13. Adverse Reproductive Effects on Plasma Vitellogenin and Sex Steroid Levels, and Gonadosomatic Index in Juvenile Common Carps (*Cyprinus carpio*) Exposed to 17β-Estradiol and D-2-Ethylhexyl Phthalate

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Abstract

Environmental estrogens are natural or synthetic substances present in the aquatic environment, especially in effluent from sewage treatment. However, the adverse effects of these estrogenic substances on fish reproduction are unknown. Di-2-ethylhexyl phthalate (DEHP) is the most common phthalate, which is used as a plasticizer in polyvinylchloride (PVC), and it is widespread in the environment and has been found in aquatic organisms and sediments. Therefore, juvenile common carps (*Cyprinus carpio*) were exposed to nominal concentrations of 17β-estradiol (E2) (0.5, 5, 50 μg/L) and DEHP (10, 100, 500 μg/L) for 21 days, to determine the adverse reproductive effects of these compounds on plasma vitellogenin (VTG) induction, sex steroid level, and gonad weight. Electrophoresis (SDS-PAGE) revealed that much of VTG was induced in fish exposed to 5 and 50 E2 μg/L, but none of DEHP exposure showed induction. Enzyme-linked immunosorbent assay (ELISA) revealed that VTG was significantly induced in fish exposed to 5 and 50 E2 μg/L, and combination of 50 E2 μg/L with 10 and 500 DEHP μg/L, but none of DEHP exposure showed induction. Analysis of sex steroid levels in some fish revealed that testosterone (T) was detected in both male and female fish of the control and DEHP exposures, but none of fish exposed to E2 concentrations had detectable testosterone level. On the other hand, E2 exposure induced 17β-estradiol in plasma of male fish, but there was no induction of 17β-estradiol in plasma of male fish exposed to DEHP. Comparison of gonadosomatic index (GSI) revealed that maximal E2 exposure inhibited ovarian growth, but maximal DEHP exposure stimulated testicular growth. The results indicated that those comparisons can be a useful bio-indicator for determining adverse reproductive effect of endocrine disrupting chemicals (EDCs).

I. Introduction

Wide range of chemicals introduced into the environment by civilization or industrialization producing industrial waste, sewage, and agricultural effluent cause adverse effects internationally on human and ecosystem. Although the diversity of these compounds suggests that a variety of mechanism may be involved, many appear to be endocrine disrupting chemicals (EDCs). Therefore, researches on monitoring existence of EDCs in the environment have been increased recently (Tolar et al. 2001).

Estrogenic chemicals such as 17β-estradiol (E2) and plasticizers are discharged from sewage treatment works, and are accumulated in aquatic organisms, and can adversely influence their reproductive system (Kang et al. 2002).

A useful biomarker for determining if chemicals have any estrogenic activity to fish has been the measurement of their blood plasma levels of vitellogenin (VTG) (Sumpter and Jobling 1995). Levels of VTG rise steadily in female fish during sexual maturation. In contrast, little or no VTG can be detected in the plasma of males and immature females. Male fish do carry the VTG gene, but it is thought that circulating levels of estradiol are too low to trigger its expression (Flouriot et al. 1993).

Gonadal growth and maintenance are also known to be under endocrine control (Fostier et al. 1983). Although it is not sensitive as vitellogenin, a correlation has been demonstrated between inhibition of gonadal growth and potency of estrogenic chemicals (Jobling et al. 1996).

The present study investigated the adverse reproductive effect of 17β-estradiol (E2) and di-2-ethylhexyl phthalate (DEHP) on plasma vitellogenin and sex steroid levels, and gonadosomatic index of juvenile common carp (*Cyprinus carpio*).

II. Materials and Methods

Test	Rapid (<21 day) *in vivo* screening assay
Species	Juvenile common carp (less than 100 mm TL)
Exposure conc. and Chemicals	Control Solvent control (acetone, less than 100 mg/L) 17β-estradiol (E2, 0.5, 5, 50 μg/L)

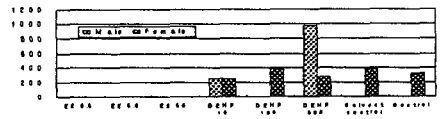
	Di-2-ethylhexyl phthalate (DEHP, 10, 100, 500 μg/L)
Sample collection	Blood, Gonad
Analysis	VTG induction by electrophoresis and ELISA Sex steroid hormone by Radioimmunoassay Gonadosomatic index (GSI)

Parameter	Condition
Test type	Semi-static renewal (every 48 hrs)
Water temperature	24 ± 1℃
Dissolved oxygen	6.0 ± 1 mg/L
pH	7.0 ± 0.5
Conductivity	160 ± 20 μS/cm
Photoperiod	16 hr light : 8 hr dark
Test solution volume	40 Liter
Initial number of fish	30
Diet	Daily (2% of body weight)

III. Results

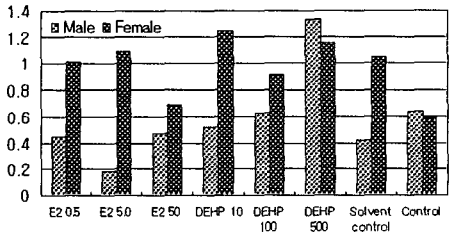
Sex steroid hormone level

Testosterone (pg/ml of plasma)

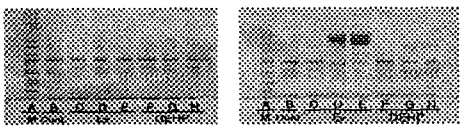


17β-Estradiol (pg/ml of plasma)

Gonadosomatic index (GSI)



Vitellogenin induction

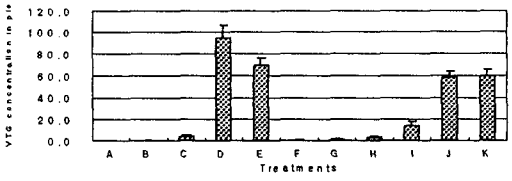


1st day of exposure　　21st day of exposure

A: Marker, B: Control,

C, D, E: E_2 exposure (0.5, 5, 50 ppb)

F, G, H: DEHP exposure (10, 100, 500 ppb)



A: Control, B: Solvent control,
C, D, E: E_2 exposure (0.5, 5, 50 ppb)
F, G, H: DEHP exposure (10, 100, 500 ppb)
I: E_2 0.5 + DEHP 500 ppb, J: E_2 50 + DEHP 10 ppb
K: E_2 50 + DEHP 500 ppb

IV. Conlusion

Although vitellogenesis can be used as a biomonitor of estrogenic pollution, it

is also necessary to ask whether this has any relevance to the fertility or health of the fish, whether high vitellogenin alters the quality or quantity of the eggs produced, and what effect it has on the males and juveniles which normally never produce vitellogenin.

Further research is needed to evaluate potential endocrine disruptors within the ecological risk assessment. The strategy for ecological effects of EDCs also needs to be integrated into the exposure characterization of a risk-based laboratory and field approach.

V. References

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