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Steroid hormones in biosolids and poultry litter: A comparison of potential environmental inputs

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ABSTRACT

Steroid hormones can act as potent endocrine disruptors when released into the environment. The main sources of these chemicals are thought to be wastewater treatment plant discharges and waste from animal feeding operations. While these compounds have frequently been found in wastewater effluents, few studies have investigated biosolids or manure, which are routinely land applied, as potential sources. This study assessed the potential environmental contribution of steroid hormones from biosolids and chicken litter. Hormone concentrations in samples of limed biosolids collected at a waste treatment plant over a four year period ranged from <2.5 to 21.7 ng/g dry weight for estrone (E1) and <2.5 to 470 ng/g dry weight for progesterone. Chicken litter from 12 mid-Atlantic farms had averages of 41.4 ng/g dry weight E1, 63.4 ng/g dry weight progesterone, and 19.2 ng/g dry weight E1-sulfate (E1-S). Other analytes studied were 17β-estradiol (E2), estriol (E3), 17α-ethinylestradiol (EE2), testosterone, E2-3-sulfate (E2-3-S), and E2-17-sulfate (E2-17-3).

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1. Introduction

Steroid hormones are a class of biological chemicals derived from cholesterol that play a variety of roles in vertebrate systems. Within this classification are estrogens, androgens, and gestagens. Estrogens and androgens are generally considered the "female" and "male" sex hormones, respectively, while gestagens, namely progesterone, are largely responsible for initiation and maintenance of pregnancy. Certain synthetic forms of these chemicals have also been developed for contraception or hormone replacement therapies; the synthetic estrogen 17α-ethynylestradiol is one of the most commonly used. While most hormones are naturally occurring, wastewater treatment plants (WTPs) and large livestock operations produce more concentrated releases of these chemicals to soil and water through effluents or land application of solid byproducts. Increased environmental concentrations of steroid hormones may contribute to endocrine disruption in various species, especially aquatic species such as fish or turtles (Irwin et al., 2001; Jobling et al., 1998). As hormones are naturally bioactive compounds they are much more potent than other synthetic compounds of concern. For instance, estrogens are at least 1000× more potent than industrial chemicals such as nonylphenol ethoxylates or bisphenol A, which are established as potential endocrine disruptors at ppb levels (Metcalfe et al., 2001). Intersex male fish have been observed after exposure to concentrations of E1 and E2 as low as 10 ng/L (Metcalfe et al., 2001).

Approximately 8.2 million tons of biosolids and more than 13 million tons of chicken litter will be produced in the U.S. in 2010 (Penn State, 2010; USEPA, 1999). Both materials are rich in nutrients and useful as soil amendments or fertilizers on agricultural fields. However, there are currently no standards regarding concentrations of organic microconstituents in biosolids or poultry litter. There is also little information available on environmental concentrations or fate for this class of compounds. This gap in understanding and lack of current regulatory diligence are raising concerns about land application of these materials. Knowledge about the occurrence of steroid hormones in both biosolids and chicken litter is very limited. Studies on biosolids have mainly focused on concentrations of the estrogens E1, E2, and EE2 determined using GC–MS/MS or HPLC–MS/MS. On the other hand, research on poultry litter has been largely conducted using bioassay techniques. Therefore, the literature mainly reports values for assays of E2 and testosterone; very little data is found on any other compound. While these bioassay methods are sensitive for detecting biological responses, they also suffer from cross-reactivity to similar chemicals and introduce questions as to which compounds exactly could produce the responses shown and are therefore represented in the total concentrations.

The overall goal of this study was to assess the relative inputs of steroid hormones to agricultural land from two soil amendments,

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biosolids and chicken litter. To date few, if any, studies have compared these materials using analytical techniques. We determined the presence and concentrations of 17β-estradiol (E2), estrone (E1), estriol (E3), 17α-ethinylestradiol (EE2), progesterone, and testosterone, and the conjugated hormones E1-sulfate (E1-S), E2-3-sulfate (E2-3-S), and E2-17-sulfate (E2-17-3) in biosolids and poultry litter that were produced in the mid-Atlantic region of the U.S. The WTP studied is the largest in this area of the country and contributes a large fraction of the land-applied biosolids to the region. Similarly, poultry litter was obtained from a number of farms throughout some of the largest poultry-producing counties. The sulfated compounds were included in the analysis because, while inactive in their current form, they can dissociate to reform the free, active hormone. We then compared the two materials as potential sources of steroid hormones to agricultural environments.

2. Materials and methods

2.1. Biosolid sample collection and preparation

Biosolid samples were collected every 3 months over 4 years (July 2005 to August 2009) from a large municipal wastewater treatment plant in the mid-Atlantic region of the U.S. The plant has a raw wastewater treatment capacity of 1.4 million cubic meters (370 million gallons) per day. Treatment includes primary treatment, activated sludge secondary treatment, nitrification/denitrification, effluent filtration, chlorination/dechlorination and post aeration. Biosolids produced are a combination of sludge from primary, activated sludge, and nitrification treatments that are dewatered and treated with lime to approximately 15% based on dry weight to produce a Class B product. These biosolids are supplied as soil amendments to many mid-Atlantic farms. Samples were collected from the treatment line after dewatering and lime mixing, put in amber glass jars (60–250 mL) and stored at −20 °C until processing.

Prior to extraction, three-gram aliquots of each sample were freeze-dried to produce final weights of ~1 g. The fluffy, cotton-like texture of the freeze-dried biosolids made grinding impractical and therefore samples were cut up with scissors to break up large masses, reduce particle sizes and to achieve relative homogeneity.

2.2. Chicken litter sample collection and preparation

Chicken litter samples were collected at 12 broiler farms located in Maryland and Delaware on the Delmarva Peninsula. Each house had held a minimum of four flocks since the last complete clean-out of litter. For each house, 30 samples of fresh litter were taken throughout the house, 12 in the brooding area and 18 in the rest of the house. Samples were collected with bulb planters from the surface to the depth where litter removal occurs. Once all samples were collected from the house, the litter was mixed well, spread out thinly and squared off into 16 quadrants. Litter (250 g) was taken from the middle of each quadrant to compose the final sample.

Samples were dried in a forced-draft oven at 50 °C and ground to pass through a 2 mm screen. They were then stored in plastic bags and refrigerated until analysis following previously published protocols (Hemmings and Hartel, 2006; Hakk et al., 2005). While these are not necessarily ideal drying conditions prior to chemical analysis, elevated temperatures are not uncommon on a poultry house floor and mineralization of both E1 and E2 has been shown to be lower in litter at higher temperatures (Hemmings and Hartel, 2006). Therefore, the litter was considered acceptable for our study.

2.3. Standards, reagents and materials

The following hormone standards were obtained from Sigma-Aldrich Corp. (St. Louis, MO, USA): E1 and E3 (VETRANAL® analytical standards, 99.5% and 99.9% respectively), E2 (Sigma Reference Standard, >97%), and E1-S (sodium salt). Progesterone (99.0%), testosterone (99.5%), and EE2 (99.5%) were obtained from EQ Laboratories Inc. (Atlanta, GA, USA). E2-3-S and E2-17-S were kindly provided by Dr. Michael Meyer at the USGS Kansas Water Science Center (Lawrence, KS). Internal standards E1-2, 4, 16, 16-d_4 (95%), E1-3-sulfate-2,4,16,16-d_4 (95%), and E2-2,3,4-^{13}C (99%) were obtained from Sigma-Aldrich Corp. (St. Louis, MO, USA), and progesterone-3,4-^{13}C (90%) came from Cambridge Isotope Laboratories, Inc. (Andover, MA, USA).

All solvents were high purity pesticide grade. Ammonium hydroxide was acquired from Fisher Scientific (Pittsburg, PA, USA). The alumina was neutral, 0.05–0.15 mm mesh, Brockmann Activity I obtained from Sigma-Aldrich Corp. (St. Louis, MO, USA). Carbon free, deionized water (DI water) was produced with a NANOpure system (Barnstead International, Dubuque, IA, USA). Sand, obtained from JT Baker (Mallinckrodt Baker, Inc., Phillipsburg, NJ, USA), and all glassware was baked at 400 °C for 4 h to drive off organic materials.

2.4. Pressurized liquid extraction

Extraction and clean-up methods for the biosolids and poultry litter were adapted from a method previously published by Nieto et al. (2008). We utilized an ASE 300 accelerated solvent extraction system ((Dionex Corp. Sunnyvale, CA, USA) and in-line alumina clean-up. Nieto et al. (2008) used alumina as well; however the exact alumina selected were not provided in their method. Our in-line clean-up and post-extraction concentration steps were as follows. In-line clean-up was incorporated into the extraction method by packing 5 g of neutral aluminum oxide (alumina), baked for 24 h at 120 °C, on top of two glass fiber filters that were placed in the bottom of each 33 mL extraction cell. Sand was mixed with 1 g of sample until the sample was evenly dispersed and the total volume was approximately 20 mL. This mixture was then poured into the extraction cell on top of the alumina plug. The remaining cell volume was topped off with sand. Following ASE extraction (conditions given in Nieto et al. (2008)), the methanol/acetone fractions, ~25 mL, were concentrated under nitrogen to a volume <5 mL, combined with second elution fraction of methanol/water, also ~25 mL, and these were further concentrated on a hotplate set to the lowest setting (~80 °C) until volumes reached <5 mL. Extracts were transferred to centrifuge tubes and diluted to 5 mL with deionized water and then further diluted to 10 mL with methanol. The final solutions were centrifuged for 7 min at 4500 rpm and the supernatant saved and analyzed by LC/MS/MS. These improvements allowed for increased contact time between the sample extract and the alumina, optimized the ratio of alumina to sample weights, and also yielded more concentrated extracts. With these changes, we achieved lower detection limits than the original method.

2.5. LC/MS/MS analysis

Separation of the analytes was achieved using a Waters 2695 LC (Waters Corp., Milford, MA) with a Waters XTerra C-18 column (5 μm MS C18 column — 150 × 2.1 mm) heated to 50 °C at a flow rate of 200 μL/min. Injection volumes were typically 20 μL. Two elution solvents were used: solvent A was a 0.1% solution of ammonium hydroxide in water and solvent B was a 0.1% solution of ammonium hydroxide in acetonitrile. Addition of ammonium hydroxide was found to be important to aiding in the ionization of analytes and to increasing sensitivity in negative ionization mode. Both solutions were made fresh before the start of each run. For the gradient elution of the analytes, the initial conditions of 90% A:10% B were held static for 5 min, then shifted by linear gradient to 40% A:60% B by 20 min, and then 20% A:80% B by 30 min. The LC column was then rapidly returned to initial conditions and equilibrated for 10 min before the next run.

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Tandem mass spectrometry analysis was performed using a triple–quadrupole mass spectrometer (Quattro Ultima from Micromass Ltd., Manchester, U.K., now Waters Corp., Milford, MA) with an electrospray ionization source that was operated in negative ionization mode for all compounds except testosterone and progesterone, which were analyzed in positive ionization mode. Acquisitions were done in Multiple Reaction Monitoring (MRM) mode and peak integration and quantitation were performed using MassLynx v4.0 software (Micromass Ltd., Manchester, U.K.). The primary parent–daughter mass transitions and the mass spectrometer settings are listed in Table 1. Isotope dilution quantitation was used in order to account for matrix suppression of analytes. Isotope pairs used were ^{13}C-E2 for E2, E1-d$_4$ for E1, E1-S-d$_4$ for E1-S, and ^{13}C-progesterone for progesterone. These isotopes were also used as internal standards as follows: ^{13}C-progesterone was used to quantitate testosterone, E1-S-d$_4$ was used for the conjugated hormones, and ^{13}C-E2 was used for E3 and EE2. Data obtained from this analysis was processed using Microsoft Excel and SigmaPlot (Systat Software Inc, San Jose, CA).

2.6. Quality control/quality assurance

Samples were run in groups of 5, with each sample being run in duplicate, and each group being run with a solvent blank to test for contamination and two samples that were spiked with a mixture of all free and conjugated hormones to test method recovery. Labeled E2, E1, progesterone, and E1-S were added as internal standards to all samples prior to extraction. Recoveries of all tested analytes were all >78% (the lowest was for testosterone in chicken litter), while the range for the reported analytes was from 85 to 109%.

Limits of quantitation (LOQ) were defined as peaks with a signal-to-noise ratio of ~10:1; values less than the LOQ were not reported. Due to the variable nature of the samples and the interferences present, the 10:1 threshold varied but was approximately 5 ng/g for quantitated compounds. Results were reported as averages of these duplicate values unless one value was below the LOQ, in which case only the quantitated value was reported. For statistical purposes, concentrations in samples where both duplicate values were below the LOQ were set at half of the lowest standard, 2.5 ng/g; otherwise they were reported as <2.5 ng/g.

3. Results

3.1. Biosolids analysis

Fig. 1 shows the concentrations of hormones found in limed biosolids over four years of collection. Progesterone and E1 were found in all samples, although not always above the LOQ. Traces of E3 and testosterone were seen in some samples, but not above the LOQ. Neither E2, EE2, nor any of the conjugated hormones were detected.

Table 1
MS conditions used for the detection of hormones and conjugated hormones.

Compound	Parent (Da)	Daughter (Da)	Retention time (min)	Cone (V)	Collision (eV)	Dwell (s)	Ion Mode
E2	271.0	183.0	21.7	100	46	0.2	ES-
E1	269.0	145.0	22.9	110	43	0.2	ES-
E3	287.4	171.4	16.5	112	50	0.2	ES-
EE2	295.0	145.2	22.7	105	44	0.2	ES-
Progesterone	315.0	108.9	26.2	65	31	0.2	ES+
Testosterone	289.0	108.9	22.4	62	31	0.2	ES+
E1-S	349.0	269.0	17.8	105	32	0.2	ES-
E2-3-S	351.0	271.0	16.7	105	35	0.2	ES-
E2-17-S	351.0	96.7	16.6	115	45	0.2	ES-
^{13}C-E2	274.1	185.4	21.7	150	45	0.2	ES-
E1-d$_4$	273.0	147.0	22.9	105	38	0.2	ES-
^{13}C-Prog	317.0	98.9	26.2	75	31	0.2	ES+
E1-S-d$_4$	352.0	272.0	17.8	105	32	0.2	ES-

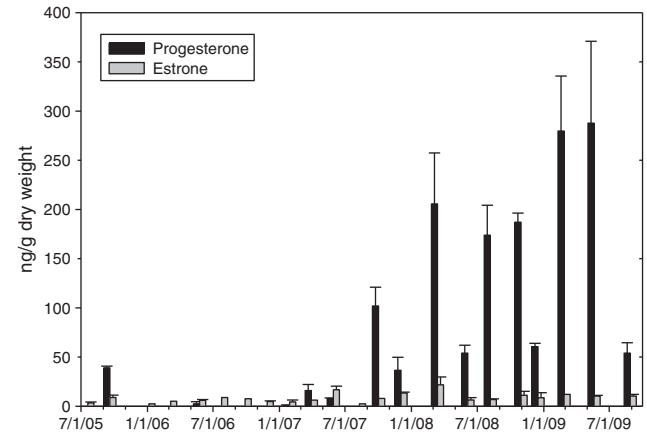

Fig. 1. Concentrations of estrone and progesterone in biosolids over 4 years. Error bars = standard error of mean (SEM). No error bar indicates one value below LOQ, only one value used for the graph.

Progesterone values show minimal fluctuation in samples collected up to August 2007, with an average concentration of 8.52 ng/g and a range of <2.5–39.1 ng/g. After this point, values increase sharply and fluctuate widely, with an average concentration of 157 ng/g and a range of 36.5–470 ng/g. Values of E1 are overall much lower, with an average over four years of 8.53 ng/g. Instead of an abrupt shift around August 2007, the concentrations instead show a slightly increasing trend over the four years of sampling. These differing trends are illustrated in Fig. 2.

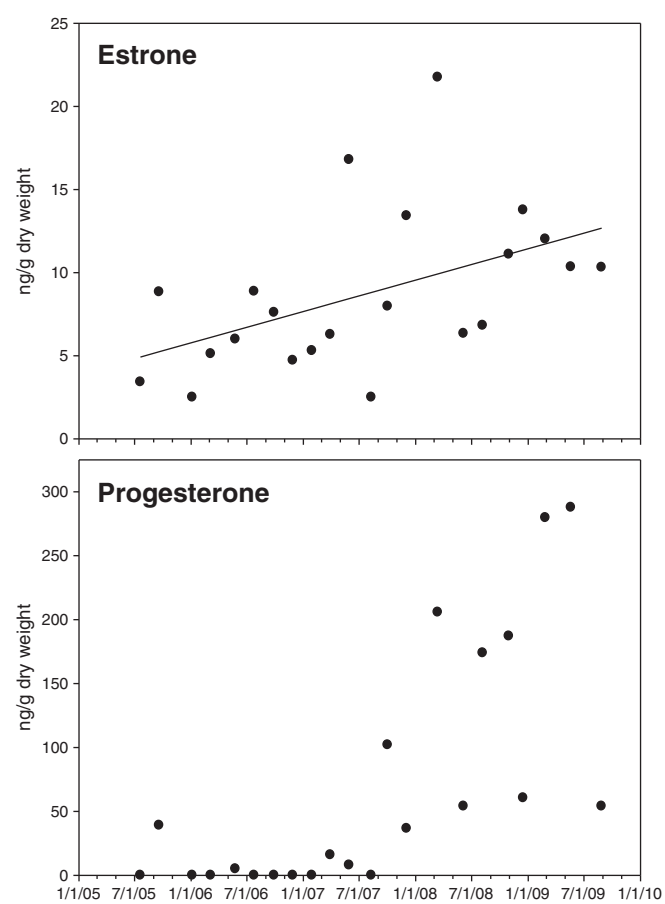

Fig. 2. Trends in estrone and progesterone values over 4 years. Note the difference in scales used. R^2 for E1 regression is 0.83.

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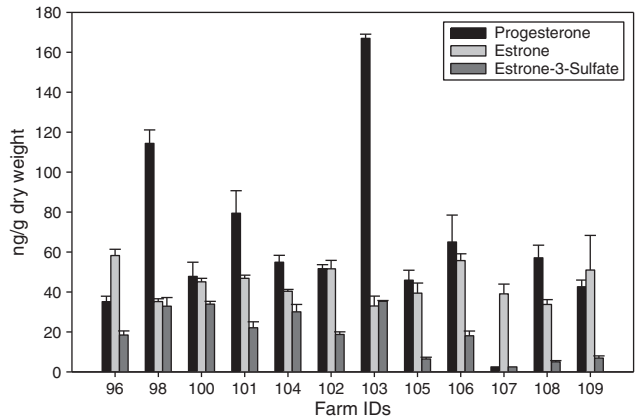

Fig. 3. Concentrations of steroid hormones in chicken litter. Error bars = SEM. No error bar (Farm 107) indicates peaks seen below LOQ. Nominal bar shown for this farm at half the lowest standard, 2.5 ng/g.

3.2. Chicken litter analysis

Fig. 3 shows the concentrations of hormones found in chicken litter samples. Progesterone and E1 were again found in all samples, and these samples also contained E1-S. All three compound peak area responses were above the LOQ in every extract, with the exception of the progesterone and E1-S peaks for Farm 107. The extract from this farm was unique in that it had an unusually large amount of matrix interference compared to other chicken litter extracts, which appears to have caused this poor quantitation. The average concentrations for progesterone and E1 over all farms were 63.4 ng/g and 44.1 ng/g, respectively. The average concentration of E1-S was 19.2 ng/g. Traces of E3 and testosterone were again seen in some samples, but all were below the LOQ. No E2 was seen. EE2 was also not seen, however this would not be expected in these samples, as chickens should not be exposed to this synthetic hormone.

4. Discussion

4.1. Biosolids analysis

Biosolids are a very heterogeneous material; among the factors that can cause variation in the compounds present in biosolids from different wastewater treat plants are the sources of wastewater loading into the plant, the attributes of the population that the plant serves, and the processes the plant uses for treatment of solids. When comparing the hormone levels detected in this study to those found in previous studies, the potential for inherent variability in the studied material needs to be taken into account. In the literature, only the EPA's Targeted National Sewage Sludge Survey (TNSSS), a broad study of sludge produced at 74 publically-owned treatment works, is known to contain treatment plants which produce limed biosolids, however the number of plants employing this method and the hormone levels associated with their biosolids was not disclosed (USEPA, 2009). This study adds new data to be compared to existing information on solids produced from other treatment processes.

Table 2 shows the steroid hormone concentrations reported in this study and in those previously published. None of these studies, except that by the EPA, include information on progesterone or testosterone (Andersen et al., 2003; USEPA, 2009; Muller et al., 2008; Nieto et al., 2008; Pothitou and Voutsa, 2008; Ternes et al., 2002). Of those compounds in common, variability in values may be attributed to a different number of factors such us wastewater source, type of treatment and storage conditions. For one, example the addition of lime to the sludge in our study would raise the pH to above 12 and adding lime to sludge raises the pH to 12 or more; these conditions favor the formation of E1 as it has a ketone group at the 17 position instead of a hydroxyl group as in the structure of the other estrogens. This may help explain why E2 was not seen in this study while it was detected in dewatered and digested biosolids (Andersen et al., 2003; Muller et al., 2008; Ternes et al., 2002). Along these lines, dried biosolids, such as those sampled by Pothitou and Voutsa (2008), may have lower hormone levels because there is extra time for the compounds to degrade compared to other methods, especially if the material is exposed to UV radiation and heat from the sun (Pothitou and Voutsa, 2008). Differences in which hormones are detected could also be a result of differences in detection limits. For example, many of the previously reported values for E2 are between 1 and 10 ng/g, which is at or below the limit of sensitivity in this analysis (Andersen et al., 2003; Muller et al., 2008; Pothitou and Voutsa, 2008; Ternes et al., 2002).

In addition to these treatment- or analytical-based possibilities, differences in hormone profiles could arise due to difference in the populations served by the selected WTP. For example, whereas E1, E2, and E3 are naturally occurring and always being excreted by human and other animal populations, EE2 is a pharmaceutical and its rate of detection may be affected by how frequently it is used by the population served by the studied treatment plant. Similarly, the ratio of domestic to industrial wastewater will also affect the concentrations of steroid hormones which enter the plant, and therefore also the concentrations in the final effluent and sludge. These demographic possibilities may help explain the lack of EE2 in our results despite it being engineered to be a more highly persistent molecule than the natural hormones that we did detect.

We did not expect to detect any of the conjugated hormones we screened for in the biosolids, due to the high degree of processing and harsh conditions they are subjected to during treatment prior to our sampling point. Our negative findings are in agreement with those of

Table 2
Concentrations of steroid hormones found in biosolids.

Authors	Treatment	Concentration (ng/g dry wt)					
		E1	E2	E3	EE2	P	T
Ternes et al. (2002)	Digestion[a]	16, ND[b,c]	9, 49[c]	NA[b]	17, 2[c]	NA	NA
Andersen et al. (2003)	Digestion[a]	22.8–27.8	4.9–5.4	NA	ND	NA	NA
Muller et al. (2008)	Dewatered	2–8	1–10	ND	1–16	NA	NA
Nieto et al. (2008)	Unknown	ND-200[d]	ND	ND-406	ND	NA	NA
Pothitou and Voutsa (2008)	Dried	ND	ND	ND	NA	NA	NA
USEPA (2009)[e]	Varied	26.7–965	22–355	7.56–232	ND	143–1290	30.8–2040
Current study	Liming	**<2.5–21.7**	**ND**	**ND**	**ND**	**<2.5–470**	**ND**

[a] Type (anaerobic versus aerobic) not specified.
[b] NA = not analyzed, ND = not detected.
[c] Only two samples analyzed.
[d] Exact number not given, value extracted from a graph.
[e] Ranges given for samples where hormones were detected. E1 was detected in 60/84 samples, E2 in 11/84, E3 in 18/84, P in 19/84, and T in 17/84.

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Muller et al. (2008). Nieto et al. (2008), however, did find sulfated hormones at levels up to 7 ng/g but they do not specify what prior treatment the solids had gone through. Again, differing processes may allow for some conjugated hormones to persist, or this may reflect a higher concentration of residential wastewater entering the plant and therefore a higher total amount of conjugated hormones present in the initial influent and therefore the final effluent as well.

The advantage this study has over other studies is that our samples were collected and analyzed over a 4-year period illustrating potential variability over time. The results shown in Fig. 1 raise the question of what occurred around August 2007 in order to increase the values of progesterone so abruptly and cause large fluctuation in concentrations after this point. A group of randomly selected samples were re-extracted and re-analyzed and the originally observed trends were reproduced, thus ruling out variations in analytical method performance as the source of these trends. We believe that the most likely cause for the increase in concentrations was a change in the treatment process at the studied WTP. After consultation with staff at the WTP, we learned that around the time when the shift occurred, the plant changed its supply of lime from one with a fine texture to one with a courser texture. A previous study at this plant had shown that while no pH changes occurred in biosolids based on lime texture, the coarser lime resulted in an increase in the levels of *E. coli* that were detected (North et al., 2008). The authors of that study believed that the coarser lime may have left larger pockets of biosolids between lime particles where the pH did not get quite as high and therefore more bacteria were able to survive (North et al., 2008). While this is not a direct comparison, we speculate that lime texture may have had an effect on the progesterone concentrations as well and perhaps to a lesser extent, on the E1 concentrations. While our observations and the report by North et al. suggest that characteristics of the lime and the contact time do indeed affect hormone concentrations in the wastewater solids, additional research is needed to more carefully test this hypothesis and to investigate the reasons behind those effects.

4.2. Chicken litter analysis

In previous studies of chicken litter, reported concentrations of E2 ranged from approximately 1 ng/g (Jenkins et al., 2006) to 904 ng/g (Finlay-Moore et al., 2000). Likewise, testosterone values ranged from approximately 0.05 ng/g (Jenkins et al., 2006) to 254 ng/g (Shore et al., 1993). Our findings did not indicate the presence of E2 and indicated only possible traces of testosterone. Much of the difference is likely due to differences in hormone detection methodologies. Whereas previous studies used bioassays that quantify overall biological responses to hormonally active compounds present in the material, and these were designed to target E2 or testosterone receptors, we employed more precise chemical identification LC/MS/MS methods which allowed specific chemical forms of the hormones to be identified. The lack of E2 in our results could indicate that conditions within the sampled houses favored the conversion of E2 to E1. Average concentrations for E1 (44.1 ng/g) and for E1-S (19.2 ng/g), or both combined, fall within the range of values reported previously as E2 by bioassay methods in broiler litter. It is possible, and even likely, that at least a part of the biological activity these reported values represent were in fact caused by the presence of E1. Shore et al. (1993) acknowledged this in his reported concentrations by ascribing them to the concentration of "estrogen," a combination of E2 and E1. Similarly, the lack of testosterone in our samples could be an indication that there are other androgenic compounds in the litter which contribute to a testosterone-like response.

Analyses were performed for three possible conjugated hormones and found that E1-S was present in the chicken litter samples at concentrations as high as 35.4 ng/g. This compound could contribute to the total estrogenicity of the chicken litter if conditions favor deconjugation. These results support the findings of Yonkos (2005) that approximately half of the E2 response came from conjugated forms of estrogens, especially as many other conjugation possibilities exist other than just E1-S and E2-S. For example, glucuronide substituted forms of these hormones, which we did not look for, are also common.

Variability was seen in measured concentrations of hormones in chicken litter, but no distinct trends were apparent. This was likely due to the fact that there was no systematic study of sampling effects and sample collection was somewhat random and carried out more as an initial screening survey. Some factors potentially contributing to variability include the number of flocks that had been in the house since the last complete clean-out, the age of the current flock, the type of chickens raised, and litter treatment or prolonged storage of litter. Of these factors, data was available for the age of the current flock and the number of flocks raised in the houses since the last clean-out. When the samples were divided based on the current flock age, no significant difference could be seen between houses with flocks <10 days old (n = 4) and those with flocks >25 days old (n = 6). All houses but two had the same number of flocks in them since the last clean-out, making that comparison less meaningful. To obtain data with this kind of detail, a much larger sample set would be needed, as well as samples from houses chosen specifically for various characteristics.

4.3. Comparison of environmental inputs by biosolids and poultry litter

In order to give some perspective on possible environmental inputs, the average hormone concentrations determined in our analysis were used to estimate the potential annual load applied by both chicken litter and biosolids for the state of Maryland. This state was chosen both because it is the region from which the samples were obtained and because the large population density and large poultry farm density, especially on the Eastern Shore, make the issue particularly relevant.

Potential annual loads were calculated based on rates of 188,220 wet tons of biosolids and 345,000 wet tons of chicken litter applied to Maryland farmland per year (Penn State, 2010; MDE, 2009a; USDA, 2007). The resulting estimates are shown in Table 3. These calculations suggest that land application of chicken litter represents a larger source of steroid hormones to the environment than land application of biosolids. This was the expected trend considering the more extensive processing that takes place in a WTP, which should result in lower concentrations of hormones persisting in this final product, as well as the smaller total weight of biosolids that are land applied versus poultry litter. However, this distribution is a statewide average and the actual ratio of inputs by biosolids versus chicken litter will vary by location. The majority of poultry farming takes places on Maryland's Eastern Shore, and due to high costs of shipping, most of

Table 3
Two comparisons of contributions of steroid hormones to Maryland agricultural soil from biosolids and chicken litter. Potential yearly hormone inputs were calculated based on yearly statewide application rates of each material. Initial Predicted Environmental Concentration ($PEC_{(ini)}$) is the approximate soil concentration immediately after amendment with the specified material.

Compound	Hormone inputs (kg/year)	$PEC_{(ini)}$ (ng/kg)
Progesterone		
Chicken litter	16	340
Biosolids	8.0	310
Estrone		
Chicken litter	11	230
Biosolids	1.9	21
Estrone-sulfate		
Chicken litter	5.0	100
Biosolids	0	0

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the litter is applied to farms near where it was produced. This makes inputs from chicken litter much more likely in these areas compared to farms elsewhere in the state that are more likely to receive biosolids.

The fate of steroid hormones after land application depends on rates and pathways of degradation as well as processes such as sorption/desorption, diffusion into soil, and leaching or runoff into water bodies after rain events. These processes, and therefore the environmental fate of these hormones, will vary depending on the conditions at the application site. In spite of these uncertainties it is still useful to estimate how much of these hormones would potentially be available in surface soil at the time of application. To accomplish this, an estimate of the initial Predicted Environmental Concentration (PEC_{ini}) in soil upon land application was calculated for progesterone, E1, and E1-S using Eq. (1) (Jackson and Eduljee, 1994).

$$PEC_{ini} = C_{soil(0)} + \frac{C_{amendment} \times AR_y}{D \times S_z \times CF} \quad (1)$$

$C_{soil(0)}$ is the background concentration of the specific hormone in soil, $C_{amendment}$ is the mean concentration of the hormone in chicken litter or biosolids (average of the last two years), AR_y is the application rate of the soil amendment, D is the soil density (1.4×10^{-3} kg/cm^3), S_z is soil depth (taken to be 10 cm), and CF is the conversion factor (1×10^8 cm^2/ha). For these calculations, the average concentrations determined for each hormone were converted into wet weight based on 70% moisture in biosolids and 20% moisture in chicken litter. Given the very limited data on hormone concentrations in soil, $C_{soil(0)}$ was assumed to be 0 for these compounds. This is in agreement with the findings of Beck et al. (2008) that estrogens were not detected in soils studied that did not have a history of manure amendment. An average for AR_y of 9200 kg/ha was determined from the total amount of biosolids applied in Maryland 2008 (188,220 tons) and the number of acres (46,000) that received biosolid application in 2008 (MDE, 2009a; MDE, 2009b). As the nitrogen content of chicken litter is approximately the same as that of biosolids, the same application rate was used. This amendment rate is in agreement with other studies involving chicken litter or biosolid application to agricultural land in this region (Jenkins et al., 2009; Jenkins et al., 2008; Andrade et al., 2010; Lozano et al., 2010).

The predicted values for PEC_{ini} after land application of biosolids or chicken litter calculated for each hormone are shown in Table 3. Few studies have directly measured hormone levels in soils, but these predictions fit in with the data that are available. Finlay-Moore et al. (2000) measured soil concentrations of E2 and testosterone by bioassay methods and found increased levels of both hormones after amendment with poultry litter. In samples collected immediately after application, concentrations of E2 rose to 675 and 260 ng/kg in pasture and hayed plots respectively (compared to 110 and 130 ng/kg in controls) (Finlay-Moore et al., 2000). In a similar study, Jenkins et al. (2006) found E2 levels (measured by bioassay) in soil to vary between 65.3 and 636 ng/kg on plots amended with poultry litter. Beck et al. (2008) employed GC–MS to detect specific estrogens in soils with a history of manure amendment and found background values for 17α-E2, 17β-E2, E1, and E3 in the low ng/kg range. Their values for E1 concentrations were nearly 10× greater than those for 17β-E2 (25 and 12 ng/kg for cropland and pasture, respectively, compared to 3 and 2 ng/kg). While not comparable to the Finlay–Moore data or our predictions for poultry litter, this does compare well with our prediction for E1 input from biosolids and supports our observation that E1 is the more dominant estrogen present.

These numbers give us an idea of how much of each hormone is potentially available in the amended soil; however there is too little information available both on the fate of these compounds after reaching the soil and on their potency in soil versus water for an estimate to be made of overall estrogenic potency. Information is especially lacking on the potential biological activity of progesterone; it is unlikely to add to the overall estrogenicity of the soil but could cause other reproductive effects on its own or produce synergistic effects with the estrogens that are present (Besse and Garric, 2009). We can say that as the potency of E1 is approximately 0.5 that of E2, the corresponding estimated E2 equivalents from this compound in soil would be 11 and 115 ng/kg after application of biosolids and poultry litter (Johnson and Sumpter, 2001). Additionally, the poultry litter contained E1-S, which will degrade to form an active hormone whereas the parent is inactive (Scherr et al., 2008). When poultry litter is applied, E1 concentrations could increase nearly 50% as E1-S is deconjugated, increasing the E2 equivalents and overall estrogenicity of the soil.

Hormones, mainly E2 and testosterone, have been detected in runoff from poultry litter- or sludge-amended fields at concentrations shown to have the potential to affect aquatic populations, especially when the runoff event occurs shortly after amendment (Busheé et al., 2008; Nichols et al., 1997; Finlay-Moore et al., 2000; Yonkos, 2005). Our calculated and predicted hormone concentrations fit into the range of values reported in these studies, indicating the potential exists to affect water bodies** adjacent amended fields. Any actual effects would depend on factors such as hydrologic conditions and the species present in the waterway (Yonkos, 2005).

5. Conclusion

This study presents a profile and comparison of steroid hormones seen in biosolids and chicken litter, two materials which are both applied to agricultural land. E1 and progesterone were frequently detected in both materials and E1-S was consistently found in chicken litter samples but not in biosolids. Continued work is needed to determine the risks that may be associated with the presence of these hormones. This is especially true of progesterone, for which there is extremely little data available. Additionally, future studies to increase our understanding of how treatment processes such as liming, aerobic or anaerobic digestion, and composting affect the concentrations of steroid hormones in biosolids and poultry litter would be beneficial. A greater knowledge of the risks associated with microconstituents in these valuable, nutrient rich materials and the ways these risks can be mitigated can make the recycling of biosolids and poultry litter a more environmentally acceptable and truly sustainable practice.

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