

Decrease in Water-Soluble 17 β -Estradiol and Testosterone in Composted Poultry Manure with Time

Heldur Hakk,* Patricia Millner, and Gerald Larsen

ABSTRACT

Little attention has been paid to the environmental fate of the hormones 17 β -estradiol and testosterone excreted in animal waste. Land application of manure has a considerable potential to affect the environment with these endocrine disrupting compounds (EDCs). Composting is known to decompose organic matter to a stable, humus-like material. The goal of the present study was to quantitatively assess levels of water-soluble 17 β -estradiol and testosterone in composting chicken manure with time. Chicken layer manure was mixed with hay, straw, decomposed leaves, and starter compost, adjusted to approximately 60% moisture, and placed into a windrow. A clay-amended windrow was also prepared. Windrows were turned weekly, and temperature, oxygen, and CO₂ in the composting mass were monitored for either 133 or 139 d. Commercial enzyme immunoassay kits were used to quantitate the levels of 17 β -estradiol and testosterone in aqueous sample extracts. Water-soluble quantities of both hormones diminished during composting. The decrease in 17 β -estradiol followed first-order kinetics, with a rate constant $k = -0.010/\text{d}$. Testosterone levels declined at a slightly higher rate than 17 β -estradiol (i.e., $k = -0.015/\text{d}$). Both hormones could still be measured in aqueous extracts of compost sampled at the conclusion of composting. The decline in water-soluble 17 β -estradiol and testosterone in extracts of clay-amended compost was not statistically different from normal compost. These data suggest that composting may be an environmentally friendly technology suitable for reducing, but not eliminating, the concentrations of these endocrine disrupting hormones at concentrated animal operation facilities.

CURRENT SOCIOECONOMIC FORCES have increased the animal numbers per farm operation, but have limited the distance manure or litter can be economically transported. Hence, considerable amounts of manure generated at concentrated animal feeding operations (CAFOs) are stockpiled, lagooned, or composted before being applied to adjoining farmland. Transport of the manure or contaminants in the manure into surface waters can readily occur after heavy rains. Exposure of livestock or wildlife to these compounds during key stages of development may play a role in subsequent developmental and/or reproductive problems.

17 β -Estradiol and testosterone are classified as EDCs when found in the environment. Animal manures are a potentially significant source of sex hormones in the environment because they are directly applied to land in

relatively high amounts (Callantine et al., 1961; Knight, 1980). Egg-laying chickens excrete high levels of 17 β -estradiol and testosterone, 50 and 250 ng/g dry manure/d, respectively (Shemesh and Shore, 1994; Shore et al., 1988, 1995a). In addition, the endogenous concentration of 17 β -estradiol in cattle urine averages 13 ng/L (Erb et al., 1977). However, the excreted concentrations can be even higher, because 17 β -estradiol, in the benzoate or palmitate ester forms, is frequently administered as a growth hormone to increase muscle mass and decrease fat deposition (Popp et al., 1997).

Several studies have demonstrated that sex hormones appear in soil, surface water, and ground water as a result of manure application (Nichols et al., 1997, 1998; Finlay-Moore et al., 2000; Peterson et al., 2000). Exposure to EDCs in the environment has been associated with widespread physiological and reproductive disorders in a variety of wildlife (Colburn et al., 1993) and in humans (i.e., increased breast cancer; David and Bradlow, 1995). The U.S. Department of Health and Human Services (National Institute of Environmental Health Sciences, 1994) has classified 17 β -estradiol as a carcinogen, based on its link to breast cancer (Dickson et al., 1986). Brown trout gonad development and feeding were halted at 50 to 300 $\mu\text{g/L}$ 17 β -estradiol (Ashby, 1957). 17 β -Estradiol levels of 2000 ng/L in water resulted in 84 to 100% feminization of salmon (Nakamura, 1984), while 250 to 5000 ng/L resulted in fish deaths (Nakamura, 1984; Kramer et al., 1998). 17 β -Estradiol in poultry litter fed to heifers caused premature udder development (Shore et al., 1988). 17 β -Estradiol has also been shown to have an effect on plant growth. At concentrations of 5.4 to 544 ng/L, 17 β -estradiol increased alfalfa growth, while at concentrations of 54.4 to 544 $\mu\text{g/L}$, decreased alfalfa growth was observed (Shore et al., 1992). Testosterone is excreted into the environment from the same sources and in comparable amounts as 17 β -estradiol (Shore et al., 1995b). To the best of our knowledge, there are no published papers on the adverse effects of testosterone in the environment, despite measured levels of up to 200 ng/L (Kolpin et al., 2002).

Little is known about the transport and fate (metabolism and degradation) of these hormones in field settings or in hydrological systems. Studies by individuals involved in this study have shown that 17 β -estradiol and testosterone are strongly sorbed to soils (Casey et al., 2003a, 2003b). The lack of data on the transport and fate of these hormones is interesting given the fact that they are vastly more potent in interacting with estrogen and androgen receptors than most anthropogenic EDCs. In vitro studies have reported that 17 β -estradiol has a 10⁴

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Abbreviations: EDC, endocrine disrupting compound; EIA, enzyme immunoassay.

to 10⁶-fold greater affinity for the estrogen receptor than alkylphenol ethoxylates (Jobling and Sumpter, 1993).

Composting is a self-heating, aerobic process that accelerates the degradation of organic materials by the successive action of a diverse group of microorganisms, including mesophiles, thermophiles, bacteria, actinomycetes, and fungi (Kaiser, 1996; Insam et al., 1996). Composting is a beneficial residuals management option that stabilizes organic by-products, reduces their weight, destroys pathogens and weed seeds, and produces a low-odor soil conditioner that also has some fertilizer value (Rynk, 1992; Willson et al., 1983). Active composting generates considerable heat (>60°C) during the 3- to 4-wk peak-heating phase of the process, during and after which the organic matter may ultimately be converted into gaseous CO₂ and water vapor. It can also provide an economic, effective, and practical solution for managing hazardous waste or remediating contaminated soil (Eitzer et al., 1997). For instance, >99% degradation was achieved when 2,4,6-trinitrotoluene-contaminated soil was composted for 160 d (Williams and Keehan, 1993). Depending on the level of degradation achieved, the costs for disposal of remediated soil resulting from composting are far lower than from incineration or land-filling (Goldstein, 1985).

The purpose of the present study was to evaluate the water extractability of the potent endogenous hormones, 17 β -estradiol and testosterone, during the course of aerobic composting. Results will be useful in evaluating the efficacy of composting in decreasing the environmental load of these hormones originating from farms, particularly concentrated animal feeding operations.

MATERIALS AND METHODS

Composting

Chicken layer manure was obtained from a commercial layer operation in Pennsylvania and transported to the Composting Research Facility at the Beltsville Agricultural Research Center (Beltsville, MD) where composting was performed. The composting facility handles about 14 500 m³ per year from residuals generated at the 3000-ha farm and research center. The operational surface of the composting facility consisted of an outdoor 7200-m² pad, which was a 21-cm-thick lime-stabilized soil pad that included coal combustion ash, cement kiln dust, quicklime, and existing Christiana clay (fine, kaolinitic, mesic Typic Paleudults) subsoil. An 8-ha orchard grass (*Dactylis glomerata* L.) runoff buffer surrounds the composting pad. The site is located in the Anacostia River watershed, which is part of the Chesapeake Bay system. Manure was analyzed for moisture and carbon (C) and nitrogen (N), from which a C to N ratio was calculated. Water was added to produce moisture content in the range of 50 to 60 g/kg. The initial C to N ratio was adjusted to 30 by addition of hay, straw, leaves, and starter compost. The standard compost composition was old hay (2 parts by weight), 3-wk-old peak heat starter compost made with no poultry, dairy, or swine manure (2 parts), layer manure (3.3 parts), autumn leaves (4 parts), and straw (2 parts). In a separate compost pile, an additional part of crushed Christiana clay was added. Each windrow was approximately 53 m long, 1.7 m wide, and 0.16 m high. After the first week of composting the windrows were turned once a week to aerate and facilitate mixing and exposure of all parts of

the mass to the high-temperature core regions of the windrows. Windrows were routinely monitored at three sites for temperature, oxygen, and carbon dioxide using a Compost Pro datalogger (Morgan Scientific, Haverhill, MA) and the data were averaged.

Sampling

Three composite samples were collected on Days 0, 6, and 13 and two were collected on Days 27, 55, 69, 83, 111, and 139 and placed into plastic bags. A composite sample consisted of three subsamples equidistant from a selected site within the windrow, and which were mixed to assure nonpreferential sampling. Samples were frozen at -20°C and shipped overnight in coolers with frozen blue-ice gel inserts to maintain samples until delivery to the Biosciences Research Laboratory (Fargo, ND). They were immediately frozen at -30°C and stored until analyzed. In general, analyses were performed within two weeks of receipt. One replicate windrow each of normal and clay-amended compost was constructed 6 d after the initiation of the first two windrows. Three composite samples were removed from the replicate windrows at Days 0 and 7, and two composites were sampled at Days 21, 49, 63, 77, 105, and 133. The data from the replicate windrows were combined for the analyses.

Sample Preparation

Frozen compost samples were removed from storage, placed into a blender (Waring, Torrington, CT) containing dry ice, and blended until homogeneity was achieved (5 min). Blended samples were placed into a plastic bag and dry ice was allowed to evaporate in a -20°C freezer. An aliquot (1.0-1.2 g wet wt.) was removed, weighed, and dried in a 50°C oven until a constant weight was obtained. This dry weight was used for all calculations of hormone concentration presented in the tables or figures. Two or three determinations of hormone activity were made on composite samples obtained from the first three time points, while one determination was made on samples from later time points.

Hormone Assays

A standard procedure for extracting field samples was adopted. An approximately 200-mg aliquot of the homogenized sample was placed into a 125-mL Erlenmeyer flask with 50 mL of double distilled water. The mixture was shaken horizontally at 25°C for 2 h in a reciprocating water bath. The sample was centrifuged at 1124 \times g and the aqueous layer was decanted and used for all assays.

Estradiol and testosterone enzyme immunoassay (EIA) kits were purchased from Cayman Chemical (Ann Arbor, MI) and used according to the manufacturer's instructions. The EIA analyses were run in triplicate or quadruplicate with blanks and standards, and both nonspecific and maximum binding were determined. The 17 β -estradiol EIA kit cross-reactivities were 17% for 17 β -estradiol-3-glucuronide, 4% for estrone, 0.57% for estriol, 0.1% for testosterone, and 5 α -dihydrotestosterone, and less than 0.1% for all other steroids. Cross reactivities for the testosterone kit were 21% for 5 α -dihydro testosterone, 12.4% for 11-keto testosterone, 10% for 5 β -dihydro testosterone, 3.6% for androstenedione, 1.2% for 11-hydroxy testosterone, and less than 1% for all other steroids. Quantitation was performed using a Victor Model 1420 multilabel counter (Wallac, Turku, Finland) by measuring the amount of 5-thio-2-nitrobenzoic acid released from added substrate in an enzymatic reaction at 405 nm. Blank EIA analyses for straw, hay,

leaves, starter compost, and Christiana clay were performed in the same manner.

Recovery Analyses

Recovery data on the extractability of the hormones from the compost matrix were performed in two ways: (i) extraction with water (50 mL \times 3) to simulate three successive rainfall events, or (ii) successive 50 mL extractions with water, methanol, and acetone to determine the total recovery. In both cases, three and five aliquots, respectively, of approximately 0.5 g of dried compost were weighed and spiked with [14 C] hormones (0.33 μ g; 0.66 ppm; [14 C]17 β -estradiol or [14 C]testosterone, 1.7×10^9 Bq/mmol and 1.9×10^9 Bq/mmol, respectively, >98% radiochemical purity; American Radiolabeled Chemicals, St. Louis, MO) in ethanol and allowed to air-dry. The compost was then extracted in each solvent for 2 h at 25°C on a shaker bath, centrifuged, decanted, and assayed immediately for radioactivity. Each extract was spotted onto silica TLC plates and developed with 1:1:2 ethyl acetate to tetrahydrofuran to hexane with standards to quantitate the amount of parent material and any possible metabolites formed.

Statistical Analyses

The combined data from replicate windrows were log-transformed and fitted to a simple linear regression model. A mixed model analysis (PROC MIXED; SAS Institute, 2004) was used to test whether a difference existed between normal and clay-amended compost.

RESULTS

The average temperature of the interior of the normal composting windrow indicated that thermophilic conditions (>40°C) were achieved by Day 3, and remained there until at least Day 41 (Fig. 1A). Mesophilic temper-

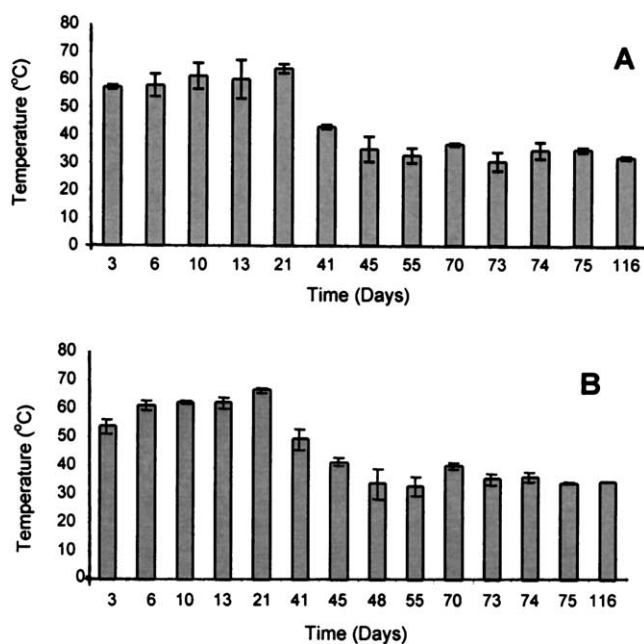


Fig. 1. Average temperature (°C) of composting chicken layer manure windrows during the first 116 d of composting for the (A) normal and (B) clay-amended windrows. Means represent three measurements taken from two windrow core regions and one windrow end region.

atures (10–40°C) characterized the interior of the normal composting windrow between Days 45 and 139 (temperatures beyond Day 116 not shown in Fig. 1A). Similar results were observed in the clay-amended composting windrow, except that thermophilic temperatures were still measured at Day 45 (Fig. 1B). Replicate windrows displayed similar temperature profiles as their paired windrow.

The percent concentration of oxygen in the interior of the normal composting windrow was measured and indicated that microbial oxygen consumption was high from Day 0 through Day 10. On Day 0, the percent oxygen was 1.6 g/kg, and recovered steadily to approximately 20 g/kg at Day 41 (Fig. 2), a level that was maintained through Day 139 (percent gas concentrations beyond Day 116 not shown in Fig. 2). The high initial consumption of oxygen was accompanied by a high evolution of carbon dioxide. The observed percent concentration decline of CO₂ over 10 d complemented the increase in the percent concentration of oxygen. The initial concentration of CO₂ was 24 g/kg, but declined to <5 g/kg by Day 21, a concentration that was maintained throughout the remainder of the study (Fig. 2). The gas concentration data were the same for the clay-amended windrow and each of the replicate windrows.

17 β -Estradiol and testosterone displayed incomplete recovery from spiked compost following a 2-h water extraction. Mean aqueous recovery of [14 C]-spiked 17 β -estradiol from compost was 51.9%, and cumulative recovery increased to 67.6 and 75.5% after a second and third 2-h extraction period (Table 1). The recovery of [14 C]-spiked testosterone under the same conditions was 61.8, 76.7, and 82.9%, respectively. Quantitative recovery of the spiked dose would have yielded an aqueous hormone concentration of 0.0026 and 0.0027 mg/L for 17 β -estradiol and testosterone, respectively. The aqueous solubility of 17 β -estradiol and testosterone is 3.6 and 23.4 mg/L (Mansell et al., 2003), respectively. Therefore, the recovery studies were conducted well below the aqueous solubility limits of the two hormones. Recovery of the spiked radiolabeled hormones from compost was also investigated after lengthening the aqueous extraction time to 24 h and decreasing the hormone concentrations; spikes would have yielded maximal aqueous concentrations of 0.00010 mg/L for 17 β -estradiol and 0.000068 mg/L for testosterone. Neither variable affected the recovery of spiked hormone from the results presented above: 24-h

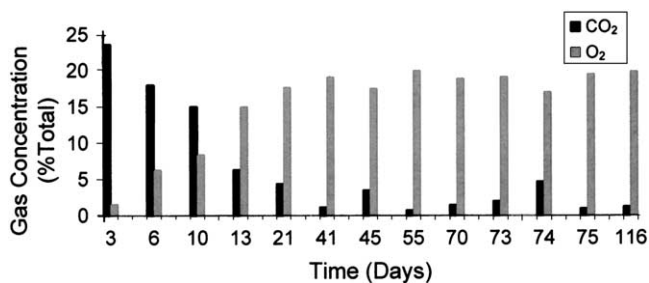


Fig. 2. Average CO₂ and O₂ concentrations (as a percent of total gaseous concentration) from a normal composting chicken layer manure windrow. Means represent three measurements each taken from two windrow core regions and one windrow end region.

Table 1. Percent recovery of spiked [^{14}C]17 β -estradiol or [^{14}C]testosterone (0.33 μg) into an aliquot of Day 0 layer manure compost (414–558 mg). Recoveries were measured following successive water extractions (50 mL \times 3; n = 3) or successive water, methanol, and acetone extractions (50 mL \times 5; n = 5).[†]

Extraction solvent	[^{14}C]17 β -estradiol-spiked compost	[^{14}C]testosterone-spiked compost
	%	
Water #1	51.9 \pm 1.9 \ddagger	61.8 \pm 2.0 \ddagger
Water #2	15.7 \pm 0.7	14.9 \pm 0.3
Water #3	7.9 \pm 0.6	6.2 \pm 0.8
Cumulative	75.5 \pm 0.9	82.9 \pm 2.2
Water	43.4 \pm 3.9	54.5 \pm 1.7
Methanol	54.6 \pm 3.1	55.6 \pm 2.8
Acetone	1.5 \pm 0.4	1.0 \pm 0.7
Cumulative	99.5 \pm 6.5	111.1 \pm 2.5

[†] Values are means \pm SD.

[‡] Replicate, 24-h aqueous extraction recoveries (n = 3): 17 β -estradiol (48.2 \pm 4.8%) and testosterone (59.6 \pm 4.2%). Replicate, low hormone concentration aqueous extraction recoveries (n = 3): 17 β -estradiol (49.1 \pm 4.9%) and testosterone (59.8 \pm 11.1%).

extraction recoveries were 49.1 and 59.8% and low concentration recoveries were 48.2 and 59.6% for 17 β -estradiol and testosterone, respectively (Table 1).

Recovery of [^{14}C]17 β -estradiol and [^{14}C]testosterone from spiked compost following successive 2-h extractions with water, methanol, and acetone yielded incomplete recovery with water for 17 β -estradiol and testosterone, 43.4% and 54.5%, respectively (Table 1). Subsequent extraction of each aliquot with methanol then acetone resulted in an additional 54.6 and 55.6% and 1.5 and 1.0% recovery of the two hormones, respectively.

At the initiation of the field composting study (Day 0), after the layer manure had been diluted with hay, starter compost, leaves, and straw, an estimated average of 83 ng of 17 β -estradiol was extractable with water per gram of compost (dry weight) using the standard extraction conditions (200 mg compost/50 mL water; ng/g). A general decrease in the concentration of water-soluble 17 β -estradiol in the poultry manure compost was observed during the course of the study. The decrease in 17 β -estradiol was modeled with a first-order with time expression:

$$dC/dt = kC \quad [1]$$

where C is the concentration of 17 β -estradiol (ng/g compost dry wt.) and t is time (d). The rate expression was integrated and written in linear form (Eq. [2]):

$$\ln C = \ln C_0 + kt \quad [2]$$

where C is the EIA-measured concentration of 17 β -estradiol at the sampling times, and C_0 is the initial concentration of 17 β -estradiol in the compost. The rate constant k for the decrease in water-soluble 17 β -estradiol was determined by estimating the slope of the best fit for Eq. [2] (Fig. 3A). After 139 d, the estimated rate constant for the decrease in water-soluble 17 β -estradiol in composted chicken manure was $-0.010 \pm 0.001/\text{d}$, and the estimate for the 17 β -estradiol half-life was 69 d. The amount of removable 17 β -estradiol in composted layer manure did not reach 0 ng/g (dry weight) at the conclusion of the 139-d study period. The background levels of removable 17 β -estradiol in the hay, starter compost,

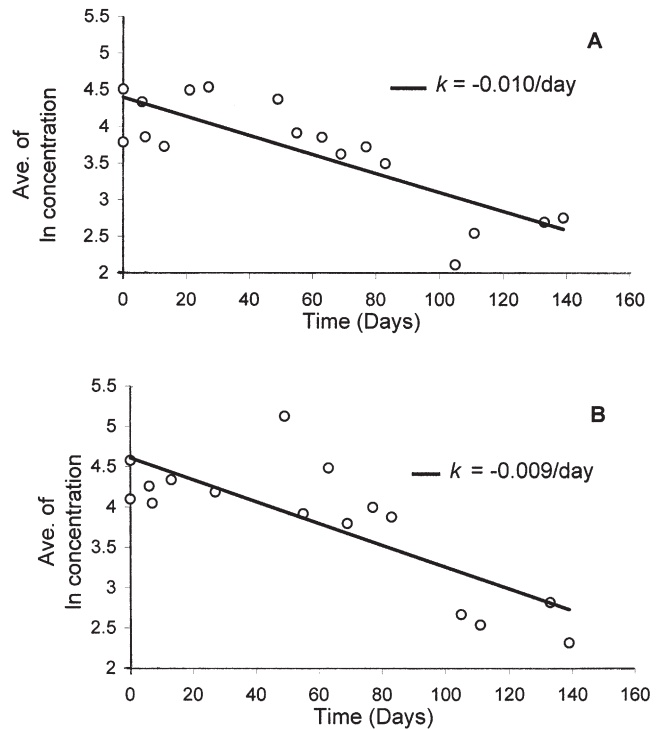


Fig. 3. The average of the natural logarithm (ln) of measured concentration of water-extractable 17 β -estradiol in (A) normal and (B) clay-amended windrows with time during chicken layer manure composting. The decrease in 17 β -estradiol was modeled with a first-order with time expression (Eq. [2]) to yield the degradation rate constants, k .

leaves, straw, and clay on a dry weight basis were 1.5, 0, 1.0, 4.8, and 0 ng/g, respectively.

Similarly, at the initiation of the field composting study, following dilution of layer manure with hay, starter compost, leaves, and straw, an estimated average of 115 ng of testosterone was extractable with water per gram of compost (dry weight) using the standard extraction conditions. The average water-soluble testosterone concentration displayed a general decrease throughout the study period. The concentration of testosterone in the aqueous extract of compost did not reach zero during active composting by EIA analysis. As with 17 β -estradiol, the decrease of testosterone was modeled with a first order with time expression (Fig. 4A). During the 139 d of the study, the estimated rate constant (k) for the decrease in water-soluble testosterone in poultry compost was $-0.015 \pm 0.001/\text{d}$, and the estimate for testosterone half-life was 46 d. The background concentration of water-soluble testosterone in the hay, starter compost, leaves, straw, and clay was 1.7, 0.4, 1.0, 4.8, and 0 ng/g dry wt., respectively.

Christiana clay was added to compost prepared in these studies to meet the needs for use in strawberry fields, where a defined topsoil texture is required. The addition of one part clay to the compost mixture resulted in approximately the same estimated rate of decline of water-soluble 17 β -estradiol or testosterone during composting. The rate constant k for the decrease in water-soluble 17 β -estradiol with time was $-0.009 \pm 0.002/\text{d}$ (Fig. 3B). The rate constant k for the decrease in water-soluble

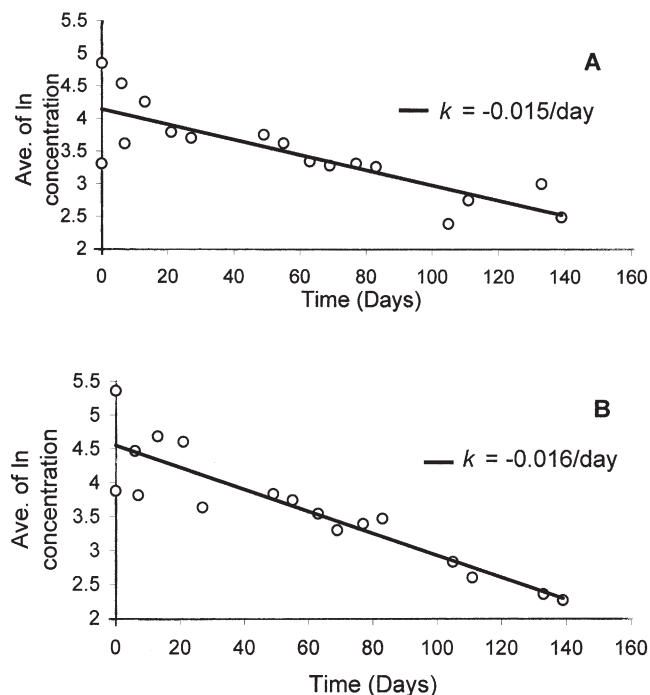


Fig. 4. The average of the natural logarithm (ln) of measured concentration of water-extractable testosterone in (A) normal and (B) clay-amended windrows with time during chicken layer manure compost. The decrease in testosterone was modeled with a first-order with time expression (Eq. [2]) to yield the degradation rate constants, k .

testosterone was $-0.016 \pm 0.001/\text{d}$ for compost containing clay (Fig. 4B). These rate constants were not statistically different from the values obtained from the decrease in water-soluble hormones in normal compost (Fig. 3A and 4A). Half-life estimates for 17 β -estradiol and testosterone in clay-amended compost were 77 and 43 d, respectively.

DISCUSSION

The results of the present study demonstrated that aerobic composting reduced the amount of 17 β -estradiol and testosterone that could be extracted with water from poultry manure compost. Initial, average concentrations of 17 β -estradiol and testosterone in water extracts of compost from replicate windrows were 83 and 115 ng/g compost dry wt., respectively, and diminished to 13 and 11 ng/g by Day 139 (Fig. 3 and 4). This represents an 84 and 90% reduction, respectively, in the water-soluble levels of these potent hormones during aerobic composting. However, composting of poultry manure for 139 d was insufficient to produce compost in which no 17 β -estradiol or testosterone could be detected in water-soluble extracts under the standardized conditions selected. The impact of these results is that water-soluble levels of hormones can be reduced during composting, but not completely eliminated.

Many factors can influence the composting process itself, and the methods and feedstock presently used may make the present report unique; however, the same is true of any composting operation. Therefore, the pres-

ent results have to be understood within the constraints we selected. Fully aerated conditions must be maintained continuously to avoid the onset of anaerobic conditions, which will lead to degradation of organic materials by a different, slower pathway (Rynk, 1992). Moisture content must also be maintained between 40 and 65% to maintain thermophilic degradation. In addition, C to N ratios of 25:1 to 40:1 are needed to provide an adequate feed source for the microorganisms. Porosity and particle size of the composting material can affect the degradation of organic materials by limiting air movement and providing too small a surface area for decomposition.

Very little data has been produced discussing the fate or transport of excreted steroid hormones following their field application in manure. A previous report indicated that soil bacteria are incapable of degrading 17 β -estradiol (Zondek and Sulman, 1943). Although not within the scope of the present study, it can be suggested, based on the investigations of others, that the chemical fate of 17 β -estradiol and testosterone during aerobic composting was either to extractable or non-extractable products, or the complete mineralization to carbon dioxide. Extractable products could include the parent compound or its metabolites. Non-extractable products may have been the result of covalent bond formation, sequestration, or hydrophobic partitioning with the matrix (Gevao et al., 2000), and would have resulted in the incorporation of the hormones into humic substances.

Many organic compounds have been studied under aerobic composting conditions. Aromatic compounds are seldom used as a sole source of energy by microorganisms found in compost. Therefore, these substances are often partially degraded to water-soluble metabolites rather than fully oxidized to carbon dioxide (Sims and Overcash, 1983). Under composting conditions, the partial degradation of aromatic explosives (Williams and Myler, 1990; Griest et al., 1993; Kaplan and Kaplan, 1982), polycyclic aromatic hydrocarbons (Hogan et al., 1988), Arochlor 1232 (Hogan et al., 1988), chlorophenols (Benoit and Barriuso, 1995; Michel et al., 1995), and persistent pesticides (Lemmon and Pylypiw, 1992) has been demonstrated.

The aromatic steroid hormone 17 β -estradiol has on some occasions been reported to be environmentally persistent (Shore et al., 1993), and at other times, readily degraded (Ternes et al., 1999a). Where degradation has been studied, it appears that transformation occurs readily on the D-ring (Fig. 5), and that A-ring conversions are more difficult. Most studies on 17 β -estradiol transformation report on the facile formation of estrone (hydroxyl on D-ring oxidized to ketone; Fig. 5). Colucci et al. (2001) has shown that 17 β -estradiol can be readily oxidized to estrone in agricultural soil. This conversion was shown to occur in both native and autoclaved soil, suggesting an abiotic cause. Any further metabolism of estrone required microbial action. Lee and Liu (2002) demonstrated that 17 β -estradiol could be rapidly oxidized (22 h) to estrone by aerobic degradation with an estradiol-degrading bacterial culture. Lee et al. (2003)

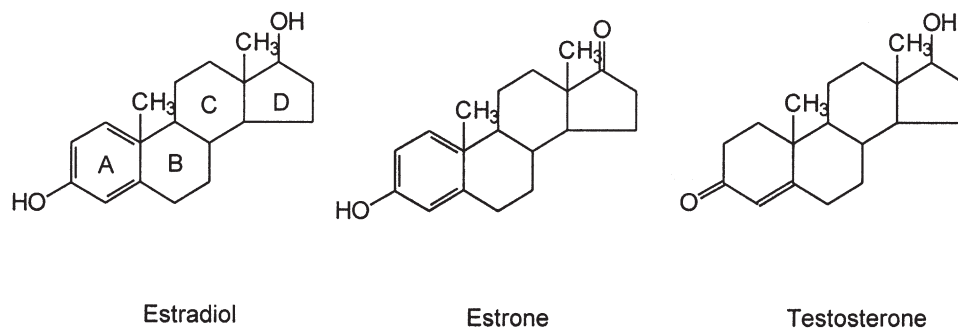


Fig. 5. Chemical structures of 17 β -estradiol, estrone, and testosterone. 17 β -Estradiol and testosterone are excreted in chicken layer manure, and estrone is a prominent metabolite of 17 β -estradiol.

also showed in soil batch equilibrium studies that the major transformation compound of 17 β -estradiol was estrone. Other metabolites of the microbial degradation of 17 β -estradiol are estriol, 16 α -hydroxyestrone, 2-methoxyestradiol, and 2-methoxyestrone (Lee and Liu, 2002). A-ring catechols of 17 β -estradiol were observed in rat hepatocytes (Rathahao et al., 2000), which can undergo further oxidation to quinones, highly reactive electrophilic compounds known to react with DNA to form adducts.

Testosterone (Fig. 5) metabolism has been studied in the estuarine mysid *Neomysis integer* (Verslycke et al., 2002). Eleven metabolites were identified following exposure in the aqueous medium; nine were characterized by liquid chromatography (LC)-mass spectrometry (MS) as monohydroxylated metabolites (2 α , 6 α , 6 β , 7 α , 11 α , 11 β , 15 α , 16 α , and 16 β). The gram-negative bacteria *Comamonas testosteroni* could metabolize testosterone as the sole carbon source (Horinouchi et al., 2001). The bacteria use dehydrogenation, desaturation, hydroxylation, and meta-cleavage reactions to accomplish the degradation of testosterone. Intermediates in the pathway are 4-androstene-3,17-dione, 1,4-androstadiene-3,17-dione, 3-hydroxy-9,10-secoandrosta-1,3,5(10)-triene-9,17-dione, and 3,4-dihydroxy-9,10-secoandrosta-1,3,5(10)-triene-9,17-dione. In soil systems (Lee et al., 2003), the dissipation of testosterone yielded only two compounds: androstenedione (confirmed by gas chromatography [GC]-MS) and androsta-4-ene-3-one-16,17-diol (suspected).

Non-extractable residues of 17 β -estradiol and testosterone may also have formed during the process of aerobic composting. This commonly occurs when organic compounds enter soil and form tight complexes with the humic substances of soil (i.e., humic acid, fulvic acid, and humin). Humic acids are complex aromatic macromolecules containing amino acid, amino sugar, peptide, and aliphatic monomeric units and which are insoluble under acid conditions but soluble at higher pH. Fulvic acids are of lower molecular weight than humic acids, water soluble at all pH values, and contain a high proportion of oxygen-bearing polar functional groups. Humin is that portion of soil and/or compost that is not soluble at any pH and has a very high molecular weight (approximately 300 000). It has been demonstrated that metabolism of organic substances to hydroxylated metabolites was followed by covalent coupling to humic

and fulvic acids in soil (Richnow et al., 1994; Calderbank, 1989). This led to long-term immobilization, but not the complete destruction of these compounds. Non-extractable organic compounds, when bound to humic substances, tend to lose their inherent biological activity (Calderbank, 1989). The presence of non-extractable residues of 17 β -estradiol and testosterone in compost should be investigated and quantitated in future studies by utilizing radiolabeled precursors and determining complete mass balances.

Despite the putative degradation of both hormones via composting, a critical issue requiring additional study is whether a lack of full degradation or the possible formation of non-extractable metabolites is a satisfactory endpoint for these hormones. Colucci et al. (2001) demonstrated a rapid oxidation of 17 β -estradiol to estrone in loam, silt loam, and sandy loam soils. Facile conversions of 17 β -estradiol to estrone have been observed by other researchers (Ternes et al., 1999a; Raman et al., 2001). Estrone is also estrogenic, although in a yeast estrogenicity assay, it is only one-half as potent as 17 β -estradiol (Colucci et al., 2001).

The measured rate of decline in the water extractability of testosterone from normal composted layer manure over 139 d in the present study was approximately 50% faster than that observed with 17 β -estradiol ($k = -0.015/\text{d}$ and $-0.010/\text{d}$, respectively; Fig. 3A and 4A), although the difference was shown not to be statistically significant. The relative rate of degradation of testosterone has been previously compared with 17 β -estradiol and was shown to be more rapid, perhaps due to microbial resistance in degrading the aromatic A-ring of estradiol. The removal and mineralization of 17 β -estradiol and testosterone from the aqueous phase of a municipal wastewater treatment plant (WWTP) were measured, and it was reported that testosterone was degraded approximately two times faster than 17 β -estradiol (Layton et al., 2000). Adaptation of the bacteria to the hormone was reported to be an important factor in the mineralization of 17 β -estradiol and testosterone. Municipal WWTP microbial populations mineralized 17 β -estradiol by 84% over 72 h, while bacteria from industrial biosolids only degraded 4% of the 17 β -estradiol under identical conditions. Testosterone degradation was not as affected by bacterial adaptation, in that only slight differences were noted when comparing the two systems, that is, 65 vs. 55%, respectively. Differences in the

degradation of these hormones can also arise from different microbe populations present in differing treatment processes. A Brazilian WWTP reported >99% degradation of 17 β -estradiol in its waste stream, while only 64% of 17 β -estradiol was removed in a German WWTP (Ternes et al., 1999b). Microbe populations may also differ in poultry compost obtained from different regions of the country due to antibiotic administration or other management practices. Additional compost degradation studies with radiolabeled hormones should be performed to assess this potential difference, and to determine the extent to which microbial versus abiotic degradation of 17 β -estradiol and testosterone are occurring.

It was theoretically possible that the compost extracts may have contained compounds that cross-reacted in the immunoassay, which would have resulted in the over-estimation of 17 β -estradiol or testosterone in the aqueous extracts. However, only related steroid hormones would be likely to cross-react with the antibodies contained in the assay kits.

Cumulative recoveries of [¹⁴C] hormones spiked into compost were shown to increase with subsequent aqueous extractions (Table 1). The implication of these results for a field setting is that rain would remove only a portion of the residual hormones in compost due to the variable volume of each rainfall event and to mass losses. These data are agreement with work done with simulated rainfall on fields where poultry litter had been applied (Nichols et al., 1997). Second-storm runoff concentrations of 17 β -estradiol were 66% less than with the first-storm runoff conducted 7 d earlier. The potential environmental impact of the hormone-bearing runoff into surface or ground waters can be reduced when fields were surrounded with grass filter strips (Nichols et al., 1998).

CONCLUSIONS

In summary, this study demonstrated that the endogenous hormones 17 β -estradiol and testosterone, excreted in poultry manure, were extractable into water to a decreasing extent with time while undergoing aerobic, thermophilic composting. This decline was observed to be approximately the same for the two hormones over 139 d. Both hormones could still be detected in aqueous extracts of compost by EIA analyses at the conclusion of the thermophilic and mesophilic periods. The observed decline in the water extractability of the hormones was nearly the same with or without Christiana clay, which is often included as a soil amendment. Composting of layer manure, as an agricultural management tool, may provide an effective and practical means of reducing, but not eliminating, the introduction of these potent hormones into the environment. It was also concluded that, in a field setting, the first rainfall event on fields amended with composted layer manure would not lead to complete removal of residual 17 β -estradiol and testosterone. Rather, the hormone levels in runoff would decrease steadily due to mass losses from each previous rainfall event, rainfall water volume, and/or biological degradation.

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