Abstract

Sex ratios in catches of wild adult fish are often affected by sex related differences in growth, behaviour and survival. Therefore, such sex ratios may not reflect the relative number of males and females that are recruited to the population. However, this can be circumvented by studying unborn fish embryos. We have found skewed sex ratios among embryos from the viviparous eelpout (*Zoarces viviparus*) which could indicate exposure to endocrine disrupting chemicals. The sex ratio was close to 50/50 at two presumably clean sites from Kattegat/Skagerrak and two from the Baltic Sea. On the Swedish Baltic coast near a large pulp mill the relative number of female embryos was significantly lower (42%; $P = 0.006$) but approached 50% further south in the discharge gradient. Treatment of females during early pregnancy with methyltestosterone inhibited oocyte development in all embryos, and instead testis-like tissue was formed. Thus, masculinization found in the field could be caused by exposure to androgen mimics or substances interfering with steroid-synthesis, activity or excretion. This may arise from exposure to the pulp mill effluents, since endocrine disruption in fish has been reported earlier near North American mills. Irrespectively of the cause, reduced numbers of female offspring may have negative impacts on the recruitment capacity of a population.

PII: S0141-1136(00)00172-0

Environmental estrogens: dose–response relationships for vitellogenin formation and reproductive toxicity in male rainbow trout

I.R. Schultz [a], P.N.N.L. Battelle [a], W.A. Richland [a], A.S. Drum [a], M.S.L. Battelle [a], W.A. Sequim [a], W.L. Hayton [a], G.A. Orner [b]

[a] *Ohio State Univeristy, Columbus, Ohio, USA*
[b] *Oregon State University, Corvallis, Oregan, USA*

Abstract

The appearance of the egg-yolk protein vitellogenin (Vg) in plasma of male fish is a sensitive indicator of exposure to estrogenic compounds. We have been studying the kinetics of Vg formation and excretion in rainbow trout with a goal towards developing an integrated pharmacokinetic–pharmcodynamic (PK–PD) model to quantitatively relate cumulative estrogenic exposure of fish to the expression and appearance of Vg in plasma. We administered graded doses of ethynylestradiol (EE2), *o,p*-DDT, DDD and DDE and octylphenol to male rainbow trout via a dorsal aortic cannula which allowed repetitive blood sampling from individual fish for up to 48 days after injection. The plasma concentrations of the xenobiotics and Vg were simultaneously quantified using ELISA and GC–MS or GC–ECD. In separate experiments, sexually mature trout were exposed to graded water concentrations of EE2 for 3 months and various parameters indicative of the functional status of the male reproductive

system determined. These parameters included tissue-somatic indices, histopathological evaluation, spermatocrit, sperm motility (quantified using computer-assisted-motion analysis) and viability of semen based on fertilization assays using eggs harvested from untreated trout. Results from fertilization assays indicated that 12 week exposure to EE2 concentrations of 10 and 100 ng/l caused a 50% reduction in the fertilization rate of semen harvested from exposed trout. PK–PD modeling strategies proved valuable tools for linking chemical exposures to Vg formation.

PII: S0141-1136(00)00173-2

Analysis of vitellogenin mRNA by quantitive reverse transcription polymerase chain reaction (RT–PCR) in juvenile fish exposed for 12 months to nonylphenol

K. Fent [a,*], G.E. Ackermann [a], J. Schwaiger [b]

[a] *Swiss Federal Institute for Environmental Science and Technology (EAWAG) and Swiss Federal Institute of Technology (ETH Zurich), CH-8600 Dubendorf, Switzerland*
[b] *Bavarian Institute for Water Research, Demollstrasse 32, D-82407 Wielenbach, Germany*

Abstract

Nonylphenol (NP) is a degradation product of surfactants that enter aquatic systems mainly via sewage treatment plants. It has been shown that the estrogenic activity of NP results in the induction of vitellogenin (VG) in male fish after short-term exposure, but the effects after long-term exposure to environmentally relevant concentrations remain unclear. Vitellogenin is a precursor of egg yolk proteins, synthesized in the liver under the control of the female sex hormone estradiol and transferred to the ovaries via the blood. Induction of VG can therefore serve as a biomarker for estrogenicity. In this study, rainbow trout eggs were exposed after fertilization to NP concentrations of 1 and 10 μg/l. Exposure occurred throughout the embryonic, larval and juvenile period under controlled laboratory conditions. After 12 months, induction of VG mRNA was analyzed in the liver by quantitative RT–PCR, and VG protein using polyclonal antibodies in Western blots. The development of quantitative RT–PCR included primer design, competitive PCR using heterologous standards and titration. Both VG mRNA and protein were induced in NP-exposed rainbow trout in a dose-dependent manner. In male fish, increases in VG mRNA and protein were already observed at 1 μg/l NP. This study shows that chronic exposure of fish early life stages to environmentally realistic concentrations of NP leads to induction of vitellogenin.

* Present address: Wacht 24, CH-8630 Rüti, Switzerland.

PII: S0141-1136(00)00174-4