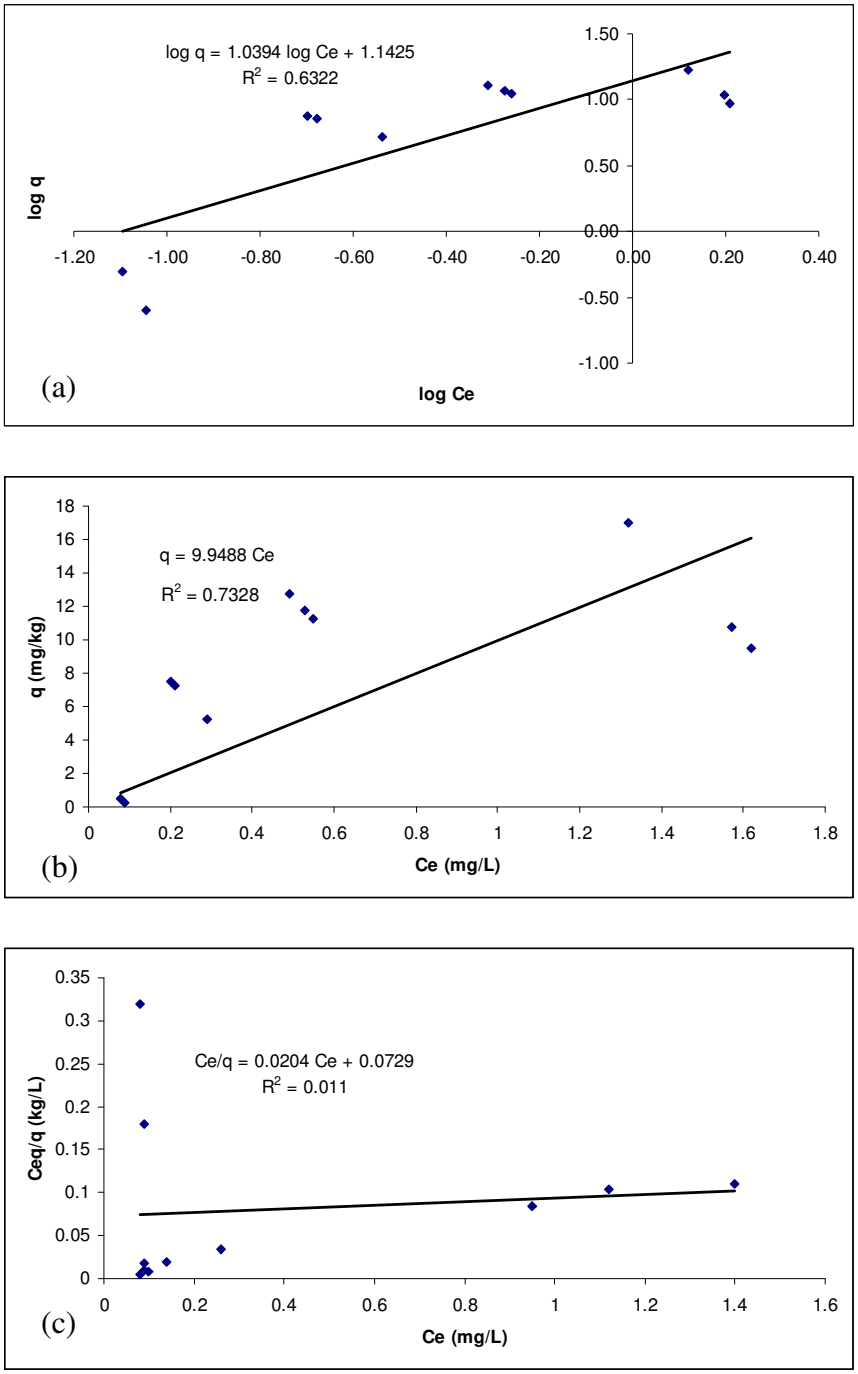


Figure 4-13. Isotherms for sorption of E2 to Cecil Bt horizon soil. (a) Freundlich, (b) Linear (c) Langmuir. Ce is the equilibrium aqueous E2 concentration (mg/L) and q is the equilibrium E2 sorbed concentration (mg/kg).

There are a number of reasons why isotherms may not describe the data for the sorption of E2 to Cecil Bt soil. First, with the exception of the Langmuir isotherm, the sorption isotherms are empirically based. With only four data points, it is difficult to assess if an isotherm adequately models the data. This is a possible issue for all of the isotherms developed in the study, because the data from the 0.05 mg/L initial concentration had to be discarded. The addition of more data points would have allowed for a more thorough examination of the fit of the developed isotherm to measured data. The major difference between the Cecil Bt soil and the other soils, specifically the Cecil soil from the plow layer, is the very low (0.1 %) organic carbon content. It is possible that the organic carbon content controls the sorption of E2 for the other soils and another sorption mechanism is taking place for Cecil Bt horizon.

4.6.5 Comparison of Sorption Isotherms

The linear isotherm fit the E2 sorption data best for Myatt, Cecil, and Cecil Bt horizon (Table 4-9), and fit well for Groseclose soil data. For the Groseclose soil, the non-linear form of the Freundlich isotherm fit the data best. The Langmuir isotherm did not describe the data for Cecil, Cecil Bt horizon or Myatt soils. Although the R^2 value (0.88) was relatively high for the linear regression of the Langmuir isotherm using data from Groseclose soil, it is unlikely that the assumptions listed in Chapter 2 for the Langmuir isotherm describe sorption of E2 to Groseclose soil. The Langmuir isotherm was developed initially to describe the adsorption of gas molecules by a solid surface and the assumptions are not generally applicable to soil systems (Essington, 2004).

Table 4-9. Summary of R^2 values and parameter estimates for sorption isotherms for Groseclose, Myatt, and Cecil soils.

	Linear		Freundlich			Langmuir		
Soil	**R^2**	**K (L/kg)**	**R^2**	**K**	**n**	**R^2**	**K (L/kg)**	**b (mg/kg)**
Groseclose	0.93	22.2	0.98	23.0	0.71	0.88	1.59	33.8
Myatt	0.96	21.7	0.91	30.1	1.48	0.26	nc	nc
Cecil	0.94	15.9	0.81	12.0	0.95	0.02	nc	nc
Cecil Bt	0.73	9.95	0.63	11.0	1.14	0.01	nc	nc

nc- not calculated because of the very low R^2 value.

Although past E2 sorption studies have employed Freundlich isotherms (Lai et al., 2000; Casey et al., 2003; Lee et al., 2003; Yu et al., 2004), the linear isotherm is a special case of the

Freundlich isotherm with n = 1. The sorption behavior of hydrophobic organic compounds, such as E2, is often described by linear isotherms (Essington, 2004). Although Groseclose was the only soil with a best fit for the Freundlich isotherm, all the soils in this experiment were compared to other studies that used the Freundlich isotherm. The Freundlich isotherms developed for Groseclose, Myatt and Cecil soils were compared to other Freundlich isotherms from this study (Figure 4-14) and from the literature (Figure 4-15). These isotherms were at the lower end of the scale for the published Freundlich isotherms.

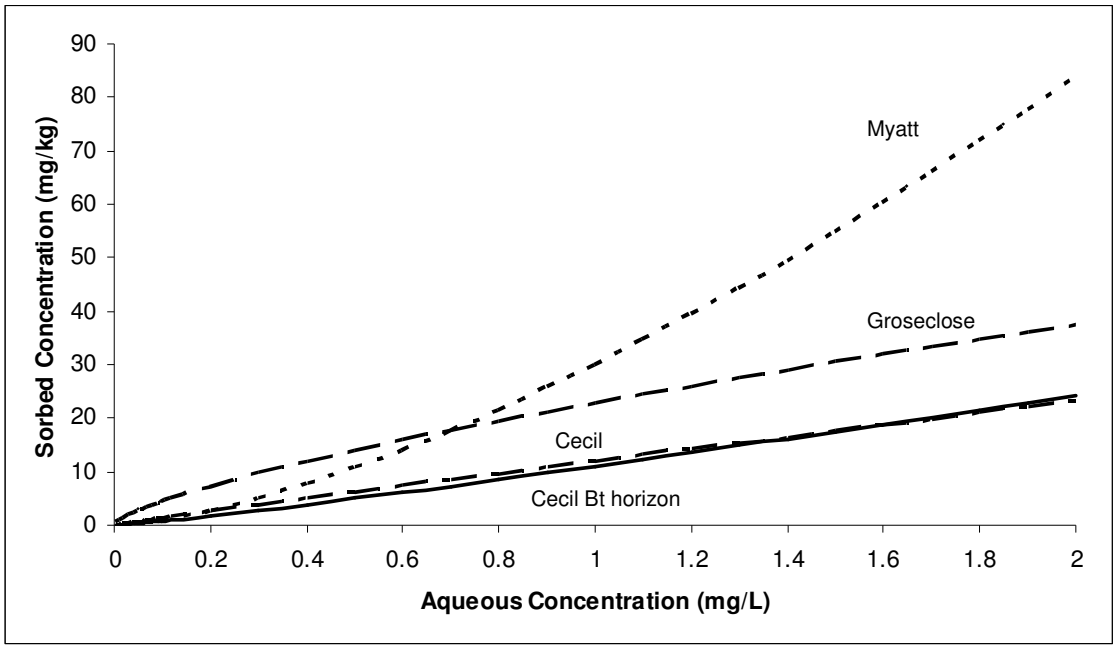


Figure 4-14. Freundlich isotherms for sorption of E2 to Groseclose, Myatt and Cecil soils.

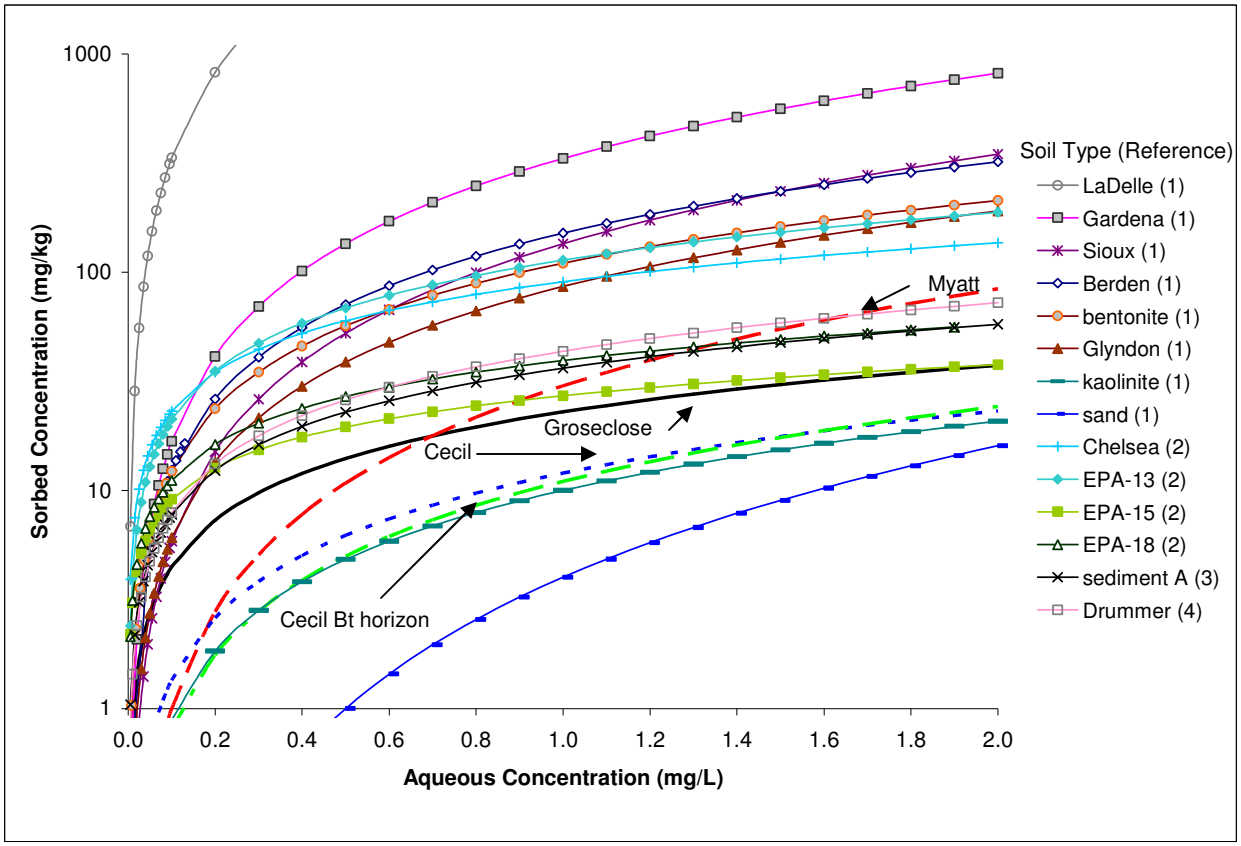


Figure 4-15. Comparison of Freundlich isotherm equations for soils from this study and from the literature. (1) Casey et al. (2003); (2) Yu et al. (2004); (3) Lai et al. (2000); (4) Lee et al. (2003). Descriptions of soil characteristics can be found in Table 2-6.

The Freundlich isotherms shown in Figure 4-15 can be deceiving because of the log scale for the y-axis. The isotherms developed by Casey et al. (2003) had n values greater than one for all soils other than bentonite (Table 4-10). As seen in Figure 4-14 for Myatt soil, a value of n greater than one results in an isotherm that does not asymptotically approach a maximum sorbed concentration. When the sorption experiments for the LaDelle soil were revisited by Casey et al. (2005), linear isotherms were chosen rather than Freundlich. Figure 4-16 compares the Freundlich isotherms developed for soils with n values less than one in this study and in others. For these soils, the Freundlich isotherms followed a general trend in which the soils with the higher organic carbon content resulted in higher sorbed E2 concentrations (Table 4-10 and Figure 4-16).

Table 4-10. Freundlich isotherm parameters for sorption E2 compared to organic carbon content of soils and sediment.

Soil	K_d ($L^n \cdot mg^{n-1}/kg$)	n	OC %	Reference
Groseclose	22.9	0.71	2.2[b]	
Cecil	12.0	0.95	1.8[b]	
Myatt	30.1	1.48	1.3[b]	
Cecil Bt horizon	11.0	1.14	0.11[b]	
Berden	151[a]	1.088	7.5	(Casey et al., 2003)
Gardena	332[a]	1.297	5.3	(Casey et al., 2003)
Glydon	86[a]	1.151	3.3	(Casey et al., 2003)
LaDelle	6670[a]	1.299	9.2	(Casey et al., 2003)
Sioux	135[a]	1.363	7.5	(Casey et al., 2003)
bentonite	110[a]	0.954	0.0	(Casey et al., 2003)
kaolinite	10[a]	1.053	0.0	(Casey et al., 2003)
Sand	4[a]	2.0	0.0	(Casey et al., 2003)
Chelsea	90[a]	0.593	5.5[c]	(Yu et al., 2004)
EPA-13 sediment	113[a]	0.728	4.5[c]	(Yu et al., 2004)
EPA-15 sediment	27[a]	0.475	0.95[c]	(Yu et al., 2004)
EPA-18 sediment	39[a]	0.55	0.66[c]	(Yu et al., 2004)
Sediment	36.3	0.67	1.1[c]	(Lai et al., 2000)
Drummer	43.4	0.74	2.9	(Lee et al., 2003)

[a] published K_d values were converted to units of $L^n \cdot mg^{n-1}/kg$ for comparison
[b] % organic carbon was estimated as OM / 1.724 (Richardson and Veparaskas, 2001)
[c] measured as TOC (wt %)

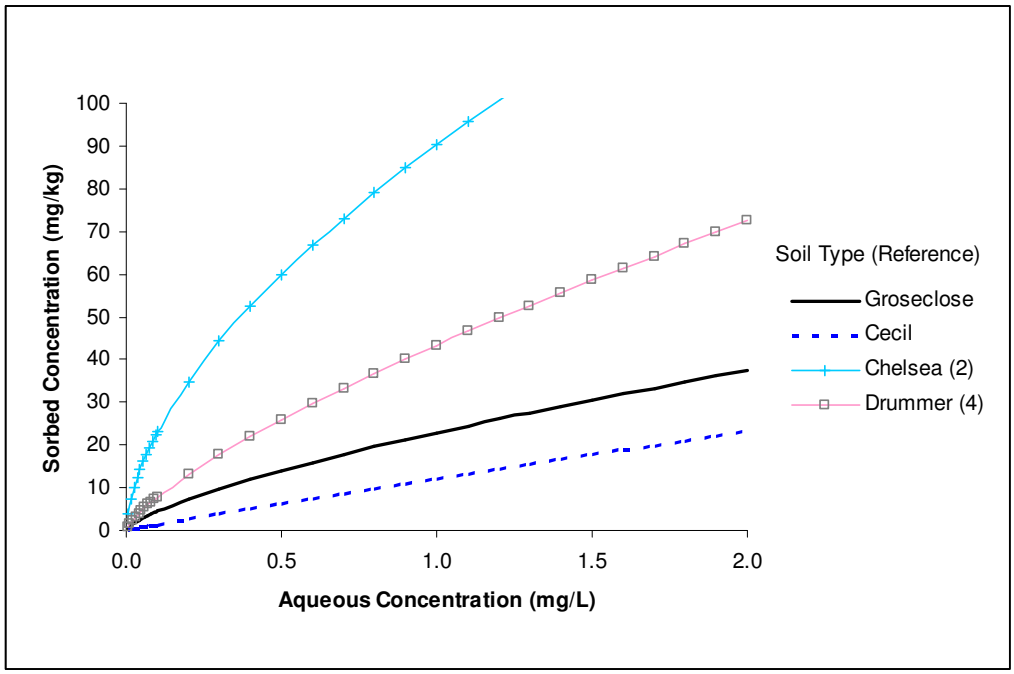


Figure 4-16. Comparison of Freundlich isotherm equations for soils from this study and others with n-values < 1. (2) Yu et al. (2004); (4) Lee et al. (2003).

When compared to the other two soils, Chelsea and Drummer, E2 was sorbed less strongly to Groseclose. This was expected if hydrophobic partitioning into organic carbon is the dominant sorption mechanism for E2 sorption to soil. The organic carbon content was higher for Chelsea soil (5.45%) and Drummer (2.91%) than for Groseclose soil (2.2%).

The linear isotherms developed for Groseclose, Cecil, Myatt and Cecil Bt soils were compared to each other in Figure 4-17. The slope of the developed linear isotherms did not increase in the order of increasing organic carbon content. Although Groseclose soil with the highest organic carbon content showed the strongest sorption of these soils, the linear isotherm developed for Myatt soil was very similar. Cecil soil has larger carbon content than Myatt soil yet the slope of the linear isotherm developed for the Cecil soil is less than the slope of the linear isotherm developed for Myatt soil. Therefore, linear partitioning to organic carbon may be the dominant mechanism, but the systems cannot be completely represented by this mechanism.

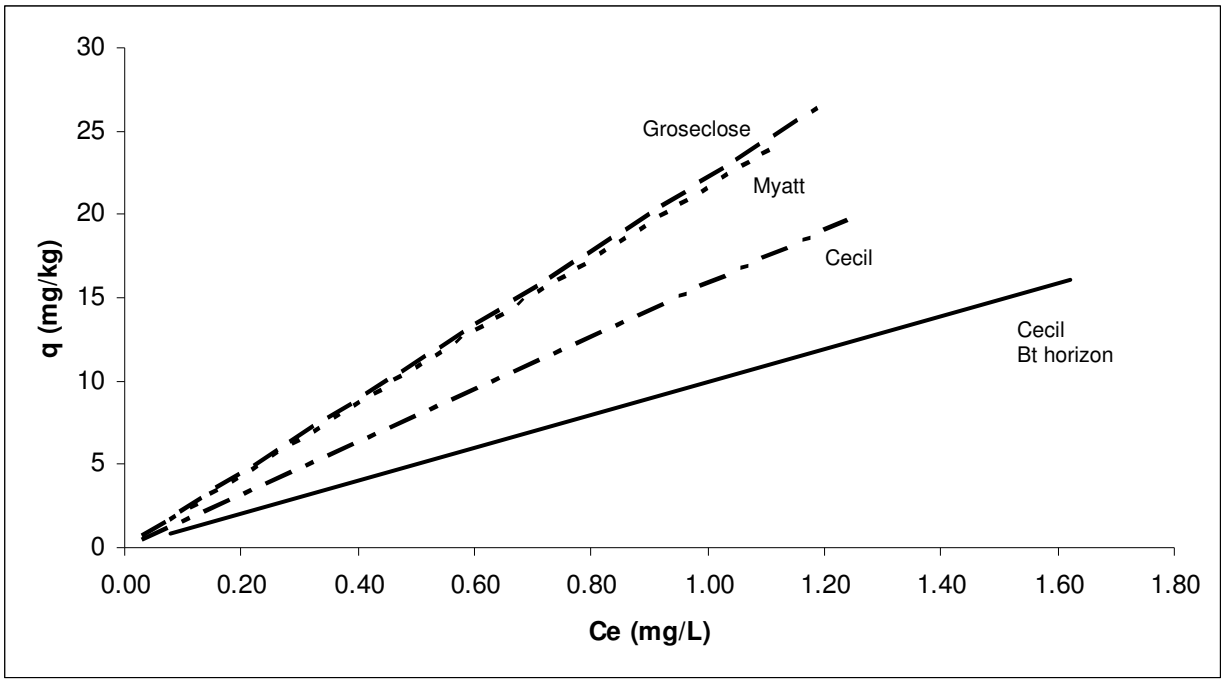


Figure 4-17. Linear isotherms for sorption of E2 to Groseclose, Cecil, Myatt, and Cecil Bt horizon soils.

Linear isotherms were also used to model the sorption of estrogens to natural soils by Casey et al. (2005) and Lee et al. (2003), but it is difficult to compare the studies because the soil-water systems were sampled at various times to create isotherms that changed over time. The systems were not sterilized, but measured estrone (E1) was included as E2. The linear isotherms from these studies are compared to those developed in this study in Figure 4-18. Casey et al. (2005) calculated parameters at different times. For the purpose of comparison, the parameters developed for soil-water systems sampled at 24 hrs were used in Figure 4-18.

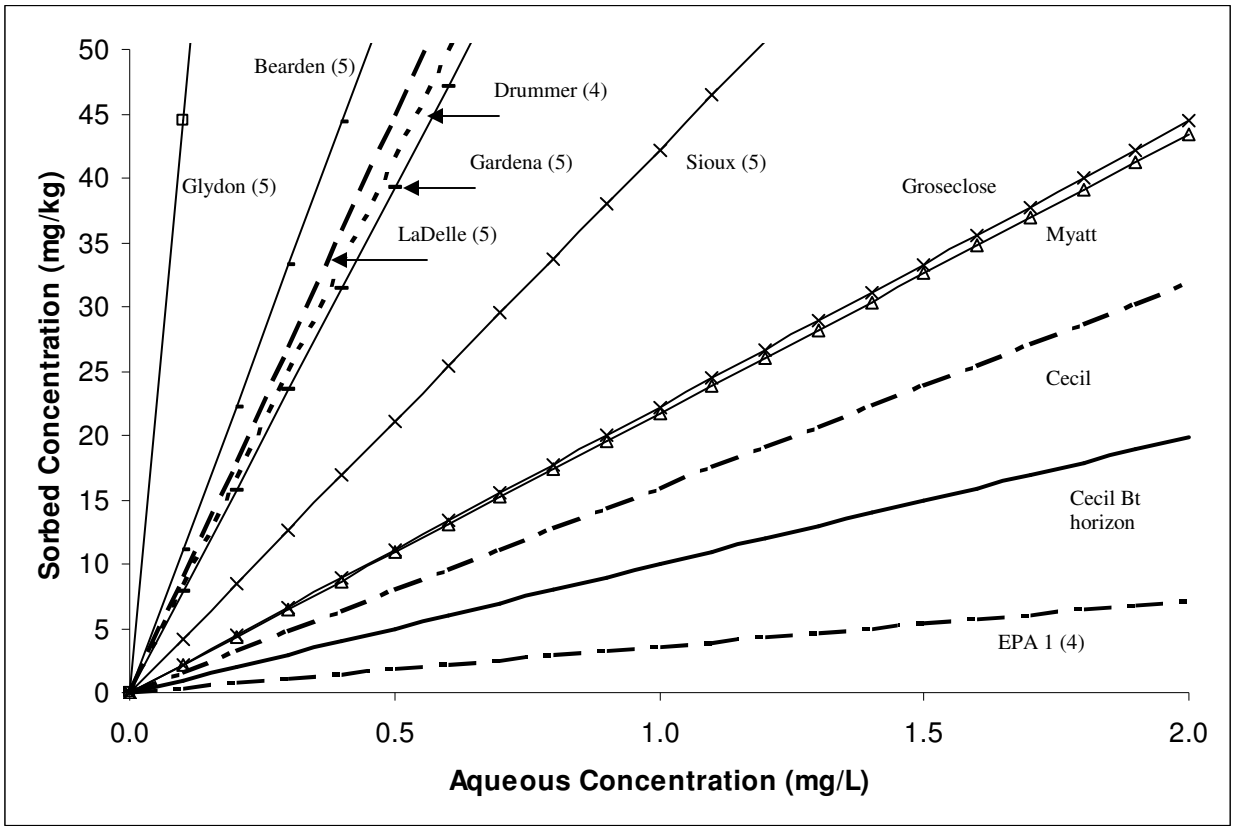


Figure 4-18. Linear isotherms for sorption of E2 to soils and sediments. (4) Lee et al. (2003); (5) Casey et al. (2003). Descriptions of soil characteristics can be found in Tables 2-6 and 4-11.

After 24 hrs, the K value for the partitioning of E2 onto the LaDelle silt loam used in the Casey et al. (2005) study was 89.47, much higher than the K values determined in this study (Table 4-11). However, the organic carbon content of the LaDelle silt loam (9.2%) is much higher than the organic carbon content of the soils used in this study. When the partitioning coefficient is normalized using the organic C content, the log K_{oc} coefficients for this study (Table 4-11) become comparable those values for LaDelle soil. Casey et al. (2005) reported log K_{oc} values that ranged from 2.27 to 2.97 from 0.5 h to 24 h, respectively. Log K_{oc} values for Drummer soil and EPA 1 sediment reported by Lee et al. (2003) were 2.46 and 3.21, respectively (Table 4-11). When the log K_{oc} values are compared within each study, the smallest values of OC% result in the largest log K_{oc} values. There is not a general correlation across studies, but Casey et al. (2005) reported a negative correlation between organic carbon content and log K_{oc} values (correlation coefficient = -0.872) indicating that hydrophobic partitioning is not the only

sorption mechanism. As organic carbon content decreases, the contribution of non-hydrophobic processes appears to increase. One possible non-hydrophobic sorption mechanism was proposed by Yu et al. (2004) who suggested that the polar groups at position C-17 can readily interact with mineral surfaces. In order to determine the specific mechanisms of sorption of E2 to soils, methods such as nuclear magnetic resonance spectroscopy need to be utilized.

Table 4-11. Summary of partitioning coefficients (K) and the organic carbon (OC) normalized (log K_{oc}) values for batch equilibrium E2 sorption experiments.

Soil	K (L/kg)	OC %	Log K_{oc}	Reference
Groseclose	22.2	2.2[a]	2.53	
Myatt	21.7	1.3[a]	2.73	
Cecil	15.9	1.8[a]	2.48	
Cecil Bt horizon	9.95	0.1[a]	3.53	
LaDelle	89.5[b]	9.2	2.97[b]	(Casey et al., 2005)
Bearden	110.9[c]	7.5	3.17[b]	(Casey et al., 2005)
Gardena	78.4[c]	5.3	3.17[b]	(Casey et al., 2005)
Glydon	445.2[c]	3.3	4.13[b]	(Casey et al., 2005)
Sioux	42.2[c]	7.5	2.75[b]	(Casey et al., 2005)
Drummer	83.2	2.91	2.46	(Lee et al., 2003)
EPA 1	3.56	0.22	3.21	(Lee et al., 2003)

[a] % organic carbon was estimated as OM / 1.724 (Richardson and Vepraskas, 2001)
[b] parameters calculated from samples taken at 24-hrs
[c] K was calculated using published K_{oc} and % OC values.

4.7. Relating Partitioning to Organic Carbon Content

Soil organic matter or soil organic carbon content is highly correlated to the extent of sorption for hydrophobic compounds (USEPA, 1998b; Essington, 2004). The organic matter can be converted to organic carbon content by dividing by a conversion factor of 1.724 (Richardson and Vepraskas, 2001). In this study, the soil with the largest organic carbon content, Groseclose (% OM = 3.8; % OC = 2.2), had the greatest sorption of E2, and the soil with the lowest organic carbon content, Cecil Bt horizon (% OM = 0.2; % OC = 0.1), had the least sorption of E2. The Cecil soil had a larger organic carbon content (% OM = 3.1; % OC = 1.8) than the Myatt soil (% OM = 2.3; % OC = 1.3), but had a smaller partitioning coefficient. The partitioning coefficient is the slope of the linear isotherm (Figure 4-17). A steeper slope, or higher partitioning coefficient, indicates greater sorption of E2.

Lai et al. (2000) reported correlation coefficients ranging from 0.86 to 0.94 for the sorption of free estrogens, including E2 and E1, to the total organic carbon (TOC) content of various sediments. Casey et al. (2003) found a correlation coefficient of r = 0.82 between the sorption of E2 and percent organic carbon. A similar relationship was found for the three soils and the Cecil Bt horizon used in this study and the organic matter content of the soils (Figure 4-19). The organic matter content of the soils used in this study is listed in Table 4-12. A correlation analysis was run in SAS between E2 sorption and soil organic matter, pH, and CEC for the data used for isotherm development at an initial concentration of 2.0 mg/L (Table 4-13). The Pearson correlation coefficient for the relationship between organic matter content and sorption was 0.82 with a p-value of 0.0021.

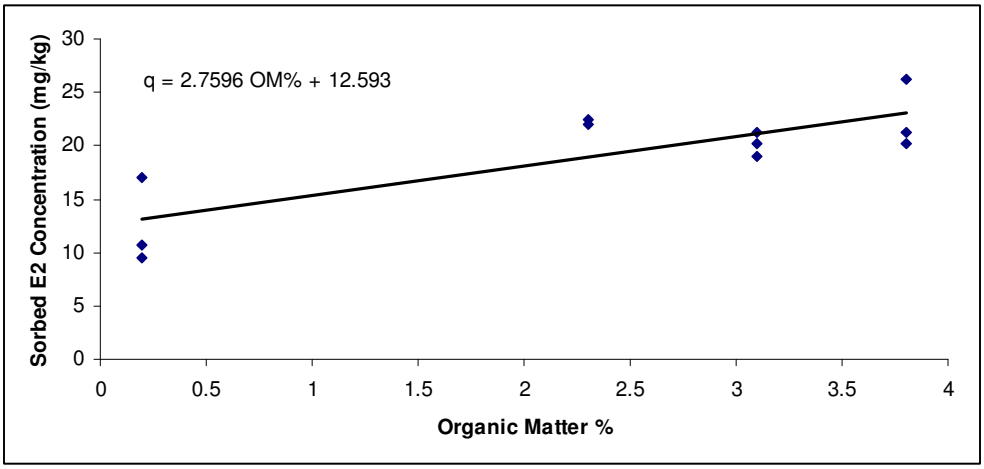


Figure 4-19. Correlation between sorbed E2 concentration and organic matter content (%). Initial concentration was 2.0 mg/L in 150 mL of background solution and 6.0 g soil.

Table 4-12. Soil characterization: pH, organic matter, and cation exchange capacity (CEC) (Lawrence, 2000). Also includes measured pH of soil-water systems (150 mL:6 g soil).

Soil Type	pH (1:1)	Organic Matter (%)	Organic Carbon[a] (%)	CEC	Measured pH
Groseclose	6.7	3.8	2.2	5.45	5.7
Cecil	6.6	3.1	1.8	4.61	6.2
Cecil Bt Horizon	ND	0.2	0.1	ND	5.4
Myatt	6.1	2.3	1.3	3.76	4.4

ND- not determined; [a] % organic carbon determined by dividing % OM by 1.724 (Richardson and Vepraskas, 2001)

The correlation between organic matter and E2 sorption could be complicated by a correlation between the pH for each soil reported by Lawrence (2000) and E2 sorption because pH and organic carbon content were highly correlated (r = 0.93, p-value 0.0007; Table 4-13). Organic carbon and measured pH were also found to be highly correlated (r = 0.90, p-value 0.0002; Table 4-13). Values of pH for each soil (1:1) are given in Table 4-12. The measured pH was recorded in each bottle at various times in the experiment and is the pH of 150 mL of E2 solution (0.005 M $CaCl_2$ and 100 mg/L NaN_3) mixed with 6 g of soil. The reported pH is not correlated to sorption of E2 (r = 0.82). There is a correlation between the measured pH and sorbed E2 concentration with a Pearson correlation coefficient, r, of 0.72 with a p-value of 0.013.

Table 4-13. Correlation analysis with soil characteristics and sorbed and aqueous E2 concentrations. Initial concentration was 2.0 mg/L E2.

	Pearson's Correlation Coefficient, r					
	OM%	**Reported pH**	**Measured pH**	**CEC**	**q**	**Ceq**
Reported pH*	0.93					
p-value	0.0007					
Measured pH**	0.90	0.61				
p-value	0.0002	0.11				
CEC	1.0	0.91	0.25			
p-value	<0.0001	0.0012	0.56			
q	0.82	-0.091	0.72	0.13		
p-value	0.0021	0.83	0.013	0.76		
Ceq	-0.82	0.091	-0.72	-0.13	-1.00	
p-value	0.0021	0.83	0.013	0.76	<0.0001	
Temperature	-0.55	0.28	-0.20	-0.12	-0.64	0.64
p-value	0.08	0.50	0.55	0.78	0.032	0.032

q is E2 sorbed concentration in mg/kg. Ceq is equilibrium aqueous E2 concentration in mg/L.
*pH reported in Lawrence (2000)
**pH measured in the soil-water systems containing 150 mL aqueous solution and 6 g soil.

No significant correlation between CEC and sorption (r = 0.13, p-value 0.76) was found, but there was a significant correlation between CEC and OM (r = 1.0, p-value <0.0001). However, the CEC values used for this analysis were only for Cecil, Groseclose, and Myatt soils. No information regarding the CEC value of Cecil Bt soil was available. It is possible that the CEC value of Cecil Bt soil could be very high compared to a very low organic carbon content.

Therefore, despite the relationship between pH and sorption, it is likely that the sorption is related to OM because of the hydrophobic behavior of E2, and the correlation to measured pH was coincidental.

4.8. Limitations of Isotherm Development Procedures

The sorption of organic compounds can depend on a variety of environmental conditions such as temperature, pH, and soil type. In these experiments, the pH was not buffered, and the temperature varied by ± 2 ^{o}C. Care must be taken when comparing these results to other studies such as Yu et al. (2004) where the pH of the soil-water systems was buffered to 6.8. To date, there are no studies examining the effect of pH on the sorption of E2 to soils. Variations associated with soil type such as clay type and content and organic carbon content can affect sorption of E2. In this study, a correlation was found between E2 sorption and organic carbon content and the isotherms created were unique to each soil. Therefore, the isotherms developed in this study should not be applied to the sorption of E2 to other soils. The study of the sorption of estrogens can be further complicated by degradation by microorganisms, photolysis, and abiotic catalysis. If only liquid concentrations are measured, it can be difficult to distinguish between the amount of estradiol sorbed and the amount degraded to estrone and other byproducts. Although efforts were made to prevent degradation of E2 by microbial or photolysis mechanisms for these experiments, some E1 was found in many samples. This is a major limitation of attempting to isolate the sorption process from the overall environmental fate of a relatively easily degraded compound such as E2. In a natural system, both sorption and degradation would serve as processes to remove E2 from the soil solution.

4.9. Hypothesis Testing

One assumption for creating isotherm equations is that a measurable amount of the compound of interest is sorbed to the soil. Figure 4-20 shows the percent of E2 sorbed to each soil for the initial concentrations used in this study. The amount of E2 sorbed was calculated by mass balance using the initial concentration added to each soil-water system. The initial concentration of 0.05 mg/L was not included because the final equilibrium concentration in the aqueous phase for each soil water system fell below the MDL of 0.03 mg/L. For the 0.1 mg/L initial concentration soil water system of Cecil Bt soil, only 7% of the E2 added was sorbed.

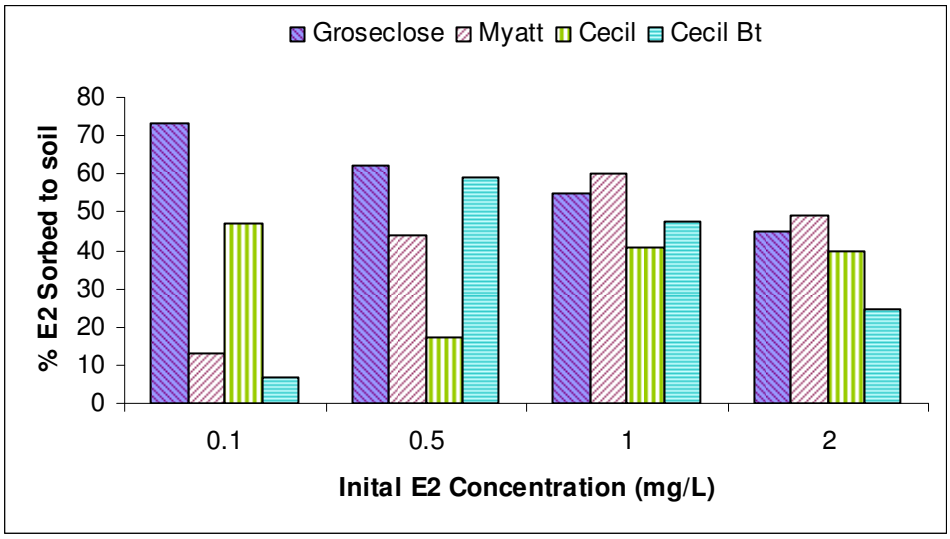


Figure 4-20. % of E2 sorbed to each soil calculated by a mass balance from the equilibrium aqueous concentration.

In order to determine if a significant amount of E2 sorbed to each soil, a Tukey's multiple comparison test ($\alpha = 0.05$) was run in SAS to compare the equilibrium concentration for each soil-water system to the initial E2 concentration (SAS code in Appendix A). This test was used to determine if the equilibrium concentration was significantly different than the initial concentration of E2 for each soil. Because the sorbed concentration is calculated by mass balance, a significant difference between initial and equilibrium concentration indicates that a significant amount of E2 is sorbed to the soil. The detailed SAS output is located in Appendix D. Table 4-14 summarizes the results of the comparison for each initial concentration and soil. The first research hypothesis was that a significant amount of estrogen sorbs to each soil. Using the multiple comparison results, this hypothesis was rejected for Myatt and Cecil Bt horizon soils at an initial concentration of 0.1 mg/L and for Cecil and Myatt soils at an initial concentration of 0.5 mg/L. While 44% of the E2 added in the 0.5 mg/L solution was sorbed to Myatt soil, the variability of the equilibrium concentrations was high, masking any differences. The hypothesis that a significant amount of E2 is sorbed to soil is only valid for the higher concentrations in this experiment. Therefore, future experiments should evaluate a larger range of initial concentrations to gain an accurate estimation of isotherm parameters.

Table 4-14. Summary of Tukey's multiple comparison test (α = 0.5). Means with the same letter in each column are not significantly different.

	Mean Concentration (mg/L)			
Initial	**0.1[a]**	**0.5[a]**	**1.0[a]**	**2.0[a]**
Cecil Bt	0.0933[a]	0.233[b]	0.523[bc]	1.503[b]
Cecil	0.0533	0.417[ab]	0.553[b]	1.193[c]
Myatt	0.0867[a]	0.280[ab]	0.400[c]	1.097[c]
Groseclose	0.0300	0.193[b]	0.447[bc]	1.027[c]

The second research hypothesis was that the aqueous concentration of E2 is related to the sorbed concentration of E2 and that isotherm equations can be developed. The null hypothesis is rejected because the high correlation (r = -1.00) of the equilibrium E2 concentration to the sorbed E2 concentration has a p-value of <0.001. However, because the sorbed concentration was calculated by a mass balance from the aqueous concentration, this correlation should be used with caution. Without measuring the sorbed concentration directly it is impossible to evaluate the first part of this hypothesis. The second part, or the creation of isotherms for each soil, is not rejected for Groseclose, Cecil and Myatt soils, but is rejected for the Cecil Bt soil. No isotherms adequately modeled the data obtained from the Cecil Bt experiment. This indicates that sorption isotherms work well for modeling the sorption of nonpolar E2 to soil when the organic carbon content of the soils is high. At low organic carbon concentrations, a different form of isotherm may need to be used. In order to evaluate this statement, more experiments using a wide range of concentrations and organic carbon contents would need to be conducted.

The third research hypothesis, that the sorption of conjugated estrogens is significantly less than the sorption of free estrogens to soil, could not be evaluated because the methanolysis procedure attempted to deconjugate estrogens was not successful. Future attempts to quantify conjugated estrogens should examine other methods of deconjugation such as enzymatic hydrolysis combined with GC/MS or use a method such as LC-MS/MS that does not require deconjugation.

The sorption of free estrogens was positively correlated to the organic carbon content of the soils used in this research. Therefore, the fourth research hypothesis, that the sorption of estrogens is positively correlated to the organic carbon content of soil, is not rejected. The null-hypothesis that there is no correlation was rejected with a p-value of 0.0021. The correlation

between organic carbon and the sorption of E2 is important because it provides insight into the dominant sorption mechanism of the nonpolar E2 molecule. Because of the linear behavior of the sorption isotherms and the correlation of sorption to OM, it appears that linear partitioning into the soil organic carbon is the dominant sorption mechanism. However, the nonlinear behavior of the Groseclose isotherm and the inability to develop an isotherm for Cecil Bt indicate that linear partitioning is not the only sorption mechanism.

Chapter 5: Summary and Conclusions

5.1. GC/MS measurement of estrogens

The first objective of this study was to develop a method using gas chromatography/mass spectrometry (GC/MS) to measure aqueous free and conjugated estrogen concentrations. Overall, the GC/MS method proved to be an acceptable method to measure estrogens in aqueous phases when combined with a derivatization procedure. The method detection level (MDL) for the procedures used in this study was 0.03 mg/L. Approximately 90% of E2 was recovered and measured by the GC/MS. An advantage to using GC/MS instead of an enzyme immunoassay (EIA) is that each sample can be checked for estrone and other degradation products. One of the issues with the GC/MS method was the time intensive sample preparation that involved extraction and derivatization for each sample. For a study where a large number of samples are involved, an alternate method such as EIA should be considered. Also, although the derivatization procedure was evaluated with a percent recovery of almost 90%, some samples had a "double peak" or two overlapping peaks instead of one for derivatized E2. For these samples, the concentration of E2 was much lower than expected. It was hypothesized that the additional peak was a result of underivatized E2. All sample chromatograms were checked for these double peaks and those that had them were discarded.

5.2. Isotherm Equations

The second objective of this research, to develop isotherm equations to describe the sorption of estrogen to soil, was accomplished by using batch equilibrium experiments. The data were analyzed using linear regression in SAS. Linear, Langmuir, and Freundlich isotherm models were considered, and the linear isotherm was selected as the best description of the experimental data for Myatt, Cecil and Cecil Bt horizon soils. The R^2 values for the linear regression to fit a linear isotherm were 0.96, 0.94, and 0.73 for Myatt, Cecil, and Cecil Bt horizon soils, respectively. The general form of the Freundlich isotherm gave the best fit for Groseclose soil ($R^2 = 0.98$).

While the high R^2 factors for Myatt, Cecil, and Groseclose soils indicate that isotherms can represent the sorption of E2, this experiment simplified the complex environmental conditions

that would determine the aqueous E2 concentration by using sodium azide to minimize biodegradation and by covering to minimize photolysis. In a field environment, degradation rates would play a role in the amount of E2 that is available for sorption.

The first research hypothesis stated that a significant amount of estrogen was expected to sorb to each soil. This hypothesis was not able to be tested for the lowest initial concentration level of 0.05 mg/L. The measured aqueous phase concentrations for soil water systems with initial concentrations of 0.05 mg/L fell on or below the MDL of 0.03 mg/L for the methods used in this experiment and had to be discarded as data points. All soils except for the Bt horizon of Cecil soil were shown to sorb a significant amount of E2 at all initial concentration levels above 0.05 mg/L. For the Bt horizon of Cecil soil, a mean of only 7% of the E2 added was sorbed to the soil at lower initial concentration of 0.1 mg/L. The major difference between the other soils in this study and the Cecil Bt horizon is the organic carbon content. It is hypothesized that the low organic carbon content of this soil is responsible for the difference in sorption behavior. However, more research needs to be done on soils with varying organic carbon contents and more initial concentrations to create isotherm equations for low organic carbon soils.

The second research hypothesis stated that the aqueous concentration of E2 is related to the sorbed concentration of E2 and can be described by an isotherm equation. This hypothesis was confirmed for Groseclose, Cecil, and Myatt soils by the successful creation of sorption isotherms. However, for the Bt horizon of Cecil soil, it was not possible to develop an isotherm equation using the data obtained in this study.

5.3. Conjugated Estrogens

The objectives of this research included the analysis of the sorption of conjugated estrogens to agricultural soils. Because Objective 1, developing an analytical procedure for the analysis of conjugated estrogens, could not be met in regard to conjugated estrogens, Objective 2, developing isotherm equations for conjugated estrogens, could also not be accomplished. The method used to attempt to deconjugate estradiol in this study, methanolysis, did not produce reproducible results and had to be abandoned. Measurable free E2 was only recovered from the conjugated E2 standard in a few samples.

5.4. Organic carbon

The sorption of E2 was correlated to organic carbon content (Pearson's correlation coefficient 0.82). This correlation is important because it supports the hydrophobic partitioning theory suggested by Lee et al. (2003) as the dominant sorption mechanism for E2. The high correlation coefficient supports the fourth research hypothesis that states that the sorption of estrogens is correlated to the organic carbon content.

5.5. Conclusions

1. GC/MS combined with liquid/liquid extraction and deconjugation was an appropriate method to measure estrogen concentrations in soil-water solutions (MDL of 0.03 mg/L and approximately 90% recovery of E2).
2. The methanolysis procedure is not an appropriate method of deconjugation of conjugated E2 in a soil-water solution.
3. Linear isotherms provided a good fit ($R^2 > 0.9$) for soils with high organic carbon content (% OC > 1.5).
4. The sorption of E2 to soil was related to the organic carbon content. In general, the higher the organic carbon content of the soil, the higher the sorption of E2.
5. Hydrophobic partitioning can be assumed to be the dominant sorption mechanism of E2 to agricultural soils. However, hydrophobic partitioning does not fully describe the sorption of E2 to soils especially for soils with low (≤ approximately 0.1 %) organic carbon content, indicating that other sorption mechanisms may be masked by hydrophobic partitioning behavior in soils with high organic carbon content (> approximately 1.0 %).

5.6. Suggestions for Future Research

While this study was directed at the free estrogen, 17β-estradiol, information on the sorption and degradation of conjugated estrogens is missing when looking at the environmental fate of estrogens. This study attempted to analyze estradiol glucuronides and sulfates by deconjugation, derivatization, and GC/MS analysis following the methanolysis deconjugation procedure described in Tang and Crone (1989). However, the attempt to use this method was unsuccessful. Future work should examine other methods such as enzymatic hydrolysis to deconjugate conjugated estrogens if GC/MS or EIA is to be used. Another possibility is to use

LC/MS methods to analyze conjugated estrogens as this eliminates the need to deconjugate. Conjugated estrogens have been largely ignored in sorption and degradation studies, but they are important because they are expected to be much more soluble and, therefore, more easily transported.

As the sorption of E2 varied greatly depending on the soil used, future studies should examine the mechanism of sorption and degradation of E2 to attempt to predict the behavior in different soils. The results of this study indicated that the organic carbon content of the soil plays an important role in determining the amount of sorption. Based on the linear behavior of the isotherms and the correlation of organic carbon content to the sorption, it is hypothesized that linear partitioning is the dominant sorption mechanism of E2 to agricultural soils. Future work needs to be done to examine the actual behavior of E2 in relation to soil organic carbon.

Although the soil water systems in this study were chemically sterilized with sodium azide, some E2 did degrade to E1 suggesting that there is an abiotic mechanism of degradation of E2. Because the bottles were covered with foil while on the shaker, photolysis is unlikely. The degradation of estrogen in the soil environment needs to be studied further with a variety of soil characteristics to adequately model the environmental fate of estrogen from land applied manure.

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Appendix A: SAS Code used to analyze E2 sorption data

A.1. Method Detection Level

```
data conc;
input Cf;
lines;
[data]
;
run;

proc means data = conc;
var Cf;
run;
```

A.2. Percent Recovery

```
proc means data = work.jlk;
var recovery;
run;

proc means data = work.jlk lclm uclm alpha = 0.05;
var recovery;
run;

proc corr data = work.jlk;
var ci recovery;
run;
```

A.3. Linear Regression for Isotherm Development

data sorption;
input x y;
lines;
[data]
;
run;

proc reg data = sorption; Linear regression intercept ≠ 0
model y = x /clb clm cli;
run;

proc reg data = cecil; Linear regression intercept = 0
model y = x /clb clm cli NOINT;
run;

A.4. Correlation Analysis

Proc corr data = work.jlk;
Var OM pH measpH CEC Ceq q T;
Run;

A.5. Tukey's Multiple Comparison Test

```
data com;
input soil $ conc;
lines;
[data]
;
run;

proc glm data = com;
class soil;
model conc=soil;
means soil/tukey;
run;
quit;
```

Appendix B: Experimental Conditions Data

Table B-1. Room temperature (°C) data for all experiments.*

Experiment	Preliminary Concentration Solution A	Preliminary Concentration Solution B	Cecil Isotherm (1)	Groseclose Isotherm (2)	Preliminary Time to Equilibrium	Myatt Isotherm
Soil(s)	Groseclose/Cecil	Groseclose/Cecil	Cecil	Groseclose	Cecil/Cecil Bt/Myatt	Myatt
Day 0	1/6/05	1/7/05	2/14/05	3/7/05	4/13/05	5/25/05
Sample Day 1			2/15/05	3/8/05	4/14/05	
T at sample time			24.0	22.5	23.5	
High			24.0	23.5	23.5	
Low			21.0	21.0	20.0	
Sample Day 2			2/16/05	3/9/05		
T at sample time			23.0	22.5		
High			23.5	23.0		
Low			22.5	21.0		
Sample Day 3					4/16/05	
T at sample time					24.0	
High					24.0	
Low					21.0	
Sample Day 4				3/11/05		
T at sample time				23.0		
High				23.0		
Low				21.0		
Sample Day 5					4/18/05	
T at sample time					23.5	
High					24.0	
Low					20.0	
Sample Day 7	1/13/05	1/14/05	2/21/05	3/14/05	4/20/05	6/1/05
T at sample time	22.5	22.0	23.5	23.5	23.5	22.5
High	23.5	23.5	23.5	23.5	24.0	25
Low	21.0	21.0	21.0	21.0	22.0	21.5
Sample Day 9					4/22/05	6/3/05
T at sample time					23.5	23
High					24.0	25
Low					21.5	21
Sample Day 10			2/24/05	3/17/05		
T at sample time			21.5	22.5		
High			23.5	23.5		
Low			21.5	21.5		

*Temperatures (T) were recorded at sample times and the high and low temperatures between sample times were recorded.

Table B-2. pH data for preliminary concentration experiment.

Date	Soil Mass (g)	pH	
		Cecil	Groseclose
1/7/2005	0.5	6.4	6.4
	1	6.1	6.2
	3	6.0	5.9
1/14/2005	0.5	6.5	6.5
Solution B	1	6.6	6.3
	3	6.0	6.1
1/14/2005	0.5	6.6	6.5
Solution A	1	6.3	6.3
	3	6.0	6.1
Average	0.5	6.5	6.4
	1	6.3	6.3
	3	6.0	6.0

Table B-3. pH data for time to equilibrium experiment for Cecil, Cecil Bt, and Myatt soils.

Date	Sample	pH			
		Cecil	CBT	Myatt	Control
4/14/2005	0.05 A	5.7	4.5	5.3	6.5
(day 1)	0.05 B	5.7	4.5	5.3	6.6
	0.05 C	5.7	4.5	5.3	6.5
	2.0 A	5.6	4.5	5.3	6.5
	2.0 B	5.7	4.5	5.3	6.5
	2.0 C	5.6	4.5	5.3	6.5
Average		5.7	4.5	5.3	6.5
4/18/2005	0.05 A	6.0	4.5	5.3	6.4
(day 5)	0.05 B	6.0	4.4	5.3	6.5
	0.05 C	6.0	4.4	NM	6.4
	2.0 A	5.8	4.4	5.3	6.3
	2.0 B	5.9	NM	5.2	6.3
	2.0 C	5.7	4.3	5.2	6.3
Average		5.9	4.4	5.2	6.4
4/24/2005	0.05 A	6.1	4.6	5.6	6.4
(day 11)	0.05 B	6.1	4.6	5.5	6.4
	0.05 C	6.1	4.6	5.5	6.4
	2.0 A	6.1	4.7	5.4	6.4
	2.0 B	6.0	4.6	5.4	6.4
	2.0 C	NM	4.6	5.4	6.4
Average		6.1	4.6	5.5	6.4

NM indicates no measurement taken; A, B, C indicate individual replications.

Table B-4. pH data for Groseclose experiments.

		pH	
Date	**C1**	**Groseclose**	**Control**
3/8/2005	0.05 A	5.7	6.4
(day 1)	0.05 B	5.8	6.4
	0.05 C	5.8	6.4
	0.1 A	5.8	N
	0.1 B	5.7	N
	0.1 C	5.7	N
	0.5 A	5.7	N
	0.5 B	5.6	N
	0.5 C	5.6	N
	1.0 A	5.6	N
	1.0 B	5.6	N
	1.0 C	5.6	N
	2.0 A	5.6	6.4
	2.0 B	5.6	6.4
	2.0 C	5.6	6.4
Average		5.7	6.4
3/17/2005	0.05 A	6.0	6.6
(day 10)	0.05 B	6.0	6.6
	0.05 C	6.2	6.6
	0.1 A	6.0	N
	0.1 B	6.0	N
	0.1 C	6.0	N
	0.5 A	6.0	N
	0.5 B	6.1	N
	0.5 C	6.1	N
	1.0 A	6.0	N
	1.0 B	6.0	N
	1.0 C	6.2	N
	2.0 A	6.0	6.6
	2.0 B	6.0	6.6
	2.0 C	6.0	6.6
Average		6.0	6.6

N = no control for corresponding E2 concentration
A, B, C indicate individual replications.

Table B-5. pH Data for Cecil isotherm experiment.

		pH	
Date	**C1**	**Cecil**	**Control**
2/24/2005	0.05 A	6.1	6.9
(day 10)	0.05 B	6.2	7.0
	0.05 C	6.1	6.8
	0.1 A	6.1	N
	0.1 B	6.1	N
	0.1 C	6.3	N
	0.5 A	6.1	N
	0.5 B	6.2	N
	0.5 C	6.2	N
	1.0 A	6.3	N
	1.0 B	6.2	N
	1.0 C	6.2	N
	2.0 A	6.1	6.7
	2.0 B	6.2	6.7
	2.0 C	6.2	6.8
Average		6.2	6.8

N = no control for corresponding E2 concentration
A, B, C indicate individual replications.

Table B-6. pH Data for Cecil Bt isotherm experiment.

		pH	
Date	**C1**	**CBT**	**Control**
5/6/2005	0.05 A	4.5	--
(day 7)	0.05 B	4.5	--
	0.05 C	4.4	--
	0.1 A	4.4	N
	0.1 B	4.4	N
	0.1 C	4.4	N
	0.5 A	4.4	N
	0.5 B	4.4	N
	0.5 C	4.3	N
	1.0 A	4.3	N
	1.0 B	4.4	N
	1.0 C	4.4	N
	2.0 A	4.5	--
	2.0 B	4.5	--
	2.0 C	4.5	--
Average		4.4	
5/11/2005	0.05 A	4.4	7.8
(day 10)	0.05 B	4.4	7.2
	0.05 C	4.4	7.4
	0.1 A	4.4	N
	0.1 B	4.4	N
	0.1 C	4.4	N
	0.5 A	4.4	N
	0.5 B	4.3	N
	0.5 C	4.3	N
	1.0 A	4.4	N
	1.0 B	4.4	N
	1.0 C	4.3	N
	2.0 A	4.4	6.7
	2.0 B	4.4	7.0
	2.0 C	4.4	6.9
Average		4.4	7.2

-- indicates no measurement taken; N = no control for this concentration
A, B, C indicate individual replications**.**

Table B-7. pH data for Myatt isotherm experiment.

		pH	
Date	**C1**	**Myatt**	**Control**
6/1/2005	0.05 A	5.5	6.5
day 7	0.05 B	5.6	6.5
	0.05 C	5.4	6.5
	0.1 A	5.4	N
	0.1 B	5.4	N
	0.1 C	5.4	N
	0.5 A	5.4	N
	0.5 B	5.5	N
	0.5 C	5.3	N
	1.0 A	5.3	N
	1.0 B	5.5	N
	1.0 C	5.3	N
	2.0 A	5.4	6.5
	2.0 B	5.4	6.5
	2.0 C	5.4	6.5
average		5.4	6.5
6/3/2005	0.05 A	5.5	6.4
day 9	0.05 B	5.6	6.5
	0.05 C	5.4	6.5
	0.1 A	5.4	N
	0.1 B	5.4	N
	0.1 C	5.4	N
	0.5 A	5.4	N
	0.5 B	5.5	N
	0.5 C	5.4	N
	1.0 A	5.4	N
	1.0 B	5.5	N
	1.0 C	5.3	N
	2.0 A	5.4	6.5
	2.0 B	5.3	6.5
	2.0 C	5.3	6.6
average		5.4	6.5

N = no control for this concentration
A, B, C indicate individual replications.

Appendix C: GC/MS Raw Data

Table C-1. Calibration Curve Data. GC/MS responses.

Date	Standard Concentration	Mirex Response	E1 Response	E2 Response	E2:Mirex Concentration Ratio	E2:Mirex Response Ratio
01/20/05	0.1	429840	0	61555	0.2	0.14
	0.1	422746	0	64073	0.2	0.15
	0.5	473475	0	439501	1	0.93
	0.5	480250	0	484697	1	1.01
	1	NI	NI	NI	NI	NI
	1	413378	0	815649	2	1.97
	2	466586	0	1914748	4	4.10
	2	416264	0	1793631	4	4.31
03/02/05	0.1	59942	0	111048	0.2	1.85
	0.1	60773	0	57246	0.2	0.94
	0.5	57185	0	1027481	1	17.97
	0.5	NI	NI	NI	NI	NI
	1	58950	0	2377590	2	40.33
	1	61010	0	2374563	2	38.92
	2	58827	0	3583988	4	60.92
	2	56651	0	3801831	4	67.11
03/29/05	0.1	87768	0	135931	0.2	1.55
	0.1	88721	0	166919	0.2	1.88
	0.5	86115	564	839034	1	9.74
	0.5	85273	1037	937524	1	10.99
	1	84237	504	1704774	2	20.24
	1	86356	54	995234	2	23.05
	2	85962	2241	5329093	4	61.99
	2	87270	415	4100531	4	46.99
05/03/05	0.1	31410	0	64129	0.2	2.04
	0.1	32306	0	22321	0.2	0.69
	0.5	31664	0	167540	1	5.29
	0.5	33096	0	532504	1	16.09
	1	31174	0	1233901	2	39.58
	1	32938	0	1238315	2	37.60
	1*	30625	0	1246263	2	40.69
	2	35278	3384	2015540	4	57.13
05/17/05	0.05	40192	0	0	0	0**
	0.05	40083	0	42613	0.1	1.06
	0.1	39133	0	72236	0.2	1.85
	0.1	41786	0	117001	0.2	2.80
	0.5	41541	0	677069	1	16.30
	0.5	40853	0	680419	1	16.66
	1	41817	0	1609237	2	38.48
	1	41208	361	1584729	2	38.46
	2	43525	0	3830185	4	88.00
	2	43035	0	3664564	4	85.15
	0.5***	42675	0	703674	1	16.49
	0.5***	42394	0	652530	1	15.39

Table C-1 (cont.)

Date	Standard Concentration	Mirex Response	E1 Response	E2 Response	E2:Mirex Concentration Ratio	E2:Mirex Response Ratio
05/31/05	0.1	31496	0	25743	0.2	0.82
	0.1	33112	0	74414	0.2	2.25
	0.5	33838	0	569944	1	16.84
	0.5	31815	0	78121	--	--
	1	33109	0	1171454	2	35.38
	1	33446	0	1248071	2	37.32
	2	33018	114	2522945	4	76.41
	2	32107	55	2497281	4	77.78
06/07/05	0.05	69427	0	101292	0.1	1.46
	0.05	71488	0	79935	0.1	1.12
	0.1	71962	0	163760	0.2	2.28
	0.1	67754	0	227704	0.2	3.36
	0.5	71457	0	1191014	1	16.67
	0.5	70018	0	1356610	1	19.38
	1	69857	0	2994651	2	42.87
	1	70575	0	2928835	2	41.50
	2	70297	0	6144518	4	87.41
	2	67917	0	6200116	4	91.29
	0.5	70180	0	1261635	1	17.98
	0.5	69905	0	1232989	1	17.64
07/06/05	0.1	33189	0	15417	0.2	0.46
	0.1	34653	0	51607	0.2	1.49
	0.5	34171	1546	392811	1	11.50
	0.5	31032	61	156241	1	10.07
	1	35019	3515	939990	2	26.84
	1	36531	4638	1045793	2	28.63
	2	38221	7550	2359656	4	61.74
	2	39229	1197	1979340	4	50.46
07/13/05	0.1	49722	0	42111	0.2	0.85
	0.1	48356	0	80432	0.2	1.66
	0.5	49552	292	533430	1	10.77
	0.5	49038	2388	544411	1	11.10
	1	49133	1887	1149899	2	23.40
	1	47978	372	1371216	2	28.58
	2	52323	2054	3215735	4	61.46
	2	54798	2005	3331358	4	60.79

Table C-2. Method Detection Limit Data. E2 concentration and GC/MS responses.

Rep	Sample	Date	E2 (mg/L)	E2 Response	E1 Response	Mirex Response
1	0.05 spike	6-Jul-05	0.10	29432	0	37227
2	0.05 spike	6-Jul-05	0.11	45313	0	38942
3	0.05 spike	6-Jul-05	0.11	40767	0	37468
4	0.05 spike	6-Jul-05	0.11	48121	0	41743
5	0.05 spike	6-Jul-05	0.10	34615	0	38441
6	0.05 spike	6-Jul-05	0.09	22231	0	38897
7	0.05 spike	6-Jul-05	0.09	27960	0	39294

Table C-3. Percent recovery of E2 in Groseclose soil matrix data.

Sample	GC/MS Date[+]	Cf (mg/L)	Avg. Cf	Cs	% recovery	Avg. recovery	E2 Response	E1 Response	Mirex Response
unknown	13-Jul-05	0.58	0.52				761053	1839	51951
unknown	13-Jul-05	0.45					551698	1983	52142
unknown	13-Jul-05	0.34*					353911	297	51666
unk + 0.5 spike	13-Jul-05	0.77	0.77	0.36	71.6	71.6	1030516	0	50138
unk + 0.5 spike	13-Jul-05	0.6*		--	--		790554	3270	51958
unk + 0.5 spike	13-Jul-05	0.39*		--	--		440698	1194	50860
unk + 1.0 spike	13-Jul-05	1.27	1.22	0.86	85.8	81.1	1835838	1281	50252
unk + 1.0 spike	13-Jul-05	1.26		0.85	84.8		1844320	590	50706
unk + 1.0 spike	13-Jul-05	1.14		0.73	72.8		1667196	1886	51120
unk + 2.0 spike	13-Jul-05	2.57	2.52	2.16	107.9	105.2	4212405	482	54006
unk + 2.0 spike	13-Jul-05	2.42		2.01	100.4		3940613	998	53683
unk + 2.0 spike	13-Jul-05	2.56		2.15	107.4		3827321	422	54335

* double peaks (discarded); [+]samples prepared on 7/12/2005; Cf = measured E2 concentration; Cs = recovered spike concentration

Table C-4. Concentration Range Experiment Raw Data.

Sample	Date	E2 Ci (mg/L)	V (mL)	Soil Mass (g)	Soil	GC/MS Date	E2 Cf (mg/L)	Avg. Cf	% sorbed	GC/MS Response		
										E2	E1	Mirex
A Control	13-Jan-05	0.1	50	no soil	None	14-Jan-05	0.18	0.20		86764	0	308147
A Control	13-Jan-05	0.1	50	no soil	None	14-Jan-05	0.21			109140	0	294494
A 0.5 G	13-Jan-05	0.1	50	0.5	Groseclose	14-Jan-05	0.14	0.16	21%	65077	0	301271
A 0.5 G	13-Jan-05	0.1	50	0.5	Groseclose	14-Jan-05	0.17			79734	0	302387
A 1.0 G	13-Jan-05	0.1	50	1.0	Groseclose	14-Jan-05	0.10	0.10	51%	38375	0	299707
A 1.0 G	13-Jan-05	0.1	50	1.0	Groseclose	14-Jan-05	0.09			30494	0	296839
A 3.0 G	13-Jan-05	0.1	50	3.0	Groseclose	14-Jan-05	0.07	0.07	64%	15832	0	308746
A 3.0 G	13-Jan-05	0.1	50	3.0	Groseclose	14-Jan-05	0.07			14447	5588	307510
A 0.5 C	13-Jan-05	0.1	50	0.5	Cecil	14-Jan-05	0.14	0.14	28%	64056	0	298125
A 0.5 C	13-Jan-05	0.1	50	0.5	Cecil	14-Jan-05	0.14			62892	0	305877
A 1.0 C	13-Jan-05	0.1	50	1.0	Cecil	14-Jan-05	0.11	0.13	33%	45151	0	295994
A 1.0 C	13-Jan-05	0.1	50	1.0	Cecil	14-Jan-05	0.15			66938	0	293079
A 3.0 C	13-Jan-05	0.1	50	3.0	Cecil	14-Jan-05	0.08	0.08	62%	21227	0	303538
A 3.0 C	13-Jan-05	0.1	50	3.0	Cecil	14-Jan-05	0.07			20208	0	311645
B Control	14-Jan-05	1.0	50	no soil	None	20-Jan-05	1.40	1.51		965771	0	334253
B Control	14-Jan-05	1.0	50	no soil	None	20-Jan-05	1.62			1009405	0	298920
B 0.5 G	14-Jan-05	1.0	50	0.5	Groseclose	20-Jan-05	1.23	1.27	16%	624846	0	313287
B 0.5 G	14-Jan-05	1.0	50	0.5	Groseclose	20-Jan-05	1.31			719724	0	330899
B 1.0 G	14-Jan-05	1.0	50	1.0	Groseclose	20-Jan-05	0.98	0.99	34%	813975	0	301485
B 1.0 G	14-Jan-05	1.0	50	1.0	Groseclose	20-Jan-05	1.00			747620	0	295612
B 3.0 G	14-Jan-05	1.0	50	3.0	Groseclose	20-Jan-05	0.53	0.51	67%	479093	0	321287
B 3.0 G	14-Jan-05	1.0	50	3.0	Groseclose	20-Jan-05	0.48			547813	0	330957
B 0.5 C	14-Jan-05	1.0	50	0.5	Cecil	20-Jan-05	1.06	1.02	33%	595891	0	292448
B 0.5 C	14-Jan-05	1.0	50	0.5	Cecil	20-Jan-05	0.97			518213	0	259158
B 1.0 C	14-Jan-05	1.0	50	1.0	Cecil	20-Jan-05	0.82	0.78	48%	263149	0	313328
B 1.0 C	14-Jan-05	1.0	50	1.0	Cecil	20-Jan-05	0.74			300965	0	330710
B 3.0 C	14-Jan-05	1.0	50	3.0	Cecil	20-Jan-05	0.47	0.46	70%	276518	14279	294228
B 3.0 C	14-Jan-05	1.0	50	3.0	Cecil	20-Jan-05	0.44			305940	0	296517

Table C-5. Cecil, Cecil Bt, and Myatt Time to Equilibrium Data.

Sample	Soil	Date	Day	Ci (mg/L)	V (mL)	Soil Mass (g)	GC/MS Date	Cf (mg/L)	Avg. Cf	Sorbed (mg/kg)	Response		
											E2	E1	Mirex
0.05 control	NS	14-Apr-05	1	0.05	150	6.0	19-Apr-05	0.08	0.08		61	0	28584
0.05 control	NS	14-Apr-05	1	0.05	150	6.0	19-Apr-05	0.08			3409	0	31076
0.05 control	NS	14-Apr-05	1	0.05	150	6.0	19-Apr-05	0.08			955	0	29896
0.05 control	NS	16-Apr-05	3	0.05	150	6.0	19-Apr-05	0.08	0.08		3109	0	36206
0.05 control	NS	16-Apr-05	3	0.05	150	6.0	19-Apr-05	0.09			8579	0	29311
0.05 control	NS	16-Apr-05	3	0.05	150	6.0	19-Apr-05	0.08			3159	0	30122
0.05 control	NS	18-Apr-05	5	0.05	150	6.0	24-Apr-05	0.10	0.10		34243	0	33564
0.05 control	NS	18-Apr-05	5	0.05	150	6.0	24-Apr-05	0.11			33327	0	31724
0.05 control	NS	18-Apr-05	5	0.05	150	6.0	24-Apr-05	0.09			9656	0	30913
0.05 control	NS	20-Apr-05	7	0.05	150	6.0	24-Apr-05	0.10	0.10		26134	0	34860
0.05 control	NS	20-Apr-05	7	0.05	150	6.0	24-Apr-05	0.11			36781	0	29949
0.05 control	NS	20-Apr-05	7	0.05	150	6.0	24-Apr-05	0.10			25315	0	33214
0.05 control	NS	22-Apr-05	9	0.05	150	6.0	24-Apr-05	0.10	0.10		31137	0	33815
0.05 control	NS	22-Apr-05	9	0.05	150	6.0	24-Apr-05	0.10			28715	0	34053
0.05 control	NS	22-Apr-05	9	0.05	150	6.0	24-Apr-05	0.10			29478	0	34327
2.0 control	NS	14-Apr-05	1	2.0	150	6.0	19-Apr-05	1.59	1.32		2161164	0	32319
2.0 control	NS	14-Apr-05	1	2.0	150	6.0	19-Apr-05	1.21			1622286	0	32454
2.0 control	NS	14-Apr-05	1	2.0	150	6.0	19-Apr-05	1.17			1348319	0	28073
2.0 control	NS	16-Apr-05	3	2.0	150	6.0	19-Apr-05	1.78	1.83		2900575	0	38558
2.0 control	NS	16-Apr-05	3	2.0	150	6.0	19-Apr-05	1.97			2961695	0	35347
2.0 control	NS	16-Apr-05	3	2.0	150	6.0	19-Apr-05	1.75			2602753	0	35261
2.0 control	NS	18-Apr-05	5	2.0	150	6.0	24-Apr-05	*	--		2069653	73	34871
2.0 control	NS	18-Apr-05	5	2.0	150	6.0	24-Apr-05	*			1687727	0	31712
2.0 control	NS	18-Apr-05	5	2.0	150	6.0	24-Apr-05	*			1471569	0	32916
2.0 control	NS	20-Apr-05	7	2.0	150	6.0	24-Apr-05	1.83	1.72		2642137	6978	34048
2.0 control	NS	20-Apr-05	7	2.0	150	6.0	24-Apr-05	1.63			2421177	6426	35203
2.0 control	NS	20-Apr-05	7	2.0	150	6.0	24-Apr-05	1.70			1464405	27072	32306
2.0 control	NS	22-Apr-05	9	2.0	150	6.0	24-Apr-05	1.53	1.61		2237946	0	34780
2.0 control	NS	22-Apr-05	9	2.0	150	6.0	24-Apr-05	1.68			2227987	6523	31252
2.0 control	NS	22-Apr-05	9	2.0	150	6.0	24-Apr-05	--			--	--	--
0.05	C	14-Apr-05	1	0.08	150	6.0	19-Apr-05	0.08	0.08	0.00	82	0	26679
0.05	C	14-Apr-05	1	0.08	150	6.0	19-Apr-05	0.08		0.00	2516	0	32427
0.05	C	14-Apr-05	1	0.08	150	6.0	19-Apr-05	<MDL		--	0	0	29592
0.05	C	16-Apr-05	3	0.08	150	6.0	19-Apr-05	0.08	0.08	0.08	5809	0	32492
0.05	C	16-Apr-05	3	0.08	150	6.0	19-Apr-05	<MDL		--	2355929	0	35821
0.05	C	16-Apr-05	3	0.08	150	6.0	19-Apr-05	<MDL		--	8979	0	35021
0.05	C	18-Apr-05	5	0.10	150	6.0	24-Apr-05	0.09	0.08	0.25	31018	0	32302
0.05	C	18-Apr-05	5	0.10	150	6.0	24-Apr-05	0.08		0.50	3002	0	31482
0.05	C	18-Apr-05	5	0.10	150	6.0	24-Apr-05	0.08		0.50	20601	0	32630
0.05	C	20-Apr-05	7	0.10	150	6.0	24-Apr-05	0.08	0.08	0.58	11177	0	32942
0.05	C	20-Apr-05	7	0.10	150	6.0	24-Apr-05	0.09		0.33	9045	0	33883
0.05	C	20-Apr-05	7	0.10	150	6.0	24-Apr-05	0.08		0.58	15414	0	34951
0.05	C	22-Apr-05	9	0.10	150	6.0	24-Apr-05	0.09	0.09	0.25	25556	0	31921
0.05	C	22-Apr-05	9	0.10	150	6.0	24-Apr-05	0.09		0.25	34542	0	36024
0.05	C	22-Apr-05	9	0.10	150	6.0	24-Apr-05	0.09		0.25	32781	0	36400
2.0	C	14-Apr-05	1	1.3	150	6.0	19-Apr-05	0.68	0.61	16.08	734093	0	27719
2.0	C	14-Apr-05	1	1.3	150	6.0	19-Apr-05	0.72		15.08	870575	938	30905
2.0	C	14-Apr-05	1	1.3	150	6.0	19-Apr-05	0.42		22.58	423845	52	27992
2.0	C	16-Apr-05	3	1.8	150	6.0	19-Apr-05	0.92	0.88	22.83	1271728	15373	34214
2.0	C	16-Apr-05	3	1.8	150	6.0	19-Apr-05	0.95		22.08	1496064	17099	38658
2.0	C	16-Apr-05	3	1.8	150	6.0	19-Apr-05	0.77		26.58	1171137	11296	38519

Table C-5. (cont.)

Sample	Soil	Date	Day	Ci (mg/L)	V (mL)	Soil Mass (g)	GC/MS Date	Cf (mg/L)	Avg. Cf	Sorbed (mg/kg)	Response		
											E2	E1	Mirex
2.0	C	18-Apr-05	5	1.3	150	6.0	24-Apr-05	0.84	0.84	10.58	1102706	21242	32719
2.0	C	18-Apr-05	5	1.3	150	6.0	24-Apr-05	*		--	443302	1724	35464
2.0	C	18-Apr-05	5	1.3	150	6.0	24-Apr-05	*		--	543177	10715	35699
2.0	C	20-Apr-05	7	1.5	150	6.0	24-Apr-05	0.90	0.84	15.50	1097584	31704	30177
2.0	C	20-Apr-05	7	1.5	150	6.0	24-Apr-05	0.80		18.00	1085226	27726	33748
2.0	C	20-Apr-05	7	1.5	150	6.0	24-Apr-05	0.83		17.25	1179206	34884	35225
2.0	C	22-Apr-05	9	1.6	150	6.0	24-Apr-05	0.75	0.77	21.38	1010935	33708	33964
2.0	C	22-Apr-05	9	1.6	150	6.0	24-Apr-05	0.80		20.13	1192702	43666	37564
2.0	C	22-Apr-05	9	1.6	150	6.0	24-Apr-05	0.77		20.88	1037442	38792	33694
0.05	CBt	14-Apr-05	1	0.08	150	6.0	19-Apr-05	0.08	0.08	0.00	82	0	26679
0.05	CBt	14-Apr-05	1	0.08	150	6.0	19-Apr-05	0.08		0.00	2516	0	32427
0.05	CBt	14-Apr-05	1	0.08	150	6.0	19-Apr-05	<MDL			0	0	29592
0.05	CBt	16-Apr-05	3	0.08	150	6.0	19-Apr-05	0.09	0.09	-0.17	5809	0	32492
0.05	CBt	16-Apr-05	3	0.08	150	6.0	19-Apr-05	1.57a			2355929	429	35821
0.05	CBt	16-Apr-05	3	0.08	150	6.0	19-Apr-05	0.09		-0.17	8979	0	35021
0.05	CBt	18-Apr-05	5	0.10	150	6.0	24-Apr-05	0.10	0.09	0.00	31018	0	32302
0.05	CBt	18-Apr-05	5	0.10	150	6.0	24-Apr-05	0.08		0.50	3002	0	31482
0.05	CBt	18-Apr-05	5	0.10	150	6.0	24-Apr-05	0.10		0.00	20601	0	32630
0.05	CBt	20-Apr-05	7	0.10	150	6.0	24-Apr-05	0.09	0.09	0.33	11177	0	32942
0.05	CBt	20-Apr-05	7	0.10	150	6.0	24-Apr-05	0.09		0.33	9045	0	33883
0.05	CBt	20-Apr-05	7	0.10	150	6.0	24-Apr-05	0.09		0.33	15414	0	34951
0.05	CBt	22-Apr-05	9	0.10	150	6.0	24-Apr-05	0.10	0.10	0.00	25556	0	31921
0.05	CBt	22-Apr-05	9	0.10	150	6.0	24-Apr-05	0.10		0.00	34542	0	36024
0.05	CBt	22-Apr-05	9	0.10	150	6.0	24-Apr-05	0.10		0.00	32781	0	36400
2.0	CBt	14-Apr-05	1	1.3	150	6.0	19-Apr-05	0.80	1.12	13.08	961665	0	30195
2.0	CBt	14-Apr-05	1	1.3	150	6.0	19-Apr-05	1.50		-4.42	2240165	0	35750
2.0	CBt	14-Apr-05	1	1.3	150	6.0	19-Apr-05	1.07		6.33	1417908	0	32381
2.0	CBt	16-Apr-05	3	1.8	150	6.0	19-Apr-05	1.56	1.20	6.83	2491879	773	37920
2.0	CBt	16-Apr-05	3	1.8	150	6.0	19-Apr-05	*		31.08	864039	0	38610
2.0	CBt	16-Apr-05	3	1.8	150	6.0	19-Apr-05	1.45		9.58	2229438	0	36658
2.0	CBt	18-Apr-05	5	1.3	150	6.0	24-Apr-05	*	1.41	--	1743558	5968	34059
2.0	CBt	18-Apr-05	5	1.3	150	6.0	24-Apr-05	1.65		-9.67	2266946	6303	32522
2.0	CBt	18-Apr-05	5	1.3	150	6.0	24-Apr-05	1.16		2.58	1629386	160	34003
2.0	CBt	20-Apr-05	7	1.5	150	6.0	24-Apr-05	1.60	1.48	-2.00	2430576	7198	36033
2.0	CBt	20-Apr-05	7	1.5	150	6.0	24-Apr-05	1.45		1.75	2154719	5831	35477
2.0	CBt	20-Apr-05	7	1.5	150	6.0	24-Apr-05	1.38		3.50	1993020	5925	34527
2.0	CBt	22-Apr-05	9	1.6	150	6.0	24-Apr-05	1.54	1.48	1.63	1984715	5791	30638
2.0	CBt	22-Apr-05	9	1.6	150	6.0	24-Apr-05	--		--	--	--	--
2.0	CBt	22-Apr-05	9	1.6	150	6.0	24-Apr-05	1.41		4.88	1611380	4759	27258
0.05	M	14-Apr-05	1	0.08	150	6.0	19-Apr-05	<MDL	0.09	--	0	0	30246
0.05	M	14-Apr-05	1	0.08	150	6.0	19-Apr-05	0.08		0.00	2190	0	29607
0.05	M	14-Apr-05	1	0.08	150	6.0	19-Apr-05	0.09		-0.25	3964	0	29952
0.05	M	16-Apr-05	3	0.08	150	6.0	19-Apr-05	0.09	0.09	-0.17	6199	696	31220
0.05	M	16-Apr-05	3	0.08	150	6.0	19-Apr-05	0.09		-0.17	8521	1583	37644
0.05	M	16-Apr-05	3	0.08	150	6.0	19-Apr-05	<MDL		--	0	0	32250
0.05	M	18-Apr-05	5	0.10	150	6.0	24-Apr-05	0.08	0.08	0.50	2998	347	32886
0.05	M	18-Apr-05	5	0.10	150	6.0	24-Apr-05	0.08		0.50	3599	217	33247
0.05	M	18-Apr-05	5	0.10	150	6.0	24-Apr-05	<MDL		--	0	0	31357
0.05	M	20-Apr-05	7	0.10	150	6.0	24-Apr-05	0.08	0.08	0.58	3873	1556	34401
0.05	M	20-Apr-05	7	0.10	150	6.0	24-Apr-05	0.08		0.58	3309	202	36540
0.05	M	20-Apr-05	7	0.10	150	6.0	24-Apr-05	0.09		0.33	5148	55	35749

Table C-5. (cont.)

Sample	Soil	Date	Day	Ci (mg/L)	V (mL)	Soil Mass (g)	GC/MS Date	Cf (mg/L)	Avg. Cf	Sorbed (mg/kg)	Response		
											E2	E1	Mirex
0.05	M	22-Apr-05	9	0.10	150	6.0	24-Apr-05	0.09	0.09	0.25	7061	54	34804
0.05	M	22-Apr-05	9	0.10	150	6.0	24-Apr-05	0.09		0.25	6034	136	35368
0.05	M	22-Apr-05	9	0.10	150	6.0	24-Apr-05	0.09		0.25	12912	68	35150
2.0	M	14-Apr-05	1	1.3	150	6.0	19-Apr-05	1.05	0.81	6.83	1373261	1752	32059
2.0	M	14-Apr-05	1	1.3	150	6.0	19-Apr-05	0.61		17.83	697168	72	29699
2.0	M	14-Apr-05	1	1.3	150	6.0	19-Apr-05	0.77		13.83	1114420	1383	36537
2.0	M	16-Apr-05	3	1.8	150	6.0	19-Apr-05	1.13	1.14	17.58	1719299	12178	37067
2.0	M	16-Apr-05	3	1.8	150	6.0	19-Apr-05	1.14		17.33	1830947	13304	39025
2.0	M	16-Apr-05	3	1.8	150	6.0	19-Apr-05	1.15		17.08	1976966	15570	41774
2.0	M	18-Apr-05	5	1.3	150	6.0	24-Apr-05	*	--	--	749336	7337	32605
2.0	M	18-Apr-05	5	1.3	150	6.0	24-Apr-05	*		--	639874	4924	32512
2.0	M	18-Apr-05	5	1.3	150	6.0	24-Apr-05	*		--	530623	4721	34350
2.0	M	20-Apr-05	7	1.5	150	6.0	24-Apr-05	1.10	1.05	10.50	2571751	7257	35708
2.0	M	20-Apr-05	7	1.5	150	6.0	24-Apr-05	1.06		11.50	1530289	26789	35088
2.0	M	20-Apr-05	7	1.5	150	6.0	24-Apr-05	0.98		13.50	1191637	20038	29674
2.0	M	22-Apr-05	9	1.6	150	6.0	24-Apr-05	0.92	1.00	17.13	1284005	27240	34330
2.0	M	22-Apr-05	9	1.6	150	6.0	24-Apr-05	1.07		13.38	1479796	33239	33695
2.0	M	22-Apr-05	9	1.6	150	6.0	24-Apr-05	1.02		14.63	1425870	31045	34274

*double peaks; -- samples not analyzed; [a]suspected contamination; C-Cecil; CBt-Cecil Bt, M-Mahan

Table C-6. Groseclose Time to Equilibrium Data.

Sample	Soil	Date	Day	Ci (mg/L)	V (mL)	Soil Mass (g)	GC/MS Date	Cf (mg/L)	Avg Cf	Sorbed (mg/kg)	Response		
											E2	E1	Mirex
0.05	G	8-Mar-05	1	0.05	150	6.0	21-Mar-05	<MDL	<MDL		11580	476	45484
0.05	G	8-Mar-05	1	0.05	150	6.0	21-Mar-05	<MDL			7920	494	46178
0.05	G	8-Mar-05	1	0.05	150	6.0	21-Mar-05	<MDL			8744	512	45880
0.05	G	9-Mar-05	2	0.05	150	6.0	21-Mar-05	<MDL	<MDL		1540376	0	52069
0.05	G	9-Mar-05	2	0.05	150	6.0	21-Mar-05	<MDL			1849482	117	51763
0.05	G	9-Mar-05	2	0.05	150	6.0	21-Mar-05	<MDL			3104334	0	51281
0.05	G	11-Mar-05	4	0.05	150	6.0	22-Mar-05	<MDL	<MDL		11278	1625	53530
0.05	G	11-Mar-05	4	0.05	150	6.0	22-Mar-05	<MDL			290	0	52817
0.05	G	11-Mar-05	4	0.05	150	6.0	22-Mar-05	<MDL			9378	3084	52529
0.05	G	14-Mar-05	7	0.05	150	6.0	22-Mar-05	<MDL	<MDL		10021	4455	52489
0.05	G	14-Mar-05	7	0.05	150	6.0	22-Mar-05	<MDL			15694	7630	55023
0.05	G	14-Mar-05	7	0.05	150	6.0	22-Mar-05	<MDL			10142	4754	53205
0.05	G	17-Mar-05	10	0.05	150	6.0	29-Mar-05	0.07	0.06	-0.50[a]	19656	8629	78197
0.05	G	17-Mar-05	10	0.05	150	6.0	29-Mar-05	0.06		-0.25[a]	2000	1167	80866
0.05	G	17-Mar-05	10	0.05	150	6.0	29-Mar-05	0.06		-0.25[a]	15249	2006	81850
0.1	G	8-Mar-05	1	0.10	150	6.0	21-Mar-05	<MDL	<MDL		10038	147	49565
0.1	G	8-Mar-05	1	0.10	150	6.0	21-Mar-05	<MDL			17050	1258	49722
0.1	G	8-Mar-05	1	0.10	150	6.0	21-Mar-05	--		--	--	--	--
0.1	G	9-Mar-05	2	0.10	150	6.0	21-Mar-05	0.03	0.03	1.75	685368	5196	52100
0.1	G	9-Mar-05	2	0.10	150	6.0	21-Mar-05	<MDL			732930	3977	52247
0.1	G	9-Mar-05	2	0.10	150	6.0	21-Mar-05	0.03		1.75	753390	4229	51847
0.1	G	11-Mar-05	4	0.10	150	6.0	22-Mar-05	<MDL	<MDL		15016	1883	51824
0.1	G	11-Mar-05	4	0.10	150	6.0	22-Mar-05	<MDL			23828	4329	52256
0.1	G	11-Mar-05	4	0.10	150	6.0	22-Mar-05	<MDL			21904	3754	53591
0.1	G	14-Mar-05	7	0.10	150	6.0	22-Mar-05	<MDL	<MDL		26112	8410	55746
0.1	G	14-Mar-05	7	0.10	150	6.0	22-Mar-05	<MDL			31135	11676	56757
0.1	G	14-Mar-05	7	0.10	150	6.0	22-Mar-05	<MDL			36334	12193	53363
0.1	G	17-Mar-05	10	0.10	150	6.0	29-Mar-05	0.06	0.06	1.00	566	1845	81874
0.1	G	17-Mar-05	10	0.10	150	6.0	29-Mar-05	0.06		1.00	4182	1550	83175
0.1	G	17-Mar-05	10	0.10	150	6.0	29-Mar-05	0.06		1.00	599	2841	83426
0.5	G	8-Mar-05	1	0.50	150	6.0	21-Mar-05	0.16	0.17	8.50	1484727	9157	49887
0.5	G	8-Mar-05	1	0.50	150	6.0	21-Mar-05	0.17		8.25	273335	4782	51127
0.5	G	8-Mar-05	1	0.50	150	6.0	21-Mar-05	--		--	--	--	--
0.5	G	9-Mar-05	2	0.50	150	6.0	21-Mar-05	0.19	0.19	7.75	312913	10516	52048
0.5	G	9-Mar-05	2	0.50	150	6.0	21-Mar-05	0.19		7.75	313479	10338	54100
0.5	G	9-Mar-05	2	0.50	150	6.0	21-Mar-05	0.20		7.50	330037	11154	53388
0.5	G	11-Mar-05	4	0.50	150	6.0	22-Mar-05	0.17	0.15	8.25	282355	17524	52349
0.5	G	11-Mar-05	4	0.50	150	6.0	22-Mar-05	0.18		8.00	291447	16050	52259
0.5	G	11-Mar-05	4	0.50	150	6.0	22-Mar-05	0.09		10.25	139316	7788	52766
0.5	G	14-Mar-05	7	0.50	150	6.0	29-Mar-05	0.15	0.13	8.75	159698	8684	72462
0.5	G	14-Mar-05	7	0.50	150	6.0	29-Mar-05	0.11		9.75	98798	518	77166
0.5	G	14-Mar-05	7	0.50	150	6.0	29-Mar-05	0.12		9.50	124115	6977	75906
0.5	G	17-Mar-05	10	0.50	150	6.0	29-Mar-05	0.13	0.11	9.25	153592	9220	82487
0.5	G	17-Mar-05	10	0.50	150	6.0	29-Mar-05	0.13		9.25	137644	9951	82187
0.5	G	17-Mar-05	10	0.50	150	6.0	29-Mar-05	0.06		11.00	801	53	81929
1.0	G	8-Mar-05	1	1.0	150	6.0	21-Mar-05	0.32	0.29	17.00	497623	6370	50229
1.0	G	8-Mar-05	1	1.0	150	6.0	21-Mar-05	0.29		17.75	290915	3762	48158
1.0	G	8-Mar-05	1	1.0	150	6.0	21-Mar-05	0.27		18.25	380204	7009	44815

Table C-6. (cont.)

Sample	Soil	Date	Day	Ci (mg/L)	V (mL)	Soil Mass (g)	GC/MS Date	Cf (mg/L)	Avg Cf	Sorbed (mg/kg)	Response		
											E2	E1	Mirex
1.0	G	9-Mar-05	2	1.0	150	6.0	21-Mar-05	0.42	0.45	14.50	46152	12484	53232
1.0	G	9-Mar-05	2	1.0	150	6.0	21-Mar-05	0.45		13.75	40325	13656	53502
1.0	G	9-Mar-05	2	1.0	150	6.0	21-Mar-05	0.47		13.25	37075	13408	47114
1.0	G	11-Mar-05	4	1.0	150	6.0	22-Mar-05	0.44	0.44	14.00	727633	23597	53639
1.0	G	11-Mar-05	4	1.0	150	6.0	22-Mar-05	0.42		14.50	699818	22751	54060
1.0	G	11-Mar-05	4	1.0	150	6.0	22-Mar-05	0.46		13.50	794265	23345	55009
1.0	G	14-Mar-05	7	1.0	150	6.0	29-Mar-05	0.23	0.21	19.25	320697	8295	75153
1.0	G	14-Mar-05	7	1.0	150	6.0	29-Mar-05	0.31		17.25	471011	15203	76682
1.0	G	14-Mar-05	7	1.0	150	6.0	29-Mar-05	0.10		22.50	84333	1259	78201
1.0	G	17-Mar-05	10	1.0	150	6.0	29-Mar-05	0.23	0.35	19.25	355359	144	86950
1.0	G	17-Mar-05	10	1.0	150	6.0	29-Mar-05	0.23		19.25	351615	8186	83734
1.0	G	17-Mar-05	10	1.0	150	6.0	29-Mar-05	0.59		10.25	1070604	48490	84523
2.0	G	8-Mar-05	1	2.0	150	6.0	21-Mar-05	1.10	1.08	22.50	243646	5376	49554
2.0	G	8-Mar-05	1	2.0	150	6.0	21-Mar-05	1.16		21.00	1812478	12081	50230
2.0	G	8-Mar-05	1	2.0	150	6.0	21-Mar-05	0.98		25.50	1482048	10356	48577
2.0	G	9-Mar-05	2	2.0	150	6.0	21-Mar-05	0.95	1.10	26.25	67980	17111	52228
2.0	G	9-Mar-05	2	2.0	150	6.0	21-Mar-05	1.15		21.25	55748	20607	52426
2.0	G	9-Mar-05	2	2.0	150	6.0	21-Mar-05	1.19		20.25	27851	1171	52597
2.0	G	11-Mar-05	4	2.0	150	6.0	21-Mar-05	1.07	1.09	23.25	3466960	33613	54252
2.0	G	11-Mar-05	4	2.0	150	6.0	21-Mar-05	1.11		22.25	3422964	31691	54314
2.0	G	11-Mar-05	4	2.0	150	6.0	21-Mar-05	1.08		23.00	3506133	31224	55137
2.0	G	14-Mar-05	7	2.0	150	6.0	29-Mar-05	0.99	1.03	25.25	1746438	37627	79241
2.0	G	14-Mar-05	7	2.0	150	6.0	29-Mar-05	1.07		23.25	1824297	39192	75820
2.0	G	14-Mar-05	7	2.0	150	6.0	29-Mar-05	*		--	751386	1183	76574
2.0	G	17-Mar-05	10	2.0	150	6.0	29-Mar-05	1.14	1.12	21.50	2125010	65666	83066
2.0	G	17-Mar-05	10	2.0	150	6.0	29-Mar-05	--		--	--	--	--
2.0	G	17-Mar-05	10	2.0	150	6.0	29-Mar-05	1.10		22.50	2037642	55098	82961
0.05 control	G	8-Mar-05	1	0.05	150	6.0	21-Mar-05	0.04	0.05		32078	54	47195
0.05 control	G	8-Mar-05	1	0.05	150	6.0	21-Mar-05	0.05			40290	66	49270
0.05 control	G	8-Mar-05	1	0.05	150	6.0	21-Mar-05	0.05			46589	0	49983
0.05 control	G	9-Mar-05	2	0.05	150	6.0	21-Mar-05	0.04	0.04		3337396	4018	51361
0.05 control	G	9-Mar-05	2	0.05	150	6.0	21-Mar-05	0.04			3240888	2933	51178
0.05 control	G	9-Mar-05	2	0.05	150	6.0	21-Mar-05	--			--	--	--
0.05 control	G	11-Mar-05	4	0.05	150	6.0	22-Mar-05	0.05	0.05		46180	66	52391
0.05 control	G	11-Mar-05	4	0.05	150	6.0	22-Mar-05	0.05			48664	0	52804
0.05 control	G	11-Mar-05	4	0.05	150	6.0	22-Mar-05	0.04			39244	117	52977
0.05 control	G	14-Mar-05	7	0.05	150	6.0	22-Mar-05	0.04	0.04		64418	0	55459
0.05 control	G	14-Mar-05	7	0.05	150	6.0	22-Mar-05	0.04			59196	0	53758
0.05 control	G	14-Mar-05	7	0.05	150	6.0	22-Mar-05	0.05			42214	0	54805
0.05 control	G	17-Mar-05	10	0.05	150	6.0	29-Mar-05	0.06	0.07		6064	0	78340
0.05 control	G	17-Mar-05	10	0.05	150	6.0	29-Mar-05	0.07			33485	0	80661
0.05 control	G	17-Mar-05	10	0.05	150	6.0	29-Mar-05	0.07			22255	0	82744
2.0 control	G	8-Mar-05	1	2.0	150	6.0	21-Mar-05	2.22	2.18		3480824	1188	50598
2.0 control	G	8-Mar-05	1	2.0	150	6.0	21-Mar-05	2.11			3319121	1201	50751
2.0 control	G	8-Mar-05	1	2.0	150	6.0	21-Mar-05	2.21			3389908	815	49422
2.0 control	G	9-Mar-05	2	2.0	150	6.0	21-Mar-05	1.95	2.03		23461	699	53757
2.0 control	G	9-Mar-05	2	2.0	150	6.0	21-Mar-05	2.09			20108	1519	52006
2.0 control	G	9-Mar-05	2	2.0	150	6.0	21-Mar-05	2.04			20284	1747	52917

Table C-6. (cont.)

Sample	Soil	Date	Day	Ci (mg/L)	V (mL)	Soil Mass (g)	GC/MS Date	Cf (mg/L)	Avg Cf	Sorbed (mg/kg)	Response		
											E2	E1	Mirex
2.0 control	G	11-Mar-05	4	2.0	150	6.0	22-Mar-05	2.08	2.06		3466960	1112	53737
2.0 control	G	11-Mar-05	4	2.0	150	6.0	22-Mar-05	2.03			3422964	1206	54144
2.0 control	G	11-Mar-05	4	2.0	150	6.0	22-Mar-05	2.06			3506133	1030	54743
2.0 control	G	14-Mar-05	7	2.0	150	6.0	22-Mar-05	2.04	2.30		3685308	875	78156
2.0 control	G	14-Mar-05	7	2.0	150	6.0	22-Mar-05	2.36			4249210	1256	78311
2.0 control	G	14-Mar-05	7	2.0	150	6.0	22-Mar-05	2.51			4587659	1024	79055
2.0 control	G	17-Mar-05	10	2.0	150	6.0	29-Mar-05	2.28	2.13		4435413	1291	84587
2.0 control	G	17-Mar-05	10	2.0	150	6.0	29-Mar-05	1.90			3725953	3092	85872
2.0 control	G	17-Mar-05	10	2.0	150	6.0	29-Mar-05	2.20			4231199	1389	83592

[a] The mass of sorbed E2 was calculated by mass balance. Negative numbers at low initial concentrations are indicative of variations in the GC/MS method and calibration curves.

Table C-7. Groseclose Isotherm Data.

Sample	Date	Ci (mg/L)	V (mL)	Soil Mass (g)	GC/MS Date	Cf (mg/L)	Avg Cf	Sorbed (mg/kg)	% sorbed	% E1	Response		
											E2	E1	Mirex
0.05	9-Mar-05	0.05	150	6	21-Mar-05	<MDL	<MDL				1540376	0	52069
0.05	9-Mar-05	0.05	150	6	21-Mar-05	<MDL					1849482	117	51763
0.05	9-Mar-05	0.05	150	6	21-Mar-05	<MDL					3104334	0	51281
0.1	9-Mar-05	0.1	150	6	21-Mar-05	0.03	0.03	1.83	73	0%	685368	5196	52100
0.1	9-Mar-05	0.1	150	6	21-Mar-05	<MDL					732930	3977	52247
0.1	9-Mar-05	0.1	150	6	21-Mar-05	0.03				0%	753390	4229	51847
0.5	9-Mar-05	0.5	150	6	21-Mar-05	0.19	0.19	7.75	62	2%	312913	10516	52048
0.5	9-Mar-05	0.5	150	6	21-Mar-05	0.19				1%	313479	10338	54100
0.5	9-Mar-05	0.5	150	6	21-Mar-05	0.20				2%	330037	11154	53388
1	9-Mar-05	1.0	150	6	21-Mar-05	0.42	0.45	13.83	55	13%	46152	12484	53232
1	9-Mar-05	1.0	150	6	21-Mar-05	0.45				18%	40325	13656	53502
1	9-Mar-05	1.0	150	6	21-Mar-05	0.47				20%	37075	13408	47114
2	9-Mar-05	2.0	150	6	21-Mar-05	0.95	1.10	22.58	45	14%	67980	17111	53757
2	9-Mar-05	2.0	150	6	21-Mar-05	1.15				25%	55748	20607	52006
2	9-Mar-05	2.0	150	6	21-Mar-05	1.19				3%	27851	1171	52917

Table C-8. Myatt Isotherm Data.

Sample	Date	Ci (mg/L)	V (mL)	Soil Mass (g)	GC/MS Date	Cf (mg/L)	Avg	Sorbed (mg/kg)	% sorbed	% E1	Response		
											E2	E1	Mirex
0.1	1-Jun-05	0.10	150	6.0	7-Jun-05	0.08	0.09	0.33	13	20%	22612	4711	62503
0.1	1-Jun-05	0.10	150	6.0	7-Jun-05	0.10[a]				12%	80766	8097	63816
0.1	1-Jun-05	0.10	150	6.0	7-Jun-05	0.08				31%	11523	3742	64407
0.5	1-Jun-05	0.50	150	6.0	7-Jun-05	0.24	0.27	5.88	47	3%	523320	31178	66181
0.5	1-Jun-05	0.50	150	6.0	7-Jun-05	0.29				0%	809168	2811	82715
0.5	1-Jun-05	0.50	150	6.0	7-Jun-05	**							
1.0	1-Jun-05	1.00	150	6.0	7-Jun-05	0.51	0.46	13.42	54	3%	1587355	67539	78515
1.0	1-Jun-05	1.00	150	6.0	7-Jun-05	0.48				2%	1206619	34822	64172
1.0	1-Jun-05	1.00	150	6.0	7-Jun-05	0.40				2%	1083763	42397	72987
2.0	1-Jun-05	2.00	150	6.0	7-Jun-05	**	1.11	22.25	45				
2.0	1-Jun-05	2.00	150	6.0	7-Jun-05	1.10				0%	3978807	2062	84255
2.0	1-Jun-05	2.00	150	6.0	7-Jun-05	1.12				0%	4128409	1827	85426

** samples discarded, double peaks

[a]not included in isotherm development. Freundlich and Langmuir isotherm models could not accommodate zero sorption.

Table C-9. Cecil Isotherm Data.

Sample	Date	Ci (mg/L)	V (mL)	Soil Mass (g)	GC/MS Date	Cf (mg/L)	Avg	Sorbed (mg/kg)	% sorbed	% E1	Response		
											E2	E1	Mirex
0.05	21-Feb-05	0.05	150	6.0	1-Mar-05	0.03	0.04	0.38	30	70%	11383	11227	58261
0.05	21-Feb-05	0.05	150	6.0	1-Mar-05	0.04				74%	14557	11481	59059
0.05	21-Feb-05	0.05	150	6.0	1-Mar-05	<MDL					11940	9978	59408
0.1	21-Feb-05	0.1	150	6.0	1-Mar-05	0.05	0.05	1.17	47	45%	17201	13093	57444
0.1	21-Feb-05	0.1	150	6.0	1-Mar-05	0.05				18%	86035	26737	58572
0.1	21-Feb-05	0.1	150	6.0	1-Mar-05	0.06				44%	20090	12551	60975
0.5	21-Feb-05	0.5	150	6.0	1-Mar-05	0.42	0.42	2.08	17	22%	126686	27984	60919
0.5	21-Feb-05	0.5	150	6.0	1-Mar-05	0.39				18%	162988	32834	57705
0.5	21-Feb-05	0.5	150	6.0	1-Mar-05	0.44				7%	822068	54653	59845
1.0	21-Feb-05	1.0	150	6.0	1-Mar-05	0.60	0.59	10.33	41	4%	978839	56085	52377
1.0	21-Feb-05	1.0	150	6.0	1-Mar-05	0.64				5%	825032	55451	60944
1.0	21-Feb-05	1.0	150	6.0	1-Mar-05	0.52				4%	965399	62281	60307
2.0	21-Feb-05	2.0	150	6.0	1-Mar-05	1.15	1.19	20.17	40	3%	2137420	93003	60059
2.0	21-Feb-05	2.0	150	6.0	1-Mar-05	1.19				3%	2294198	98328	62133
2.0	21-Feb-05	2.0	150	6.0	1-Mar-05	1.24				3%	2324549	91761	60335

Table C-10. Cecil Bt Horizon Isotherm Data.

Sample	Date	Ci (mg/L)	V (mL)	Soil Mass (g)	GC/MS Date	Cf (mg/L)	Avg	Sorbed (mg/kg)	% sorbed	% E1	Response		
											E2	E1	Mirex
0.05	11-May-05	0.1	150	6.0	17-May-05	0.08	0.09	-1.00	-80	0%	1941	0	40532
0.05	11-May-05	0.1	150	6.0	17-May-05	0.09				0%	14153	0	40833
0.05	11-May-05	0.1	150	6.0	17-May-05	0.10				0%	25372	0	40718
0.1	11-May-05	0.1	150	6.0	17-May-05	0.08	0.09	0.17	7	0%	2560	0	40905
0.1	11-May-05	0.1	150	6.0	17-May-05	0.09				0%	14437	0	39577
0.1	11-May-05	0.1	150	6.0	17-May-05	0.11[a]				0%	49541	0	38995
0.5	11-May-05	0.5	150	6.0	17-May-05	0.21	0.21	7.38	59	0%	236798	0	43367
0.5	11-May-05	0.5	150	6.0	17-May-05	0.20				0%	226560	150	43122
0.5	11-May-05	0.5	150	6.0	17-May-05	0.29				--	404995	257	43694
1	11-May-05	1.0	150	6.0	17-May-05	0.53	0.52	11.92	48	0%	875764	1822	44504
1	11-May-05	1.0	150	6.0	17-May-05	0.49				--	795689	1172	43789
1	11-May-05	1.0	150	6.0	17-May-05	0.55				--	888854	2040	43031
2	11-May-05	2.0	150	6.0	17-May-05	1.57	1.50	12.42	25	0%	2926896	4027	44216
2	11-May-05	2.0	150	6.0	17-May-05	1.32				--	2395118	3839	43828
2	11-May-05	2.0	150	6.0	17-May-05	1.62				--	3053127	6446	44784

[a]data point not included in isotherm development because calculated sorbed E2 was negative.

Table C-11. Data for Correlation Analyses. These data are from the data used to develop sorption isotherms at an initial concentration of 2.0 mg/L E2.

Soil	OM (%)	pH	Experimental pH	CEC	C_{eq} (mg/L)	q (mg/kg)	Temperature (^{o}C)
Groseclose	3.8	6.7	5.7	5.45	0.95	26.25	23
Groseclose	3.8	6.7	5.7	5.45	1.15	21.25	23
Groseclose	3.8	6.7	5.7	5.45	1.19	20.25	23
Cecil	3.1	6.6	6.2	4.61	1.15	21.25	24
Cecil	3.1	6.6	6.2	4.61	1.19	20.25	24
Cecil	3.1	6.6	6.2	4.61	1.24	19.00	24
Mahan	2.3	6.1	5.4	3.76	1.10	22.50	23
Mahan	2.3	6.1	5.4	3.76	1.12	22.00	23
Cecil Bt	0.2		4.4		1.57	10.75	24
Cecil Bt	0.2		4.4		1.32	17.00	24
Cecil Bt	0.2		4.4		1.62	9.50	24

Appendix D: SAS Outputs

D.1. Method Detection Level

The MEANS Procedure

Analysis Variable : cf

N	Mean	Std Dev	Minimum	Maximum
7	0.1014286	0.0089974	0.0900000	0.1100000

D.2. Percent Recovery

The MEANS Procedure

Analysis Variable: recovery

N	Mean	Std Dev	Minimum	Maximum
7	88.4571429	13.6979143	71.6	107.9

The MEANS Procedure

Analysis Variable: recovery

Lower 95% CL for Mean	Upper 95% CL for Mean
75.7886850	101.1256007

The CORR Procedure

2 Variables: Ci recovery

Simple Statistics

Variable	N	Mean	Std Dev	Sum	Minimum	Maximum	Label
Ci	7	1.35714	0.62678	9.50000	0.50000	2.000000	Ci
Recovery	7	88.45714	13.69791	619.20000	71.60000	107.90000	recovery

Pearson Correlation Coefficients, N = 7
Prob > |r| under H0: Rho=0

	Ci	recovery
Ci	1.00000	0.91737
Ci		0.0036
recovery	0.91737	1.00000
recovery	0.0036	

D.3. Groseclose Freundlich Regression

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	1.57511	1.57511	413.12	<.0001
Error	9	0.03431	0.00381		
Corrected Total	10	1.60943			

Root MSE	0.06175	R-Square	0.9787
Dependent Mean	0.96496	Adj R-Sq	0.9763
Coeff Var	6.39894		

Parameter Estimates

Variable	DF	Parameter Estimate	Standard Error	t Value	Pr > |t|	95% Confidence Limits	
Intercept	1	1.35952	0.02690	50.55	<.0001	1.29867	1.42036
x	1	0.70863	0.03486	20.33	<.0001	0.62976	0.78750

D.4. Groseclose Linear Regression

NOTE: No intercept in model. R-Square is redefined.

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	2139.55734	2139.55734	126.97	<.0001
Error	10	168.50516	16.85052		
Uncorrected Total	11	2308.06250			

Root MSE	4.10494	R-Square	0.9270
Dependent Mean	12.34091	Adj R-Sq	0.9197
Coeff Var	33.26285		

Parameter Estimates

Variable	DF	Parameter Estimate	Standard Error	t Value	Pr > |t|	95% Confidence Limits	
x	1	22.16526	1.96706	11.27	<.0001	17.78238	26.54814

D.5. Groseclose Langmuir Regression

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	0.00166	0.00166	90.74	<.0001
Error	9	0.00016470	0.00001830		
Corrected Total	10	0.00183			

Root MSE	0.00428	R-Square	0.9098
Dependent Mean	0.03238	Adj R-Sq	0.8997
Coeff Var	13.20971		

Parameter Estimates

Variable	DF	Parameter Estimate	Standard Error	t Value	Pr > |t|	95% Confidence Limits	
Intercept	1	0.01795	0.00199	9.02	<.0001	0.01345	0.02245
x	1	0.03012	0.00316	9.53	<.0001	0.02297	0.03728

D.6. Cecil Freundlich Regression

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	4.32155	4.32155	52.43	<.0001
Error	12	0.98919	0.08243		
Corrected Total	13	5.31075			

Root MSE	0.28711	R-Square	0.8137
Dependent Mean	0.51094	Adj R-Sq	0.7982
Coeff Var	56.19288		

Parameter Estimates

Variable	DF	Parameter Estimate	Standard Error	t Value	Pr > |t|	95% Confidence Limits	
Intercept	1	1.07822	0.10967	9.83	<.0001	0.83928	1.31716
x	1	0.95034	0.13125	7.24	<.0001	0.66436	1.23631

D.7. Cecil Linear Regression

NOTE: No intercept in model. R-Square is redefined.

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	1474.72049	1474.72049	210.32	<.0001
Error	13	91.15451	7.01189		
Uncorrected Total	14	1565.87500			

Root MSE	2.64800	R-Square	0.9418
Dependent Mean	7.28571	Adj R-Sq	0.9373
Coeff Var	36.34505		

Parameter Estimates

Variable	DF	Parameter Estimate	Standard Error	t Value	Pr > |t|	95% Confidence Limits	
x	1	15.87812	1.09487	14.50	<.0001	13.51281	18.24344

D.8. Cecil Langmuir Regression

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	0.00124	0.00124	0.20	0.6634
Error	12	0.07452	0.00621		
Corrected Total	13	0.07576			

Root MSE	0.07880	R-Square	0.0163
Dependent Mean	0.09698	Adj R-Sq	-0.0656
Coeff Var	81.25631		

Parameter Estimates

Variable	DF	Parameter Estimate	Standard Error	t Value	Pr > |t|	95% Confidence Limits	
Intercept	1	0.10776	0.03204	3.36	0.0056	0.03794	0.17757
x	1	-0.02212	0.04957	-0.45	0.6634	-0.13013	0.08589

D.9. Myatt Freundlich Regression

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	3.06128	3.06128	74.49	<.0001
Error	7	0.28767	0.04110		
Corrected Total	8	3.34895			

Root MSE	0.20272	R-Square	0.9141
Dependent Mean	0.77820	Adj R-Sq	0.9018
Coeff Var	26.05005		

Parameter Estimates

Variable	DF	Parameter Estimate	Standard Error	t Value	Pr > |t|	95% Confidence Limits	
Intercept	1	1.47883	0.10562	14.00	<.0001	1.22908	1.72859
x	1	1.47684	0.17111	8.63	<.0001	1.07223	1.88146

D.10. Myatt Linear Regression

NOTE: No intercept in model. R-Square is redefined.

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	1543.61094	1543.61094	202.39	<.0001
Error	8	61.01406	7.62676		
Uncorrected Total	9	1604.62500			

Root MSE	2.76166	R-Square	0.9620
Dependent Mean	10.83333	Adj R-Sq	0.9572
Coeff Var	25.49223		

Parameter Estimates

Variable	DF	Parameter Estimate	Standard Error	t Value	Pr > |t|	95% Confidence Limits	
x	1	21.72876	1.52734	14.23	<.0001	18.20670	25.25081

D.11. Myatt Langmuir Regression

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	0.00580	0.00580	2.50	0.1581
Error	7	0.01628	0.00233		
Corrected Total	8	0.02208			

Root MSE	0.04822	R-Square	0.2629
Dependent Mean	0.06858	Adj R-Sq	0.1576
Coeff Var	70.31615		

Parameter Estimates

Variable	DF	Parameter Estimate	Standard Error	t Value	Pr > |t|	95% Confidence Limits	
Intercept	1	0.10160	0.02637	3.85	0.0063	0.03925	0.16395
x	1	-0.06912	0.04375	-1.58	0.1581	-0.17256	0.03433

D.12. Cecil Bt Horizon Freundlich Regression

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	2.30001	2.30001	15.47	0.0034
Error	9	1.33795	0.14866		
Corrected Total	10	3.63796			

Root MSE	0.38557	R-Square	0.6322
Dependent Mean	0.72898	Adj R-Sq	0.5914
Coeff Var	52.89127		

Parameter Estimates

Variable	DF	Parameter Estimate	Standard Error	t Value	Pr > |t|	95% Confidence Limits	
Intercept	1	1.14248	0.15674	7.29	<.0001	0.78792	1.49705
x	1	1.03939	0.26425	3.93	0.0034	0.44162	1.63716

D.13. Cecil Bt Horizon Linear Regression

NOTE: No intercept in model. R-Square is redefined.

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	775.78057	775.78057	27.42	0.0004
Error	10	282.90693	28.29069		
Uncorrected Total	11	1058.68750			

Root MSE	5.31890	R-Square	0.7328
Dependent Mean	8.52273	Adj R-Sq	0.7061
Coeff Var	62.40842		

Parameter Estimates

Variable	DF	Parameter Estimate	Standard Error	t Value	Pr > |t|	95% Confidence Limits	
x	1	9.94877	1.89986	5.24	0.0004	5.71562	14.18193

D.14. Cecil Bt Horizon Langmuir Regression

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	0.00104	0.00104	0.10	0.7590
Error	9	0.09348	0.01039		
Corrected Total	10	0.09452			

Root MSE	0.10192	R-Square	0.0110
Dependent Mean	0.08111	Adj R-Sq	-0.0989
Coeff Var	125.64787		

Parameter Estimates

Variable	DF	Parameter Estimate	Standard Error	t Value	Pr > |t|	95% Confidence Limits	
Intercept	1	0.07294	0.04014	1.82	0.1026	-0.01786	0.16375
x	1	0.02042	0.06457	0.32	0.7590	-0.12565	0.16649

D.15. Correlation Analysis

The CORR Procedure

7 Variables: OM pH measpH CEC Ceq q T

Simple Statistics

Variable	N	Mean	Std Dev	Sum	Minimum	Maximum
OM	11	2.35455	1.47876	25.90000	0.20000	3.80000
pH	8	6.51250	0.25877	52.10000	6.10000	6.70000
measpH	11	5.42727	0.71985	59.70000	4.40000	6.20000
CEC	8	4.71250	0.70486	37.70000	3.76000	5.45000
Ceq	11	1.23636	0.19971	13.60000	0.95000	1.62000
q	11	19.09091	4.99284	210.00000	9.50000	26.25000
T	11	23.54545	0.52223	259.00000	23.00000	24.00000

Pearson Correlation Coefficients
Prob > |r| under H0: Rho=0
Number of Observations

	OM	pH	measpH	CEC	Ceq	q	T
OM	1.00000	0.93343	0.89749	0.99929	-0.81733	0.81733	-0.54739
OM		0.0007	0.0002	<.0001	0.0021	0.0021	0.0813
	11	8	11	8	11	11	11
pH	0.93343	1.00000	0.60767	0.91930	0.09104	-0.09104	0.28000
pH	0.0007		0.1100	0.0012	0.8302	0.8302	0.5018
	8	8	8	8	8	8	8
measpH	0.89749	0.60767	1.00000	0.24607	-0.71639	0.71639	-0.20313
measpH	0.0002	0.1100		0.5569	0.0131	0.0131	0.5491
	11	8	11	8	11	11	11
CEC	0.99929	0.91930	0.24607	1.00000	-0.12817	0.12817	-0.12042
CEC	<.0001	0.0012	0.5569		0.7623	0.7623	0.7764
	8	8	8	8	8	8	8
Ceq	-0.81733	0.09104	-0.71639	-0.12817	1.00000	-1.00000	0.64414
Ceq	0.0021	0.8302	0.0131	0.7623		<.0001	0.0324
	11	8	11	8	11	11	11
q	0.81733	-0.09104	0.71639	0.12817	-1.00000	1.00000	-0.64414
q	0.0021	0.8302	0.0131	0.7623	<.0001		0.0324
	11	8	11	8	11	11	11
T	-0.54739	0.28000	-0.20313	-0.12042	0.64414	-0.64414	1.00000
T	0.0813	0.5018	0.5491	0.7764	0.0324	0.0324	
	11	8	11	8	11	11	11

D.16. Tukey's Multiple Comparison Tests

Table D-1. Symbols for each soil and intitial concentration used in Tukey's multiple comparison.

Symbol	Treatment
NO	Initial Concentration (no soil)
CE	Cecil
CB	Cecil Bt
MY	Myatt
GR	Groseclose

D.16.a E2 concentration of 0.1 mg/L

ANOVA Table:

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	0.00874286	0.00218571	24.59	<.0001
Error	9	0.00080000	0.00008889		
Corrected Total	13	0.00954286			

R-Square	Coeff Var	Root MSE	conc Mean
0.916168	12.45219	0.009428	0.075714

Tukey's Studentized Range (HSD) Test for conc

NOTE: This test controls the Type I experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	9
Error Mean Square	0.000089
Critical Value of Studentized Range	4.75541

Comparisons significant at the 0.05 level are indicated by ***.

soil Comparison	Difference Between Means	Simultaneous 95% Confidence Limits		
NO - CB	0.006667	-0.019219	0.032552	
NO - MY	0.013333	-0.012552	0.039219	
NO - CE	0.046667	0.020781	0.072552	***
NO - GR	0.070000	0.041059	0.098941	***
CB - NO	-0.006667	-0.032552	0.019219	
CB - MY	0.006667	-0.019219	0.032552	
CB - CE	0.040000	0.014115	0.065885	***
CB - GR	0.063333	0.034393	0.092274	***
MY - NO	-0.013333	-0.039219	0.012552	
MY - CB	-0.006667	-0.032552	0.019219	
MY - CE	0.033333	0.007448	0.059219	***
MY - GR	0.056667	0.027726	0.085607	***
CE - NO	-0.046667	-0.072552	-0.020781	***
CE - CB	-0.040000	-0.065885	-0.014115	***
CE - MY	-0.033333	-0.059219	-0.007448	***
CE - GR	0.023333	-0.005607	0.052274	
GR - NO	-0.070000	-0.098941	-0.041059	***
GR - CB	-0.063333	-0.092274	-0.034393	***
GR - MY	-0.056667	-0.085607	-0.027726	***
GR - CE	-0.023333	-0.052274	0.005607	

D.16.b E2 concentration of 0.5 mg/L

ANOVA Table:

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	0.20037333	0.05009333	6.82	0.0065
Error	10	0.07340000	0.00734000		
Corrected Total	14	0.27377333			

R-Square	Coeff Var	Root MSE	conc Mean
0.731895	26.38823	0.085674	0.324667

Tukey's Studentized Range (HSD) Test for conc

NOTE: This test controls the Type I experimentwise error rate, but it generally has a higher Type II error rate than REGWQ.

Alpha	0.05
Error Degrees of Freedom	10
Error Mean Square	0.00734
Critical Value of Studentized Range	4.65429
Minimum Significant Difference	0.2302

Means with the same letter are not significantly different.

Tukey Grouping		Mean	N	soil
	A	0.50000	3	NO
B	A	0.41667	3	CE
B	A	0.28000	3	MY
B		0.23333	3	CB
B		0.19333	3	GR

D.16.c E2 concentration of 1.0 mg/L

ANOVA Table:

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	0.69117333	0.17279333	56.10	<.0001
Error	10	0.03080000	0.00308000		
Corrected Total	14	0.72197333			

R-Square	Coeff Var	Root MSE	conc Mean
0.957339	9.492203	0.055498	0.584667

Tukey's Studentized Range (HSD) Test for conc

NOTE: This test controls the Type I experimentwise error rate, but it generally has a higher Type II error rate than REGWQ.

Alpha	0.05
Error Degrees of Freedom	10
Error Mean Square	0.00308
Critical Value of Studentized Range	4.65429
Minimum Significant Difference	0.1491

Means with the same letter are not significantly different.

Tukey Grouping		Mean	N	soil
	A	1.00000	3	NO
	B	0.55333	3	CE
C	B	0.52333	3	CB
C	B	0.44667	3	GR
C		0.40000	3	MY

D.16.d E2 concentration of 2.0 mg/L

ANOVA Table:

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	1.91489333	0.47872333	36.64	<.0001
Error	10	0.13066667	0.01306667		
Corrected Total	14	2.04556000			

R-Square	Coeff Var	Root MSE	conc Mean
0.936122	8.380463	0.114310	1.364000

Tukey's Studentized Range (HSD) Test for conc

NOTE: This test controls the Type I experimentwise error rate, but it generally has a higher Type II error rate than REGWQ.

Alpha	0.05
Error Degrees of Freedom	10
Error Mean Square	0.013067
Critical Value of Studentized Range	4.65429
Minimum Significant Difference	0.3072

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	soil
A	2.00000	3	NO
B	1.50333	3	CB
C	1.19333	3	CE
C	1.09667	3	GR
C	1.02667	3	MY