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Biomagnification of endocrine disrupting chemicals (EDCs) by *Pleuronectes yokohamae*: Does *P. yokohamae* accumulate dietary EDCs?

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HIGHLIGHTS

- This study aimed to investigate the bioaccumulation of selected EDCs through dietary route using *Pleuronectes yokohamae*.
- BMF values suggesting biomagnification not occurred which in contrast to high assimilation percentages.
- Glucuronidation was studied to investigate the possible metabolism and excretion of EDCs by *P. yokohamae*.

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ABSTRACT

We evaluated the potential for biomagnification of endocrine disrupting chemicals (EDCs) such as nonylphenol (NP), octylphenol (OP), bisphenol A (BP), and natural estrogens such as estrone (E1) and 17β-estradiol (E2) in a benthic fish, *Pleuronectes yokohamae*. The assimilation efficiencies (AE) of most EDCs ranged from 88 to 96% suggesting that they were efficiently incorporated and assimilated into *P. yokohamae*, except for NP (50%). However, the biomagnification factor (BMF) values were <1.0 suggesting that the compounds were not biomagnifying. Additionally, three of the target EDCs were not detected (BP, E1 and E2). Glucuronidation activity towards BP (11.44 ± 2.5 nmol/mg protein/min) and E2 (12.41 ± 3.2 nmol/mg protein/min) was high in the intestine suggesting that EDCs were glucuronidated prior to excretion into bile. Thus, we conclude that biomagnification of dietary EDCs is reduced in *P. yokohamae* because of effective glucuronidation.

1. Introduction

The presence of endocrine disrupting chemicals (EDCs) in the aquatic environment becoming a great concern due to the interference of these xenobiotics on the normal functioning of aquatic life endocrine systems. A number of estrogenic compounds have been detected in the environment include natural steroid hormones estrone (E1) and 17β-estradiol (E2). Industrial chemicals with estrogenic properties such as nonylphenol (NP), octylphenol (OP) (used as polyethoxylates in industrial detergents and emulsifiers) and bisphenol A (BP; used to produce epoxy resins and polycarbonate plastics) were detected as well (Lange et al., 2002). EDCs reach aquatic ecosystem mainly through sewage effluents thereby posing a potential threat to the aquatic organisms (Matthiessen and Sumpter, 1998).

The concentrations of nonylphenol (NP), octylphenol (OP), bisphenol A (BP), estrone (E1), and 17β-estradiol (E2) were measured in the sediment and the wild polychaete, *Paraprionospio* sp. from Osaka Bay. The result surprisingly showed the higher concentration of these EDCs in the *Paraprionospio* sp. than in the sediment (Koyama et al., 2013; Nurulnadia et al., 2014). Thus, we hypothesized that these compounds were being biomagnified in *Paraprionospio* sp. from the sediment. Furthermore, because polychaetes are an important component of the diet of benthic fish, the accumulated EDCs may be transferred into fish, and potentially humans, through the food chain. Thus, there is a need to understand the pathways of bioaccumulation and biomagnification of these compounds (Kristensen and Tyle, 1990; Lai et al., 2000; Wang et al., 1996).

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The aims of the present study were to examine 1) the assimilation efficiency, 2) biomagnifications, and 3) possible metabolism of EDCs in *Pleuronectes yokohamae*. Assimilation efficiency (AE) provides a measure of the fraction of ingested compounds that are incorporated into biological tissues (Jones et al., 2001; Olsen and Olsen, 2008). After organic contaminants are assimilated into fish tissues they may either biomagnify or be metabolized (Coldham et al., 1998). To investigate the biomagnification, *P. yokohamae* was exposed to EDCs through dietary route. EDCs structures often include phenols and aromatic groups (e.g. alkylphenols, phthalates and bisphenols), hence conjugation of polar moieties by phase II degradation enzymes is expected (Coldham et al., 1998; James, 2011; Thibaut et al., 1998; Thibaut and Porte, 2004; Yokota et al., 2002). The addition of glucuronyl group to the parent molecule is believed to increase the water solubility and facilitating the excretion (Giroud et al., 1998). Glucuronidation is one of the major metabolic pathways involved in the elimination of endogenous and exogenous compounds in fish (Dutton, 1980; Stegeman et al., 2010; Yokota et al., 2002). Therefore, to examine another possible process after assimilation of EDCs is metabolism by glucuronidation.

2. Materials and methods

2.1. Chemicals

EDCs standards were obtained from the Kanto Chemical Co. Inc., Japan (*p*-NP), Tokyo Chemical Ind. Co., Japan (OP), Nacalai Tesque, Inc. Kyoto, Japan (E2), Wako Pure Chemical Ind., Japan (BP, E1, E2-16,16,17-d3, and *p*-n-NP-d4), and Sigma Aldrich, United States of America (Bisphenol A mono-β-D-glucuronide, β-estradiol 17-β-D-glucuronide sodium salt, β-estradiol 3-β-D-glucuronide sodium salt, and UDPGA trisodium salt). Pesticide grade hexane, dichloromethane (DCM), diethyl ether, and florisil were purchased from Wako Pure Chemical Ind. Co., Japan. HPLC grade (methanol, isopropyl alcohol, ammonium acetate, and acetonitrile), high purity grade (magnesium chloride, potassium chloride, and sodium chloride), poisonous metal analysis grade (hydrochloric acid, perchloric acid, and nitric acid), Wako first grade (aprotinin from bovine lung, tris(2-amino-2hydroxymethyl)-1,3-propanediol, and chromium (III) oxide), and Wako special grade (2-phenoxyethanol and triethylamine) were also purchased from Wako Pure Chemical Ind. Co., Japan. Protein lysis solution CHAPs (3-[(3-Cholanidopropyl)dimethylammonio]-1-propanesulfonate) and ethylenediaminetetraacetic acid disodium salt (EDTA 2NA) were purchased from Dojindo Molecular Technologies, Inc., Japan.

2.2. Test organism (*P. yokohamae*)

P. yokohamae is a benthic marine fish that is distributed from southern Hokkaido to Oita Prefecture in Kyushu, in the Yellow Sea, the Gulf of Bohai, and the northern part of the East China Sea (Sakamoto, 1984). *P. yokohamae* specimens were purchased from the Regional Government Aquaculture Center in Yamaguchi, Japan. They were reared under laboratory conditions for six months until they obtained the desired body size around 10 g (juvenile) and fed a commercial diet (Nippon Formula Feed Manufacturing Co., Japan).

2.3. Experimental design

2.3.1. Assimilation efficiency of EDC

We used an inert marker of chromium (III) oxide (0.5% in the diet) to estimate the assimilation efficiency of EDCs following the method described in Austreng et al. (2000). The diet was prepared by grinding 100 g of commercial fish diet with a pestle and mortar and mixing with 0.5 g of Cr (III) oxide. We then added sufficient water (10 mL per addition) so that the diet could be pelletized on a stainless tray using a syringe. The diet was dried under sunlight for 2 d and cut into the appropriate size for feeding. The mixture of EDCs was dissolved in diethyl ether and spiked into 50 g of the commercial diet containing 0.5% Cr (III) oxide in a stainless tray to aid the drying process. The spiked diet was left overnight in a draft chamber.

P. yokohamae were exposed to dietary EDCs and Cr (III) oxide in a glass aquarium (15 × 25 × 18 cm) with semi-static, well aerated water. The seawater was replaced once daily. The aquariums were placed in a heated water bath to maintain the temperature at 20 °C. Each aquarium was stocked with a single *P. yokohamae* (total of 9 aquaria) and the fish were starved for 2 d to clear the gut. After this, the fish in all tanks were fed the diet containing Cr (III) oxide for 3 d at a daily feeding rate 2% of the body weight. Feeding occurred twice daily and any food that was uneaten after 30 min was removed immediately. The diet was then switched to the EDC spiked diet (containing Cr (III) oxide) on days 4–6. During this period, feces were collected using siphon after naturally excreted by fish prior to feeding. The feces were then centrifuged at 760× g at 4 °C for 10 min. After removing the supernatant, the feces were stored in a freezer (−18 °C) until analysis. The body weight of individual *P. yokohamae* ranged from 17.0 g to 26.0 g at the beginning of the exposure experiments.

2.3.2. Biomagnifications of EDC

The concentration level was decided based on the measured EDCs in polychaete from Osaka Bay (Nurulnadia et al., 2014) as medium level, and 5 times lower and higher concentration to represent low and high levels, respectively. EDC standards were prepared for each compound at low, medium, and high concentrations. Each EDC was dissolved in diethyl ether and spiked into 50 g commercial diet (Nippon Formula Feed Manufacturing Co., Japan) in a stainless tray. The spiked diet was left overnight in a draft chamber. Sixteen aerated flow-through tanks (35 × 48 × 18 cm, 3 concentrations for each EDC and 1 control) were used for the exposure test of individual EDCs. Twenty-four of *P. yokohamae* were placed in each tank, including a control tank. The test water (30 L) was completely replaced 300 L/10 times/d with natural sand-filtered seawater. On day 0, three *P. yokohamae* individuals were sampled as a baseline. *P. yokohamae* in each tank were then administered one of the spiked diets at a daily feeding rate of 2% of their body weight for 14 d. Five fish in each tank were sampled on days 2, 4, 7 and 14. The average body weight of individual *P. yokohamae* was 2.95 ± 0.38 g and 3.51 ± 0.49 g, for the control and exposure groups, respectively, at the beginning of the experiment. Non-consumed food residue was removed daily. During sampling, *P. yokohamae* were anaesthetized in 0.05% 2-phenoxyethanol. After measuring the body length and weight, the fish were stored in a freezer (−18 °C) until EDC analysis.

2.4. Chemical analysis

2.4.1. Quality assurance

The recovery test was performed using the fish diet spiked with 100 ng/g of each EDC standard and both external and internal standards (*p*-n-NP-d4 and E2-16,16,17-d3). The EDC standard extraction and measurement were performed using the procedures described above. Mean percent recovery, calculated based on the amount added and recovered, was between 60 and 104%. All analyses were performed in triplicate (n = 3) with the error of all analysis within ±25% (Table 1).