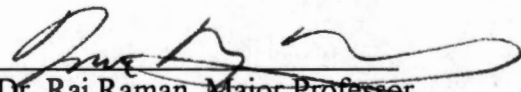
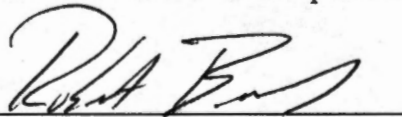


To the Graduate Council:

I am submitting herewith a thesis written by Elizabeth Louise Williams entitled "Survey of Estrogen Concentrations in Dairy and Swine Waste Storage and Treatment Facilities in and around Tennessee". I have examined the final paper copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the Master of Science, with a major in Biosystems Engineering Technology.


Dr. Raj Raman, Major Professor

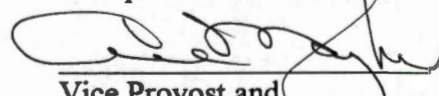
We have read this thesis
and recommend its acceptance:







Accepted for the Council:


Vice Provost and
Dean of Graduate Studies

**Survey Of Estrogen Concentrations In Dairy And Swine Waste Storage
And Treatment Facilities In And Around Tennessee**

A Thesis
Presented for the
Master of Science
Degree
The University of Tennessee, Knoxville

Elizabeth Louise Williams
December 2002

Thesis
2002
W946

DEDICATION

To Larry and Nancy Williams

For their unfailing love and support.

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ABSTRACT

Naturally occurring estrogens in animal wastes may cause negative environmental impacts in some watersheds. However, there is little published data regarding the concentrations of these estrogenic compounds in full-scale animal waste treatment and storage structures, thus making risk assessment difficult. To address this knowledge gap, the research described herein explores estrogen concentrations at 19 animal waste storage and treatment structures at dairy and swine production facilities in and around Tennessee during the winter and summer months of one year. Samples were collected from eight dairy and eleven swine facilities, representing a range of waste storage and treatment structures typical of Tennessee, and much of the southeastern US. Swine waste samples were collected from both swine farrowing operations that are populated by farrowing sows and piglets, and swine finishing operations that are populated by growing, non-pregnant male and female pigs. Dairy waste samples were collected from dairy operations populated by lactating cows in various stages of their hormonal and reproductive cycles. The 17β -estradiol concentrations in all samples were assayed in triplicate serial dilutions by means of an enzyme linked immunosorbent assay (ELISA). In addition, concentrations of 17β -estradiol, 17α -estradiol, and estrone were determined by gas-chromatography mass-spectroscopy (GC-MS). All samples were also assayed for total solids, volatile solids, ash, total Kjeldahl nitrogen (TKN), total phosphorus, potassium, and chemical oxygen demand. An estrogen to macronutrient (N, P, and K)

mass ratio was determined for all waste samples. This was done because the raw waste value does represent the true estrogen pollution potential.

Several tentative conclusions have been drawn from the data presented here, as follow: (1) Using ELISA to assay 17β -estradiol (E2), there were statistically significant differences ($\alpha=0.05$) between all the farm types sampled (dairy, swine farrowing, and swine finishing) for E2/N, E2/P, and E2/K ratios (where N, P, and K represent nitrogen, phosphorus, and potassium respectively). Swine farrowing operations showed the highest estrogen to macronutrient mass ratios, averaging 19.2 ppt E2/ppm N (1.1 sd), 65.6 ppt E2/ppm P (4.7 sd), and 24.3 ppt E2/ppm K (1.7 sd). Average swine finishing and dairy ratios were approximately 2 and 5 times lower, respectively. The same trend was seen in GC-MS 17β -estradiol, estrone, and total estrogen ratios (the sum of 17β -estradiol, 17α -estradiol, and estrone concentrations). In contrast, dairy operations had a significantly ($\alpha=0.05$) higher estrogen to macronutrients mass ratio for GC-MS 17α -estradiol as compared to swine farrowing, averaging 16.5 ppt 17α /ppm N (5.7 sd), 62.6 ppt 17α /ppm P (6.9 sd), and 30.9 ppt 17α /ppm K (4.1 sd).

(2) Dairy waste structures (holding ponds, dry stack-solid, and semisolid) did not have statistically significantly different ($\alpha=0.05$) for ELISA 17β -estradiol and GC-MS 17β -estradiol and estrone ratios. However, dairy dry stack-semisolid waste had significantly ($\alpha=0.05$) higher estrogen to macronutrients mass ratios for GC-MS 17α -estradiol than holding ponds and dry stack-solid structures. Dairy dry stack-semisolid showed the highest ratios for 17α -estradiol to P, which were 85.2 ppt/ppm (8.5 sd). Dairy dry stack-semisolid also had statistically significantly higher total estrogen to

macronutrient ratios ($\alpha=0.05$) than did the other structures. The highest values were for P, averaging 129.2 ppt total estrogen/ppm (27.1 sd).

(3) Swine finishing lagoon structures had statistically significantly higher ($\alpha=0.05$) estrone/N and estrone/P ratios than did swine finishing hoop structures. For example, swine finishing lagoons had P ratios of 202 ppt E1/ppm P (40.8 sd) as compared to 13.2 ppt E1/ppm P (47 sd) observed in swine finishing hoop structures. In contrast, ELISA 17β -estradiol ratios were not statistically significantly different for any of the swine finishing structures. GC-MS 17β -estradiol and 17α -estradiol concentrations were below the detection limits for the structures.

These results suggest that it is probable that wastes from swine farrowing facilities pose the greatest threat as environmental estrogen pollutants. Specifically, swine farrowing wastes had statistically significantly higher ($\alpha=0.05$) ratios of 17β -estradiol and estrone to the macronutrients N and P than did swine finishing and dairy waste. (Although dairy wastes had the highest ratios of 17α -estradiol to macronutrients, 17α -estradiol is not as potent an estrogen as 17β -estradiol and estrone.) Ultimately, the actual threat of estrogenic pollution posed by these wastes must be determined by additional runoff studies, supplemented by watershed-scale studies. This thesis helps identify the wastes most likely to cause problems, and therefore most worthy of additional study.

Phosphorus-based application of the manures studied would result in lower mass application rates of estrogen to fields (ca. 2 – 3x). In this sense, the increasing trend toward P-based application will help mitigate the likelihood of estrogen pollution from the land application of animal manures.

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Chapter 1

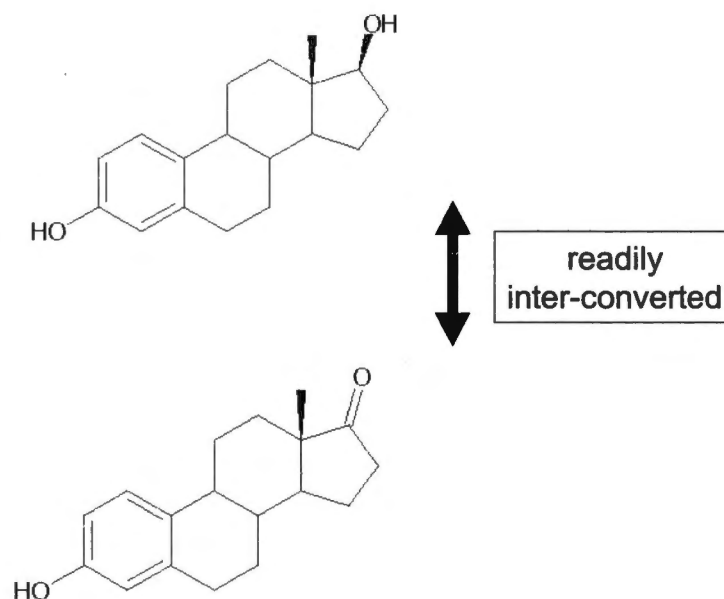
INTRODUCTION

Background and Justification

Estrogens are a broad group of steroidal hormones present in fish, birds and mammals. 17β -estradiol (E2) is the most potent, naturally occurring estrogenic compound (Nichols et al., 1998) and estrone (E1) is the ketone form of 17β -estradiol (Arcand-Hoy et al., 1998) (Figure 1). 17β -estradiol and estrone are emitted into the environment through municipal wastewater, (Routledge et al., 1998) and animal manure (Monk et al., 1975; Shore et al., 1993). These estrogens can be transported in runoff from land receiving manure application (Bushee et al., 1998; Nichols et al., 1997; Dyer, 2001). The emission of estrogens into the environment could cause wildlife and public health concerns, such as male reproductive tract disorders and female breast cancer (Nichols et al., 1997; Davis et al., 1995).

The presence of estrogens in the environment has been a cause for concern for many years (Nichols et al., 1997). One concern is the presence of estrogens in the effluent of sewage treatment works. Belfroid et al. (1999) found that women excrete 2-12 μg of 17β -estradiol and estrone per person each day. When these estrogens are added to synthetic hormones (e.g. contraceptives), the amount of estrogens present in wastewater treatment facilities can be enough to result in the sex reversal of some male fish (Purdom et al., 1994; Sumpter et al., 1995). In fish, an indication of feminization is an increase of vitellogenin, a blood protein required for fish to develop ovaries (Sumpter et al., 1995). It

17 β -estradiol (E2)



Estrone (E1)

Figure 1. Structure of 17 β -estradiol and estrone compounds.

is produced naturally by the liver in females but can also be produced by females and males when exposed to estrogens (Purdom et al., 1994). Purdom et al. (1994) placed male rainbow trout in the effluent of sewage treatment works and found a pronounced increase in their vitellogenin concentrations. Male fish begin to produce vitellogenin when exposed to water containing estrogens with concentrations between 10 and 100 ppt (Routledge et al., 1998).

Nakamura (1984) examined the effects of masu salmon (*Oncorhynchus masou*) immersed in water containing 17 β -estradiol. The salmon were placed in the water at various concentrations (250-200,000 ppt) for 18 days starting 5 days after hatching. It

was shown that 500-5,000 ppt water concentrations were sufficient in inducing feminization in masu salmon and levels of 10,000 to 200,000 ppt resulted in death shortly after treatment (Nakamura, 1984). The study suggests that the reduction in estrogens in waterways may be beneficial in protecting wildlife populations (Nichols et al., 1997).

High levels of estrogens introduced to mammals can cause problems. Premature udder development and lactation has been observed in heifers fed poultry litter (Nichols et al., 1997). Animals excrete large quantities of estrogens into the environment (Shore et al., 1993). It has been shown that sows excrete an elevated amount of hormones early in pregnancy (Vos, 1996). Milk from cows also contains estrogen at approximately 1,000 ppt but there are no known effects on humans (Shore et al., 1993). Total estrogen emission rates in manure have been estimated at 1000 ppt per cow per day (Raman et al., 2001). With over 9×10^6 adult dairy cows in the U.S. the amount of estrogen excreted from cows could exceed 9 kg d^{-1} (Raman et al., 2001). However, these numbers are estimates based on scattered literature data, and do not account for post excretion losses of estrogen in waste treatment and storage facilities. By sampling waste storage structures directly, this thesis directly explores the concentrations of naturally excreted estrogens in dairy and swine farming operations. Such information is not readily available in the literature.

Previous work (Dyer, 2001) demonstrated the presence of potentially biologically relevant concentrations of 17β -estradiol in runoff from plots receiving dairy manure at rates sufficient to meet crop nitrogen requirements. Furthermore, Dyer's work suggested that mass application rates of 17β -estradiol and estrone correlated with mass losses of these compounds in runoff, at least during the first runoff event after application.

Specifically, Dyer observed 17β -estradiol in runoff when 17β -estradiol application rates exceeded 5.5 g ha^{-1} .

While scattered reports of 17β -estradiol and estrone concentrations in animal wastes exist in the literature (Nichols et al., 1998; Nichols et al., 1997; Knight 1980; Monk et al., 1975), there is no comprehensive survey of these compounds in concentrated animal wastes in storage and treatment structures typical of full-scale animal production systems. Such data is needed to estimate the estrogen-pollution risk associated with various waste types, and to mitigate those risks if needed. This thesis addresses these issues in two ways. First, by determining estrogen to macronutrient concentrations in dairy and swine manure and the relative risk of these two types of wastes can be estimated. The macronutrients N, P, and K will be used in this analysis. N and P are typically used in determining land application rates. Second, by assessing estrogen emission from wet and dry manure, the impact of waste phase (wet or dry) on estrogens can be determined. The work addresses two hypotheses, as follow.

Macronutrient-Related Hypotheses

Based on the idea that areal mass application rates of estrogen are the best indicators of the potential for estrogen pollution from a waste. These hypotheses are based on the mass ratio of estrogen to application-rate-limiting macronutrients (N, P, and K), rather than simply on the raw estrogen concentration in the waste. This ratio will be referred to as the estrogenicity of the waste. The following null hypotheses were tested:

Hypothesis 1: There is no difference in the estrogenicity of wastes from dairy, swine farrowing, and swine finishing operations.

Hypothesis 2: There is no difference in the estrogenicity of wastes due to manure handling and storage methods.

If hypotheses one and two are accepted then one may conclude that estrogen emission potential is independent of animal species and waste storage methods. To determine the validity of these hypotheses, dairy and swine waste was collected from various storage structures, and assayed for estrogen and macronutrient concentrations.

Chapter 2

MATERIALS AND METHODS

Overview

The estrogen concentrations in eight dairy and eleven swine facilities were tested for estrogen concentrations during the winter and summer months over a one-year period. All facilities were in the state of Tennessee, or in adjacent states within fifty miles of Tennessee. Three wet dairy systems (holding ponds) and five dry stacks with varying moisture content were sampled. Five wet swine farrowing systems (four pits and one lagoon) three wet swine finishing systems (all lagoons) and three dry swine finishing systems (hoop structure) were sampled (Table 1). These structures represent all major types of dairy and swine facilities employed in the southeast, except for hoop structures which are not a predominant system of any region in the U.S. (Burns, 2002). The 17 β -estradiol concentrations in all samples were assayed in a triplicate serial dilution by means of an enzyme linked immunosorbent assay (ELISA) (Assay Designs, Inc., Ann Arbor, MI). Concentrations of 17 β -estradiol, 17 α -estradiol, and estrone were assayed by gas-chromatography mass-spectroscopy (GC-MS). All samples were also assayed for total solids, volatile solids, ash, total Kjeldahl nitrogen (TKN), total phosphorus, potassium, and chemical oxygen demand.

Location Information

Prior to sampling, the facility owner/manager was contacted to determine if their farm could be used for sampling. An on-site data collection sheet was completed for each farm (Figure 2). The collection sheet was used to record pertinent information during

Table 1. Facility identification. Each farm was given an identification code to provide a description of the facility. The first letters distinguish between dairy (D), swine farrowing (SFR), or swine finishing (SFI) operations. The type of storage facility is shown by a one or two letter abbreviation as follows, pit (P), lagoon (L), hoop structure (H), dry stack-solid (DS), and dry stack-semisolid (WS). The index number, following the structure abbreviation, distinguishes between otherwise identical structures.

ID	Swine			Dairy	
	Finishing		Farrowing		
	Dry	Wet	Wet	Dry	Wet
SFR-P1			pit		
SFR-P2			pit		
SFR-P3			pit		
SFR-P4			pit		
SFR-L1			lagoon		
SFI-L1		lagoon			
SFI-L2		lagoon			
SFI-L3		lagoon			
SFI-H1	hoop structure				
SFI-H2	hoop structure				
SFI-H3	hoop structure				
D-HP1					holding pond
D-HP2					holding pond
D-HP3					holding pond
D-DS1				dry stack	
D-DS2				dry stack	
D-WS1				wet-dry stack	
D-WS2				wet-dry stack	
D-WS3				wet-dry stack	

On-Site Data Collection Form			
Name of Farm	ID#	Proprietor	
Location		Extension Agent	
Date		Phone Number	
Dairy ____ Swine ____ Farrowing ____ Finishing ____ Dry ____ Wet ____ Pit ____ Lagoon ____ H.Pond ____ Dry Stack ____ Wet Stack ____ Hoop ____			
Number of Animals:			
Average Weight of Animals:			
Breed of Animal:		Bedding Type:	
Last Pump Out (lagoon, h.pond, pit):			
Confinement Period of Animals (time):			
Storage Intervals of Manure (dry, wet stacks, hoop):			
Dimensions of Storage Facilities:			
Estimated Age of Manure: New:		Old:	
Area (ft ²) collects rainwater and drains in area:			
Volume of fresh water added to system daily (gal):			
Sample Name	Depth of Sample	Temperature	Sample Weight
Time: Original pH of Sample: pH After Acidification: Amount of Acid Added: Strength of Acid: Diagram (including sample locations): Comments:			

Figure 2. On-site identification form.

sampling, including sample location and temperature. A diagram was included in the notes to indicate the location and a depth of samples (Appendix). The operator was asked during the second sampling if any process changes had occurred since the initial sampling, and any such changes were noted.

Storage Facility Description

Lagoons and holding ponds

Samples were collected from non-agitated holding ponds and lagoons. {PRIVATE "TYPE=PICT;ALT="}A lagoon is a liquid-based manure management system, characterized by waste residing in water to a depth of at least six feet for a period ranging between 180-365 days. They are designed for anaerobic treatment of waste and hold sludge for 2-25 years. In contrast, holding ponds are designed to hold manure and dilution water for 180-365 days but are not explicitly designed to promote waste biodegradation. If the farm used a two-stage storage system, then samples were collected from the primary cell only. In each liquid storage system, a total of eight samples were collected, as follows: (1) four 1-L samples were collected along a depth profile in the center of the liquid storage area, using a RPV (remotely piloted vehicle) designed and built for this project (Figure 3). The top and bottom samples were taken 0.3 m (1 ft) from the surface and from the bottom; the others were taken at one-third and two-thirds the total depth. (2) Four additional samples were collected from surface locations: one sample from each corner of the area, if accessible. When the influent pipe was readily accessible, one sample was collected from the area immediately adjacent to the pipe, instead of from the closest corner to the pipe. The

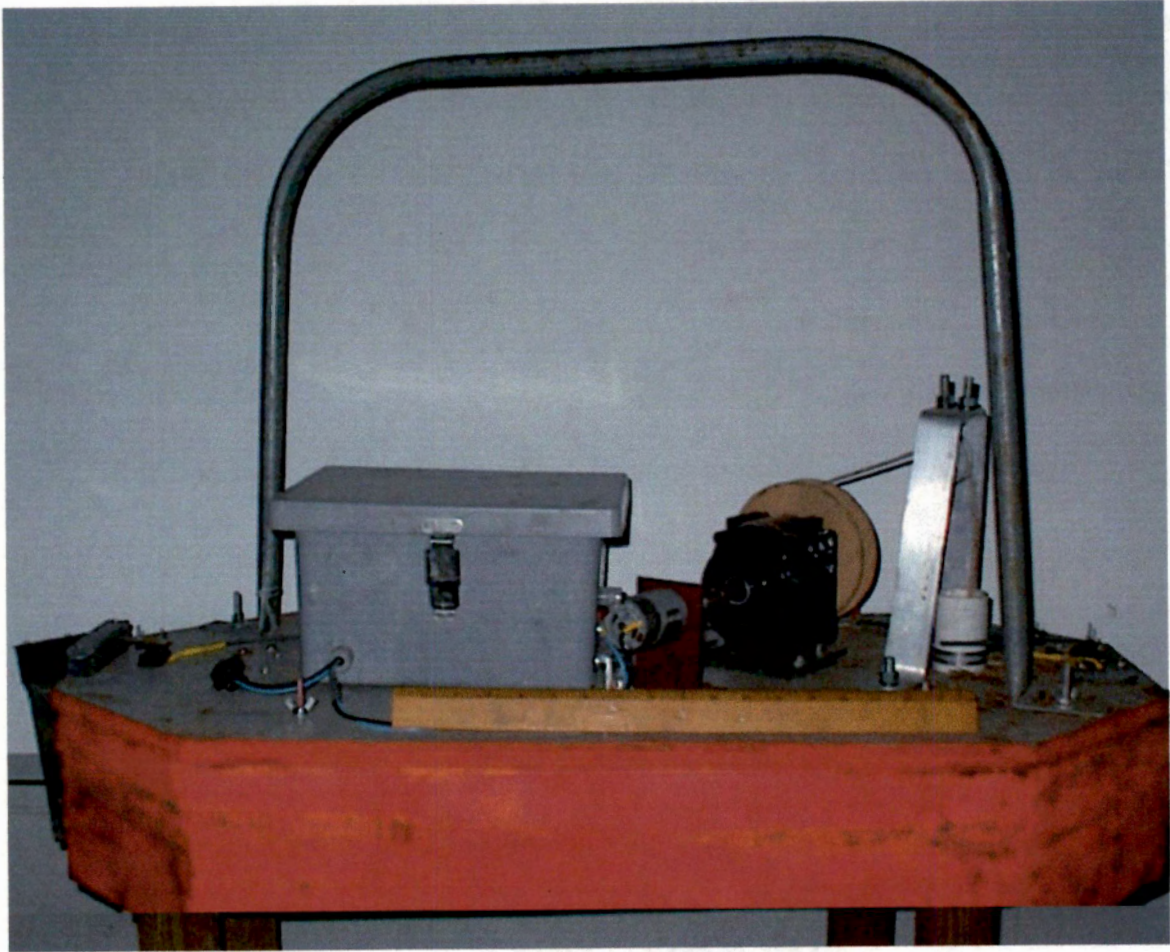


Figure 3. Remotely Piloted Vehicle (RPV).

surface samples were collected with a 500-mL polyethylene dipper (14-242-5, Fisherbrand, USA).

Pits under slatted floor

Samples were collected from non-agitated pits under slatted floors in swine farrowing facilities. When possible, samples were collected close to the pit outlet. Four 1-L samples were collected along a depth profile in the pit, using a remotely triggered water sampler (JT-1, LaMotte Co., Chestertown, MD) (Figure 4). The top and bottom samples were taken 0.3 m (1 ft) from the surface and from the bottom; the others were taken at one-third and two-thirds the total depth.

Hoop structures

For each swine structure, two representative locations were identified in the waste area. Representative locations were visually determined to be typical of the overall structure. At each of these locations, 500-g samples were collected from the top, middle, and bottom of the material, by means of a shovel and portable scale. The two samples from each depth were combined to form a composite sample for that depth.

Dry stack-solid

Dairy dry stack-solid structures were divided into new and old regions, and the age of the manure in the new and old regions was estimated by the facility owner/operator. Three representative sample locations were visually identified within the old and the new areas that were typical of the overall structure. At each of these three locations, a 500-g sample from the top, middle, and bottom of the stack was taken using a shovel (sometimes assisted by a front-end loader); then the three samples were combined to form a composite sample for that depth.

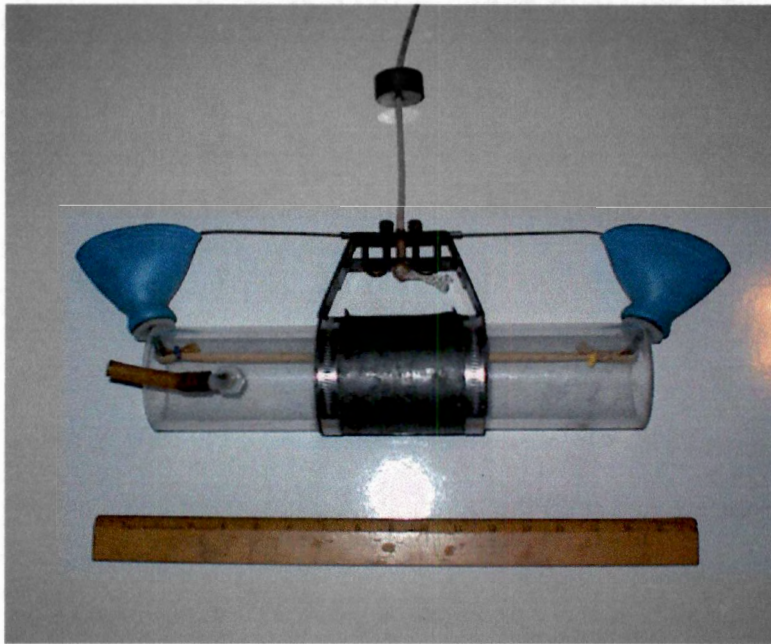


Figure 4. LaMotte Water Sampler. Ruler indicates length of approximately 18".

Sampling locations were determined as described for dry stack-solid systems above. Samples were collected with a 1-L container attached to the end of a 3-m (9-ft) pole (Figure 5). This device was designed and built by Mr. Adam Daugherty and Dr. Raj Raman. An upward-opening door allowed semisolid materials to push up through the sampler as the sampler moved down through the stack. As the sampler was raised, the door shut, thereby collecting a sample from the desired depth. If the sampler was unable to reach the bottom of the area then the deepest depth possible was collected and the depth was noted.

General Sample Handling Procedure

Measuring estrogen concentrations can be difficult because of degradation. Therefore, specific collection and storage of samples must be followed to preserve the estrogen concentrations for measurement procedures. Raman et al. (2001) showed that acidifying the samples to a pH of 2 and storage at 5°C reduced 17 β -estradiol losses 15% and total estrogen losses 17%. In contrast, unacidified samples lost 90% of 17 β -estradiol and 40% of total estrogens (Raman et al., 2001). On-site acidification of samples was conducted to maintain sample integrity.

Immediately after each sample was collected, its temperature was measured by using a temperature gauge (30/25 FT, YSI Inc., Yellow Springs, OH) and recorded. The pH was determined by using colorimetric pH paper (09-876-17, Fisherbrand, USA). Next, approximately 25 mL of 4N H₂SO₄ was added to the sample, to acidify the sample to a pH of approximately 2. The pH was checked again, and more acid was added if



Figure 5. Sampler used for dry stack-semisolid.

necessary. Samples were placed in a cooler filled with ice and transported back to the lab, which took anywhere from 1 to 36 h. Liquid samples were placed in 500-mL plastic bottles for acidification, transport and storage. Hoop structure, dry stack-solid, and Dry stack-semisolid samples were placed in plastic bags for acidification and transport. They were then homogenized in the laboratory with a blender (ProBlend BL-675, Black & Decker, USA) and stored in 500-mL plastic bottles.

Extraction Procedure

Samples were extracted in triplicate. In the case of liquid samples, a 10-mL sub-sample was placed in a 40-mL glass vial, along with 10 mL of deionized water, approximately 100 μ L of 6N NaOH (NaOH to neutralize the samples), and 10 mL of ethyl ether. For dry samples, a 5-g sub-sample was placed in 40-mL glass vial, along with 5 mL of deionized water, 3 drops of 6N NaOH, and 5 mL of ethyl ether. For each structure, water blank, spike blank, and three matrix spikes were assayed on an ELISA plate. Water blanks consisted of deionized water and ethyl ether. Spike blanks consisted of deionized water, ethyl ether, and concentrations of 17 β -estradiol (100,000 ppt) and estrone (500,000 ppt for winter samples; 250,000 ppt for summer samples). Matrix spikes consisted of deionized water, ethyl ether; of 17 β -estradiol (100,000 ppt) and estrone (500,000 ppt for winter samples; 250,000 ppt for summer samples), combined with a sub-sample from the structure sampled. Wet and dry samples were shaken for 2 h on a vertical shaker and centrifuged for approximately 10 min at 2300 g to separate the ether layer. One mL of ether was extracted from each triplicate sample and blown down with N₂ gas. Original samples were refrigerated at 4°C and pellets were placed in a freezer to prevent degradation. Both dry and wet samples were tested for 17 β -estradiol by using an

enzyme linked immunoassay (ELISA) kit (Assay Designs, Inc., Ann Arbor, MI), per the manufacturer instructions.

Assay Procedure

Pellets were warmed to room temperature, re-suspended with 100 μ L of reagent alcohol, and vortexed, for approximately 30 seconds. An additional 500 μ L of assay buffer was added to the solution, then the mixture was vortexed again. A four-step serial dilution was performed for each triplicate sample (1:1, 1:3, 1:9, and 1:27) and for spike blanks and matrix spikes (1:8, 1:16, 1:32, and 1:64) to ensure that the samples would be in the range of the assay plate.

ELISA Analysis

Data analysis was performed by using Microsoft Excel (Microsoft, 1997). Standards were run to fit a four-point parameter calibration curve,

$$y = \frac{y_0 + a}{1 + e^{\frac{-(x-x_0)}{b}}}$$

and r^2 values were recorded. Data that fell outside the calibration curve were not used. An average was calculated for the triplicate sample dilutions, as well as, the standard deviation and coefficient of variation. The binding values of the samples along with the sample mass, ether volume, and extraction buffer volume were used to calculate the amount of 17 β -estradiol in the original sample.

Data Analyses

Estrogen and nutrient concentrations for each sample were recorded on a master spreadsheet. The spreadsheet was organized by facility type and further sub-divided by farm. The samples were identified by using the identification code. The spreadsheet included winter and summer collection data.

Chemical Oxygen Demand, Total Solids, Volatile Solids, and Ash

Samples were homogenized by shaking (liquid samples) or by using a blender (ProBlend BL-675, Black & Decker, USA) (semi-solid and solid samples). Sub-samples were taken for analysis by using a Finnpiquette, (4501-110, Fisherbrand, USA) for liquid samples, or by using a scoopula, (14-357, Fisherbrand, USA) for solid samples. All samples were measured for chemical oxygen demand (COD) by using the Reactor Digestion Method (0-1,500 range) (method #8000, HACH Company, Loveland, CO.) Total solids, volatile solids, and ash were measured in accordance with Standard Methods (American Public Health Association et al., 1998).

Total Kjeldahl Nitrogen (TKN), Total Phosphorous (TP), and Potassium

TKN, TP, and potassium analysis were conducted at the University of Tennessee Water Quality Laboratory, with the assistance of Galina Melnichenko.

Total Kjeldahl nitrogen (TKN) and total phosphorous (TP) were performed in accordance to LACHET Quick Chem Methods 13-107-06-2-D and 13-115-01-1-B (LACHET Instruments, Milwaukee, WI). Samples were digested with sulfuric acid in a block digester (BUCHI K-435). For N, the digested sample is read with a LACHET Colorimeter where the sample is heated with salicylate and hypochlorite to produce a blue color. The color is read with the LACHET Colorimeter (LACHET Instruments,

Milwaukee, WI). Phosphorous is handled similarly; the digested samples are heated with ammonium molybdate to produce a blue color.

Potassium was determined with the Perkin-Elmer standard flame emission conditions and using an Atomic Absorption-5000 (Perkin-Elmer Instruments, Norwalk, CT). Samples were extracted with ammonium acetate acid and measured at a wavelength of 766.5nm.

GC-MS Analysis

All samples were measured for 17 β -estradiol, estrone, and 17 α -estradiol by gas-chromatography mass-spectroscopy (GC-MS) at the Center of Biotechnology at the University of Tennessee by Jim Easter. Derivatized samples were analyzed on a HP 6890 Series gas chromatograph with a DB-5MS capillary column (J&W Scientific; 30 m X 0.25 mm i.d.; 0.25 μ L film thickness) using a HP 5973 mass selective detector. Samples were analyzed twice. The first analysis was for compound identification and the second analysis was used to quantify 17 β -estradiol and estrone. The primary ions selected for quantification were m/z 416 for 17 β -estradiol and m/z 342 for estrone. Standard curves of at least five points, ranging from 1-1000 ppb, were used for calculation of estrogen concentrations. Estrogen standards were from Sigma (St.Louis, MO). Raman et al. (2001) has further details of the GC-MS analysis.

Statistical Analysis

Statistical analysis were performed with SAS version 8.12 (1999, SAS Institute, Cary, NC). A statistical significance of $\alpha=0.05$ was used. Least square means for multiple comparisons were performed. Multiple comparisons were done using Tukey-Kramer

comparisons. Estrogen concentrations that were below the detection limit were not included in the analysis.

Chapter 3

RESULTS AND DISCUSSION

Spike Matrix Results

A spike matrix test was conducted for each farm during both sample collections. A known concentration of 17 β -estradiol and estrone were added to one sample per farm. ELISA 17 β -estradiol samples were spiked with 100,000 ppt and the GC-MS 17 β -estradiol and estrone samples were spiked with 500,000 ppt for the winter sampling cycle and 250,000 ppt for the summer sampling. These concentrations were chosen to best fit the range for the GC-MS column. Three farms did not have matrix spikes run for at least one of the estrogen tests, yielding a total of 35 ELISA 17 β -estradiol spike matrix results and 37 GC-MS estrone matrix spike results.

To assess recovery efficiency, the matrix spike concentration from each farm was subtracted from the sample concentration used in the same matrix spike. The difference was divided by the water spike concentration from the same farm to determine the recovery ratio. In samples without interference, the spike recovery ratio should be 1. Average recovery ratios for 17 β -estradiol by ELISA, 17 β -estradiol by GC-MS, and estrone (by GC-MS) are presented for both summer and winter sampling events, in Figure 6. The results indicate that the 17 β -estradiol ELISA method was unbiased for the winter data set. However, the summer data set may be overestimated by a factor of 1.5. The GC-MS results appeared generally less biased. The GC-MS E2 spike recovery ratios are very similar to the ELISA ratios. Therefore, the higher E2 values may represent a slight interference.

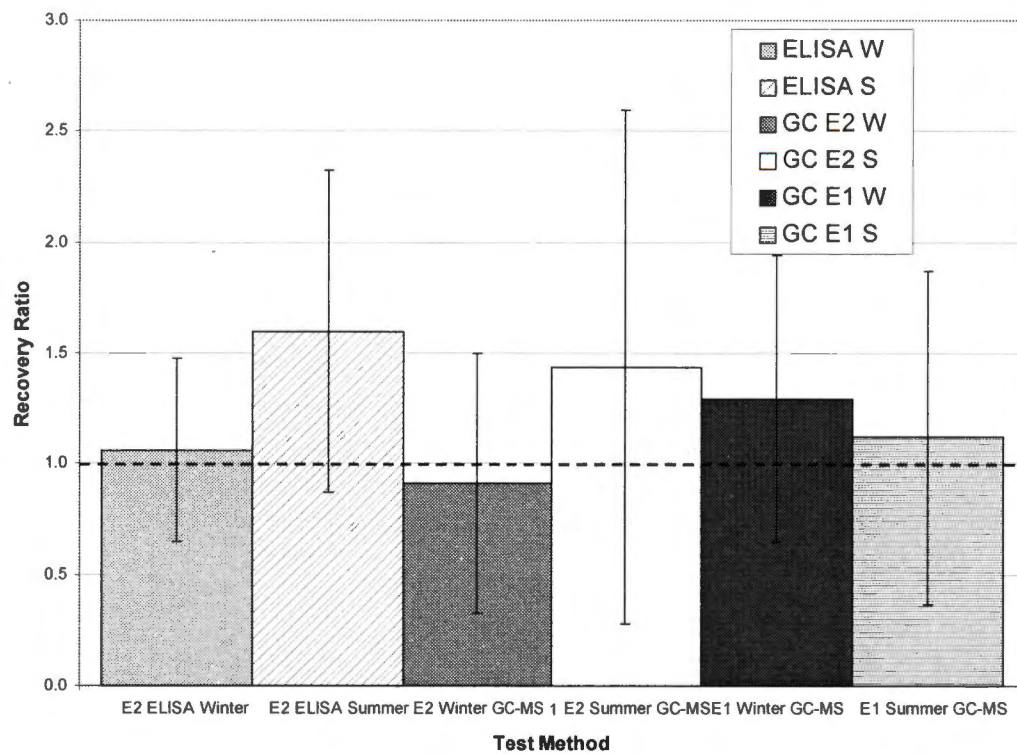


Figure 6. Recovery ratios based on spike matrix and spike blank data for each experiment. Horizontal line indicates a 1:1 ratio. Error bars reflect standard deviations.

Figure 7 demonstrates the spike recovery ratios by farm types. There is an overall lack of pattern, therefore Figure 7 is critical, as it shows that the subsequent data does not simply reflect assay biases.

Two problems were encountered when running the spike tests. First, the solvent appeared to volatilize over time leading to higher concentrations of the spike compounds. To address this problem, fresh solvent was used to reduce volatilization. Furthermore, by computing the recovery ratio by the water spike, this problem was corrected. By dividing by the water spike, you are effectively correcting for this error. Second, the ether carrier solvent appeared to absorb into the dry samples within one to two days. This made it more difficult to remove the ether for analysis. This problem occurred most often during the GC-MS tests, because more time elapsed prior to the tests.

Statistical Analysis

Samples were collected during the winter and summer months of a one year period. Nineteen waste storage structures were sampled in and around Tennessee. A total of 222 samples were collected and analyzed (Tables 2 and 3). It should be noted that all estrogen pollution potential values were determined by and estrogen to macronutrient mass ratios and not on raw waste values. For each sample, ratios of E2 (ELISA and GC-MS), estrone, and 17 α -estradiol to macronutrients (N, P, and K) were computed. These ratios were statistically analyzed by animal and structure type.

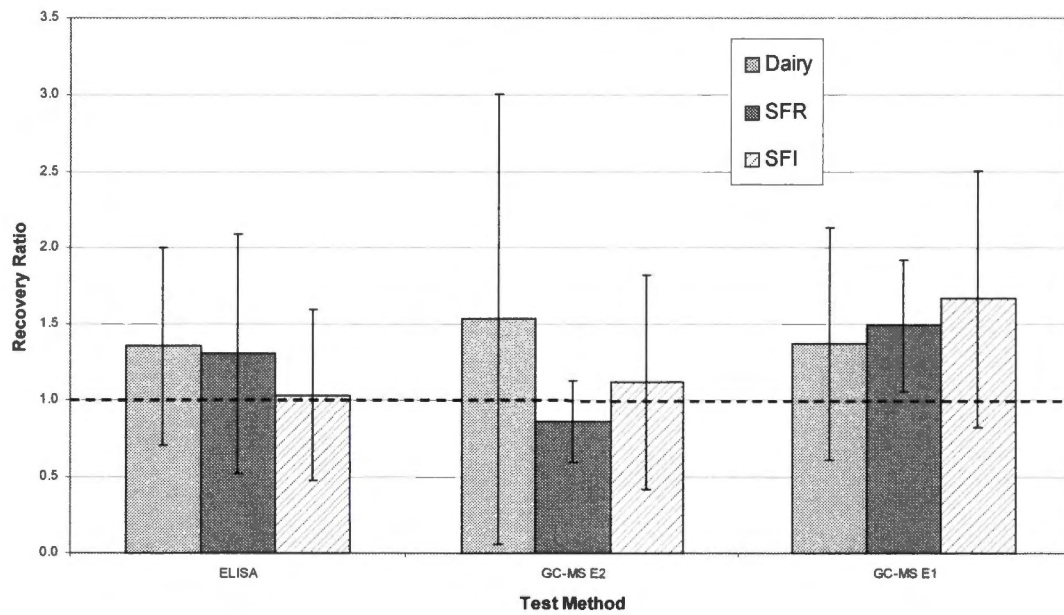


Figure 7. Recovery ratio by farm operation type. The horizontal line indicates 1:1 ratio. Error bars reflect the standard deviations. Key: SFR = swine farrowing; SFI = swine finishing (SFI).

Table 2. The average analyzed values for all tests conducted for each structure type.

Includes winter, summer, and an overall average of the two sampling cycles.

Structure Type	ELISA 17 β -estradiol (ppt)						GC-MS 17 β -estradiol (ppt)					
	Winter	Winter-Std	Summer	Summer-Std	Overall Average	Overall-Std	Winter	Winter-Std	Summer	Summer-Std	Overall Average	Overall-Std
D-HP	1506	97	1489	86	1497	91	3560	833	2300	17	3350	425
D-DS	8249	391	13479	943	10864	9139	27667	3578	17675	675	23670	13934
D-WS	12539	1013	29062	792	20801	15791	25341	5267	53722	9606	39937	36547
SFI-H	40516	2782	66952	1740	53734	2261	35467	5867	BDL	BDL	35467	5867
SFI-L	2337	163	3027	98	2682	130	BDL	BDL	BDL	BDL	BDL	BDL
SFR-P	23396	1012	21641	450	22519	731	18067	2193	13143	5183	15794	3573
SFR-L	3528	147	4356	171	3942	159	BDL	BDL	BDL	BDL	BDL	BDL

Structure Type	GC-MS Estrone (ppt)						GC-MS 17 α -estradiol (ppt)					
	Winter	Winter-Std	Summer	Summer-Std	Overall Average	Overall-Std	Winter	Winter-Std	Summer	Summer-Std	Overall Average	Overall-Std
D-HP	8938	4075	6618	3467	7595	3771	3320	892	6350	800	5185	831
D-DS	51450	6117	30578	13160	42505	36799	88517	4900	17975	2142	60300	43293
D-WS	36300	5583	72706	13944	58897	57227	75529	11161	122278	18878	101825	60566
SFI-H	72633	10778	11367	12500	48127	11467	BDL	BDL	BDL	BDL	BDL	BDL
SFI-L	14888	3313	15132	4009	15067	3823	BDL	BDL	BDL	BDL	BDL	BDL
SFR-P	71115	3513	51814	7581	61465	5547	13933	1333	9804	1738	11574	1564
SFR-L	10357	1343	BDL	BDL	10357	1343	BDL	BDL	BDL	BDL	BDL	BDL

Structure Type	• Total Solids (%)						Volatile Solids (%)					
	Winter	Winter-Std	Summer	Summer-Std	Overall Average	Overall-Std	Winter	Winter-Std	Summer	Summer-Std	Overall Average	Overall-Std
D-HP	1.5	0.7	1.3	0.6	1.4	0.6	65.1	13.7	62.9	9.4	64.0	11.7
D-DS	16.8	6.8	25.0	5.1	20.9	7.2	63.8	15.7	66.2	20.5	65.0	17.9
D-WS	15.6	1.9	18.2	4.0	16.9	3.4	65.2	8.6	65.3	10.7	65.2	9.6
SFI-H	16.1	2.7	28.3	9.6	22.2	9.3	76.9	8.3	63.7	11.0	70.3	11.6
SFI-L	1.1	0.2	1.0	0.7	1.0	0.5	78.6	9.0	72.2	12.0	75.4	11.0
SFR-P	1.2	0.3	1.4	0.7	1.3	0.5	71.1	20.6	64.6	8.7	67.9	15.9
SFR-L	0.7	0.2	0.9	0.2	0.8	0.2	80.4	5.6	76.3	11.2	8.8	8.8

Table 3. The average analyzed values for all tests conducted for each structure type.

Includes winter, summer, and an overall average of the two sampling cycles.

Structure Type	Ash (%)						COD (ppm)					
	Winter	Winter-Std	Summer	Summer-Std	Overall Average	Overall-Std	Winter	Winter-Std	Summer	Summer-Std	Overall Average	Overall-Std
D-HP	35.0	13.6	37.1	9.4	36.1	11.7	8430	4996	5101	3738	6766	4678
D-DS	36.2	15.7	33.8	20.5	35.0	17.9	67381	34710	47767	30918	57574	33671
D-WS	34.8	8.6	34.7	10.7	34.8	9.6	75879	29905	65789	13320	70834	23382
SFI-H	23.1	8.3	36.3	11.0	29.7	11.6	72754	25197	57178	45335	64966	36472
SFI-L	21.6	9.0	27.8	12.0	24.6	11.0	1403	743	3156	4420	2280	3258
SFR-P	29.0	20.6	35.4	8.7	32.2	15.9	9434	3962	9333	4878	9383	4381
SFR-L	19.6	5.6	23.7	11.2	21.7	8.8	1574	2150	1135	1252	1354	1714

Structure Type	Nitrogen (ppm)						Phosphorous (ppm)					
	Winter	Winter-Std	Summer	Summer-Std	Overall Average	Overall-Std	Winter	Winter-Std	Summer	Summer-Std	Overall Average	Overall-Std
D-HP	652	266	616	373	634	321	122	51	111	47	117	49
D-DS	4174	2210	5447	2257	4810	2279	1479	1271	1522	490	1501	942
D-WS	6343	3757	4853	1103	5598	2832	1362	236	1349	339	1355	288
SFI-H	6409	1395	9471	4192	7940	3416	2270	536	3836	1881	3053	1565
SFI-L	397	111	613	428	505	328	106	63	246	400	176	292
SFR-P	1580	508	1275	695	1428	618	338	156	429	433	383	324
SFR-L	126	45	206	112	166	92	80	45	97	71	88	58

Structure Type	Potassium (ppm)						Temperature (°C)					
	Winter	Winter-Std	Summer	Summer-Std	Overall Average	Overall-Std	Winter	Winter-Std	Summer	Summer-Std	Overall Average	Overall-Std
D-HP	567	117	801	350	684	284	8.9	4.6	23.3	4.5	16.1	8.5
D-DS	4642	3673	4748	2727	4695	3164	17.6	11.1	33.2	7.4	25.4	12.2
D-WS	977	731	3757	1637	2367	1884	10.2	6.5	26.9	2.4	18.6	9.7
SFI-H	2644	393	5519	2158	4082	2110	18.7	2.2	33.0	2.4	25.9	7.6
SFI-L	410	168	460	125	435	148	12.1	1.7	24.4	3.0	18.2	6.7
SFR-P	911	232	760	139	835	203	17.2	2.4	21.7	3.8	19.4	3.9
SFR-L	180	43	503	617	341	454	10.4	0.3	19.7	0.5	15.1	4.8

Macronutrient-Related Hypotheses

Hypothesis 1: There is no difference in estrogenicity of wastes from dairy, swine farrowing, and swine finishing operations.

ELISA 17 β -estradiol

To test this hypothesis, all 222 samples collected during the experiment were grouped by farm type and analyzed using ELISA 17 β -estradiol data. Specifically, all dairy, swine farrowing, and swine finishing samples were used, regardless of moisture content. A bar graph representing the ELISA 17 β -estradiol to macronutrient mass ratios, by farm types, is shown in Figure 8.

The concentrations of ELISA 17 β -estradiol to N and P mass ratios were statistically significantly different ($\alpha=0.05$) between all farm types. Swine farrowing showed the highest ELISA 17 β -estradiol to N ratios: 19.2 ppt E2/ppm N (1.1 sd). This ratio was over 2 times greater than swine finishing and 5 times greater than dairy samples. Swine farrowing also had the highest ELISA 17 β -estradiol to P ratio: 65.5 ppt E2/ppm P (4.7 sd). This ratio was again twice as high as swine finishing and 5 times as high as dairy operations. Thus for N and P, Hypothesis 1 was rejected. Instead, it concluded that swine farrowing waste has significantly higher estrogen to macronutrient mass ratios than dairy and swine finishing.

Swine farrowing mass ratios of ELISA 17 β -estradiol to K were significantly higher ($\alpha=0.05$) than that of swine finishing and dairy facilities. However, there was no significant difference between swine finishing and dairy operations. Thus, for K,

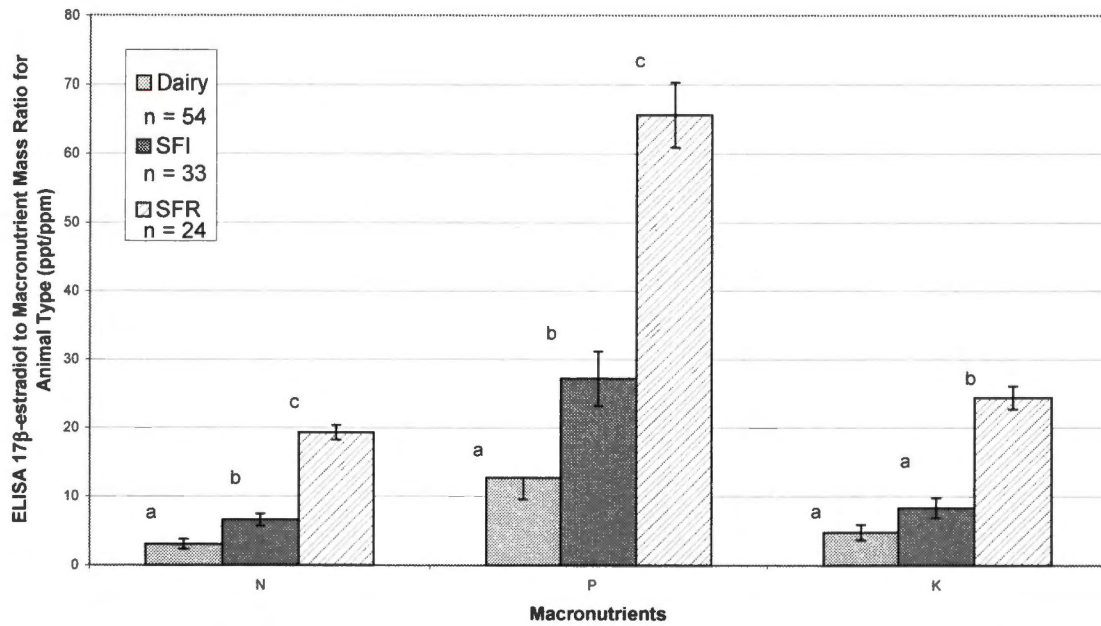


Figure 8: Mass ratio of ELISA 17β-estradiol to nitrogen (N), phosphorous (P), and potassium (K) for the three farm types sampled. Key: SFR = swine farrowing; SFI = swine finishing. Error bars reflect standard deviations. Letters (a, b, and c) indicate significant differences within each macronutrient at $\alpha=0.05$.

Hypothesis 1 was rejected; and it was concluded that swine farrowing waste has higher estrogen to macronutrient mass ratios than dairy and swine finishing wastes.

It is possible that swine farrowing operations have the highest estrogen to macronutrients mass ratios because the samples are from structures that house pregnant sows. The sows release high amounts of estrogens during pregnancy (Vos, 1996).

GC-MS 17 β -estradiol

Hypothesis 1 was also tested using GC-MS data including, 17 β -estradiol, estrone, 17 α -estradiol, and the sum of the three, termed total estrogen. For GC-MS 17 β -estradiol, only dairy and swine farrowing farm types were analyzed because the swine finishing samples were below detection limits. The ratios of GC-MS 17 β -estradiol to N, P, and K were statistically significantly different ($\alpha=0.05$) for both dairy and swine farrowing. Swine farrowing had the highest N, P, and K ratios (12.6 ppt 17 β /ppm N (2.6 sd), 42.6 ppt 17 β /ppm P (5.6 sd), and 20.2 ppt 17 β /ppm K (3.6 sd)) while dairy had the lowest, (7.63 ppt 17 α /ppm N (1.9 sd), 29.2 ppt 17 α /ppm P (4.1 sd), and 16.5 ppt 17 α /ppm K (2.6 sd)). Thus, Hypothesis 1 for GC-MS 17 β -estradiol was rejected for N, P, and K.

GC-MS Estrone

The concentration of GC-MS estrone to macronutrient N mass ratios were statistically significantly different ($\alpha=0.05$) for all animal types. Swine farrowing had the highest ratios (40.9 ppt E1/ppm N (2.9 sd)). Dairy had the lowest ratios for all farm types (8.6 ppt E1/ppm N (2.1 sd)). There were statistically significant differences ($\alpha=0.05$) for P mass ratios. Swine farrowing and swine finishing were significantly greater than dairy. Swine farrowing had the highest values (208.6 ppt E1/ppm P (26.8 sd)). There was not a

significant difference ($\alpha=0.05$) between dairy and swine finishing. However, swine farrowing was statistically significantly different between swine finishing and dairy for K mass ratios. Swine farrowing had the highest ratios of (68.9 ppt E1/ppm K (5.3 sd)) and dairy had the lowest ratios (13.9 ppt E1/ppm K (3.8 sd)). Therefore, this hypothesis was rejected for N, P, and K ratios because swine farrowing was significantly greater than one or both of the farm types analyzed.

GC-MS 17 α -estradiol

For GC-MS 17 α -estradiol, only dairy and swine farrowing farm types were analyzed because the swine finishing samples were below detection limit. The ratios of GC-MS 17 α -estradiol to N, P, and K mass ratios were statistically significantly different ($\alpha=0.05$) between dairy and swine farrowing (Figure 9). Dairy had the highest ratios for N, P, and K (16.5 ppt 17 α /ppm N (2.3 sd), 62.6 ppt 17 α /ppm P (6.9 sd), and 30.9 ppt 17 α /ppm K (4.1 sd)) while swine farrowing had the lowest ratios (6.4 ppt 17 α /ppm N (5.7 sd), 42.4 ppt 17 α /ppm P (16.9 sd), and 12.3 ppt 17 α /ppm K (10 sd)). Therefore, Hypothesis 1 for GC-MS 17 α -estradiol was rejected for N, P, and K.

GC-MS Total Estrogen

Swine farrowing farm types were statistically significantly different ($\alpha=0.05$) for N, P, and K mass ratios for GC-MS total estrogen (Figure 10). There was no statistically significant difference between dairy and swine finishing for N and P ratios. However, there was a statistically significant difference ($\alpha=0.05$) between dairy and swine finishing for K mass ratios. Swine farrowing had the highest GC-MS total estrogen for N, P, and K

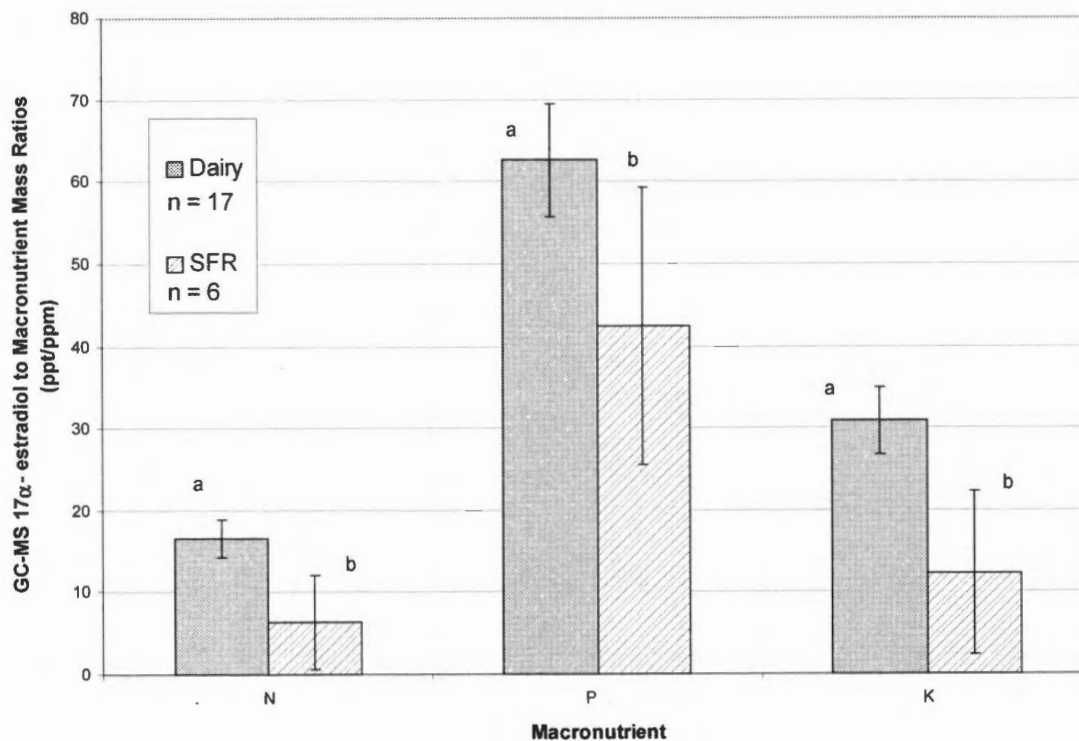


Figure 9. Mass ratio of GC-MS 17 α -estradiol to N, P, and K for the three farm types sampled. Key: SFR = swine farrowing and SFI = swine finishing. Error bars reflect standard deviations. Letters (a and b) indicate significant differences with macronutrient at $\alpha=0.05$.

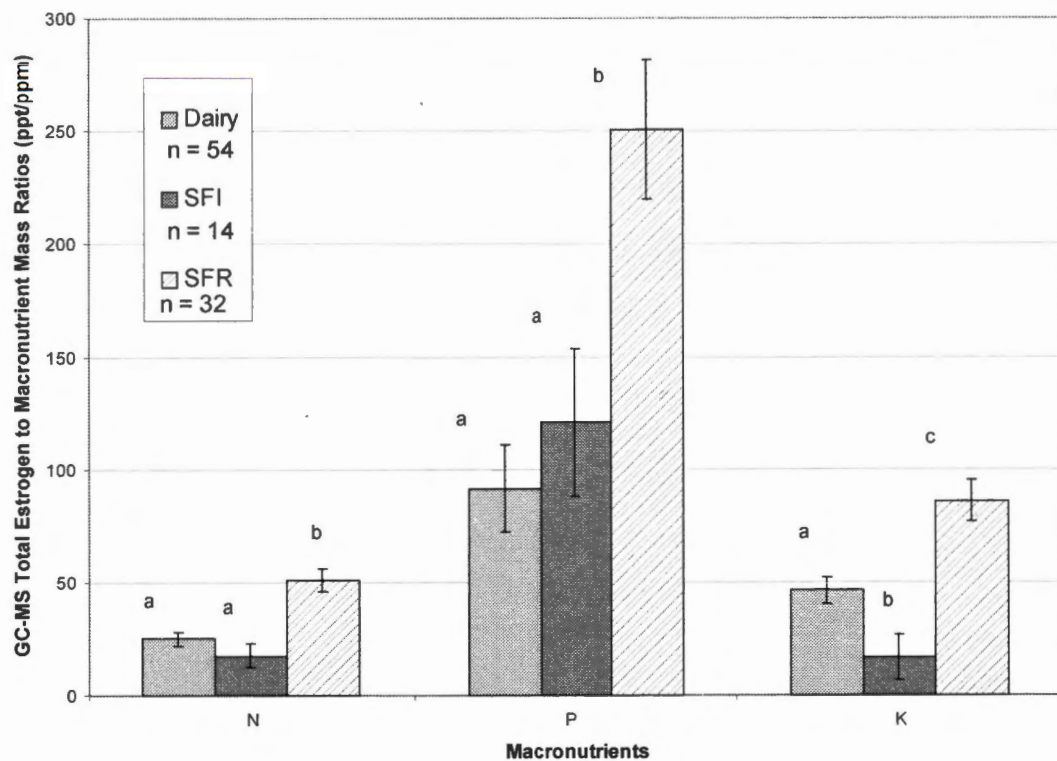


Figure 10. Mass ratio of GC-MS total estrogen to N, P, and K for the three farm types sampled. Key: SFR = swine farrowing and SFI = swine finishing. Error bars reflect standard deviations. Letters (a, b, and c) indicate significant differences within each macronutrient at $\alpha=0.05$.

mass ratios (51.2 ppt TE/ppm N (4.9 sd), 250.6 ppt TE/ppm P (30.9 sd), 86.2 ppt TE/ppm K (9.4 sd)). Swine finishing had the lowest ratios for N and K (17.6 ppt TE/ppm N (5.3 sd) and 16.9 ppt TE/ppm K (10 sd)). Dairy had the lowest ratios for P ratios (91.8 ppt TE/ppm P (19.5 sd)). Therefore, Hypothesis 1 was rejected for N, P, and K mass ratios.

A summary of the results of testing Hypothesis 1 is presented in Table 4.

Hypothesis 2: There is no difference in estrogenicity of wastes due to manure handling and storage methods.

To test Hypothesis 2, dairy and swine finishing structures were grouped for analysis. Swine farrowing structures were not included in this analysis because no dry swine farrowing operations were sampled. However, a bar graph showing average 17β :N and 17β :P ratios by structure type is provided in Figure 11. Analyses were run using both ELISA 17β -estradiol and GC-MS data.

Dairy-ELISA 17β -estradiol, GC-MS 17β -estradiol, and estrone

For ELISA 17β -estradiol, GC-MS 17β -estradiol, and estrone, there were no significant differences ($\alpha=0.05$) between any of the structures analyzed. Thus, the hypothesis was accepted (Figure 12).

Dairy-GC-MS 17α -estradiol

The GC-MS 17α -estradiol to N mass ratios of dairy dry stack-semisolid were statistically significantly different ($\alpha=0.05$) from dairy holding ponds and dairy dry stack-solid structures. Dairy dry stack-semisolid had the highest ratios

Table 4. Summary of results, Hypothesis 1. Key: SFR = swine farrowing, SFI = swine finishing.

Hypothesis 1 - There is no difference in the estrogenicity of wastes from dairy, swine farrowing, and swine finishing operations.		
ELISA 17β-estradiol		
N	rejected	SFR significantly higher than Dairy and SFI
P	rejected	SFR significantly higher than Dairy and SFI
K	rejected	SFR significantly higher than Dairy and SFI
GC-MS 17β-estradiol		
N	rejected	SFR significantly higher than Dairy and SFI
P	rejected	SFR significantly higher than Dairy and SFI
K	rejected	SFR significantly higher than Dairy and SFI
GC-MS Estrone		
N	rejected	SFR significantly higher than Dairy and SFI
P	rejected	SFR significantly higher than Dairy and SFI
K	rejected	SFR significantly higher than Dairy and SFI
GC-MS 17α-estradiol		
N	rejected	Dairy significantly higher than SFR
P	rejected	Dairy significantly higher than SFR
K	rejected	Dairy significantly higher than SFR
GC-MS Total Estrogen		
N	rejected	SFR significantly higher than Dairy and SFI
P	rejected	SFR significantly higher than Dairy and SFI
K	rejected	SFR significantly higher than Dairy and SFI

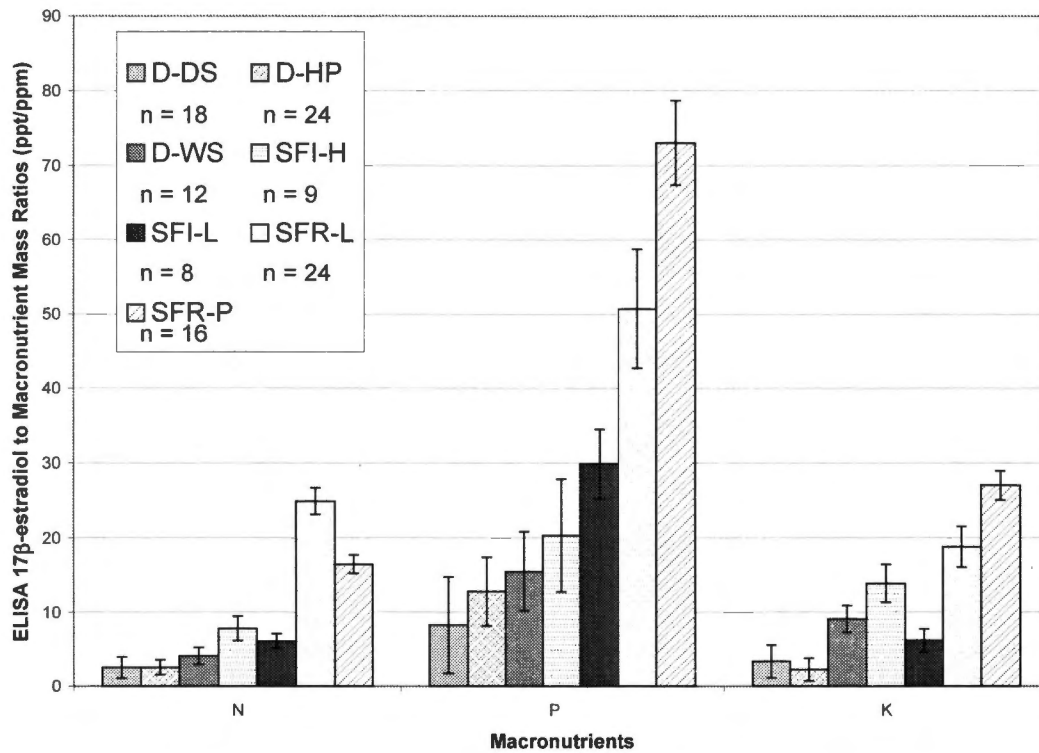


Figure 11. Mass ratio of ELISA 17 β -estradiol to N, P, and K for all seven types of structures sampled. Key: SFR-L = swine farrowing lagoon; SFR-P = swine farrowing; SFI-L = swine finishing lagoon; SFI-H = swine finishing hoop; D-HP = dairy holding pond; D-DS = dairy dry stack-solid; and D-WS = dairy dry stack-semisolid. Error bars reflect standard deviations.

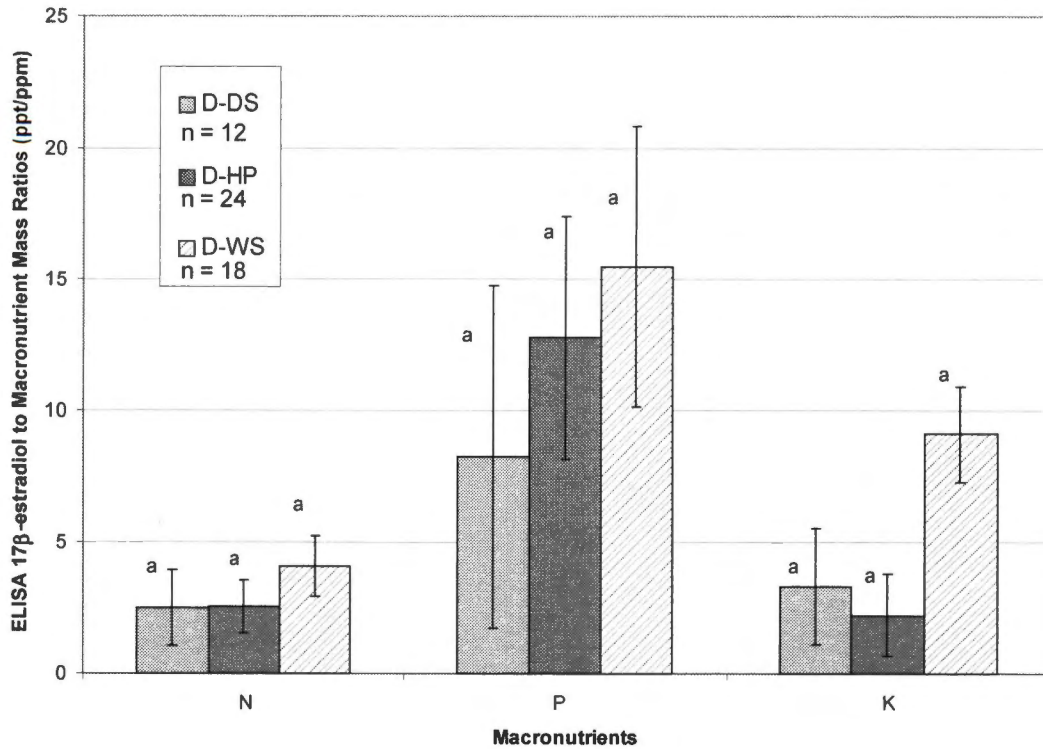


Figure 12. Mass ratio of ELISA 17 β -estradiol to N, P, and K for dairy facilities sampled.

Key: D-HP = dairy holding pond; D-DS = dairy dry stack-solid; and D-WS = dairy dry stack-semisolid. Error bars reflect standard deviations. No significant differences were observed between facility type at $\alpha=0.05$.

(22.7 ppt 17α /ppm N (3.0 sd)) while dairy holding ponds had the lowest ratios (5.96 ppt 17α /ppm N (4.2 sd)). The ratios in dairy dry stack-solid were statistically greater ($\alpha=0.05$) from those in dairy holding ponds as well. When expressed in a P or K mass ratio, dairy dry stack-semisolid was statistically greater ($\alpha=0.05$) than dairy holding ponds and dairy dry stack-solid structures. However, there was no significant difference ($\alpha=0.05$) in 17α -estradiol to macronutrient mass ratios between dairy holding ponds and dairy dry stack-solid structures.

Dairy-GC-MS Total Estrogen

For dairy structures, the mass ratios of total estrogen (by GC-MS) to N and K were statistically significantly different ($\alpha=0.05$) for dairy dry stack-semisolid (Figure 13). Dairy dry stack-semisolid had the highest ratios (33.9 ppt TE/ppm N (4.3 sd) and 74.6 ppt TE/ppm K (7.6 sd)) while dairy holding ponds had the lowest ratios (12.7 ppt TE/ppm N (5.7 sd) and 14.82 ppt TE/ppm K (10.1 sd)). There was no significant difference ($\alpha=0.05$) between any of the dairy structures for total estrogen to P mass ratio. Therefore, this hypothesis was rejected for N and K ratios and accepted for P ratios.

Table 5 summarizes the statistical results from hypothesis 2-dairy for ELISA 17β -estradiol and GC-MS data.

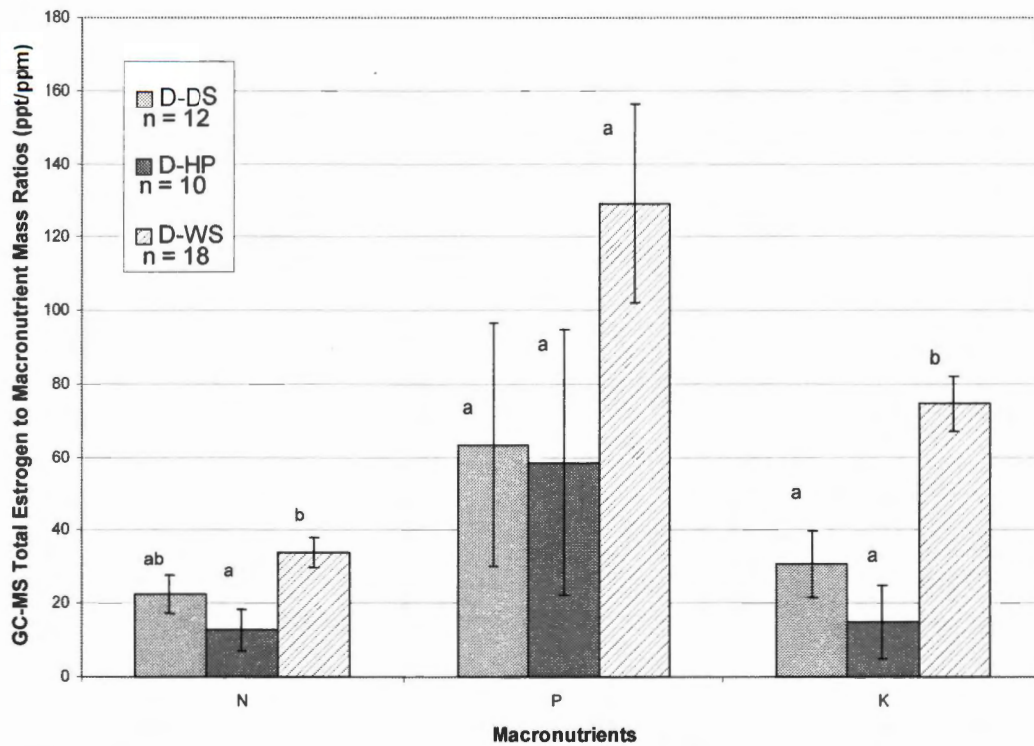


Figure 13. Mass ratio of GC-MS total estrogen to N, P, and K for dairy facilities sampled. Key: D-HP = dairy holding pond; D-DS = dairy dry stack-solid; D-WS = dairy dry stack-semisolid. Error bars reflect standard deviations. Letters (a, b, and ab) indicate significant differences at $\alpha=0.05$.

Table 5. Summary of Hypothesis 2 results for dairies.

Hypothesis 2 -Dairy-There is no difference in the estrogenicity of wastes due to manure handling and storage methods.		
ELISA 17β-estradiol		
N	accepted	no difference
P	accepted	no difference
K	accepted	no difference
GC-MS 17β-estradiol		
N	accepted	no difference
P	accepted	no difference
K	accepted	no difference
GC-MS Estrone		
N	accepted	no difference
P	accepted	no difference
K	accepted	no difference
GC-MS 17α-estradiol		
N	rejected	D-WS significantly greater than other structures
P	rejected	D-WS significantly greater than other structures
K	rejected	D-WS significantly greater than other structures
GC-MS Total Estrogen		
N	rejected	D-WS significantly greater than other structures
P	accepted	no difference
K	rejected	D-WS significantly greater than other structures

Swine Finishing-ELISA 17 β -estradiol, GC-MS 17 β -estradiol, and GC-MS 17 α -estradiol

There were no significant differences ($\alpha=0.05$) between swine finishing structures for ELISA 17 β -estradiol (Figure 14). However, swine finishing structures were not analyzed for GC-MS 17 β -estradiol, nor 17 α -estradiol, because values were below the detection limit for the analyses. Therefore, Hypothesis 2 was accepted for ELISA 17 β -estradiol.

Swine Finishing-GC-MS Estrone

The GC-MS estrone concentrations to N and P mass ratios were statistically significantly different ($\alpha=0.05$) for both swine finishing hoop structures and lagoons (Figure 15). Specifically, swine finishing lagoons had the highest ratios (26.9 ppt E1/ppm N (3.7 sd), 202.2 ppt E1/ppm P (34.8 sd)) while swine finishing hoops had the lowest ratios (5.23 ppt E1/ppm N (4.2 sd), 13.2 ppt E1/ppm P (40.1 sd)). There was no significant difference ($\alpha=0.05$) between the structures for estrone to K mass ratios. Therefore, Hypothesis 2 was rejected for N and P ratios and accepted for K ratios.

Swine Finishing-GC-MS Total Estrogen

When using GC-MS, derived values, swine finishing lagoons and swine finishing hoop structures were statistically significant different ($\alpha=0.05$) for total estrogen to P mass ratios. Swine finishing lagoons had the highest ratios (202.2 ppt TE/ppm P (40.8 sd)) and swine finishing hoop structures had the lowest ratios (13.2 ppt TE/ppm P (47 sd)). In contrast, there was no significant difference ($\alpha=0.05$) between the structures for total estrogen to N

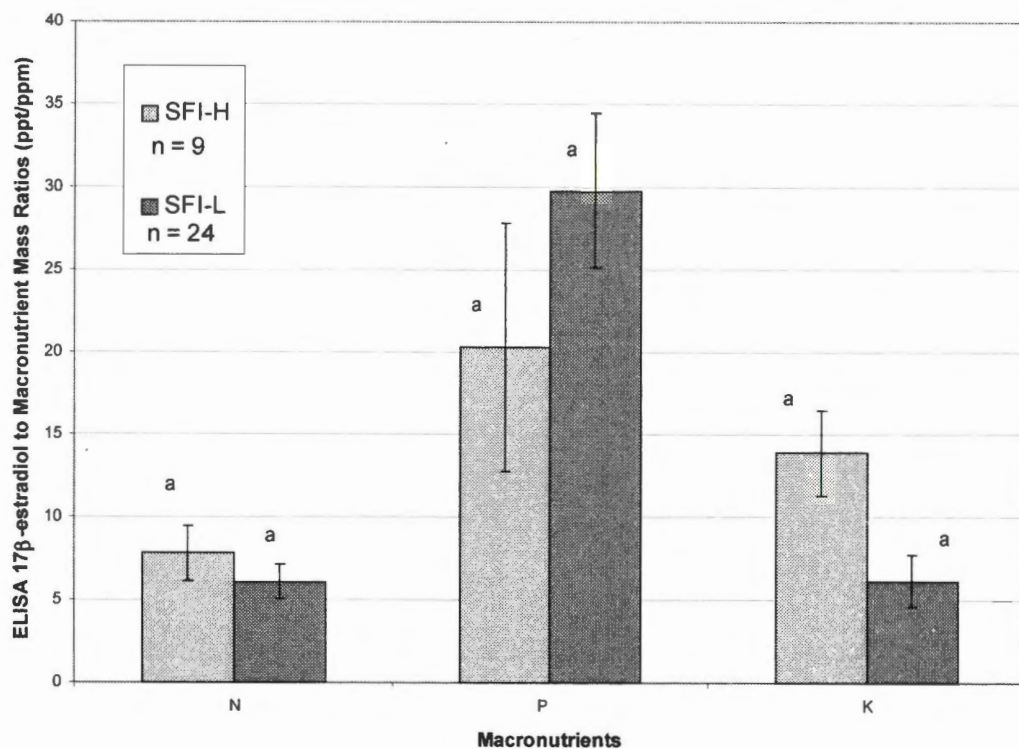


Figure 14. Mass ratio of ELISA 17 β -estradiol to N, P, and K for swine finishing facilities sampled. Key: SFI-L = swine finishing lagoon; SFI-H = swine finishing hoop. Error bars reflect standard deviations. No significant differences observed at $\alpha=0.05$.

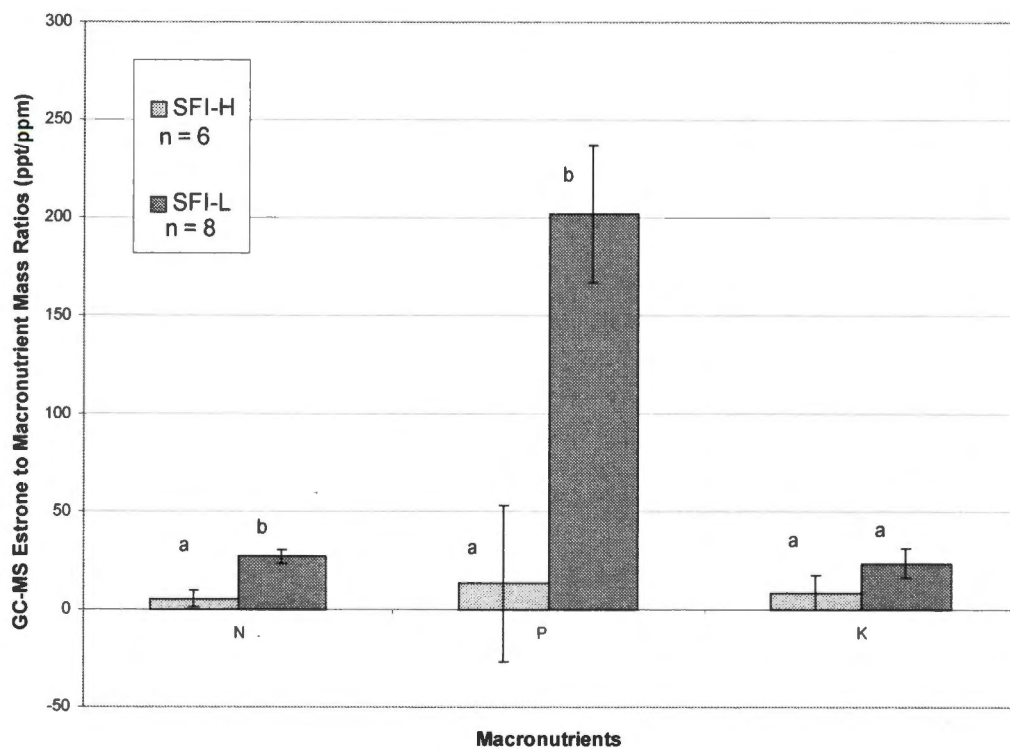


Figure 15. Mass ratio of GC-MS estrone to N, P, and K for swine finishing facilities sampled. Key: SFI-L = swine finishing lagoon; SFI-H = swine finishing hoop. Error bars reflect standard deviations. Letters (a and b) indicate significant differences at $\alpha=0.05$.

and K ratios. Thus, the hypothesis 2 was rejected for P and accepted for N and K (results are summarized in Table 6).

Table 6. Summary of Hypothesis 2 results for swine finishing wastes.

Hypothesis 2 -Swine Finishing-There is no difference in the estrogenicity of wastes due to manure handling and storage methods.		
ELISA 17β-estradiol		
N	accepted	no significant difference
P	accepted	no significant difference
K	accepted	no significant difference
GC-MS 17β-estradiol		
N	N/A	GC-MS results below the detection limit
P	N/A	GC-MS results below the detection limit
K	N/A	GC-MS results below the detection limit
GC-MS Estrone		
N	rejected	SFI-L significantly greater than SFI-H
P	rejected	SFI-L significantly greater than SFI-H
K	accepted	no significant difference
GC-MS 17α-estradiol		
N	N/A	GC-MS results below the detection limit
P	N/A	GC-MS results below the detection limit
K	N/A	GC-MS results below the detection limit
GC-MS Total Estrogen		
N	accepted	no significant difference
P	rejected	SFI-L significantly greater than SFI-H
K	accepted	no significant difference

Potential Estrogen Pollution Emission

It is important to determine if estrogen to macronutrient mass ratio concentrations could have an environmental impact as a source of estrogen pollution.

To do this, I refer to the work of Dyer (2001), who conducted a runoff study at The University of Tennessee Experiment Station located in Lewisburg, Tennessee. In her work, Dyer established nine runoff plots, and applied dairy manure to six of the nine plots: three plots received manure on N application basis, and three received manure on a P application basis. These rates were applied during the winter and summer. Dyer observed biologically relevant 17β -estradiol concentrations in runoff in excess of 2.2 g/ha. The estrogens to macronutrient mass ratios reported in this thesis can be compared to the critical E2 application rate of 2.2 g/ha if a specific N or P application rate is assumed. To do this, literature application rates for N and P were used (The University of Tennessee, Agricultural Extension Service, PB 1635 and PB 1510) (Table 7).

The estrogen application rates were calculated with the following formula:

$$(\text{Estrogen / Macronutrient Ratio}) \frac{\text{Macronutrient Application Rate}}{\text{Availability Factor}}$$

Table 7. Nitrogen and phosphorous application and crops used for dairy and swine. The availability factor represents the amount of nutrients in the waste that is available to the plants. Modified from The University of Tennessee, Agricultural Extension Service, PB 1635 and PB 1510.

Nitrogen				
	Crop Type	Yield Level	Application Rate	Availability Factor
Dairy	corn silage	19-25 tons	168 kg/ha	0.4
Swine	grain corn	125-150 bu/ac	168 kg/ha	1

Phosphorous Removal				
	Crop Type	Yield Level	Application Rate	Availability Factor
Dairy	corn silage	15 tons	3.36 kg/ha	1
Swine	grain corn	150 bu/ac	0.58 kg/ha	1

N Farm Type

For nitrogen based application rates, swine farrowing and swine were above the critical value of 2.2 g/ha for ELISA 17 β -estradiol (Figure 16). Swine farrowing and swine finishing had values of 8.1 g/ha (3.9 sd) and 2.8 g/ha (2.0 sd), respectively. When considering GC-MS 17 β -estradiol, swine farrowing and dairy were greater than 2.2 g/ha with values of 5.3 g/ha (4.1 sd) and 3.2 g/ha (2.7 sd), respectively.

P Farm type

As might be expected, phosphorus based manure application yielded lower estrogen mass application rates (Figure 17). Swine farrowing was above the critical value for ELISA 17 β -estradiol with a value of 2.5g/ha (1.6). None of the farm types were over the critical value of 2.2 g/ha for GC-MS 17 β -estradiol.

N Structure Type

For nitrogen, based manure application, swine farrowing pits and lagoons, and swine finishing lagoons and hoop structures were above the critical value of 2.2 g/ha for ELISA 17 β -estradiol (Figure 18). Swine farrowing lagoons had the highest rate of 10.4 g/ha (4.0 sd) and swine finishing lagoons had the lowest rates of 2.6 g/ha (1.5 sd). For GC-MS 17 β -estradiol, swine farrowing pits and dairy dry stacks-solid and semisolid had values above the critical limit. Swine farrowing pits were the highest values (5.3 g/ha) (4.1 sd). Dairy dry stack-solid and semisolid had similar values of approximately 3.3 g/ha (0.4 sd).

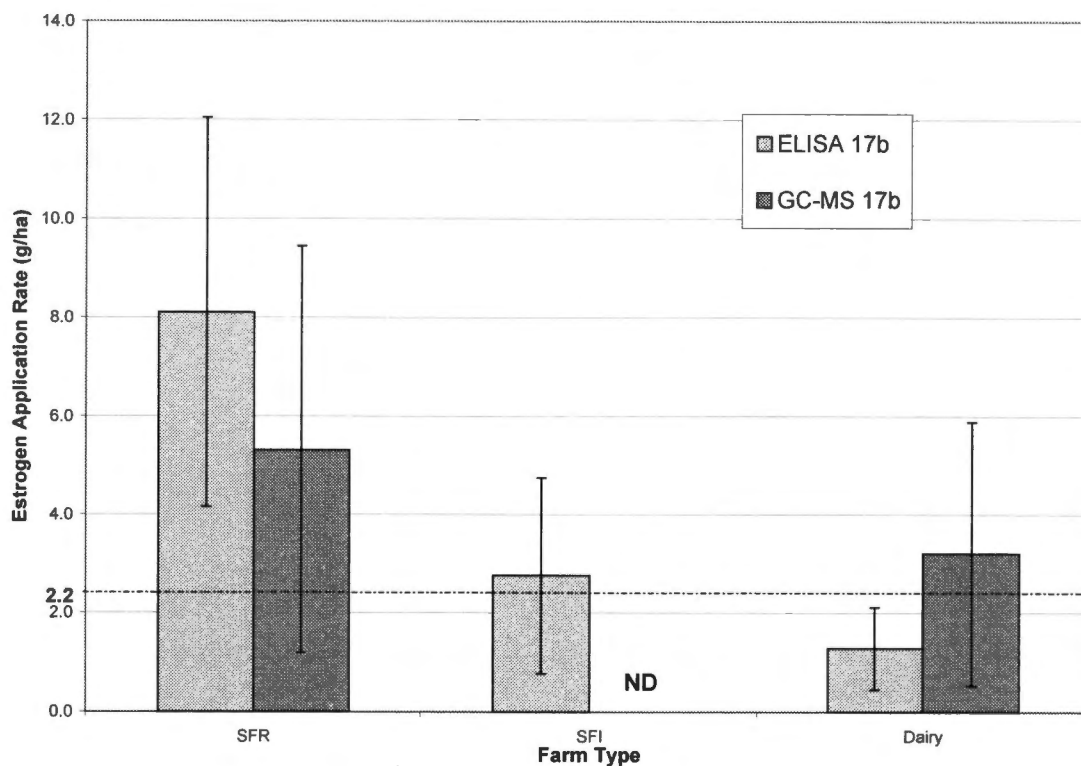


Figure 16. Estrogen application rates for N for farm types. Key: SFI-L = swine finishing lagoon; SFI-H = swine finishing hoop; ND = no data. Error bars reflect standard deviations. Swine finishing does not have GC-MS 17 β -estradiol application rates because the derived values were below the detection limit for the GC-MS analysis. The horizontal line represents the critical value of 2.2 g/ha.

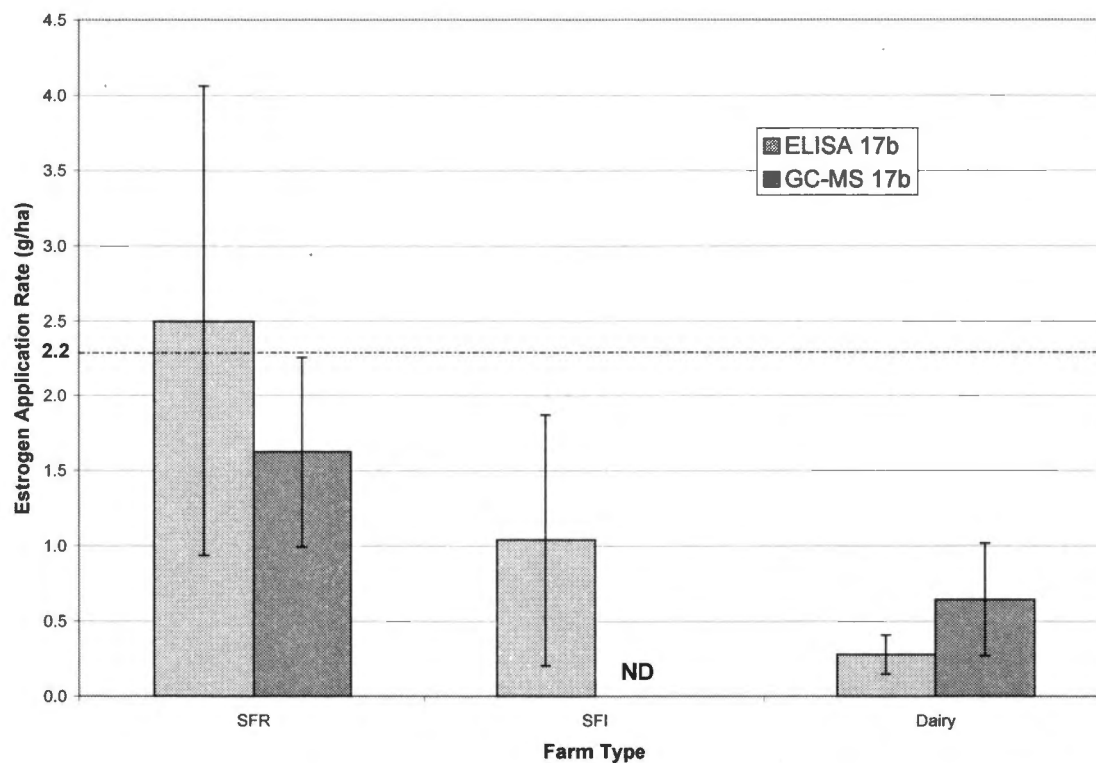


Figure 17. Estrogen application rates for P for farm types. Key: SFI-L = swine finishing lagoon; SFI-H = swine finishing hoop; ND = no data. Error bars reflect standard deviations. Swine finishing does not have GC-MS 17 β -estradiol application rates because the derived values were below the detection limit for the GC-MS analysis. The horizontal line represents the critical value of 2.2 g/ha.

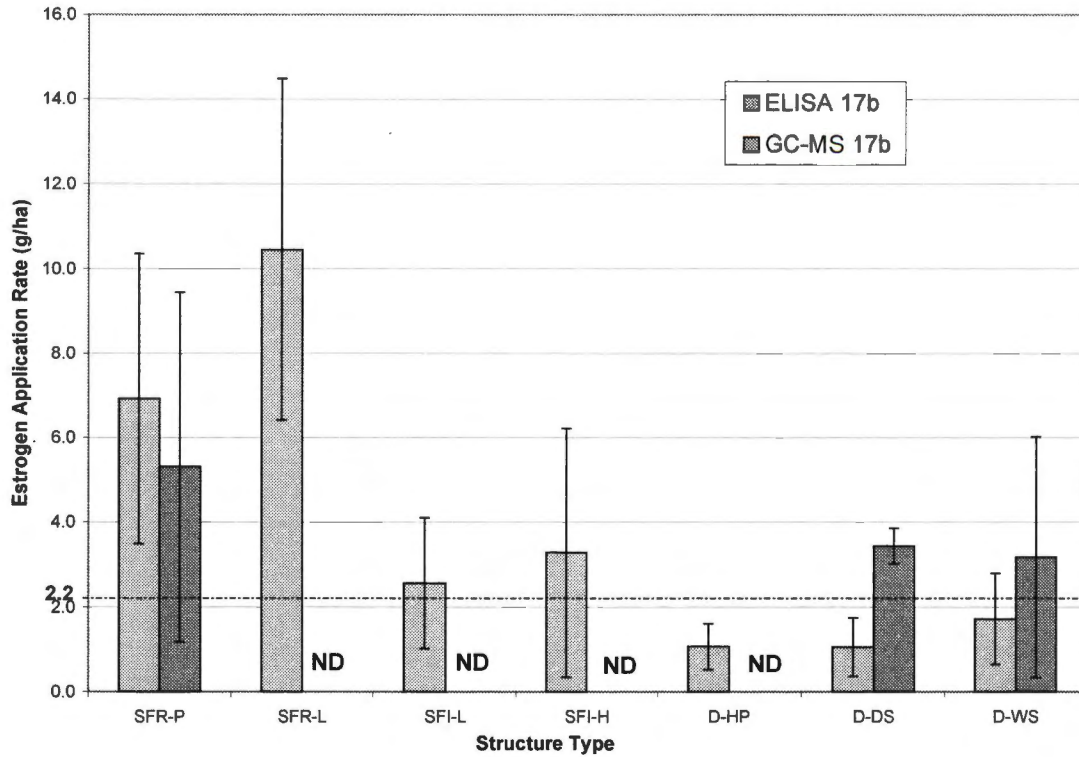


Figure 18. Estrogen application rates for N for structure types. Key: SFR-L = swine farrowing lagoon; SFR-P = swine farrowing; SFI-L = swine finishing lagoon; SFI-H = swine finishing hoop; D-HP = dairy holding pond; D-DS = dairy dry stack-solid; D-WS = dairy dry stack-semisolid; ND = no data. Error bars reflect standard deviations. Structure with columns missing had values that were below the detection limit for the GC-MS analysis. The horizontal line represents the critical value of 2.2 g/ha.

P Structure Type

For phosphorous, based manure application, swine farrowing pits were above the critical value of 2.2 g/ha for ELISA 17 β -estradiol (Figure 19). Swine farrowing pits had rates of 2.8 g/ha (1.8 sd). For GC-MS 17 β -estradiol derived rates there were no structures that were above the critical limit.

Swine farrowing pits had the highest potential for exceeding the critical estrogen manure application rate on an N and P basis. N-based application yields predicted greater estrogen mass application rates than P-based applications. However, the 2.2 g/ha limit must be used with great caution. It should be stated that this critical estrogen concentration comes from one runoff study that contained one soil type, on one slope, and one type of manure. Additional runoff work will need to be conducted to determine the most accurate estrogen manure application rate.

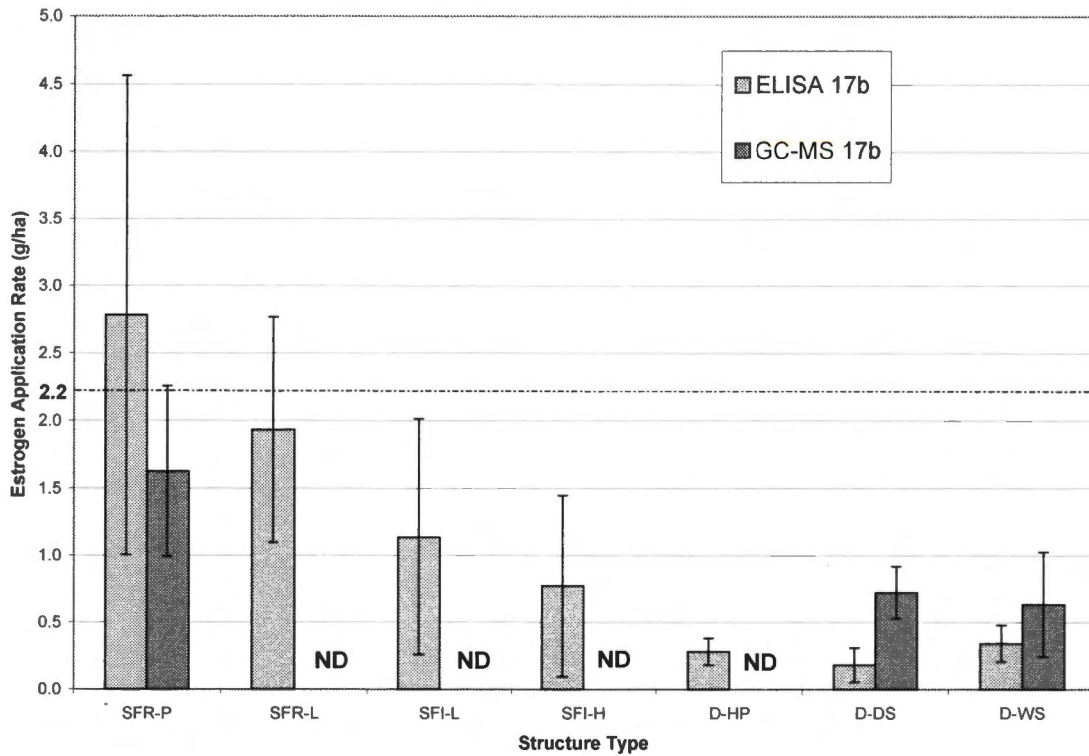


Figure 19. Estrogen application rates for P for structure types. Key: SFR-L = swine farrowing lagoon; SFR-P = swine farrowing; SFI-L = swine finishing lagoon; SFI-H = swine finishing hoop; D-HP = dairy holding pond; D-DS = dairy dry stack-solid; D-WS = dairy dry stack-semisolid; ND = no data. Error bars reflect standard deviations. Structure with columns missing had values that were below the detection limit for the GC-MS analysis. The horizontal line represents the critical value of 2.2 g/ha.

Chapter 4

CONCLUSIONS AND FUTURE WORK

Conclusions

This research explored the concentrations of estrogens contained in animal waste at dairy and swine production facilities, through a comprehensive survey of 19 waste storage structures in and around Tennessee. Approximately 220 samples were collected from eight dairy and eleven swine facilities during the winter and summer months of a one year period. Three wet dairy systems (holding ponds) and five dry stacks with varying moisture content were sampled. Five wet swine farrowing systems (four pits and one lagoon), three wet swine finishing systems (lagoons), and three dry swine finishing systems (hoop structures) were sampled.

For each sample, the mass ratios of estrogen to nitrogen (N), phosphorus (P), and potassium (K) were determined. The data were then analyzed by farm type (dairy, swine farrowing, and swine finishing) and by structure type (swine farrowing pits and lagoons, swine finishing lagoons and hoop structures, dairy holding ponds, dry stack-solid and dry-stack semisolid).

Swine farrowing operations had significantly higher ($\alpha=0.05$) ratios of 17β -estradiol to N, P, and K than did swine finishing and dairy structures sampled. A similar trend was observed in GC-MS 17β -estradiol, estrone, and total estrogen. In contrast, dairy waste showed statistically significantly higher ($\alpha=0.05$) ratios of 17α -estradiol to macronutrients than did swine finishing and dairy wastes.

Ratios of GC-MS 17α -estradiol to N, P, and K were significantly higher ($\alpha=0.05$) in dairy dry stack-semisolid than in the other dairy structures. In addition, dry stack-semisolid had statistically higher ($\alpha=0.05$) ratios of GC-MS total estrogen to N and K. In contrast, there were no statistically significant differences between any of the dairy structures for ratios of ELISA 17β -estradiol, GC-MS 17β -estradiol, and estrone to macronutrients.

Swine finishing lagoons had significantly higher ($\alpha=0.05$) ratios of GC-MS estrone to N and P than did hoop structures. There were no statistically significant differences between swine finishing lagoons and hoop structures in ratios of ELISA 17β -estradiol to N, P, and K. However, lagoons had statistically higher ratios ($\alpha=0.05$) of GC-MS total estrogen to P. GC-MS 17β -estradiol and 17α -estradiol were below detection limits.

Based upon the ratios of 17β -estradiol and estrone to N, P, and K, the estrogenicity of the wastes studied was as follows: swine farrowing > swine finishing > dairy. In contrast, 17α -estradiol was greatest in dairy wastes. However, since 17α -estradiol is not particularly potent, the concentrations of 17β -estradiol and estrone appear to be the best predictors of estrogen pollution potential, thus, the estrogen pollution potential of the wastes studied is also: swine farrowing > swine finishing > dairy.

Mass application rates of estrogenic compounds to land are likely to be indicators of the risk of environmental pollution due to runoff of these compounds. Nitrogen-based manure application yields the highest mass application rates of estrogens. In particular, N-based application of swine farrowing waste yielded 17β -estradiol application rates

roughly, 4x greater than that needed to cause biologically relevant concentrations of 17 β -estradiol in runoff (Dyer, 2001). In contrast, P-based application, which is increasingly encouraged to address other water quality concerns, yielded 17 β -estradiol application rates generally below the threshold noted in Dyer's work. While the actual threshold value needs to be supported by more research, the general trend that P-based manure application will reduce estrogen mass application rates holds true.

Future Work

Future areas of research on this topic should include a greater exploration of estrogen concentrations in waste storage structures throughout the southeast and the United States. It is possible that storage structures in other geographic regions would have varying estrogen concentrations. It would be of interest to compare the same type of structures from warmer and colder climate regions.

Additional runoff studies are needed to determine a more accurate critical estrogen manure application rate. This would entail runoff studies using a variety of waste types, soil types, and slopes. Based on this work, swine farrowing waste would be a strong candidate for such studies, as it appears to have the greatest potential for estrogen pollution.

More detail in the spatial variability and the estrogen concentrations within structure types would also be of interest. For example, a better understanding of the relationship between depth of sample and estrogen content, and between organic matter and estrogen content, would be valuable. The data collected herein should be analyzed to address these issues.

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Swine Farrowing-Pit Operations

Table A1. Data from on-site information sheet for SFR-P1.

Farm ID	Number of Animals	Average Weight (lb)	Breed
SFR-P1	24 sows + pigs	400-450	hampshire land race and yorkshire land race
Last Flush - Winter	Last Flush - Summer	Dimensions	Months of Sample Collection
1 week prior	2 weeks prior	1.2m x 18.3m x 1.2m	March and May
Volume of Water Added	Bedding Type	Area that Collects Rainwater	Confinement Period
6-8" Deep	none	none	24 hrs

X - Represents Area of Sample Collection
for Winter and Summer
- Depth of Structure is 0.10 m

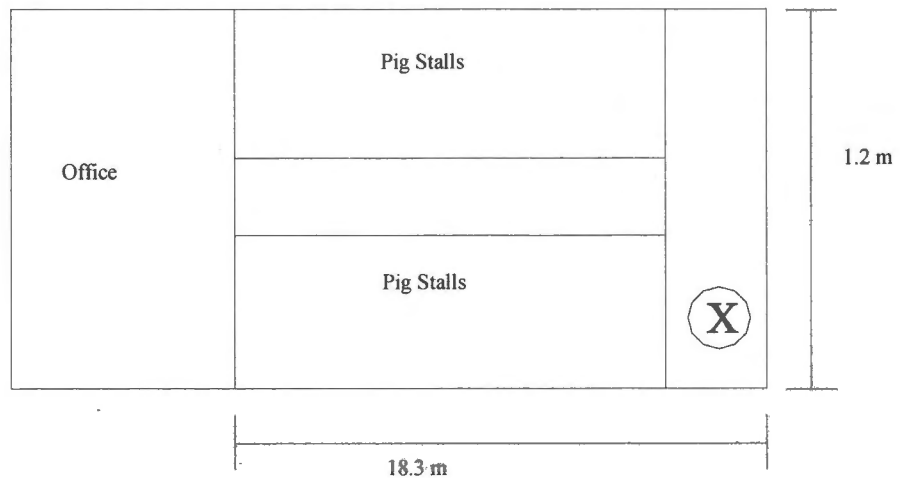


Figure A1. Diagram of SFR-P1 storage facility. Including the sample locations and dimensions.

Table A2. Data from on-site information sheet for SFR-P2.

Farm ID	Number of Animals	Average Weight (lb)	Breed
SFR-P2	20 sows + pigs	400-450	yorkshire land race
Last Flush - Winter	Last Flush - Summer	Dimensions	Months of Sample Collection
1 month prior	1 week prior	12.2m x 21.3m x 1.2m	January and June
Volume of Water Added	Bedding Type	Area that Collects Rainwater	Confinement Period
none	none	none	24 hrs

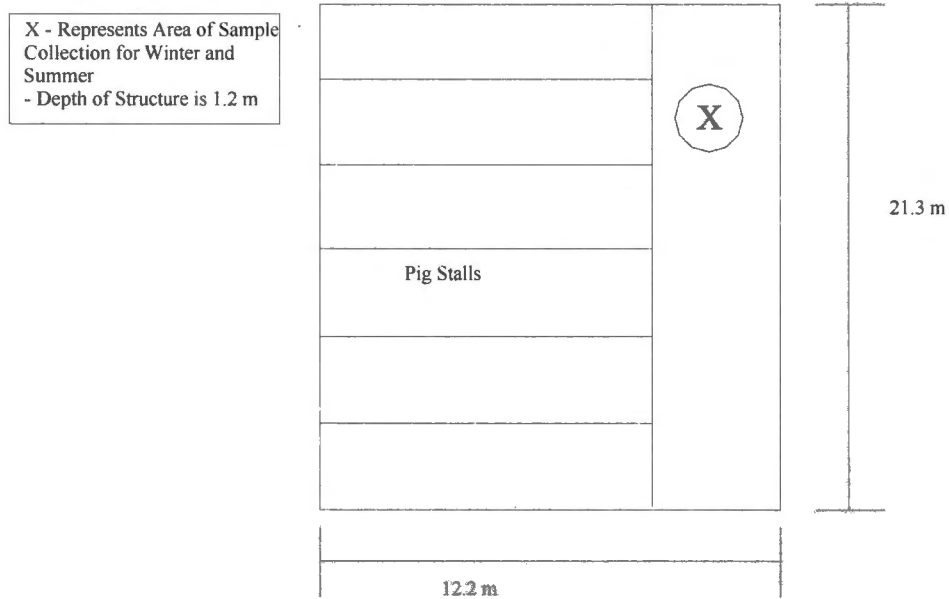


Figure A2. Diagram of SFR-P2 storage facility. Including the sample locations and dimensions.

Table A3. Data from on-site information sheet for SFR-P3.

Farm ID	Number of Animals	Average Weight (lb)	Breed
SFR-P3	12 sows + pigs	450	PIC Line 22
Last Flush - Winter	Last Flush - Summer	Dimensions	Months of Sample Collection
3 weeks prior	3 weeks prior	2.1m x 22.9m x 0.6m	January and June
Volume of Water Added	Bedding Type	Area that Collects Rainwater	Confinement Period
none	none	none	24 hrs

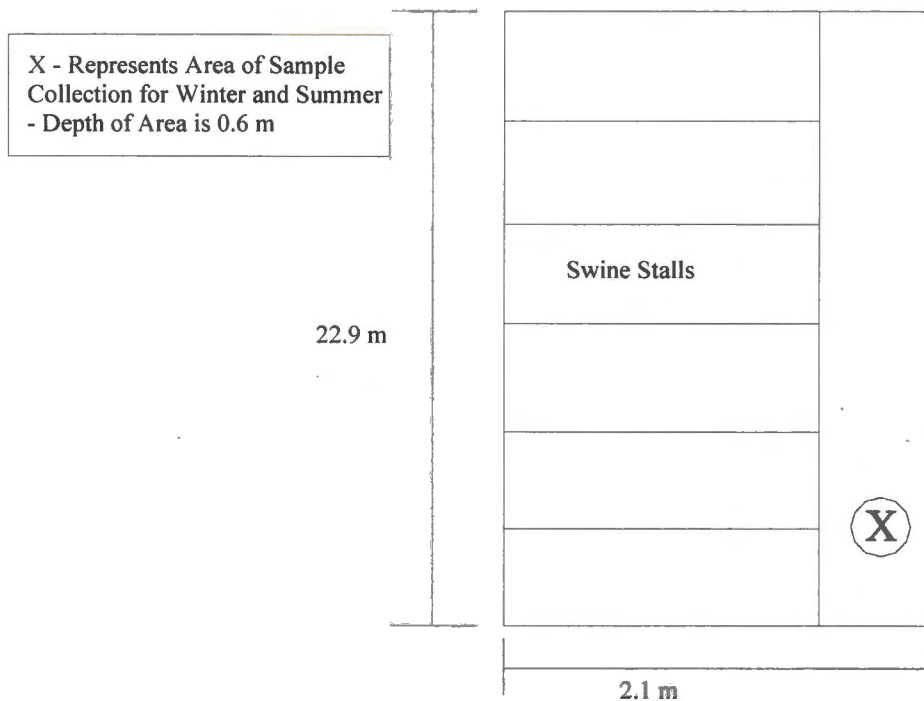


Figure A3. Diagram of SFR-P3 storage facility. Including the sample locations and dimensions.

Table A4. Data from on-site information sheet for SFR-P4.

Farm ID	Number of Animals	Average Weight (lb)	Breed
SFR-P4	10-12 sows + pigs	500	yorkshire landrace
Last Flush - Winter	Last Flush - Summer	Dimensions	Months of Sample Collection
3 weeks prior	1 month prior	2(2.7m x 9.1m x 1.2m)	January and June
Volume of Water Added	Bedding Type	Area that Collects Rainwater	Confinement Period
6" Deep	none	none	24 hrs

X - Represents Area of Sample Collection
for Winter and Summer
- Depth of Structure is 1.2 m

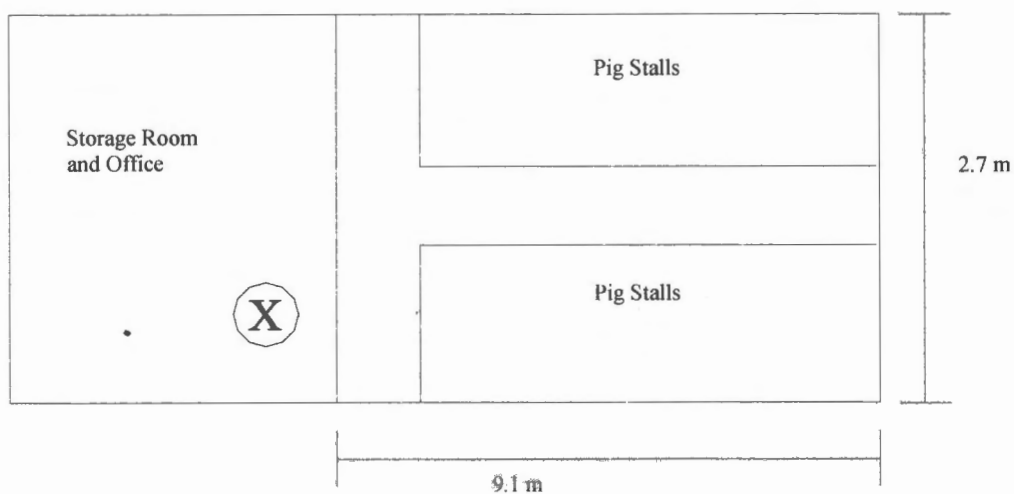


Figure A4. Diagram of SFR-P4 storage facility. Including the sample locations and dimensions.

Swine Farrowing-Lagoon Operation

Table A5. Data from on-site information sheet for SFR-L1.

Farm ID	Number of Animals	Average Weight (lb)	Breed
SFR-L1	3000-3500	200	PIC-C22
Last Pump Out - Winter	Last Pump Out - Summer	Dimensions	Months of Sample Collection
5 months prior	2 months prior	39.6m x 144.8m x 2.4m	March and May
Volume of Water Added	Bedding Type	Area that Collects Rainwater	Confinement Period
minimal recycled	none	area of lagoon	24 hrs

X - Represents Areas of
Sample Collection for
Winter and Summer
- Depth of Area is 2.4 m

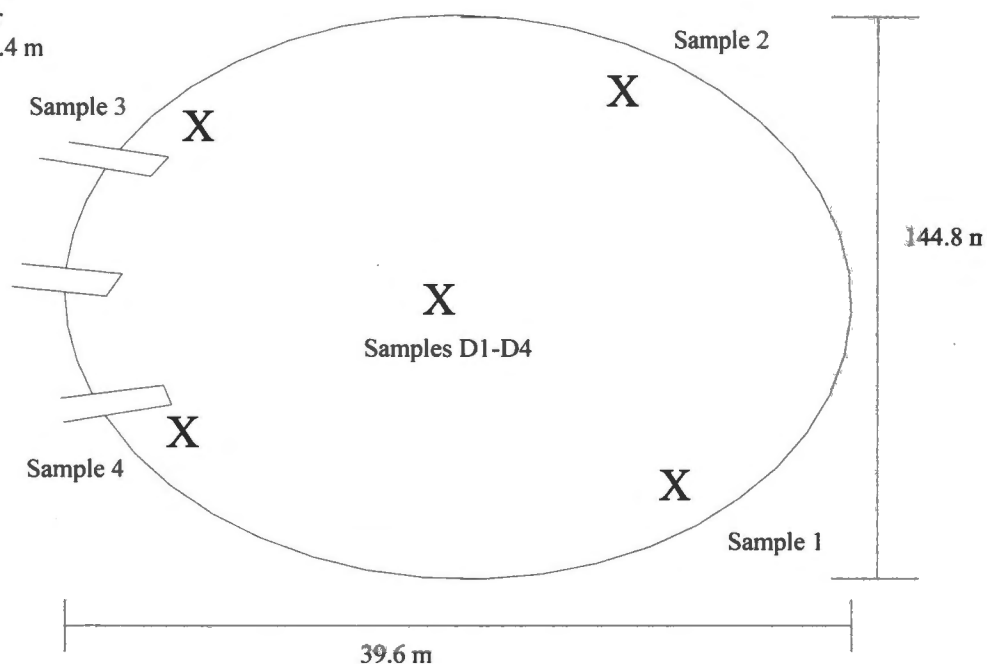


Figure A5. Diagram of SFR-L1 storage facility. Including the sample locations and dimensions.

Swine Finishing-Lagoon Operation

Table A6. Data from on-site information sheet for SFI-L1.

Farm ID SFI-L1	Number of Animals 500	Average Weight (lb) 145	Breed hampton land race and yorkshire land race
Last Pump Out - Winter 6 months prior	Last Pump Out - Summer 8 months prior	Dimensions 33.5m x 67.1m x 2.7m	Months of Sample Collection March and May
Volume of Water Added pit recharge once a week	Bedding Type none	Area that Collects Rainwater area of lagoon	Confinement Period 24 hours

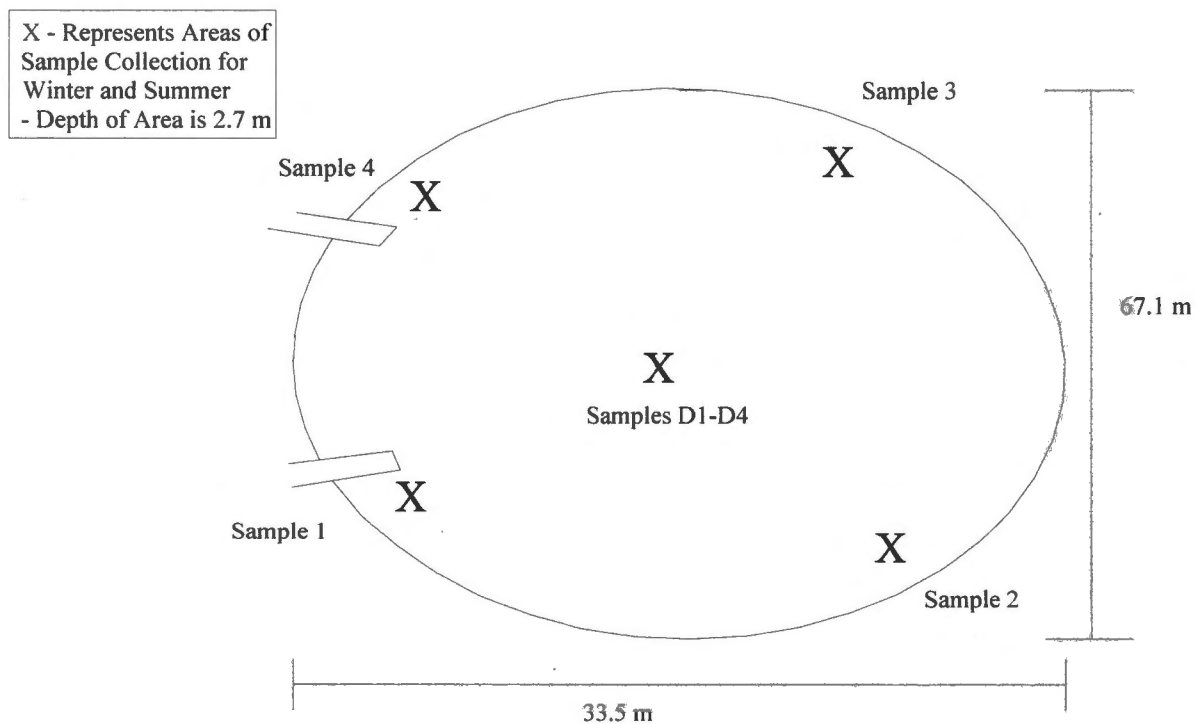


Figure A6. Diagram of SFI-L1 storage facility. Including the sample locations and dimensions.

Table A7. Data from on-site information sheet for SFI-L2.

Farm ID	Number of Animals	Average Weight (lb)	Breed
SFI-L2	10,000-13,000	150	yorkshire land race
Last Pump Out - Winter	Last Pump Out - Summer	Dimensions	Months of Sample Collection
6 months prior	1 months prior	83.8m x 99.1m x 2.1m	March and May
Volume of Water Added	Bedding Type	Area that Collects Rainwater	Confinement Period
recycled only	none	area of lagoon	24 hours

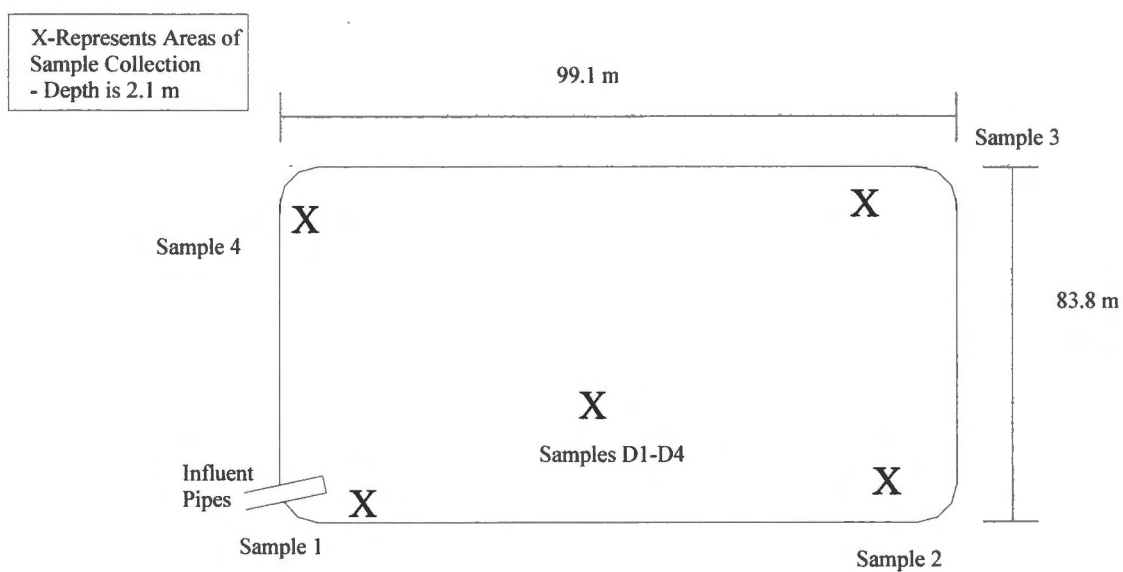


Figure A7. Diagram of SFI-L2 storage facility. Including the sample locations and dimensions.

Table A8. Data from on-site information sheet for SFI-L3.

Farm ID	Number of Animals	Average Weight (lb)	Breed
SFI-L3	10,000-11,500	150	PIC
Last Pump Out - Winter	Last Pump Out - Summer	Dimensions	Months of Sample Collection
8 months prior	10 months prior	123.7m x 291.4m x 2.4m	March and May
Volume of Water Added	Bedding Type	Area that Collects Rainwater	Confinement Period
recycled only	none	area of lagoon	24 hours

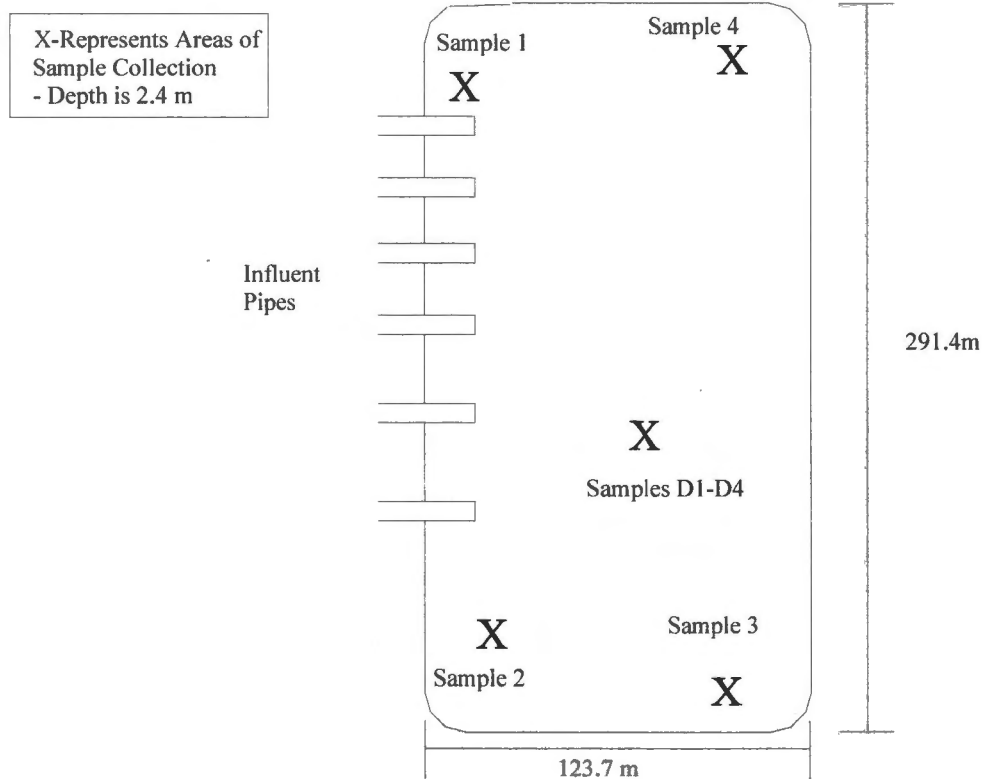


Figure A8. Diagram of SFI-L3 storage facility. Including the sample locations and dimensions.

Swine Finishing-Hoop Operation

Table A9. Data from on-site information sheets for SFI-H1 and SFI-H2.

Farm ID	Number of Animals	Average Weight (lb)	Breed
SFI-H1	100-200	250	cross
Last Scrape Out - Winter	Last Scrape Out - Summer	Dimensions	Months of Sample Collection
2 months prior	3 months prior	9.1m x 25.9m x 1.2m	January and June
Estimated Age of Manure	Bedding Type	Area that Collects Rainwater	Confinement Period
winter - 1 day to 2 months summer - 1 day to 3 months	wheat straw	none	24 hours

Farm ID	Number of Animals	Average Weight (lb)	Breed
SFI-H2	225	200-250	cross
Last Scrape Out - Winter	Last Scrape Out - Summer	Dimensions	Months of Sample Collection
5 months prior	2 months prior	9.1m x 25.9m x 1.2m	March and June
Estimated Age of Manure	Bedding Type	Area that Collects Rainwater	Confinement Period
winter - 1 day to 5 months summer - 1 day to 2 months	wheat straw	none	24 hours

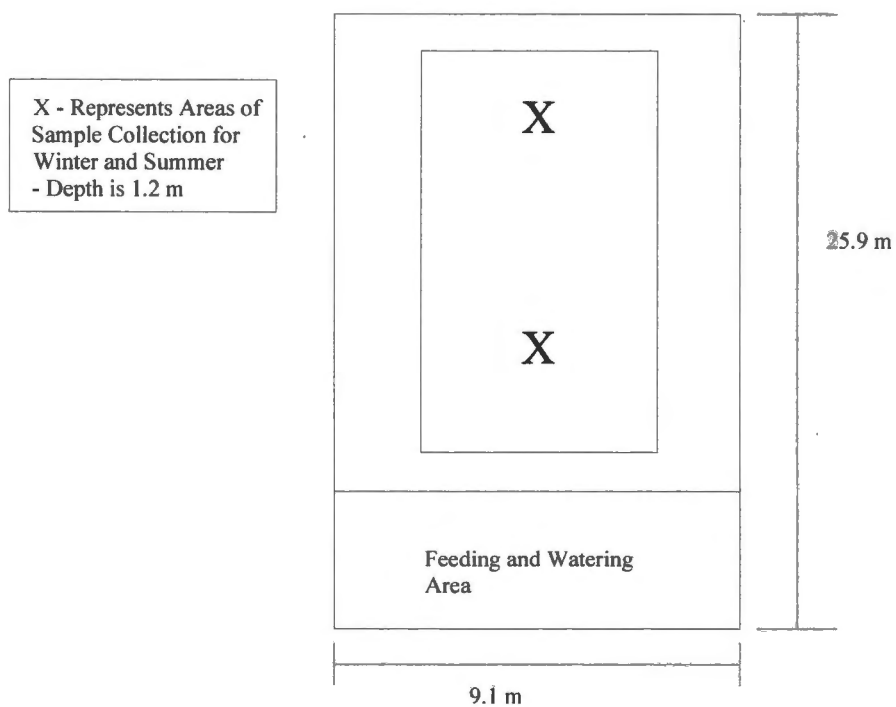


Figure A9. Diagram of SFI-H1 and SFI-H2 storage facilities. Including the sample locations and dimensions.

Table A10. Data from on-site information sheet for SFI-H3.

Farm ID	Number of Animals	Average Weight (lb)	Breed
SFI-H3	170	160	cross
Last Scrape Out - Winter	Last Scrape Out - Summer	Dimensions	Months of Sample Collection
2 months prior	4 months prior	12.2m x 18.3m	January and June
Estimated Age of Manure	Bedding Type	Area that Collects Rainwater	Confinement Period
winter - 1 day to 2 months summer - 1 day to 4 months	straw	none	24 hours

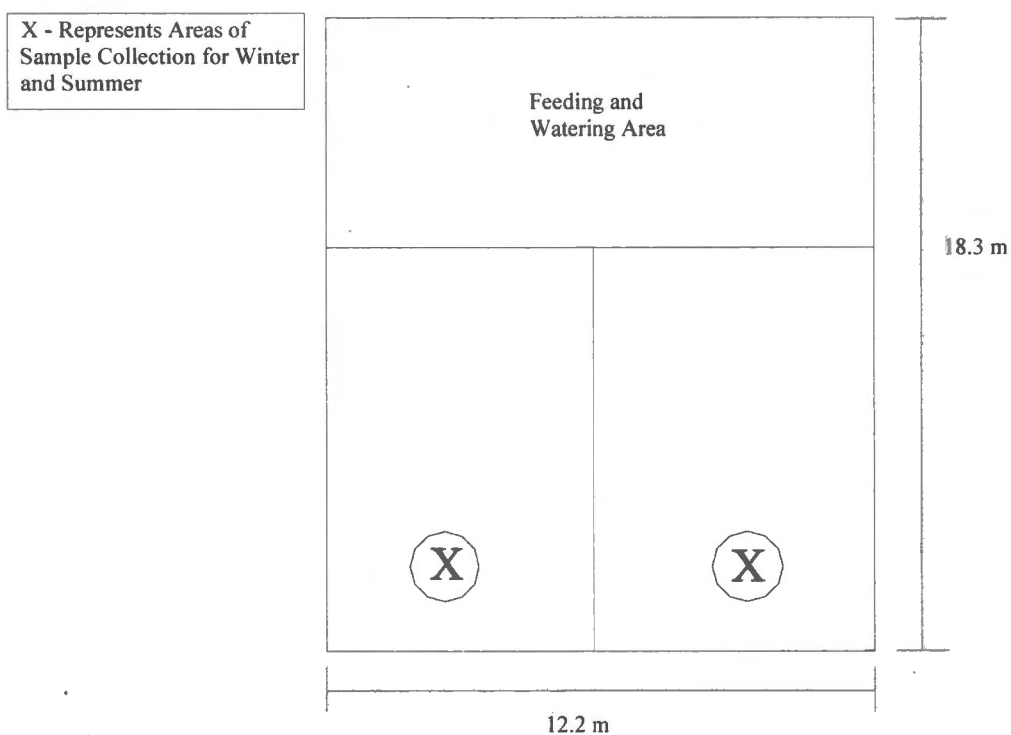


Figure A10. Diagram of SFI-H3 storage facility. Including the sample locations and dimensions.

Dairy-Holding Pond Operations

Table A11. Data from on-site information sheet for D-HP1.

Farm ID	Number of Animals	Average Weight (lb)	Breed
D-HP1	160	1000	Jersey
Last Pump Out - Winter	Last Pump Out - Summer	Dimensions	Months of Sample Collection
10 months prior	13 months prior	73.7m x 42.7m x 2.4m	January and May
Volume of Water Added	Bedding Type	Area that Collects Rainwater	Confinement Period
~ 350 gal/day	recycled manure and sawdust	area of lagoon	winter - 12-16 hrs/day summer - none

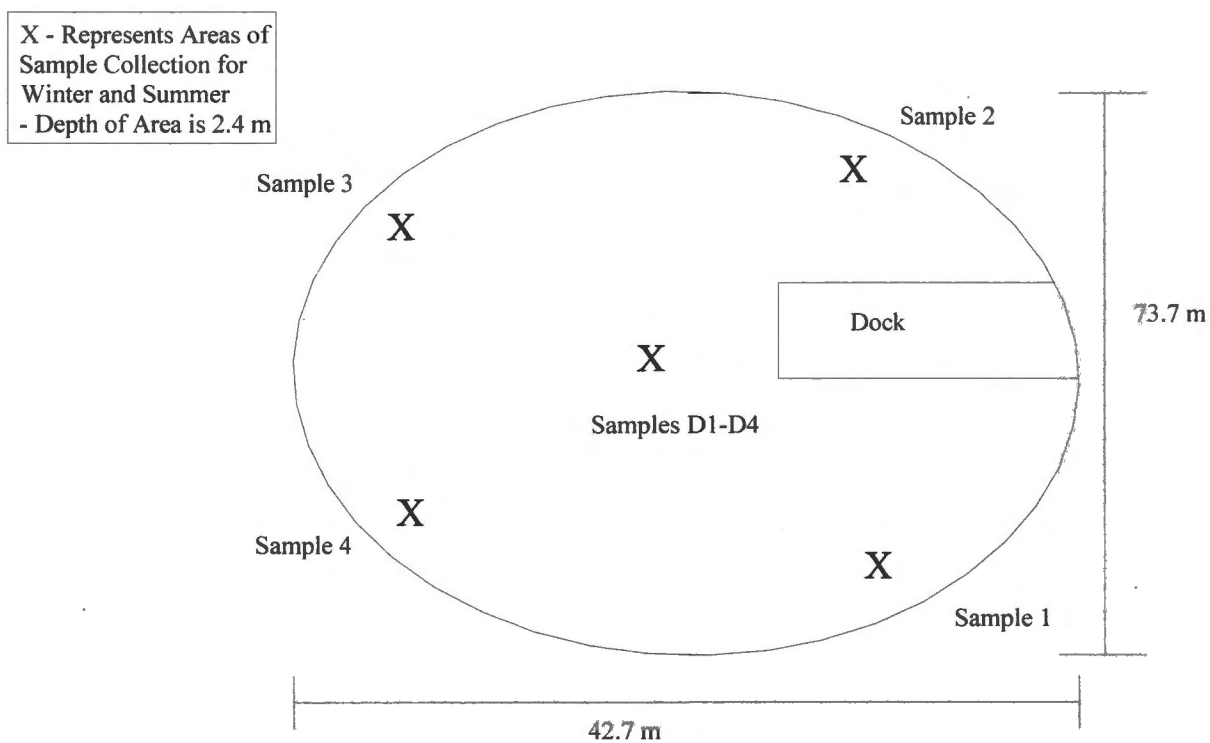


Figure A11. Diagram of D-HP1 storage facility. Including the sample locations and dimensions.

Table A13. Data from on-site information sheet for D-HP2.

Farm ID	Number of Animals	Average Weight (lb)	Breed
D-HP2	100	1300	Holstein
Last Pump Out - Winter	Last Pump Out - Summer	Dimensions	Months of Sample Collection
2 months prior	4 months prior	45.1m x 33.5m x 2.4m	February and July
Volume of Water Added	Bedding Type	Area that Collects Rainwater	Confinement Period
none	pine shavings	area of lagoon	winter - 50% of time summer - free choice

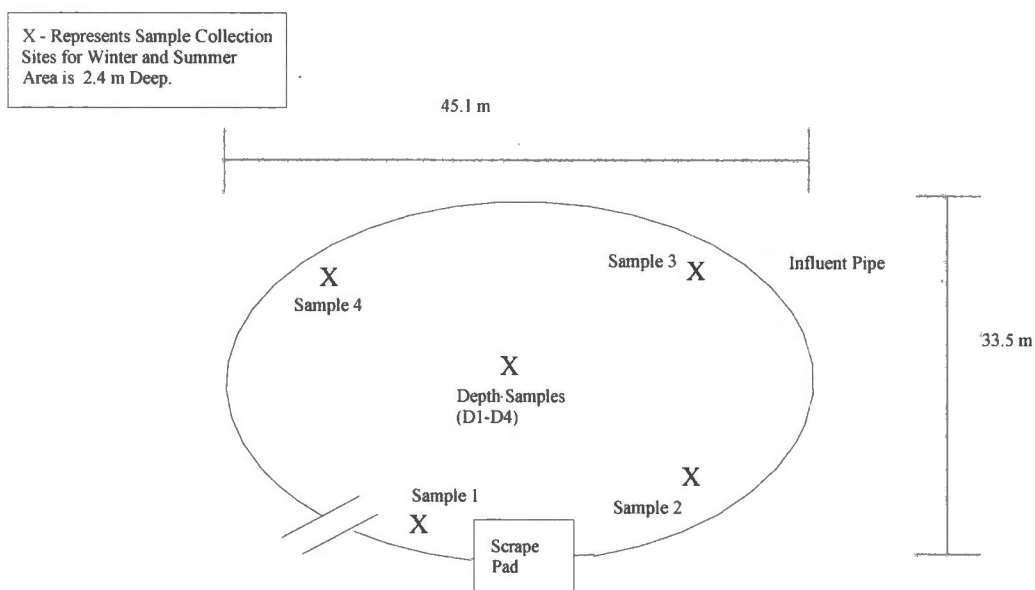


Figure A13. Diagram of D-HP2 storage facility. Including the sample locations and dimensions.

Table A12. Data from on-site information sheet for D-HP3.

Farm ID	Number of Animals	Average Weight (lb)	Breed
D-HP3	400-500	1300-1600	Holstein
Last Pump Out - Winter	Last Pump Out - Summer	Dimensions	Months of Sample Collection
3 months prior	2 months prior	45.1m x 33.5m x 2.4m	December and May
Volume of Water Added	Bedding Type	Area that Collects Rainwater	Confinement Period
33,000 gal/day from secondary pond	sawdust	area of holding pond	24 hours

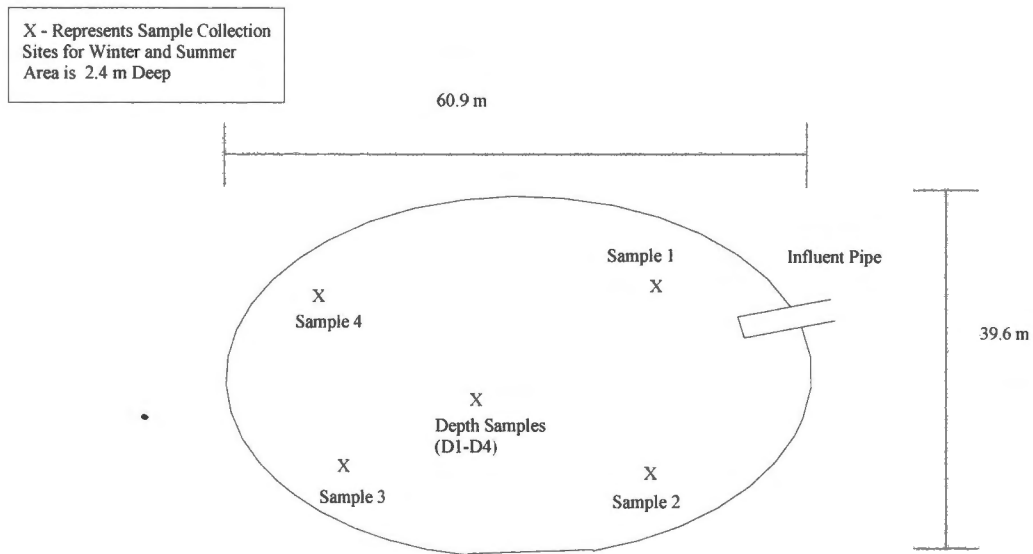


Figure A12. Diagram of D-HP3 storage facility. Include the sample locations and dimensions.

Dairy Dry Stack-Solid

Table A14. Data from on-site information sheet for D-DS1.

Farm ID	Number of Animals	Average Weight (lb)	Breed
D-DS1	40	1400-1500	Holstein
Last Scrape Out - Winter	Last Scrape Out - Summer	Dimensions	Months of Sample Collection
1 month prior	1 month prior	11m x 11m	January and July
Estimated Age of Manure	Bedding Type	Area that Collects Rainwater	Confinement Period
winter - 1-7 days to 1 month summer - 1 day to 1 month	pine shavings	none	by choice

X - Represents Areas of Sample Collection for Winter and Summer

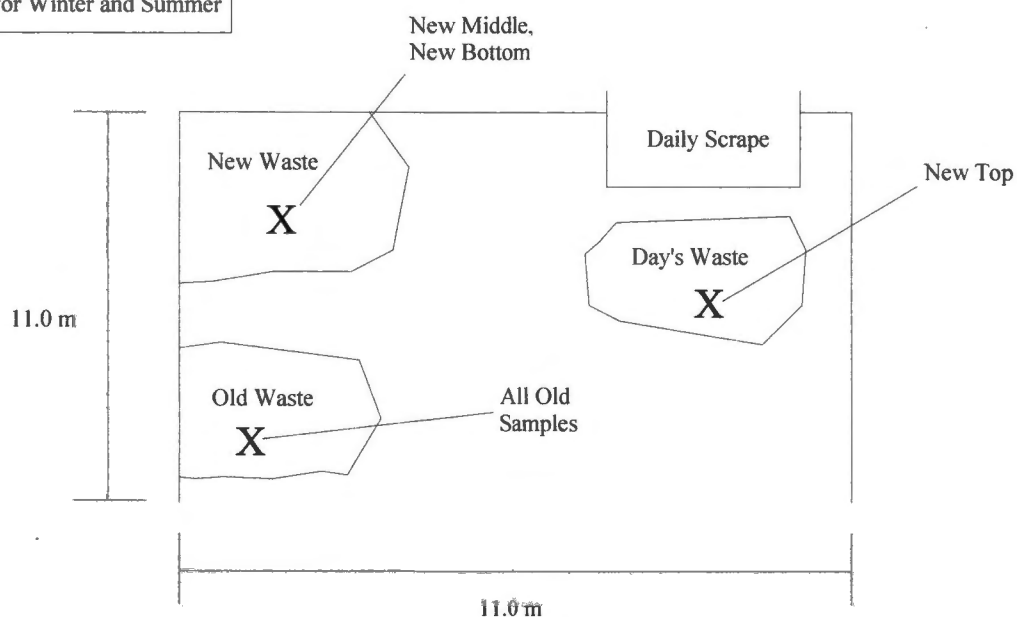


Figure A14. Diagram of D-DS1 storage facility. Including the sample locations and dimensions.

Table A15. Data from on-site information sheet for D-DS2.

Farm ID	Number of Animals	Average Weight (lb)	Breed
D-DS2	25-35	1200	Holstein and Jersey
Last Scrape Out - Winter	Last Scrape Out - Summer	Dimensions	Months of Sample Collection
8 months prior	6 months prior	13.7m x 19.8m x 2.7m	January and June
Estimated Age of Manure	Bedding Type	Area that Collects Rainwater	Confinement Period
winter - 1 week to 8 months summer - 1 day to 6 months	sawdust and hay	none	by choice

X - Represents Areas of Sample Collection for Winter and Summer
- Depth of Area is 2.7 m

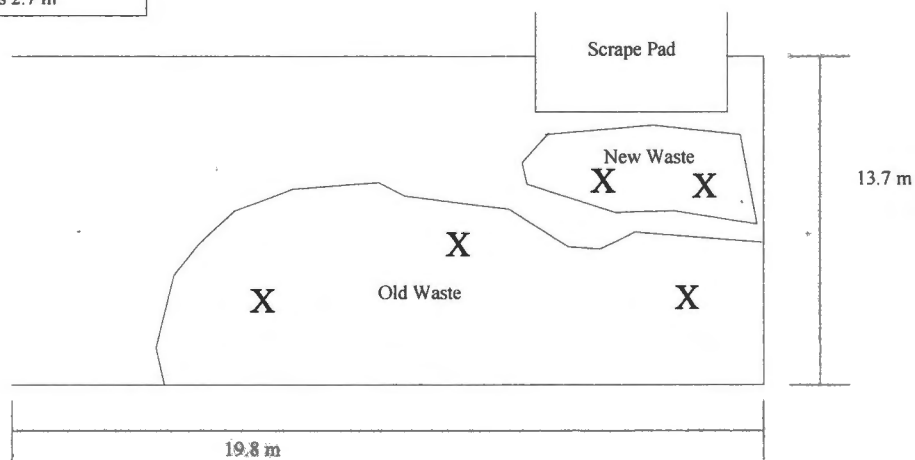


Figure A15. Diagram of D-DS2 storage facility. Including the sample locations and dimensions.

Dairy-Dry Stack-Semisolid Operation

Table A16. Data from on-site information sheet for D-WS1.

Farm ID	Number of Animals	Average Weight (lb)	Breed
D-WS1	90-100	1300-1400	Holstein
Last Scrape Out - Winter	Last Scrape Out - Summer	Dimensions	Months of Sample Collection
2 months prior	3 months prior	9.1m x 32.0m x 2.7m	December and May
Estimated Age of Manure	Bedding Type	Area that Collects Rainwater	Confinement Period
winter - 1 day to 2 months summer - 1 day to 3 months	pine shavings	none	winter - by choice summer - 10 hrs/day

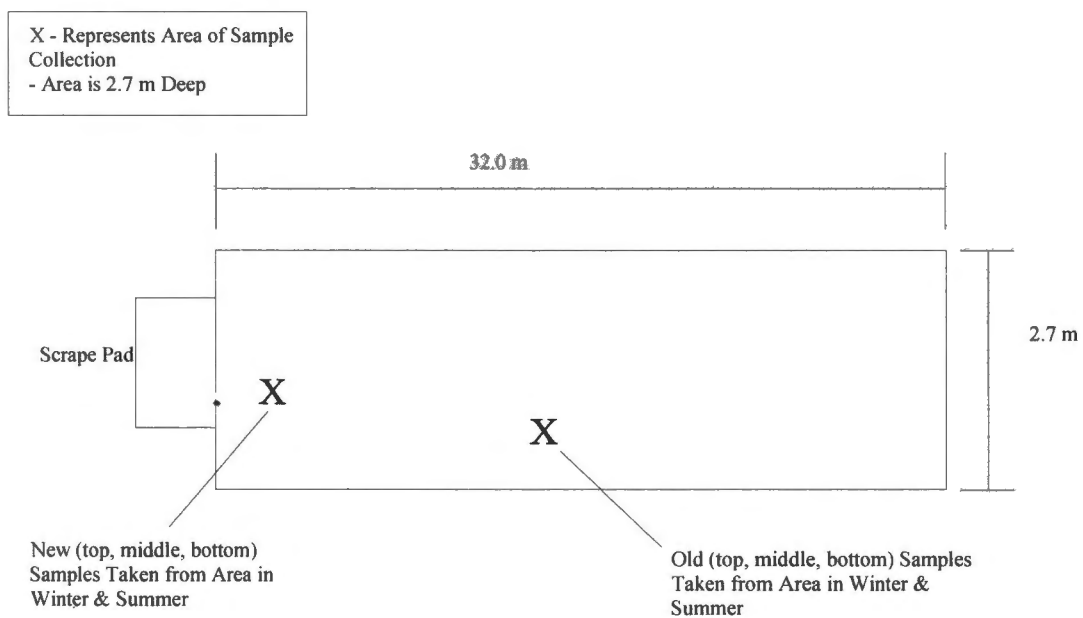


Figure A16. Diagram of D-WS1 storage facility. Including the sample locations and dimensions.

Table A17. Data from on-site information sheet for D-WS2.

Farm ID	Number of Animals	Average Weight (lb)	Breed
D-WS2	250	1300	Holstein
Last Scrape Out - Winter	Last Scrape Out - Summer	Dimensions	Months of Sample Collection
2 weeks prior	2 months prior	9.1m x 27.4m x 2.1m	December and July
Estimated Age of Manure	Bedding Type	Area that Collects Rainwater	Confinement Period
winter - 1 day to 2 weeks summer - 1 day to 2 months	pine shavings and lime	none	24 hours

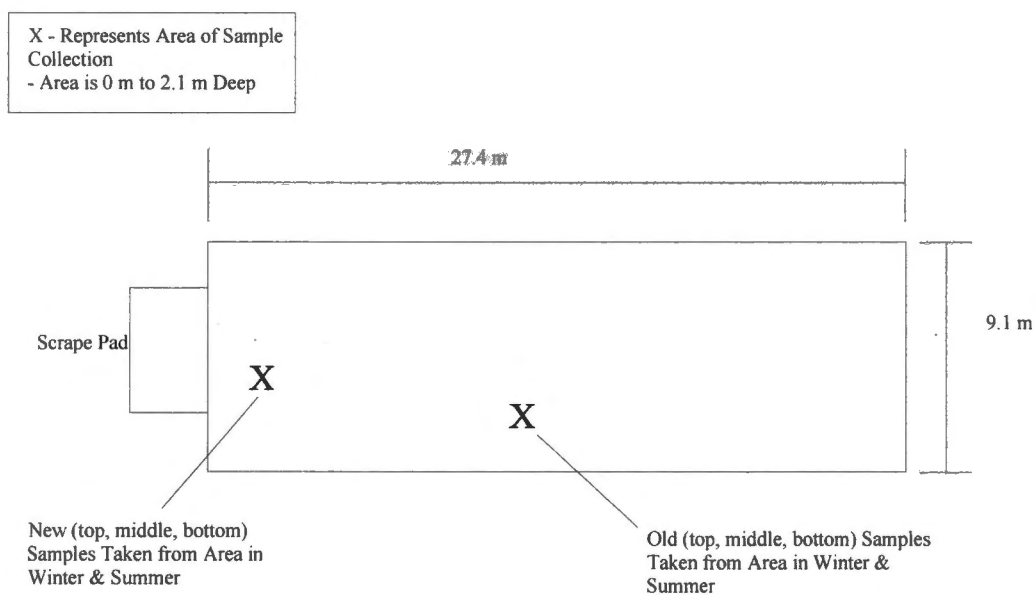


Figure A17. Diagram of D-WS2 storage facility. Including the sample locations and dimensions.

Table A18. Data from on-site information sheet for D-WS3.

Farm ID	Number of Animals	Average Weight (lb)	Breed
D-WS3	35-40	1400-1500	Holstein and Jersey
Last Scrape Out - Winter	Last Scrape Out - Summer	Dimensions	Months of Sample Collection
3 months prior	1 month prior	7.1m x 15.8m x 2.4m	January and June
Estimated Age of Manure	Bedding Type	Area that Collects Rainwater	Confinement Period
winter - 1 day to 1 month summer - 1 day to 1 month	lime	none	winter - 19-20 hrs/day summer - 18 hrs/day

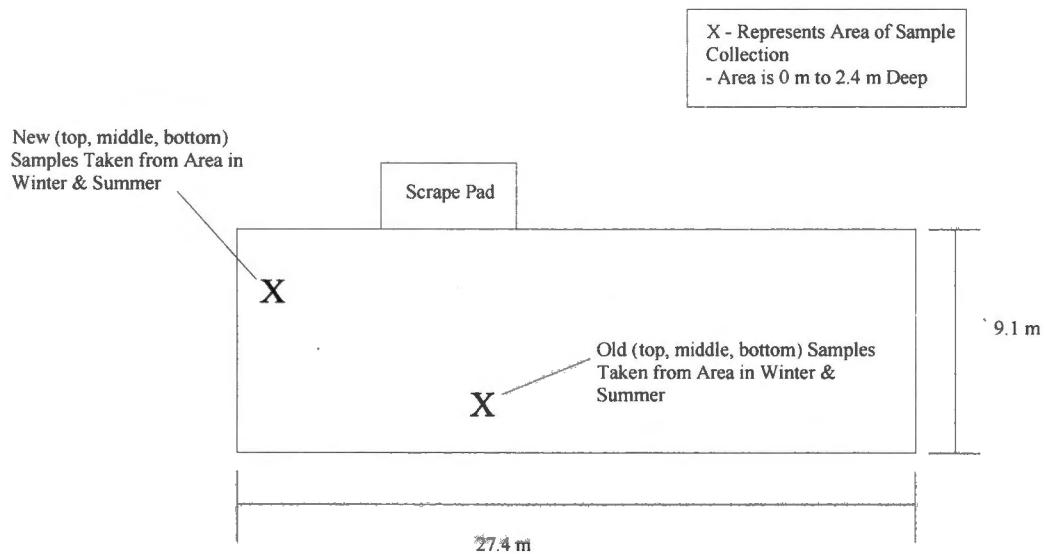


Figure A18. Diagram of D-WS3 storage facility. Including the sample locations and dimensions.

Table A 19. ELISA and GC-MS 17 β -estradiol values for swine farrowing operations sampled.

Farm ID	Sample ID	Elisa 17 β -estradiol (ppt)				GC-MS 17 β -estradiol (ppt)			
		Winter	Winter-Std	Summer	Summer-Std	Winter	Winter-Std	Summer	Summer-Std
SFR-P1	SFR-P1-T-W	27427	578	7404	15	11328	900	13400	1500
	SFR-P1-M1-W	36896	365	7406	14	13259	1900	13400	1400
	SFR-P1-M2-W	35268	643	7388	18	16796	2000	11500	2800
	SFR-P1-B-W	46156	723	9841	9	42857	11800	24000	2900
SFR-P2	SFR-P2-T-W	3956	289	39982	1562	BDL	BDL	20911	3800
	SFR-P2-M1-W	5760	121	9841	317	BDL	BDL	3900	2600
	SFR-P2-M2-W	15652	361	15475	246	15200	1800	8200	1400
	SFR-P2-B-W	11156	339	13735	463	13200	1300	8700	1300
SFR-P3	SFR-P3-T-W	23619	283	48143	332	12400	700	10400	5100
	SFR-P3-M1-W	28098	748	28398	233	20500	2300	7700	14100
	SFR-P3-M2-W	21420	707	43981	753	15800	1300	10200	6700
	SFR-P3-B-W	42180	710	96484	2319	22700	1400	25400	18600
SFR-P4	SFR-P4-T-W	16380	253	3657	122	18300	1800	BDL	BDL
	SFR-P4-M1-W	22133	9439	2734	144	14400	1900	BDL	BDL
	SFR-P4-M2-W	21297	258	3813	109	18900	600	BDL	BDL
	SFR-P4-B-W	16935	369	7982	547	17300	1000	BDL	BDL
SFR-L1	SFR-L1-D1-W	3122	96	2910	211	BDL	BDL	BDL	BDL
	SFR-L1-D2-W	3195	130	2323	109	BDL	BDL	BDL	BDL
	SFR-L1-D3-W	3451	62	3533	174	BDL	BDL	BDL	BDL
	SFR-L1-D4-W	6980	567	8613	292	BDL	BDL	BDL	BDL
	SFR-L1-S1-W	2371	68	2278	77	BDL	BDL	BDL	BDL
	SFR-L1-S2-W	3591	124	2523	492	BDL	BDL	BDL	BDL
	SFR-L1-S3-W	2698	45	6434	5	BDL	BDL	BDL	BDL
	SFR-L1-S4-W	2817	81	6232	9	BDL	BDL	BDL	BDL

Table A 20. GC-MS estrone and 17 α -estradiol values for swine farrowing operations sampled.

Farm ID	Sample ID	GC-MS Estrone (ppt)				GC-MS 17 α -estradiol (ppt)			
		Winter	Winter-Std	Summer	Summer-Std	Winter	Winter-Std	Summer	Summer-Std
SFR-P1	SFR-P1-T-W	14840	1900	37100	5400	BDL	BDL	BDL	BDL
	SFR-P1-M1-W	16300	3800	40000	6200	BDL	BDL	BDL	BDL
	SFR-P1-M2-W	18600	1100	37000	4800	BDL	BDL	BDL	BDL
	SFR-P1-B-W	31300	9500	53500	5800	BDL	BDL	BDL	BDL
SFR-P2	SFR-P2-T-W	30300	3400	117129	17600	BDL	BDL	10036	1000
	SFR-P2-M1-W	30900	2400	23400	7000	BDL	BDL	3600	1900
	SFR-P2-M2-W	90400	6600	35900	500	6500	1100	4600	1400
	SFR-P2-B-W	90000	6600	38800	6600	6200	1000	3700	1200
SFR-P3	SFR-P3-T-W	143800	300	80000	11300	17400	1600	11000	2500
	SFR-P3-M1-W	173300	2600	70000	9500	20500	1000	11100	1500
	SFR-P3-M2-W	169700	2600	102400	1100	18500	1600	13600	800
	SFR-P3-B-W	121900	3500	148900	26800	14500	1700	20800	3600
SFR-P4	SFR-P4-T-W	47900	6400	10600	6900	BDL	BDL	BDL	BDL
	SFR-P4-M1-W	41800	4300	5400	4100	BDL	BDL	BDL	BDL
	SFR-P4-M2-W	62500	2800	9300	5500	BDL	BDL	BDL	BDL
	SFR-P4-B-W	54300	1000	19600	2200	BDL	BDL	BDL	BDL
SFR-L1	SFR-L1-D1-W	14700	1800	BDL	BDL	BDL	BDL	BDL	BDL
	SFR-L1-D2-W	13400	400	BDL	BDL	BDL	BDL	BDL	BDL
	SFR-L1-D3-W	9300	1900	BDL	BDL	BDL	BDL	BDL	BDL
	SFR-L1-D4-W	12600	3100	BDL	BDL	BDL	BDL	BDL	BDL
	SFR-L1-S1-W	6700	600	BDL	BDL	BDL	BDL	BDL	BDL
	SFR-L1-S2-W	8000	600	BDL	BDL	BDL	BDL	BDL	BDL
	SFR-L1-S3-W	7800	1000	BDL	BDL	BDL	BDL	BDL	BDL
	SFR-L1-S4-W	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL

Table A 21. TS, VS, ash, and COD values for swine farrowing operations sampled.

Farm ID	Sample ID	Total Solids (%)		Volatile Solids (%)		Ash (%)		COD (ppm)	
		Winter	Summer	Winter	Summer	Winter	Summer	Winter	Summer
SFR-P1	SFR-P1-T-W	1.1	1.1	1.1	63	99	38	6648	7970
	SFR-P1-M1-W	1.2	1.0	67	64	33	36	7024	6180
	SFR-P1-M2-W	1.4	1.1	85	67	15	33	7143	6260
	SFR-P1-B-W	1.7	1.4	68	67	32	33	12036	11720
SFR-P2	SFR-P2-T-W	0.88	3.4	85	55	15	45	5010	15820
	SFR-P2-M1-W	0.71	1.5	64	77	36	23	5350	4380
	SFR-P2-M2-W	1.9	2.0	82	56	18	44	11640	5490
	SFR-P2-B-W	1.6	2.0	88	61	13	39	8660	7000
SFR-P3	SFR-P3-T-W	0.88	1.1	62	73	38	27	14900	4800
	SFR-P3-M1-W	1	0.9	67	78	33	22	14985	3590
	SFR-P3-M2-W	1	1.1	86	58	14	42	15854	4760
	SFR-P3-B-W	0.87	1.8	85	68	15	32	14763	8950
SFR-P4	SFR-P4-T-W	1.3	0.8	68	71	32	29	8350	16700
	SFR-P4-M1-W	0.96	0.8	79	60	21	40	4390	14600
	SFR-P4-M2-W	1.3	0.8	74	71	26	29	7460	17700
	SFR-P4-B-W	1.2	1.2	76	45	24	55	6730	13400
SFR-L1	SFR-L1-D1-W	0.67	0.9	90	77	10	23	630	660
	SFR-L1-D2-W	0.54	0.8	75	73	25	27	600	550
	SFR-L1-D3-W	0.68	0.9	80	83	20	17	6700	510
	SFR-L1-D4-W	0.95	1.3	79	67	21	33	2250	4050
	SFR-L1-S1-W	0.48	1.0	86	86	14	14	580	1740
	SFR-L1-S2-W	0.54	1.0	75	86	25	14	590	530
	SFR-L1-S3-W	0.82	0.9	83	85	17	15	630	420
	SFR-L1-S4-W	0.82	0.8	75	55	25	45	610	620

Table A 22. N, P, K, and temperature values for swine farrowing operations sampled.

Farm ID	Sample ID	Nitrogen (ppm)		Phosphorous (ppm)		Potassium (ppm)		Temperature (°C)	
		Winter	Summer	Winter	Summer	Winter	Summer	Winter	Summer
SFR-P1	SFR-P1-T-W	1058	673	320	444	668	599	14.2	16.7
	SFR-P1-M1-W	1152	682	324	361	625	631	14.2	16.8
	SFR-P1-M2-W	1097	706	335	343	642	598	14.1	16.7
	SFR-P1-B-W	1334	406	601	542	655	598	14.1	16.8
SFR-P2	SFR-P2-T-W	1629	2869	177	1778	1019	991	20.7	28
	SFR-P2-M1-W	1955	1411	207	263	1032	898	20.7	26.4
	SFR-P2-M2-W	2017	1872	737	964	1032	943	20.4	26.4
	SFR-P2-B-W	1884	1813	514	534	1023	941	21.2	27.1
SFR-P3	SFR-P3-T-W	2172	1479	288	180	1221	873	16.6	22.5
	SFR-P3-M1-W	2253	1401	296	128	1210	779	17.1	21.1
	SFR-P3-M2-W	2120	1616	272	223	1202	865	16.6	21.6
	SFR-P3-B-W	2183	2323	285	632	1229	730	16.8	21.5
SFR-P4	SFR-P4-T-W	610	710	141	51	776	659	16.8	21.1
	SFR-P4-M1-W	1373	801	257	87	698	641	16.8	21
	SFR-P4-M2-W	1192	748	373	80	800	673	17	21
	SFR-P4-B-W	1252	894	280	252	745	735	17.2	22
SFR-L1	SFR-L1-D1-W	127	177	55	64	163	193	10.2	19.7
	SFR-L1-D2-W	113	148	56	58	152	1485	10.2	18.9
	SFR-L1-D3-W	91	225	53	63	216	1517	10.3	20
	SFR-L1-D4-W	206	445	162	251	192	207	10.4	20.4
	SFR-L1-S1-W	83	280	62	156	157	157	11	19.7
	SFR-L1-S2-W	162	106	139	53	141	155	10.5	19.3
	SFR-L1-S3-W	151	141	42	57	267	156	10.5	19.7
	SFR-L1-S4-W	76	125	68	76	153	150	10.4	20

Table A 23. ELISA and GC-MS 17 β -estradiol values for swine finishing lagoon operations sampled.

Farm ID	Sample ID	Elisa 17 β -estradiol (ppt)				GC-MS 17 β -estradiol (ppt)			
		Winter	Winter-Std	Summer	Summer-Std	Winter	Winter-Std	Summer	Summer-Std
SFI-L1	SFR-L1-D1-W	404	18	3276	88	BDL	BDL	BDL	BDL
	SFR-L1-D2-W	589	34	4646	157	BDL	BDL	BDL	BDL
	SFR-L1-D3-W	381	27	4047	88	BDL	BDL	BDL	BDL
	SFR-L1-D4-W	661	106	5501	137	BDL	BDL	BDL	BDL
	SFR-L1-S1-W	355	22	2011	55	BDL	BDL	BDL	BDL
	SFR-L1-S2-W	298	42	1942	75	BDL	BDL	BDL	BDL
	SFR-L1-S3-W	6104	523	1756	64	BDL	BDL	BDL	BDL
	SFR-L1-S4-W	5513	570	874	64	BDL	BDL	BDL	BDL
SFI-L2	SFR-L2-D1-W	1088	51	1614	62	BDL	BDL	BDL	BDL
	SFR-L2-D2-W	719	24	3724	64	BDL	BDL	BDL	BDL
	SFR-L2-D3-W	863	74	1756	22	BDL	BDL	BDL	BDL
	SFR-L2-D4-W	861	55	7075	264	BDL	BDL	BDL	BDL
	SFR-L2-S1-W	1191	38	1061	33	BDL	BDL	BDL	BDL
	SFR-L2-S2-W	1037	58	2827	127	BDL	BDL	BDL	BDL
	SFR-L2-S3-W	978	78	1898	79	BDL	BDL	BDL	BDL
	SFR-L2-S4-W	1110	41	1705	58	BDL	BDL	BDL	BDL
SFI-L3	SFR-L3-D1-W	3602	34	2815	117	BDL	BDL	BDL	BDL
	SFR-L3-D2-W	3002	106	2736	74	BDL	BDL	BDL	BDL
	SFR-L3-D3-W	2502	81	2777	72	BDL	BDL	BDL	BDL
	SFR-L3-D4-W	10679	1115	6247	252	BDL	BDL	BDL	BDL
	SFR-L3-S1-W	2865	102	3040	118	BDL	BDL	BDL	BDL
	SFR-L3-S2-W	3461	149	2220	68	BDL	BDL	BDL	BDL
	SFR-L3-S3-W	5225	510	4301	88	BDL	BDL	BDL	BDL
	SFR-L3-S4-W	2602	49	2800	125	BDL	BDL	BDL	BDL

Table A 24. GC-MS estrone and 17 α -estradiol values for swine finishing lagoon operations sampled.

ID	Sample ID	GC-MS Estrone (ppt)				GC-MS 17 α -estradiol (ppt)			
		Winter	Std	Summer	Std	Winter	Std	Summer	Std
SFI-L1	SFR-L1-D1-W	BDL	BDL	19100	3000	BDL	BDL	BDL	BDL
	SFR-L1-D2-W	BDL	BDL	18900	2100	BDL	BDL	BDL	BDL
	SFR-L1-D3-W	BDL	BDL	16000	2800	BDL	BDL	BDL	BDL
	SFR-L1-D4-W	BDL	BDL	20300	6900	BDL	BDL	BDL	BDL
	SFR-L1-S1-W	BDL	BDL	22500	3400	BDL	BDL	BDL	BDL
	SFR-L1-S2-W	BDL	BDL	14500	3000	BDL	BDL	BDL	BDL
	SFR-L1-S3-W	BDL	BDL	20100	6500	BDL	BDL	BDL	BDL
SFI-L2	SFR-L1-S4-W	BDL	BDL	19300	4000	BDL	BDL	BDL	BDL
	SFR-L2-D1-W	BDL	BDL	6500	3600	BDL	BDL	BDL	BDL
	SFR-L2-D2-W	BDL	BDL	9900	1800	BDL	BDL	BDL	BDL
	SFR-L2-D3-W	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
	SFR-L2-D4-W	BDL	BDL	28100	9500	BDL	BDL	BDL	BDL
	SFR-L2-S1-W	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
	SFR-L2-S2-W	BDL	BDL	13900	1400	BDL	BDL	BDL	BDL
SFI-L3	SFR-L2-S3-W	BDL	BDL	6200	6100	BDL	BDL	BDL	BDL
	SFR-L2-S4-W	BDL	BDL	4200	2500	BDL	BDL	BDL	BDL
	SFR-L3-D1-W	12100	1200	8900	3000	BDL	BDL	BDL	BDL
	SFR-L3-D2-W	10700	1800	9400	1200	BDL	BDL	BDL	BDL
	SFR-L3-D3-W	15600	4000	13900	2000	BDL	BDL	BDL	BDL
	SFR-L3-D4-W	29300	7600	28200	6200	BDL	BDL	BDL	BDL
	SFR-L3-S1-W	11900	2900	15200	4900	BDL	BDL	BDL	BDL
	SFR-L3-S2-W	10400	1700	13200	4200	BDL	BDL	BDL	BDL
	SFR-L3-S3-W	13500	3400	12300	4200	BDL	BDL	BDL	BDL
	SFR-L3-S4-W	15600	3900	12300	5900	BDL	BDL	BDL	BDL

Table A 25. TS, VS, ash, and COD values for swine finishing lagoon operations sampled.

Farm ID	Sample ID	Total Solids (%)		Volatile Solids (%)		Ash (%)		COD (ppm)	
		Winter	Summer	Winter	Summer	Winter	Summer	Winter	Summer
SFI-L1	SFR-L1-D1-W	1.2	0.8	89	82	11	18	1176	700
	SFR-L1-D2-W	0.96	0.6	86	88	14	13	1142	700
	SFR-L1-D3-W	0.96	0.6	86	89	14	11	1108	710
	SFR-L1-D4-W	1.2	1.0	76	86	24	14	2608	3060
	SFR-L1-S1-W	1.3	0.8	84	82	16	18	1381	730
	SFR-L1-S2-W	1.2	0.6	82	78	18	22	1244	780
	SFR-L1-S3-W	1.1	0.6	94	78	6.3	22	1193	700
	SFR-L1-S4-W	1	0.7	80	90	20	10	1142	710
SFI-L2	SFR-L2-D1-W	0.68	1.1	70	69	30	31	790	3700
	SFR-L2-D2-W	0.89	2.1	85	57	15	43	850	13900
	SFR-L2-D3-W	1.1	1.0	81	86	19	14	1700	3400
	SFR-L2-D4-W	1.2	3.7	76	62	24	38	1640	18800
	SFR-L2-S1-W	0.94	0.9	86	83	14	17	1500	1700
	SFR-L2-S2-W	1.2	1.7	71	58	29	42	1040	7400
	SFR-L2-S3-W	1	1.1	80	60	20	40	1180	3300
	SFR-L2-S4-W	1	1.1	73	64	27	36	1100	3000
SFI-L3	SFR-L3-D1-W	0.88	0.6	62	67	38	33	1150	1070
	SFR-L3-D2-W	1.1	0.6	69	75	31	25	1210	1190
	SFR-L3-D3-W	1	0.7	80	80	20	20	1200	1120
	SFR-L3-D4-W	1.4	1.1	75	60	25	40	4483	3920
	SFR-L3-S1-W	0.96	0.8	71	55	29	46	1180	1650
	SFR-L3-S2-W	0.89	0.6	69	63	31	38	1210	1100
	SFR-L3-S3-W	0.96	0.8	64	55	36	46	1180	1210
	SFR-L3-S4-W	1.17	0.7	97	70	2.7	30	1260	1200

Table A 26. N, P, K, and temperature values for swine finishing lagoon operations sampled.

Farm ID	Sample ID	Nitrogen		Phosphorous		Potassium		Temperature	
		Winter	Summer	Winter	Summer	Winter	Summer	Winter	Summer
SFI-L1	SFR-L1-D1-W	328	166	73	63	637	385	10.4	25.1
	SFR-L1-D2-W	284	266	72	70	315	348	10.5	21.5
	SFR-L1-D3-W	265	276	71	70	342	365	10.4	21.3
	SFR-L1-D4-W	317	458	131	161	329	391	10.5	20.2
	SFR-L1-S1-W	297	239	74	64	311	359	10.6	28.4
	SFR-L1-S2-W	252	342	76	74	326	334	10.8	29.6
	SFR-L1-S3-W	288	269	73	69	327	350	10.7	26
	SFR-L1-S4-W	353	240	77	68	312	339	10.9	27.3
SFI-L2	SFR-L2-D1-W	440	546	124	225	267	369	14.2	26
	SFR-L2-D2-W	401	1134	129	878	238	409	13.9	23.2
	SFR-L2-D3-W	619	554	221	202	268	388	13.3	25.1
	SFR-L2-D4-W	469	1948	271	1824	250	429	14.8	26.3
	SFR-L2-S1-W	436	412	112	87	265	394	13.3	27
	SFR-L2-S2-W	405	1096	113	801	256	381	14.2	27.6
	SFR-L2-S3-W	502	557	157	309	221	432	14.3	28.4
	SFR-L2-S4-W	432	629	141	278	250	377	15.6	28.2
SFI-L3	SFR-L3-D1-W	388	425	31	49	613	697	10.9	20.9
	SFR-L3-D2-W	378	231	56	95	619	604	10.7	21.6
	SFR-L3-D3-W	698	693	104	50	603	614	10.6	21.7
	SFR-L3-D4-W	549	907	235	159	612	714	10.6	22
	SFR-L3-S1-W	352	703	54	127	667	574	11.8	21.3
	SFR-L3-S2-W	351	1298	38	49	601	609	12.5	23.1
	SFR-L3-S3-W	406	351	57	76	600	601	12.2	21.4
	SFR-L3-S4-W	314	978	52	52	603	590	12.5	22

Table A 27. ELISA and GC-MS 17 β -estradiol, estrone, 17 α -estradiol, TS, VS, ash, COD, N, P, K, and temperature values for swine finishing hoop structures sampled.

Farm ID	Sample ID	Elisa 17 β -estradiol (ppt)				GC-MS 17 β -estradiol (ppt)			
		Winter	Winter-Std	Summer	Summer-Std	Winter	Winter-Std	Summer	Summer-Std
SFI-H1	SFI-H1-T-W	30003	1875	41968	2712	24600	6200	BDL	BDL
	SFI-H1-M-W	31066	911	33481	1155	13500	4300	BDL	BDL
	SFI-H1-B-W	35302	109	42342	2125	14600	1700	BDL	BDL
SFI-H2	SFI-H2-T-W	55721	3746	82546	2054	36400	9700	BDL	BDL
	SFI-H2-M-W	47844	12660	50014	1955	12800	1500	BDL	BDL
	SFI-H2-B-W	43111	3338	270137	2606	17900	1200	BDL	BDL
SFI-H3	SFI-H3-T-W	35318	1060	26289	1244	63700	8900	BDL	BDL
	SFI-H3-M-W	38940	315	22858	1492	56500	4400	BDL	BDL
	SFI-H3-B-W	47338	1023	32933	318	79200	14900	BDL	BDL

Farm ID	Sample ID	GC-MS Estrone (ppt)				GC-MS 17 α -estradiol (ppt)			
		Winter	Std	Summer	Std	Winter	Std	r	Std
SFI-H1	SFI-H1-T-W	29700	1300	7000	5000	BDL	BDL	BDL	BDL
	SFI-H1-M-W	21800	5000	10800	11400	BDL	BDL	BDL	BDL
	SFI-H1-B-W	37700	7900	11500	12800	BDL	BDL	BDL	BDL
SFI-H2	SFI-H2-T-W	80700	13700	9100	8600	BDL	BDL	BDL	BDL
	SFI-H2-M-W	58100	6600	20700	28700	BDL	BDL	BDL	BDL
	SFI-H2-B-W	96000	14400	9100	8500	BDL	BDL	BDL	BDL
SFI-H3	SFI-H3-T-W	128400	24400	BDL	BDL	BDL	BDL	BDL	BDL
	SFI-H3-M-W	100400	12100	BDL	BDL	BDL	BDL	BDL	BDL
	SFI-H3-B-W	100900	11600	BDL	BDL	BDL	BDL	BDL	BDL

Farm ID	Sample ID	Total Solids (%)		Volatile Solids (%)		Ash (%)		COD (ppm)	
		Winter	Summer	Winter	Summer	Winter	Summer	Winter	Summer
SFI-H1	SFI-H1-T-W	14.5	25.4	58.3	67	41.7	33	93883	19600
	SFI-H1-M-W	19.1	35.0	76.5	64	23.5	36	58253	22600
	SFI-H1-B-W	20.7	19.2	70.4	58	29.6	42	54502	47900
SFI-H2	SFI-H2-T-W	18	22.0	84	69	16	31	53100	35600
	SFI-H2-M-W	15	15.9	83	79	17	21	35700	28800
	SFI-H2-B-W	16	23.6	75	69	25	31	61500	24900
SFI-H3	SFI-H3-T-W	13	29.1	81	64	19	36	102407	112400
	SFI-H3-M-W	13	42.8	82	66	18	34	103941	76000
	SFI-H3-B-W	16	41.6	82	38	18	62	91496	146800

Farm ID	Sample ID	Nitrogen (ppm)		Phosphorous (ppm)		Potassium (ppm)		Temperature (°C)	
		Winter	Summer	Winter	Summer	Winter	Summer	Winter	Summer
SFI-H1	SFI-H1-T-W	9497	9392	3466	3498	2416	7475	16.7	31.2
	SFI-H1-M-W	6747	11583	2261	7720	2294	9554	16.8	31.1
	SFI-H1-B-W	7458	6462	2630	2662	2178	3738	17.1	31.1
SFI-H2	SFI-H2-T-W	6372	4780	2308	2107	2186	4972	17.4	33.6
	SFI-H2-M-W	5913	4055	2240	1672	3293	4275	17.5	32
	SFI-H2-B-W	5690	7196	1821	3204	2792	5171	18.4	31
SFI-H3	SFI-H3-T-W	5776	14180	2193	3960	2944	2265	22	34.5
	SFI-H3-M-W	5511	16135	1790	5741	2744	6714	21.5	34.2
	SFI-H3-B-W	4721	11461	1725	3964	2948	5508	21.2	38

Table A 28. ELISA and GC-MS 17 β -estradiol values for dairy holding ponds sampled.

Farm ID	Sample ID	Elisa 17 β -estradiol (ppt)				GC-MS 17 β -estradiol (ppt)			
		Winter	Winter-Std	Summer	Summer-Std	Winter	Winter-Std	Summer	Summer-Std
D-HP1	D-HP1-D1-W	594	23	676	32	BDL	BDL	BDL	BDL
	D-HP1-D2-W	800	48	885	48	BDL	BDL	BDL	BDL
	D-HP1-D3-W	615	17	1643	45	BDL	BDL	BDL	BDL
	D-HP1-D4-W	2322	79	773	31	BDL	BDL	BDL	BDL
	D-HP1-S1-W	371	36	484	22	BDL	BDL	BDL	BDL
	D-HP1-S2-W	481	24	973	48	BDL	BDL	BDL	BDL
	D-HP1-S3-W	702	39	409	34	BDL	BDL	BDL	BDL
	D-HP1-S4-W	483	29	868	20	BDL	BDL	BDL	BDL
D-HP2	D-HP2-D1-W	1334	42	1140	159	5400	5000	BDL	BDL
	D-HP2-D2-W	1488	43	1961	94	3000	2400	BDL	BDL
	D-HP2-D3-W	3810	124	3112	252	2500	1700	BDL	BDL
	D-HP2-D4-W	3173	99	1695	111	2100	900	BDL	BDL
	D-HP2-S1-W	1486	91	1314	89	BDL	BDL	BDL	BDL
	D-HP2-S2-W	1115	86	1002	105	BDL	BDL	BDL	BDL
	D-HP2-S3-W	1257	540	1542	79	BDL	BDL	BDL	BDL
	D-HP2-S4-W	763	134	1558	26	BDL	BDL	BDL	BDL
D-HP3	D-HP3-D1-W	1912	139	1523	47	BDL	BDL	2300	200
	D-HP3-D2-W	1781	105	1520	60	BDL	BDL	BDL	BDL
	D-HP3-D3-W	1983	56	2851	166	BDL	BDL	BDL	BDL
	D-HP3-D4-W	2444	106	3176	243	BDL	BDL	BDL	BDL
	D-HP3-S1-W	1641	69	2809	227	4800	BDL	BDL	BDL
	D-HP3-S2-W	1676	151	1117	32	BDL	BDL	BDL	BDL
	D-HP3-S3-W	1942	39	1301	33	BDL	BDL	BDL	BDL
	D-HP3-S4-W	1975	220	1401	50	BDL	BDL	BDL	BDL

Table A29. GC-MS estrone and 17 α -estradiol values for dairy holding ponds sampled.

ID	Sample ID	GC-MS Estrone (ppt)				GC-MS 17 α -estradiol (ppt)			
		Winter	Std	Summer	Std	Winter	Std	Summer	Std
D-HP1	D-HP1-D1-W	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
	D-HP1-D2-W	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
	D-HP1-D3-W	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
	D-HP1-D4-W	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
	D-HP1-S1-W	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
	D-HP1-S2-W	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
	D-HP1-S3-W	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
	D-HP1-S4-W	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
D-HP2	D-HP2-D1-W	16800	12800	3000	2400	3200	1500	BDL	BDL
	D-HP2-D2-W	7600	4700	8300	2400	2500	1600	5100	1800
	D-HP2-D3-W	10500	7300	10000	6200	2700	1900	9600	100
	D-HP2-D4-W	9000	7300	4000	2100	3100	2600	BDL	1500
	D-HP2-S1-W	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
	D-HP2-S2-W	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
	D-HP2-S3-W	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
	D-HP2-S4-W	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
D-HP3	D-HP3-D1-W	BDL	BDL	5300	5800	BDL	BDL	BDL	500
	D-HP3-D2-W	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
	D-HP3-D3-W	BDL	BDL	8300	8100	BDL	BDL	4600	2200
	D-HP3-D4-W	BDL	BDL	8900	7400	BDL	BDL	8000	4800
	D-HP3-S1-W	5900	4300	11600	800	BDL	BDL	10700	1900
	D-HP3-S2-W	5500	3500	4100	3700	BDL	BDL	3700	2100
	D-HP3-S3-W	5600	3800	5500	3600	5100	3100	5800	3300
	D-HP3-S4-W	10600	12500	3800	1500	BDL	BDL	3300	1000

Table A30. TS, VS, ash, and COD values for dairy holding ponds sampled.

Farm ID	Sample ID	Total Solids (%)		Volatile Solids (%)		Ash (%)		COD (ppm)	
		Winter	Summer	Winter	Summer	Winter	Summer	Winter	Summer
D-HP1	D-HP1-D1-W	0.63	0.7	33.3	70	66.7	30	3000	1910
	D-HP1-D2-W	0.69	0.9	90	62	10	39	2790	3510
	D-HP1-D3-W	0.51	0.9	42.9	69	57.1	31	4380	3820
	D-HP1-D4-W	1.5	1.0	53.8	64	46.2	36	10930	3970
	D-HP1-S1-W	0.45	0.5	50	86	50	14	3270	1500
	D-HP1-S2-W	0.48	0.6	57.1	78	42.9	22	4780	1530
	D-HP1-S3-W	0.52	0.6	40	67	60	33	3120	1550
	D-HP1-S4-W	0.44	0.6	66.7	67	33.3	33	2580	1500
D-HP2	D-HP2-D1-W	2.34	1.1	85.7	56	14.3	44	5520	3920
	D-HP2-D2-W	2.31	1.4	70.5	70	29.5	30	4920	7160
	D-HP2-D3-W	2.44	2.0	74.5	75	25.5	25	6110	13100
	D-HP2-D4-W	2.61	1.6	72.5	64	27.5	36	6320	6900
	D-HP2-S1-W	1.75	1.2	70.6	59	29.4	41	5550	4710
	D-HP2-S2-W	2.01	1.1	77	63	23.1	38	5150	3070
	D-HP2-S3-W	2.11	1.2	78	59	22	41	7211	3120
	D-HP2-S4-W	2.03	1.1	70	56	30	44	7518	3030
D-HP3	D-HP3-D1-W	1.8	1.4	65	40	35	60	14930	5160
	D-HP3-D2-W	1.7	2.0	64	67	36	33	14660	4820
	D-HP3-D3-W	1.7	2.2	64	59	36	41	14690	9240
	D-HP3-D4-W	1.7	2.9	68	55	32	45	15780	16000
	D-HP3-S1-W	1.7	1.8	75	60	25	40	14730	10590
	D-HP3-S2-W	1.6	1.5	61	57	39	43	14760	4150
	D-HP3-S3-W	1.6	1.5	70	54	30	46	14590	3990
	D-HP3-S4-W	1.7	1.4	64	53	36	47	15040	4170

Table A31. N, P, K, and temperature values for dairy holding ponds sampled.

Farm ID	Sample ID	Nitrogen (ppm)		Phosphorous (ppm)		Potassium (ppm)		Temperature (°C)	
		Winter	Summer	Winter	Summer	Winter	Summer	Winter	Summer
D-HP1	D-HP1-D1-W	554	111	261	85	389	456	4.6	18.4
	D-HP1-D2-W	328	327	83	130	407	471	3.6	18.3
	D-HP1-D3-W	274	219	87	121	471	1107	3.3	17.8
	D-HP1-D4-W	246	164	79	113	385	613	3.1	16.8
	D-HP1-S1-W	295	170	102	82	435	473	3.2	18.3
	D-HP1-S2-W	604	110	84	85	363	472	1.4	18.1
	D-HP1-S3-W	635	106	97	102	441	462	3.2	17.9
	D-HP1-S4-W	363	103	85	92	453	1835	1.8	18.1
D-HP2	D-HP2-D1-W	604	676	84	87	618	593	13.5	23
	D-HP2-D2-W	635	867	97	140	578	569	13.1	22.9
	D-HP2-D3-W	363	1271	85	248	618	583	13.1	24.4
	D-HP2-D4-W	826	960	223	136	621	594	12.8	23.8
	D-HP2-S1-W	620	717	89	69	589	546	13.4	23.1
	D-HP2-S2-W	625	586	95	75	602	592	13.3	22.5
	D-HP2-S3-W	497	684	79	117	593	609	13.2	22.8
	D-HP2-S4-W	461	597	75	96	603	612	15.4	23.3
D-HP3	D-HP3-D1-W	978	695	157	92	656	963	9.8	29.2
	D-HP3-D2-W	981	658	162	80	643	977	8.4	28.7
	D-HP3-D3-W	963	938	160	161	702	1088	8.7	28.4
	D-HP3-D4-W	962	1097	173	226	749	991	9.5	29
	D-HP3-S1-W	1097	1264	178	139	646	1267	9.8	28.4
	D-HP3-S2-W	849	854	161	69	698	1094	12.8	28.4
	D-HP3-S3-W	906	846	131	69	705	1089	12.3	28.5
	D-HP3-S4-W	978	773	98	60	643	1165	11.3	29.2

Table A32. ELISA and GC-MS 17 β -estradiol values for dairy dry stacks-solid and semisolid sampled.

Farm ID	Sample ID	Elisa 17 β -estradiol (ppt)				GC-MS 17 β -estradiol (ppt)			
		Winter	Winter-Std	Summer	Summer-Std	Winter	Winter-Std	Summer	Summer-Std
D-WS1	D-WS1-NT-W	14254	995	15142	88	45500	7800	47600	9300
	D-WS1-NM-W	18373	2697	14330	99	38100	4400	61300	21400
	D-WS1-NB-W	10470	465	20296	919	28600	5300	53100	6200
	D-WS1-OT-W	14934	1309	13599	278	34500	5500	77400	18800
	D-WS1-OM-W	12209	963	8931	112	26000	5500	29600	17900
D-WS2	D-WS1-OB-W	9542	690	11802	113	21300	6500	40700	13900
	D-WS2-NT-W	13000	521	25255	991	20800	7200	19900	600
	D-WS2-NM-W	18602	2219	32417	537	23800	3600	21400	2600
	D-WS2-NB-W	16330	521	26788	3883	30400	6600	26600	2800
	D-WS2-OT-W	19333	1073	27981	845	28500	9600	21600	2200
D-WS3	D-WS2-OM-W	18035	1644	18824	1728	26300	7200	5900	8100
	D-WS2-OB-W	20116	1070	20646	581	24900	900	20400	1600
	D-WS3-NT-W	4468	244	84805	151	13100	3900	219800	26600
	D-WS3-NM-W	6420	689	36898	540	20100	10800	61900	14400
	D-WS3-NB-W	5627	408	37474	656	19400	5700	52400	7800
D-DS1	D-WS3-OT-W	7900	1775	48487	1076	15400	2900	65300	11100
	D-WS3-OM-W	7879	507	26785	467	14100	1400	66200	5500
	D-WS3-OB-W	8214	442	52663	1199	BDL	0.0	75900	2100
	D-DS1-NT-W	7802	305	17195	2995	36400	6100	BDL	BDL
	D-DS1-NM-W	11166	565	22178	1159	50300	4800	BDL	BDL
D-DS2	D-DS1-NB-W	7808	301	33326	2123	37500	6400	28600	4100
	D-DS1-OT-W	3363	487	22923	1065	BDL	BDL	18200	1000
	D-DS1-OM-W	9266	559	24050	1330	BDL	BDL	11700	1400
	D-DS1-OB-W	5916	377	20531	1526	BDL	BDL	12200	1600
	D-DS2-NT-W	15727	926	11466	601	9900	3900	BDL	BDL
	D-DS2-NM-W	17850	378	3405	222	20800	2800	BDL	BDL
	D-DS2-NB-W	14688	562	2412	113	11100	8200	BDL	BDL
	D-DS2-OT-W	3223	111	1318	55	BDL	BDL	BDL	BDL
	D-DS2-OM-W	390	21	777	45	BDL	BDL	BDL	BDL
	D-DS2-OB-W	1792	97	2163	77	BDL	BDL	BDL	BDL

Table A33. GC-MS estrone and 17 α -estradiol values for dairy dry stack-solid and semisolid sampled.

Farm ID	Sample ID	GC-MS Estrone (ppt)				GC-MS 17 α -estradiol (ppt)			
		Winter	Std	Summer	Std	Winter	Std	Summer	Std
D-WS1	D-WS1-NT-W	BDL	BDL	37000	17200	100800	23800	86700	64800
	D-WS1-NM-W	BDL	BDL	43500	11600	81900	11900	128800	18200
	D-WS1-NB-W	36200	23800	43600	17000	69500	26000	149700	23700
	D-WS1-OT-W	109600	17100	60600	39500	104400	16500	158100	24600
	D-WS1-OM-W	93200	22900	41700	18600	83500	13300	94000	10100
	D-WS1-OB-W	80000	15600	58200	28500	68200	14600	136000	17600
D-WS2	D-WS2-NT-W	4800	2900	14300	1900	79000	18400	38500	5300
	D-WS2-NM-W	12900	3900	19800	3700	80900	13200	47200	8100
	D-WS2-NB-W	17500	10100	28900	6000	75000	23900	53600	4700
	D-WS2-OT-W	17900	10100	7600	0	97500	13200	56000	7000
	D-WS2-OM-W	3500	2000	7400	5300	97400	18000	27600	1500
	D-WS2-OB-W	7600	0	17900	0	95700	800	49400	9100
D-WS3	D-WS3-NT-W	BDL	BDL	128900	1400	BDL	BDL	249800	39200
	D-WS3-NM-W	BDL	BDL	187200	25900	BDL	BDL	231000	17400
	D-WS3-NB-W	BDL	BDL	184200	12600	BDL	BDL	237500	32800
	D-WS3-OT-W	16100	2200	151700	37100	20100	3700	132700	29100
	D-WS3-OM-W	BDL	BDL	128900	12900	3500	3600	153800	10800
	D-WS3-OB-W	BDL	BDL	147300	11800	BDL	BDL	170600	15800
D-DS1	D-DS1-NT-W	43000	10700	9300	7500	66900	13300	BDL	BDL
	D-DS1-NM-W	59900	7700	5100	6700	92300	4200	BDL	BDL
	D-DS1-NB-W	43800	3000	14000	7200	55600	9300	36100	15200
	D-DS1-OT-W	17900	1700	7500	6400	BDL	BDL	14900	3600
	D-DS1-OM-W	106400	17500	12400	1900	BDL	BDL	10400	3300
	D-DS1-OB-W	64100	4200	13600	1700	BDL	BDL	10500	3600
D-DS2	D-DS2-NT-W	62300	5500	94500	47600	70400	5100	BDL	BDL
	D-DS2-NM-W	71900	10800	24300	5000	112700	6900	BDL	BDL
	D-DS2-NB-W	114900	10000	BDL	BDL	133200	5300	BDL	BDL
	D-DS2-OT-W	17900	0	BDL	BDL	BDL	BDL	BDL	BDL
	D-DS2-OM-W	1600	0	BDL	BDL	BDL	BDL	BDL	BDL
	D-DS2-OB-W	13700	2300	94500	47600	BDL	BDL	BDL	BDL

Table A34. TS, VS, ash, and COD values for dairy dry stack-solid and semisolid sampled.

Farm ID	Sample ID	Total Solids (%)		Volatile Solids (%)		Ash (%)		COD (ppm)	
		Winter	Summer	Winter	Summer	Winter	Summer	Winter	Summer
D-WS1	D-WS1-NT-W	14.6	14.8	67.3	76	32.7	24	112295	63900
	D-WS1-NM-W	14.7	16.9	68.9	77	31.2	23	64390.1	58600
	D-WS1-NB-W	14.9	14.3	68.2	72	31.2	28	83995.3	58000
	D-WS1-OT-W	13.7	17.5	59	69	41	31	51604.1	50800
	D-WS1-OM-W	13.2	12.9	59.1	66	40.9	34	49387.8	70800
	D-WS1-OB-W	11.9	13.8	59.4	67	40.6	33	57229.9	71100
D-WS2	D-WS2-NT-W	15.7	22.5	78	46	22	54	87745.8	63900
	D-WS2-NM-W	17.8	20.6	70	47	30	53	114682	58600
	D-WS2-NB-W	16.9	19.7	63.5	53	37	47	103771	58000
	D-WS2-OT-W	18.2	24.8	75.5	63	25	37	74959.8	50800
	D-WS2-OM-W	14.1	10.9	80	59	20	41	83654.3	70800
	D-WS2-OB-W	14.5	24.1	79.4	45	21	55	143834	71100
D-WS3	D-WS3-NT-W	13.5	19.3	62.4	75	37.6	25	80244.7	87600
	D-WS3-NM-W	17.7	16.5	53.9	71	46.1	29	87063.9	76700
	D-WS3-NB-W	17.2	17.4	52.3	73	47.7	27	53990.8	46600
	D-WS3-OT-W	18.4	19.8	58.2	72	41.8	28	29100.7	97100
	D-WS3-OM-W	16.5	17.3	59.3	69	40.7	31	37454.2	76800
	D-WS3-OB-W	16.6	24.0	59.8	74	40.2	26	50410.7	53000
D-DS1	D-DS1-NT-W	13.1	30.9	80.2	78	19.8	22	71038.8	123900
	D-DS1-NM-W	13.2	26.1	78.9	85	21.1	15	30976	77600
	D-DS1-NB-W	14.2	21.2	79.5	85	20.5	15	69845.4	69400
	D-DS1-OT-W	16.2	24.3	67.4	87	32.6	13	64731	36500
	D-DS1-OM-W	24.5	25.4	69.9	90	30.1	10	58252.8	46500
	D-DS1-OB-W	18.2	25.7	71	87	29	13	57059.4	55400
D-DS2	D-DS2-NT-W	14.1	24.4	64	53	36.1	47	39159	32900
	D-DS2-NM-W	10.2	30.0	66.4	40	33.6	60	47854	14000
	D-DS2-NB-W	11.5	33.8	70.7	40	29.3	60	46660	11300
	D-DS2-OT-W	35.1	24.4	43	49	56.9	51	52030	32700
	D-DS2-OM-W	14.3	17.7	39.1	45	60.9	55	118262	37600
	D-DS2-OB-W	17	16.1	35.9	55	64.1	45	152699	35400

Table A35. N, P, K, and temperature values for dairy dry stack-solid and semisolid sampled.

Farm ID	Sample ID	Nitrogen (ppm)		Phosphorous (ppm)		Potassium (ppm)		Temperature (°C)	
		Winter	Summer	Winter	Summer	Winter	Summer	Winter	Summer
D-WS1	D-WS1-NT-W	5109	4561	1133	1136	388	3647	13.4	24.1
	D-WS1-NM-W	4980	7199	1150	1723	364	5513	14.2	24.8
	D-WS1-NB-W	4772	6687	1084	1527	371	6129	14.5	29.3
	D-WS1-OT-W	5955	5375	1525	1333	543	3240	13.5	23.7
	D-WS1-OM-W	5010	4004	1481	1170	518	3263	14.4	25.8
	D-WS1-OB-W	4911	5784	1231	1506	442	4524	14.5	26.4
D-WS2	D-WS2-NT-W	5729	3736	1687	850	1823	1897	15.9	29.1
	D-WS2-NM-W	6315	3918	1617	1022	1907	1826	15.7	29.9
	D-WS2-NB-W	6912	4055	1903	1030	2082	1902	16.8	30.5
	D-WS2-OT-W	6049	5492	1479	1440	2123	2565	13.2	27
	D-WS2-OM-W	6264	2954	1323	729	1867	1762	14.8	30.5
	D-WS2-OB-W	5922	3483	1293	948	2031	1298	14.4	30.6
D-WS3	D-WS3-NT-W	2033	5079	1133	1428	569	3940	0.6	24.6
	D-WS3-NM-W	6485	5553	1150	1888	538	6003	0.4	24.7
	D-WS3-NB-W	8011	4825	1084	1677	517	5431	0.8	24.6
	D-WS3-OT-W	20360	5479	1525	1750	493	5815	2.6	26.5
	D-WS3-OM-W	6383	4846	1481	1651	395	3886	2.5	25.8
	D-WS3-OB-W	2980	4326	1231	1478	606	4987	1.7	26.2
D-DS1	D-DS1-NT-W	3047	10785	553	1466	1961	11342	21.6	31.5
	D-DS1-NM-W	2874	8428	592	1982	2021	6700	20.8	31.7
	D-DS1-NB-W	3281	5550	674	1808	1947	2748	19.7	28.6
	D-DS1-OT-W	4285	5272	1076	1311	4157	2740	38.7	30.2
	D-DS1-OM-W	2719	4776	896	1446	4500	3049	32.6	33
	D-DS1-OB-W	2689	6511	833	1095	3690	4838	26.1	33.8
D-DS2	D-DS2-NT-W	2143	4080	3232	919	2019	2868	3.5	50
	D-DS2-NM-W	2554	3041	3681	1044	2071	2163	5.2	44.2
	D-DS2-NB-W	6621	3916	3516	932	3481	2565	6.1	36.7
	D-DS2-OT-W	9771	5802	1892	2301	14147	6258	10.6	27.8
	D-DS2-OM-W	4985	3789	413	2246	8091	7243	14.7	25.5
	D-DS2-OB-W	5117	3412	392	1714	7616	4463	11.5	25.4

VITA

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