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# Occurrence and removal of free estrogens, conjugated estrogens, and bisphenol A in manure treatment facilities in East China

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ABSTRACT

The occurrence of four free estrogens, four conjugated estrogens, and bisphenol A (BPA) was investigated in three cow farms, four swine farms, and five chicken farms. The daily total estrogen (free and conjugated) excretions of a cow were 145.23–179.27 μg/d mainly through feces (92%), while swine excreted 42.56–219.25 μg/d of estrogens mainly through urine (98–99%). Estrogen conjugates contributed 14.6–48.8% to the total estrogen excretions in cow feces and more than 98% in swine urine. A chicken excreted 0.66–12.78 μg/d of total estrogens through feces, among which 34.2–100% was contributed by conjugated estrogens. The total estrogen removal efficiencies of manure anaerobic digesters and composters were 14.7–21.8% and less than 70.1%, respectively. Estrogens (E1, 17β-E2, E1-3S, and E2-3S) still existed in treated manure at concentrations up to 2695 ± 181 ng/L (anaerobic digestate) and at contents up to 80.8 ± 6.0 ng/g (compost). BPA was found in feces and compost samples at similar contents (nd–25 ng/g), and approximately 60–70% of BPA was removed in wastewater treatment facilities.



## 1. Introduction

Since the late 1990s, there has been an increasing concern for the presence of endocrine disrupting compounds (EDCs) in the environment, including natural estrogens produced by humans and animals, and synthetic chemicals known as xenoestrogens (Belfroid et al., 1999; Panter et al., 2000). Natural estrogens, such as estrone (E1), 17α-estradiol (17α-E2), 17β-estradiol (17β-E2), and estriol (E3), can reach the environment through discharge from sewage treatment plants (STPs) and livestock manure disposal units (Ternes et al., 1999; Raman et al., 2004; Combalbert et al., 2011). Although estrogens are only detected at the ng/L levels in the aquatic environment, they are the major compounds responsible for the feminization of male fish (Ternes et al., 1999; Andersen et al., 2003; Jobling et al., 2003). As an important source of natural estrogens in the environment, animals excrete different types and amounts of estrogens depending on animal species, sex, age, circadian cycle, and reproductive state (Hanselman et al., 2003; Combalbert and Hernandez-Raquet, 2010). Estrogens are excreted in urine and feces in either biologically active free

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forms or inactive forms conjugated with glucuronide and/or sulfate groups which can deconjugate to free forms (Panter et al., 1999; Hutchins et al., 2007). Previous studies have reported relatively accurate concentrations and daily excretion of free estrogens, while conjugated estrogens have been rarely investigated due to the difficulty in measurement (Hanselman et al., 2003).

Before applied to the farmland as organic fertilizer, fresh excreta are treated by manure treatment facilities. In China, prior to land application, liquid manure is often stored in lagoons or anaerobic ponds to produce biogas, and solid manure is composted in windrows on cement pads or directly stacked on the ground. Some small-scaled livestock farms don't even have proper waste treatment facilities. Previous studies have shown that free estrogens are incompletely removed in lagoons and composting systems (Raman et al., 2004; Hutchins et al., 2007; Gadd et al., 2010; Fine et al., 2003; Combalbert et al., 2011). Consequently, estrogens from livestock waste can reach surrounding groundwater and surface water, through compost applied on the land or surface runoff (Belfroid et al., 1999; Bartelt-Hunt et al., 2011; Dutta et al., 2012). However, up to date, there have been no studies focused on the estrogen conjugates in manure treatment facilities. As conjugated estrogens might be deconjugated into free forms, re-activating estrogenic activity, it is necessary to investigate the occurrence and fate of conjugated estrogens in livestock manure treatment facilities. This requires that it is essential to develop an efficient and accurate method to analyze estrogens and their conjugates in high-matrix samples.

In addition to natural estrogens, synthetic estrogens such as 17α-ethinylestradiol (EE2), used for human contraception purposes, are still detected in wastewater discharged from Chinese concentrated animal feeding operation (CAFO) facilities and nearby receiving rivers (Chen et al., 2010; Liu et al., 2012), despite they have been prohibited in animal-feed additives by the Ministry of Agriculture in China in 1999 (MOA, 1999). Moreover, the estrogenic activity of EE2 is 2.46 times greater than that of E2 (Pillon et al., 2005). Therefore, it is important to identify whether EE2 detected in CAFO wastewater is from animal excreta and can be removed in manure treatment facilities. Other EDCs in animal manure have also been detected, such as bisphenol A (BPA), which may be released from the materials coating the inner surface of food containers or manure-storage tanks (Fromme et al., 2002).

The aims of the present study were (i) to develop a feasible and accurate method to detect E1, 17β-E2, E3, EE2, estrone-3-sulfate sodium salt (E1-3S), 17β-estradiol-3-sulfate sodium salt (E2-3S), estrone-3-glucuronide sodium salt (E1-3G), 17β-estradiol-3-glucuronide sodium salt (E2-3G), and BPA in fresh animal excreta and compost; (ii) to investigate the occurrence of the above nine compounds contained in fresh excreta of livestock; (iii) to calculate the daily estrogen excretion of different animal species; and (iv) to assess the removal of the nine compounds in manure treatment facilities installed in the East China.

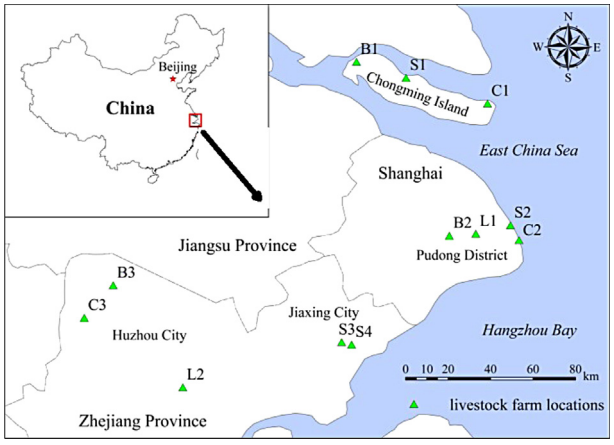


**Fig. 1 – Locations of sampled livestock farms in Shanghai City and Zhejiang Province.**

## 2. Materials and methods

### 2.1. Chemicals and materials

E1, E2, E3, E1-3S, E2-3S, E1-3G, E2-3G, EE2, and BPA were purchased from Sigma–Aldrich, USA, and had purities >98%, which satisfied the experimental requirements. Stock solutions of the nine compounds were prepared in HPLC-grade acetonitrile and were stored at −20 °C. Working standards (1–100 ng/mL) were prepared by serial dilution of the stock solutions with acetonitrile and Milli-Q water (1:1) and were stored at 4 °C.

The organic solvents acetone, acetonitrile (ACN), methanol (MeOH), n-hexane, dichloromethane (DCM), and ethylacetate (EAC) (HPLC grade) as well as anhydrous sodium sulfate and sodium hydroxide (analytical reagent grade) were obtained from Mallinckrodt, USA. The cleanup and extraction of the samples were performed using a Florisil cartridge (500 mg, 6 mL, Agela), Cleanert $NH_2$ cartridge (500 mg, 6 mL, Agela), Oasis HLB cartridge (200 mg, 6 mL, Waters), and nylon filter (0.22 μm, Sigma–Aldrich). Wastewater filtration was performed with GF-F glass-fiber filters (0.7 μm, Whatman). All glassware used in the experiment was soaked in acetone, placed in an ultrasonic bath for 2 h, rinsed with Milli-Q water, and heated at 450 °C for 4 h.

### 2.2. Sampling sites and sample collection

Shanghai City and Zhejiang Province in China were selected as the study area. Samples in Shanghai were collected from two swine farms (S1 and S2), two cattle farms (C1 and C2), two broiler farms (B1 and B2), and one layer farm (L1), as shown in Fig. 1. B1, S1, and C1 are located on Chongming Island. Samples in Zhejiang Province were collected from two swine farms (S3 and S4), two cattle farms (C3 and C4), one broiler farm (B3), and one layer farm (L2), as shown in Fig. 1. Detailed information regarding the sampling areas and CAFOs are listed in the Supplementary Material.

Feces and urine samples were collected immediately after excretion and then added into pre-cleaned 500-mL glass bottles. Three parallel excreta samples were collected from every animal, and three animals were chosen for each animal type and age. Compost, lagoon and anaerobic digestate samples were also collected in pre-cleaned 500-mL glass bottles. A