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Sexually disrupting effects of nonylphenol and diethylstilbestrol on male silver carp (*Carassius auratus*) in aquatic microcosms

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Abstract

Based on detected nonylphenol (NP) levels in aquaculture water, this study investigated sexually disrupting effects in mature male silver carp (*Carassius auratus*) exposed to NP and a positive control diethylstilbestrol (DES). The combined evidences of steroid hormone (17β-estradiol, estrone and testosterone) levels and hispathological pictures showed that NP ($\geqslant$10 μg/L) and DES could exert estrogenic effects through indirect mechanisms [i.e. increased estrogens levels (up to two times) and decreased androgen level in serum (down to 20–30%)], which might subsequently induce vitellogenin synthesis in liver. Environmental realistic concentrations of NP might be on the verge of inducing significant estrogenic effects in male silver carps. High amounts of NP and DES might be accumulated in fish serum, and the uptake by fish was possibly responsible for their quick attenuation in experimental tank water. NP and DES might have different metabolic mechanisms, the estrogenic effects of DES were more significant than those of NP.

Keywords: Nonylphenol (NP); Diethylstilbestrol (DES); Silver carps; Steroid hormones; Aquaculture

1. Introduction

Endocrine disrupting chemicals (EDCs), which include a diverse range of synthetic and natural compounds, have received increasing attention recently due to their ability to disrupt or alter the functions of endocrine system and consequently their adverse health effects in an intact organism. Among them, nonylphenol (NP) is the most critical metabolite of nonylphenol polyethoxylates (NPEOs), which are the major groups of non-ionic surfactants widely used commercially in the production of plastics, textiles, and agricultural chemicals, and in household applications such as detergents, paints, pesticides and cosmetics (reviewed in Ying et al., 2002). It has been shown that NP has estrogenic, toxic, and carcinogenic effects in fish, birds, and mammals; and enhanced resistance towards biodegradation; potential ability to bio-accumulate in aquatic organisms (Servos, 1999).

Diethylstilbestrol (DES), a synthetic estrogen used for fattening cattle as well as in humans to prevent miscarriages in the mid-20th century, is the first recognized EDC due to its marked effects. For example, infants of women prescribed with DES had to battle against severe diseases: daughters developed vaginal cancer, sons suffered from malfunctions of the sexual organs such as sperm anomalies, hypospadias, and ectopic testes (Klip et al., 2002). These diseases were caused by pre-natal exposure to DES during sensitive stages of sexual differentiation of the developing fetus. The mechanisms of action of DES in humans and wildlife have been well studied, and DES serves as a model compound for xenoestrogen actions (Walker, 1989).

Substantial amounts of EDCs including NP and DES originate from a variety of sources including plants, by-products of manufacturing, agricultural run-off, and sewage and wastewater treatment plants. Consequently, as the ultimate reservoir, environmental problems associated with EDCs in aquatic environment are of great concern. It has been reported that concentrations of NP in treated wastewater effluents were high up to 369 μg/L levels

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(Blackburn and Waldock, 1995; Ying et al., 2002), and were relatively low at 0–10 μg/L levels in most rivers (Ahel et al., 1994; Kolpin et al., 2002; Snyder et al., 1999). However, information on NP and DES concentrations in fish culture water has been seldom documented.

Fish, perhaps more than any other vertebrates, may experience lifelong systemic exposure to a wide variety of EDCs due to contamination of their habitats. Henceforth, fish may provide an effective "early warning system" for the presence and effects of EDCs in aquatic environments. For example, EDCs levels detected in fish tissues, a high incidence of intersexuality, significant alterations of endocrine system, elevated levels of vitellogenin (VTG) and abnormal steroid hormone ratios were found in fish collected at the pollution site compared with those found at reference locations (Folmar et al., 1996; Jobling et al., 1998; Kannan et al., 2003; Sole et al., 2003).

Steroid hormones are a group of low molecular weight lipids naturally synthesized from cholesterol and play an important role in biological homeostasis. There have been reports of the adverse effects of EDCs, including the disruption of the normal balance of sex steroid hormones levels in blood (Folmar et al., 2001; Guillette et al., 1996). However, the harmful effects of NP on sex hormones in fish were controversial (Giesy et al., 2000; Harris et al., 2001; Matsumura et al., 2005; Schwaiger et al., 2002; Villeneuve et al., 2002), Histology is a powerful tool in the analysis of endocrine disrupting effects on fish. For instance, changes in Sertoli cells and germ cell syncytia, reduced testicular growth, intersex (testis–ova) induction, hepatocytes hypertrophy, and severe kidney pathology were widely reported in males of several important laboratory fish models after NP exposure (Christiansen et al., 1998; Gray and Metcalfe, 1997; Jobling et al., 1996; Miles-Richardson et al., 1999; Weber et al., 2003; Zha et al., 2007). However, these studies failed to provide detailed information and systemic analysis on histo-cytopathological alterations, and most of all, the linkages between the multipurpose effects induced in different tissues.

Methods for the simultaneous determination of EDCs and steroid hormones were developed in our previous studies (Yang et al., 2006a, b). The automated method was rapid, simple, sensitive, easy to operate, and provided a powerful tool for the study of sexually disrupting effects of EDCs on steroid hormones in fish body. By using these methods, the present study aims to: (1) investigate the current NP and DES pollution conditions in fish culture waters in Guangdong, China; (2) study the kinetics of NP and DES in tank water and fish serum in aquatic microcosms; (3) compare the effects of NP to DES, a positive EDC control, on serum sex steroid hormones and histo-cytological changes in the liver and testes of carps; and (4) analyze the possible linkages between the multi-purpose effects induced in different tissues.

Sexually mature male silver carp (*Carassius auratus*) were chosen as the test organism in this study. Carps are commercially available, relatively easy to maintain in the laboratory, and large enough to provide sufficient blood volumes for hormones analysis. Moreover, the reproductive biology of carps has been well studied. This species has been often used as a fish model for the studies of disruptive effects of EDCs under field (Folmar et al., 1996; Sole et al., 2003) and laboratory conditions (Villeneuve et al., 2002).

2. Materials and methods

2.1. Chemicals and reagents

HPLC-grade methanol and diethyl ether were supplied by Merck (Darmstadt, Germany). Nonylphenol technical grade (*t*-NP) was purchased from Sigma-Aldrich (St. Louis, MO, USA). DES (99%), steroid hormones including testosterone (*T*) (99 + %) and estrone (E_1) (99 + %) were obtained from Acros (New Jersey, USA). 17β-estradiol (E_2) was obtained from Sigma-Aldrich (St. Louis, MO, USA). The derivatization reagent N,O-bis(trimethylsilyl) trifluoroacetamide (BSTFA) (98 + %) was from Sigma-Aldrich (St. Louis, MO, USA). Sodium chloride (NaCl) (AR) and hydrochloric acid (HCl) (GR) were from Guangzhou chemical reagent factory.

2.2. Field samples collection and pretreatment

A total of 26 river water and fish culture water samples were collected from three main culture villages in Zhongshan Sanjiao (Guangdong, China) in December 2005. Samples were taken using pre-cleaned glass bottles and then filtered after returning to the laboratory to remove suspended particulate matter (SPM). Sample pretreatment followed the procedures described in our previous paper (Yang et al., 2006a, b).

2.3. Laboratory fish treatment

Sexually mature male mono-sex silver carp (*Carassius auratus*) during active spermatogenesis (2–3 years old, body weight 90 ± 15 g) were supplied by NanHai Aquafarms (Guangdong, China). Upon arrival, every 15th fish per treatment from overall 180 fish was randomly placed into one of 12 fiberglass tanks (120 L, 40 × 50 × 60 cm) containing 100 L aerated tap water and acclimatized for 7 days before the treatment. Air was delivered with a pump equipped with a 12-channel pump head. Water quality parameters were regularly monitored including temperature (25 ± 2 °C), dissolved oxygen (6 ± 1 mg/L), pH (7.7–7.8) and hardness (21.3°dGH). The photoperiod was maintained in a 12-h light:12-h dark regime. The fish were fed daily and excreted waste and uneaten food were removed daily. Half of the tank water was renewed every two days after the first 72 h exposure. Stock solutions of NP and DES were separately prepared in methanol. Aliquots of these stock solutions were diluted and added to tank water every two days to produce four treatment groups with nominal concentrations of NP at 1, 10, and 100 μg/L, and DES at 10 μg/L, with methanol concentrations fixed at 0.01%. Dilution water control (DWC) and solvent water control (SWC, 0.01% methanol) were also set up.

2.4. Laboratory samples collection and pretreatment

Water sample (100 mL) in each exposure tank was collected at 0, 12, 24, 48, 72 h after the administration of NP and DES. To minimize variability due to diurnal fluctuations in plasma steroid concentrations, all fish samples were sampled between the hours of 8:30 and 10:30 AM. Four to five fish were collected from each treatment group at exposure day 7, 14, and 21. Blood collection and samples pretreatment followed the procedures as described in our previous paper (Yang et al., 2006a, b).