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# Assessment of environmental estrogens and the intersex/sex reversal capacity for chinook salmon (*Oncorhynchus tshawytscha*) in primary and final municipal wastewater effluents

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**Abstract**

A trickling filter/solid contact (TF/SC) biological secondary treatment plant with chlorine disinfection serving a suburban population of 740,000 was assessed for environmental estrogens. Weekly grab samples were taken at established sampling points and analyzed for various pertinent environmental estrogens including industrial chemicals, and natural and synthetic steroidal estrogens. Additionally, human estrogen receptor (hER) activity and capacity to elicit intersex/sex reversal for the wastewater was monitored using a recombinant yeast assay and whole fish exposures, respectively. hER activity levels varied from 76 to 106 ng/L E2 equivalents in the primary effluent, and were reduced by 25% by biological treatment. For the primary and final effluent no evidence of sex reversal or intersex was apparent in any of the treatment groups (1%, 3%, 10%, or 100%) based on genetic sex determinations and histological examination of the gonads in alevin from 28 d exposed chinook salmon (*Oncorhynchus tshawytscha*) eggs.


*Keywords:* Environmental estrogens; *Oncorhynchus tshawytscha*; Intersex/sex reversal

## 1. Introduction

Environmental estrogens are a subset of chemicals which make up a group of environmentally important compounds known as endocrine disrupting compounds (EDCs) and include industrial chemicals, natural and synthetic steroidal estrogens, and various naturally occurring phytoestrogens (Johnson and Sumpter, 2001; Kolpin et al., 2002). One of the largest bodies of evidence illustrating endocrine disruption in wild organisms due to an anthropogenic stressor is the scientific literature reporting reproductive effects in several fish species exposed to municipal wastewater effluents and pulp/paper industry discharges (Denton et al., 1985; Jobling et al., 1998; reviewed in CSTEE, 1999). In North America, Nagler and colleagues (Nagler et al., 2001; Chowen and Nagler, 2004) found a high proportion of physiological female chinook salmon from three naturally spawning populations in the Columbia River tested positive for a male-specific DNA marker. From these results, the authors hypothesized that endocrine disruption may be playing a role in the sex determination of these fish. Such sex reversal effects have not been observed in British Columbia populations (Devlin et al., 2005), but laboratory experiments have provided some evidence for sex reversal in this species arising from exposure to municipal and industrial effluents (Afonso et al., 2002). Thus, a possible mechanism for sex reversal of genetically male salmon involves exposure to environmental estrogens. Domestic and industrial wastewater are well known primary point-source inputs for many contaminants of concern including EDCs in aquatic environments (Liu and Lipták, 1999).

The compounds shown in Fig. 1 represent typical environmental estrogens which have been identified as likely culprits of reproductive disruption in aquatic organisms in the vicinity of wastewater discharge zones. Nonylphenol (NP) the ultimate breakdown product of non-ionic surfactants nonylphenol ethoxylates (NPEOs) used in domestic and industrial soaps and detergents has

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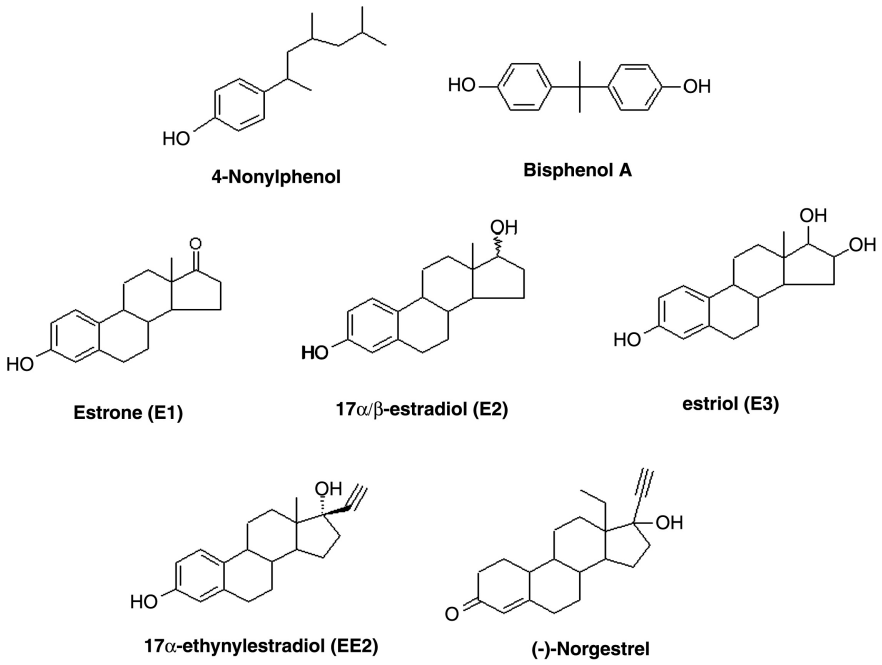


Fig. 1. Structures and common names for typical environmental estrogens found in domestic wastewater.

shown to be estrogenic via *in vitro* yeast assays, rat uterotrophic assay and exposed fish (Routledge and Sumpter, 1996; Odum et al., 1997; Gray and Metcalfe, 1997). Bisphenol A (BPA) is an environmentally ubiquitous industrial chemical used to make plastic/epoxy resins and other products and a known endocrine disruptor (Krishnan et al., 1993). BPA has shown to be acutely toxic to freshwater and marine aquatic organisms at concentrations of 1–10 mg/L (Alexander et al., 1988). However, it is the sublethal toxicity of BPA which is of greater environmental concern. BPA has been shown to induce ovotestes at concentrations as low as 10 μg/L in Japanese medaka (*Oryzias latipes*) (Metcalfe et al., 2001) and inhibit the development of secondary sexual characteristics in swordtails (*Xiphophorus helleri*) over a concentration range from 0.2 to 20 μg/L (Kwak et al., 2001). Synthetic and biogenic steroidal estrogens in sewage are also of concern due to their high potency and thus capacity to cause adverse effects to the reproductive status of exposed wildlife. All three endogenous female estrogens, estrone (E1), 17β-estradiol (E2) and estriol (E3) (see Fig. 1) along with commonly used synthetic estrogens in birth control formulations such as 17α-ethynylestradiol (see Fig. 1) have all been shown to contribute significantly to the estrogenicity of wastewater effluents (Ternes et al., 1999).

In this work, the concentrations of these important environmental estrogens are assessed using a gas chromatography–high resolution mass spectrometry (GC–HRMS) based method. Additionally, in order to capture any estrogens which may not have been included in our list of targets, estrogenic activity was assessed using an *in vitro* recombinant yeast assay (RYA). These analytical techniques were applied to primary and final effluents for one of the largest trickling filtration solid contact (TF/SC) municipal wastewater treatment plants in North America. The TF/SC plant features conventional primary settling tanks, a hybridized trickling filter–activated sludge biological reactor followed by conventional secondary settling and finally tertiary chlorine disinfection. The final effluent with BOD and TSS of ~12 mg/L is discharged into the Fraser River which is frequented by Pacific salmon including chinook salmon runs. Additionally, during periods of high plant influent flows (i.e. large rain events), it is possible that primary effluent would be bypassed and discharged directly into the river. To determine if estrogens in effluent from the TF/SC plant could alter sex determination in salmon chinook salmon eggs were exposed to several dilutions of the wastewater, including environmentally realistic concentrations, from the eyed stage of development to 28-days posthatch.

## 2. Materials and methods

### 2.1. Sampling

The trickling filter/solid contact (TF/SC) biological secondary treatment with chlorine disinfection had a daily average flow of 455 ML per day (MLD), mean hydraulic retention time of 0.3–2 h (bioreactor) and served a suburban population of 740,000. The TF/SC process consists of a high rate attached growth reactor (trickling filter) followed by a short solids residence time aerobic suspended growth reactor (solid contact tank) for the purpose of improving the settling characteristics of biomass wasted from trickling filters as well as providing additional oxidation of carbonaceous organic material (Slezak et al., 1998). Four liter grab samples were taken in the morning at established sampling points (by plant personnel) to provide enough material for chemical, *in vitro* and *in vivo* experiments. The TF/SC plant was sampled weekly from established sampling points directly downstream of the primary sedimentation process and from the final plant effluent each Tuesday for eight weeks from December to February, 2002–03. The weekly effluent samples were split, where a fraction of the sample went for chemical and *in vitro* analysis and a much larger fraction remained in a cold room and was used in the static renewal experiments. In an attempt to minimize the impact of on-going biological and physical (i.e. UV) degradation in the samples, same-day sample collection and extraction was applied whenever possible (however due to shipping time longer delays of up to 48 h may have occurred during which samples were stored on ice). Finally, aliquots of each sample were frozen at −20 °C unpreserved, whereas the bulk (~3 L) was preserved with 5% formalin (chemistry samples only) and stored at 4–6 °C for archive purposes.