Science of the Total Environment 494–495 (2014) 58–64

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Potential bioactivity and association of 17β-estradiol with the dissolved and colloidal fractions of manure and soil

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HIGHLIGHTS

- Dissolved and colloidal sized fractions were filtered from swine manure and soil.
- Estrogen preferred the dissolved rather than colloidal fractions of soils and manures.
- Bioassay assessed the bioactivity of estrogens in soil and manure solutions.
- Estrogens remained potentially bioactive in soil and manure dissolved fractions.
- Estrogen associated with colloids was not bioactive.

ARTICLE INFO

Article history:
Received 28 April 2014
Received in revised form 19 June 2014
Accepted 27 June 2014
Available online xxxx

Editor: Adrian Covaci

Keywords:
Colloid
Dissolved
Estrogen
Bioavailability
Soil
Manure

ABSTRACT

The dissolved (DF) and colloidal fractions (CF) of soil and manure play an important role in the environmental fate and transport of steroidal estrogens. The first objective of this study was to quantify the association of 17β-estradiol (E2) with the DF and CF isolated from (i) liquid swine manure (LSM), (ii) a soil:water mixture (soil), and (iii) a LSM:soil:water mixture (Soil + LSM). The appropriate CF and DF size fractions of the Soil, Soil + LSM, and LSM media were obtained by first filtering through a 0.45 μm filter, which provided the combined DF and CF (DF/CF). The DF/CF from the three media was spiked with carbon-14 ([^{14}C]) radiolabeled E2 ([^{14}C]-E2), and then ultrafiltered to isolate the CF (<0.45 μm and >1 kDa) from the DF (<1 kDa). The average recoveries of the [^{14}C] associated with the DF were 67%–72%, 67%–79%, and 76%–78% for the Soil, Soil + LSM and LSM, respectively. For the CF that was retained on the 1 kDa filter, organic carbon and [^{14}C]-E2 were dislodged with subsequent water rinses the Soil + LSM and LSM, but not the Soil. The second objective was to evaluate whether the E2 associated with the various fractions of the different media could still bind the estrogen receptor using an E2 receptor (17β-ER) competitor assay, which allowed E2 equivalent concentrations to be determined. The estrogen receptor assay results indicated that E2 present in the DF of the Soil and Soil + LSM solutions could still bind the estrogen receptor. Results from this study indicated that E2 preferentially associated with the DF of soil and manure, which may enhance its dissolved advective transport in surface and subsurface water. Furthermore, this study indicated that E2 associated with DF solutions in the environment could potentially induce endocrine responses through its interactions with estrogen receptor.

1. Introduction

Endocrine disrupting chemicals (EDC) mimic endogenous hormones, interacting with hormone receptors, and altering the natural pattern of hormone synthesis and/or metabolism (Mills and Chichester, 2005). Endocrine disrupting chemicals include a variety of synthetic organic chemicals and naturally occurring estrogenic hormones, such as E2, estrone (E1), and estriol (E3) (Jiang et al., 2005). Numerous reports have implicated EDCs in the cause of abnormal physiological development, altered reproductive capabilities, and

Abbreviations: [^{14}C], carbon-14 radiolabel; [^{14}C-E2, radiolabeled 17β-estradiol; 17β-ER, 17β-estradiol receptor; CF, colloidal size fraction (<0.45 μm and >1 kDa); DF, dissolved size fraction (<1 kDa); DF/CF, dissolved and colloidal size fractions together (<0.45 μm); E1, estrone; E2, 17β-estradiol; E3, estriol; EDC, endocrine disrupting compound; EEQ, 17β-estradiol equivalent concentration; FP, fluorescence polarization; LSC, liquid scintillation counter; LSM, liquid swine manure; TLC, thin layer chromatography; TOC, total organic carbon.

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http://dx.doi.org/10.1016/j.scitotenv.2014.06.121
0048-9697/

abnormal behaviors of various organisms, especially in aquatic organisms (Trudeau and Tyler, 2007).

The fate and transport of estrogenic compounds are potentially influenced by their strong associations with dissolved and colloidal fractions of soil and manures (Bowman et al., 2002; Holbrook et al., 2004; Lee et al., 2011a; Zhao et al., 2010; Zhou et al., 2007). For example, Thompson et al. (2009) frequently detected E2 in soil leachate and shallow ground water beneath plots that received swine manure (*Sus scrofa domesticus*), and found E2 concentrations were significantly correlated to the DF and CF size fractions (≤0.45 μm) of total organic carbon (Thompson et al., 2009). Furthermore, in wastewater, 60% of the aqueous E2 was estimated to be associated with the CF, suggesting preferential association of E2 to the CF (Holbrook et al., 2004). Additionally, soil batch sorption experiments revealed that aqueous layer E2 persistence was greater and sorption to soil decreased in the presence of liquid swine manure (Zitnick et al., 2011).

Multiple EDCs are suspected of mimicking natural hormones by binding to estrogen receptors either as agonists or antagonists, thereby disrupting endocrine systems. It is possible to assess the binding affinity of a chemical to the estrogen receptor using in-vitro assays (Lee et al., 2011a). In-vitro assays are used to assess and identify EDCs from human activities and intensive farming practices (Rodriguez-Mozaz et al., 2004). These assays provide an efficient and cost effective way to evaluate the potential of EDCs to produce an estrogenic response by quantifying estrogen receptor binding; however, they cannot mimic whole animal uptake, distribution, and metabolism in an organism (Cespedes et al., 2004; Petrovic et al., 2004). Nonetheless, in-vitro assays are effective for initial screening and provide a rough estimate of the estrogenic potential (Li et al., 2012).

Although natural dissolved and colloidal fractions play an important role in the fate and transport of estrogens in the environment, little is known about how these fractions influence the bioavailability of estrogenic EDCs. The objectives of this study were (i) to quantify the association of E2, a naturally-occurring EDC, to soil- and manure-derived dissolved and colloidal fractions; and (ii) to estimate the bioactivity of E2 associated with DF and CF. Soil and LSM samples used in the study were preserved to minimize complications associated with E2 transformation in biologically active systems. Estrogen receptor-β competitor binding assays were used to quantify the potential affinity of the ligand (either as free E2, or as E2 associated with DF/CF) to bind to the estrogen receptor, providing an estimate of bioactivity.

2. Materials and methods

An overview of the experimental procedures and analytical methods used to achieve the two study objectives is provided in Fig. 1.

2.1. Soil and liquid swine manure sample preparation and characterization

The LSM was obtained from a facility that housed approximately 4000 animals, which included swine at all stages of development (Shelver et al., 2010). The manure slurry was initially filtered using cheesecloth that was folded to create 10 layers (~3 mm thick), and then filtered using #2, Qualitative filter paper (Whatman International Ltd., Maidstone, England) under vacuum to remove any large debris. The DF/CF fraction was obtained by filtering the LSM through a 0.45 μm nitrocellulose filter (Millipore Ltd., Co. Cork, Ireland). The manure slurry was characterized for chemical and physical parameters by Servi-Tech Laboratories (Hastings, NE) using their lagoon analysis method (Table 1). Background concentrations of E2 in the DF/CF of the LSM were quantified by liquid chromatography tandem mass spectrometry. Chromatography (Alliance 2695 Separation Model; Waters, Beverly, MA) and spectrometry are described by Shappell et al. (2008) and use a Waters Ultima API-US quadrupole-time-of-flight mass spectrometer in negative ion-electrospray ionization mode. The LSM DF/CF samples used in further analyses were preserved with 0.02% sodium azide, which inhibit microbial growth (Lichstein and Soule, 1944). Sodium azide was used as the preservative because it did not interfere with the 17β-ER competitor assay compared to other preservatives tested that included mercurous chloride (0.005 mg/mL) and formaldehyde (2.5%).

The Soil DF and CF were extracted from the surface (0–15 cm) of a Ulen soil series (Sandy, mixed, frigid Aeric Calciaquolls), which was obtained near Embden, North Dakota, USA. Soil particle size distribution was determined using a hydrometer method (Gee and Bauder, 1979), organic matter content was determined by loss on ignition, pH (Table 2) and electrical conductivity were determined using saturated paste and ion selective electrode, and cation exchange capacity was determined using methods described in Burt (2004) (Table 3).

The soil was first dried and passed through a 2 mm sieve before obtaining the DF and CF fractions. Air-dried soil and deionized water was then mixed at a ratio of 1:2 (15 g soil: 30 ml deionized water), and tumbled, end-over-end, at approximately 110 rpm for 30 min. After tumbling, the mixture was filtered using a 0.45 μm filter paper. The filtrate was then preserved with 0.02% sodium azide. The Soil + LSM media was processed the same way, except a 2% solution of LSM and deionized water (0.6 mL of LSM: 29.4 mL of deionized water) was used with 15 g of soil. The 2% LSM solution was based on the recommended soil application rates of LSM for N and P requirements (Hernandez and Schmitt, 2012), which was 12.2 L m^{-2} for a depth of 15 cm.

2.2. Association of [^{14}C]-17β-estradiol with colloidal and dissolved fractions

Size segregation between DF and CF was based on other studies (Holbrook et al., 2004; Zitnick et al., 2011), where the CF was defined as <0.45 μm but retained by a 1 kDa filter, and anything passing through a 1 kDa filter was considered DF. The 1 kDa (~0.05 μm pores) ultrafiltration disk membranes (cellulose, 25 mm, Millipore Corporation, Billerica, MA) were used with a 3 mL ultrafiltration unit (Amicon Model 8003 stirred cell apparatus; Millipore Corporation, Bedford, MA). Samples of LSM, Soil, and Soil + LSM media were spiked with two concentrations of [^{14}C]-E2 referred to as high and low dose (Table 4) before filtering.

Prior to filtration, all filters were washed to remove the manufacturer's glycerin preservative according to Holbrook et al. (2005). Sample volumes of 3 mL were filtered by applying pressure (65 kPa nitrogen) to the ultrafiltration unit for approximately 1 h or until filtered to dryness. After ultrafiltration, three 100 μL aliquots were assayed for total organic carbon (TOC) and [^{14}C]. The TOC was determined using a Total Organic Carbon Analyzer (Shimadzu Corporation, Japan) for all medias. The [^{14}C] was assayed by liquid scintillation counting (LSC) for 10 min using a Packard 2300 TR scintillation analyzer (Meriden, CT). Aliquots (100 μL) for LSC analysis were placed in 6 mL LSC vials with 4 mL of EcoLite™ scintillation cocktail (MP Biomedicals, LLC, Solon, OH). Blanks were also assayed using LSC for each sample set to account for background radioactivity. The ultrafiltation unit was then carefully dismantled, and using forceps, the filter was removed for quantification of the TOC and [^{14}C] of the CF. The [^{14}C] retained on the filter was determined using a combustion analyzer (Packard Model 307; Downers Grove, IL) after air-drying for 24 h (Zitnick et al., 2011). Ultrafiltration was replicated three times for all samples. Between each run, the ultrafiltration unit was dismantled, rinsed with methanol, washed with a 1% solution of Liquinox detergent (Alconox, Jersey City, NJ), and then rinsed 3 consecutive times with deionized water.

After the initial ultrafiltration of all three media, the filtration experiments were repeated and this time the filters containing the CF were rinsed three additional times with nanopure water (E-pure Ultrapure; Barnstead/Thermolyne, Dubuque, IA) to assess whether the TOC and/or the [^{14}C]-E2 could be dislodged from the particles retained on the filter. Additionally, non-specific binding of radioactivity to the apparatus and filters was assessed by filtering blank solutions. The blank solutions