



## WATER RESOURCES RESEARCH GRANT PROPOSAL

**Title:** Assessing The Effects Of Endocrine Disrupters (Edcs) From A St. Paul Sewage Treatment Plant On Sperm Viability And Testicular Development In Fish: Adding A New Dimension To An Existing Project

**Keywords:** Endocrine Disruption, 17 $\beta$ -Estradiol, Sperm Viability, Fish

Duration: March 1999 to February 2001

Federal Funds: \$ 14,490 each year for two (2) years

Non-Federal Funds: \$ 29,000 each for two (2) years

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Congressional District: 5

Statement of Critical Regional or State Water Problems:

One of the fastest growing water quality issues worldwide over the past decade has been observation that many species of fish and wildlife - and perhaps even humans - suffer from disrupted endocrinological function when exposed various types of agricultural, industrial, and sewage effluents. Although neither the extent nor significance of this issue is well understood, the observation that male fish found in the effluent of modern sewage treatment plants in Europe commonly experience sex reversal, (Harries et al. 1998), has clearly established that public officials should be concerned that even tertiary sewage treatment plants may be discharging large quantities of endocrine disrupting compounds (EDCs) which mimic the actions of the female hormone estrogen. Recently this problem came home to roost in the United States; last year it was discovered that common carp living downstream of the Pig's Eye Sewage Treatment Plant in St. Paul (MSTP) were also experiencing signs of feminization (vitellogen induction; Folmar et al. 1996). Similar observations have also now been made with walleye (Folmar, in press). At this time, neither the identity of the compounds responsible, the extent of their distribution, nor the biological threat imposed is understood at all but many public officials have expressed great concern and several meetings and public forums have already been held. One consequence of these meetings has been a pledge by the Metropolitan Council Environmental Services (MCES) to award a University of Minnesota research team (Swackhamer, Adelman, Sorensen) \$200,000 to begin documenting the local problem. The award is merely intended to identify the chemical composition of local estrogenic EDCs, and to conduct a preliminary characterization of whether gametes obtained from male fish exposed to possible endocrine disrupting compounds (EDCs) are less able to fertilize eggs. At present, funding is extremely restricted and does not permit an examination of whether or why the fertility of male fish is impaired. The study proposed here is intended to answer this extremely important question, thereby greatly enhancing

the power of the MCES study. Together, these studies will allow for the most comprehensive study of endocrine disruption to date in the United States.

### **Statement of Results or Benefits**

This study will determine if fish exposed to compounds found in the effluent of the MSTP experience sex reversal and suffer from reduced sperm viability as a result of exposure to EDCs. In addressing these issue, we will develop a bioassays (ability to gauge sperm viability) which can be readily applied to other local waterways, or indeed the United States, to determine if they too are experiencing similar problems. Also, simply by proving funds, this project will allow the investigating team to achieve a critical mass by hiring a post-doctoral fellow, establishing the only research group in the upper mid-west with expertise in this growing issue.

### **Nature and Objectives of this Research**

#### 1.1 Introduction and Objectives Statement:

This study will determine if 17 $\beta$ -estradiol (estradiol), a suspected steroidal endocrine disrupter (EDC) in the effluent of the Metropolitan Sewage Treatment Plant (MSTP), has significant adverse effects on fertility of male fishes. Estradiol has been identified as the likely EDC in effluent of tertiary sewage treatment plants in the UK (Jobling et al. 1998), and it seems highly likely to be the case in St. Paul as well. That is present (and in what quantity) will in any case be verified in the first year of the study. We will examine sperm viability and motility using standard means to measure male fertility. The first year of study is devoted to developing these techniques in the laboratory, the second to applying them to wild fish from the Mississippi River. Goldfish are the laboratory model as they are great experimental subjects with which we have over a decade of experience, and are very closely related to common carp, with which they will actually hybridize on occasion. This study will complement and extend a study proposed to the Metropolitan Council Environmental Services (MCES) (see Appendix A). Briefly, the latter study will:

- 1) fractionate and identify EDCs in local waters,
- 2) evaluate fertility of wild fishes by determining the ability sperm to fertilize eggs, and
- 3) determine spawning success of lab fish exposed to leading EDC candidates.

The present study proposes two objectives to establish the relationship between EDCs and reproductive success. They are

- (1) Determine the effects of estradiol exposure on sperm viability in laboratory fishes. It is very likely that estrogenic compounds influence testicular function, and in particular sperm viability. Sperm viability is critical to external fertilizers, and very small differences in sperm viability have been shown to have drastic effects on male fertility (DeFraipont and Sorensen 1993 ; Zheng et al. 1997). Further, technology exists for

monitoring this parameter so it can easily be transferred to many sites (Kime et al. 1996). The link between cause (estradiol exposure) and effect (reduced sperm viability) must be established if the source of the chemical is to be prevented or remediated. Techniques will be established in a controlled laboratory setting in the first year to exclude factors which might effect sperm viability in the field. During this time, we will also determine as part of the MCES contract the quantities of estradiol actually found in MSTP plant. The latter should be very straightforward as many stand antibody based kits exist for the detection of this female hormone.

(2) Determine sperm viability in wild fish upstream and downstream from MSTP effluent in the Mississippi river. These experiments are a logical extension of the laboratory studies (Objective #1) and will establish if estradiol exposure causes adverse effects on the reproductive success of wild fish populations. Exposure may result in a decrease of reproductive ability and subsequent reduction in the genetic diversity of the population, which may affect the overall health of the population.