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## An Enzyme-Linked Immunosorbent Assay for Carp (*Cyprinus carpio*) Vitellogenin and Assay for Oestrogenic Chemicals

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### Abstract

The egg-yolk precursor vitellogenin(VTG) is secreted by the liver of female and male fish, in response to estrogenic compound. Carp(*Cyprinus carpio*) vitellogenin of one major protein is 160kDa, two minor proteins is 110kDa and 170kDa. We were induced vitellogenin by inject of 17-estradiol and purified in a two step procedure by separating it from plasma protein precipitated by 35% saturated (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> and then from the remainder by Mono-Q chromatography. We found major carp(*Cyprinus carpio*) vitellogenin band at 160kDa. Effect of different concentration of oestrogen on vitellogenin synthesis in carp(*Cyprinus carpio*) exposed for 4 weeks. Show differential effects on vitellogenin synthesis 7 days after treatment. Plasma vitellogenin was measured 3 times for each by an enzyme linked immunosorbent assay(ELISA). ELISA was developed for the detection of the egg yolk precursor vitellogenin in plasma of carp(*Cyprinus carpio*). The ELISAs performance was optimized and characterized.

*Keyword* : Vitellogenin, Oestrogen, Carp (*Cyprinus carpio*), 17-estradiol

### 1. Introduction

There is increasing concern about man-made chemicals in the aquatic environment that mimic oestrogens because they may disrupt reproductive function.<sup>1</sup> Vitellogenin(VTG) is the precursor for the major yolk protein phosvitin and ovitellin, this called a lipid-rich phosphoglycoprotein.<sup>2</sup> A precursor of egg-yolk, Vitellogenin in fish may be used as a biomarker for oestrogen exposure. In the mid-1980s, effluents from sewage treatment works discharge into rivers in England and Wales were found to be estrogenic, due to their ability to induce the production of a female specific egg- yolk protein pre cursor, Vitellogenin, in male fish. Subsequent field studies, in which caged fish were placed at varying distances downstream of sewage treatment works effluent discharges, demonstrated that the estrogenic activity persisted in some

receiving waters for considerable distances downstream from the point of sewage effluent input. Recently, populations of wild fish inhabiting English rivers have been shown to be intersex, a manifestation of endocrine disruption, which can be induced by exposure to 17-estradiol. Chemical analysis of sewage treatment works effluent has identified natural steroidal oestrogens, and in some cases the synthetic oestrogen, 17-ethinyloestradiol, at concentration sufficient to induce the oestrogenic responses observed in the caged fish studies. In sewage treatment works receiving influent from wool scouringmills. Alkylphenolic chemicals(biodegradation products alkylphenol poly ethoxylates) were found to be the major estrogenic contaminants and they were present at concentration high enough to induce feminizing effects in fish (Vitellogenin induction and suppression of testis growth in males).<sup>3</sup> This study investigated the use of an enzyme-linked immunosorbent assay developed to carp(*Cyprinus carpio*) vitellogenin to measure vitellogenin of them, especially laboratory studies on estrogenic xenobiotics.

## 2. Materials and Method

The carp(*Cyprinus carpio*) were from the Ju-nam lake fish of Changwon city. Reagent VTG was induced in 1-year-old male and female(200-500g). Ten adult male and female fish were given a series of weekly intraperitoneal(i.p.) injection( $1\text{mgkg}^{-1}$ ) of  $17\beta$ -estradiol(sigma) dissolved in a solution 0.5mg distilled water. After a 7-21day expose at 8-10°C water.

## 3. Result and Discussion

The time course of vitellogenin in carp(*Cyprinus carpio*) that were injected with a dose of  $17\beta$ -estradiol( $1\text{mgkg}^{-1}$ ) is presented a strong response after 14 days. The endocrine system can be disrupted by complex and interacting mechanism. Recent attention has focused on the ability of xenobiotic chemicals to mimic the structure and receptor binding properties of endogenous hormones such as the females sex hormone  $17\beta$ -estradiol.

## References

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