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Changes in estrogen/anti-estrogen activities in ponded secondary effluent

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Abstract

Total estrogenic activity, measured using the yeast estrogen screen reporter gene bioassay, decreased from 60 pM (equivalent 17α-ethinylestradiol concentration) to an estimated 1.4 pM during a 24-hour period in which secondary effluent was held in a shallow infiltration basin. Over the same period, anti-estrogenic activity, measured as an equivalent concentration of tamoxifen, increased from 35 to 260 nM, suggesting that antagonists produced during secondary effluent storage played a role in the apparent loss of estrogenic activity. Androgenic activity, measured over the same 24-hour period using the yeast androgen screen, was near or below the method detection limit (0.7 pM as testosterone). However, the same pond samples were clearly anti-androgenic. When whole-sample extracts were separated via adsorption and stepwise elution in alcohol/water solutions consisting of 20, 40 and 100% ethanol, the sum of estrogenic activities in derived fractions was always lower than the measured estrogenic activity in the whole-sample extracts. Summed anti-estrogenic activities in the same fractions, however, always exceeded values for corresponding whole-sample extracts. Results reinforce the importance of sample preparation steps (concentration of organics followed by estrogen/anti-estrogen separation) when measuring endocrine-related activities in chemically complex samples such as wastewater effluent. The potential complexity of relationships among estrogens, anti-estrogens and matrix organics suggests that additive models are of questionable validity for estimating whole-sample estrogenic activity from measurements involving sample fractions.

Keywords: Yeast estrogen screen; Estrogens; Anti-estrogens; Androgens; Anti-androgens; Wastewater treatment

1. Introduction

Estrogens and estrogen mimics such as 17β-estradiol (E_2), 17α-ethinylestradiol (EE_2), estrone (E_1), estriol (E_3); nonylphenol, and octylphenol (Table 1) are present in all municipal wastewaters and survive, to a degree, during conventional wastewater treatment (D'Ascenzo et al., 2003; Desbrow et al., 1998; Lee and Peart, 1998; Snyder et al., 1999). Each compound was present in a significant fraction of samples taken in the recent USGS survey of United States surface waters (Kolpin et al., 2002). These chemicals are probably responsible for elevated incidence of intersex characteristics in freshwater fish (Colborn et al., 1993; Gray and Metcalfe, 1997; Jobling and Sumpter, 1993; Jobling et al., 1998; Routledge et al., 1998; Tyler et al., 1998).

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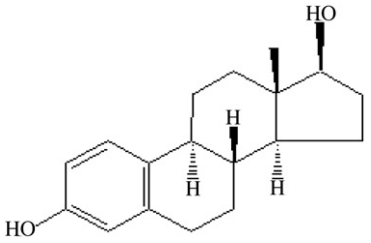
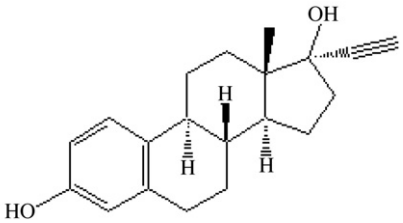
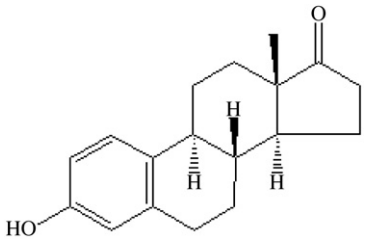
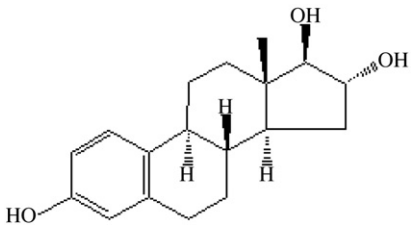
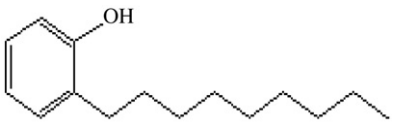
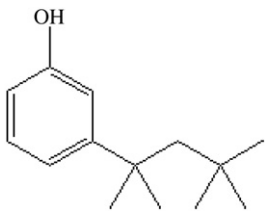
Table 1
Structures and properties of wastewater compounds with estrogenic behavior

Chemical	Structure	Molecular weight	Log K_{ow} [a]	Relative estrogenic activity (YES bioassay)
17β-estradiol (E_2)		272.39	3.94	1.0
17α-ethinyl estradiol (EE_2)		296.40	4.15	1.32
Estrone (E_1)		270.37	3.43	0.20
Estriol (E_3)		288.39	2.81	0.01
Nonylphenol (NP)		220.35	4.48 [b]	2×10^{-4}
Octylphenol (OP)		206.33	4.12 [b]	3×10^{-4}

[a] Lai et al. (2000).
[b] Ahel and Giger (1993).