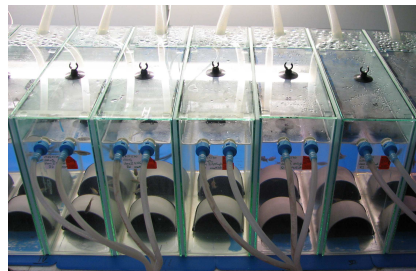


The 21-day OECD Fish Screening Assay with the Fathead Minnow and three Model Substances

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Conclusions

Species selection

Male Fathead minnows guard the spawning ground and are strongly territorial during the spawning period. This behaviour stresses the animals on top of possible stress of a chemical. Feasibility of the testing design should be better with non-territorial species.

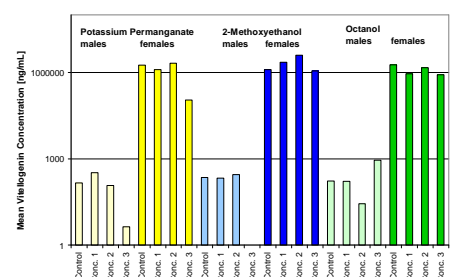
Mortality

High mortality reduces the data for endocrine endpoints. Therefore selection of sublethal concentrations is necessary to achieve meaningful results.

Evaluation of Endpoints

Overall, the histological assessment gave the most detailed information about the status of the fish. However, for screening purposes histology seems to need too much efforts.

Regarding the limited results of these studies, the vitellogenin content seems to fit best to the results of the gonadal histology. Also the expectations for the model substances (no endocrine effects) are reflected mostly by the vitellogenin contents.



Apart from the vitellogenin content and the histological assessments the other endpoints showed partially unexpected results compared to the control. E.g. fish exposed to Potassium permanganate showed reduced spawning, decreased number and score of tubercles and loss of territorial behaviour. These effects seem to result from a toxic potential of the substances rather than from a primary endocrine effect, as the histological assessment indicated.

Introduction

The 21 day fish screening assay to detect endocrine active substances has recently been developed within the OECD. IBACON was asked to contribute to the validation of this method by performing three studies in the Fathead minnow (*Pimephales promelas*) using substances at given concentrations which do not affect the endocrine system. One of those "negative substances" was a toxicant known to damage testis via a non-endocrine mode of action.

Substances

Potassium permanganate (0.1125, 0.225, 0.45 mg/L), should not affect the endocrine system of fish.

Octanol (0.32, 1.0, 3.2 mg/L), should not affect the endocrine system of fish.

2-Methoxyethanol (1.0, 10, 100 mg/L), should have toxic effects on fish testis. The assessment of this substance should clarify, if non-endocrine active substances could mimic endocrine-like effects.

Fish Species and Design

Sexually mature Fathead minnow (*Pimephales promelas*).

Four replicates with two male and four female fish were exposed for 21 days in a flow-through test system.

Endpoints

Daily assessments:

- mortality
- symptoms of intoxication
- days of spawning, number of eggs
- secondary sex characteristics

Assessments at the end of the study:

- body wet weight and total length
- number of nuptial tubercles
- plasma vitellogenin
- histopathology of gonads

Results

Potassium permanganate

0.45mg/L of this substance caused more than 50% mortality and a decrease of nuptial tubercles. Vitellogenin content in the male's plasma was also reduced. Spawning and number of eggs per female were reduced at the two highest concentrations.

Vitellogenin concentrations in females were not affected.

The test substance was not stable but formed MnO₂ and sub-sequently degraded to pyrolusit building brown deposits. Accordingly the substance is supposed to have caused physical damages to the fish (e.g. gills).

Octanol

Octanol did neither cause significant mortality to the fish nor changes of the secondary sex characteristics. Substance related effects on spawning, number of eggs per female, on plasma vitellogenin and histomorphology of gonads were also not observed. However, Octanol underwent a rapid biodegradation in the flow-through system which made it necessary to clean the aquaria twice a day. It is obvious that these are not the optimal conditions investigating territorial fish at their spawning ground.

Methoxyethanol

The recommended test concentrations of nominal 10 and 100 mg test item/ L were too high and resulted in intoxication and high mortality. Effects on secondary sex characteristics or fecundity were not observed down to the lowest test concentration (1mg/L). As a consequence of the intoxication and mortality, the analysis of the toxic effect of 2-Methoxyethanol to the gonads of the test fish was limited.