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Estrogenic activity of alkylphenols, bisphenol S, and their chlorinated derivatives using a GFP expression system

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Abstract

Alkylphenol ethoxylates, widely used non-ionic surfactants, are biodegraded into alkylphenols such as nonylphenol (NP) and *t*-octylphenol (OP), short-chain ethoxylates such as NP-monoethoxylate (NP1EO) and NP-diethoxylate (NP2EO), and alkylphenoxy carboxylic acids such as 4-*t*-octylphenoxyacetic acid (OP1EC). Bisphenol S (BPS) is more heat-stable and photo-resistant than bisphenol A (BPA), and therefore replaces BPA. These chemicals could be chlorinated during wastewater treatment. We synthesized these compounds and their chlorinated derivatives to estimate their estrogenic activities using a GFP expression system. The EC_{50} ranking of NP-related compounds was NP > ClNP > diClNP > NP1EO > ClNP1EO > NP2EO. The estrogenic activity of OP1EC was 10 times less potent than parent OP. Furthermore, BPS showed comparable estrogenic activity with BPA. The EC_{50} ranking of BPS-related compounds was BPA $\geq$ BPS > triClBPS > diClBPS > ClBPS. Other tested BPS derivatives had no estrogenic activity. Chlorination of the tested chemicals did not enhance their estrogenic activity, in contrast to certain chlorinated BPAs. Thus, our results demonstrated that chlorinated derivatives of NP, OP, and BPS, even if artificially produced during wastewater processing, were less estrogenic than their parent chemicals, known as endocrine disruptors.

Keywords: Alkylphenol ethoxylates; Bisphenol S; Biodegradation products; Chlorinated derivatives; Estrogenic activity; GFP

1. Introduction

It is known that certain industrial chemicals such as bisphenol A (BPA, 2,2-bis(4-hydroxyphenyl) propane), 4-nonylphenol (NP), and 4-*t*-octylphenol (OP) show estrogenic activity (Krishnan et al., 1993; Soto et al., 1991, 1995; White et al., 1994; Hossaini et al., 2001; Seki et al., 2003). These chemicals may modulate the endocrine system contributing to adverse health, reproduction, and developmental effects in humans and wildlife (McLachlan et al., 1980; Guillette et al., 1994), and, therefore, are commonly referred to as endocrine disruptors. Due to widespread uses and manufacture of them, they are discharged into the terrestrial, aquatic, and marine environments as wastes and wastewaters.

Alkylphenol ethoxylates (APEs) have been in use for more than 40 years as detergents, emulsifiers, wetting agents, and dispersing agents in household products and in agricultural and industrial applications. Most APEs enter the aquatic environment after disposal in wastewater. APEs are nonionic surfactants made up of a branched-chain alkylphenol such as NP and OP that has been reacted with ethylene oxide to produce an ethoxylate chain (Warhurst, 1995). Biodegradation accomplished by stepwise shortening of the ethoxylate chain creates a complex soup of compounds that can be divided into three main groups: short-chain ethoxylates, alkylphenoxy carboxylic acids, and alkylphenols such as NP and OP (Warhurst, 1995; Petrovic et al., 2001). Several studies have confirmed their presence in raw sewage, final effluent, sludge, sediments, fish, mussels, and even in surface and drinking water (Naylor et al.,

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1992; Ahel et al., 1994a,b; Di Corcia et al., 1994; Field and Reed, 1996; Bennie et al., 1997; La Guardia et al., 2001; Staples et al., 2001; Sabik et al., 2003, Shao et al., 2003; Petrovic et al., 2004). Todorov et al. (2002) reported that surfactant metabolites might be contributing to the estrogenic effects observed in larval fish, because of the higher concentrations of nonylphenol-ethoxylate metabolites in the effluents from sewage treatment plants. Jobling et al. (1993, 1996) reported that nonylphenol diethoxylate (NP2EO) and 4-nonylphenoxy carboxylic acid as well as NP and OP are all weakly estrogenic. In this study, we synthesized several biodegradation products of APEs, namely 2-hydroxyethyl 4-nonylphenyl ether (nonylphenol monoethoxylate, NP1EO), 2-(2-hydroxyethoxy)ethyl 4-nonylphenyl ether (nonylphenol diethoxylate, NP2EO), 4-*t*-octylphenoxyacetic acid (OP1EC), and their chlorinated derivatives which were produced during wastewater treatment (Petrovic et al., 2001), and investigated their estrogenic activity.

BPA is a raw material used in the polymer and plastic industries, and is also used to produce paper, such as thermal paper and carbonless copy paper. BPA readily reacts with sodium hypochlorite, which is used as a bleaching agent in paper factories, and mono-, di-, tri-, and tetrachlorinated BPAs have been detected in the final effluent from waste-paper recycling plants using sodium hypochlorite (Fukazawa et al., 2001, 2002). Polychlorinated BPAs stably contaminate the final effluent since they are not easily biodegraded (Fukazawa et al., 2001). Certain chlorinated BPAs were more active at lower concentrations than parent BPA (Kuruto-Niwa et al., 2002). Several chemicals structurally similar to BPA are also utilized in the manufacture of resin and plastics. One of them is bisphenol S (BPS, bis(4-hydroxyphenyl) sulfone), whose two phenolic rings are joined together with sulfur. BPS has excellent stability against high temperature and resistance to sunlight. The production of BPS increases year by year (Jin and Zhao, 1997). BPS, as well as 4-isopropoxyphenyl 4-hydroxyphenyl sulfone (IPBPS), is used as a developer in dyes for thermal paper in Japan (Watanabe et al., 2004). Therefore, the contamination of BPS and its derivatives will increase in the environment. We also estimated their possible estrogenic activity.

A number of in vitro assays have been developed to identify and assess the estrogenicity of chemicals (Zacharewski, 1998). Among these assays, an estrogen response element (ERE)-regulated reporter gene assay seems to be the most commonly used (Pons et al., 1990; Klinge et al., 1997; Legler et al., 1999). We devised a simple green fluorescent protein (GFP) expression system to detect estrogenic chemicals using a quantitative fluorescent imaging system, in which a GFP reporter vector regulated by an ERE was constructed and transfected into human breast carcinoma MCF7 cells which are estrogen-responsive (Kuruto-Niwa et al., 2002). In the present study, estrogenic activity of the chemicals described above was investigated using the GFP expression system to assess their environmental impacts.

2. Materials and methods

2.1. Materials

17β-Estradiol (E_2), NP (mixture of chain isomers), and BPS were purchased from Tokyo Kasei Kogyo Co. Ltd. (Tokyo, Japan). BPA and OP were purchased from Kanto Chemical Co. Inc. (Tokyo, Japan). Other compounds used in this study were synthesized as described below.

2.2. Synthesis of the compounds used in this study

2.2.1. Synthesis of chlorinated derivatives of 4-nonylphenol (mixture of chain isomers)

An aqueous solution of sodium hypochlorite (minimum 2.5%, 12 ml) was added dropwise to a solution of NP (1.1 g) in 50% methanol (50 ml). The solution was stirred for 3 h and an aqueous solution of sodium sulfite was added. The mixture acidified with 2 M hydrochloric acid was extracted with diisopropyl ether. The organic solution was dried over magnesium sulfate. After removal of the solvent under reduced pressure, the residue was subjected to column chromatography on silica gel (2% tetrahydrofuran in hexane) to give 2-chloro-4-nonylphenol (ClNP, 250 mg) and 2,6-dichloro-4-nonylphenol (diClNP, 120 mg). Their structures are shown in Fig. 1A. Their mass chromatograms are shown in Fig. 2.

2.2.2. Synthesis of 4-nonylphenyl 2-hydroxyethyl ether and its chlorinated derivatives

A solution of NP (1.1 g) in DMF (5 ml) was added dropwise to a mixture of sodium hydride (0.6 g of 60% dispersion in mineral oil) in DMF (20 ml) with stirring. After the mixture had been stirred for 30 min, a solution of 2-benzyloxyethyl bromide (1.3 g) in DMF (5 ml) was added dropwise at room temperature. The mixture was stirred for 5 h, poured into an ice-water and extracted with diisopropyl ether. The organic solution was washed with brine and dried over magnesium sulfate. After removal of the solvent, the oily residue was subjected to column chromatography on silica gel (10% ethyl acetate in hexane) to give an oily product, 2-benzyloxyethyl 4-nonylphenyl ether (0.83 g). This product was added to a mixture of 5% paradium on charcoal (100 mg) in ethanol (50 ml) and the mixture was stirred overnight under a hydrogen atmosphere. After removal of the catalyst by filtration, the filtrate was condensed under reduced pressure to give an oily product, NP1EO (0.7 g). Its structure is shown in Fig. 1A. The mass chromatogram is shown in Fig. 2D.

2-Chloro-4-nonylphenyl 2-hydroxyethyl ether (ClNP1EO) and 2,6-dichloro-4-nonylphenyl 2-hydroxyethyl ether (diClNP1EO) were synthesized from ClNP and diClNP,