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Received December 3, 2009. Revised manuscript received February 2, 2010. Accepted February 9, 2010.

The impact of a confinement dairy operation (>2000 head) using best management practices for land application of animal wastes, on estrogenic activity (E-Screen), estrogens, and nutrients of associated surface waters and tile drain runoff were evaluated. Farm tile drain and creek samples were collected from the drainage region: above and below a municipal wastewater treatment plant located upstream from the dairy; and downstream from animal housing, parlor, and fields receiving applied wastes. Fifty-four thousand tons of waste (from ~1000 milking head) were applied to ~800 ha from April to July. Maximum estradiol equivalents (E2Eqs) present in tile drain samples (<2057 ng/L) were 2-fold maximum creek E2Eqs, but only 25% of the proposed no observable effect concentration for E2 (1 ng/L). Relative manure slurry estrogen concentrations were estrone >17α-E2 >17β-E2. Creek nutrient concentrations were similar above and below the dairy, with higher concentrations found in tile drain samples: tile ammonia ranged from <0.05 to 0.70 mg/L, nitrate/nitrite from 1.2 to 14 mg/L, and total phosphorus from 0.04 to 0.34 mg/L. No differences in estrogenic activity or nitrate/nitrite, ammonia, and phosphorus concentrations were detected in surface waters downstream of a large confinement dairy facility and measured nutrients were within regional norms.

Introduction

The steroid hormone content of animal waste has long been recognized (1, 2). The potential environmental impact of these steroids has only recently become of concern, as large confined animal operations have become more common, and documentation of endocrine disruption caused by pollutants has surfaced (3). There have been some literature reports of steroid hormones (both androgens and estrogens) and or estrogenic activity in runoff or surface waters associated with animal agriculture (4–6). Few data have been published on estrogenic activity or steroid contents of surface waters associated with large livestock confinement operations that employ best management practices (BMPs) for the application of animal wastes to crop-land.

Specifically, BMPs for handling manure include: composite soil testing for nutrient levels every three years; yearly testing of manure from each storage structure; manure and/or additional nutrient application plan based on crop rotations, actual yield history and soil tests, applying no more nitrogen and phosphorus than crop needs for next growing season; timing of manure application dependent on rainfall predictions (not when >50% chance of rainfall exceeding 1/2 in./1.3 cm); monitoring of soils to assess potential for preferential flow, pooling or runoff of liquid manure; no more than 1/2 in./1.3 cm of liquid manure applied per acre for fields that are tile drained; 100 ft/30.5 m buffer (or 30 foot/9.1 m vegetated buffer) between manure application and state waters; and complete record keeping of analyses and date, rate and method of application (USDA-NRCS, Code 633 and 500, refs 7, 8). The objectives of this study were to determine (1) estimates of nutrient and estrogen loading from dairy waste application, (2) the magnitude and timing of area precipitation events, and (3) nutrient and estrogenicity contents of surface waters from surrounding creek and tile drain water samples from the drainage of a large commercial dairy that employed best management practices for land application of dairy waste. This project was possible through a partnership established with a large confinement dairy operator, a federal agency (U.S. Department of Agriculture, Agricultural Research Service), and a state department of agriculture.

Experimental Section

System Description. Surface waters surrounding a large confinement dairy operation (Figure 1; >2000 milking head) located in the midwestern United States were sampled in April, June, and July in a period when dairy wastewater was land applied. Parlor wash waste, press effluents, and solids were applied to fields by center pivot irrigation, drag-hose application and incorporation, and manure spreader in April and May of 2008. Center pivot irrigation using parlor wash wastes continued through July. Rates of application were in compliance with best management practices for nutrient loading (7, 8) and each field received only a single application by entrenchment. Fields were characterized by degree slopes (i.e., a 2% slope is a 2 ft/0.61 m change in elevation over 100 ft/30.5 m) and soil type. Fields consisted predominantly of Blount loams on 0–2% and 2–6% slopes, followed by fields of Pewamo silt loamy sands on 0–2% slopes, and small areas of Glynnwood loams on 2–6% slopes. A creek running parallel to the dairy farm was sampled upstream 10.9 km from the dairy farm both above (C1; Figure 1) and below (C2) a municipal wastewater treatment plant (0.105 million gallons/day or 0.397 million liters/da), and downstream from the dairy facility and fields receiving dairy wastewater (C3 and C4, Figure 1). There are no allowed agricultural wastewater discharges or agricultural return flows from irrigation in this watershed. Field tile drain water was sampled at two locations (TD1 and TD2). Tile drain water enters the creek between C3 and C4, with a contribution of less than 0.1% of creek volume at that location. Waste was applied no closer than 100 ft/30.5 m from the tile drain. A 12 foot (3.6 m) vegetative buffer zone ran parallel between tile drain and planted crops. The Tile Drain 1 site (TD1) was 150 ft/45 m from the tile system’s origin, with receiving water coming from one 60 acre·/0.243 km² field and possible drainage from a domestic septic system. Fields associated with TD1 received application of
waste on 5/17/08. The Tile Drain 2 site (TD2) was a continuation of the tile system on which TD1 was located, but was approximately 1 mile/1.6 km downstream of TD1 and 0.55 mile/0.9 km above the creek entrance. Fields associated with TD2 (358 acres/1.45 km²) received dairy waste applications from 5/1 to 5/10. The field emptying into TD1 received \( \sim 50\% \) of dry matter per acre that the TD2 acreage received (Figure 2). Applied wastes in Figure 2 represent waste from \( \sim 1000 \) of the cows in residence, field-applied to 1996 acres/8.1 km². Fields did not receive manure from grazing cattle because all cows were maintained in a free-stall barn and did not have access to fields. Manure stored in three concrete lined storage units, press effluent stored in two clay-lined storage units and animal housing were \( \sim 0.4 \) km from the creek. Sampling sites were marked by rebar placed in the banks of the creek or tile drain. Water temperature, width, column height (depth measured across the width at \( \geq 3 \) places (with the exception of TD 1 and 2 on 7/15 because of insufficient water), and estimated flow rate based on measuring water flow and cross-sectional path (9) were recorded.

**Sampling, Extraction, and Analyses.** The farmer, accompanied by an agent of the Federal Fish and Wildlife Services, collected triplicate water samples from each site on 4/20/08, 4/30/08, 6/4/08, and 7/15/08. Samples were collected by hand, in 2 L HDPE bottles (precleaned to Environmental Protection Agency (EPA) standards, EP, VWR International, West Chester, PA) filled six inches below the water surface (exception, tile drains when water was too shallow) while standing in the creek or beside the tile drainage ditch, with container submerged upstream from the collector. After agitation, samples were subdivided into two 500 mL HDPE sample bottles (EP, A. Daigger, Vernon Hills, IL) which were then frozen for estrogen analyses; the remaining one-liter portion was preserved with acid for nitrate/ite, ammonia, and total phosphorus determinations (stored at 4 °C prior to shipment, U.S. EPA methods 353.1, 350.1, and 365.4, respectively (10)).

Waste materials were collected from the drag hose applicator during field application on 5/16/08 (at three separate times). The material being applied to the fields represented a collection of wastes accumulated during the previous 6 months. Processing, to remove bedding sand, was via a McLanahan sand manure separator. Briefly, wastes were screw pressed through a 500 \( \mu \)m screen with the resulting liquid fraction held in a long-term (primary) storage lagoon. Lagoon waste (primary and secondary lagoons) and pressed solids were also analyzed by a commercial lab for dry matter, total Kjeldahl nitrogen, phosphorus and potassium using the methods described by Peters (11).

**Waste Samples.** Water samples were extracted and extracts were evaluated for estrogenicity by E-Screen as described by Shappell (12). Briefly, samples were thawed, shaken, and 250 mL filtered through packed glass wool, followed by solid phase extraction on a cartridge containing a mixture of hydrophobic and hydrophilic packing (OASIS HLB, Waters, Milford, MA). Extracts were eluted with a series...
of organic solvents, evaporated to dryness under N2, and the residue resuspended in water for analysis using the E-Screen assay. The MCF-7 BOS, estrogen-dependent cell line (derived from a human mammary epithelial carcinoma) was used to evaluate sample extract estrogenicity relative to 17-β-estradiol. Extracts were tested over a wide range of concentrations (15-fold concentrated to 128-fold diluted from original sample/environmental concentration). Specificity of estrogenicity in positive samples was confirmed by E2-receptor antagonist ICI 182,780 (Tocris, Ellisville, MO) (13, 14).

Surface water samples with the highest 17-β-estradiol equivalents (E2Eqs) and nanopure extraction blanks were analyzed by LC MS/MS as previously described (ref 15 text and Supporting Information). The same sample extracts prepared for E-Screen were prepared for injection (20 µL of 781 fold original concentration) to contain internal standards (deuterated 17-β-E2, estrone, and estriol at final concentrations of 10 pg/µL and ethinyl estradiol at 20 pg/µL). Parent and three fragment ions were monitored in the ESI mode using a quadrupole-time-of-flight mass spectrometer (LC MSMS using sample extracts concentrated 1.7 fold original concentrations), and for chemical composition (LC MSMS using sample extracts concentrated 1.7–50 times those of the original concentrations). The instrument limits of quantitation (LOQ) were 2 pg/µL on column for all but estriol, which was 5 pg/µL. Limits of detection were 50% of respective LOQs. Limit of detection in original samples depend on the matrix and sample concentration factors.

**Statistical Methods.** Data analyses for this observational study was conducted using SAS/STAT software (17). A general linear model was fitted to observed concentrations of phosphorus, nitrate, ammonia, and E2Eqs using the PROC MIXED procedure. The REPEATED statement was used with the GROUP option to account for heterogeneity of variance among dates and between creek and tile sites. The fitted model included Date, Site and the interaction of Date and Site. The CONTRAST statement was used to compare specific groups of sites (e.g., Tile vs Creek) across dates and for each sampling date. Tukey’s Multiple Comparison procedure was used to provide post hoc comparisons of all possible pairs of site means for each date using a macro written and described by Saxton (18).

**Results and Discussion**

**Estrogens in Surface Water.** Farms using best management practices apply animal wastes to fields to meet N and P needs, and must monitor tile drains to ensure that runoff is not occurring during application. The timing of field application of waste material is dependent on a number of variables including snowmelt, field moisture content, weather forecast, proposed planting date, and availability of personnel. As a consequence, wastes are typically applied over an extended period of time, rather than in a brief discrete time frame. Figure 2 shows the rainfall and schedule of field application of waste (tons of dry matter, grouped by the upstream-most creek site or tile drain into which the fields would drain). In an experimental world where all parameters could be optimized, and timing, duration, and intensity of precipitation events could be predicted, an additional sampling point in mid-May would have been included. Estrogenic activities of surface waters measured by E-Screen (E2Eqs) and rates of water discharge are plotted in Figure 3. All estrogenic activity was confirmed as E2 receptor dependent, because coincu...
Again, while E2Eqs were detected in all but one sampling site but the reverse for the July sampling, TD1 > PROC MIXED was used. Statistical analyses comparing E2Eqs variance, the GROUP option in the REPEATED statement of material in samples. To allow for this heterogeneity of inclusion to varying amounts of sediment and particulate zero for the July sampling. The low volume resulted in discharge rates for tile drain samples were essentially from each site was fairly low, with a mean of 0.05, peaking at this time.

In making statistical comparisons of the TD1 and TD2 samples, the variability of E-screen values should be considered. Overall, the variability for the triplicate samples taken from each site was fairly low, with a mean of all sample sites’ COVs of 28%. In contrast, variability was higher in tile drain samples for the June and July sampling (44%) when the depth of water in the ditches draining the tile drain was limited. The discharge rates for tile drain samples were essentially zero for the July sampling. The low volume resulted in inclusion to varying amounts of sediment and particulate material in samples. To allow for this heterogeneity of variance, the GROUP option in the REPEATED statement of PROC MIXED was used. Statistical analyses comparing E2Eqs from TD1 to TD2 found TD1 > TD2 for both April samples, but the reverse for the July sampling, TD1 < TD2 (P < 0.001). Again, while E2Eqs were detected in all but one sampling site in July, all values from all dates and sample sites were below the pNOEC of 100 pg/L for estriol.

Analyses by LC MSMS were used to identify the chemicals responsible for the estrogenicity detected by the E-Screen assay. Prior to LC MSMS analyses, possible concentrations of estrogens were calculated based on E2Eqs measured by E-Screen and the relative estrogenicity of each estrogen, in order to determine if concentrations would fall within our instrument detection limits. While the E-Screen reflects a sum of biological activity of all estrogens present, calculations were performed assuming that 100% of the estrogenic activity of a given samples was caused by each estrogen separately. Thus, the greatest observed E2Eqs (0.257 ng/L) expressed as 17β-E2 equivalents would represent only ~0.4 pg/μL in an extract concentrated approximately 1500 times; this concentration of 17β-E2 is below the limit of detection by LC MSMS. If estrone or 17α-E2 alone were responsible for the observed E2Eqs, then we would expect to find 100 times the amount of 17β-E2 because estrone and 17α-E2 have only 1% of the estrogenic potency of 17β-E2 (in the E-Screen application). The resulting value, ~40 pg/μL, was well within the limit of quantitation by LC MSMS. Samples from nanopure water control extractions, and sites C2, TD1, and TD2 were analyzed by LC-MSMS. Matrix interference caused ion suppression and samples were diluted to 750 fold original concentrations, at which point deuterated internal standards were then quantifiable. Twenty microliter injection volumes failed to yield any detectable estrogens, suggesting that 17β-E2 may have been the predominant source of E2Eqs present. This data does not rule out the presence of other estrogenic compounds.

Estrogens in Applied Waste. In order to ensure that, in fact, dairy waste contained estrogenic activity at the time of application, waste samples collected at application were extracted by the method of Hanselman et al. (16). The mean E2Eq of the three dairy waste samples was 1417 ng/L, or ~60 µg/kg dry matter (DM, Table 1). Using the method reported by Hanselman et al. (16), estrogens were analyzed only in the final eluent collected after three sequential SPE steps using three different SPE chemistries. However, we found that estrogenic activity was present (50~70% of the final eluent) in the first SPE wash phase (Carbograph). The estrogenic activity in the Carbograph wash could have been the result of unretained estrogen conjugates present in the matrix or due to overloading the cartridges. Subsequent work by H. Hakk, using radiolabeled 17β-E2 in the absence of matrix, has indicated that 50% of the applied radioactivity was also lost in the first Carbograph column washes (personal communication), indicating overloading was not a factor in the incomplete recoveries of estradiol.

Literature reports of estrogen concentrations in dairy waste were converted to E2Eqs for comparison to study results. Hanselman et al (16) evaluated flushed dairy manure wastewater sampled on their university research farm on five consecutive days (above method). Converting the mean estrogen concentrations they determined to E2Eqs, their wastewater contained 625 ng/L E2Eqs. Assuming that the concentrations reported by Hanselman et al (16) underestimate the actual E2Eqs (~40% due to loss on Carbograph washes), then their adjusted mean concentration should have been approximately 1062 ng/L E2Eqs. This value is similar to the 1417 ng/L we reported. Converting chemical data of Raman et al. (21) to estrogenic equivalents, the sum of estrogens in an analysis of dairy waste holding pond material would be 2150 ng/L E2Eqs. Other reports have yielded chemical concentrations of estrogens that result in lower E2Eqs, ranging from 30 to 319 ng/L for dairy lagoon waste (22). However, the report by Sarmah et al. (22), is from dairies in New Zealand, where management practices are very different from those in the United States, including a seasonal milking schedule. Regardless of differences in management practices, it is obvious that the amount of wash water used will seriously impact the reported concentrations. Just as enzyme activity is reported on a mg of protein and unit of time basis, reporting E2Eqs concentrations in animal wastes on a dry matter basis will help compensate for differences due to water content. On a dry matter basis, the mean E2Eqs of the dairy wastewater from Hanselman’s (16) study was 77 µg/kg DM, a value highly consistent with the 60 µg/kg DM reported here. In a review paper of steroids in animal waste (23), 17β-E2 ranged from 113 to 239 µg/kg DM for waste from dairy holding ponds, solid and semisolid dry stacked wastes, with coefficients of variations of 12 and 92%, respectively (n > 20 per mean). These data indicate some degree of consistency of estrogen concentrations in waste on a DM basis, with means varying no more than 2-fold.

| TABLE 1 Estimated Estradiol Equivalents of Applied Dairy Waste |
|-------------------|----------|---------|--------------|----------|
| dairy waste*     | E2Eq ng/L | SD ± c  | fold pLOEC  | dry matter % ± SD*| E2Eq DM µg/kgf |
| grab 1           | 1476     | 84      | 148         | 2.85% ± 0.014  | 51.8       |
| grab 2           | 1742     | 253     | 174         | 3.03% ± 0.042  | 57.5       |
| grab 3           | 1032     | 89      | 103         | 1.76% ± 0.015  | 58.6       |

Waste samples were collected on 5/16/08, at three times over the application period. * Sum of E-screen found in both the Carbograph wash fraction and the ethyl acetate elution from the final column (ODS). a Standard deviations are based on the highest COV from either extract tested. Based on the 10 ng/L pLOEC (19). d Dry matters were based on quadruplicate measurements. Assumption that E2Eqs are constant per unit of dry matter (DM).
Using these data as support for an assumption of relative constancy for E2Eqs on a dry matter basis, the application rate of E2Eqs in our study was 32 mg E2Eqs/acre (7.91 g/km²), or 63 g E2Eqs applied to 1996 acres (8.08 km²). In comparison, values from dairy manure (21, 24) when converted to E2Eqs results in approximately 381 mg E2Eqs per acre (DM basis). Johnson’s value is ten times greater than the value reported here, and reflects the difference in estrogenic activity of fresh manure versus processed or aged waste (25). Similar findings were mirrored in the concentration change of 17β-E2 in fresh manure (153 µg/kg DM) versus dairy waste held for 3 months (18 µg/kg DM) (25).

The relative abundances of specific estrogens present in applied dairy waste were measured by LC MSMS (Table 2). These values do not include estrogens that eluted in the Carboxograph wash fraction. Ion suppression due to matrix effects required dilution of samples to ~2× pre-extract concentrations. Only estrone and 17α-E2 were detected in these samples, with estrone present at greater concentrations. No 17β-E2 was detected. Others (25, 26), have reported similar findings in treated dairy waste, indicating the variability of 17β-E2. Colucci et al. (27) reported conversion of 17β-E2 to estrone, even in the presence of autoclaved soil. When dairy waste is fresh, higher concentrations of 17α-E2 have been reported than in aged or processed wastes; in addition, fresh dairy waste will contain measurable 17β-E2, but no estradiol has been reported (16, 21, 22). Hutchins et al. (28) found lower concentrations of estrone and 17α-E2 in dairy lagoons than those reported in Table 2, but also found α- and β-E2 isomers to be present at similar concentrations. It is unclear if extraction method differences, maturity of waste lagoons, and/or dry matter content account for most of these differences. No attempts were made to analyze for estrogen conjugates, due to their relatively limited estrogenicity (from 500 to 80 000 lower activity than 17β-E2 as assessed by E-Screen) (29).

**Water Nutrient Concentrations.** Nutrient content of wastes effects potential rate of application. The nutrient content of applied wastes is presented in Supporting Information (SI) Table S1. Application rates of N, P, and K were all at, or below commonly recommended rates. Nutrient data, water temperature, and cross sectional area of sampling sites are presented in Figure 4. Water temperatures at creek sites were consistently warmer than those measured in tile drain samples. This is expected, as tile drains are fed by subsurface water flow. Discharge rates were similar to those reported by Dodds and Oakes (30) for creeks and prairie streams, ranging from 1 to 100 ft³/sec and 0 to 0.3 ft³/sec (0.0283 to 2.83 m³ and 0 to 0.0085 m³), respectively (Figure 3). Nitrate/ite concentrations were higher in samples from tile drains than creek samples across all dates (P < 0.0001, maxima of 14.8 versus 0.8 mg/L for tile drain versus creek, respectively). Maximal nitrate/ite concentrations were well below concentrations which would raise environmental alarm. For example, the EPA maximum contaminant level goal for nitrate/ite in drinking water is 10 mg/L (EPA CFR 40) (31), and the highest tile drain samples were 14.8 mg/L, and 10.4 mg/L in TD1 and TD2, respectively, collected in

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**TABLE 2. Estimated Concentrations of Estrogens Detected by LC MSMS.**

<table>
<thead>
<tr>
<th>Dairy Waste</th>
<th>17α-E2 ng/L</th>
<th>Estrone ng/L</th>
<th>17α-E2 µg/kg DM</th>
<th>Estrone µg/kg DM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grab 1</td>
<td>700</td>
<td>3200</td>
<td>25</td>
<td>112</td>
</tr>
<tr>
<td>Grab 2</td>
<td>1100</td>
<td>3900</td>
<td>36</td>
<td>129</td>
</tr>
<tr>
<td>Grab 3</td>
<td>700</td>
<td>1000</td>
<td>39</td>
<td>58</td>
</tr>
</tbody>
</table>

* 17 β-estradiol, estriol, and ethinyl estradiol were not detected. Carbograph extracts were not analyzed. Values are provided to give relative concentrations, rather than absolute concentrations, as matrix effects and detection limits hindered LC MSMS performance.

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**FIGURE 4. Water chemistry, water temperature, and cross sectional area of sampling sites.** Nitrate/ite, ammonia, and total phosphorus concentrations (means ± standard deviations from three separate samples) and cross-sectional areas of creek or tile drain at time of sampling. Temperature in degrees C. Limits of quantitation were 0.1, 0.05, and 0.01 mg/L for nitrate/ite, ammonia, and phosphorus, respectively. Within a sampling date, bars with different letters are different based on analyses of least-squares means (P < 0.05).

June: Kolodziej et al. (5) reported a range of 9.5–350 mg/L for nitrate in tile drain samples found on dairy farms. While nitrate/ite were consistently higher in tile drain samples than creek samples, both within and across dates (P < 0.05); ammonia results varied by sampling date and site (P < 0.05). Ammonia was below assay detection limits (0.05 mg/L) at all sites sampled in April except TD1. The June sampling yielded detectable ammonia concentrations at all sites, and these were higher (P < 0.05) in ammonia concen-
trations than other dates across all sites except July TD1. Mean creek ammonia concentrations fell back to below assay limits in July, while tile drain samples were still elevated (0.70 TD1 > 0.15 TD2, P < 0.05). Peak ammonia concentration from tile drain was 0.7 mg/L versus 0.2 mg/L in creek samples. Limits for ammonia concentrations in aquatic warm water habitat streams are dependent on duration of exposure, pH, and water temperature, and are cited as 4.16 mg/L at 20 °C, and pH 7.5 (32). While July tile drain values rose to 0.7 mg/L ammonia, the permitting limits for summer release of ammonia by some US MWTPs is 19 mg/L. Sample pH values are reported in SI Table S2.

Phosphorus concentrations ranged from 0.025 to 0.340 mg/L and were similar to those previously reported in creeks (0.013 to 0.234 mg/L) (30). For both April sampling dates, all creek phosphorus concentrations were lower than TD1 (P < 0.05), and TD2 phosphorus was intermediate to both creek and TD1. In June, tile drain values were higher than creek (P < 0.05). The greatest values were measured in July, at times of lowest discharge rates. While July phosphorus values for TD1 and TD2 were not different, the mean for C3 was equivalent to TD2 and higher than all other creek concentrations (P < 0.05). Phosphorus concentrations mirrored those of nitrate/ite, in that TD1 were higher than TD2, with the exception of the June phosphorus sample. Limits for phosphorus in surface water and discharge concentration limits for MWTPs have yet to be established in the U.S. Typically regional permits designate total maximum daily loads based on total phosphorus loading and monitored phosphorus concentrations. Warm water goals of 1 mg/L are typically met, and even the highest P values from tile drains (0.34 mg/L) were ~30% of the mean for 25 Canadian MWTP (1.3 mg/L) (33) and 10% of typical discharge concentrations from a local municipal wastewater treatment plant (3 mg/L, MWTP, Fargo, ND). Creek P values were in line with reported 25% values for the decade in Ecoregion VI of 0.076 mg/L (EPA, Table 2, Median stream values, 2000; ref 34).

In conclusion, no differences in estrogenic activity or nitrate/ite, ammonia, and phosphorus concentrations were detected in surfaces waters upstream and downstream of a large confinement dairy facility. The highest E2Eqs detected were measured in tile drain samples, but concentrations of E2Eqs in those samples were approximately 1/3 lower than the proposed no effect concentration for estradiol. Water quality (nitrate/ite, ammonia, and total phosphorus) were similar regardless of sampling site along the creek, and were within regional norms. It appears that use of best management practices for land application of dairy waste results in negligible nutrient and estrogenic contributions to surface waters associated with large dairy operations.

Acknowledgments
Appreciation is extended to Dr. Ana Soto and Dr. Carlos Sonnenschien, who graciously provided cells and access to their laboratories to learn E-screen methodology. We would like to acknowledge the dedicated efforts of Mr. Lloyd Billey in sample extraction and E-screen analyses; excellent technical skills of Grant Harrington in operation of the LC/MS-MS; and Dr. Hedur Hak’s pioneering efforts in method development for quantification of steroids by LC/MS-MS. Mention of trade names or commercial products in this article is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture.

Supporting Information Available
Methodology for estimation of flow rates for creek and tile drains; N, P, and K concentrations of applied dairy waste, and pH of water samples are provided in Supporting Information. This material is available free of charge via the Internet at http://pubs.acs.org.

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