

SUPPORTING INFORMATION
Hormone Discharges from a Midwest Tile-Drained
Agroecosystem Receiving Animal Wastes

Heather E. Gall[†], Stephen A. Sassman[‡], Linda S. Lee^{‡}, Chad T. Jafvert[†]*

Purdue University, [†]School of Civil Engineering
[‡]Department of Agronomy, West Lafayette, IN 47907

*Corresponding Author: Telephone: (765) 494-8612; Fax (765) 496-2926
E-mail: lslee@purdue.edu

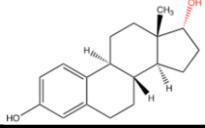
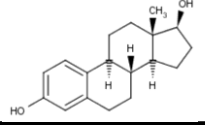
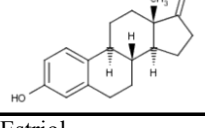
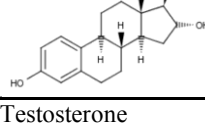
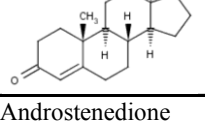
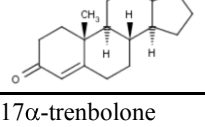
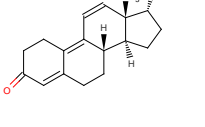
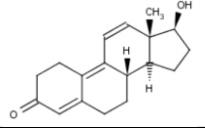
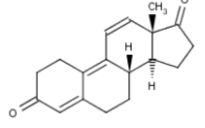
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1. Hormone Properties

Table SI-1. Properties of selected estrogens and androgens

Hormone	Molecular weight, (g mol ⁻¹)	Log K _{ow}	Log K _{oc}	Microbial Degradation Range in Half lives (t _{1/2} , d)
17α-estradiol 	272.4	3.73 ^a	2.97 ± 0.13 ^d	--
17β-estradiol 	272.4	3.76 ^a 4.01 ^b	3.14 ± 0.16 ^d 3.34 ± 0.18 ^e 3.03 ± ~0.2 ^f	0.8 – 9.7 ^g 0.4 – 2 ^j 0.3 – 0.5 ^k
Estrone 	270.4	3.53 ^a 3.38 ^c 4.54 ^b	3.20 ± 0.02 ^e 3.35 ± ~0.2 ^f	0.7 – 4.8 ⁱ 0.6 – 1.7 ^k
Estriol 	288.4	2.55 ^c 3.24 ^b	3.16 ± ~0.2 ^f	--
Testosterone 	288.4	3.22 ^c 3.84 ^b	3.32 ± 0.18 ^g	0.3 – 7.3 ^g 2 – 14 ^j
Androstenedione 	286.4	4.36 ^b	3.72 ± 0.05 ^g	0.7 – 3.2 ⁱ
17α-trenbolone 	270.4	2.65 ^a	2.77 ± 0.12 ^h	0.5 – 4.2 ^l
17β-trenbolone 	270.4	2.65 ^a 2.53 ^b	3.08 ± 0.10 ^h 3.37 ± 0.81 ⁱ	0.6 – 3.6 ^l
Trendione 	268.4	2.63 ^a 3.06 ^b	3.38 ± 0.19 ^h	1 – 4.2 ^l

^a Measured, Qiao et al. (2011); ^b MarvinSketch[®] 5.2.0, www.chemaxon.com; ^c Calculated by Nuez and Yalkowsky (1997); ^d Average log of OC-normalized sorption coefficients (log K_{OC}) and SD for seven soils, Mashtare et al. (2011); ^e Average log K_{OC} and standard deviation (SD) for two soils, Lee et al. (2003); ^f Caron et al. (2010); ^g Batch slurry studies (1:35 g/mL to 3:35 g/mL) with 2 to 5 soil types, Lee et al. (2003); ^h Average and SD of the log K_{OC} for five soils from Khan et al. (2009a); ⁱ Schiffer et al. (2004); ^j Column studies, Das et al. (2003); ^k Moist soil microcosms, Colucci et al. (2001); ^l Moist soil microcosms, Khan et al. (2009b; 2010).

2. Study Site Details

The study was conducted at Purdue University's Animal Science Research and Education Center (ASREC), which covers approximately 600 ha of tile-drained cropland in North Central Indiana and is both a working farm and an EPA-designated Concentrated Animal Feed Operation (CAFO). The ASREC mission is to provide animals, facilities, technical assistance and labor to conduct basic to applied research involving nutrition, physiology, behavior, genetics, reproduction, animal health, and product quality. In 2009, ASREC's animal inventory consisted of beef, dairy, poultry, sheep, swine, and Ossabaw¹ swine units with 618, 449, 2488, 534, 1929, and 168 animals, respectively. The study area annually produces approximately 360, 120, 80, and 50 ha respectively of corn, soybeans, alfalfa, and grass hay.

The site is located on the Tipton Till Plains approximately 15 km northwest of Purdue University's West Lafayette campus. The dominant soil types at the study site are typical of poorly drained agricultural soils in the Midwestern United States and include Drummer silty clay loam (fine-silty, mixed, superactive, mesic Typic Endoaquoll), Toronto silt loam (fine-silty, mixed, superactive, mesic Udollic Epiaqualfs) and Fincastle silt loam (fine-silty, mixed, superactive, mesic Aeric Epiaqualfs). The soil parent materials are loess and glacial till, and a confining layer of glacial till exists approximately 2 m below the soil surface. Due to the presence of these poorly drained soils, subsurface tile drains were installed in the early-1990s (see **Figure SI-1** for a map of the tile lines). The tile drains are located approximately one meter below the soil surface and range in diameter from 10 to 61 cm.

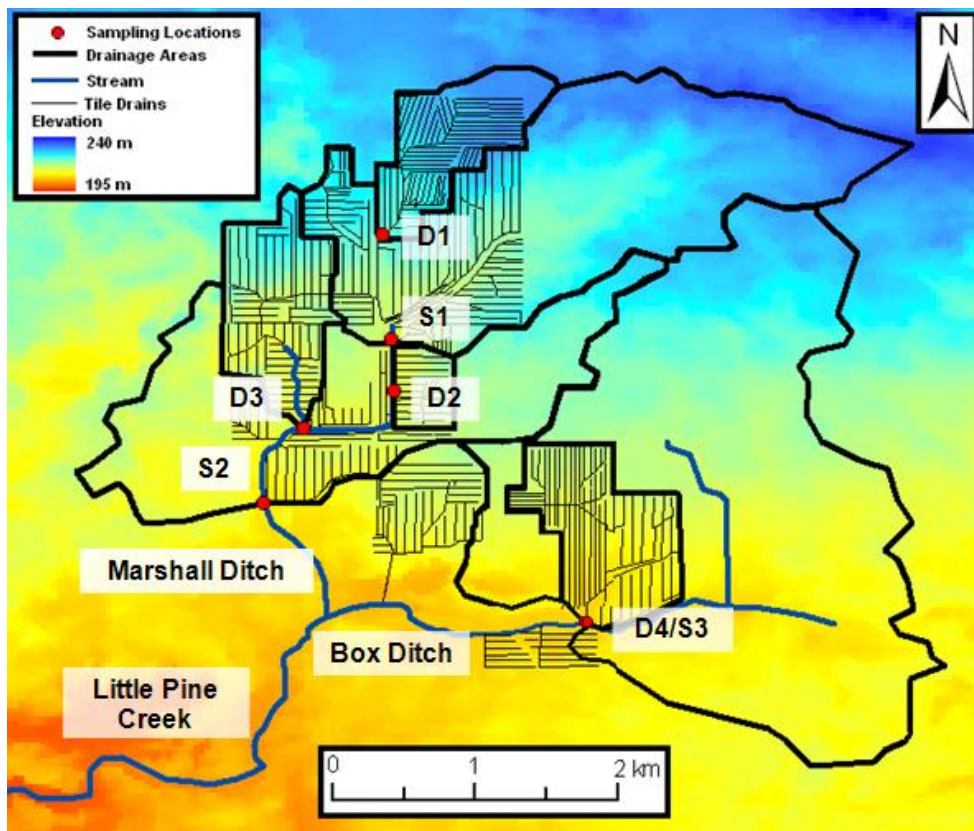
Animal wastes are stored on-site and then land-applied via pivot irrigation (effluent from on-site lagoons and retention ponds), solids broadcasting (dewatered bedding/manure wastes), or subsurface injection (liquid slurry from above ground slurry storage units). Beef, dairy, swine, and poultry each have their own open lagoon system consisting of a series of cells with the last cell typically used for effluent irrigation. In addition, dairy and the Ossabaw-specific swine units have above ground slurry storage units for some of their barns. Further details regarding the lagoon system are provided by Huang and Lee (2001). The beef and dairy bedding/manure wastes are regularly scraped out of the barns and stacked for dewatering near each of the respective units. The dairy hard pack stack, which includes solids from the dairy wastes separator, dewater into the dairy retention pond and is routed to the dairy lagoon system for storage until irrigation. Poultry bedding wastes are relatively small at this farm and are stacked with dairy bedding wastes. The beef hard pack dewater to the beef retention pond for storage until irrigation.

Seven monitoring stations were installed to monitor real-time flow rates and collect water samples from tile drains and ditches within ASREC (schematic and aerial image shown in **Figures SI-1 and SI-2**, respectively). Stations were located to capture each major animal waste application practice based on ASREC's manure management plan: (i) beef and dairy effluent (D1 and S1); (ii) beef and dairy effluent and annual applications of dairy solids (D3 and S2); (iii) poultry and swine effluent (D4 and S3). For fields drained by D2, beef and dairy manure solids had been routinely applied up to 2007, but not during our study period. Although the major waste sources did align with our station plan, detailed waste application records provided in each subsequent year revealed some application of animal waste types outside the original management plan for a given field. Details regarding all

¹ Ossabaw pigs kept at Purdue's facilities are specifically used for medical research. They are used to conduct research related to obesity, diabetes, and heart problems. (YouTube video on Purdue's Ossabaw swine unit and related research: <http://www.youtube.com/watch?v=0HTNpkkzsQo>)

1 applications are provided in **Tables SI-2 to SI-8**. Early in our study, an interconnect system between
2 lagoons was installed as an additional safety measure to minimize lagoon overflow potential. The
3 timing of waste rerouting was not well recorded since it was not required by the EPA. In addition, two
4 valves in the interconnect system did not have a proper seal. This caused north to south leakage between
5 lagoon systems, thus moving beef wastes to the dairy, swine, and poultry lagoon systems. Therefore,
6 intentional and unintentional routing of wastes between animal-specific lagoon systems and deviations
7 from the expected management plan prevented an explicit delineation of hormone release between
8 waste types in this study.

9 Drainage areas for each monitoring station were delineated in ArcGIS by considering both
10 topography (1.5 m digital elevation models) and known tile drain locations (maps provided by ASREC
11 personnel). Each station's drainage area and the overall amount and type of animal waste applied to
12 fields within the drainage areas are summarized in **Table SI-2**. Detailed effluent and manure
13 applications to fields within D1, D3, D4, S1, S2, and S3 drainage areas are summarized in **Tables SI-3**
14 **through SI-8**, respectively. For completeness, animal waste applications for the fall and winter months
15 prior to our study period (September - December 2008) are included in these tables. Fields monitored by
16 D2 received no manure or effluent applications during the study period. It should be noted that the
17 application amounts (**Table SI-2**) and rates (**Tables SI-3 through SI-8**) are estimated by ASREC
18 personnel based on machinery ratings. These values have not been confirmed through field
19 measurements, as ASREC personnel do not have appropriate equipment to assess the machinery
20 performance and quantify deviations from expected performance standards. Also included for reference
21 is the nutrient content of various animal waste samples sent to commercial laboratories as part of routine
22 farm operations for assessing the general nutrient value of animal wastes (**Table SI-9**).



23
24 **Figure SI-1.** Purdue Animal Science Research and Education Center. Study site map, including
25 monitoring stations (S = stream; D = drain), subsurface tile drain locations, and the drainage area for
26 each station.
27

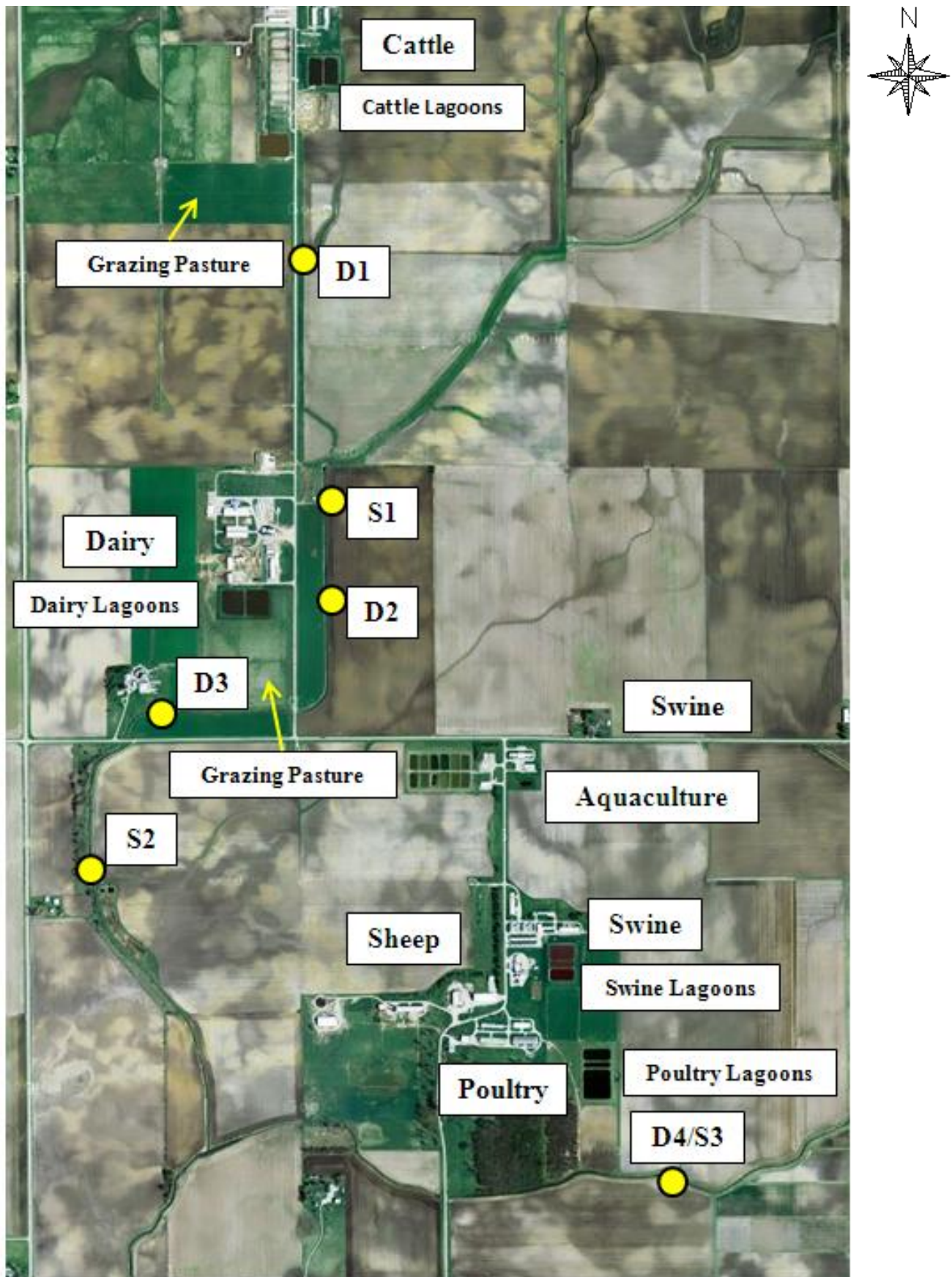


Figure SI-2. Animal Science Research and Education Center Aerial Image. Map shows animal unit and monitoring station locations. Aerial image retrieved on April 5, 2011 from <http://maps.google.com>.

1 **Table SI-2.** Summary of total amounts of manure applied to fields within each monitoring station's
2 drainage area from September 2008 through January 2010. (Detailed applications provided in **Tables**
3 **SI-3** through **SI-8**).

Station ID	Drainage Area (ha)	Application Source	Application Amount	Units
D1	62	Secondary Beef Lagoon	7,313	m ³
		Beef Retention Pond	11,764	m ³
		Primary Dairy Lagoon	4,435	m ³
		Secondary Dairy Lagoon	27,820	m ³
		Ossabaw Swine Slurry Storage Unit #1	361	m ³
		Ossabaw Swine Slurry Storage Unit #2	1,456	m ³
		Dairy Solids	143	tonnes
D2	26.7	No wastes applied since before 2008	--	--
S1	367	Secondary Beef Lagoon	12,219	m ³
		Beef Retention Pond	15,672	m ³
		Primary Dairy Lagoon	7,070	m ³
		Secondary Dairy Lagoon	43,340	m ³
		Ossabaw Swine Slurry Storage Unit #1	361	m ³
		Ossabaw Swine Slurry Storage Unit #2	1,456	m ³
		Dairy Solids	143	tonnes
D3	77	Secondary Beef Lagoon	8,357	m ³
		Beef Retention Pond	9,447	m ³
		Primary Dairy Lagoon	6,540	m ³
		Secondary Dairy Lagoon	29,032	m ³
		Dairy Solids	1,018	tonnes
S2	597	Secondary Beef Lagoon	15,670	m ³
		Beef Retention Pond	21,211	m ³
		Primary Dairy Lagoon	10,976	m ³
		Secondary Dairy Lagoon	57,988	m ³
		Ossabaw Swine Slurry Storage Unit #1	361	m ³
		Ossabaw Swine Slurry Storage Unit #2	1,456	m ³
		Dairy Solids	1,161	tonnes
D4	64	Tertiary Poultry Lagoon	46,419	m ³
		Tertiary Swine Lagoon	27,130	m ³
		Swine Finish Room Slurry	750	m ³
		Dairy Slurry	3,465	m ³
		Boar Lagoon	63.5	m ³
		Dairy Solids	376	m ³
		Beef Solids	130	tonnes
		Sheep Pit	146	tonnes
		Sheep Solids	95.3	tonnes
S3	612	Tertiary Poultry Lagoon	46,419	m ³
		Tertiary Swine Lagoon	27,130	m ³
		Swine Finish Room Slurry	750	m ³
		Dairy Slurry	3,465	m ³
		Boar Lagoon	63.5	m ³
		Dairy Solids	1,010	m ³
		Beef Solids	130	tonnes
		Sheep Pit	146	tonnes
		Sheep Solids	95.3	tonnes

1 **Table SI-3.** Detailed animal waste applications to fields within D1's drainage area.

Application Date	Source	Application Rate
9/8/08	Secondary Dairy Lagoon	65.5 m ³ /ha
9/17/08	Secondary Dairy Lagoon	65.5 m ³ /ha
9/24/08	Beef Retention Pond	65.5 m ³ /ha
9/24/08	Dairy Hard Pack	22.4 tonnes/ha
9/26/08	Beef Retention Pond	65.5 m ³ /ha
10/27/08	Ossabaw Slurry Storage Unit #1	98.6 m ³ /ha
10/28/08	Ossabaw Slurry Storage Unit #1	108 m ³ /ha
10/31/08	Ossabaw Slurry Storage Unit #2	98.3 m ³ /ha
11/1/08	Ossabaw Slurry Storage Unit #2	102 m ³ /ha
11/3/08	Ossabaw Slurry Storage Unit #2	102 m ³ /ha
11/12/08	Primary Dairy Lagoon	65.5 m ³ /ha
11/13/08	Primary Dairy Lagoon	6.6 m ³ /ha
3/17/09	Secondary Dairy Lagoon	65.5 m ³ /ha
3/18/09	Secondary Dairy Lagoon	65.5 m ³ /ha
3/23/09	Secondary Dairy Lagoon	65.5 m ³ /ha
3/24/09	Secondary Dairy Lagoon	65.5 m ³ /ha
3/26/09	Secondary Dairy Lagoon	65.5 m ³ /ha
3/27/09	Secondary Dairy Lagoon	65.5 m ³ /ha
5/23/09	Secondary Dairy Lagoon	65.5 m ³ /ha
5/25/09	Secondary Dairy Lagoon	65.5 m ³ /ha
6/25/09	Secondary Dairy Lagoon	65.5 m ³ /ha
6/26/09	Secondary Dairy Lagoon	65.5 m ³ /ha
6/27/09	Secondary Dairy Lagoon	65.5 m ³ /ha
7/6/09	Secondary Beef Lagoon	65.5 m ³ /ha
7/7/09	Secondary Beef Lagoon	65.5 m ³ /ha
7/8/09	Secondary Beef Lagoon	65.5 m ³ /ha
7/10/09	Secondary Beef Lagoon	65.5 m ³ /ha
7/13/09	Secondary Beef Lagoon	65.5 m ³ /ha
7/15/09	Beef Retention Pond	65.5 m ³ /ha
7/16/09	Beef Retention Pond	65.5 m ³ /ha
7/17/09	Beef Retention Pond	65.5 m ³ /ha
7/20/09	Beef Retention Pond	65.5 m ³ /ha
7/21/09	Beef Retention Pond	65.5 m ³ /ha
9/8/09	Secondary Dairy Lagoon	65.5 m ³ /ha
9/9/09	Secondary Dairy Lagoon	65.5 m ³ /ha
9/10/09	Secondary Dairy Lagoon	65.5 m ³ /ha
9/11/09	Secondary Dairy Lagoon	65.5 m ³ /ha
9/29/09	Ossabaw Slurry Storage Unit #2	102 m ³ /ha
9/30/09	Ossabaw Slurry Storage Unit #2	102.5 m ³ /ha
10/1/09	Ossabaw Slurry Storage Unit #2	100 m ³ /ha
10/5/09	Ossabaw Slurry Storage Unit #1	64.7 m ³ /ha
10/15/09	Dairy Hard Pack	32.5 tonnes/ha
11/4/09	Secondary Dairy Lagoon	65.5 m ³ /ha
11/6/09	Secondary Dairy Lagoon	65.5 m ³ /ha
11/7/09	Secondary Dairy Lagoon	65.5 m ³ /ha
11/8/09	Secondary Dairy Lagoon	65.5 m ³ /ha
11/9/09	Primary Dairy Lagoon	65.5 m ³ /ha
11/10/09	Primary Dairy Lagoon	65.5 m ³ /ha
11/11/09	Primary Dairy Lagoon	65.5 m ³ /ha
11/24/09	Beef Retention Pond	65.5 m ³ /ha
11/25/09	Beef Retention Pond	65.5 m ³ /ha

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1 **Table SI-4.** Detailed animal waste applications to fields within D3's drainage area.

Application Date	Source	Application Rate
9/8/08	Secondary Dairy Lagoon	74.8 m ³ /ha
9/12/08	Dairy Hard Pack	22.4 tonnes/ha
9/18/08	Dairy Hard Pack	22.4 tonnes/ha
9/19/08	Dairy Hard Pack	22.4 tonnes/ha
9/19/08	Secondary Dairy Lagoon	74.8 m ³ /ha
9/21/08	Dairy Hard Pack	22.4 tonnes/ha
9/24/08	Dairy Hard Pack	22.4 tonnes/ha
9/25/08	Beef Retention Pond	74.8 m ³ /ha
11/12/08	Primary Dairy Lagoon	74.8 m ³ /ha
11/13/08	Primary Dairy Lagoon	74.8 m ³ /ha
3/17/09	Secondary Dairy Lagoon	74.8 m ³ /ha
3/18/09	Secondary Dairy Lagoon	7.5 m ³ /ha
3/23/09	Secondary Dairy Lagoon	74.8 m ³ /ha
3/24/09	Secondary Dairy Lagoon	74.8 m ³ /ha
3/26/09	Secondary Dairy Lagoon	74.8 m ³ /ha
3/27/09	Secondary Dairy Lagoon	74.8 m ³ /ha
5/23/09	Secondary Dairy Lagoon	74.8 m ³ /ha
5/25/09	Secondary Dairy Lagoon	74.8 m ³ /ha
5/29/09	Secondary Dairy Lagoon	74.8 m ³ /ha
6/25/09	Secondary Dairy Lagoon	74.8 m ³ /ha
6/26/09	Secondary Dairy Lagoon	74.8 m ³ /ha
6/27/09	Secondary Dairy Lagoon	74.8 m ³ /ha
7/6/09	Secondary Beef Lagoon	74.8 m ³ /ha
7/7/09	Secondary Beef Lagoon	74.8 m ³ /ha
7/8/09	Secondary Beef Lagoon	74.8 m ³ /ha
7/10/09	Secondary Beef Lagoon	74.8 m ³ /ha
7/13/09	Secondary Beef Lagoon	74.8 m ³ /ha
7/15/09	Beef Retention Pond	74.8 m ³ /ha
7/16/09	Beef Retention Pond	74.8 m ³ /ha
7/17/09	Beef Retention Pond	74.8 m ³ /ha
7/20/09	Beef Retention Pond	74.8 m ³ /ha
7/21/09	Beef Retention Pond	74.8 m ³ /ha
9/8/09	Secondary Dairy Lagoon	74.8 m ³ /ha
9/9/09	Secondary Dairy Lagoon	74.8 m ³ /ha
9/10/09	Secondary Dairy Lagoon	74.8 m ³ /ha
9/11/09	Secondary Dairy Lagoon	74.8 m ³ /ha
11/4/09	Secondary Dairy Lagoon	74.8 m ³ /ha
11/6/09	Secondary Dairy Lagoon	74.8 m ³ /ha
11/7/09	Secondary Dairy Lagoon	74.8 m ³ /ha
11/8/09	Secondary Dairy Lagoon	74.8 m ³ /ha
11/9/09	Primary Dairy Lagoon	74.8 m ³ /ha
11/10/09	Primary Dairy Lagoon	74.8 m ³ /ha
11/11/09	Primary Dairy Lagoon	74.8 m ³ /ha
12/11/09	Dairy Hard Pack	5.9 tonnes/ha
12/12/09	Dairy Hard Pack	5.9 tonnes/ha
12/16/09	Dairy Hard Pack	5.9 tonnes/ha
12/18/09	Dairy Hard Pack	5.9 tonnes/ha
1/6/10	Dairy Hard Pack	5.9 tonnes/ha

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1 **Table SI-5.** Detailed animal waste applications to fields within D4's drainage area.

Application Date	Source	Application Rate
9/1/08	Tertiary Swine Lagoon	65.5 m ³ /ha
9/4/08	Boar Lagoon	65.5 m ³ /ha
9/9/08	Tertiary Swine Lagoon	65.5 m ³ /ha
9/10/08	Tertiary Swine Lagoon	65.5 m ³ /ha
9/11/08	Tertiary Swine Lagoon	65.5 m ³ /ha
9/16/08	Tertiary Swine Lagoon	65.5 m ³ /ha
9/18/08	Tertiary Swine Lagoon	65.5 m ³ /ha
9/22/08	Tertiary Swine Lagoon	65.5 m ³ /ha
9/23/08	Tertiary Swine Lagoon	65.5 m ³ /ha
10/20/08	Dairy Slurry Storage Unit	132 m ³ /ha
10/21/08	Dairy Slurry Storage Unit	116 m ³ /ha
10/22/08	Dairy Slurry Storage Unit	108 m ³ /ha
2/28/09	Beef Hard Pack	22.4 tonnes/ha
3/1/09	Beef Hard Pack	22.4 tonnes/ha
3/3/09	Sheep Pit	22.4 tonnes/ha
3/3/09	Sheep Pit	17.9 tonnes/ha
3/4/09	Sheep Hard Pack	22.4 tonnes/ha
5/11/09	Dairy Hard Pack	22.4 tonnes/ha
5/12/09	Dairy Hard Pack	22.4 tonnes/ha
5/22/09	Dairy Hard Pack	22.4 tonnes/ha
5/23/09	Dairy Hard Pack	22.4 tonnes/ha
5/29/09	Tertiary Swine Lagoon	65.5 m ³ /ha
6/25/09	Tertiary Swine Lagoon	65.5 m ³ /ha
6/26/09	Tertiary Swine Lagoon	65.5 m ³ /ha
6/29/09	Tertiary Swine Lagoon	65.5 m ³ /ha
7/6/09	Tertiary Poultry Lagoon	65.5 m ³ /ha
7/7/09	Tertiary Poultry Lagoon	65.5 m ³ /ha
7/8/09	Tertiary Poultry Lagoon	65.5 m ³ /ha
7/10/09	Tertiary Poultry Lagoon	65.5 m ³ /ha
7/13/09	Tertiary Poultry Lagoon	65.5 m ³ /ha
7/15/09	Tertiary Poultry Lagoon	65.5 m ³ /ha
7/16/09	Tertiary Poultry Lagoon	65.5 m ³ /ha
7/17/09	Tertiary Poultry Lagoon	65.5 m ³ /ha
7/20/09	Tertiary Poultry Lagoon	65.5 m ³ /ha
7/21/09	Tertiary Poultry Lagoon	65.5 m ³ /ha
7/22/09	Tertiary Poultry Lagoon	65.5 m ³ /ha
7/23/09	Tertiary Poultry Lagoon	65.5 m ³ /ha
7/24/09	Tertiary Poultry Lagoon	65.5 m ³ /ha
7/27/09	Tertiary Poultry Lagoon	65.5 m ³ /ha
7/29/09	Tertiary Poultry Lagoon	65.5 m ³ /ha
7/30/09	Tertiary Poultry Lagoon	65.5 m ³ /ha
7/31/09	Tertiary Poultry Lagoon	65.5 m ³ /ha
8/5/09	Tertiary Poultry Lagoon	65.5 m ³ /ha
8/7/09	Tertiary Poultry Lagoon	65.5 m ³ /ha
8/10/09	Tertiary Poultry Lagoon	65.5 m ³ /ha
8/11/09	Tertiary Poultry Lagoon	65.5 m ³ /ha
8/12/09	Tertiary Poultry Lagoon	65.5 m ³ /ha
8/13/09	Tertiary Poultry Lagoon	65.5 m ³ /ha
8/17/09	Tertiary Poultry Lagoon	65.5 m ³ /ha
8/18/09	Tertiary Poultry Lagoon	65.5 m ³ /ha
8/19/09	Tertiary Poultry Lagoon	65.5 m ³ /ha
8/20/09	Tertiary Poultry Lagoon	65.5 m ³ /ha
8/25/09	Tertiary Poultry Lagoon	65.5 m ³ /ha
8/26/09	Tertiary Poultry Lagoon	65.5 m ³ /ha
8/28/09	Tertiary Poultry Lagoon	65.5 m ³ /ha
8/31/09	Tertiary Poultry Lagoon	65.5 m ³ /ha
9/8/09	Tertiary Swine Lagoon	65.5 m ³ /ha
9/9/09	Tertiary Swine Lagoon	65.5 m ³ /ha

1 **Table SI-5.** Detailed animal waste applications to fields within D4's drainage area. *(continued)*

Application Date	Source	Application Rate
9/10/09	Tertiary Swine Lagoon	65.5 m ³ /ha
9/11/09	Tertiary Swine Lagoon	65.5 m ³ /ha
11/4/09	Tertiary Swine Lagoon	65.5 m ³ /ha
11/5/09	Tertiary Swine Lagoon	65.5 m ³ /ha
11/6/09	Tertiary Swine Lagoon	65.5 m ³ /ha
11/7/09	Tertiary Swine Lagoon	65.5 m ³ /ha
11/9/09	Tertiary Poultry Lagoon	65.5 m ³ /ha
11/10/09	Tertiary Poultry Lagoon	65.5 m ³ /ha
11/11/09	Tertiary Poultry Lagoon	65.5 m ³ /ha

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4 **Table SI-6.** Detailed animal waste applications to fields within S1's drainage area.

Application Date	Source	Application Rate
9/8/08	Secondary Dairy Lagoon	65.5 m ³ /ha
9/8/08	Secondary Dairy Lagoon	74.8 m ³ /ha
9/17/08	Secondary Dairy Lagoon	65.5 m ³ /ha
9/19/08	Secondary Dairy Lagoon	74.8 m ³ /ha
9/24/08	Beef Retention Pond	65.5 m ³ /ha
9/24/08	Dairy Hard Pack	22.4 tonnes/ha
9/25/08	Beef Retention Pond	74.8 m ³ /ha
9/26/08	Beef Retention Pond	65.5 m ³ /ha
10/27/08	Ossabaw Slurry Storage Unit #1	98.6 m ³ /ha
10/28/08	Ossabaw Slurry Storage Unit #1	108 m ³ /ha
10/31/08	Ossabaw Slurry Storage Unit #2	98.3 m ³ /ha
11/1/08	Ossabaw Slurry Storage Unit #2	102 m ³ /ha
11/3/08	Ossabaw Slurry Storage Unit #2	102 m ³ /ha
11/12/08	Primary Dairy Lagoon	74.8 m ³ /ha
11/12/08	Primary Dairy Lagoon	65.5 m ³ /ha
11/13/08	Primary Dairy Lagoon	6.5 m ³ /ha
11/13/08	Primary Dairy Lagoon	74.8 m ³ /ha
3/17/09	Secondary Dairy Lagoon	65.5 m ³ /ha
3/17/09	Secondary Dairy Lagoon	74.8 m ³ /ha
3/18/09	Secondary Dairy Lagoon	7.5 m ³ /ha
3/18/09	Secondary Dairy Lagoon	65.5 m ³ /ha
3/23/09	Secondary Dairy Lagoon	65.5 m ³ /ha
3/23/09	Secondary Dairy Lagoon	74.8 m ³ /ha
3/24/09	Secondary Dairy Lagoon	65.5 m ³ /ha
3/24/09	Secondary Dairy Lagoon	74.8 m ³ /ha
3/26/09	Secondary Dairy Lagoon	65.5 m ³ /ha
3/26/09	Secondary Dairy Lagoon	74.8 m ³ /ha
3/27/09	Secondary Dairy Lagoon	74.8 m ³ /ha
3/27/09	Secondary Dairy Lagoon	65.5 m ³ /ha
5/23/09	Secondary Dairy Lagoon	65.5 m ³ /ha
5/23/09	Secondary Dairy Lagoon	74.8 m ³ /ha
5/25/09	Secondary Dairy Lagoon	74.8 m ³ /ha
5/25/09	Secondary Dairy Lagoon	65.5 m ³ /ha
5/29/09	Secondary Dairy Lagoon	65.5 m ³ /ha
5/29/09	Secondary Dairy Lagoon	74.8 m ³ /ha
6/25/09	Secondary Dairy Lagoon	65.5 m ³ /ha
6/25/09	Secondary Dairy Lagoon	74.8 m ³ /ha
6/26/09	Secondary Dairy Lagoon	74.8 m ³ /ha
6/26/09	Secondary Dairy Lagoon	65.5 m ³ /ha
6/27/09	Secondary Dairy Lagoon	65.5 m ³ /ha
6/27/09	Secondary Dairy Lagoon	74.8 m ³ /ha

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1 **Table SI-6.** Detailed animal waste applications to fields within S1's drainage area. *(continued)*

Application Date	Source	Application Rate
7/6/09	Secondary Beef Lagoon	65.5 m ³ /ha
7/6/09	Secondary Beef Lagoon	74.8 m ³ /ha
7/7/09	Secondary Beef Lagoon	65.5 m ³ /ha
7/7/09	Secondary Beef Lagoon	74.8 m ³ /ha
7/8/09	Secondary Beef Lagoon	74.8 m ³ /ha
7/8/09	Secondary Beef Lagoon	65.5 m ³ /ha
7/10/09	Secondary Beef Lagoon	65.5 m ³ /ha
7/10/09	Secondary Beef Lagoon	74.8 m ³ /ha
7/13/09	Secondary Beef Lagoon	65.5 m ³ /ha
7/13/09	Secondary Beef Lagoon	74.8 m ³ /ha
7/15/09	Beef Retention Pond	74.8 m ³ /ha
7/15/09	Beef Retention Pond	65.5 m ³ /ha
7/16/09	Beef Retention Pond	65.5 m ³ /ha
7/16/09	Beef Retention Pond	74.8 m ³ /ha
7/17/09	Beef Retention Pond	65.5 m ³ /ha
7/17/09	Beef Retention Pond	74.8 m ³ /ha
7/20/09	Beef Retention Pond	74.8 m ³ /ha
7/20/09	Beef Retention Pond	65.5 m ³ /ha
7/21/09	Beef Retention Pond	65.5 m ³ /ha
7/21/09	Beef Retention Pond	74.8 m ³ /ha
9/8/09	Secondary Dairy Lagoon	65.5 m ³ /ha
9/8/09	Secondary Dairy Lagoon	74.8 m ³ /ha
9/9/09	Secondary Dairy Lagoon	74.8 m ³ /ha
9/9/09	Secondary Dairy Lagoon	65.5 m ³ /ha
9/10/09	Secondary Dairy Lagoon	65.5 m ³ /ha
9/10/09	Secondary Dairy Lagoon	74.8 m ³ /ha
9/11/09	Secondary Dairy Lagoon	65.5 m ³ /ha
9/11/09	Secondary Dairy Lagoon	74.8 m ³ /ha
9/29/09	Ossabaw Slurry Storage Unit #2	102 m ³ /ha
9/30/09	Ossabaw Slurry Storage Unit #2	103 m ³ /ha
10/1/09	Ossabaw Slurry Storage Unit #2	100 m ³ /ha
10/5/09	Ossabaw Slurry Storage Unit #1	64.7 m ³ /ha
10/15/09	Dairy Hard Pack	32.5 tonnes/ha
11/4/09	Secondary Dairy Lagoon	65.5 m ³ /ha
11/4/09	Secondary Dairy Lagoon	74.8 m ³ /ha
11/6/09	Secondary Dairy Lagoon	74.8 m ³ /ha
11/6/09	Secondary Dairy Lagoon	65.5 m ³ /ha
11/7/09	Secondary Dairy Lagoon	65.5 m ³ /ha
11/7/09	Secondary Dairy Lagoon	74.8 m ³ /ha
11/8/09	Secondary Dairy Lagoon	65.5 m ³ /ha
11/8/09	Secondary Dairy Lagoon	74.8 m ³ /ha
11/9/09	Primary Dairy Lagoon	74.8 m ³ /ha
11/9/09	Primary Dairy Lagoon	65.5 m ³ /ha
11/10/09	Primary Dairy Lagoon	65.5 m ³ /ha
11/10/09	Primary Dairy Lagoon	74.8 m ³ /ha
11/11/09	Primary Dairy Lagoon	65.5 m ³ /ha
11/11/09	Primary Dairy Lagoon	74.8 m ³ /ha
11/24/09	Beef Retention Pond	65.5 m ³ /ha
11/25/09	Beef Retention Pond	65.5 m ³ /ha

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1 **Table SI-7.** Detailed animal waste applications to fields within S2's drainage area.

Application Date	Source	Application Rate
9/8/08	Secondary Dairy Lagoon	74.8 m ³ /ha
9/8/08	Secondary Dairy Lagoon	65.5 m ³ /ha
9/12/08	Dairy Hard Pack	22.4 tonnes/ha
9/17/08	Secondary Dairy Lagoon	65.5 m ³ /ha
9/18/08	Dairy Hard Pack	22.4 tonnes/ha
9/19/08	Secondary Dairy Lagoon	74.8 m ³ /ha
9/19/08	Dairy Hard Pack	22.4 tonnes/ha
9/19/08	Secondary Dairy Lagoon	74.8 m ³ /ha
9/21/08	Dairy Hard Pack	22.4 tonnes/ha
9/24/08	Beef Retention Pond	65.5 m ³ /ha
9/24/08	Dairy Hard Pack	22.4 tonnes/ha
9/24/08	Dairy Hard Pack	22.4 tonnes/ha
9/25/08	Beef Retention Pond	65.5 m ³ /ha
9/26/08	Beef Retention Pond	65.5 m ³ /ha
10/27/08	Ossabaw Slurry Storage Unit #1	98.6 m ³ /ha
10/28/08	Ossabaw Slurry Storage Unit #1	108 m ³ /ha
10/31/08	Ossabaw Slurry Storage Unit #2	102 m ³ /ha
11/1/08	Ossabaw Slurry Storage Unit #2	102 m ³ /ha
11/3/08	Ossabaw Slurry Storage Unit #2	102 m ³ /ha
11/12/08	Dairy Lagoon # 1	74.8 m ³ /ha
11/12/08	Dairy Lagoon # 1	65.5 m ³ /ha
11/13/08	Dairy Lagoon # 1	74.8 m ³ /ha
11/13/08	Dairy Lagoon # 1	6.6 m ³ /ha
3/17/09	Secondary Dairy Lagoon	74.8 m ³ /ha
3/17/09	Secondary Dairy Lagoon	65.5 m ³ /ha
3/18/09	Secondary Dairy Lagoon	7.5 m ³ /ha
3/18/09	Secondary Dairy Lagoon	65.5 m ³ /ha
3/23/09	Secondary Dairy Lagoon	74.8 m ³ /ha
3/23/09	Secondary Dairy Lagoon	65.5 m ³ /ha
3/24/09	Secondary Dairy Lagoon	74.8 m ³ /ha
3/24/09	Secondary Dairy Lagoon	65.5 m ³ /ha
3/26/09	Secondary Dairy Lagoon	74.8 m ³ /ha
3/26/09	Secondary Dairy Lagoon	65.5 m ³ /ha
3/27/09	Secondary Dairy Lagoon	74.8 m ³ /ha
3/27/09	Secondary Dairy Lagoon	65.5 m ³ /ha
5/23/09	Secondary Dairy Lagoon	74.8 m ³ /ha
5/23/09	Secondary Dairy Lagoon	65.5 m ³ /ha
5/25/09	Secondary Dairy Lagoon	74.8 m ³ /ha
5/25/09	Secondary Dairy Lagoon	65.5 m ³ /ha
5/29/09	Secondary Dairy Lagoon	74.8 m ³ /ha
5/29/09	Secondary Dairy Lagoon	65.5 m ³ /ha
6/25/09	Secondary Dairy Lagoon	74.8 m ³ /ha
6/25/09	Secondary Dairy Lagoon	65.5 m ³ /ha
6/26/09	Secondary Dairy Lagoon	74.8 m ³ /ha
6/26/09	Secondary Dairy Lagoon	65.5 m ³ /ha
6/27/09	Secondary Dairy Lagoon	74.8 m ³ /ha
6/27/09	Secondary Dairy Lagoon	65.5 m ³ /ha
7/6/09	Calvert Lagoon # 2	74.8 m ³ /ha
7/6/09	Calvert Lagoon # 2	65.5 m ³ /ha
7/7/09	Calvert Lagoon # 2	74.8 m ³ /ha
7/7/09	Calvert Lagoon # 2	65.5 m ³ /ha
7/8/09	Calvert Lagoon # 2	74.8 m ³ /ha
7/8/09	Calvert Lagoon # 2	65.5 m ³ /ha
7/10/09	Calvert Lagoon # 2	74.8 m ³ /ha
7/10/09	Calvert Lagoon # 2	65.5 m ³ /ha
7/13/09	Calvert Lagoon # 2	74.8 m ³ /ha
7/13/09	Calvert Lagoon # 2	65.5 m ³ /ha
7/15/09	Calvert Ret. Pond	74.8 m ³ /ha
7/15/09	Calvert Ret. Pond	65.5 m ³ /ha

1 **Table SI-7.** Detailed animal waste applications to fields within S2's drainage area. *(continued)*

Application Date	Source	Application Rate
7/16/09	Calvert Ret. Pond	74.8 m ³ /ha
7/16/09	Calvert Ret. Pond	65.5 m ³ /ha
7/17/09	Calvert Ret. Pond	74.8 m ³ /ha
7/17/09	Calvert Ret. Pond	65.5 m ³ /ha
7/20/09	Calvert Ret. Pond	74.8 m ³ /ha
7/20/09	Calvert Ret. Pond	65.5 m ³ /ha
7/21/09	Calvert Ret. Pond	74.8 m ³ /ha
7/21/09	Calvert Ret. Pond	65.5 m ³ /ha
9/8/09	Secondary Dairy Lagoon	74.8 m ³ /ha
9/8/09	Secondary Dairy Lagoon	65.5 m ³ /ha
9/9/09	Secondary Dairy Lagoon	74.8 m ³ /ha
9/9/09	Secondary Dairy Lagoon	65.5 m ³ /ha
9/10/09	Secondary Dairy Lagoon	74.8 m ³ /ha
9/10/09	Secondary Dairy Lagoon	65.5 m ³ /ha
9/11/09	Secondary Dairy Lagoon	74.8 m ³ /ha
9/11/09	Secondary Dairy Lagoon	65.5 m ³ /ha
9/29/09	Ossabaw Slurry Storage Unit #2	102 m ³ /ha
9/30/09	Ossabaw Slurry Storage Unit #2	103 m ³ /ha
10/1/09	Ossabaw Slurry Storage Unit #2	100 m ³ /ha
10/5/09	Ossabaw Slurry Storage Unit #1	64.7 m ³ /ha
10/15/09	Dairy Hard Pack	32.5 tonnes/ha
11/4/09	Secondary Dairy Lagoon	74.8 m ³ /ha
11/4/09	Secondary Dairy Lagoon	65.5 m ³ /ha
11/6/09	Secondary Dairy Lagoon	74.8 m ³ /ha
11/6/09	Secondary Dairy Lagoon	65.5 m ³ /ha
11/7/09	Secondary Dairy Lagoon	74.8 m ³ /ha
11/7/09	Secondary Dairy Lagoon	65.5 m ³ /ha
11/8/09	Secondary Dairy Lagoon	74.8 m ³ /ha
11/8/09	Secondary Dairy Lagoon	65.5 m ³ /ha
11/9/09	Dairy Lagoon # 1	74.8 m ³ /ha
11/9/09	Dairy Lagoon # 1	65.5 m ³ /ha
11/10/09	Dairy Lagoon # 1	74.8 m ³ /ha
11/10/09	Dairy Lagoon # 1	65.5 m ³ /ha
11/11/09	Dairy Lagoon # 1	74.8 m ³ /ha
11/11/09	Dairy Lagoon # 1	65.5 m ³ /ha
11/24/09	Beef Retention Pond	65.5 m ³ /ha
11/25/09	Beef Retention Pond	65.5 m ³ /ha
12/11/09	Dairy Hard Pack	32.5 tonnes/ha
12/12/09	Dairy Hard Pack	32.5 tonnes/ha
12/16/09	Dairy Hard Pack	32.5 tonnes/ha
12/18/09	Dairy Hard Pack	32.5 tonnes/ha
1/6/10	Dairy Hard Pack	32.5 tonnes/ha

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1 **Table SI-8.** Detailed animal waste applications to fields within S3’s drainage area.*

Application Date	Source	Application Rate
1/5/09	Dairy Hard Pack	22.4 tonnes/ha
1/9/09	Dairy Hard Pack	22.4 tonnes/ha
1/12/09	Dairy Hard Pack	22.4 tonnes/ha
1/21/09	Dairy Hard Pack	22.4 tonnes/ha
1/22/09	Dairy Hard Pack	22.4 tonnes/ha
1/26/09	Dairy Hard Pack	22.4 tonnes/ha
1/27/09	Dairy Hard Pack	22.4 tonnes/ha
11/6/09	Swine Finish Room Slurry	45.1 m ³ /ha
11/7/09	Swine Finish Room Slurry	43.2 m ³ /ha
11/8/09	Swine Finish Room Slurry	43.0 m ³ /ha

2 *S3’s drainage area encompasses all fields drained by D4 and therefore the applications
3 provided in **Table SI-5** are also applicable to S3. The fields receiving the applications
4 detailed in this table are tile-drained with the outlet downstream of S3’s location. Therefore,
5 these applications could only influence water quality at S3 during surface runoff or
6 snowmelt events.

1 **Table SI-9.** Nutrient content of the various animal wastes.

Source	Total N	NH ₄ -N	Total P ₂ O ₅	Total K ₂ O	Max. Avail. N	Avail. P ₂ O ₅	Avail. K ₂ O	Units	Name of Analysis Lab
Beef Retention Pond	0.02	0.01	0.01	0.05	0.01	0.01	0.05	kg/m ³	A&L Great Lakes
Primary Beef Lagoon	1.53	0.28	1.44	0.46	0.72	1.44	0.46	kg/m ³	Holmes Lab
Secondary Beef Lagoon	0.42	0.23	0.35	0.20	0.30	0.35	0.20	kg/m ³	A&L Great Lakes
Primary Dairy Lagoon	1.20	0.30	1.20	0.50	0.61	1.20	0.50	kg/m ³	Holmes Lab
Secondary Dairy Lagoon	0.30	0.20	0.07	0.40	0.24	0.07	0.40	kg/m ³	A&L Great Lakes
Dairy Slurry Storage Unit	0.85	0.28	0.30	0.38	0.48	0.30	0.38	kg/m ³	A&L Great Lakes
Tertiary Poultry Lagoon	0.05	0.02	0.04	0.06	0.04	0.04	0.04	kg/m ³	A&L Great Lakes
Tertiary Swine Lagoon	0.22	0.14	0.06	0.28	0.17	0.06	0.28	kg/m ³	A&L Great Lakes
Swine Finish Room	0.86	0.52	1.03	0.17	0.69	1.03	0.17	kg/m ³	A&L Great Lakes
Ossabaw Swine Slurry Storage Unit #1	1.07	0.44	0.95	0.28	0.66	0.95	0.28	kg/m ³	A&L Great Lakes
Ossabaw Swine Slurry Storage Unit #2	0.32	0.22	0.29	0.14	0.25	0.29	0.14	kg/m ³	A&L Great Lakes
Boar Lagoon	0.32	0.25	0.16	0.29	0.28	0.16	0.29	kg/m ³	Holmes Lab
Dairy Hard Pack	5.35	1.07	3.00	6.01	2.55	3.00	6.01	kg/tonne	A&L Great Lakes
Beef Hard Pack	8.76	0.99	3.70	5.06	3.70	3.70	5.06	kg/tonne	A&L Great Lakes
Sheep Hard Pack	8.23	2.47	4.94	12.30	4.49	4.94	12.3	kg/tonne	A&L Great Lakes
Sheep Pit	12.7	0.7	5.6	2.4	7.9	5.6	2.4	kg/tonne	Holmes Lab

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3. Analytical Methods

Sample Collection and Processing. Water samples were generally retrieved from the ISCO samplers within 12 to 48 h after ISCO collection especially during storm events. During base flow, samples were retrieved 24 to 72 h after collected by the ISCO. Water samples were refrigerated immediately upon receipt in the lab. Samples were processed within 72 h with the majority of samples typically processed in less than 36 h. Although the maximum time for water collection from the field, filtering, and hormone extraction was each 72 h, no sample experienced the maximum holding times for each step. For example, during storm events, samples were collected from the field daily, and then may have been refrigerated longer (up to 72 h) due to the number of samples coming to the lab. In the absence of rain events, collection time from the field may have been longer than 24 h, but processing in the lab would have been rapid (< 24 h). Also once samples were filtered, storage for the 72-h maximum holding time hormone concentrations remained relatively unchanged for 72 h (detailed later associated with **Table SI-15**).

Sample Preparation of Field Samples. Samples were weighed and filtered (VWR glass fiber filter, Grade 696, 1.2 μm) through a Büchner funnel. Sample collection bottles were rinsed with methanol, which was then poured into the Büchner funnel to desorb hormones that may have sorbed onto the filter or were loosely associated with the sediments. After approximately one minute, the methanol was pulled through the filter by vacuum and combined with the water sample (final methanol content was less than 6 vol. %). Water samples with volume < 1L were diluted with water to 1L. Each filtered sample was amended with deuterated standards (6.25 ng of $17\beta\text{-E2-16,16,17-d}_3$ and 5 ng of TST-16,16,17- d_3 dissolved in 0.5 mL of methanol, purchased from CDN Isotopes (Pointe-Claire, Quebec, Canada) and Sigma-Aldrich (St. Louis, MO), and hormones extracted by solid phase extraction immediately or stored at 4 °C in the dark for no longer than 72 h.

Water samples were pre-concentrated using solid phase extraction (SPE) on a 24 port Visiprep SPE vacuum manifold (Supelco, Bellefonte, PA) by passing through the cartridges (Phenomenex SDB-L, 200 mg in a 3-mL cartridge conditioned with 4 mL of isopropanol and 4 mL of water) at < 10 mL/min. Loaded cartridges were stored at -20 °C for up to four months. Hormones appeared stable while frozen as indicated by high recoveries of both internal standards and no difference between cartridges that were frozen or eluted immediately. Cartridges were then washed sequentially with 3 mL of 90:10 Tris buffer:methanol (pH 8.5), 3 mL of 80:20 water:methanol and twice with 3 mL of water followed by drying for 5 min under vacuum to remove bulk water. Analytes were then eluted with 4 mL of methanol, and the eluant was evaporated to dryness under a gentle stream of nitrogen. Residues were reconstituted in methanol (0.5 mL) and typically analyzed within 24 h.

HPLC/MS/MS Analysis. Hormone analysis was by high performance reverse-phase liquid chromatography tandem electrospray ionization mass spectrometry (HPLC/ESI-MS/MS) using a Shimadzu HPLC system (HTA autosampler with dual SCL-10ADvp pumps) coupled to a Sciex API-3000 operated in multiple reaction monitoring mode. Estrogens were analyzed in the negative ESI mode while androgens were analyzed in the positive ESI mode. A Phenomenex Synergi RP-MAX column (150 x 2.0 mm, dp = 4 μm) was used to separate estrogens with gradient elution using 90:10 water:methanol containing 2 mM ethanolamine and acetonitrile containing 2 mM ethanolamine at a flow rate of 0.35 mL/min. Androgen separation was accomplished using a Phenomenex Hyperclone ODS(C18) column (150 x 2.0 mm, dp = 3 μm) and gradient elution with 89.8:10:0.2

water:methanol:formic acid and acetonitrile at a flow rate of 0.25 mL/min. Gradient details are provided in **Tables SI-10 and SI-11**.

Table SI-10. Shimadzu LC Column and Gradient Method for the Estrogens

Column: Phenomenex Synergi RP-MAX 150 x 2 mm, 4 um particle size

Mobile Phase: A: 90/10 H₂O/Methanol + 2 mM ethanolamine;

B: Acetonitrile + 2mM ethanolamine

Gradient

Time (min)	% A	% B
0.00	70	30
8.00	50	50
8.50	0	100
10.50	0	100
11.00	70	30

Shimadzu LC system Equilibration time = 2.0 min

Injection volume: 25 µL Flow Rate: 0.35 mL/min

Table SI-11. Shimadzu LC Column and Gradient Method for the Androgens

Column: Phenomenex Hyperclone ODS(C18) 250 x 2.0mm, particle size = 3 um

Mobile Phase: A: 90/10 H₂O/Methanol + 0.1% formic acid;

B:Acetonitrile

Gradient

Time (min)	% A	% B
0.00	60	40
6.00	30	70
6.50	5	95
9.00	5	95
9.50	60	40
11.5	60	40

Shimadzu LC system Equilibration time = 2.0 min

Injection volume: 15 µL Flow Rate: 0.25 mL/min

Limit of Detection/Quantitation. The method limit of detection (LOD) and quantitation (LOQ) for the steroid hormones under investigation were evaluated by extracting water samples using SPE, reconstituting the resulting extracts in methanolic solution containing varying concentrations of hormones, and analyzing the spiked samples by HPLC/ESI-MS/MS. Twenty five liters of water were collected in 1 L polyethylene bottles at S2 over the course of ~ 30 minutes using an ISCO unit. The water samples were brought to the lab and combined in a 25 L carboy and mixed thoroughly. The water was returned to the 1 L bottles in 950 mL portions to yield 25 presumably homogeneous samples. The water samples were then filtered and subjected to SPE using the same protocol that was used for field samples. After evaporation of the extracts to dryness, the samples were re-dissolved in 0.5 mL of methanolic solution containing steroid hormones at varying concentration (replicates of 5 for each concentration). The method LOD was calculated as 3 times the standard deviation (n = 5) at the lowest concentration where there were no non-detects in five replicates for samples which were spiked with a low concentration standard after SPE extraction of the matrix. The method LOQ was calculated as 10 times the standard deviation (n = 5) at the lowest concentration where there were no non-detects in five

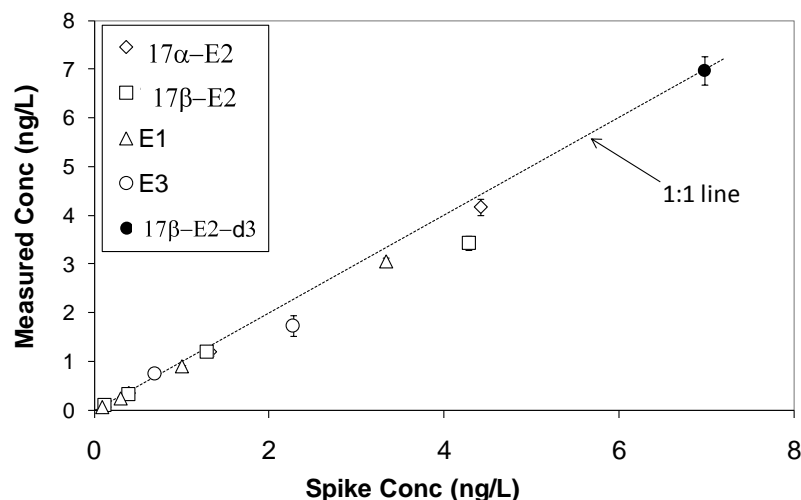
replicates for samples which were spiked with a low concentration standard after SPE extraction of the matrix. Retention times, ions monitored, and the limits of detection (LOD) and quantitation (LOQ) for each hormone in the aqueous samples are summarized in **Table SI-12**.

Table SI-12. Method limits of detection (LOD) and quantitation (LOQ) for each hormone in filtered stream water, and ion transitions monitored by the MS.

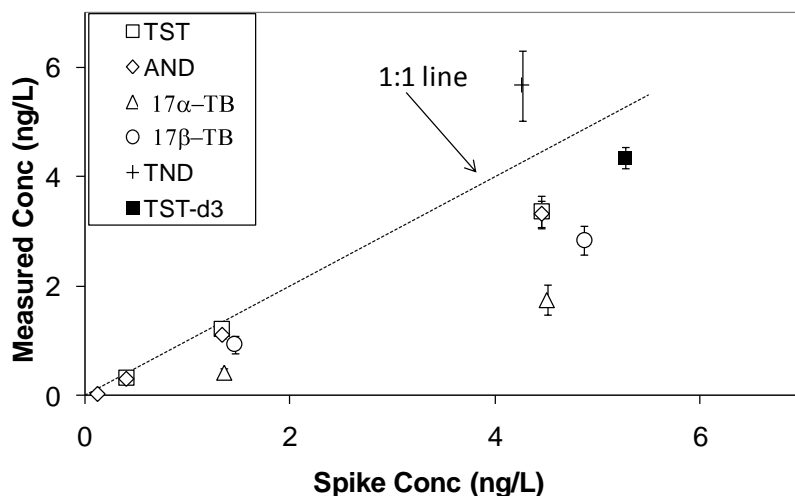
Compound	Compound ID	LOD (ng/L)	LOQ (ng/L)	Retention Time (min)	Transition Monitored Precursor Ion > Product Ion
17 β -estradiol	17 β -E2	0.06	0.21	7.5	271 > 145
17 α -estradiol	17 α -E2	0.09	0.29	8.1	271 > 145
Estrone	E1	0.05	0.16	8.9	269 > 145
Estriol	E3	0.64	2.1	3.1	287 > 145
Testosterone	TST	0.17	0.58	5.5	287 > 97
Androstendione	AND	0.12	0.41	6.1	287 > 97
17 β -trenbolone	17 β -TB	0.47	1.6	4.4	271 > 199
17 α -trenbolone	17 α -TB	0.22	0.74	4.7	271 > 199
Trendione	TND	1.9	6.4	5.4	269 > 225

Effect of Sample Matrix on HPLC/ESI-MS/MS Response. ESI-MS/MS response for many compounds is known to be affected by co-eluting sample matrix components. The sample matrix effect for the steroid hormones under investigation was evaluated by extracting water samples using SPE, reconstituting the resulting extracts in methanolic solution containing varying concentrations of hormones, and analyzing the spiked samples by HPLC/ESI-MS/MS. S2 ditch water samples were homogenized, filtered, extracted and spiked using the same protocol that was used to evaluate the LOD/LOQ.

In general, the presence of the sample matrix resulted in some signal suppression for all the hormones except TND. For the estrogens (negative ESI mode), signal suppression was small and similar between the non-isotopically labeled compounds relative to the deuterated internal standard 17 β -E2-d₃ (**Figure SI-3**). For the androgens (positive ESI mode), signal suppression was more pronounced. TST-16,16,17-d₃ (the internal standard), TST and AND had similar signal suppression (**Figure SI-4**) with the degree of signal reduction greatest for 17- α TB and 17- β TB. On the contrary, enhanced responses to TND were observed in the presence of the sample matrix. These results suggest that using 17 β -E2-d₃ to correct for matrix effects is reasonable for the estrogens with only a minor underestimation of the actual concentration for the estrogen in some cases. For the androgens, TST-d₃ would provide a reasonable correction factor for TST and AND, but would tend to underestimate the concentration of 17- α TB and 17- β TB and overestimate the concentration of TND. Optimally, having deuterated internal standards for all compounds of interest would be best. Deuterated internal standards are currently not available commercially for TND or 17- α TB.



1 **Figure SI-3.** Effect of sample matrix on LC-negative ESI-MS/MS response for estrogens.



2 **Figure SI-4.** Effect of sample matrix on LC-positive ESI-MS/MS response for androgens.

3

4 **Extraction Recovery of Hormones.** Recovery of the hormones during the filtration and extraction
5 procedure was evaluated by spiking water samples prior to extraction by SPE and analysis by LC-ESI-
6 MS/MS. Fifteen liters of water were collected in 1 L polyethylene bottles at S2 over the course of ~ 30
7 minutes using an ISCO unit. The water samples were brought to the lab, combined in a 25 L carboy and
8 mixed thoroughly. Then the water was returned to the 1 L bottles in 950 mL portions to yield 15
9 presumably homogeneous samples. Five of the samples were amended with 0.5 mL of a standard
10 solution containing all hormones dissolved in methanol at a concentration range of 4 – 10 ng/mL.
11 Another five samples were amended with 0.5 mL of a standard solution containing all hormones
12 dissolved in methanol at a concentration range of 0.4 – 0.9 ng/mL. The remaining five samples were not
13 fortified with hormones. The water samples were then filtered, extracted, and analyzed using the same
14 procedure that was used for field samples. Extraction recovery was estimated by comparing the
15 concentrations measured in extracted samples to standards dissolved in methanol. If the presence of
16 endogenous hormones was detected in the non-fortified water samples, the average concentration (n =
17 5) was subtracted from the measured concentration for spiked samples.

Table SI-13 summarizes the extraction recovery for all hormones along with the recoveries obtained after correcting for matrix effects by dividing the absolute recovery of each individual hormone by the magnitude of signal suppression or enhancement measured as described above. For E1 and 17 β -E2, which were detected at concentrations above the method LOD in unfortified samples, the concentrations of natural hormones were subtracted from the measured value before calculating extraction recovery. Recovery did not appear to be concentration dependent for estrogens with matrix effect corrected recoveries ranging from 81-102%. Average recovery for androgens was generally lower. Upon correcting for matrix effects, the recovery for most of the androgens was improved to acceptable levels. When hormones were extracted from de-ionized water using the same procedure, recovery was nearly 100% for all hormones (data not shown).

Table SI-13. Hormone recovery (%) during sample preparation using field samples fortified with hormones at < 1 ng/L and 4 to 10 ng/L with and without matrix corrections and the % proximity estimated based on the use of deuterated compounds from extraction recoveries and matrix effects in field samples.

Analyte	< 1 ng/L (n=5)	4 to 201 ng/L (n=5)	% Proximity to <i>True Concentration</i> estimated using deuterated compounds to estimate recovery and matrix effect in Field Samples ^c
	Recovery corrected for compound-specific matrix effects ^a (%)	Recovery corrected for compound-specific matrix effects ^a (%)	
17 α -E2	97 \pm 24	88 \pm 6	105
17 β -E2	84 \pm 23	82 \pm 5	88
E1	87 \pm 24	85 \pm 5	90
E3	ND	82 \pm 14	91
TST	90 \pm 20	76 \pm 4	99
AND	89 \pm 24	72 \pm 3	100
17 α -TB	< LOD	118 \pm 12	60
17 β -TB	< LOD	89 \pm 5	79
TND	< LOD	50 \pm 6	97

^a matrix correction used is for the specific compound (**Figure SI-3 and 4**) and not based on the internal standard

^b < LOD = below limits of detection

^c 17 β E2-d₃ for estrogens and TST-16,16,17-d₃ for androgens.

Extraction recovery and matrix effects are likely dependent upon several environmentally variable solution parameters including dissolved organic carbon, pH, and sediment load. Differences in hydrophobicity within the estrogens and androgens were considered to be within a relatively small range (e.g., range in log K_{oc} in **Table SI-13**) with regards to impacts on recovery dynamics. Therefore, we chose to add the internal standard prior to extraction allowing it to function as a correction for both extraction efficiency and signal suppression by the sample matrix. Also shown in **Table SI-13** is an estimate of the % proximity to the true concentrations (i.e., using compound specific recoveries and matrix effects) that is obtained using the deuterated compounds from extraction recoveries and matrix effects in field samples.

Recoveries of internal standards including corrections for matrix effects for all the field samples are categorically summarized in **Table SI-14**. Overall recoveries were in the range expected for large field studies. As noted previously (**Figures SI-3 and SI-4**), signal suppression varied for the individual hormone especially for the androgens. For the estrogens and endogenous androgens, recovery and matrix effects were similar to the deuterated internal standards ($17\beta\text{E}2\text{-d}_3$ for estrogens and TST-16,16,17- d_3 for androgens) such that internal standard corrections estimated concentrations within 10% of what the concentration estimates may have been if compound-specific recovery and matrix effects had been applied. For the synthetic androgens, the potential error using the internal standard TST-16,16,17- d_3 to correct for both recovery and matrix effects is greater with the highest being for $17\alpha\text{-TB}$.

Table SI-14. Summary of the internal standard recoveries for $17\beta\text{-E}2\text{-16,16,17-}\text{d}_3$ and TST-16,16,17- d_3 .

Internal Standard	Total # of points	Recovery Range	% Data in this Range	Average %	% SD ^a
$7\beta\text{-E}2\text{-16,16,17-}\text{d}_3$	3559	50 to 150 %	84.8	91.1	18.3
		60 to 140 %	82.6	91.2	16.9
		70 to 130 %	77.9	91.7	14.8
TST-16,16,17- d_3	3688	50 to 150 %	78.4	73.1	19.6
		60 to 140 %	63.9	77.9	17.4
		70 to 130 %	45.4	84.3	14.5

^a standard deviation

Assessing Sorption of hormones to ISCO Polyethylene Collection Bottles. Tests were performed to assess the potential for hormone sorption to the polyethylene collection bottles and amber glass bottles. A 0.5 mL aliquot of an acetonitrile stock solution containing representative hormones (E2, E3, E1, AND, and TST) was added to each bottle filled with deionized water to achieve an approximate concentration of 2 ng/L of each hormone. Bottles were mixed end-over-end for a few minutes and then stored at 4 °C in the dark or extracted right away for a time zero measurement. Hormone concentrations were measured by destructive sampling in duplicate at 0, 24 h, 48 h, and 72 h. Hormone masses recovered between polyethylene and glass bottles were not significantly different over time (0 to 72 h). The observed difference in hormone concentrations were typically less than 5% and were not statistically different at the 95% confidence level between polyethylene and glass bottles over time (0 to 72 h).

Hormone Stability in Field Samples. Hormones are subject to microbial degradation from the time of ISCO collection to the loading of the aqueous samples onto the solid phase cartridges. We estimated the potential loss of hormones during sample processing by adding known amounts of hormones to S2 ditch water collected with the ISCO and monitoring under conditions representative of what the average sample was expected to experience. Conditions included filtered and unfiltered S2 ditch water at 4 °C and ~ 23 °C as detailed below.

Hormone Stability in Hormone-fortified Unfiltered S2 Ditch Water. To determine the stability of hormones in S2 ditch water collected with the ISCO automated sampling unit, field samples were spiked and hormone concentration measured at 0, 26, 50, 74, and 146 h. Fifteen bottles of water were collected in 1 L polyethylene bottles at S2 over the course of ~ 30 minutes using an ISCO unit. The

1 water samples were brought to the lab, combined in a 25 L carboy and mixed thoroughly. Then the
2 water was returned to the 1 L bottles in 950 mL portions to yield 15 presumably homogeneous samples.
3 Samples were fortified with 0.5 mL of a standard hormone mixture in methanol to give a concentration
4 range of 4 – 10 ng/mL and allowed to incubate at 4 °C and 23 ± 2 °C in the dark for the prescribed time
5 interval. After incubation, the water samples were filtered, extracted, and analyzed using the same
6 procedure that was used for all field samples. The experiments conducted at the two temperatures were
7 conducted at different times, and S2 ditch water for collected fresh for each.

8 Of the estrogens, 17α -E2 and 17β -E2 concentrations decreased to $47 \pm 6\%$ for 17β -E2 and $71 \pm$
9 5% for 17α -E2 over a 74-h period at the warmer temperature (**Figure SI-5**). E1 increased over the first
10 74 h of incubation most likely due to microbial conversion of 17α -E2 and 17β -E2 to E1 during the
11 storage period before appearing to decrease. Concentrations of E3, a secondary metabolite, decreased
12 slowly likely because as the initial E3 degraded, additional E3 was produced from degradation of E2
13 and E1. These results demonstrate that, in particular, the E2 isomers have the potential to be partially
14 transformed to E1 during storage of samples under field conditions. This suggests that E1
15 concentrations at the time of ISCO collection may be overestimated while 17α -E2 and 17β -E2 may be
16 underestimated depending on the storage times prior to sample filtration. The transformation rate for the
17 androgens TST and AND was faster than the estrogens with $> 40\%$ degraded within the first 24 h and
18 only $7 \pm 2\%$ and $5 \pm 0.5\%$, respectively, remaining after 74 hours. This rapid dissipation suggests that
19 levels of TST and AND may be greatly underestimated in the majority of samples. The synthetic
20 androgens 17α -TB and 17β -TB decreased at a much slower rate with $67 \pm 18\%$ and $55 \pm 14\%$ still
21 remaining at 146 h, respectively.

22 Although data presented for degradation in amended S2 ditch water samples (**Figure SI-5** and
23 **SI-6**) exemplifies substantial degradation potential for some of the hormones especially the endogenous
24 androgens within the first 24 h, results are not consistent with trends (or lack thereof) for samples
25 collected during base flow over a 24-72 h period and with varying holding times in the laboratory.
26 Although no 2 field samples are expected to be identical even when collected back to back,
27 concentrations for samples collected over an extended period defined as near steady-state based on field
28 conditions would be expected to be higher for the later collection times compared to the early collection
29 times if degradation rates as observed for laboratory-amended S2 ditch samples were applicable. This
30 trend was not observed in samples collected within a 72 h period in the summer months (15 to 30 °C)
31 suggesting that the laboratory-derived rates are not directly applicable to the field samples in storage
32 prior to filtration. We suspect that the enhanced aeration of the ditch water during homogenization
33 immediately prior to hormone amendment greatly elevated aerobic microbial degradation rates (Lim et
34 al., 2007; Ying et al., 2008). In addition, the methanol carrier introduced into the system upon
35 amendment (hormone solution was in methanol) is a readily available food source to microbes, thus
36 potentially able to further elevate microbial degradation rates if concentrations are not too high (Lim et
37 al., 2007).

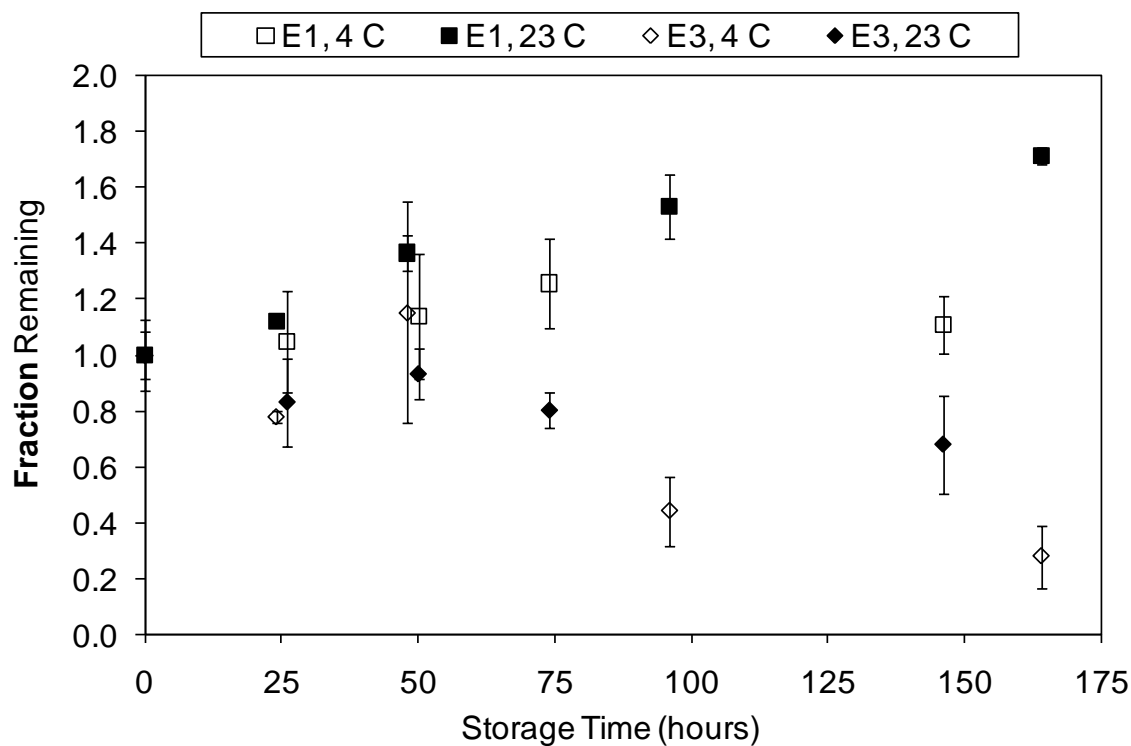
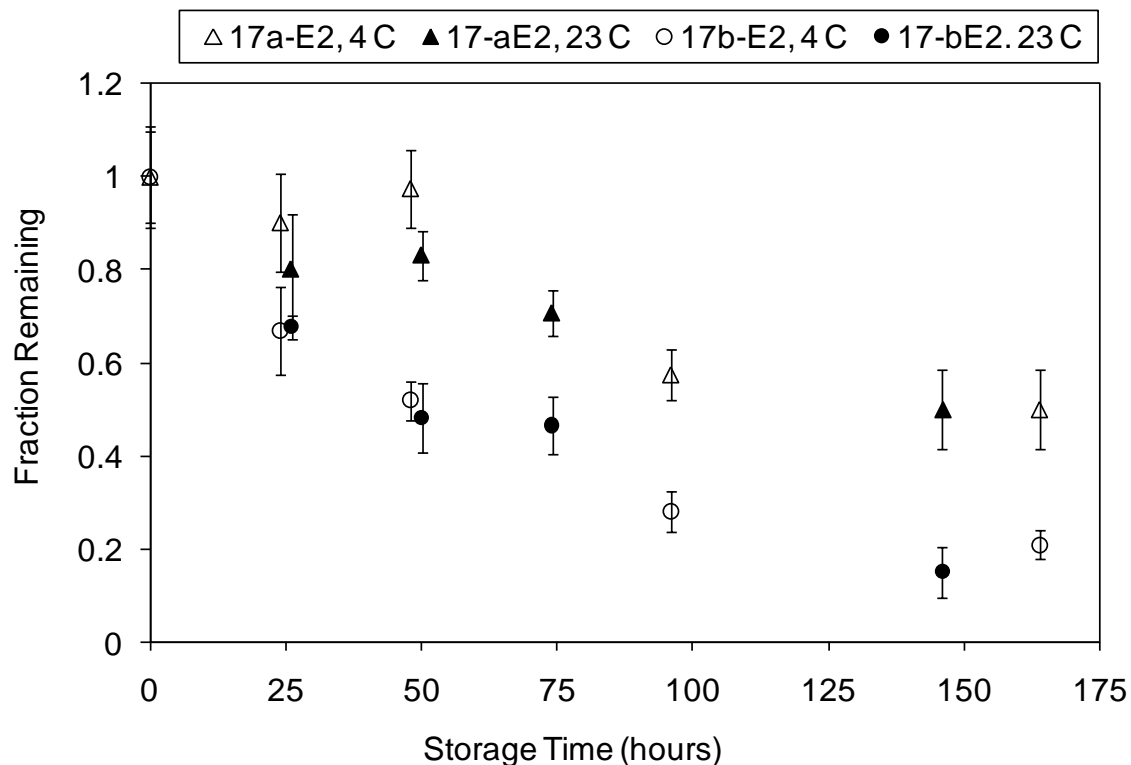


Figure SI-5. Stability of estrogens in unfiltered S2 ditch water amended with a mix of 17α -E2, 17β -E2, E1, and E3 and stored at 4 °C and 23 ± 2 °C. Fraction remaining is based on the amount of that hormone on day 0. E1 and E3 are both known metabolites of E2 degradation. The 4 °C and 23 ± 2 °C temperature studies were conducted at different times, thus the S2 ditch water used for each was collected at different times.

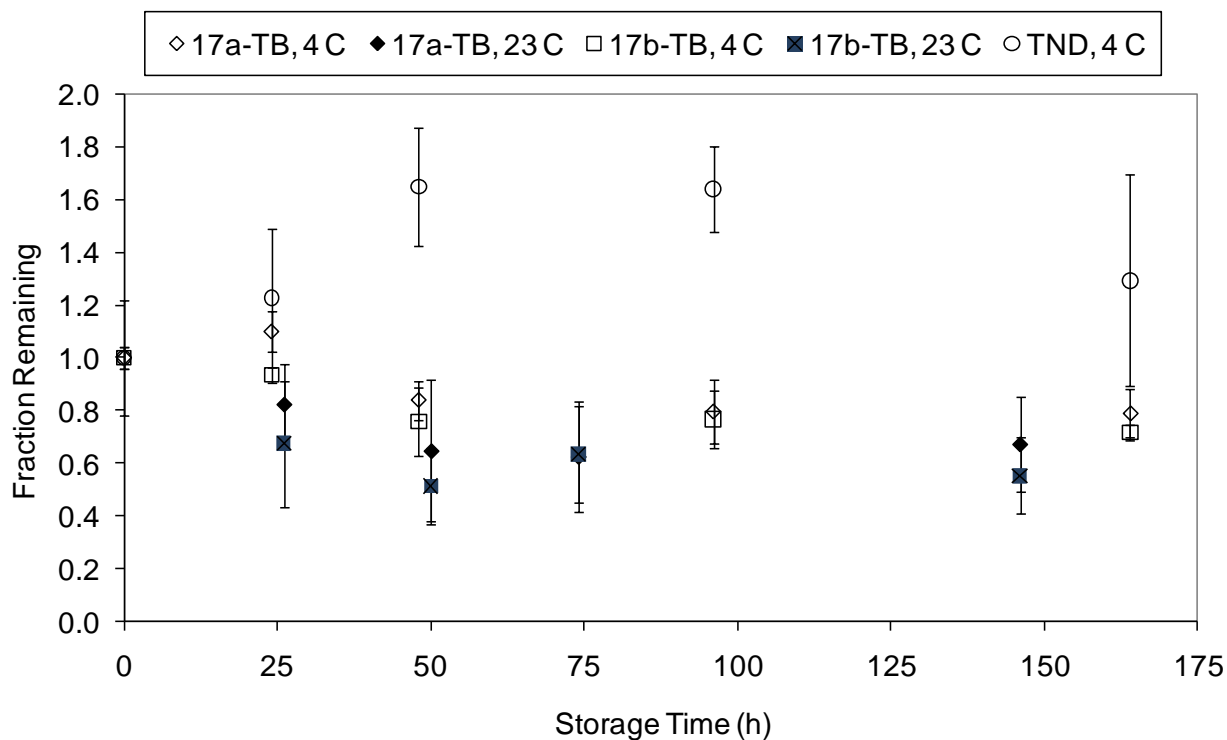
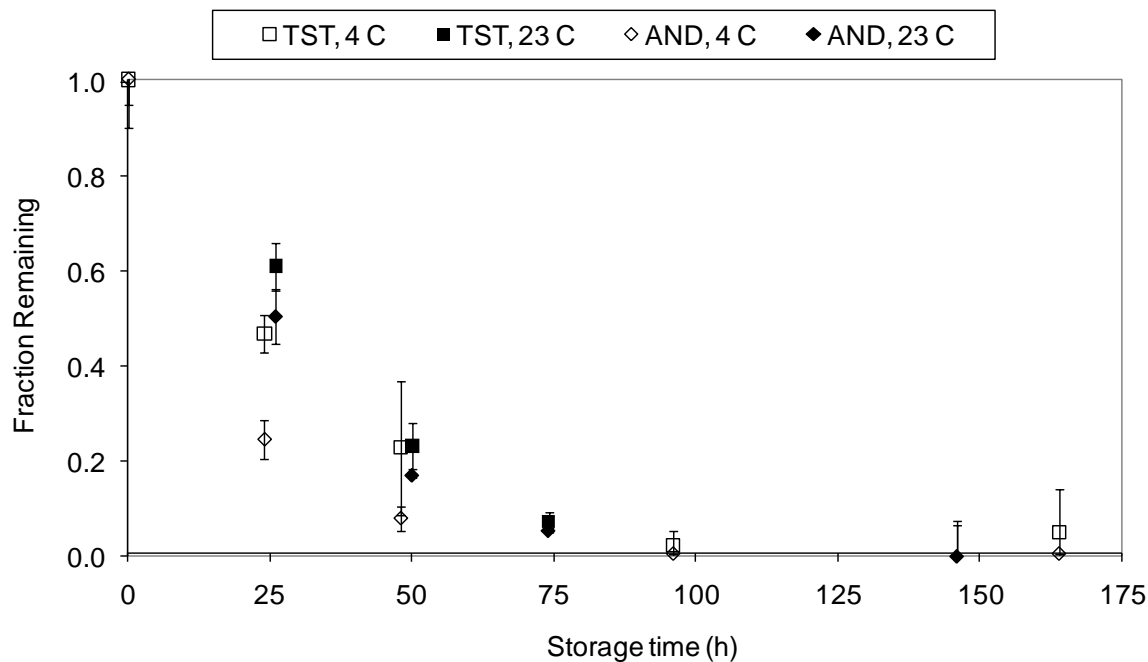


Figure SI-6. Stability of androgens in unfiltered S2 ditch water amended with a mix of TST, AND, 17 α -TB, 17 β -TB, and TND and stored at 4 °C and 23 \pm 2 °C. Fraction remaining is based on the amount of that hormone on day 0. AND and TND are known metabolites of TST and TB degradation, respectively. The 4 °C and 23 \pm 2 °C temperature studies were conducted at different times, thus the S2 ditch water used for each was collected at different times.

Hormone Stability in Hormone-fortified Filtered S2 Ditch Water. Because filtration with 1.2 μm exclusion size glass fiber filters should remove most bacterial cells and increase stability of the hormones, field samples were typically filtered as soon as possible and stored at 4 °C after filtration until they could be extracted by SPE. Stability testing for filtered and unfiltered S2 ditch water was conducted simultaneously. The procedure was identical (described above) except samples were filtered immediately before being fortified with the hormone mixture. **Table SI-15** shows the fraction of each hormone remaining after incubation at 23 ± 2 °C for 26 and 74 h. Limited hormone loss after filtration indicates that once field samples were filtered, hormone concentrations were stable during additional storage, which was never longer than 72 h. Preservation of samples by acidification to pH = 2 using mineral acid has been used by numerous authors and was considered for this work (Labadie and Budzinski, 2005; Baronti et al., 2000; Fine, et al., 2003). However, acidification caused protonation of dissolved organic substances resulting in very darkly colored extracts and subsequent precipitation during storage of prepared samples. Therefore, no sample preservation was employed and instead, the lag time between ISCO collection and retrieval from the field was minimized as much as possible as previously described. Upon receipt in the lab, water samples were refrigerated immediately and typically processed within 36 h.

Table SI-15. Stability of hormones in filtered S2 ditch water at 23 ± 2 °C.

Analyte	Initial Concentration (ng/L)	Fraction Remaining (%) ^a	
		26 h	74 h
17 α -E2	4.4	96 \pm 3	85 \pm 11
17 β -E2	4.3	89 \pm 7	92 \pm 15
E1	3.3	110 \pm 4	109 \pm 15
E3	2.3	87 \pm 8	84 \pm 18
TST	4.5	115 \pm 4	102 \pm 10
AND	4.5	93 \pm 3	92 \pm 13
17 α -TB	4.5	115 \pm 25	94 \pm 16
17 β -TB	4.9	69 \pm 27	106 \pm 12
TND	4.3	49 \pm 13	63 \pm 17

4. Additional Data, Hydrographs, and Hormone Chemographs

Control Plot at the Purdue's Water Quality Field Station (WQFS). The Water Quality Field Station (WQFS) neighbors the east boundary of the ASREC site. The WQFS is located within the Tipton Till Plain region of North Central Indiana, which is subject to perched water tables for extended periods throughout the year. The WQFS was constructed in 1992 to assess the effect of common crop management practices on discharge of nutrients and contaminants and was set up as a randomized complete block design, replicated four times. The WQFS is an approximately 4-ha site where 48 drainage lysimeter plots were constructed and grouped into a random four block design. Each block is subdivided into two groups of six plots. Each rectangular plot is 10 m wide by 48.5 m long, and its long axis is oriented in a north-south direction along a gently downward sloping gradient of the same orientation. Bentonite clay slurry walls 0.3 m thick and 1.6 m deep isolate the middle 24 m of each plot and the north and south ends of each plot. The WQFS has a leachate collection system allowing for the recovery of soil drainage water into subsurface tile lines from a hydrologically isolated volume of soil. Plastic agricultural tile lines (0.1 m d) are placed at 0.9 m depth and cross the length of the collection box. The tiles are perforated for the length of this drainage lysimeter allowing collection of water from within only the lysimeter area. The collection tile is coupled to a non-perforated transfer tile that extends into an instrumented hut. In each instrument hut, stainless steel tipping buckets measure hourly flow volumes. Each tipping bucket is fitted with a magnetic sensor switch (Honeywell SR3C-A1, Sacramento, CA) to determine tips. Tip count data is collected using a Campbell CR10 data logger (Campbell Scientific, Logan, UT) and summarized every hour. Each tipping bucket is individually calibrated for volume per tip and these correction factors are used to convert hourly tips to hourly drain flow volumes. Each side of the tipping bucket measures approximately one liter of water. Once it is full, it tips over, sends a signal to a data logger, and allows the other side of the bucket to begin filling. The Campbell data collection system is also used to trigger ISCO samplers and refrigeration units. The 48 plots receive different types of crop rotations and fertilizer treatment along with four plots that routinely receive subsurface injection of swine manure each fall and spring, respectively. Daily flow-weighted average concentrations of nutrients and dissolved organic carbon have been measured at these plots. We attempted to monitor these plots with intensive monitoring similar to the ASREC site. Our data from this site indicates rapid transport to the tile drains; however, we had frequent equipment failures that left us with incomplete data sets. Also the manure slurries applied to these plots are about 10 times more dilute than manure slurries applied at ASREC. Since they are long-term plots, we were not at liberty to change the manure source being applied. Regardless, we successfully monitored Plot E30 as a control plot from Aug 14, 2009 - May 16, 2010. Plot E30 has received only commercial N-fertilizer for over 10 years and never had any animal wastes manure intentionally applied to the field. However, the plots 15 meters to the east and west of Plot E30 have been receiving subsurface injection of swine manure each fall and spring, respectively. The hormone data (n=22) collected during this period are summarized in **Table SI-16**.

1 **Table SI-16.** Data for control plot E30 at the Water Quality Field Station immediately neighboring the
2 ASREC site. This plot has received only commercial N-fertilizer for over 10 years. Water samples
3 collected with storm events between Aug 14, 2009 and May 16, 2010. A total of 22 samples were taken
4 during this period.

Hormone	Values > LOQ	
(LOD, LOQ)^a	Concentration range^b	Values < LOD
17β-E2	2	19
(0.06, 0.21)	(0.36 - 3.13 ng/L)	
E1	5	10
(0.05, 0.16)	(0.17 - 0.38 ng/L)	
17α-E2	0	19
(0.09, 0.29)		
E3	0	22
(0.64, 0.21)		
17β-TB	0	22
(0.47, 1.6)		
TND	0	22
(1.9, 6.4)		
17α-TB	0	22
(0.22, 0.74)		
TST	0	22
(0.17, 0.58)		
AND	0	22
(0.12, 0.41)		

5 ^a LOQ and LOD are the method limits of quantitation and detection, respectively.

6 ^b Concentration range for the values > the method LOQ

7

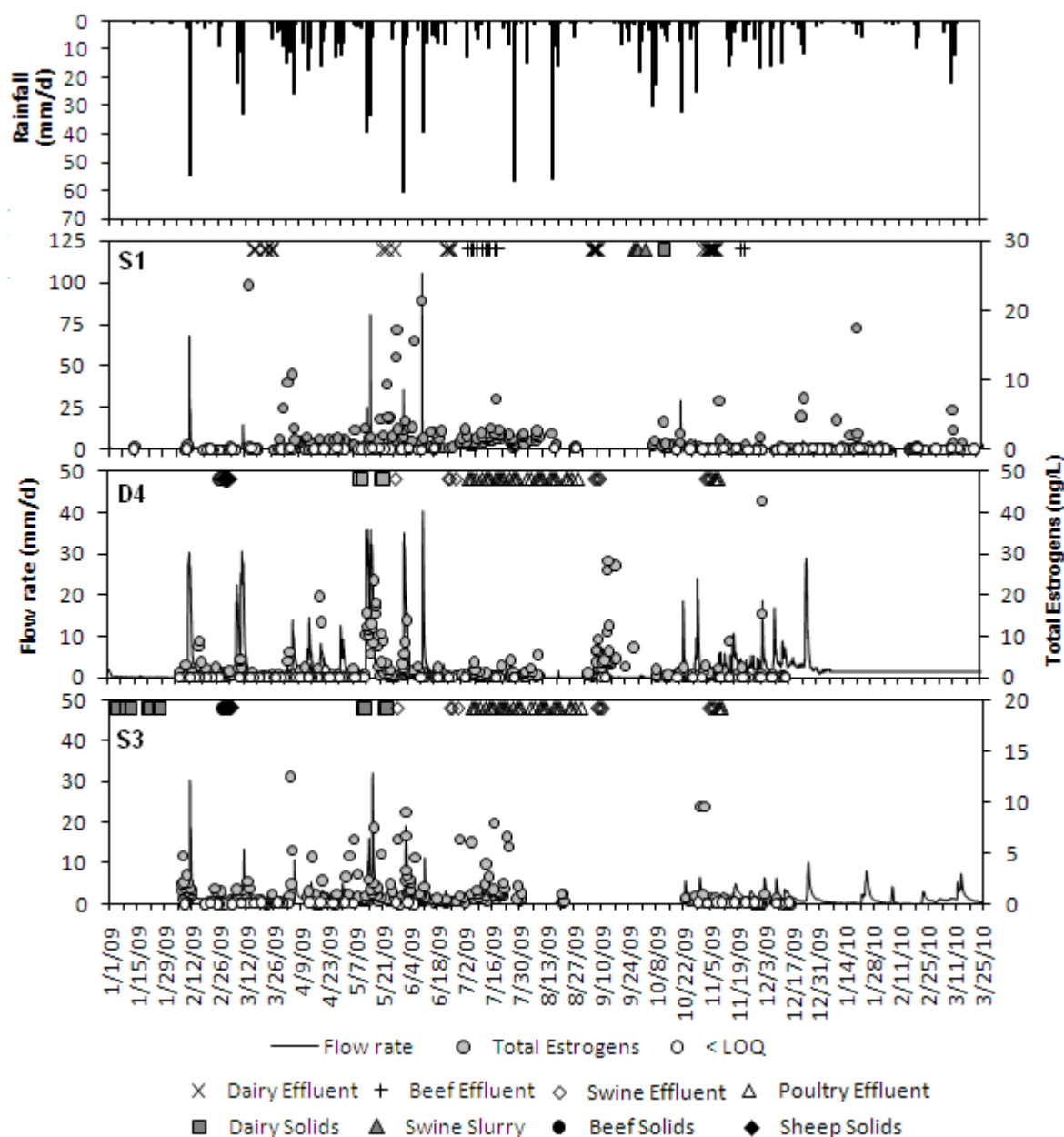


Figure SI-7. Hyetograph, hydrographs, and total estrogen chemographs (● E1 + E2 + E3) for the study period at D4, S1, and S3. Animal waste applications are shown across the top of each figure (× dairy effluent irrigation, + beef effluent irrigation, ■ dairy solids, and ▲ subsurface injection of swine slurry). Application rates are provided in **Tables SI-5, SI-6, and SI-8**. Samples containing estrogen concentrations below the limit of quantitation are depicted as open circles (○ < LOQ) with concentrations below the limit of detection plotted as 0 ng/L. The values plotted in the rainfall hyetograph represent total daily rainfall (*i.e.*, not intensity). Equipment-related errors at D4 resulted in inaccurate flow rate measurements after Jan. 9, 2010 and ISCO sampler-related errors at S3 prevented samples from being collected after late Dec. 2009.

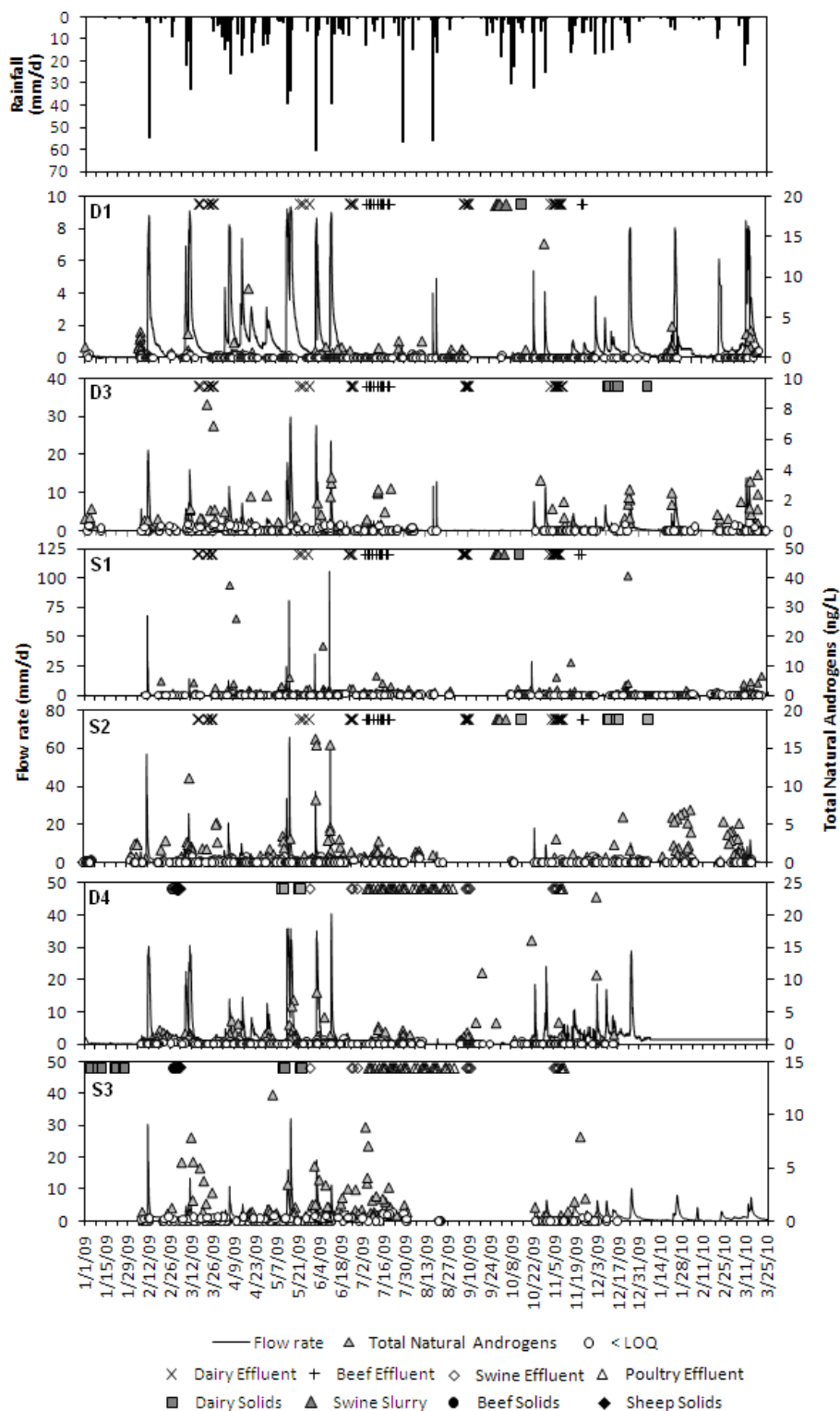


Figure SI-8. Hyetograph, hydrographs, and total natural androgens chemographs (\blacktriangle TST + AND) for the study period at D1, D3, D4, S1, S2, and S3. Animal waste applications are shown across the top of each figure (\times dairy effluent irrigation, $+$ beef effluent irrigation, \blacksquare dairy solids, and \blacktriangle subsurface injection of swine slurry). Application rates are provided in **Tables SI-3 through SI-8**. Samples containing estrogen concentrations below the limit of quantitation are depicted as open circles ($\circ < \text{LOQ}$) with concentrations below the limit of detection plotted as 0 ng/L. Equipment-related errors at D4 resulted in inaccurate flow rate measurements after Jan. 9, 2010 and ISCO sampler-related errors at S3 prevented samples from being collected after late Dec. 2009.

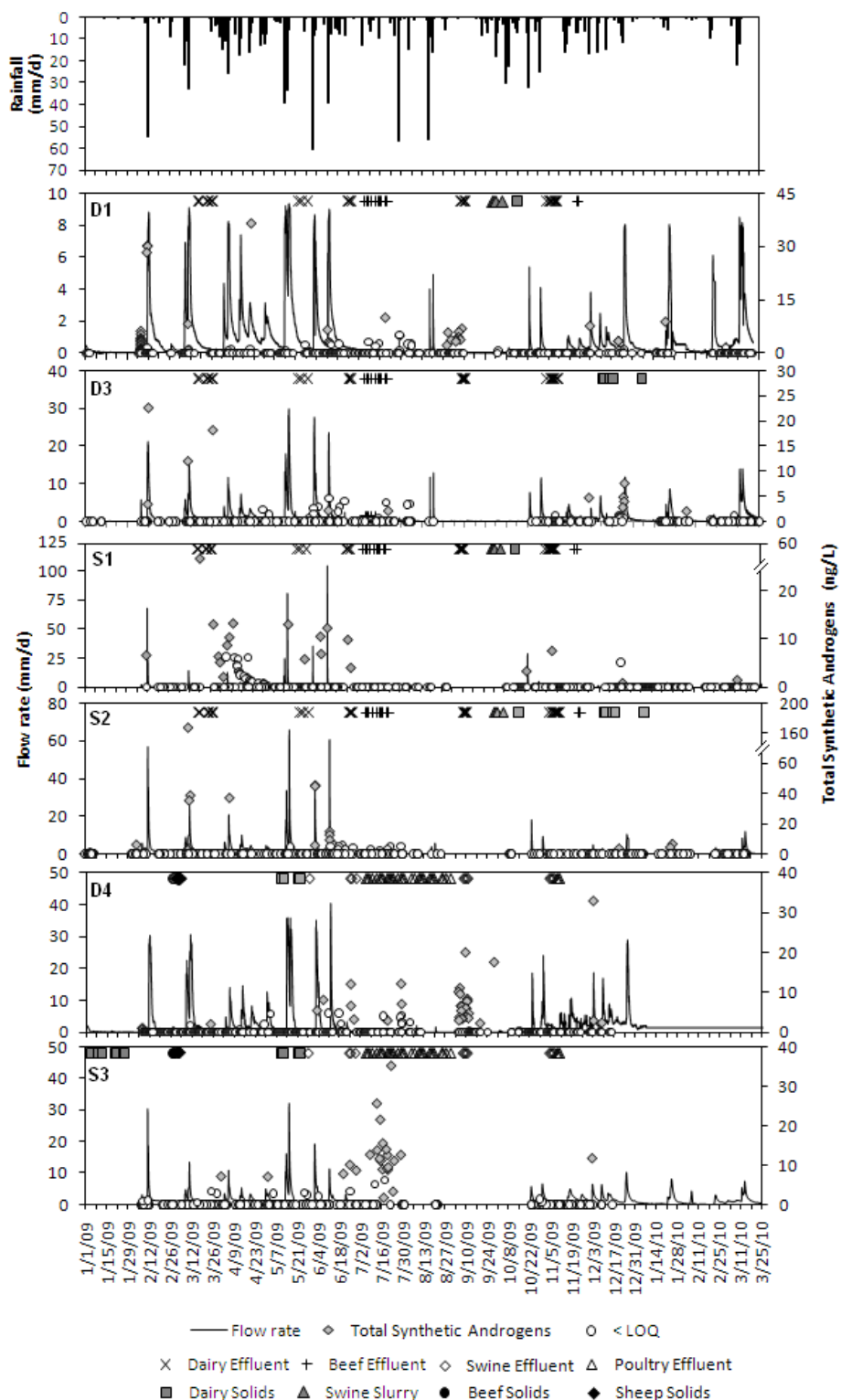
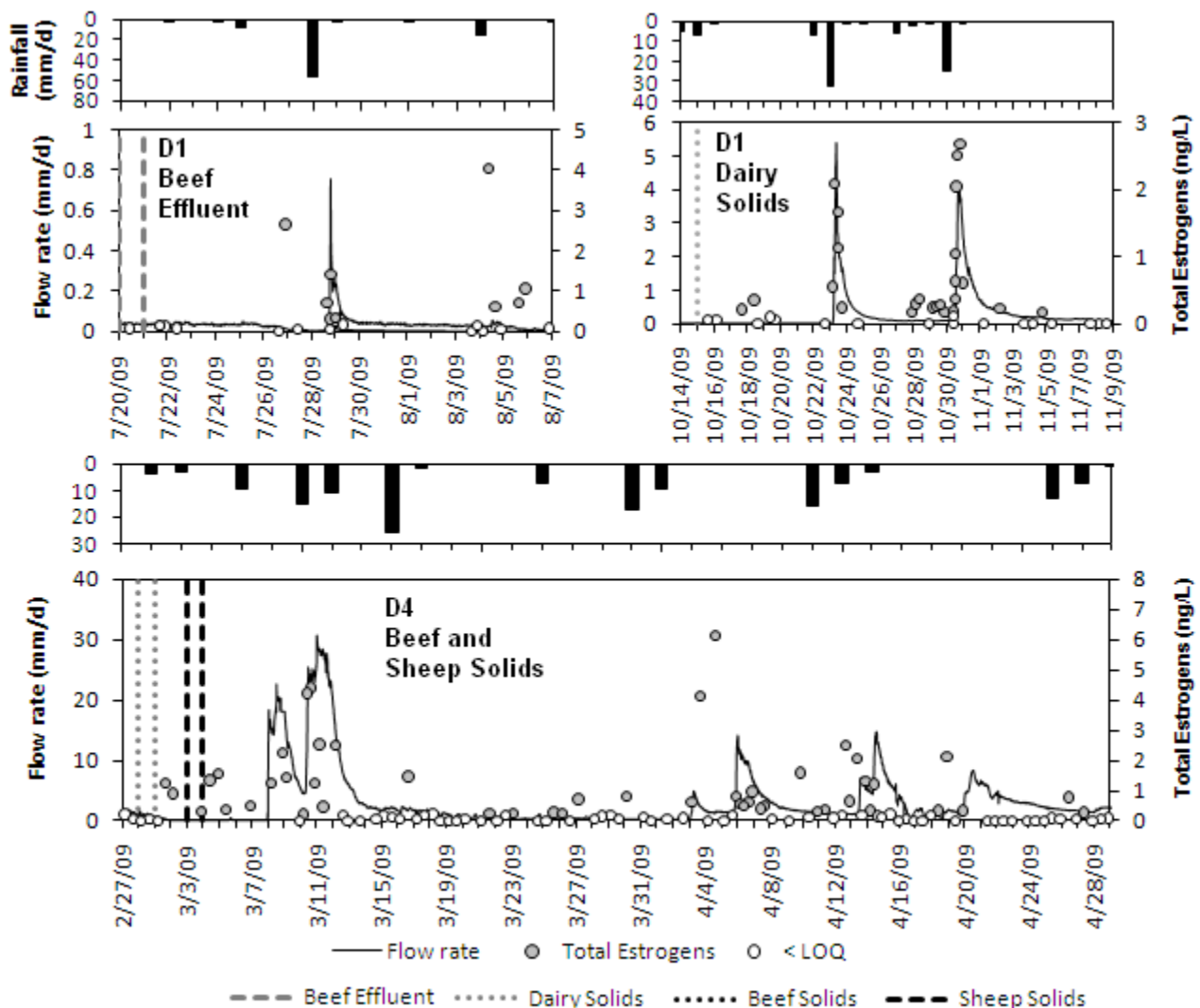


Figure SI-9 Hyetograph, hydrographs, and total synthetic androgens chemographs (◆ 17α -TB + 17β -TB + TND) for the study period at D1, D3, D4, S1, S2, and S3. Animal waste applications are shown across the top of each figure (× dairy effluent irrigation, + beef effluent irrigation, ■ dairy solids, and ▲ subsurface injection of swine slurry). Application rates are provided in **Tables SI-3 through SI-8**. Samples containing estrogen concentrations below the limit of quantitation are depicted as open circles (○ < LOQ) with concentrations below the limit of detection plotted as 0 ng/L. The values plotted in the rainfall hyetograph represent total daily rainfall (*i.e.*, not intensity). Equipment-related errors at D4 resulted in inaccurate flow rate measurements after Jan. 9, 2010 and ISCO sampler-related errors at S3 prevented samples from being collected after late Dec. 2009.

1



2

3 **Figure SI-10.** Hyetograph, hydrographs, and total estrogen (● E1 + E2 + E3) chemographs following
 4 beef effluent irrigation at D1 (top left), dairy solids application at D1 (top right), and beef and sheep
 5 solids at D4 (bottom). Application events (rates given in **Table SI-3** for D1 and **SI-5** for D4) are shown
 6 as vertical lines (--- beef effluent irrigation, dairy solids, beef solids, and --- sheep
 7 solids). Note that all scales are different for each figure for visual clarity of all data. Hormone
 8 concentrations below the limit of quantitation are depicted as open circles (○ < LOQ) with
 9 concentrations below the limit of detection plotted as 0 ng/L.

10

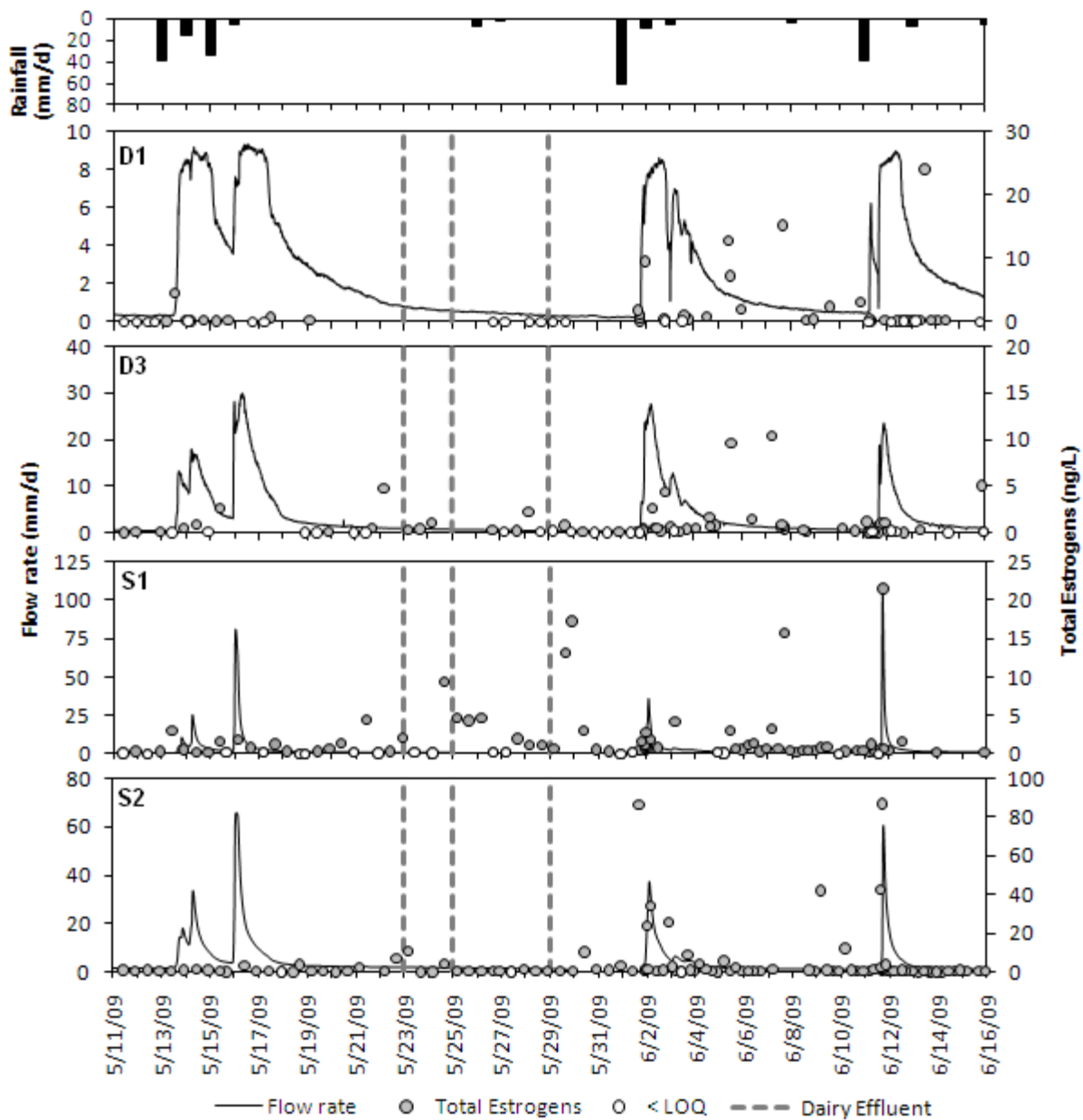


Figure SI-11. Hyetograph, hydrographs and total estrogen (E1 + E2 + E3) chemographs following dairy effluent irrigation. Dairy effluent irrigations events (rates of 65.5 m³/ha) are shown as dashed vertical lines (---). Note that all scales are different for each figure for visual clarity of all data.

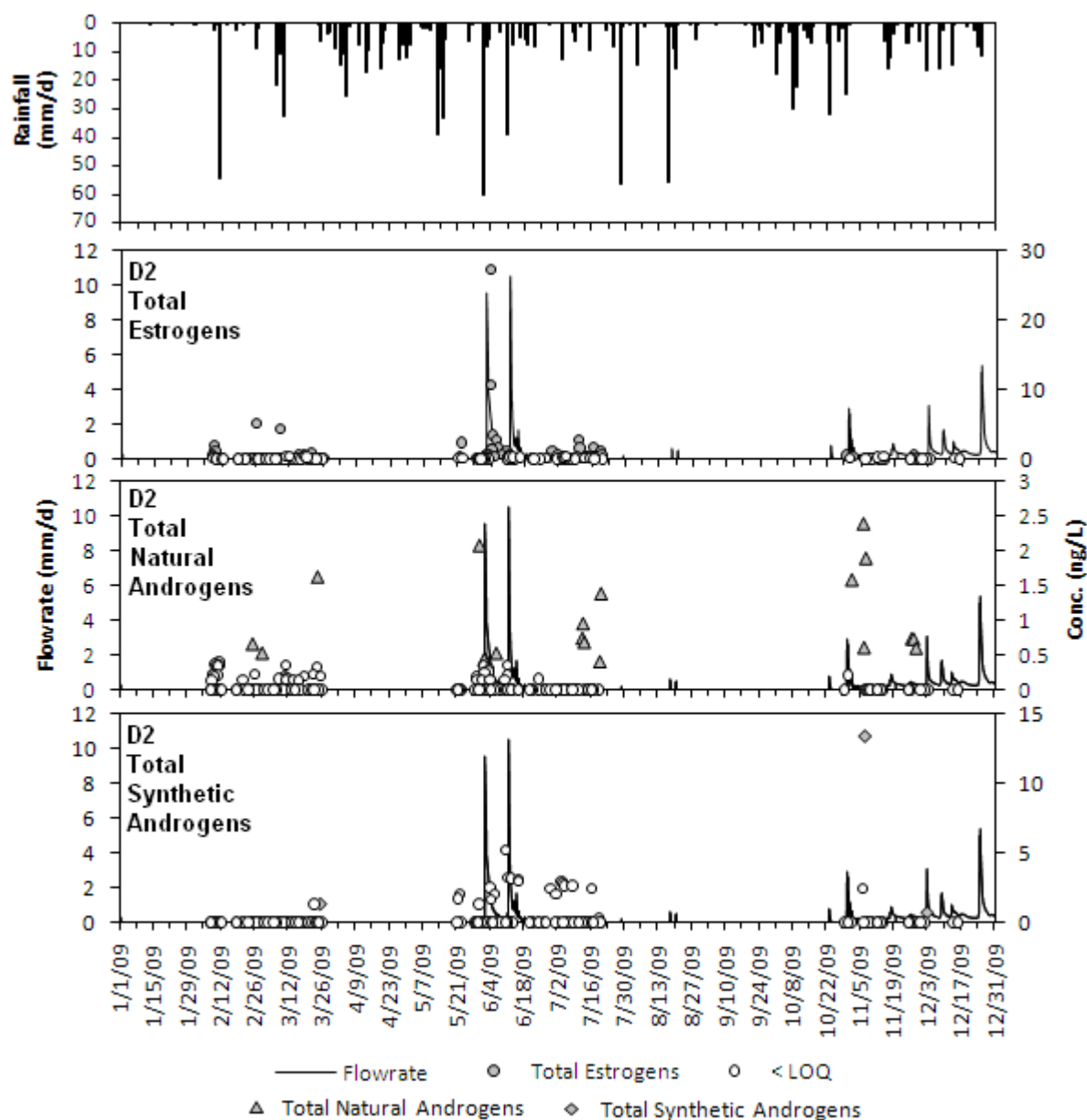


Figure SI-12. Hyetograph, hydrographs, total estrogen chemographs (● E1 + E2 + E3), total natural androgens chemographs (▲ TST + AND), and total synthetic androgens chemographs (◆ 17 α -TB + 17 β -TB + TND) at D2. 2008-2010 application records indicate that no animal wastes were applied on the fields drained by tiles monitored by D2 since before 2008. Hormones in tile drains are likely due to run off from adjacent fields, as the increased concentrations observed at D2 coincide with runoff events (rain or snow melt). The exception is the increased concentrations in July, which do not coincide with runoff but do coincide with effluent irrigation at D1. For graphical clarity, high total estrogen concentrations are shown using a broken-axis notation. Hormone concentrations below the limit of quantitation are depicted as open circles (○ < LOQ) with concentrations below the limit of detection plotted as 0 ng/L.

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