

**EFFECTS OF SWINE SLURRY ON SORPTION OF 17 $\beta$ -ESTRADIOL TO SOIL**

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Title

Effects of Liquid Swine Manure on Sorption of 17 $\beta$ -estradiol to Soil

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By

Kimberly Korthauer Zitnick

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## ABSTRACT

Zitnick-Anderson , Kimberly Korthauer; M.S.; Program of Soil Science; College of Agriculture, Food Systems, and Natural Resources; North Dakota State University; July 2010. Effects of Swine Slurry on Sorption of 17 $\beta$ -estradiol to Soil. Major Professors: Dr. Francis Casey and Dr. Thomas DeSutter.

17 $\beta$ -estradiol (E2) is a potent endocrine disrupting compound that is found in swine manure. Liquid swine manure or otherwise known as swine slurry is commonly used as a form of fertilizer in agricultural practices. Laboratory studies have demonstrated that E2 binds readily and strongly to soil and degrades within hours. However, field studies detect E2 in the environment at frequencies that suggest its moderate mobility and persistence. The objective of this study was to determine if colloidal organic carbon (COC; < 1 kDa) and dissolved organic carbon (DOC; > 1 kDa to < 0.45 $\mu$ m) from swine slurry affect the sorption and persistence of E2 in soil. Batch experiments were used to determine the sorption of <sup>14</sup>C labeled E2 in soil with slurry solution compared to the sorption of E2 in soil with only a 0.01M CaCl<sub>2</sub> solution. Samples were quantified for total radioactivity using liquid scintillation counting (LSC), and thin layer chromatography (TLC) was used to identify the formation of any E2 metabolites. Oxidation analysis was also used to determine the quantitative amounts of extractable and non-extractable E2 and metabolites at each time point in the aqueous and soil-bound phases. To determine if E2 preferentially associated with a manure organic carbon fraction (DOC or COC), ultrafiltration was performed. Although E2 was present in both the slurry and CaCl<sub>2</sub> solution phase after 14 d, the fractional recovery for E2 in the slurry solution was 12% and only 8% for the CaCl<sub>2</sub> solution. 17 $\beta$ -estradiol persisted in the parent form and did not convert to its metabolite,

estrone (E1) in the slurry solution. In the  $\text{CaCl}_2$  solution, conversion of E2 to E1 was complete after 3 d. Ultrafiltration results indicated that E2 preferentially associated with the COC fraction of the slurry. Results suggest that the suspended COC fraction facilitates the persistence and potential mobility of E2 in the soil environment.

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.....

I want to say thanks to the love of my life Matt Anderson for being my willing partner in my chaotic whirlwind that is my life. To my friends from the past to the present, thanks for being supportive when I was weak and making me laugh. Thank you to my family for being patient, understanding, and offering solutions to problems I thought were hopeless. Most importantly thanks for teaching me that nothing is impossible.

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## INTRODUCTION

Estrogens can be naturally occurring hormones and synthetic and are classified as endocrine disrupting chemicals (EDC) (Lee et al., 2007). 17 $\beta$ -estradiol (E2) is one of the most potent estrogens and has a relative potency of 10<sup>4</sup> to 10<sup>6</sup> times that of six estrogenically active alkylphenol-polyethoxylates (Jobling et al., 1998). When concentrations are as low as 1 to 7 ng L<sup>-1</sup>, E2 can have modifying effects on the sexual and neurological development in amphibians, reptiles, fish, and waterfowl (Bogi et al., 2003; Condeca and Canario, 2001; Fry, 1995).

Past evidence has implicated the role of organic carbon (OC) fractions as a possible contributor to facilitating the transport and persistence of some contaminants, such as metals, within the soil environment (Ashworth et al., 2004; Jardine et al., 1989; Jonge et al., 2000; Saiers and Lenhart, 2003; Sprague et al., 2000). Multiple laboratory studies have observed E2 adsorbing to soil and degrading rapidly (Casey et al., 2003; Casey et al., 2005). However, field studies have shown detections of E2 in the environment up to 300 d after application (Lægsmann et al., 2009; Minamiyama et al., 2008; Overcash et al., 2005; Thompson et al., 2009) and the association between E2 and OC fractions has been suggested as contributing to the estrogen persistence and mobility as a result of binding to OC fractions (Lægsmann et al., 2009; Minamiyama et al., 2008; Overcash et al., 2005; Thompson et al., 2009). Some field studies have shown that E2 can be transported to greater depths and allowed to persist for longer periods of time (Lægsmann et al., 2009; Minamiyama et al., 2008; Thompson et al., 2009).

The two OC fractions associated with contaminant transport are the colloidal (COC) and dissolved (DOC) (Ashworth et al., 2004; Jacobsen et al., 2005; Jonge et al.,

2000; Lægsmann et al., 2009; Minamiyama et al., 2008; Overcash et al., 2005; Saiers and Lenhart, 2003; Thompson et al., 2009). Some have tried to classify COC and DOC based on different size fractions; however, due to a continuous transition from particulate to colloidal to dissolved OC, separation of the fractions based on size is hard to determine (Villholth et al., 2000). Ultimately, the distinguishing factor between the OC fractions is that COC is suspended within a solution whereas DOC is incorporated into the solution (Chen et al., 2005).

17 $\beta$ - estradiol has been detected in waterways (Kolpin et al., 2002), and a possible source could be untreated livestock manure. There is a potential that untreated livestock manures deposit relatively high amounts of E2 in comparison to the amount found in municipal waste (Sarmaha et al., 2006). For livestock, the free estrogen has been measured within swine (*Sus domesticus*) slurry at concentrations as high as 21,000 ng L<sup>-1</sup>, and up to 6,000  $\mu$ g kg<sup>-1</sup> for dried swine manure (Hutchins et al., 2007 (supporting info. Table SI-1); Lorenzen et al., 2004), whereas the estrogen concentration in cattle (*Bos primigenius*) and poultry (*Gallus gallus domesticus*) manure has been measured up to 5,000 mg kg<sup>-1</sup> (Lorenzen et al., 2004).

To date the OC fraction which E2 binds with has been variable. Studies have suggested OC fractions can facilitate the transport and persistence of E2 within the environment (Lægdsmand et al., 2009; Minamiyama et al., 2008). One study observed 60% of E2 sorbed to COC and 40% sorbed to DOC in municipal wastewater (Holbrook et al., 2004). There is a general idea (or theory) that organic compounds need to be detached from the soil surface so that microbes can degrade organic compounds, such as E2 (Harms and Bosma, 1997) and therefore, it is important to identify which organic fraction that E2 is

most likely to associate. The objectives of this study were to 1) determine if COC and DOC within swine slurry affect the sorption and/or potential mobility of E2 to soil and 2) to identify the OC fraction that E2 is most likely to associate with.

## LITERATURE REVIEW

### Physiochemical Properties of Steroidal Estrogens

Estrogens are naturally occurring hormones that have been found within aquatic and soil environments (Axelson et al., 1984). There are three main unconjugated estrogens forms: 17 $\beta$ -estradiol (E2), estrone (E1), and estriol (E3); with molecular weights of 272.4, 270.35 and 288.4 (g mol<sup>-1</sup>), respectively (Figure 1). 17 $\beta$ -estradiol, E1, and E3 have

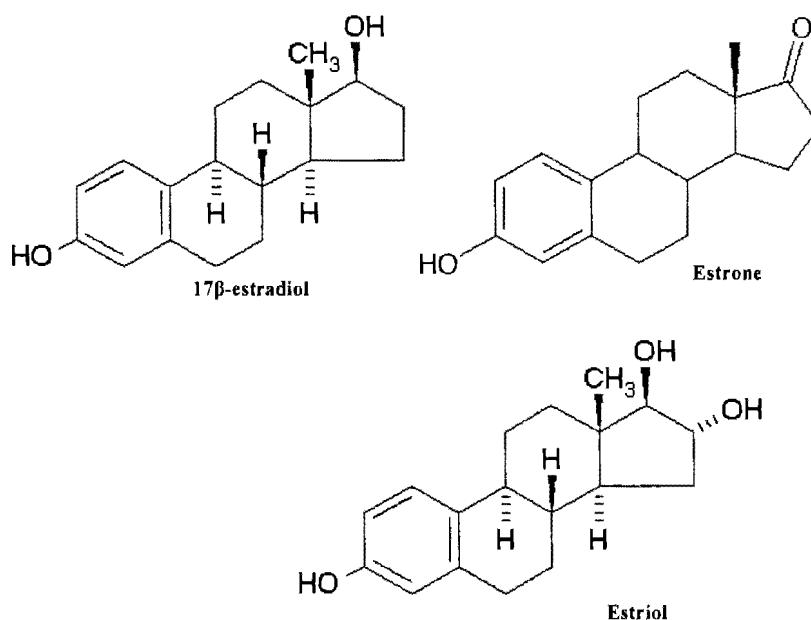


Figure 1. Chemical structures for 17 $\beta$ -estradiol, estrone and estriol (Referenced in Ying et al., 2002).

reported solubility factors that range from about 1 to 13 mg L<sup>-1</sup> and half-lives in aqueous solutions of 13, 19, and 1.5 hr, respectively. All these steroids have very low vapor pressures which indicates low volatility (Table 1). The log of the octanol-water partitioning coefficient values of these steroids are 3.94 (E2), 3.43 (E1), and 2.81 (E3), which indicates that these natural steroids are also hydrophobic (Ying et al., 2002). Therefore, from the physiochemical properties, it can be theorized that the sorption of these estrogens

Table 1. Physiochemical properties of steroidal estrogens

Property	17 $\beta$ -estradiol	Estrone	Estriol	Referenced By
formula	C <sub>18</sub> H <sub>24</sub> O <sub>2</sub>	C <sub>18</sub> H <sub>22</sub> O <sub>2</sub>	C <sub>18</sub> H <sub>24</sub> O <sub>3</sub>	Hanselman et al., 2003
MW <sup>†</sup> (g mol <sup>-1</sup> )	272.4	270.4	288.4	Hanselman et al., 2003
S <sub>w</sub> <sup>‡</sup> (mg/L)	5.4-13.3	0.8-12.4	3.2-13.3	Khanal et al., 2006
Half-life (hr.)	13	19	1.5	Ying et al., 2002; Jürgens et al., 2002
VP <sup>¶</sup> (Pa)	3 x 10 <sup>-8</sup>	3 x 10 <sup>-8</sup>	9 x 10 <sup>-13</sup>	Hanselman et al., 2003; Khanal et al., 2006
Log K <sub>ow</sub>	3.1-4.0	3.1-3.4	2.6-2.8	Hanselman et al., 2003; Khanal et al., 2006; Ying et al., 2002
pK <sub>a</sub>	10.5-10.7	10.3-10.8	10.4	Hanselman et al., 2003
Abbreviations	E2	E1	E3	Hanselman et al., 2003

† MW; molecular weight

‡ S<sub>w</sub>; solubility in water

¶ VP; vapor pressure

will likely sorb to sediment or soil rather than remaining within an aqueous solution (Ying et al., 2002).

An extensive description of the pathway of E2 synthesis is provided by Zubay et al. (1995). Estrone (E1) is developed as a by-product of E2 through hydrolysis in the liver and also classified as a steroidal hormone and endocrine disrupting chemical (EDC). An EDC is defined as being an exogenous agent that interferes with homeostasis and regulation of the developmental process. The process starts with the condensation in the cytosol of two molecules of acetyl-CoA, in a reaction catalyzed by thiolase. Through a series of enzymes the chemical is transformed into HMG-CoA (3-hydroxy-3-methyl-glutaryl-CoA reductase), an enzyme synthase. HMG-CoA is then reduced to mevalonate, the precursor to cholesterol. Once cholesterol has been formed, a series of carbon cleavage steps and hydrolysis occur within the mitochondrial matrix of the liver. In the mitochondrial matrix, cholesterol is converted by desmolase enzymes into pregnenolone and then transported to the endoplasmic reticulum for further conversion into progesterone. Progesterone is an intermediate in the synthesis of E2. Estriol is formed from the oxidation of E2 within the endoplasmic reticulum by cytochromes and are removed from E2 to form E3. Estriol is

classified as a steroidal hormone and has also been classified as an EDC. Since many steroidal hormones follow a common formation pathway, any disturbance with the activity or amount of an enzyme could lead to certain hormonal deficiencies or over abundance, which may cause adverse reactions such as pseudohermaphroditism in embryos (Zubay et al., 1995).

Estrone and E3 are both metabolites of E2. Under aerobic conditions E2 is transformed through hydrolysis and/or oxidation into E1 and E3. Conversion of E2 to its metabolites predominantly occur in aerobic conditions, but conversions can also occur under anaerobic conditions (Colucci and Topp, 2001; Mansell et al., 2004; Xu et al., 2008; Zhang et al., 2007). The abiotic transformation of E2 to E1 have been attributed to the oxidation by manganese oxides within aqueous and soil solutions (Xu et al., 2008). Municipal sewage treatment plants have used synthetic magnesium oxides to oxidize any E2 that has survived within the sewage effluent (Xu et al., 2008). Photolysis is another possible abiotic process that transforms E2 into E1 or E3 (Mansell et al., 2004; Zhang et al., 2007). When E2 is exposed to natural sunlight for duration of 24 hr, it degrades into E1 and E3 (Mansell et al., 2004; Zhang et al., 2007).

### **Hormones in the Environment**

Research has detected elevated concentrations of E2 and E1 within municipal sewage treatment plants (STPs) and livestock manure storage facilities (Table 2). For example, estrogenic steroids have been detected in the effluents of STP's in different countries at concentrations as high as 70, 64, and 18 ng L<sup>-1</sup> for E1, E2, and E3, respectively (Table 2). One reason why estrogens may not be completely biodegraded in STP's is due to



Table 2. Concentrations of hormones in the environment, municipal, and livestock manures.

Hormone	Concentration (Method of Detection)	Medium	Location	Source
<b>Environmental/ Municipal</b>				
17 $\beta$ -estradiol	64 ng L <sup>-1</sup> (A <sup>†</sup> )	STP <sup>§§</sup> effluent	Japan, Germany, Italy, Netherlands	Ying et al., 2002
17 $\beta$ -estradiol	1.9 to 6.0 ng L <sup>-1</sup> (A)	river waters	East Coast MD	Dorabawila and Gupta, 2005
17 $\beta$ -estradiol	4.2 to < 4 ng L <sup>-1</sup> (A, B <sup>‡</sup> )	soil-aquifer treatments	SouthWestern, US	Mansell and Drewes, 2004
17 $\beta$ -estradiol	12 ng kg <sup>-1</sup> (C <sup>¶</sup> )	soil conc.	Ontario, Canada	Colucci and Topp, 2002
17 $\beta$ -estradiol	93 ng L <sup>-1</sup> (D <sup>§</sup> , E <sup>#</sup> , F <sup>††</sup> )	stream samples	139 streams across US	Kolpin et al., 2002
17 $\beta$ -estradiol	2.3 to 3.2 ng L <sup>-1</sup> (A)	coastal bays	Tangier Island, MD	Dorabawila and Gupta, 2005
17 $\beta$ -estradiol	1 to 12 ng L <sup>-1</sup> (B, G <sup>‡‡</sup> )	STP effluent	Netherlands	Belfroid et al., 1999
17 $\beta$ -estradiol	48 to 0.1 ng L <sup>-1</sup> (B, G)	STP effluent	River Lea, UK	Desbrow et al., 1998
Estrone	70 ng L <sup>-1</sup> (A)	STP effluent	Japan, Germany, Italy, Netherlands	Ying et al., 2002
Estrone	51 ng L <sup>-1</sup> (D, E, F)	stream water	139 streams across US	Kolpin et al., 2002
Estrone	77 $\pm$ 14 ng L <sup>-1</sup> (B)	STP effluent	Florida Keys/Delaware	Atkinson et al., 2003
Estrone	70 ng L <sup>-1</sup> (G)	German STP	Frankfurt, Germany	Ternes et al., 1999
Estrone	< 1 to 47 ng L <sup>-1</sup> (B, G)	STP effluent	Netherlands	Belfroid et al., 1999
Estrone	48 to 0.3 ng L <sup>-1</sup> (B, F)	STP effluent	River Lea, UK	Desbrow et al., 1998
<b>Livestock</b>				
<b>Dairy</b>				
17 $\beta$ -estradiol	0.05 to 1.80 ng L <sup>-1</sup> (A)	cattle lagoons	Pendleton, SC	Irwin et al., 2001
17 $\beta$ -estradiol	370 to 550 ng L <sup>-1</sup> (G, H <sup>¶¶</sup> )	secondary dairy waste lagoons	south central, US	Hutchins et al., 2007 (supporting info. Table SI-1)
17 $\beta$ -estradiol	2x10 <sup>5</sup> ng kg <sup>-1</sup> (F)	dry-stack waste type	across the US	Hanselman et al., 2003
17 $\beta$ -estradiol	2 $\pm$ 3 x10 <sup>5</sup> ng kg <sup>-1</sup> (F)	lagoon waste	across the US	Hanselman et al., 2003
17 $\beta$ -estradiol	< 2 x10 <sup>5</sup> ng kg <sup>-1</sup> (C)	dried liquid cattle manure <sup>†††</sup>	Ontario, Canada	Lorenzen et al., 2004
<b>Swine</b>				
17 $\beta$ -estradiol	1 to 21 x10 <sup>3</sup> ng L <sup>-1</sup> (G, H)	primary swine lagoons	south central, US	Hutchins et al., 2007 (supporting info. Table SI-1)
17 $\beta$ -estradiol	3 to 6 x10 <sup>5</sup> ng kg <sup>-1</sup> (C)	dried liquid swine manure <sup>‡‡‡</sup>	Ontario, Canada	Lorenzen et al., 2004
17 $\beta$ -estradiol	2 to 2.5 x10 <sup>5</sup> ng kg <sup>-1</sup> (C)	dried liquid swine manure <sup>¶¶¶</sup>	Ontario, Canada	Lorenzen et al., 2004
17 $\beta$ -estradiol	0.15 to 2.3 x10 <sup>3</sup> ng L <sup>-1</sup> (A)	livestock manure treated field	Across US	Findlay-Moore et al., 2000
17 $\beta$ -estradiol	1.2 $\pm$ 0.275 x10 <sup>5</sup> ng kg <sup>-1</sup> (F)	farrowing pits	Across the US	Hanselman et al., 2003
17 $\beta$ -estradiol	1.8 ng L <sup>-1</sup> (G)	swine manure	Denmark	Kjær et al., 2007
Estrone	4.7 $\pm$ 0.42 x10 <sup>5</sup> ng kg <sup>-1</sup> (B, F, H)	farrowing pits	Across the US	Hanselman et al., 2003
Estrone	68.1 ng L <sup>-1</sup> (F)	swine manure	Denmark	Kjær et al., 2007
Estrone	10.8 x 10 <sup>5</sup> ng kg <sup>-1</sup> (F)	liquid swine manure	Throughout US	Khanal et al., 2006
<b>Poultry</b>				
17 $\beta$ -estradiol	33 $\pm$ 12 x 10 <sup>3</sup> ng kg <sup>-1</sup> (C)	broiler litter	across the US	Hanselman et al., 2003
17 $\beta$ -estradiol	0.9 x10 <sup>5</sup> ng kg <sup>-1</sup> (C)	broiler litter (females)	across the US	Hanselman et al., 2003
17 $\beta$ -estradiol	0.05 to 0.4 x10 <sup>5</sup> ng kg <sup>-1</sup> (G, H)	dried broiler litter	Ontario, Canada	Lorenzen et al., 2004
17 $\beta$ -estradiol	1.8 to 4 x10 <sup>3</sup> ng L <sup>-1</sup> (A)	primary poultry waste lagoons	south central, US	Hutchins et al., 2007; (supporting info. Table SI-1)
17 $\beta$ -estradiol	5 ng L <sup>-1</sup> (A, B)	livestock manure treated field	south west, US	Shore et al., 1988

† A; enzyme linked immunoassay

‡ B; high performance liquid chromatography,

¶ C; high performance liquid chromatography- radioactivity detection,

§ D; liquid chromatography/ mass spectrometry- positive ion electrospray

# E; continuous liquid-liquid extraction

†† F; gas chromatography/mass spectrometry

‡‡ G; gas chromatography-mass spectrometry/mass spectrometry

¶¶ H; liquid chromatography-mass spectrometry/mass spectrometry

§§ STP; sewage treatment plants

††† adult sows and finishing pigs

‡‡‡ weaned pigs <1 year

¶¶¶ pregnant adults

their hydrophobic nature (Kolpin et al., 2002; Ying et al., 2002).

Although municipal STPs can contribute a substantial amount of estrogens into the environment, livestock excrement has the potential to deposit a higher amount of hormones into water and soil environments (Hanselman et al., 2003; Khanal et al., 2006; Lorenzen et al., 2004; Shore et al., 1998; Ying et al., 2002). The livestock industry uses exogenous estrogens to enhance the growth of livestock as well as regulate the gestation cycle (Lange et al., 2002). Estrogens are eliminated in the wastes of treated animals once the animals are injected with estrogen. While cattle, poultry, and swine all contribute a substantial amount of estrogen into the environment, swine operations have the highest detections of E2 (Table 2).

There are multiple methods of storing livestock manure, which include dry stacking, composting, manure storage ponds (MSP's) or lagoons. Manure storage ponds contain some of the highest levels of estrogenic activity (Table 2). Many storage ponds are predominantly anaerobic, and can significantly reduce biological processes, due to the lack of oxygen (Lorenzen et al., 2004). Under anaerobic conditions, hormones such as E2 will biodegrade at slower rates resulting in greater persistence in the parent form compared to an aerobic environment (Fan et al., 2007; Lorenzen et al., 2004). The concentrations of free estrogens under anaerobic conditions were 1233 ng g<sup>-1</sup> dry wt in contrast the concentrations of free estrogens in aerobic conditions were 11.3 ng g<sup>-1</sup> dry wt suggests that anaerobic environments can facilitate the persistence of free estrogens (Lorenzen et al., 2004).

Most livestock manure is applied to agricultural land for fertilization (Lee et al., 2007). A common practice of liquid manure application is to forcibly inject the slurry into the upper 30 cm of the soil. This method of manure application is considered a best

management practice. Kjær et al. (2007) injected swine manure slurry into a field soil and through the use of GC-MS/MS, E2 and E1 was detected in the soil 300 d after application. They speculated the persistence of E2 was a result of anaerobic conditions that was created in the manure injection band.

Any animal operation has the potential to contribute hormones into the environment. The amount of hormone introduced into the environment depends predominantly on manure management practices. The conditions in the Chesapeake Bay exemplify this point (Dorabawila and Gupta, 2005). Poultry is the dominant livestock in the state of Maryland and most poultry operations are located on the eastern shore of the state (Dorabawila and Gupta, 2005). The dried litter from the poultry operations are spread across agricultural lands with the potential for contamination of waterways and coastal rivers via runoff from fields (Dorabawila and Gupta, 2005). When the eastern coastal bay waters were sampled, which are closest to poultry production, E2 concentrations varied but were as high as  $6.0 \text{ ng L}^{-1}$  (Dorabawila and Gupta, 2005). In contrast the waterways that were sampled from the locations where fewer poultry operations occurred, the concentration levels only reached  $1.7 \text{ ng g}^{-1}$  (Dorabawila and Gupta, 2005).

### **Hormones as Endocrine Disrupting Chemicals**

In recent years, the detection of hormones in the environment has become an increasingly important issue. Aquatic species are most likely to be exposed to EDC's due to such sources as urban run-off or agricultural applications (Gross et al., 2002). Endocrine disrupting chemicals can act as hormonal mimics creating disruption within certain processes that include the steroidal receptor superfamily, which include sex hormones, thyroidal hormones, and adrenal hormones (Gross et al., 2002). Abnormal gene expression,

enzyme function, and selective toxicities for endocrine active and target tissues are some examples of adverse reactions hormone mimics could cause (Gross et al., 2002).

Studies have shown that estrogens can have a significant impact on wildlife at levels as low as 1 to 7 ng L<sup>-1</sup> (Condeca and Canario, 2001; Fry, 1995; Guillette et al., 1995). 17 $\beta$ -estradiol is one of the most potent estrogens, with a relative potency of 10<sup>4</sup> to 10<sup>6</sup> times that of six estrogenic active alkylphenol-polyethoxylates (Jobling et al., 1998). The effects of short estrogenic exposure (1 to 2 hr) and various temperatures (26 to 28°C) to the common crocodile (*Crocodylus acutus*) and the common turtle (*Terrapine Carolina*) during the second third of the embryonic stage *in vivo* produced all female offspring (Fry, 1995; Guillette et al., 1995). 17 $\beta$ -estradiol levels of 2 to 15 mg kg<sup>-1</sup> can have adverse effects on certain fish species such as the Sea Bream (*Sparus aurata L.*), causing an increase in vitellogenesis (Condeca and Canario, 2001). Research conducted on various avian species showed that during the fetal development exposure to elevated estrogens, sex change was possible and could affect the formation of the structure of the ovaries and reproductive tract within birds (Fry, 1995). When exposed to elevated estrogen levels, female gulls and chickens experienced an enlargement in the development of the right oviduct, which can result in female chicks reaching adulthood and laying eggs with thin or soft shells (Fry, 1995).

### **Organic Carbon**

Organic carbon has the ability to bind with both organic and inorganic chemicals, which can facilitate their movement and persistence within the environment (Ashworth and Alloway, 2003; Holbrook et al., 2004; Jonge et al., 2000; Saiers and Lenhart, 2003; Sprague et al., 2000; Villhoth et al., 2000). A broad review of current research suggests that

once an organic compound binds with the solid soil, then the compound becomes unavailable to microbial decomposition (Harms and Zehnder, 1995; Harms and Bosma, 1997). The total OC fraction (TOC) in soils and effluents are highly decomposed forms of organic matter (Jardine et al., 1989) and is comprised of several fractions including COC and DOC. Various definitions have been used to classify COC and DOC based on size (Table 3), but due to a continuous transition from particulate to colloidal to dissolved OC, separation of fractions based on size is difficult (Villhoth et al., 2000). The only distinguishing factor between the two fractions is the suspension of COC within a solution and the incorporation of DOC into a solution (Chen et al., 2005).

Table 3. Size definitions of colloidal (COC) and dissolved organic carbon (DOC).

Author	COC Size	DOC size
Ashworth and Alloway, 2003	0.7 $\mu\text{m}$ to 0.45 $\mu\text{m}$	< 0.45 $\mu\text{m}$
Chittleborough et al., 1992	0.5 $\mu\text{m}$ to 2 $\mu\text{m}$	< 2 $\mu\text{m}$
Holbrook et al., 2004	1.5 kD <sup>†</sup> to 1.5 $\mu\text{m}$	< 1.5 kD
Chen et al., 2005	< 2 $\mu\text{m}$ to 1 $\mu\text{m}$	< 1 $\mu\text{m}$
Honeyman, 1999	1 $\mu\text{m}$ to 1 nm	< 1 $\mu\text{m}$
Villhoth et al., 2000	0.7 $\mu\text{m}$ to 2 $\mu\text{m}$	< 2 $\mu\text{m}$
Sprague et al., 2000	0.2 $\mu\text{m}$ to 2 $\mu\text{m}$	< 0.2 $\mu\text{m}$
Gustafsson et al., 2001	1 kD to 2 $\mu\text{m}$	< 1 kD

<sup>†</sup> kD; kiloDalton is a unit of mass used to express atomic and molecular masses.

Several studies have shown that DOC facilitates the transport of heavy metals and pesticides (Ashworth and Alloway, 2003; Hsu and Lo, 2000; Villhoth et al., 2000.) The composition and the pH of soil are important in the mobilization and adsorption behavior of DOC. Adsorption of DOC to certain soil matrices such as kaolinitic clays or soils containing gibbsite can significantly increase and render DOC immobile (Jardine et al., 1989). Adsorption of contaminants to DOC is based on pH values of the soil and solution. Dissolved OC has pKa values of approximately 6, which means that at a pH of 6 DOC becomes deprotonated and becomes mobile within solution making this OC fraction

available for adsorption to other positively charged contaminants (Nizeyimana and Opadeyi, 2006). For example, in two soil column studies experimenting with heavy metals Cu, Ni, and Zn, adsorption and formation of stable soluble complexes were pH dependent. When pH levels were greater than 6.3, the DOC and heavy metal levels increased in the leachate of the soil column experiments, indicating that DOC possibly facilitated the transport of these metals (Ashworth and Alloway, 2003; Hsu and Lo, 2000). Similar results have been reported for the organic contaminant N-Propyl-N-(2,4,6-trichlorophenoxy) ethyl-imidazole-1-carboxamide (Porchloraz,  $pK_a = 4$ ) where as pH increased above 4, Porchloraz preferentially bound with surface DOC soil particles within a soil environment. Therefore, when certain organic contaminants and DOC are combined, the adsorption process is likely pH dependent (Villhoth et al., 2000).

Estrogens within the environment have a  $pK_a$  value of approximately 10 (Table 1). However, a pH of 10 is highly unlikely to occur in a natural environment unless the soil environment is experiencing a serious sodium problem (Department of Primary Industries, 2010). In contrast to DOC, COC adsorption to contaminants is not pH dependent and is more dependent on saturation levels and bulk densities (Chen et al., 2005; Jonge et al., 2000; Jonge et al., 2004; Saiers and Lenhart, 2003; Sprague et al., 2000; Tong et al., 2006). is the “clumsy” fraction of OC, due the different sizes and shapes, and its ability to get physically caught or trapped in small pores allowing for the COC to become immobilized within a soil/water matrix (Tong et al., 2006). The movement of colloids, once suspended through either anthropogenic or natural methods of saturation, can move by advection and dispersion and can be redeposited by mass transfer reactions that proceed through mineral and air-water interfaces (Saiers and Lenhart, 2003). In a non-saturated system, most

colloidal materials are believed to be immobilized until the environment becomes saturated (Jonge et al., 2004). Once an environment becomes saturated or experiences an increase in moisture content the amount of mobilized COC increases (Jonge et al., 2004). Mobilized colloids can allow for low-solubility contaminants to become soluble during the adsorption to the colloidal material, thus making the COC interactions with soil particles minimal (Jonge et al., 2004). For these reasons the transport of colloids through the soil profile leaching of contaminants that are usually regarded as being immobile (Jonge et al., 2004).

Soil column studies have effectively represented the affinity between COC and various contaminants under different saturation levels and bulk densities (Chen et al., 2005; Jonge et al., 2000; Sprague et al., 2000). When COC was suspended and Cs was present, results showed that most contaminant transport occurred in the vadose zone at complete saturation (Chen et al., 2005). When atrazine (2-chloro-4-(ethylamine)-6-(isopropylamine)-s-triazine) was applied to a well saturated soil, COC particles became dislodged and were available for adsorption to atrazine (Sprague et al., 2000). Colloidal organic carbon associated atrazine has been recorded at 1 m deeper than Cs and at greater concentrations (Sprague et al., 2000). Similar to atrazine, glyphosate (N-(phosphonomethyl) glycine) can also be transported to deeper depths when associated with COC (Jonge et al., 2000). However, when the soil is unsaturated the amount of COC associated contaminants become immobilized. One soil column study observed a correlation between increased water application and an increase in mobilized COC associated glyphosate, indicating that COC was facilitating the transport of glyphosate (Jonge et al., 2000). Therefore, COC is capable of associating with contaminants under multiple circumstances, as evidenced by the previous studies (Chen et al., 2005; Jonge et al., 2000; Sprague et al., 2000).

## COC, DOC, and E2

Estrogenic hormones have been shown to have a relationship with DOC and COC. Steroidal hormones can bind to dissolved organic matter residue and studies have shown this association to be a significant fate process for estrogenic compounds in soils (Jacobsen et al., 2005). In general, the binding of hormones to soil is a result of hydrophobic adsorption (Lee et al., 2004). Once hormones bind or sorb to the soil it is likely they become unavailable for microbial degradation allowing the compounds to remain in their parent form (Harms and Bosma, 1997; Jacobsen et al., 2005). It is important to note that some studies have shown that some hormonal degradation does occur in the absence of microbial activity, but that the rate of degradation is minimal (Colucci et al., 2001; Fan et al., 2007). Further there is abiotic reduction of E2 to E1 from MnO<sub>2</sub> (Sheng et al., 2009; Xu et al., 2008).

17 $\beta$ -estradiol and E1 can preferentially adsorb to DOC or COC within either soil or aqueous solutions (Casey et al., 2003; Lai et al., 2000). The net effect of the presence of DOC/COC within aqueous solutions may be to inhibit the adsorption of hormones to solid phases and thus keep hormones suspended within solution, thereby increasing their mobility and persistence within the environment (Lai et al., 2000).

Field lysimeter studies from various locations have also suggested that DOC from soil and/or manure can facilitate hormone transport (Lædsmand et al., 2009; Minamiyama et al., 2008; Thompson et al., 2009). In a study using soil monoliths, E2 was theorized to transport through the monoliths through either preferential flow or by the association of swine manure-borne OC applied to the soil (Lædsmand et al., 2009). Results showed that there was an increase in E2 output due to one of the two theories which were: 1) that E2 is



transported through the monoliths through either preferential flow or 2) by association of swine manure-borne OC applied to the soil (Lægdsmand et al., 2009). There has also been an association between swine manure being used as plant fertilizer and the amount of DOC associated E2 (Minamiyama et al., 2008). After swine manure application, E2 was detected 2 m below the soil surface and persisted in the parent form for up to 300 d after application and irrigation (Minamiyama et al., 2008). Further research has shown E2 to leach to similar depths following a wetting front when manure-borne E2 was applied to an agricultural field (Thompson et al., 2009). The researchers of these studies all suggested that the persistence and transport E2 in the soil was likely enhanced by the presence of DOC or COC from manure or soil (Lægdsmand et al., 2009; Minamiyama et al., 2008; Thompson et al., 2009).

The association of E2 and COC from municipal sewage waste has also been determined using fluorescence detection (Holbrook et al., 2004). Binding interactions were thought to occur between  $\pi$ -electron of E2 and COC that contributes to binding reactions (Holbrook et al., 2004; Yamamoto et al., 2004). More research needs to be conducted to determine the influence of DOC/COC fraction on the fate and transport of E2 and which OC fraction is the main influence on the fate and transport of E2 in the environment.

### **Methods of Detection**

Multiple methods have been used for measuring estrogenic activity within the environment. Radioactive labeled E2 is a method that allows for the individual to determine the sorption pathway within soil. Using  $^{14}\text{C}$ -labeled E2 is also beneficial because there would be no loss of  $^{14}\text{C}$  during the transformation of E2 to its various metabolites. The radiolabel is located at the C-4 position of the A-ring, which is unaltered through transformation of E2 to E1 (Holthaus et al., 2002). However, it is important to note that  $^{14}\text{C}$

labeled E2 is only suitable and legal for lab studies and not field studies. Quantification of the labeled compound in the liquid phase requires liquid scintillation counting (LSC) analysis. The  $^{14}\text{C}$  follows total radioactivity and other methods such as TLC or HPLC are needed to discern whether metabolism of  $^{14}\text{C}$ -E2 has occurred.

Liquid scintillation counting measures the radiation from beta-emitting nuclides (University of Wisconsin - Madison, 1998). Once a sample is suspended within a cocktail solution containing aromatic solvent and scintillators, beta particles are emitted from the sample. The energy from the sample is transferred to the solvent molecules and then to scintillators. The scintillators are excited and dissipate the energy by emitting light. The emission of light is referred to as a pulse of light. These samples are usually placed in translucent or transparent vials that are loaded into a liquid scintillation counter. The counter has two photomultiplier tubes that are connected to a coincidence circuit. The coincidence circuit assures that genuine light pulses are counting and background noise is ignored.

Soil combustion analysis measures the amount of radioactivity in a dry sample. The sample is weighted out to .0011 g in a potato starch cone and covered. The samples undergo intense heat that converts the sample to water vapor and  $^{14}\text{C}$  labeled  $\text{CO}_2$ . The water vapor and  $^{14}\text{CO}_2$  are drawn into two tubes, where one tube collects the water and the second tube collects the  $\text{CO}_2$ . The labeled  $^{14}\text{CO}_2$  is trapped  $^{14}\text{CO}_2$  in NaOH solution and quantification is achieved using scintillation counting.

In order to determine the speciation of the hormone a qualitative measurement using thin layer chromatography (TLC) can be used to estimate the amount of parent material and metabolite within an aqueous phase (Rock et al., 1988). Thin layer

chromatography imaging scanners are specifically designed to work with radiolabeled compounds. Imaging scanning provides highly sensitive digital data from an entire chromatographic separation in one measurement without mechanical scanning. The detector is a windowless imaging counter that can view an entire lane simultaneously and give a complete picture of the radiolabeled compounds separated on a specific lane. The detector uses a special gas mixture to measure the ionization produced by either beta or gamma rays emitted during radioactive decay. The ionization is collected at the high voltage anode wire and sensed by the electronic circuitry. The scanner can overcome limitations inherent in counting low energy beta emitters from the surface of the TLC plates by providing a factor 100 in sensitivity. With  $^{14}\text{C}$  or higher energy isotopes, the corresponding sensitivity is 100 disintegrations per minute (DPM).

## MATERIALS AND METHODS

### Soil and Sample Description

The soils used in the batch studies came from the surface 0-15 cm and subsurface 15-30 cm depths of a Garborg loamy fine sand (Sandy mixed frigid Typic Endoaquoll), which was obtained from East-Central, North Dakota, USA. The soils were air-dried and ground to pass through a 2 mm sieve and stored frozen until the batch study started. The main differences between the two horizons are the organic matter content and electrical conductivity (Table 4).

Table 4. Physical and chemical properties of the A and B horizons of the Garborg soil used in this study.

Horizon	Depth	Sand	Silt	Clay	OM <sup>†</sup>	pH	CEC <sup>‡</sup>	EC <sup>¶</sup>
	cm	g kg <sup>-1</sup>	g kg <sup>-1</sup>	g kg <sup>-1</sup>	g kg <sup>-1</sup>		cmol <sub>(+)</sub> kg <sup>-1</sup>	dS m <sup>-1</sup>
A	0-15	73.8	14.6	11.6	21.4	7.6	10.9	0.2
B	15-30	74.0	15.1	10.6	16.0	7.5	11.3	1.07

<sup>†</sup> OM; organic matter

<sup>‡</sup> CEC; cation exchange capacity

<sup>¶</sup> EC; electrical conductivity

The solutions used for both the batch and ultrafiltration studies were swine manure slurry and 0.01M CaCl<sub>2</sub>. The lagoon manure slurry was obtained within 3 km from where the soil was collected. Slurry was obtained from a facility that housed approximately 4,000 animals, which included all stages of swine development described by Shelver et al. (2010). The manure slurry was filtered through a 0.45 µm Whatman filter to remove particulates before its use in the experiments. The slurry was characterized for chemical and physical parameters by Servi-Tech Laboratories (Hastings, NE) using their Lagoon Analysis package (Table 5).

Table 5. Physical and chemical parameters of the < 0.45  $\mu\text{m}$  fraction of the lagoon slurry used for the batch and ultrafiltration studies

Parameter	Concentration
Total N ( $\text{mg L}^{-1}$ )	162
Organic N ( $\text{mg L}^{-1}$ )	12
Ammonium-N ( $\text{mg L}^{-1}$ )	150
Nitrate-N ( $\text{mg L}^{-1}$ )	<1
Magnesium ( $\text{mg L}^{-1}$ )	3
Sodium ( $\text{mg L}^{-1}$ )	30
Zinc ( $\text{mg L}^{-1}$ )	<1
Iron ( $\text{mg L}^{-1}$ )	4
Manganese ( $\text{mg L}^{-1}$ )	<1
Copper ( $\text{mg L}^{-1}$ )	<1
EC <sup>†</sup> ( $\text{dS m}^{-1}$ )	12.6
Phosphorus ( $\text{mg L}^{-1}$ )	6
Potassium ( $\text{mg L}^{-1}$ )	67
Sulfur ( $\text{mg L}^{-1}$ )	11
Calcium ( $\text{mg L}^{-1}$ )	17
Boron ( $\text{mg L}^{-1}$ )	2
Moisture (%)	99.4
Total Solids ( $\text{mg L}^{-1}$ )	600
Organic Matter ( $\text{mg L}^{-1}$ )	300
Ash ( $\text{mg L}^{-1}$ )	300
C/N <sup>‡</sup> ratio	1:1
pH	7.4

<sup>†</sup> EC; electrical conductivity

<sup>‡</sup> C/N; carbon to nitrogen ratio

## Batch Experiments

### *Aqueous Phase*

Radiolabeled ( $^{14}\text{C}$ ) E2 (American Radiolabeled Chemicals, St. Louis, MO)

dissolved in ethanol solution was used in both the batch sorption and ultrafiltration studies.

The radiolabeled C is located on the C-4 position of the A-ring and is maintained during the transformation from E2 to its metabolite E1. Batch sorption procedures were similar to those described by Casey et al. (2005), where 1.6 g of soil and 8 mL of aqueous solution were placed into the 10 mL clear glass vials (06-406-38, Wheaton Science Products, Millville NJ). Vials were sealed using three pronged stopper plugs (06-4474, Wheaton Science Products, Millville, NJ) and metal crimp caps (06-406-15C, Wheaton Science Products, Millville NJ). Experimental controls consisted of aqueous phase without soil. All

batch vials were sterilized by irradiation for 16 hr with 8.5 kGy using a  $^{137}\text{CsCl}$  Gamma Irradiator, (Model #M38-4 Gammator, 1970). The batch studies were conducted in triplicate using four concentrations of  $^{14}\text{C}$ -labeled E2: 432.7, 185.5, 43.3, and 6.2  $\mu\text{g L}^{-1}$  with specific activities of 0.07, 0.03, 0.007, and 0.001  $\mu\text{Ci } \mu\text{mole}^{-1}$ , respectively. Although the concentrations of  $^{14}\text{C}$ -labeled E2 are above levels found in slurries that have been applied to agricultural fields (Findlay and Moore, 2000; Shore et al., 1998), the concentrations used in this experiment were due to practical considerations for instrument detection limits. To determine sorption rates from aqueous phase to solid phase, aliquots were taken at seven time points of 4 hr, 8hr, 1, 2, 3, 7, and 14 d. All batch vials were placed onto a rotating drum ( $360^{\circ}/5\text{sec.}$ ) for the duration of 14 d. In sterile soil systems the time to equilibrium is approximately 14 d. At every time point all batch vials were removed, centrifuged, sampled, and placed back onto the rotating drum. Centrifugation was accomplished using a SpeedVac centrifuge at 380 Gs for 10 min.

At each time point a cleaned syringe was inserted twice into each vial to obtain sample volumes of: (1) 100  $\mu\text{L}$  to assay for an aqueous layer radioactivity assay, and (2) 150  $\mu\text{L}$  to detect any  $^{14}\text{C}$  metabolites and observe whether E2 had transformed. The 100  $\mu\text{L}$  aliquots were injected into 5 mL of Ecolite (88247505, MP BioMedicals, Solon, OH) and then analyzed by a Beckman Model LS6001C liquid scintillation counter (Fullerton, CA). The 150  $\mu\text{L}$  aliquots were evaporated to approximately 10  $\mu\text{L}$  and spotted onto a reverse-phase TLC plate (RP-18F 266013, Analtech, Newark DE). The TLC plates were developed in 48:2 chloroform to methanol ratio in a TLC plate chamber (022.5256, CAMAG, Wilmington NC). After development the plate was analyzed by a TLC System 2000 Imaging Scanner (Bioscan, Inc., Washington, D.C.).

### *Solid Phase Extraction*

In addition to the batch vials, E2 associated with soil was analyzed from each time point (seven total vials). The lowest specific activity (0.01  $\mu\text{Ci}$ ) of  $^{14}\text{C}$ -labeled E2 was used because of the expense of radiolabeled material. At the appropriate time the vial was removed and injected with 3  $\mu\text{L}$  of formaldehyde to inhibit or stop biological transformations of E2, and frozen ( $-4.4\text{ }^{\circ}\text{C}$ ) until further analysis. Samples were thawed and centrifuged in a SpeedVac centrifuge at 380 Gs for 10 min. The supernatant was then decanted from these vials and stored in separate bottles and again frozen ( $-4.4\text{ }^{\circ}\text{C}$ ). The remaining soil in the vials was washed with 3 mL of nanopure water and sonicated for 30 min., centrifuged at 380 rpm for 10 min., and finally the supernatant was decanted into 10 mL polypropylene plastic bottles (209625, Wheaton Science Products, Millville NJ). This procedure was repeated twice with nanopure water and twice with acetone. Each wash was maintained separately; after the final acetone wash, the solid phase was air-dried evaporated complete dryness for 5 d at  $25^{\circ}\text{C}$ . Aliquots from the washings of 100  $\mu\text{L}$  of the 3 mL were removed from each supernatant bottle and assayed for  $^{14}\text{C}$  using LSC analysis. Using 0.1 g of the air dried solid phase, combustion analysis (Packard Model 307 Oxidizer, Downers Grove, IL) was used to quantify non-extractable  $^{14}\text{C}$  remaining on the solid phase by measuring  $^{14}\text{CO}_2$  in the combustion emission.

### **Ultrafiltration**

Volumes of 10 mL of i) 0.01M  $\text{CaCl}_2$ , ii) nanopure water, and iii) 0.45 $\mu\text{m}$ -filtered slurry were created, and 7  $\mu\text{L}$  of  $^{14}\text{C}$ -labeled E2 stock solution (0.001  $\mu\text{Ci } \mu\text{L}^{-1}$ ) was added to each of the three solutions. Each of the three solutions spiked with  $^{14}\text{C}$ -E2 were then ultrafiltered using a 1 kDa filter (PLAC02510, Millipore Regenerated Cellulose

Ultrafiltration Membrane, Billerica, MA) and a 10 mL Amicon stirred cell (Model 8010; Billerica, MA). The stirred cell ultrafilter was placed under 22a kPa pressure using N<sub>2</sub> gas for 4 hr. When ultrafiltration was complete, <sup>14</sup>C of three 100 μL filtrate aliquots was determined using LSC. The stirred cell was then dismantled and the 1 kDa filter was removed, and placed onto aluminum foil to air-dry at 23°C for 24h. The filter was then were cut in half, and <sup>14</sup>C retained on each filter half was determined using the combustion analysis previously described. The <sup>14</sup>C recovered from the two filter halves were summed. The above ultrafiltration procedure was replicated three times for all three solutions. The 6.35 mm diameter polypropylene tubing used for output from the Amicon stirred cell was replaced between each run to avoid cross contamination



## RESULTS AND DISCUSSION

### Persistence

Although E2 degradation is dominated by biological processes (Colucci and Topp, 2001; Fan et al., 2007), results indicate that E2 conversion to E1 occurred in abiotic sterile systems. Besides the E1, the TLC analysis detected little or no other metabolites of E2.  $17\beta$ -estradiol was observed to persist throughout the duration of the experiment in the slurry solution for both surface and subsurface soils, whereas it dissipated completely by 7 d in the  $\text{CaCl}_2$  aqueous solution for both soils (Figure 2). The transformation of E2 to E1 could be attributed to abiotic mechanisms such as the presence of manganese oxides (Mn(II)) or photolysis (Fan et al., 2007; Mansell et al., 2004; Sheng et al., 2009; Xu et al., 2008; Zhang et al., 2007).

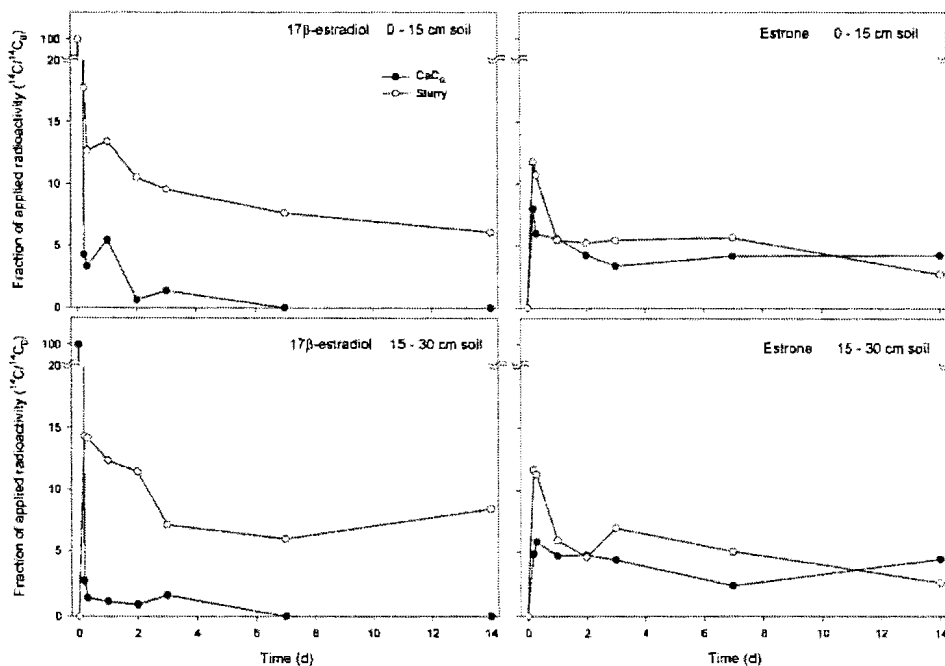


Figure 2 – The fraction of applied radioactivity recovered in the aqueous phase as  $17\beta$ -estradiol or estrone through time for the calcium chloride ( $\text{CaCl}_2$ ) and slurry solutions, in surface and subsurface soils.

Under different various environmental conditions, Sheng et al. (2009) and Xu et al. (2008) showed conversion from E2 to E1 due to the possible presence of manganese oxides. Manganese oxides can facilitate the transformation of E2 into E1 in sterile soil and aqueous systems, when oxidation of a hydroxyl group at position 17 is converted to a carbonyl (Sheng et al., 2009; Xu et al., 2008). In soil, E2 is shown to rapidly degrade to E1 in the presence of manganese oxides, when the E2 can access the surface reaction sites (Sheng et al., 2009; Xu et al., 2008). Manganese oxides have also been suspect in the degradation of E2 in non-sterile soils by Sheng et al. (2009) and Xu et al. (2008). However, sorption of other metals such as Fe (II) will compete with E2 for surface reaction sites of manganese oxides, which decreases E2's transformation to E1 (Sheng et al., 2009; Xu et al., 2008).

While the DOC-COC suspended in the aqueous phase could bind to the E2 molecule, which would reduce its sorption affinity to the soil surface; higher concentrations of suspended DOC-COC, such as in the slurry solution, would result in the E2 remaining in the aqueous layer rather than being oxidized by the manganese oxides (Xu et al., 2008). Furthermore, the DOC-COC from the slurry may compete for soil surface reaction sites, which would further reduce E2 oxidation rates (Sheng et al., 2009; Xu et al., 2008). The results presented here with those reported by Jacobsen et al. (2005), indicate that once E2 binds with a TOC fraction, it will persist unmetabolized longer in aqueous solution.

In addition to the higher concentrations of E2 suspended within the aqueous layer, E1 also persisted at higher concentrations in the aqueous solution for the slurry solution compared to the CaCl<sub>2</sub> solution for both the surface and subsurface soils (Figure 2). This

suggests that E1 remained suspended in solution for longer periods of time for the slurry due to its association with DOC-COC. Similar to E2, E1 associated DOC-COC will reduce its adsorption affinity to the soil surface, allowing for the hormone to remain in the aqueous layer (Gong et al., 2001; Holbrook et al., 2004).

Photolysis may also cause E2 to convert to E1. When E2 is in an aqueous solution and exposed to light sources, the excitation of the phenolic ring can occur (Zhang et al., 2007). Through this excitation the formation of photoproducts such as E1 arise from further reactivity onto the carbon in position 17. Ninety percent of E2 has been reported to degrade in aqueous solutions that were exposed to 5.2 hr of sunlight or light emitted at 254 nm (Mansell et al., 2004; Zhang et al., 2007). Although the batch vials in this experiment were not individually wrapped in foil, they were not exposed to direct sunlight and the transmission of 254 nm does not penetrate the glass vials (Janos Technology, 2010). Similar studies have shown no significant photodegradation in batch soil vials (Fan et al., 2007).

## **Radioactive Recoveries**

### *Aqueous Phase Recovery*

Thin layer chromatography yielded no evidence of other metabolites present within the aqueous layer besides E1 (Appendix 2). There would be no loss of  $^{14}\text{C}$  during the conversion of E2 to E1 because the radiolabeled is located at the C-4 position of the A-ring, which is unaltered in the steroidal structure during transformation (Holthaus et al., 2002). Therefore, the total recovered radioactivity from the aqueous phase through time was assumed to contain either or both E2 and E1. Taking the approach of Holthaus et al. (2002), the recovered radioactivity is assumed to represent a composite concentration of E2

and E1, and the sorption of E2 and E1 were considered identical. Holthaus et al. (2002) made this assumption based on the similarity of the E2 and E1 molecules and their physiochemical properties (e.g., molecular mass, solubility), and based on the similarities of reported E2 and E1 sorption parameters (Casey et al., 2005; Lee et al., 2003)

The initial concentrations for all experimental controls followed the same trends throughout the duration of the 14 d experiments (Figure 3). The aqueous radioactivity

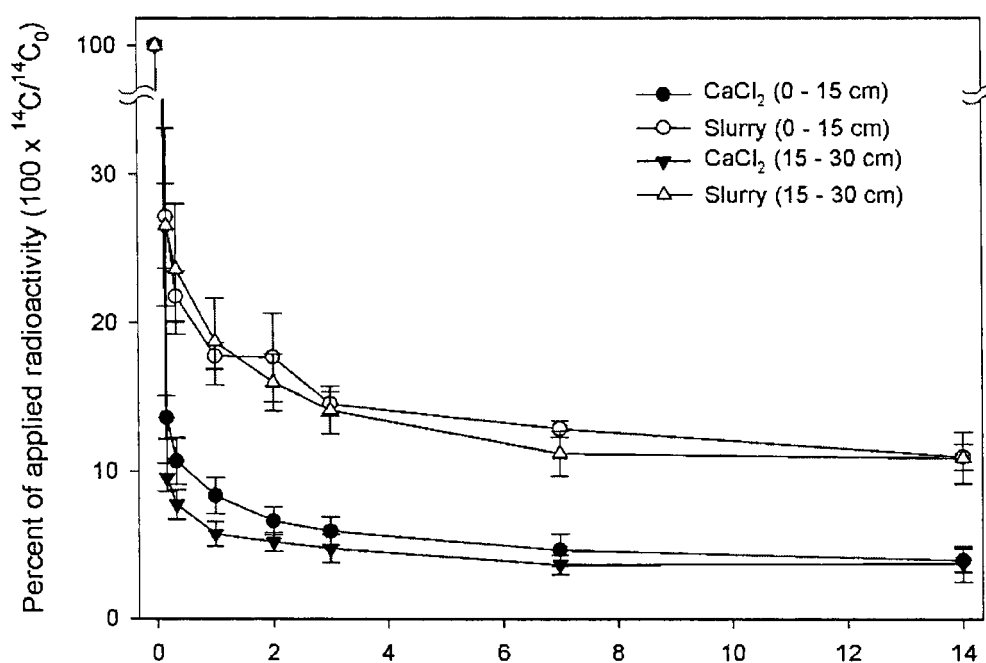


Figure 3 – The average percent of the applied radioactivity recovered from the aqueous phase through time for the calcium chloride solution (CaCl<sub>2</sub>) and slurry solution for the surface and subsurface soils. The error bars represent standard deviations of the percent applied radioactivity.

recovered from the CaCl<sub>2</sub> solution appears different between the surface and the subsurface soils, which can be attributed to hydrophobic sorption and differences in the soil organic matter contents of these two soils. The sorption of E2 and E1 is a hydrophobic process (Lee

et al., 2004), which would indicate a higher sorption affinity to soils with higher organic matter contents. In contrast, there was no observed difference for the aqueous radioactivity recovered from slurry solution for the surface and subsurface soils. Results reported here indicate 17 $\beta$ -estradiol associated with suspended DOC-COC fractions, and could explain its enhanced mobility in soils as did those of higher DOC-COC levels in the slurry solution will reduce surface soil sorption and minimize the apparent effects of hydrophobic sorption to soil surfaces.

#### *Solid Phase Recovery*

Radioactivity in the solid phase was recovered in three pools (Figure 4). Radioactivity recovered in water and acetone extracts pools were considered potentially and possibly potential exchangeable sorption fractions, respectively (Figure 4). Through soil combustion analysis, a more recalcitrant pool of radioactivity was retrieved. The partitioning of E2-E1 into variably extractable sorption sites and irreversibly bound sorption sites is consistent with previous studies (Fan et al., 2008). Irreversible sorption of organic compounds in the soil OC has been accounted for by diffusion, physical entanglement, and/or chemical or enzymatic covalent binding (Huang and Weber, 1997; Pignatello and Xing, 1996). Additionally, the functional groups of E2 and E1 could interact with humic substances or mineral surfaces of the soil via hydrogen and covalent binding (Yu et al., 2004).

Overall the radioactivity recovered from the acetone extracts and combustion analysis was greater for all CaCl<sub>2</sub> solutions compared to slurry solutions. The association of these hormones with the suspended COC-DOC can cause less partitioning to the soil in the moderately exchangeable and the non-exchangeable fractions, which could

increase its mobility in soil. This result is consistent with field lysimeter studies that have suggested DOC derived from soil and/or manure can facilitate hormone transport (Minamiyama et al., 2008).  $17\beta$ -estradiol can be transported through soil monoliths through preferential flow paths and/or by swine manure organic matter, facilitated transport (Lægdsmand et al., 2009). Furthermore, research has shown strong correlations between TOC and E2 detections in field lysimeter and well samples, which suggests TOC enhances estrogen mobility and persistence (Thompson et al. 2009).

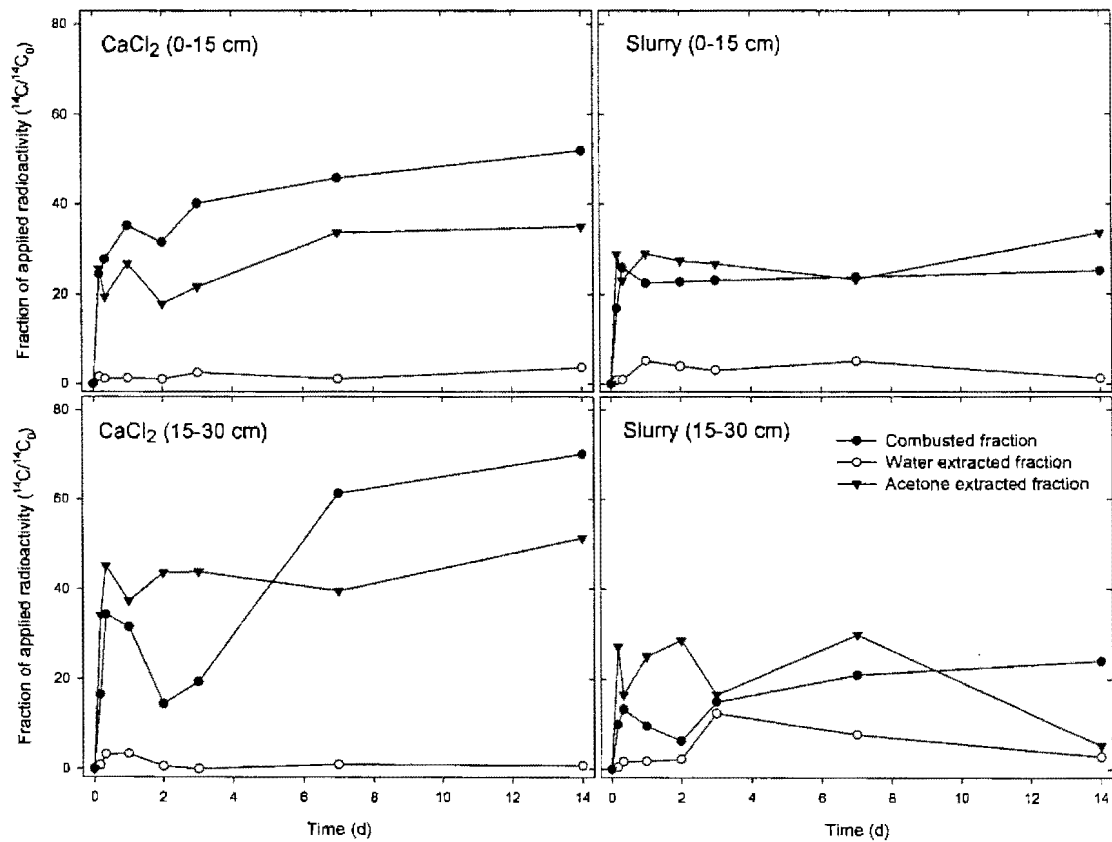


Figure 4 – The radioactivity recovered from the solid phase by combustion, water extraction, and acetone extraction through time.

## Aqueous and Sorbed Phase Distributions

As previously discussed, E2 and E1 were the only radiolabeled compounds present in the aqueous phase and a large fraction of the radioactive mass was irreversibly adsorbed. Also, the radioactivity recovered with the water and acetone extractions was too small to analyze with TLC and precisely identify any E2 or E1 peaks. Therefore, the development of independent isotherms for E2 and E1 was impossible due to this lack of quantifiable and qualifiable concentrations of E2 and E1 bound to the soil surface. However, using the approach of Holthaus et al. (2002), explained in the above section, E2 and E1 were assumed to have identical sorption properties. Furthermore, the mass of E2 and E1 bound to the soil surface was determined by mass balance difference between the originally applied radioactivity and recovered aqueous phase concentrations.

Linear sorption isotherms were developed and sorption partitioning coefficients ( $K_d$ ) were determined that represented a mixture of E2 and E1. The  $\log K_{oc}$  ( $=\log_{10}(K_d/OC)$ ) values derived from the  $K_d$  values for the  $CaCl_2$  (average = 2.89; min. = 2.31; max = 3.39) and slurry (averages = 2.52; min. = 2.05; max. = 2.99) solutions agreed well with previously reported  $\log K_{oc}$  values for soil (Casey et al., 2005, Lee et al., 2003). The fit of the linear isotherms to the data were acceptable and supported by the high correlation coefficient (Table 6), which is similar to other reported studies (Casey et al., 2005; Lee et al., 2003; Yu et al., 2004). The apparent  $K_d$  values for the subsurface soils for the  $CaCl_2$  were higher except for the last time point at 14 d (Figure 5). Higher OC surface soils are assumed to have higher sorption affinity due to hydrophobic sorption (Lee et al., 2003). However, results from the current study showed that it took 14 d for the surface soil

to have a greater sorption potential for E2 and E1 compared to the subsurface soil, which has lower OC.

Table 6. Estimates of the linear sorption distribution coefficients ( $K_d$ ) and corresponding coefficient of determination ( $r^2$ ) for the fit of the linear isotherm to the measured data. Data is based on four concentrations.

Time d	Calcium Chloride Solution				Slurry Solution			
	0-15 cm Soil		15-30 cm Soil		0-15 cm Soil		15-30 cm Soil	
	$K_d$ L kg <sup>-1</sup>	$r^2$	$K_d$ L kg <sup>-1</sup>	$r^2$	$K_d$ L kg <sup>-1</sup>	$r^2$	$K_d$ L kg <sup>-1</sup>	$r^2$
0.17	273.81	1.00	346.87	0.99	150.23	1.00	139.01	1.00
0.33	322.09	1.00	414.99	0.99	177.71	1.00	159.59	1.00
1	434.70	0.99	549.69	0.99	205.31	1.00	197.57	1.00
2	492.25	0.99	636.89	1.00	220.83	1.00	228.53	1.00
3	534.87	0.99	650.83	0.99	246.18	1.00	244.08	1.00
7	650.62	0.99	789.73	0.99	278.76	1.00	293.39	1.00
14	815.48	0.99	649.17	0.98	306.60	1.00	312.02	1.00

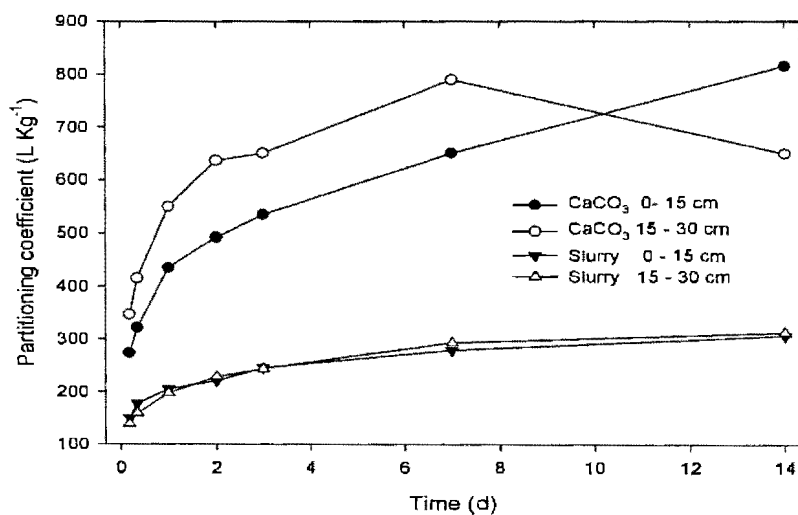


Figure 5 – The linear sorption-partitioning coefficient calculated through time for the two solutions in the two surface and subsurface soil.

The delay in sorption potential of steroidal hormones to high OC soil has been attributed to rate limited sorption processes associated with the OC fraction of the soil



(Casey et al., 2005; Lee et al., 2003). Other studies have also seen a negative correlation between the  $K_{oc}$  values and OC indicating that non-hydrophobic processes are contributing to sorption (Casey et al., 2005). One suggestion is that the polar groups at the C-17 position of E2 and E1 can interact with humic acids or mineral surfaces via hydrogen and covalent bonding causing sorption in addition to hydrophobic interactions (Lee et al., 2003).

The  $K_d$  values for the slurry solution were the same for both the surface soil and subsurface soil indicating that the DOC/COC derived from the slurry minimized the sorption potential to soil regardless of soil OC content. Ideally, the  $K_d$  values would be higher for surface soils for both E2 and E1 due to higher OC content in comparison to the subsurface soil because of the hydrophobic sorption of the estrogens. However, the DOC/COC within the slurry hindered the E2/E1 sorption potential to the soil surface, which decreased the  $K_d$ .

### **Ultrafiltration**

Ultrafiltration results indicated whether the radiolabeled E2 preferentially adsorbed to either the DOC or COC fraction in various solutions. The  $\text{CaCl}_2$  and nanopure water solutions had low recoveries of the radiolabeled E2 on the 1 kDa filter (Table 7). In contrast, the slurry solution had a third of the  $^{14}\text{C}$ -E2 retained on the 1 kD filter. The E2 in all three solutions predominantly associated with the < 1 kD fraction, but in the slurry solution 33% was associated with the > 1 kD to < 0.45  $\mu\text{m}$  fraction. The results indicate an association with > 1 kD to < 0.45  $\mu\text{m}$ , indicating that in a solution such as liquid manure,  $^{14}\text{C}$ -E2 is likely to associate with greater fractions of OC. These data supports the theory that livestock lagoon manure may facilitate the transport of E2.

In municipal wastewater, the  $K_d$  values for COC ranged from less than 1 to  $179 \times 10^3 \text{ kg L}^{-1}$  and 60% of the aqueous E2 concentrations were associated with COC (Holbrook et al., 2004). These results are also supported by other studies that have found correlations between  $K_d$  values and smaller size fractions and OC fractions in riverbed sediments (Holthaus et al., 2002; Casey et al., 2005). Concentrations of E2 and E1 have been found to increase with depth in lagoon storage ponds (Raman et al., 2004), which could be explained by the association with OC within solids that settle to the bottom and/or the decrease in their degradation based on the anaerobic conditions as depth increases.

Table 7. Ultrafiltration results showing the average radioactivity recovered in the filtrate, on the filter, and total recovered.

Solution	Average radioactivity recovered in filtrate (< 1kDa)	Average radioactivity recovered on filter (> 1kDa)	Average total radioactivity recovered
	%	%	%
Slurry	56 (11) <sup>†</sup>	33 (8)	89 [71-108] <sup>‡</sup>
CaCl <sub>2</sub>	74 (3)	6 (6)	80 [73-89]
Nanopure water	99 (3)	3 (1)	102 [98-106]

<sup>†</sup> Values in parenthesis are standard deviations

<sup>‡</sup> Values in brackets are ranges

## CONCLUSION

When in the presence of OC, E2 is less likely to degrade into its metabolite E1 and is more likely to persist in the parent form for longer periods of time. In the slurry solution, E2 will associate with OC fractions causing the E2 to stay suspended within the aqueous layer for longer periods of time and at higher concentrations. In the CaCl<sub>2</sub> solution E2 was shown to transform into E1 more readily and adsorb to the solid phase more rapidly and at higher concentrations compared to the slurry solution. Conceptually, once E2 associates with the suspended OC from the slurry solution it is unavailable for transformation because it cannot adsorb to solid surface reaction sites on the soil. The environmental implications of these results would mean that E2, which is one of the most potent estrogenic hormones, may persist longer and transport through the soil more readily. Furthermore, if E2 is biologically active while it is associated with the DOC/COC, then it could persist in the environment at concentrations that are detrimental to wildlife. For example, detections of 17 $\beta$ -estradiol have been as high as 21,000 ng L<sup>-1</sup> within finishing swine lagoon slurry (Hutchins et al., 2007 [supporting info. Table SI-1]). Using the fractional concentrations of E2 from this study, 7 d after swine slurry application to soil E2 concentrations would decrease to 10,500 ng L<sup>-1</sup> and 9,450 ng L<sup>-1</sup> for the surface and subsurface soils used in this study. If E2 is not associated with the slurry manure matrix, as observed for the CaCl<sub>2</sub> solution, then it can quickly and completely convert to E1, which has 100 x less binding strength in the hypothalamus tissue of rainbow trout (*Oncorhynchus mykiss*) (Allison and Omeljaniuk, 2000). Nonetheless, the effect of the association may interfere with the binding strength to hormone receptor sites, thus decreasing potency. Although it has been demonstrated that DOC/COC derived from manure slurry can enhance E2 persistence and

potential mobility, further research is still necessary to determine the toxicological implications of these results.

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# APPENDIX 1

## LSC Raw Data

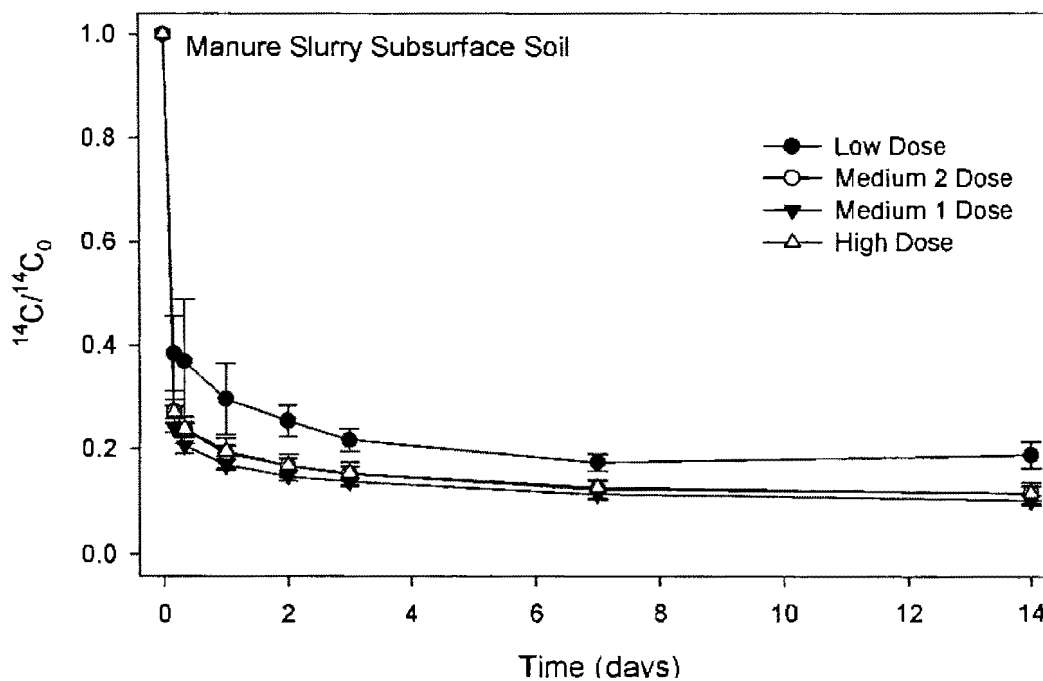
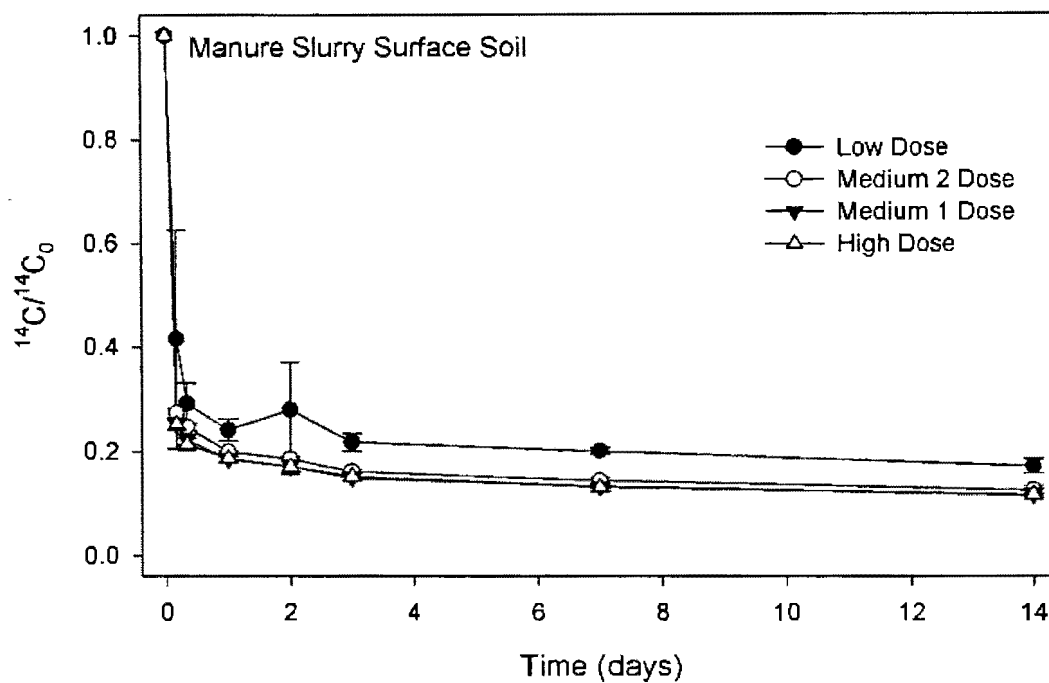


Figure 6 – Represents the fractional recovered  $^{14}\text{C}$  in the aqueous phase over time for the swine slurry solution.

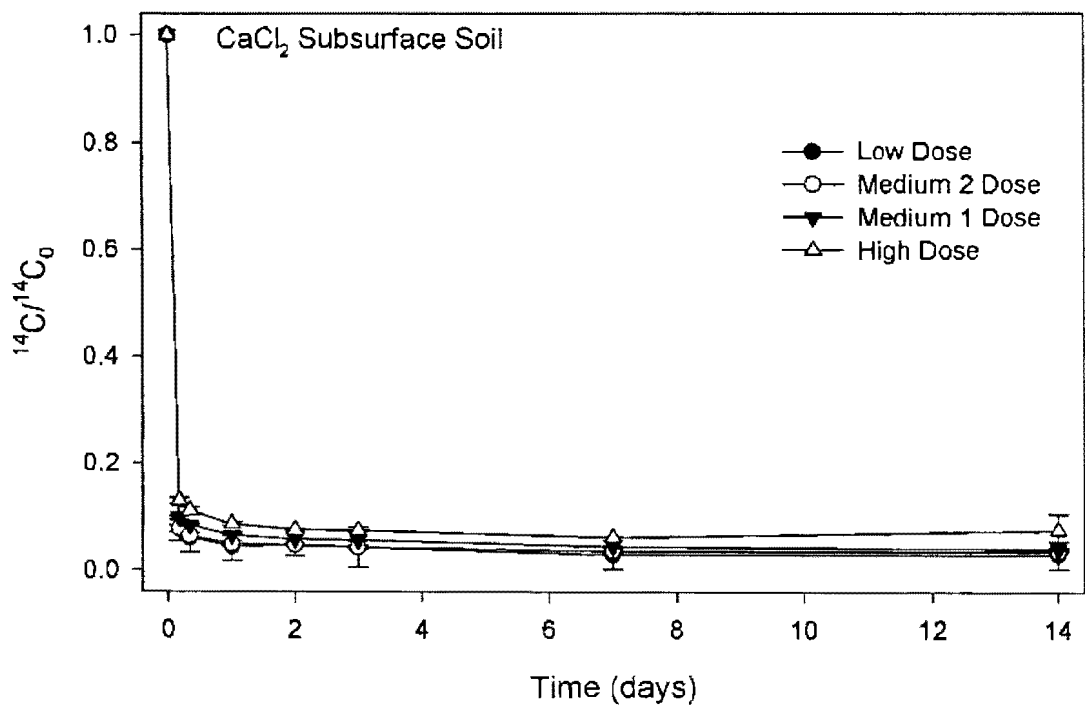
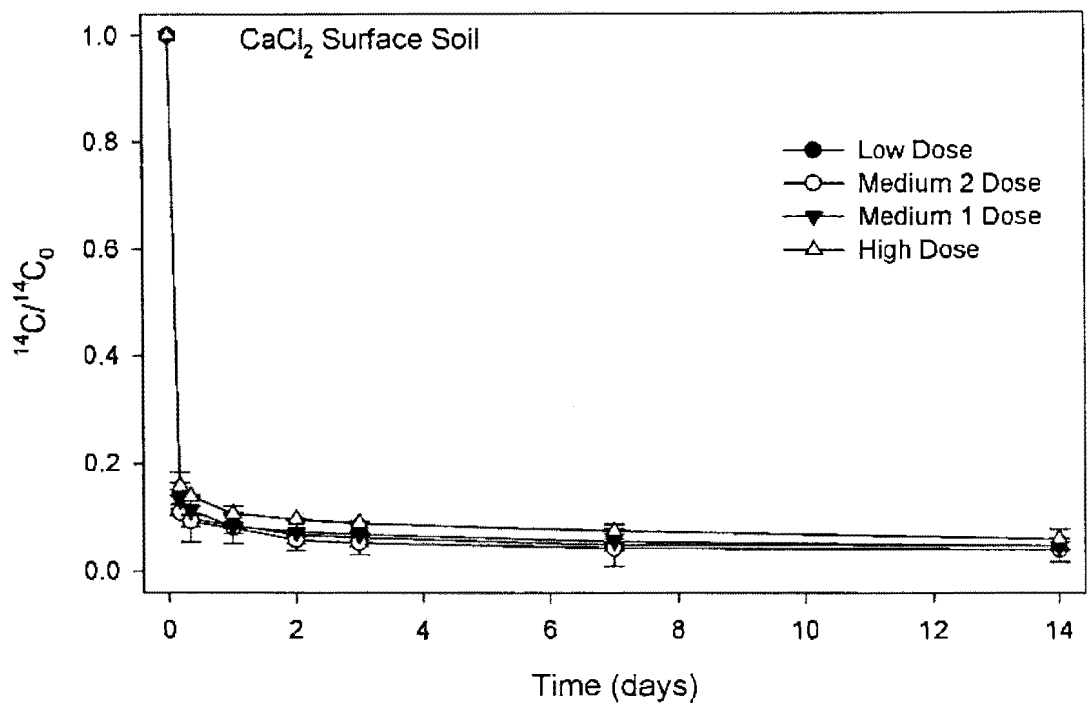


Figure 7- Represents the fractional recovered <sup>14</sup>C in the aqueous phase over time for the CaCl<sub>2</sub> solution.

## APPENDIX 2

### TLC Raw Data

**E2**  
 STANDARD 4613 CHOL3:HIGH RT110  
 Date: Mar 17 2009 Start time: 03:00 Accum time: 00:02:00  
 Data File: Plate: 3 Lane: 1  
 Slect Resolution: 105100 Amp. Range: 0 - 22471  
 Integration Parameters: Auto integration  
 Leak slope: 1.0 Min width: 0.1 Min %: 2.0  
 Total Count Region: 0.00cm to 20.00cm  
 Total Counts: 2124  
 Total CPM: 984.7

Reg. #	Start (cm)	Stop (cm)	Center (cm)	Region Counts	Region CPM	% of Tot Reg	% of Tot Cnt
1	13.74	15.98	14.86	2106	907.9	100.00	94.11
TOTAL				2106	907.9	100.00	94.11

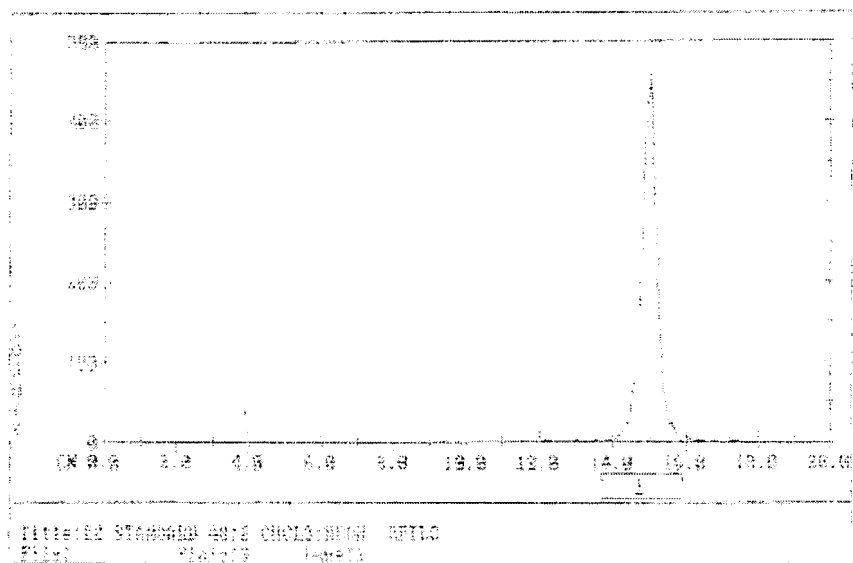


Figure 8 – Represents the TLC report for the E2 standard.

PRIMARY REPORT

RE STANDARD AND COLLECTION: E1TLC

Date: Mar 17 2009 Start time: 00:54 Accum time: 00:08:56  
 Data File: Plate: 2 Lane: 1

Elect. Resolution: 0.05061 (Amp. Range: 0 - 2047)  
 Integration Parameters: Auto Integration  
 Peak slope: 1.6 Min width: 0.1 Min %: 2.0

Total Count Region: 0.00cm to 20.00cm  
 Total Counts: 2495  
 Total CPM: 276.6

Reg. #	Start (cm)	Stop (cm)	Center (cm)	Region Counts	Region CPM	% of Tot Reg	% of Tot Cnt
1	15.50	17.50	16.73	2053	228.5	94.43	82.62
2	17.90	19.10	18.50	121	13.47	5.57	4.87
TOTAL				2174	242	100.00	87.48

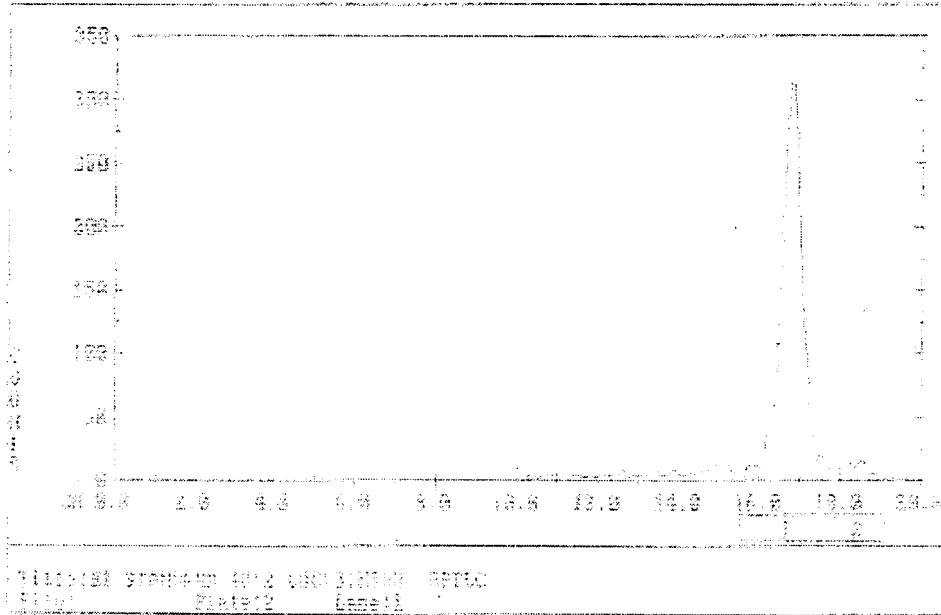


Figure 9 – Represents the TLC data for the E1 standard.

SUMMARY REPORT

~~same~~ High Dose 0 to 4hrs. Lagoon

Date: Aug 03 2009 Start time: 11:26 Accum time: 00:15:00  
 Data File: Plate: 2 Lane: 1

Elect Resolution: NORMAL (Amp. Range: 0 - 2047)  
 Integration Parameters: Auto Integration  
 Peak slope: 1.0 Min width: 0.1 Min %: 2.0

Total Count Region: 0.00cm to 20.00cm  
 Total Counts: 1011  
 Total CPM: 67.4

Reg. #	Start (cm)	Stop (cm)	Center (cm)	Region Counts	Region CPM	% of Tot Reg	% of Tot Cnt
1	2.14	2.75	2.41	22	1.467	4.26	2.18
2	9.38	10.00	9.66	26	1.733	5.04	2.57
3	14.79	16.42	15.61	336	22.4	65.12	33.23
4	17.75	18.97	18.32	132	8.8	25.58	13.06
TOTAL				516	34.4	100.00	51.04

90.7

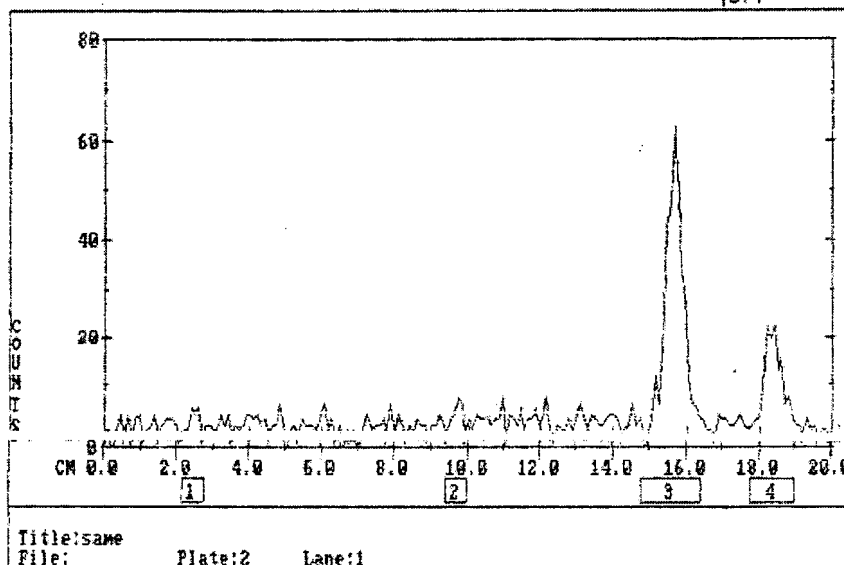


Figure 10 – Represents the TLC data for the high dose at four hrs for the surface soil and swine slurry solution.



SUMMARY REPORT

0-6 Hrs. High Dose CHCL3:MeOH 40:2

Date: Aug 05 2009 Start time: 09:54 Accum time: 00:30:00  
 Data File: Plate: 1 Lane: 1

Elect Resolution: NORMAL (Amp. Range: 0 - 2047)  
 Integration Parameters: Auto Integration  
 Peak slope: 1.0 Min width: 0.1 Min %: 2.0

Total Count Region: 0.00cm to 20.00cm  
 Total Counts: 1468  
 Total CPM: 48.93

Reg. #	Start (cm)	Stop (cm)	Center (cm)	Region Counts	Region CPM	% of Tot Reg	% of Tot Cnt
1	1.12	1.73	1.43	59	1.8	0	2.66
2	7.96	8.57	8.24	44	1.4	7.63	3.00
3	11.32	11.93	11.63	1830	8.49	8.49	3.34
4	14.69	15.30	15.00	8838	44.89	44.89	17.64
5	16.42	16.93	16.68	30	1	5.20	2.04
6	17.80	18.41	18.11	156	6.2	27.04	10.60
TOTAL				577	19.23	100	39.31

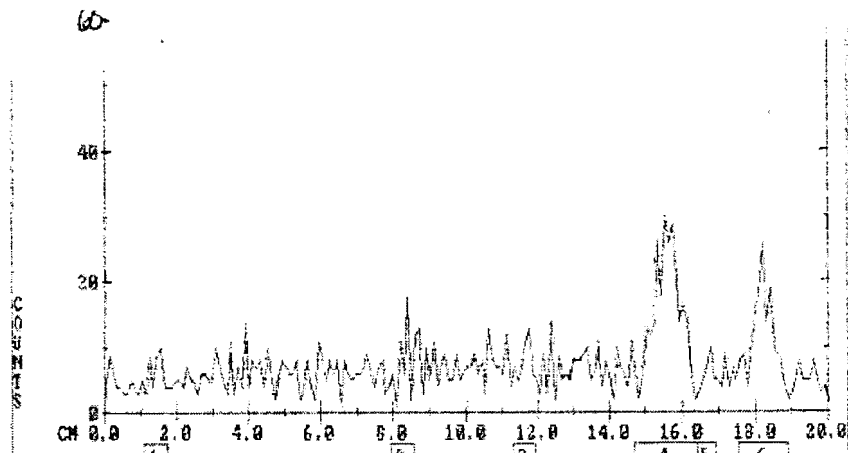


Figure 11 – Represents the TLC data for the high dose at eight hrs for the surface soil and swine slurry.

SUMMARY REPORT

0.6 High dose 1day

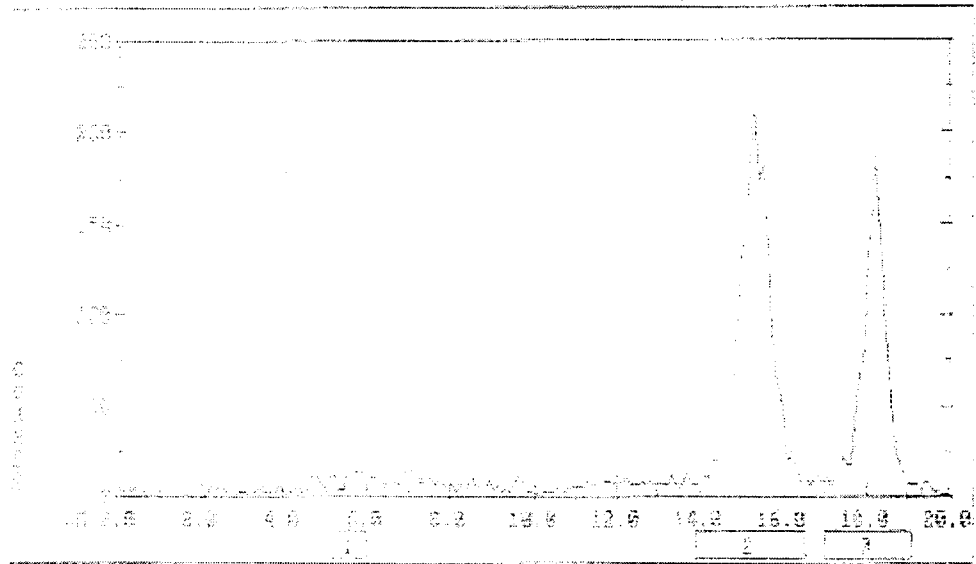
Date: Aug 21 2009 Start time: 08:34 Accum time: 00:30:00  
 Data File: 4873 Plate: 225 Lane: 1

Scan mode: NORMAL (Amp. Range: 0 - 2047)  
 Integration Parameters: Auto Integration  
 Peak slope: 1.0 Min width: 0.1 Min %: 2.0

Total Count Region: 0.000m to 20.000m  
 Total Counts: 4079  
 Total CPM: 105.6

Reg. #	Start (cm)	Stop (cm)	Center (cm)	Region Counts	Region CPM	% of Tot Reg	% of Tot Cnt
1	0.00	3.10	1.55	104	3.467	3.28	2.56
2	13.90	14.70	14.30	3079	62.63	59.31	46.17
3	17.00	18.10	17.55	1196	39.5	37.41	28.12
TOTAL				4079	105.6	100.00	77.84

96.72



File: 0.6 high dose 1day  
 Plate: 225 Lane: 1

Figure 12 – Represents the TLC data for the high dose at 1 d for the surface soil and swine slurry.

SUMMARY REPORT

*CI = 1.51*  
*RI = 1.37*

0-6 2dav high dose 48:2 CHCl3:MeOH

Date: Aug 12 2009 Start time: 13:49 Accum time: 00:35:18  
Data File: Plate: 2 Lane: 1

Elect Resolution: NORMAL (Amp. Range: 0 - 2047)  
Integration Parameters: Auto Integration  
Peak slope: 1.0 Min width: 0.1 Min %: 2.0

Total Count Region: 0.00cm to 20.00cm  
Total Counts: 3407  
Total CPM: 98.52

Reg. #	Start (cm)	Stop (cm)	Center (cm)	Region Counts	Region CPM	% of Tot Reg	% of Tot Cnt
1	10.71	11.63	11.19	116	3.286	5.12	3.40
2	13.77	15.81	14.94	1336	37.85	58.93	39.21
3	16.83	17.65	17.23	69	1.955	3.04	2.03
4	17.65	18.67	18.32	746	21.13	32.91	21.90
TOTAL				2267	64.22	100.00	66.54

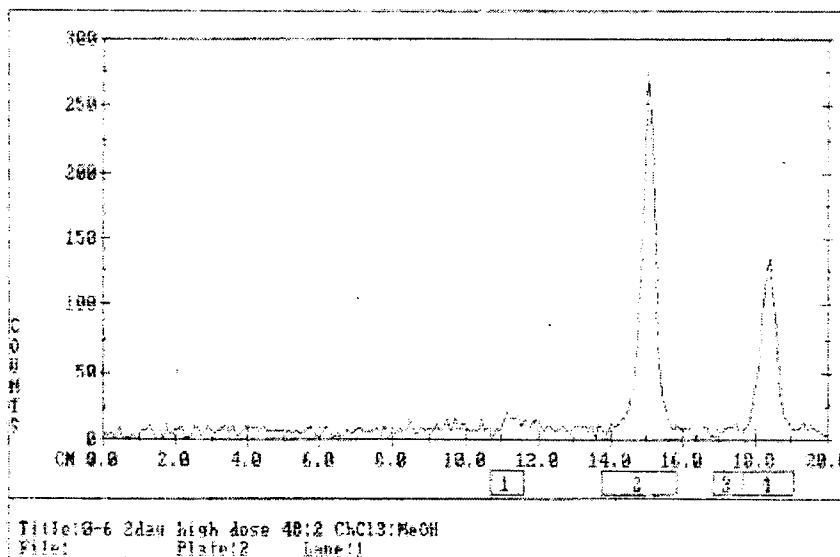


Figure 13 – Represents the TLC data for the high dose at 2 d for the surface soil and swine slurry.

7-6 ~~6-12~~ high dose 3 day 48:2 CHCl<sub>3</sub>:MeOH

SUMMARY REPORT

Date: Aug 10 2009 Start time: 15:10 Accum time: 00:45:01  
 Data File: Plate: 4 Lane: 1

Elect Resolution: NORMAL (Amp. Range: 0 - 2047)  
 Integration Parameters: Auto Integration  
 Peak slope: 1.0 Min width: 0.1 Min %: 2.0

Total Count Region: 0.00cm to 20.00cm  
 Total Counts: 3062  
 Total CPM: 68.02

Reg. #	Start (cm)	Stop (cm)	Center (cm)	Region Counts	Region CPM	% of Tot Reg	% of Tot Cnt
1	0.41	1.22	0.75	75	1.666	4.25	2.45
2	1.94	2.75	2.31	71	1.577	4.02	2.32
3	8.36	9.28	8.74	99	2.199	5.61	3.23
4	10.81	11.53	11.16	111	2.466	6.29	3.63
5	12.94	12.95	12.57	76	1.555	3.97	2.29
6	14.69	16.42	15.57	931	20.688	52.78	30.40
7	16.93	18.16	17.49	407	9.041	23.07	13.29
TOTAL				1764	39.49	100.00	57.61

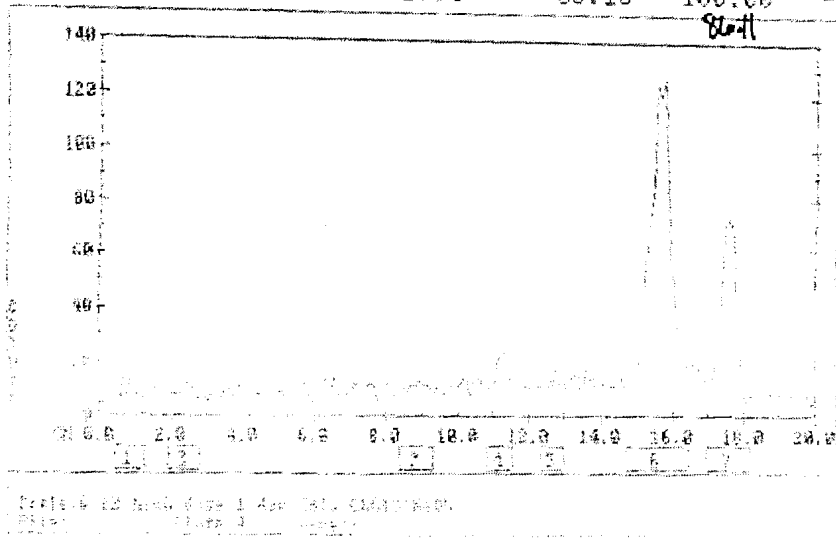


Figure 14 – Represents the TLC data for the high dose at 3 d for the surface soil and swine slurry.

SUMMARY REPORT

0-6 7 day high dose

Date: Sep 18 2009      Start time: 12:17      Accum time: 00:30:00  
 Data File:      Plate: 2      Lane: 1

Elect Resolution: NORMAL      (Amp. Range: 0 - 2047)  
 Integration Parameters: Auto Integration  
 Peak slope: 1.0      Min width: 0.1      Min %: 2.0

Total Count Region: 0.00cm to 20.00cm  
 Total Counts: 1954  
 Total CPM: 65.13

Reg. #	Start (cm)	Stop (cm)	Center (cm)	Region Counts	Region CPM	% of Tot Reg	% of Tot Cnt
1	0.20	0.92	0.56	42	1.4	3.86	2.15
2	6.73	7.45	7.08	46	1.533	4.23	2.35
3	9.38	10.10	9.67	61	2.033	5.61	3.12
4	13.97	15.50	14.73	464	15.47	<del>42.85</del>	23.75
5	17.03	17.85	17.42	73	2.4	6.63	3.68
6	17.85	19.48	18.64	408	13.48	37.04	20.62
<b>TOTAL</b>				1088	36.27	100.00	55.68

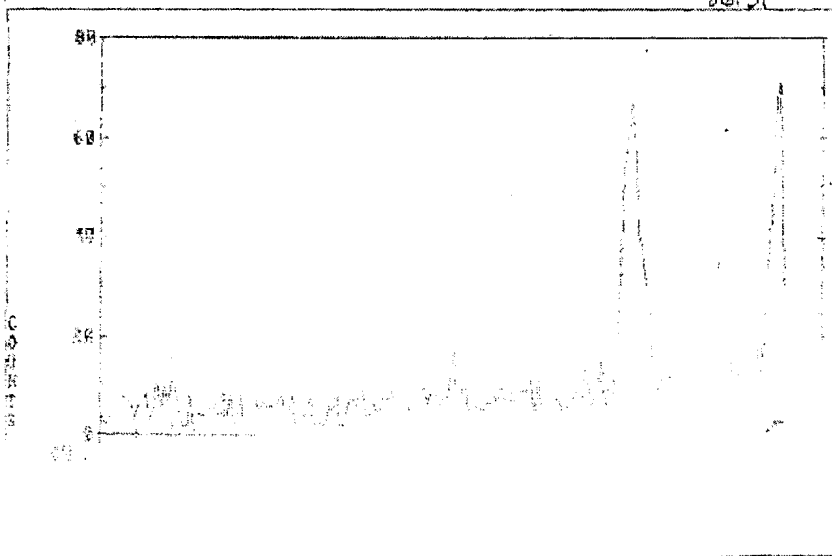


Figure 15 - Represents the TLC data for the high dose at 7 d for the surface soil and swine slurry.

4000

0-6 high dose 14 day

Date: Sep 24 2008

Time:

File:

	Area	Height	Width	Area	Height	Width	Area
1	11.00	10.71	10.00	50	1.887	5.61	8.15
2	11.00	10.24	11.58	157	5.233	13.90	8.97
3	18.36	15.10	14.97	278	9.1	41.55	17.34
4	16.73	17.34	17.05	43	1.433	6.54	3.75
5	17.55	16.97	18.55	134	4.407	20.40	8.51
TOTAL				657	21.0	100.00	41.74
						92.39	

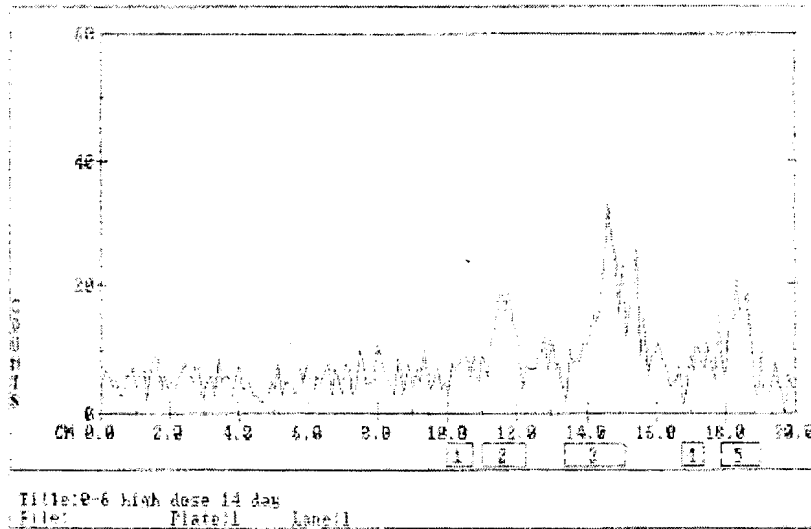


Figure 16 – Represents the TLC data for the high dose at 14 d for the surface soil and swine slurry.