DEVELOPING FISH ASSAYS FOR EVALUATING ENDOCRINE ACTIVE CHEMICALS AND PHARMACEUTICALS

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The European Chemicals Industry proposed an aquatic research programme to develop reliable screening and testing methods for evaluating endocrine active chemicals (EACs) in freshwater fish. The fathead minnow (*Pimephales promelas*) was the chosen test species because, in the US and Europe, it is commonly used for the assessment of toxicity in chemical regulation. It has also been widely employed in studies on endocrine disrupting effects of chemicals and pharmaceuticals. As part of this programme, 21-day screening assays for EACs have been successfully developed, using mixed sex juvenile and sub-adult male and female fish. These assays have been validated using a range of reference chemicals including (anti-)oestrogenic, (anti-)androgenic and aromatase inhibiting substances and employed a suite of easily measured endpoints, including gonad growth, secondary sexual characteristics (SSCs) and vitellogenin (VTG) concentrations. Current research is based on the development of fish chronic tests for EACs that provide alternatives to the fish full life-cycle test. Two tests have been proposed that focus on life periods of heightened sensitivity for endocrine disruption. The first of these is a development test, which focuses on the period of sexual differentiation in fish. The second is a reproduction test, which will detect adverse impacts on pair-breeding adult fish (and thus capture both direct and physiological impacts on gamete formation and effects on reproductive behaviour). Embryos will

also be maintained in dilution water for <90 days post hatch (dph), to provide information on transgenerational effects, which may occur in the F1 generation.

The development test has been evaluated using the weak oestrogen 4-*tert*-pentylphenol (4TPP). Fathead minnow embryo-larvae were continuously exposed to 4TPP in flow-through systems for <107dph, at 25±1°C. The exposure protocol followed OECD test guideline 210, but was extended to allow the evaluation of gonadal development, SSCs, gonadosomatic index (GSI) and plasma VTG. Nominal (mean measured) test chemical concentrations of 4TPP were 56 (57), 180 (188) and 560 (599) μg l^{-1}, with fish sampled after 30, 60 or 107 days post-hatch (dph). In addition, fish were also exposed to 180 μg 4TPP l^{-1} for periods of 0-30 or 0-60 dph, before being transferred to dilution water for the remainder of the study (107 dph). Fish sampled at 30, 60 and 107 dph, were evaluated for growth and gonadal histology. In addition, at 107 dph fish were also evaluated for SSC, GSI, and plasma VTG. At 60 dph delays in sexual differentiation and development were observed in exposed fish. At 107 dph, effects were seen on sexual differentiation with a bias towards the female gender in the exposure treatments. All fish in the highest 4TPP concentration were female and in these fish the GSI was reduced compared with the controls. In females, condition factor was also significantly reduced at concentrations at and above 56 μg 4TPP l^{-1} (180 μg 4TPP l^{-1}continuous exposure treatment only). There was no dose-related induction of VTG.

The development test is presently being evaluated using 4TPP.