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Screening estrogenic oxidized by-products by combining ER binding and ultrafiltration

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Abstract

Ozonation and chlorination of 17β-estradiol (E2), 17α-ethynylestradiol (EE2), bisphenol A (BPA), and nonylphenol (NP) were performed to evaluate the estrogenic activity of the by-products of these endocrine disrupting chemicals (EDCs). After 15 min oxidation, samples were extracted using solid phase extraction (SPE) cartridges, and tested in vitro to measure the estrogenic activities of the oxidized products. MCF-7 cell proliferation assay showed that chlorinated BPA solution displayed slightly stronger estrogenicity than BPA, while chlorinated NP retained about one-tenth of its bioactivity. The estrogenic mono-, di-, tri-, and tetra-ClBPAs and di-ClNP were screened out from the corresponding chlorinated products by a combined application of estrogen receptor (ER) binding with ultrafiltration and identified by high performance liquid chromatography coupled with mass spectrometry (LC/MS). Ozonation of the above four estrogens and chlorination of E2 and EE2 significantly decreased their estrogenic activities under the applied conditions.
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Keywords: Ozonation; Chlorination; Estrogens; ER binding; MCF-7 cell proliferation; LC/MS

1. Introduction

Endocrine disrupters are compounds known to impact the endocrine system of humans and animals. Reproductive abnormalities were reported in fishes living downstream of wastewater treatment plants in the 1990s (Purdom et al., 1994; Harries et al., 1996; Jobling et al., 1998). Since then, endocrine disrupting compounds (EDCs) have been detected globally in surface waters (Solé et al., 2000; Kolpin et al., 2002; William et al., 2003). Steroid estrogens, especially the endogenous 17β-estradiol (E2) and synthetic birth control pharmaceutical 17α-ethynylestradiol (EE2), have been determined to be the most potent EDCs in a series of in vitro assays (Desbrow et al., 1998; Snyder et al., 2001, 2003a). They were shown to induce measurable changes in fish reproduction at concentrations as low as 2 ng/l (Arcand-Hoy et al., 1998; Kramer et al., 1998; Routledge et al., 1998). Xenoestrogens bisphenol A (BPA) and 4-nonylphenol (NP) have also drawn similar attention because of their high concentrations in wastewater, though the estrogenic potencies are much lower than E2 and EE2 (Kuch and Ballschmiter, 2001; Meesters and Schröder, 2002; Han et al., 2002; Kolpin et al., 2002). BPA is a widely used monomer for epoxy resins and polycarbons, while NP is a metabolite formed in the biological wastewater treatment process from the surfactant nonylphenolethoxylates (NPEOx). Additional information related to endocrine disrupters in waters is available in a recent review (Snyder et al., 2003a).

Domestic and industrial inputs to wastewater treatment plants are important sources of natural and synthetic estrogens. Recent studies have shown evidence of endocrine disrupter removal during wastewater treatment by comparing their influent–effluent levels at wastewater treatment facilities (Leganà et al., 2000; Baronti et al., 2000; Meesters and Schröder, 2002; Petrovic et al., 2003; Snyder et al., 2003b). However, measurement based solely on the presence or absence of the primary chemical structures cannot always ensure the removal of biological activity of the EDCs since the

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reactive functional groups in some of these compounds may be transformed under conditions of chlorination and ozonation employed for disinfection in water and wastewater treatment plants (Snyder et al., 2003a). For example, mono-, di-, tri-, and tetra-chlorinated BPAs have been shown to possess estrogenic activity similar to or greater than BPA itself (Fukazawa et al., 2002; Kuruto-Niwa et al., 2002). Therefore, it is necessary to conduct chemical analysis in conjunction with bioassay testing to evaluate the estrogenic activity of post disinfection products. To date, only a few such studies have been performed.

The number and structure of chlorination products of estrogens depend on the reaction time and conditions. The products may be substituted, oxidized, or degraded depending upon the reaction period. Estrogenic activities of E2, BPA, and NP have been reported to be completely eliminated due to structural degradation after 24 h chlorination (Lee et al., 2004). But within 1 h reaction, the chlorinated E2 solution was reported to show similar, though slightly lower, estrogenic activity to that before chlorination (Hu et al., 2003). Unfortunately, the corresponding bioactive products formed in the reaction solution were not identified. Thus, it is essential to perform further investigations to identify the estrogenic oxidized by-products.

Estrogens regulate the growth, differentiation, and function of target tissues by binding to the nuclear estrogen receptor (ER). After dimerization, the estrogen/ER complex mediates estrogen response elements (EREs) in the estrogen response genes, modulates the transcription of genes, and consequently stimulates cell growth and differentiation (Gaido et al., 1999). The MCF-7 is a breast cancer cell line that is commonly used for in vitro testing of estrogenic activity of samples. The measured targets could be either proteins or genes that are stimulated by ER activation (Soto et al., 1995; Korach and McLachlan, 1995; Kuruto-Niwa et al., 2002).

MCF-7 cell proliferation assay was applied here to measure the possible estrogenic activity of by-products resulting from bench ozonation and chlorination of E2, EE2, BPA, and NP (see structures in Fig. 1). Because this assay gives only an integral result of a sample and cannot indicate specific compounds in the mixture that are causing the estrogenic activity, a combination of ER binding and ultrafiltration followed by LC/MS analysis was performed to identify the estrogenic compounds from the by-product mixtures. In addition, the first application for purification of antigens of the E2 antibody for LC/MS analysis utilizing paramagnetic Streptavidin A-beads is also introduced.

2. Materials and methods

2.1. Materials

All of the chemicals and reagents were purchased from either Sigma (St. Louis, MO) or Aldrich (Milwaukee, WI). Ozone gas was generated using an Ozone Generator from PCI Ozone & Control System, Inc. (West Caldwell, NY). LiChrolut RP-18 SPE cartridges were provided from VWR International (San Francisco, CA). Monoclonal antibodies to 17β-estradiol (1 mg, 10-E15, clone M94150) were purchased from Fitzgerald Industries International (Concord, MA). Human recombinant β-estrogen receptors (ERβ) were bought from Invitrogen Life Technologies (Carlsbad, CA). Biotinylated anti-mouse IgG (H + L) (1.5 mg, BA-2000) was purchased from Vector Laboratory (Burlingame, CA). Crypto-Giardia recovery buffer (100 ml, Dil-1002) and Streptavidin A-beads (1 ml, A010101.01) were obtained from ImmTech, Inc. (New Windsor, MD). Microcon YM-30 centrifugal filters were provided by Millipore (Bedford, MA).

2.2. Ozonation and chlorination of estrogens

One liter aliquots of distilled water were individually spiked with 1 ppm of E2, EE2, BPA, or NP and then reacted with either 5 ppm of ozone (O_3) or 7 ppm of sodium hypochlorite (NaOCl) for 15 min at room temperature. The

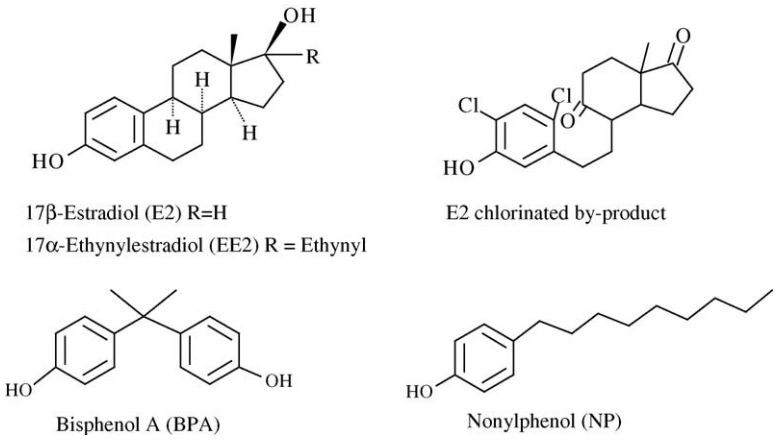

Fig. 1. Structures of E2, EE2, BPA, NP, and a major E2 chlorinated by-product.