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Endocrine disruption and metabolic changes following exposure of *Cyprinus carpio* to diethyl phthalate

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Abstract

Diethyl phthalate (DEP) enter into aquatic environment from industries manufacturing cosmetics, plastic and many commercial products and can pose potential fish and human health hazard. This experiment evaluated effects of DEP in adult male (89 g) common carp (*Cyprinus carpio*) by exposing them to fractions of LC_{50} (1/500–1/2.5) doses with every change of water for 28 days. Vitellogenin induction metabolic enzymes, somatic indices and bioaccumulation were studied on 7th, 14th, 21st and 28th day. The 96th hour LC_{50} of DEP in fingerlings was found to be 48 mg/L. Compared to control, except increase ($P < 0.01$) in alkaline phosphatase activity (EC 3.1.3.1) and liver size, there was decrease ($P < 0.01$) in activity of acid phosphatase (EC 3.1.3.2), aspartate aminotransferase (EC 2.6.1.1), alanine aminotransferase (EC 2.6.1.2) and testiculosomatic index following exposure to 1, 5 and 20 ppm DEP. Significant ($P < 0.01$) dose dependant vitellogenin induction was observed with exposure of fish to 0.1, 1 and 5 ppm DEP. The bioaccumulation of DEP in testis, liver, brain, gills and more importantly in muscle tissues of fish increased significantly ($P < 0.01$) with increase of dose from 1 to 5 ppm. Significant interaction ($P < 0.01$) of dose and duration of exposure indicated that exposure period of a week to two was sufficient to bring about changes in quantifiable parameters studied. Fish exposed to 20 ppm DEP became lethargic and discolored during onset of the 4th week. This is the first report describing metabolic changes and vitellogenin induction following exposure of *C. carpio* to DEP dose that is as low as 1/500th fraction of LC_{50}.

Keywords: Diethyl phthalate; *Cyprinus carpio*; LC_{50}; Vitellogenin; Metabolic enzymes; Bioaccumulation

1. Introduction

The endocrine disrupters are a large group of chemicals which enter into the aquatic environment from manufacture of various industrial and consumer products, agriculture and food/drug processing, wastewater treatment plants and human wastes. This group includes certain polychlorinated biphenyls, polyaromatic hydrocarbons, dioxins, furans, pesticides, alkylphenols, synthetic steroids, phthalate esters, plant sterols and parabens. Among phthalate group of chemicals, diethyl phthalate (DEP) is the one which have many industrial uses. Phthalate esters are key additives used for softening and making PVC more flexible and hence is an important constituent of many common commercial products. It is used in medical devices (intravenous devices, blood transfusion bags and pharmaceutical coatings), as a vehicle for fragrances and cosmetics, in the manufacture of celluloid, as a solvent for cellulose acetate in the manufacture of varnishes and ropes, in the denaturation of alcohol, in plastic for wrapping food, shower curtains and as a vehicle for pesticide sprays [1–3].

Phthalate esters have been suspected to be one of the estrogenic group of compounds [4–6]. These esters have been identified in all environmental compartments, water, air, sediment and biota, in the gulfs [7] and rivers [8] around the world. Contamination of food items (cheese, butter and

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cream) with phthalate ester has also been reported [9]. Biological adverse effects of high levels of phthalate esters in serum have become a concern [10]. In Indian subcontinent, DEP is also extensively used in the manufacture of incense sticks and as a perfume binder [11]. The empty DEP containers are washed in rivers and lakes and subsequently the containers are used for domestic water storage.

Among potential sources of DEP contamination and accumulation in human beings, one is cosmetic products and other is dietary meat of fish particularly from unknown contaminated sources [12,13]. DEP acts as a solvent and vehicle for fragrance and cosmetic ingredient and subsequently comes in contact with skin. Using survey (fragrance manufacturers in US in 1995–1996) figures of ~4000 metric tons of DEP production and uses as described [14] (International Fragrance Association, Geneva, the European Cosmetic, Toiletry and Perfumery Association), Api [2] reported the figures of potential exposure through cosmetics in humans as around 50 mg/day or 0.83 mg/kg/day.

Sewage fed fisheries is practiced in many countries including India with waste waters being utilized for the purpose of culturing fishes [15,16]. Endocrine disruption and accumulation of phthalate residue is more likely to occur in sewage fed fisheries. This is obvious for the reasons that wastewater from various industries and garbage containing DEP related products are released in water and DEP is found in suspended water than sediment [17]. Common carp, which spawns year around [18], has been projected as a candidate species for sewage fed aquaculture in India [15,16,19]. In this study with *Cyprinus carpio*, we studied effects of fractions of LC_{50} dose of DEP on metabolic enzymes, somatic indices and vitellogenin induction. Except for vitellogenin induction study (where 0.1 ppm or 1/500 dose of LC_{50} was used), dose range used was 1–20 ppm (1/50–1/2.5 of LC_{50}).

2. Materials and methods

2.1. Test animals

Four months old common carp (body weight 89.25 ± 0.32 g) were procured from Maritech India Pvt Ltd, Mansar (MP, India). Fish of approximately same weight were acclimatized to laboratory conditions for two weeks before initiation of experiments. Along with control (only one replicate), there were three treatment groups with three replicates in each treatment. They were reared in aquaria containing 280-L dechlorinated tap water, which were provided with continuous aeration. Each tub was stocked with 20 fish. One-fourth water was removed every 4th day and complete water was exchanged on a weekly basis. Weekly water quality parameters were measured in each system, the means of which were as: water temperature (27 °C) pH (8.0), dissolved oxygen (7.25 mg/L), negligible free CO_2, hardness (290 mg/L), nitrate (0.12 mg/L) and phosphate (0.33 μg/L). Fish were fed *ad libitum* twice a day (9.30 and 17.00) with pellets during the acclimation and experimental period. The pellets were prepared with extruder (BTPL twin screw extruder, Calcutta) at Central Institute of Fisheries Education, Mumbai and contained 35% crude protein and 4% crude fat (crude protein 35% and crude fat 4%).

2.2. Chemicals

Diethyl phthalate (1,2-Benzenedicarboxylic acid diethyl ester CAS No: 84-66-2) (Fluka Chemika, Germany) and HPLC grade acetone (Merck), anti-carp vitellogenin ELISA (Cayman Chemicals, MI, USA), enzymes (alanine transaminase, aspartate transaminase, alkaline and acid phosphatase) kits (Siddham Chemicals, Nagpur) and clove oil (average Eugenol concentration 80%) (Nav Niketan Pharmaceuticals, Mumbai, India) were procured.

2.3. LC_{50} studies

The LC_{50} studies were conducted in accordance with methods adapted from the US EPA and the American Public Health Association [20,21]. Initial range finding test was undertaken to select the maximum exposure level. Stock solution was prepared in acetone. Glass aquaria were filled with water and DEP was then added with constant aeration. Each aquarium was provided with 10-fingerling (9 g) and the experiment was conducted for 96 h. Fish were fed daily once, and the excess feed was removed every 24 h.

2.4. Sublethal exposure experiments

The LC_{50} of DEP determined as above was 48 ppm and hence male fishes were exposed to 0 (solvent control), 1 (1/48th of LC_{50}), 5 (1/9.6th of LC_{50}) and 20 (1/2.5th of LC_{50}) mg/L DEP concentration with every change of water. Because vitellogenin is normally induced with exposure to higher concentration of EDCs, apart from 1- and 5-mg/L dose, we used 0.1 mg/L DEP. Stock solutions were prepared in acetone and the experiments were conducted in glass aquaria over a period of 28 days. This period was chosen because pseudo-equilibrium of the toxicant concentrations between exposure media and fish tissues would be expected to occur within 30 days of exposure [22]. All parameters were studied on 7th, 14th, 21st and 28th day.

2.5. Behavioral monitoring

Behavioral monitoring was conducted daily throughout the 28-day exposures. The general behavior, swimming, crowding or seclusion, skin coloration and external appearance of fish were monitored, and their startle response behavior was examined by tapping the aquarium. Fish were then fed and their feeding habits were observed until either the food had been consumed or 5 min had passed. If food had not been consumed within 5 min, the aquarium was checked periodically over the next 2 h to see if the food had been consumed (non-consumed food was then removed).