

Steroid Hormone Runoff from Agricultural Test Plots Applied with Municipal Biosolids

Yun-Ya Yang, James L. Gray, Edward T. Furlong, Jessica G. Davis, Rhiannon C. ReVello, and Thomas Borch

Environ. Sci. Technol., **Just Accepted Manuscript** • DOI: 10.1021/es203896t • Publication Date (Web): 30 Jan 2012

Downloaded from <http://pubs.acs.org> on January 31, 2012

Just Accepted

“Just Accepted” manuscripts have been peer-reviewed and accepted for publication. They are posted online prior to technical editing, formatting for publication and author proofing. The American Chemical Society provides “Just Accepted” as a free service to the research community to expedite the dissemination of scientific material as soon as possible after acceptance. “Just Accepted” manuscripts appear in full in PDF format accompanied by an HTML abstract. “Just Accepted” manuscripts have been fully peer reviewed, but should not be considered the official version of record. They are accessible to all readers and citable by the Digital Object Identifier (DOI®). “Just Accepted” is an optional service offered to authors. Therefore, the “Just Accepted” Web site may not include all articles that will be published in the journal. After a manuscript is technically edited and formatted, it will be removed from the “Just Accepted” Web site and published as an ASAP article. Note that technical editing may introduce minor changes to the manuscript text and/or graphics which could affect content, and all legal disclaimers and ethical guidelines that apply to the journal pertain. ACS cannot be held responsible for errors or consequences arising from the use of information contained in these “Just Accepted” manuscripts.

1
2
3 **1 Steroid Hormone Runoff from Agricultural Test Plots Applied with Municipal Biosolids**
4

5
6 2 YUN-YA YANG,[†] JAMES L. GRAY,[‡] EDWARD T. FURLONG,[‡] JESSICA G. DAVIS,^{†,§}
7
8 3 RHIANNON C. REVELLO,[‡] AND THOMAS BORCH^{*,†,||}
9

10
11 4
12
13
14 5
15
16
17 6
18
19
20 7 [†]Department of Soil and Crop Sciences, Colorado State University, Fort Collins, Colorado
21
22 8 80523-1170.
23

24
25 9 [‡]National Water Quality Laboratory, U.S. Geological Survey, Denver Federal Center, Denver,
26
27 10 Colorado 80225-0046.
28

29
30 11 [§]Institute for Livestock and the Environment, Colorado State University, Fort Collins,
31
32 12 Colorado 80523-1170.
33

34
35
36 13 ^{||}Department of Chemistry, Colorado State University, Fort Collins, Colorado 80523-1872.
37
38

39 14
40
41
42 15 *To whom correspondence should be sent:
43

44
45 16 Thomas Borch, Department of Soil and Crop Sciences, 1170 Campus Delivery,
46
47

48
49 17 Colorado State University, Fort Collins, Colorado 80523-1170, USA
50

51
52 18 Phone: +1-970-491-6235, Fax: +1-970-491-0564, email: thomas.borch@colostate.edu
53
54

55 19
56
57

58 20
59
60

21

22 **Table of Contents (TOC) Art**

23



24

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3 25
4 26 **Abstract**
5
6

7 27 The potential presence of steroid hormones in runoff from sites where biosolids have
8
9
10 28 been used as agricultural fertilizers is an environmental concern. A study was conducted to
11
12 29 assess the potential for runoff of seventeen different hormones and two sterols, including
13
14 30 androgens, estrogens, and progestogens from agricultural test plots. The field containing the
15
16 31 test plots had been applied with biosolids for the first time immediately prior to this study.
17
18 32 Target compounds were isolated by solid phase extraction (water samples) and pressurized
19
20 33 solvent extraction (solid samples), derivatized, and analyzed by gas chromatography-tandem
21
22 34 mass spectrometry. Runoff samples collected prior to biosolids application had low
23
24 35 concentrations of two hormones (estrone <0.8 to 2.23 ng L^{-1} and androstenedione <0.8 to
25
26 36 1.54 ng L^{-1}) and cholesterol ($22.5 \pm 3.8 \text{ } \mu\text{g L}^{-1}$). In contrast, significantly higher
27
28 37 concentrations of multiple estrogens (<0.8 to 25.0 ng L^{-1}), androgens (<2 to 216 ng L^{-1}), and
29
30 38 progesterone (<8 to 98.9 ng L^{-1}) were observed in runoff samples taken 1, 8, and 35 days
31
32 39 after biosolids application. A significant positive correlation was observed between
33
34 40 antecedent rainfall amount and hormone mass loads (runoff). Hormones in runoff were
35
36 41 primarily present in the dissolved phase ($<0.7\text{-}\mu\text{m}$ GF filter), and, to a lesser extent bound to
37
38 42 the suspended-particle phase. Overall, these results indicate that rainfall can mobilize
39
40 43 hormones from biosolids-amended agricultural fields, directly to surface waters or
41
42 44 redistributed to terrestrial sites away from the point of application via runoff. Although
43
44 45 concentrations decrease over time, 35 days is insufficient for complete degradation of
45
46 46 hormones in soil at this site.
47
48

47
48 **Introduction**
49
50
51
52
53
54
55
56
57

49 49 The presence of endocrine-disrupting chemicals (EDCs) in the environment, including
50
51
52
53
54
55
56
57
58
59
60

1
2
3 50 natural and synthetic hormones, has become a growing concern because low part-per-trillion
4
5 51 concentrations of these chemicals have caused adverse impacts on aquatic organisms.^{1, 2}
6
7
8 52 Possible sources of hormones to the environment include discharges from wastewater
9
10 53 treatment plants (WWTPs),³⁻⁵ use of reclaimed water for irrigation,⁶ domestic septic
11
12 54 systems,⁷ effluents from concentrated animal feeding operations (CAFOs),⁸⁻¹⁰ and runoff
13
14 55 from agricultural fields where manure and biosolids (organic-rich solids resulting from
15
16 56 treatment of sewage sludge) are applied as fertilizers and soil amendments.¹¹⁻¹³
17
18
19

20 57 Numerous laboratory studies have investigated the persistence and degradation
21
22 58 pathways of hormones in manure,^{14, 15} WWTP-affected streams and groundwater,^{16, 17} and
23
24 59 biosolids.^{18, 19} However, most studies investigating the behavior of hormones in manure or
25
26 60 biosolids-amended soils have been conducted under laboratory rather than field conditions.
27
28 61 Jacobsen et al.¹¹ investigated the impact of biosolids amendments to soils on testosterone
29
30 62 degradation in laboratory microcosms. The observed inhibition of [¹⁴C]-testosterone
31
32 63 mineralization in a loam soil heavily amended with biosolids was suggested to result from
33
34 64 inhibition of microbial activity or increased sorption. Stumpe and Marschner²⁰ conducted
35
36 65 laboratory incubation experiments to determine the mineralization potential of 17 β -estradiol
37
38 66 (β E2) and testosterone in soils with long-term biosolids application and wastewater irrigation,
39
40 67 and found that for β E2 the mineralization rate (5-7%) was lower and the sorption affinity was
41
42 68 higher than for testosterone in all test soils. Long-term application of biosolids had no effect
43
44 69 on the 21-day hormone mineralization rates while long-term irrigation with wastewater did,
45
46 70 likely due to soluble sorbents (e.g., colloidal and dissolved organic matter) decreasing
47
48 71 bioavailability. In another recent study, mineralization of estrogens was not related to changes
49
50 72 in sorption parameters, rather it was enhanced by up to 147% or depressed by up to 50%,
51
52 73 depending on site characteristics and the type of organic waste applied (i.e., manure, biosolids,
53
54 74 wastewater).²¹ In sum, these studies show that while these hormones can be transformed in
55
56
57
58
59
60

1
2
3 75 agricultural settings, they likely persist long enough to potentially impact the quality of runoff
4
5
6 76 water.

7
8
9 77 Other studies have indicated that land application of manure can result in hormone
10
11 78 leaching and runoff.^{12, 22} Agricultural management practices such as tillage and rate of
12
13 79 manure application may also affect the eventual exports of steroid hormones in runoff.
14
15 80 Compared with raw litter and reduced tillage, no-tillage and pelletized litter treatments
16
17 81 yielded much lower exports and concentrations of estrogens, indicating that pelletized litter
18
19 82 and no-tillage could be used as best management practices to reduce estrogen exports from
20
21 83 agricultural fields.²³ Testosterone (6 to 19 ng L⁻¹) and β E2 (19 to 389 ng L⁻¹) were observed
22
23 84 in runoff following poultry litter application depending on tillage conditions,²⁴ whereas
24
25 85 another study detected β E2 (6 to 66 ng L⁻¹) in groundwater adjacent to fields amended with
26
27 86 poultry litter and cattle manure.²⁵ Nichols et al.²⁴ demonstrated that field-applied poultry
28
29 87 litter can contribute β E2 to runoff, and amending poultry litter with alum reduced mean β E2
30
31 88 concentrations. Finlay-Moore et al.²⁵ measured the β E2 (20 to 2530 ng L⁻¹) and testosterone
32
33 89 (10 to 1830 ng L⁻¹) in runoff water and soil from poultry (broiler) litter-amended grasslands.
34
35 90 In field soil, the highest observed concentration of β E2 and testosterone was 675 and 165 ng
36
37 91 kg⁻¹, respectively. In these studies, runoff concentration appeared strongly dependent on the
38
39 92 litter application rate and frequency.^{24, 25}

40
41
42
43
44
45
46
47 93 Mansell et al.¹⁰ investigated soil and runoff concentrations in simulated rainfall
48
49 94 experiments from steer feedlots. Whereas only three of the six hormones they measured were
50
51 95 detected in fresh manure, all six were detected in surficial soil after two weeks. This is likely
52
53 96 due to conversion of testosterone to androstenedione and α E2 to E1 and β E2 as the soil aged.
54
55 97 Androstenedione and progesterone in aged soils were present at levels much higher than could
56
57 98 be accounted for by antecedent levels of testosterone. Other research indicates that both of
58
59 99 these compounds can be generated from sterols that are likely to be present in manure as well
60

1
2
3 100 as in biosolids.²⁶
4
5

6 101 In the United States, it is estimated that the average WWTP produces 240 kg dry
7
8 102 weight of solids per million liters of wastewater treated, resulting in approximately 8 million
9
10 103 tons (dry weight basis) of biosolids produced per year, of which about 50% are land
11
12 104 applied.²⁷ Surprisingly, little is known about the potential for runoff of hormones after land
13
14 105 application of biosolids, although some inferences can be drawn from the existing studies
15
16 106 conducted with manure and poultry litters indicating that hormones can be mobilized from
17
18 107 solid material by rainfall. Agricultural operations both through livestock emission and
19
20 108 spreading of manure can be an important source of hormones to the environment.²⁸ Thus,
21
22 109 the main objectives of this study were to evaluate the potential for hormone (compounds
23
24 110 listed in Table S1 of the Supporting Information) runoff from agricultural test plots applied
25
26 111 with biosolids and to elucidate the major transport mechanisms.
27
28
29
30
31
32
33
34

35 113 **Experimental Section**

36 114 **Experimental Site and Sample Collection**

37
38 115 During April to June, 2008, three experimental plots (6 m²; 2 m wide by 3 m long) in
39
40 116 eastern Colorado (latitude 40°06'08"N, longitude 104°12'43"W, Figure S1) were established.
41
42 117 The experimental plots are located in a dryland winter wheat field (wheat-fallow rotation)
43
44 118 with reduced tillage, typical of the fields receiving biosolids from Denver and suburbs.
45
46 119 Rainfall simulations replicating a 100-year rainfall event (approximately 65 mm hr⁻¹) were
47
48 120 performed 5 days before, and 1, 8, and 35 days after routine biosolids application. Biosolids
49
50 121 had not been previously applied to this site. The biosolids applied to the test plots were
51
52 122 produced through anaerobic digestion and dewatering of concentrated solids from primary
53
54 123 and secondary treatment at an urban wastewater treatment plant in Colorado. The actual
55
56
57
58
59
60

1
2
3 124 application rate was about 3.5 dry metric tons of biosolids per hectare. Immediately after
4
5 125 application, the biosolids were partially incorporated into the soil with a chisel plow to a
6
7
8 126 depth of about 15 cm. The plots were established parallel to the slope (~3%), and the soil at
9
10 127 the lowest position (plot 1) had the finest texture and most gentle slope (Table S2, Figure S1).
11
12 128 Before each simulated rainfall, soil samples were collected from locations outside the
13
14 129 experimental plots in tared cans, at depths of 0 to 2, 2 to 6, and 6 to 10 cm for soil moisture
15
16 130 measurement (Figure S2). Cans were weighed, dried at 105 °C for 24 h, and then weighed
17
18 131 again to determine soil moisture. Soil was sampled at 9 randomly selected points in the 0 to
19
20 132 10 cm layer. The 9 samples were combined to yield one composite sample for soil properties
21
22 133 analysis. The soil type on the experimental site was loamy sand (% sand/silt/clay: 82/12/6) to
23
24 134 sandy loam (% sand/silt/clay: 86-88/8-9/4-5; Table S2).
25
26
27
28
29

30 135 Artificial rainfall was applied to each 6-m² plot with a rainfall simulator that used a
31
32 136 TeeJet™1/2 HH-SS50WSQ nozzle placed in the center of the simulator.²⁹ The nozzles and
33
34 137 associated water piping, pressure gauge, and electrical wiring were mounted on an aluminum
35
36 138 frame. The simulator was placed approximately 3 meters above the soil surface, and
37
38 139 hormone-free well water was used in all experiments (pH = 6.5 to 7, EC = 0.04 to 2 dS m⁻¹).
39
40 140 Metal borders were installed (0.2-cm thick and 15.2-cm wide) 7 cm above ground and 9.25
41
42 141 cm below ground to isolate surface runoff. A runoff collection gutter was installed at the
43
44 142 down-slope edge of each plot to divert runoff to the collection point. Calibration cans were
45
46 143 placed at three sides of the plot to collect rainfall in order to determine the uniformity of flow
47
48 144 over the plot. Runoff and particulate matter yields from each 6-m² plot were measured during
49
50 145 each experiment. The runoff flow rate was measured as described previously³⁰. Rainfall was
51
52 146 applied and periodic samples were collected until *ca.* steady-state runoff rate was observed
53
54 147 (30 to 60 min per test). Twelve runoff samples were collected in 1-L amber glass bottles from
55
56 148 each plot through the course off each simulated rainfall event, and then placed on ice in the
57
58
59
60

1
2
3 149 dark and transported to the laboratory. The 12 runoff samples collected from each experiment
4
5
6 150 were then composited into 3 samples using a cone splitter, representing the early, middle, or
7
8 151 late periods of the rainfall event. A total of 9 composited samples collected from three
9
10 152 experimental plots were analyzed. Isotope-dilution standards (IDSs) were added to each
11
12 153 sample (Tables S3 and S4), and samples were stored at -60°C in high-density polyethylene
13
14
15 154 (HDPE) containers until analysis. The samples were extracted and analyzed without
16
17 155 filtration, so reported concentrations include both dissolved and suspended-particle bound
18
19
20 156 hormones.

21
22
23 157 To investigate the primary phase responsible for transporting hormones, the
24
25 158 percentage of hormones partitioned to the suspended-particle bound phase and in the
26
27 159 dissolved phase were determined for a subset of unfiltered runoff samples by filtering the
28
29
30 160 runoff through a 0.7- μm (nominal) glass-fiber filter (Whatman GF/F, 142 mm diameter,
31
32 161 Whatman Inc. Piscataway, NJ). Both the filter and the filtrate were retained and analyzed.

33
34
35 162 A composite biosolids sample was collected from several points within the pile
36
37 163 applied to the field at the time of application. This composite sample was subsampled in the
38
39 164 laboratory 5 times (denoted biosolids A through E) and analyzed for the same 19 compounds
40
41
42 165 determined in the water analyses. All subsamples were taken from one jar and were intended
43
44 166 to provide an estimate of method and sampling variability and precision (Table S5).

45
46
47 167

48 49 50 168 **Sample Analysis**

51
52
53 169 Seventeen natural and synthetic hormones, comprising nine estrogens, six androgens,
54
55 170 and two progestins, and two related sterols were measured in unfiltered (combined dissolved
56
57 171 and suspended-particle bound phases) and filtered runoff, filtered suspended particles, and
58
59
60 172 biosolids samples (Table S1). Many of the hormones have previously been detected in

1
2
3 173 biosolids, so biosolids application to agricultural fields represents a viable source to the
4
5 174 environment.^{19, 31} Although the sterols (cholesterol and coprostanol) are not hormonally
6
7
8 175 active, they are typically present in biosolids at high concentration and are useful for
9
10 176 comparative purposes.

11
12
13 177 Standards and reagents are described in the SI. Prior to use, all glassware was
14
15 178 silanized. Hormones were extracted from filtered or unfiltered runoff samples using C₁₈ solid-
16
17
18 179 phase extraction (SPE) disks and isolated using Florisil cartridges; hormones in the
19
20 180 suspended-particle bound phase from filters and biosolids samples were extracted using
21
22 181 pressurized solvent extraction (PSE), and interferences were removed by isolation on OASIS
23
24 182 HLB cartridges and elution across Florisil cartridges. Both SPE and PSE extracts were
25
26 183 derivatized with activated N-methyl-(N-trimethylsilyl)trifluoroacetamide (MSTFA, Sigma-
27
28 184 Aldrich) and analyzed by gas chromatography-tandem mass spectrometry (GC/MS/MS).
29
30 185 More detail on extraction, isolation, and analysis can be found in Foreman et al.³² The PSE
31
32 186 extraction and sample cleanup was modified after the method of Burkhardt et al.³³; the only
33
34 187 difference was that Florisil cartridges were eluted with 95% dichloromethane (DCM)/5%
35
36 188 methanol rather than a DCM/diethethyl ether solvent mixture. The derivatization and
37
38 189 instrumental analysis was carried out as in the water method with the exception that 500 μ L
39
40 190 of MSTFA was used (versus 200 μ L) to minimize reagent limitations previously observed in
41
42 191 the more complex solid extract matrix.

43 44 45 46 47 192 **Quality Assurance/Quality Control**

48
49
50 193 Throughout the study, regular verification of method performance was conducted in
51
52 194 the form of spike-recovery experiments and analysis of blank samples. At least one spike
53
54 195 and one blank was analyzed per set of 10 samples, for a total of 8 of each during the course of
55
56 196 the study. For all target compounds, spike recovery averaged $93 \pm 11.6\%$, ranging from 71
57
58 197 $\pm 14.9\%$ (equilin) to $116 \pm 22.0\%$ (cholesterol) (Table S3). Seventeen of 19 compounds

1
2
3 198 were never detected in blank samples, two compounds (cholesterol and coprostanol) are
4
5 199 commonly observed in method blanks, but neither ever exceeded censoring levels. For every
6
7
8 200 sample, IDS response was evaluated independently to estimate absolute compound recovery,
9
10 201 although reported results are recovery corrected by isotope dilution. Recovery of IDS
11
12 202 compounds averaged 57.7%; for individual compounds average recovery during the study
13
14
15 203 ranged from $46 \pm 17.5\%$ (cholesterol-d7) to $71 \pm 19.1\%$ (estrone-d4) (Table S4).
16
17

18 204 **Statistical Analysis**

19
20
21 205 Pearson's correlation analysis was performed using SAS PROC CORR (version 9.2)
22
23 206 on hormone mass loads and antecedent rainfall amount.
24
25

26 207

28 29 208 **Results & Discussion**

30 31 32 209 **Steroid Hormone Runoff during a Series of Rainfall Events**

33
34
35 210 Six out of seventeen hormones were detected in the biosolid samples, representing a
36
37 211 wide range of concentrations (Table S5). Estrone was found at highest concentration (78 ± 5.8
38
39 212 ng g^{-1}) in biosolids, followed by androstenedione ($22 \pm 5.3 \text{ ng g}^{-1}$) and cis-androsterone (18
40
41 213 $\pm 1.4 \text{ ng g}^{-1}$). Twelve of the seventeen hormones, coprostanol, and cholesterol were detected in
42
43
44 214 at least one unfiltered runoff sample during the experimental period (Tables S6 and S7). Four
45
46 215 synthetic hormones, diethylstilbestrol, norethindrone, ethinyl estradiol and mestranol, were
47
48
49 216 not detected in any of the samples, nor was equilin, a natural estrogen that is used in hormone
50
51 217 replacement therapy (Tables S6 and S7). In the rainfall simulation 5 days prior to biosolids
52
53 218 application, only androstenedione (one of nine samples, 1.54 ng L^{-1}), estrone (six of nine
54
55 219 samples 0.95 to 2.23 ng L^{-1}), and cholesterol (nine of nine samples, $22.5 \pm 3.8 \text{ } \mu\text{g/L}$) were
56
57
58 220 detected in runoff (Table S6).
59
60

221 In contrast, significant concentrations of hormones were observed in runoff samples

1
2
3 222 one day after biosolids application (Figure 1). One day after biosolids application, 14 of 19
4
5 223 compounds were observed in runoff with 82.5% detection frequency among detected
6
7
8 224 compounds. The average concentrations of coprostanol and cholesterol in runoff were 153
9
10 225 and 112 $\mu\text{g L}^{-1}$, respectively. The hormones that were detected were present at parts-per-
11
12 226 trillion concentrations (ng L^{-1}). Runoff samples collected one day after biosolids application
13
14
15 227 contained significantly ($p < 0.05$) higher concentrations of estrogens, as well as androgens
16
17 228 and progesterone based on Pearson's correlation analysis (Figure S3-S5). Overall, estrogen
18
19 229 (E1, αE2 , βE2 , and estriol (E3)), androgen (testosterone, epitestosterone, 11-ketotestosterone,
20
21 230 cis-androsterone, and androstenedione), and progestogen (progesterone) concentrations one
22
23
24 231 day after biosolids application ranged from <0.8 to 15 ng L^{-1} , <2 to 220 ng L^{-1} , and 17 to 98.9
25
26
27 232 ng L^{-1} , respectively (Table S6).

28
29
30 233 Concentrations (ng L^{-1}) for each rainfall event are shown averaged across plots for
31
32 234 different time points (Figure 1), and generally decreased or remained constant for most
33
34 235 compounds from day 1 to day 35. Increased runoff rate as soil became saturated meant that
35
36
37 236 hormone load (ng min^{-1}) increased substantially over the course of each rainfall simulation
38
39 237 (Figure 2). The mass load was calculated in the following way:

40
41
42 238 **Mass load (ng min^{-1}) = Runoff rate (L min^{-1}) \times Runoff concentration (ng L^{-1})**

43
44
45 239 Furthermore, for compounds that were not detected in 100% of samples, the non-detections
46
47 240 were clustered during the early periods of runoff sampling. At 8 and 35 days after biosolids
48
49 241 application (Table S6 and Figure S3-S5), similar patterns of generally lower concentrations
50
51 242 and lower frequency of detection (42.1% detections on day 8, 58.3% on day 35) were
52
53
54 243 observed.

55
56
57 244 The runoff patterns observed for each of the hormones during the experimental period
58
59 245 are likely associated with differences in partitioning behavior, (bio)transformation, and

1
2
3 246 (de)conjugation of the individual compounds. Mean concentrations of hormones detected in
4
5 247 biosolids samples ranked in the following order: E1 > androstenedione > cis-androsterone >
6
7
8 248 progesterone. This order suggests that both the initial hormone concentration in biosolids
9
10 249 and physicochemical properties affect the observed runoff concentrations. Some observed
11
12 250 hormones may be generated microbially from sterols or through interconversion of hormones,
13
14
15 251 as other studies have observed increases of androstenedione and progesterone in manure³⁴
16
17 252 and manure-impacted soils¹⁰ as they aged. However, the concentrations of androgens in
18
19 253 runoff had decreased by day 8 and day 35, which is consistent with previous reports and
20
21
22 254 suggests that biodegradation or mass loss via leaching may also be an important factor
23
24
25 255 affecting runoff concentrations.^{11, 35}

26
27 256 Observed runoff concentrations could also be affected by differential rates of *in situ*
28
29 257 degradation for the hormones after biosolids application to the soil. Previous batch, column,
30
31
32 258 and field studies have also shown that the sorption affinity of testosterone is lower, while the
33
34 259 dissipation/transformation rate and potential for migration is higher than for β E2.^{20, 25, 35}

35
36
37 260 These results demonstrate that runoff from biosolids-amended agricultural soils can
38
39 261 transport hormones laterally off-field, and suggest that such runoff could be an important
40
41
42 262 source of hormones to receiving waters, especially in areas where application of biosolids is
43
44
45 263 common, and where a substantial portion of surface water flow is derived from such runoff.²
46
47 264 Further, runoff from these experiments had hormone concentrations that exceed biological
48
49 265 effects thresholds¹ (Figure 1), suggesting potential for effects on aquatic organisms exposed
50
51 266 to such runoff. However, the rainfall rate used in the experiments corresponds to a 100-year
52
53
54 267 rain event for eastern Colorado, so these results likely represent an upper bound on hormone
55
56 268 runoff at this site.

57
58
59 269 Among the hormones, androstenedione was found in the highest concentration in
60
270 runoff on day 1, followed by cis-androsterone and progesterone. Temporal trends in hormone

1
2
3 271 load were similar between the three plots (Figure 2). However, the runoff concentrations of
4
5 272 hormones observed at day 1 after biosolids application followed the order: androstenedione >
6
7
8 273 cis-androsterone > progesterone >> E1. The fact that testosterone, epitestosterone, 11-
9
10 274 ketotestosterone, α E2, and β E2 were observed in runoff samples but not in biosolids is likely
11
12 275 due to less sensitivity of solid versus liquid analytical methods, although microbial
13
14
15 276 production of these hormones as the soil aged is possible.

17
18 277 One day after biosolids application, the concentration of androstenedione was
19
20 278 between one and two orders of magnitude higher than E1 or β E2 (Figure 1 and Table S6). In
21
22 279 general, the highest concentrations of androgens in runoff occurred on the first day after
23
24
25 280 biosolids application. For example, in plot 1, the runoff concentrations of testosterone,
26
27 281 dihydrotestosterone, androstenedione, and cis-androsterone decreased by 50 to 75% from day
28
29
30 282 1 to day 8, and decreased again by more than 44% from day 8 to day 35 (Figure S3). Similar
31
32 283 trends were observed for runoff concentrations of coprostanol and cholesterol. In plot 1 on
33
34 284 day 1, the highest concentration of coprostanol and cholesterol were 400 and 280 $\mu\text{g L}^{-1}$,
35
36
37 285 respectively (Table S6). Conversely, in plot 1, the runoff concentrations of E1 and β E2
38
39 286 increased by more than 42% from day 1 to day 8, but decreased by more than 40% from day
40
41
42 287 8 to day 35 (Figure S3).

43
44 288 In contrast to the androgens, total estrogen (i.e., E1, α E2, and β E2) runoff
45
46 289 concentrations did not markedly decrease in the month following biosolids application.
47
48
49 290 Previous studies have suggested that the most predominant factors contributing to the fate
50
51 291 and transport of β E2 and testosterone in the field were soil-water status (i.e., soil moisture
52
53 292 percentage), organic matter content, and colloid-facilitated transport²⁰. The higher runoff rate
54
55
56 293 observed during the second simulated rainfall event (day 8) after biosolids application is
57
58
59 294 likely due to the high antecedent moisture content of the soil (Figure S2) and intervening
60
295 natural rain (25.4 mm rain between days 1 and 8). The total estrogen concentration in runoff

1
2
3 296 at day 35 still exceeds biological effects thresholds.¹ The concentrations of E1 were found to
4
5
6 297 be consistently greater than α E2 and β E2 for all simulated rainfall events.
7

8
9 298 Finally, the runoff concentrations of progesterone were reduced at least 87% at day 8,
10
11 299 and then increased to a similar level as day 1 on day 35 (Figure S3, Tables S6 and S7). The
12
13 300 trends observed in plots 2 and 3 are provided in SI (Figures S4 and S5). The concentration of
14
15 301 progesterone in day 1 runoff decreased significantly ($p < 0.0001$) for the subsequent rainfall
16
17 302 event (D-8), and then increased again on day 35 ($p = 0.0017$).
18
19

20
21 303 The runoff mass loads in nanograms per minute, of hormones and runoff rates for
22
23 304 plots 1, 2, and 3 on day 1, 8, and 35 are shown in Figure 2. The concentration of each
24
25 305 compound was multiplied by the runoff rate measured closest to each sampling time. The
26
27 306 overall trend of mass load changes is consistent between different rainfall events, and the
28
29 307 highest mass load of hormones correlated with the antecedent rainfall amount for 90% of the
30
31 308 samples ($p < 0.05$; Table S8). Hormone loads were highest towards the end of each rainfall
32
33 309 simulation. Because the simulated rainfall corresponded to an intense storm, it is likely that
34
35 310 the early runoff concentrations are more indicative of what would be observed during a
36
37 311 typical rainfall event. Significant correlations (R ranged from 0.67 to 0.98, p value < 0.05 ;
38
39 312 Table S8) were observed between antecedent rainfall amount and mass loads of androgens
40
41 313 (i.e., androstenedione, cis-androsterone, dihydrotestosterone, and testosterone), estrogens
42
43 314 (i.e., E1, α E2, and β E2), and progesterone for the three experimental plots on day 1, 8, and
44
45 315 35. This observation is in agreement with a recent study that also reported significant
46
47 316 correlations between antecedent rainfall amounts and mass exports of estrogens from poultry
48
49 317 litter amended soil and suggests that mass exports and concentrations of estrogens do not
50
51 318 necessarily monotonically decrease with successive rainfall events.²³ This observation is
52
53 319 consistent with our findings with respect to E1, which actually increased in concentration on
54
55 320 day 8 compared to day 1. Collectively, our results suggest that higher rainfall amount may
56
57
58
59
60

1
2
3 321 promote runoff with higher hormone loads from both manure- and biosolids-amended soils.
4
5

6 322 Whereas both α E2 and β E2 were observed at very low concentrations ($< \sim 2 \text{ ng L}^{-1}$) in
7
8 323 runoff, on day 35 E1 was present in runoff at much higher concentration ($> 10 \text{ ng L}^{-1}$). The
9
10 324 greater E1 concentrations on day 35 are most likely due to release from the biosolids and to a
11
12 325 smaller extent biodegradation of β E2 to E1, suggested by the observation that the
13
14 326 concentration profile of β E2 followed the profile of E1 (Figure 1). Interestingly, a recent
15
16 327 study found that E1 had a higher soil sorption coefficient ($K_d = 33 \text{ L Kg}^{-1} \text{ soil}$) and a higher
17
18 328 soil sorption coefficient per unit organic carbon ($K_{oc} = 1557 \text{ L Kg}^{-1}$) than β E2 ($K_d = 23 \text{ L Kg}^{-1}$
19
20 329 soil and $K_{oc} = 1082 \text{ L Kg}^{-1}$) based on investigations of 121 surface (0-15 cm) soils.³⁶ Thus,
21
22 330 stronger sorption of β E2 is not a likely explanation for this observation. However, it is likely
23
24 331 that some α E2 and β E2 were degraded to E1^{23, 37} in the biosolids-amended soil and may, in
25
26 332 part, have contributed to the observed concentration of E1 on the day 8 and day 35.
27
28
29
30
31
32

33 333 There was no natural precipitation between the day biosolids were applied to the field
34
35 334 and one day after biosolids application. However, natural precipitation events did occur
36
37 335 between days 1 and 8 after biosolids application (25.4 mm) and between day 8 and 35 after
38
39 336 biosolids application (10.4 mm). The intermittent wetting and drying of the soil and applied
40
41 337 biosolids throughout the entire study could influence the transport and biodegradation of
42
43 338 hormones. In WWTPs, higher removal efficiencies have been found for androgens and
44
45 339 progestogens (91-100%) than for estrogens (67-80%), with biodegradation being the major
46
47 340 removal route.³ The absence of progesterone ($< 8 \text{ ng L}^{-1}$) at day 8 and the reappearance at day
48
49 341 35 suggests either a slow desorption from the biosolids into the soil solution or microbial
50
51 342 formation, perhaps from sterols.¹⁰ Further research is needed to determine if wetting the soil
52
53 343 could induce rapid degradation of progesterone.
54
55
56
57
58

59 344 Kjær et al.²² assessed leaching of estrogens from manure-treated structured soils and
60
345 found that leaching appears to be influenced by preferential transport, fast solute transport,

1
2
3 346 and drainage water dynamics. While our results demonstrate that hormones do runoff from
4
5 347 biosolids-amended soils, additional studies are needed to address the mechanisms of transport
6
7 348 (e.g., leaching potential of hormones from biosolids-amended soils). The past history of
8
9 349 conventional-tillage on the reduced-tillage plots could have resulted in the development of
10
11 350 impeding layers, which could have reduced infiltration and enhanced runoff.³⁸ Jenkins et al.
12
13 351¹² observed higher exports of β E2 in runoff from conventional-tillage ($1300 \mu\text{g ha}^{-1}$) versus
14
15 352 no tillage ($600 \mu\text{g ha}^{-1}$) with poultry litter plots. Further research is also required to evaluate
16
17 353 the potential for hormone transport from biosolids-amended soils with different agricultural
18
19 354 management practices.

25 355 **Steroid Hormone Fractionation between Dissolved and Suspended-Particle Bound**

28 356 **Phases**

31 357 For this study, dissolved-phase hormones are operationally defined as the fraction of
32
33 358 hormones in water that pass through a $0.7\text{-}\mu\text{m}$ glass-fiber filter. The runoff samples were
34
35 359 filtered the day after collection and stored at $-60\text{ }^{\circ}\text{C}$ until analysis. Likewise, particle-bound
36
37 360 hormones are defined as the fraction of hormones that are retained on the same filter. The
38
39 361 percentages of hormones bound to the suspended-particle phase and in the dissolved phase
40
41 362 were compared (Figure 3) to determine the importance of each phase for hormone transport.
42
43 363 These comparisons were made for compounds that were present in the day 1 and 8 samples
44
45 364 from all 3 plots, and day 35 samples from plots 1 and 2.

50 365 Coprostanol (>98%) and cholesterol (> 98%) were present primarily in the suspended
51
52 366 particles (i.e., particles $>0.7 \mu\text{m}$), β E2 was present approximately 50 % bound to suspended
53
54 367 particles, while the rest of the hormones remained primarily in the dissolved phase. At 1 day
55
56 368 after biosolids application, the percentage of particle-associated hormones varied from <0.8
57
58 369 to 16% for testosterone, 6 to 17% androstenedione, 6 to 16% for cis-androsterone, 22 to 45%

1
2
3 370 for E1, and 46 to 63 % for β E2 based on the results from three experimental plots. More than
4
5 371 95% of progesterone was observed in the dissolved phase. Similar trends were observed 8
6
7
8 372 days and 35 days after biosolids application (Figure 3), but with a higher frequency of non-
9
10 373 detections.

11
12
13 374 Coprostanol and cholesterol were present on suspended particles to a greater extent
14
15 375 than estrogens, androgens, and progesterone, likely due to their hydrophobic properties. In
16
17 376 general, the mean percentage of particle-bound hormones observed in this study followed the
18
19 377 order sterols >> estrogens > androgens and progesterone. This pattern is in partial agreement
20
21 378 with previous studies^{39, 40} and may be due to the lower water solubility of estrogens ($S_w = \sim 1$
22
23 379 to 4 mg L⁻¹) than androgens ($S_w = \sim 20$ to 875 mg L⁻¹) and progesterone ($S_w = 8.81$ mg L⁻¹)
24
25 380 (Table S1). The log K_{ow} for progesterone (3.67 to 3.87) is higher than the log K_{ow} values
26
27 381 (1.92 to 3.69) for the androgens but similar to or lower than the log K_{ow} (3.13 to 4.01) of E1
28
29 382 and β E2 (Table S1).

30
31
32
33
34
35 383 Physicochemical properties could also influence distribution of hormones between
36
37 384 particles and aqueous phases. Dipole effects, which could influence particle association by
38
39 385 interaction with charged clay particles, would be stronger for estrogens than the other
40
41 386 compounds due to the presence of a phenol substituent rather than a conjugated ketone in the
42
43 387 A-ring of the steroid.³⁹ The fraction of biosolids in the soil is relatively low so there could be
44
45 388 some polar interactions occurring with clays.⁴⁰ Indeed, Khan et al.⁴¹ and Mashtare et al.⁴²
46
47 389 show differences in sorption to agricultural soils between stereoisomers of estradiol and
48
49 390 trenbolone, a synthetic androgen, with stronger partitioning observed for the 17 β isomer
50
51 391 relative to the 17 α isomer. Since stereochemical configuration does not affect K_{ow} values,
52
53 392 they attributed the observed differences to polar interactions between the hormones and soil
54
55 393 mineral phases. The present study results confirm that the 17 β stereoisomers of testosterone
56
57 394 and estradiol are more strongly associated with particulate matter than the 17 α stereoisomers
58
59
60

1
2
3 395 (Figure 3).
4
5

6 396 Finally, three compounds were observed at concentrations near their detection limit in
7
8 397 the dissolved phase and not detected in the suspended-particle phase. Therefore, for
9
10 398 progesterone, α E2, and 11-ketotestosterone the minimum fraction present in the dissolved
11
12 399 phase is reported. However, the failure to detect progesterone, 11- ketotestosterone, and α E2
13
14 400 in particulate samples, coupled with detection limits that approach their aqueous
15
16 401 concentrations, precludes reliable determination of their partitioning behavior.
17
18
19

20
21 402 Esperanza et al.⁴³ assessed the fate of sex hormones in two pilot-scale municipal
22
23 403 WWTPs and found that testosterone, androstenedione, and progesterone tended to remain in
24
25 404 the dissolved phase (filtrate; passing through 1- μ m filters) and did not partition significantly
26
27 405 to the solids. It is noted that the dissolved and suspended-particle phases were separated by a
28
29 406 standard 0.7- μ m glass-fiber filter in the present study. Interestingly, we did not detect
30
31 407 progesterone in the particulate fraction (Figure 3). Thus, it is likely that the aqueous fraction
32
33 408 contained small particles and/or colloids containing sorbed steroid hormones. Holbrook et
34
35 409 al.⁴⁴ observed that up to 60% of β E2 and EE2 in wastewater is associated with aqueous
36
37 410 colloidal material, but the log K_{oc} values and log K_{ow} values were not well-correlated with
38
39 411 estrogen sorption to colloids. Similar poor linear relationships between log K_{ow} and
40
41 412 partitioning to colloidal organic carbon for E1, β E2, and EE2 have been shown previously.⁴⁵
42
43 413 This further suggest that hydrophobic partitioning does not completely explain sorption
44
45 414 behavior of hormones and that aquatic colloids may play an important role in the
46
47 415 environmental behavior of steroid hormones. Additional research is needed to evaluate the
48
49 416 physicochemical properties of colloids that influence transport of hormones.
50
51
52
53
54
55

56
57 417 **Environmental Implications**
58
59
60

1
2
3 418 The present study assessed the runoff potential for estrogens, androgens, and
4
5
6 419 progestogens from agricultural test plots receiving a first-time application of biosolids. Three
7
8 420 factors need to be considered when evaluating this dataset: (1) the data likely represent a
9
10 421 worst-case scenario (100-year rainfall event or 65 mm h^{-1}) for runoff conditions at our field
11
12 422 site, (2) while hormones were present in runoff from biosolids-amended fields, farther
13
14 423 transport to surface waters, wetlands, or elsewhere on the landscape, requires additional
15
16 424 study, and (3) if hormones did reach receiving waters, concentrations likely would be diluted
17
18 425 and impact on aquatic organisms would thus be mitigated. Nevertheless, our findings
19
20 426 demonstrate that hormones can be present in runoff from biosolids-amended agricultural
21
22 427 fields, and that relatively high concentrations of androgens and progesterone are likely to be
23
24 428 found in the surface runoff even after multiple heavy rainfall events and over one month
25
26 429 following the original application of biosolids. The hormone mass load correlated with
27
28 430 antecedent rainfall amount, emphasizing that a heavy rainstorm event will promote a pulse of
29
30 431 hormones in runoff. This suggests that biosolids could be an important source of hormones to
31
32 432 surface waters. The concentrations of estrogens and androgens, in particular androstenedione,
33
34 433 detected in this study are higher than concentrations that have been shown to alter
35
36 434 biochemistry and behavior in susceptible fish.^{1, 46-50} The lowest observed effect level (LOEL)
37
38 435 affecting vitellogenin production in juvenile female rainbow trout was found to be as low as
39
40 436 3.3 ng E1/L .⁵¹ Furthermore, the possible impact of hormone exposure on terrestrial organisms
41
42 437 has not been documented. However, Kinney et al.⁵² demonstrated the uptake of wastewater-
43
44 438 derived compounds by earthworms and this suggests the potential for bioaccumulation of
45
46 439 these compounds in the food web. Additional research is required to evaluate the potential for
47
48 440 hormone transport from biosolids-amended soils with different soil composition, means of
49
50 441 biosolids application, and climate conditions (e.g., precipitation rate) and across longer
51
52 442 distances.
53
54
55
56
57
58
59
60

1
2
3 443
4
5
6 444**Acknowledgments**

7
8
9 445 This research was supported by the Colorado Water Institute (CWI) and, in part, by a
10
11 446 National Science Foundation (NSF) CAREER Award (EAR 0847683) to T. B. Support also
12
13 447 was provided by the USGS Toxic Substances Hydrology Program's Emerging Contaminant
14
15 448 Project. The authors thank Dana Kolpin (USGS), Tracy Yager (USGS), William Foreman
16
17 449 (USGS), Corey Stephens (USGS), Jeff Writer (USGS), Adriane Elliott (CSU), and Kathy
18
19 450 Doesken (CSU) for their invaluable technical assistance. The authors also thank Mr. Doug
20
21 451 Lingo for the access to his farm. Any use of trade, firm, or product names in the paper is for
22
23 452 descriptive purposes only and does not imply endorsement by the U.S. Government.
24
25
26
27
28 453

Supporting Information Available

29
30
31 454
32
33 455 Material supporting detailed description of the analytical methods, tables and figures
34
35 456 showing chemical structures and physico-chemical properties of target compounds (Table
36
37 457 S1), the physical and chemical properties of soils (Table S2), concentrations of target
38
39 458 compounds in biosolids (Table S5) and runoff samples (Table S6 and S7), statistical analysis
40
41 459 (Table S8), the experimental design (Figure S1), the antecedent soil moisture (Figure S2), and
42
43 460 additional results of average concentration of hormones distinguishing the three different
44
45 461 plots (Figure S3 to S5). This material is available free of charge via the Internet at
46
47 462 <http://pubs.acs.org>.
48
49
50
51
52
53
54 463
55
56
57
58
59
60

1
2
3 464
4 465 **FIGURE CAPTIONS**

5
6
7 466 **FIGURE 1.** Boxplot showing runoff concentration distribution of detected compounds in a
8
9
10 467 total of nine runoff samples collected from all three plots during simulated rainfall 5
11
12 468 days prior to biosolids application (-5) and 1, 8, and 35 (no measurements were made
13
14 469 from plot 3 on day 35; six runoff samples were analyzed) days afterwards. Boxes
15
16
17 470 indicate 25th and 75th percentile values and whiskers are drawn to the highest and
18
19 471 lowest data points that are not outliers (AND: *cis*-androsterone, ADSD:
20
21 472 androstenedione, T: testosterone, EpiT: epitestosterone, DHT: dihydrotestosterone, 11-
22
23 473 KT: 11-ketotestosterone, E1: estrone, α -E2: 17 α -estradiol, β -E2: 17 β -estradiol, E3:
24
25 474 estriol, EQN: equilenin, P: progesterone).

26
27
28
29 475
30
31
32 476 **FIGURE 2.** Hormone mass load and runoff rate (blue circles on secondary axis) from all
33
34 477 three plots 1 day, 8 days, and 35 days after biosolids application. No measurements
35
36 478 were made for plot 3 on day 35.

37
38
39 479
40
41
42
43 480 **Figure 3.** Mean (three sampling times) coprostanol, cholesterol, and hormone percentages in
44
45 481 the dissolved (including <0.7- μ m particles) and suspended-particle bound phases in
46
47 482 runoff samples collected from plots 1-3 during the rainfall events 1, 8, and 35 days
48
49 483 after biosolids application (AND: *cis*-androsterone, ADSD: androstenedione, T:
50
51 484 testosterone, EpiT: epitestosterone, DHT: dihydrotestosterone, 11-KT: 11-
52
53 485 ketotestosterone, E1: estrone, α E2: 17 α -estradiol, β E2: 17 β -estradiol, P: progesterone).
54
55 486 No measurements were made for plot 3 on day 35. *Compounds were observed at
56
57 487 concentrations near their detection limit which may lead to larger errors.
58
59
60

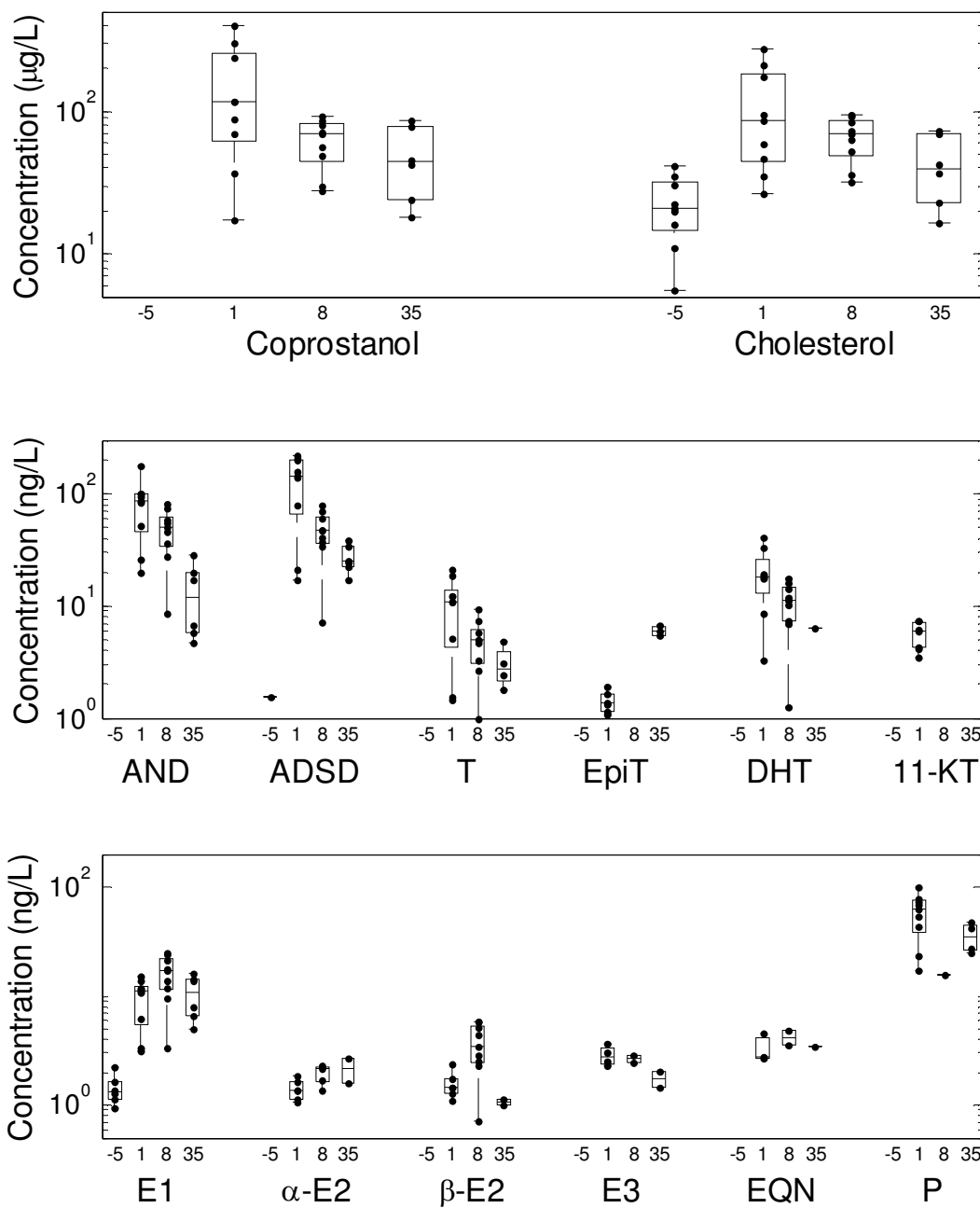


FIGURE 1.

488
489
490
491

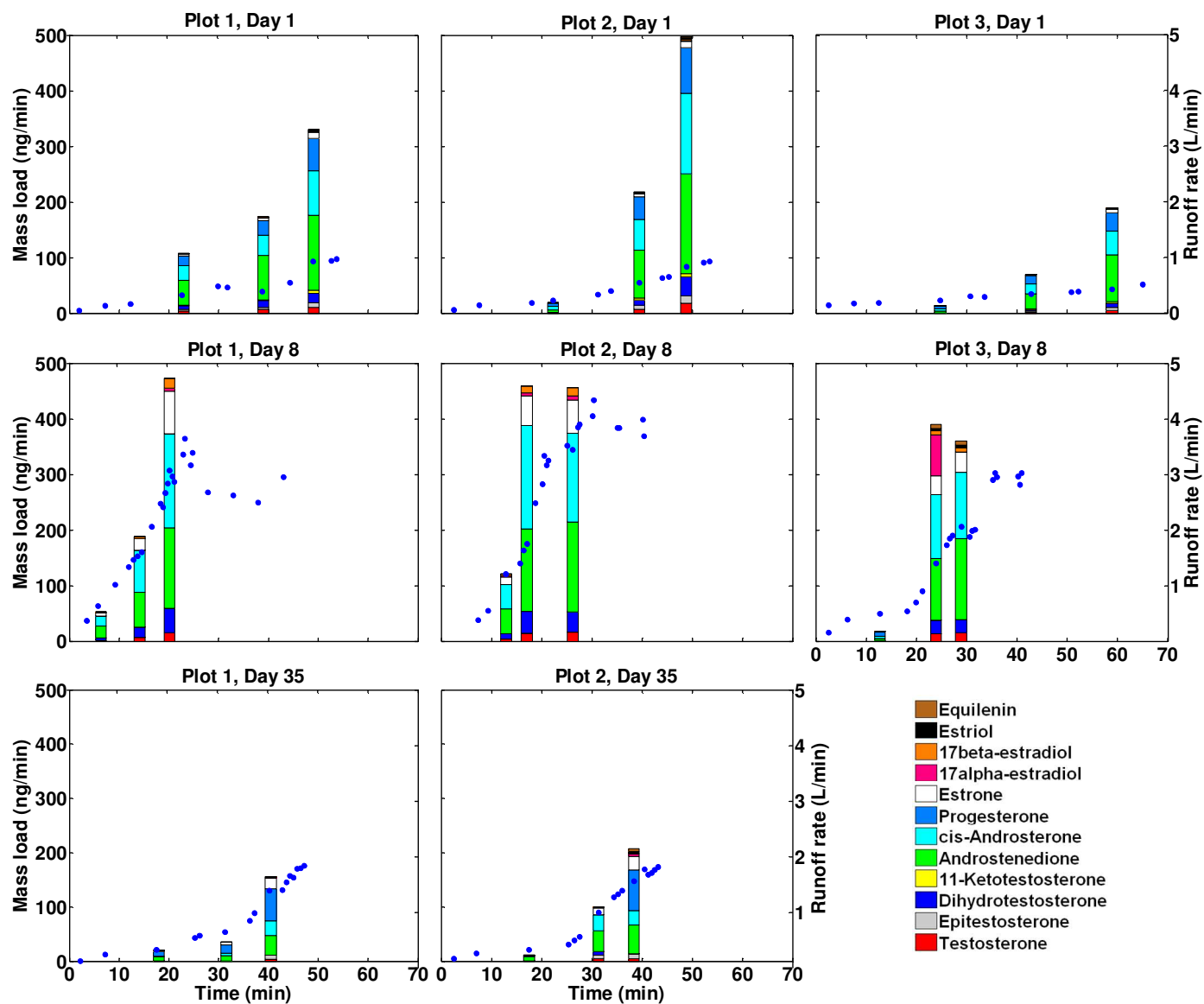


FIGURE 2

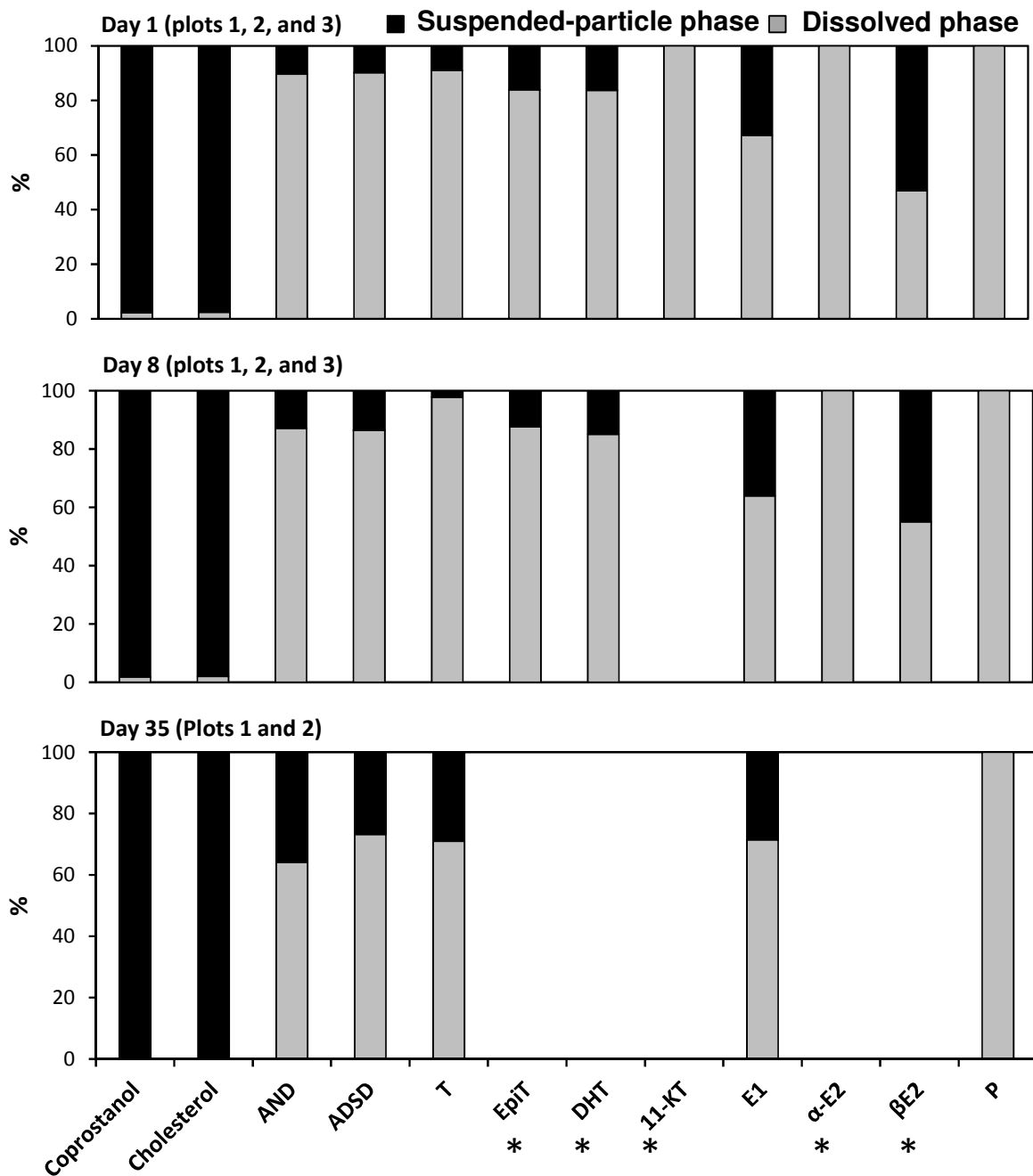


FIGURE 3

REFERENCES

- 1
2
3 505
4 506
5 507 (1) Vajda, A.M.; Barber, L.B.; Gray, J.L.; Lopez, E.M.; Woodling, J.D.; Norris, D.O.
6
7
8 508 Reproductive disruption in fish downstream from an estrogenic wastewater effluent.
9
10 509 *Environ. Sci. Technol.* **2008**, *42* (9), 3407-3414.
11
12 510 (2) Kolodziej, E.P.; Harter, T.; Sedlak, D.L. Dairy wastewater, aquaculture, and spawning
13
14 511 fish as sources of steroid hormones in the aquatic environment. *Environ. Sci. Technol.*
15
16 512 **2004**, *38* (23), 6377-6384.
17
18 513 (3) Chang, H.; Wan, Y.; Wu, S.; Fan, Z.; Hu, J. Occurrence of androgens and
19
20 514 progestogens in wastewater treatment plants and receiving river waters: Comparison
21
22 515 to estrogens. *Water Res.* **2011**, *45* (2), 732-740.
23
24
25 516 (4) Ying, G.G.; Kookana, R.S.; Kumar, A.; Mortimer, M. Occurrence and implications of
26
27 517 estrogens and xenoestrogens in sewage effluents and receiving waters from South
28
29 518 East Queensland. *Sci. Total Environ.* **2009**, *407* (18), 5147-5155.
30
31
32 519 (5) Pal, A.; Gin, K.Y.H.; Lin, A.Y.C.; Reinhard, M. Impacts of emerging organic
33
34 520 contaminants on freshwater resources: Review of recent occurrences, sources, fate
35
36 521 and effects. *Sci. Total Environ.* **2010**, *408* (24), 6062-6069.
37
38
39 522 (6) Wang, Y.Q.; Hu, W.; Cao, Z.H.; Fu, X.Q.; Zhu, T. Occurrence of endocrine-disrupting
40
41 523 compounds in reclaimed water from Tianjin, China. *Anal. Bioanal. Chem.* **2005**, *383*
42
43 524 (5), 857-863.
44
45
46 525 (7) Swartz, C.H.; Reddy, S.; Benotti, M.J.; Yin, H.F.; Barber, L.B.; Brownawell, B.J.;
47
48 526 Rudel, R.A. Steroid estrogens, nonylphenol ethoxylate metabolites, and other
49
50 527 wastewater contaminants in groundwater affected by a residential septic system on
51
52 528 Cape Cod, MA. *Environ. Sci. Technol.* **2006**, *40* (16), 4894-4902.
53
54
55 529 (8) Chen, T.S.; Chen, T.C.; Yeh, K.J.C.; Chao, H.R.; Liaw, E.T.; Hsieh, C.Y.; Chen, K.C.;
56
57 530 Hsieh, L.T.; Yeh, Y.L. High estrogen concentrations in receiving river discharge from

- 1
2
3 531 a concentrated livestock feedlot. *Sci. Total Environ.* **2010**, *408* (16), 3223-3230.
- 4
5
6 532 (9) Gadd, J.B.; Tremblay, L.A.; Northcott, G.L. Steroid estrogens, conjugated estrogens
7
8 533 and estrogenic activity in farm dairy shed effluents. *Environ. Pollut.* **2010**, *158* (3),
9
10 534 730-736.
- 11
12
13 535 (10) Mansell, D.S.; Bryson, R.J.; Harter, T.; Webster, J.P.; Kolodziej, E.P.; Sedlak, D.L.
14
15 536 Fate of endogenous steroid hormones in steer feedlots under simulated rainfall-
16
17 537 induced runoff. *Environ. Sci. Technol.* **2011**, *45* (20), 8811-8818.
- 18
19
20 538 (11) Jacobsen, A.-M.; Lorenzen, A.; Chapman, R.; Topp, E. Persistence of testosterone and
21
22 539 17 beta-estradiol in soils receiving swine manure or municipal biosolids. *J. Environ.*
23
24 540 *Qual.* **2005**, *34* (3), 861-871.
- 25
26
27 541 (12) Jenkins, M.B.; Endale, D.M.; Schomberg, H.H.; Hartel, P.G.; Cabrera, M.L. 17 beta-
28
29 542 Estradiol and testosterone in drainage and runoff from poultry litter applications to
30
31 543 tilled and no-till crop land under irrigation. *J. Environ. Manage.* **2009**, *90* (8), 2659-
32
33 544 2664.
- 34
35
36 545 (13) Gall, H.E.; Sassman, S.A.; Lee, L.S.; Jafvert, C.T. Hormone discharges from a
37
38 546 midwest tile-drained agroecosystem receiving animal wastes. *Environ. Sci. Technol.*
39
40 547 **2011**, *45* (20), 8755-8764.
- 41
42
43 548 (14) Yang, Y.-Y.; Borch, T.; Young, R.B.; Goodridge, L.D.; Davis, J.G. Degradation
44
45 549 kinetics of testosterone by manure-borne bacteria: Influence of temperature, pH,
46
47 550 glucose amendments, and dissolved oxygen. *J. Environ. Qual.* **2010**, *39* (4), 1153-
48
49 551 1160.
- 50
51
52
53 552 (15) Yang, Y.-Y.; Pereyra, L.P.; Young, R.B.; Reardon, K.F.; Borch, T. Testosterone-
54
55 553 mineralizing culture enriched from swine manure: Characterization of degradation
56
57 554 pathways and microbial community composition. *Environ. Sci. Technol.* **2011**, *45*
58
59 555 (16), 6879-6886.
- 60

- 1
2
3 556 (16) Bradley, P.M.; Barber, L.B.; Chapelle, F.H.; Gray, J.L.; Kolpin, D.W.; McMahon, P.B.
4
5
6 557 Biodegradation of 17 beta-estradiol, estrone and testosterone in stream sediments.
7
8 558 *Environ. Sci. Technol.* **2009**, *43* (6), 1902-1910.
9
10 559 (17) Barber, L.B.; Keefe, S.H.; LeBlanc, D.R.; Bradley, P.M.; Chapelle, F.H.; Meyer, M.T.;
11
12 560 Loftin, K.A.; Kolpin, D.W.; Rubio, F. Fate of sulfamethoxazole, 4-nonylphenol, and
13
14 561 17 beta-estradiol in groundwater contaminated by wastewater treatment plant effluent.
15
16 562 *Environ. Sci. Technol.* **2009**, *43* (13), 4843.
17
18 563 (18) Layton, A.C.; Gregory, B.W.; Seward, J.R.; Schultz, T.W.; Sayler, G.S. Mineralization
19
20 564 of steroidal hormones by biosolids in wastewater treatment systems in Tennessee
21
22 565 U.S.A. *Environ. Sci. Technol.* **2000**, *34* (18), 3925-3931.
23
24
25 566 (19) Citulski, J.A.; Farahbakhsh, K. Fate of endocrine-active compounds during municipal
26
27 567 biosolids treatment: A review. *Environ. Sci. Technol.* **2010**, *44* (22), 8367-8376.
28
29
30 568 (20) Stumpe, B.; Marschner, B. Long-term sewage sludge application and wastewater
31
32 569 irrigation on the mineralization and sorption of 17 beta-estradiol and testosterone in
33
34 570 soils. *Sci. Total Environ.* **2007**, *374* (2-3), 282-291.
35
36
37 571 (21) Stumpe, B.; Marschner, B. Dissolved organic carbon from sewage sludge and manure
38
39 572 can affect estrogen sorption and mineralization in soils. *Environ. Pollut.* **2010**, *158*
40
41 573 (1), 148-154.
42
43
44 574 (22) Kjaer, J.; Olsen, P.; Bach, K.; Barlebo, H.C.; Ingerslev, F.; Hansen, M.; Sorensen,
45
46 575 B.H. Leaching of estrogenic hormones from manure-treated structured soils. *Environ.*
47
48 576 *Sci. Technol.* **2007**, *41* (11), 3911-3917.
49
50
51 577 (23) Dutta, S.; Inamdar, S.; Tso, J.; Aga, D.S.; Sims, J.T. Free and conjugated estrogen
52
53 578 exports in surface-runoff from poultry litter-amended soil. *J. Environ. Qual.* **2010**, *39*
54
55 579 (5), 1688-1698.
56
57
58 580 (24) Nichols, D.J.; Daniel, T.C.; Moore, P.A.; Edwards, D.R.; Pote, D.H. Runoff of
59
60

- 1
2
3 581 estrogen hormone 17 beta-estradiol from poultry litter applied to pasture. *J. Environ.*
4
5 582
6 *Qual.* **1997**, 26 (4), 1002-1006.
7
8 583 (25) Finlay-Moore, O.; Hartel, P.G.; Cabrera, M.L. 17 beta-estradiol and testosterone in
9
10 584 soil and runoff from grasslands amended with broiler litter. *J. Environ. Qual.* **2000**, 29
11
12 585 (5), 1604-1611.
13
14 586 (26) Jenkins, R.L.; Wilson, E.M.; Angus, R.A.; Howell, W.M.; Kirk, M. Androstenedione
15
16 587 and progesterone in the sediment of a river receiving paper mill effluent. *Toxicol. Sci.*
17
18 588 **2003**, 73 (1), 53-59.
19
20 589 (27) Kinney, C.A.; Furlong, E.T.; Zaugg, S.D.; Burkhardt, M.R.; Werner, S.L.; Cahill, J.D.;
21
22 590 Jorgensen, G.R. Survey of organic wastewater contaminants in biosolids destined for
23
24 591 land application. *Environ. Sci. Technol.* **2006**, 40 (23), 7207-7215.
25
26 592 (28) Bevacqua, C.E.; Rice, C.P.; Torrents, A.; Ramirez, M. Steroid hormones in biosolids
27
28 593 and poultry litter: A comparison of potential environmental inputs. *Sci. Total Environ.*
29
30 594 **2011**, 409 (11), 2120-2126.
31
32 595 (29) Miller, W.P. A solenoid-operated, variable intensity rainfall simulator. *Soil Sci. Soc.*
33
34 596 *Am. J.* **1987**, 51 (3), 832-834.
35
36 597 (30) Davis, J.G.; Truman, C.C.; Kim, S.C.; Ascough, J.C.; Carlson, K. Antibiotic transport
37
38 598 via runoff and soil loss. *J. Environ. Qual.* **2006**, 35 (6), 2250-2260.
39
40 599 (31) Furlong, E.T.; Gray, J.L.; Quanrud, D.M.; Teske, S.S.; Werner, S.L.; Esposito, K.E.;
41
42 600 Marine, J.; Ela, W.P.; Zaugg, S.D.; Phillips, P.J.; Stinson, B. Pharmaceuticals,
43
44 601 hormones, anthropogenic waste indicators, and total estrogenicity in liquid and solid
45
46 602 samples from municipal sludge stabilization and dewatering. *U.S. Geological Survey*
47
48 603 *Open-File Report* **2011**, 1132, 77.
49
50 604 (32) Foreman, W.T.; Gray, J.L.; ReVello, R.C.; Lindley, C.E.; Losche, S.A.; Barbar, L.B.
51
52 605 Determination of steroid hormones in filtered and unfiltered water by solid-phase
53
54
55
56
57
58
59
60

- 1
2
3 606 extraction, derivatization and gas chromatography with tandem mass spectrometry.
4
5
6 607 *U.S. Geological Survey Techniques and Methods* **2011**.
7
8 608 (33) Burkhardt, M.R.; ReVello, R.C.; Smith, S.G.; Zaugg, S.D. Pressurized liquid
9
10 609 extraction using water/isopropanol coupled with solid-phase extraction cleanup for
11
12 610 industrial and anthropogenic waste-indicator compounds in sediment. *Anal. Chim.*
13
14 611 *Acta* **2005**, 534 (1), 89-100.
15
16
17 612 (34) Zheng, W.; Yates, S.R.; Bradford, S.A. Analysis of steroid hormones in a typical airy
18
19 613 waste disposal system. *Environ. Sci. Technol.* **2008**, 42 (2), 530-535.
20
21
22 614 (35) Casey, F.X.M.; Hakk, H.; Simunek, J.; Larsen, G.L. Fate and transport of testosterone
23
24 615 in agricultural soils. *Environ. Sci. Technol.* **2004**, 38 (3), 790-798.
25
26
27 616 (36) Caron, E.; Farenhorst, A.; Zvomuya, F.; Gaultier, J.; Rank, N.; Goddard, T.; Sheedy,
28
29 617 C. Sorption of four estrogens by surface soils from 41 cultivated fields in Alberta,
30
31 618 Canada. *Geoderma* **2010**, 155 (1-2), 19-30.
32
33
34 619 (37) Colucci, M.S.; Bork, H.; Topp, E. Persistence of estrogenic hormones in agricultural
35
36 620 soils: I. 17 beta-estradiol and estrone. *J. Environ. Qual.* **2001**, 30 (6), 2070-2076.
37
38
39 621 (38) Lal, R. No-tillage effects on soil properties under different crops in Western Nigeria.
40
41 622 *Soil Sci. Soc. Am. J.* **1976**, 40 (5), 762-768.
42
43
44 623 (39) Kubli-Garfias, C. Comparative study of the electronic structure of estradiol,
45
46 624 epiestradiol and estrone by ab initio theory. *Theochem-J. Mol. Struct.* **1998**, 452 175-
47
48 625 183.
49
50
51 626 (40) Hofstetter, T.B.; Neumann, A.; Schwarzenbach, R.P. Reduction of nitroaromatic
52
53 627 compounds by Fe(II) species associated with iron-rich smectites. *Environ. Sci.*
54
55 628 *Technol.* **2006**, 40 (1), 235-242.
56
57
58 629 (41) Khan, B.; Qiao, X.L.; Lee, L.S. Stereoselective sorption by agricultural soils and
59
60 630 liquid-liquid partitioning of trenbolone (17 alpha and 17 beta) and trendione. *Environ.*

- 1
2
3 631 *Sci. Technol.* **2009**, *43* (23), 8827-8833.
- 4
5
6 632 (42) Mashtare, M.L.; Khan, B.; Lee, L.S. Evaluating stereoselective sorption by soils of 17
7
8 633 alpha-estradiol and 17 beta-estradiol. *Chemosphere* **2011**, *82* (6), 847-852.
- 9
10 634 (43) Esperanza, M.; Suidan, M.T.; Marfil-Vega, R.; Gonzalez, C.; Sorial, G.A.; McCauley,
11
12 635 P.; Brenner, R. Fate of sex hormones in two pilot-scale municipal wastewater
13
14 636 treatment plants: Conventional treatment. *Chemosphere* **2007**, *66* (8), 1535-1544.
- 15
16
17 637 (44) Holbrook, R.D.; Love, N.G.; Novak, J.T. Sorption of 17-beta-estradiol and 17 alpha-
18
19 638 ethinylestradiol by colloidal organic carbon derived from biological wastewater
20
21 639 treatment systems. *Environ. Sci. Technol.* **2004**, *38* (12), 3322-3329.
- 22
23
24 640 (45) Liu, R.X.; Wilding, A.; Hibberd, A.; Zhou, J.L. Partition of endocrine-disrupting
25
26 641 chemicals between colloids and dissolved phase as determined by cross-flow
27
28 642 ultrafiltration. *Environ. Sci. Technol.* **2005**, *39* (8), 2753-2761.
- 29
30
31 643 (46) Baronti, C.; Curini, R.; D'Ascenzo, G.; Di Corcia, A.; Gentili, A.; Samperi, R.
32
33 644 Monitoring natural and synthetic estrogens at activated sludge sewage treatment
34
35 645 plants and in a receiving river water. *Environ. Sci. Technol.* **2000**, *34* (24), 5059-5066.
- 36
37
38 646 (47) Brian, J.V.; Harris, C.A.; Scholze, M.; Kortenkamp, A.; Booy, P.; Lamoree, M.;
39
40 647 Pojana, G.; Jonkers, N.; Marcomini, A.; Sumpter, J.P. Evidence of estrogenic mixture
41
42 648 effects on the reproductive performance of fish. *Environ. Sci. Technol.* **2007**, *41* (1),
43
44 649 337-344.
- 45
46
47
48 650 (48) Coe, T.S.; Hamilton, P.B.; Hodgson, D.; Paull, G.C.; Stevens, J.R.; Sumner, K.; Tyler,
49
50 651 C.R. An environmental estrogen alters reproductive hierarchies, disrupting sexual
51
52 652 selection in group-spawning fish. *Environ. Sci. Technol.* **2008**, *42* (13), 5020-5025.
- 53
54
55 653 (49) Thorpe, K.L.; Maack, G.; Benstead, R.; Tyler, C.R. Estrogenic wastewater treatment
56
57 654 works effluents reduce egg production in fish. *Environ. Sci. Technol.* **2009**, *43* (8),
58
59 655 2976-2982.

- 1
2
3 656 (50) Sorensen, P.W.; Pinillos, M.; Scott, A.P. Sexually mature male goldfish release large
4
5 657 quantities of androstenedione into the water where it functions as a pheromone. *Gen.*
6
7
8 658 *Comp. Endocrinol.* **2005**, *140* (3), 164-175.
9
10 659 (51) Thorpe, K.L.; Cummings, R.I.; Hutchinson, T.H.; Scholze, M.; Brighty, G.; Sumpter,
11
12 660 J.P.; Tyler, C.R. Relative potencies and combination effects of steroidal estrogens in
13
14 661 fish. *Environ. Sci. Technol.* **2003**, *37* (6), 1142-1149.
15
16
17 662 (52) Kinney, C.A.; Furlong, E.T.; Kolpin, D.W.; Burkhardt, M.R.; Zaugg, S.D.; Werner,
18
19 663 S.L.; Bossio, J.P.; Benotti, M.J. Bioaccumulation of pharmaceuticals and other
20
21 664 anthropogenic waste indicators in earthworms from agricultural soil amended with
22
23 665 biosolid or swine manure. *Environ. Sci. Technol.* **2008**, *42* (6), 1863-1870.
24
25
26
27 666
28
29 667
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60