
**"Fish, Prolonged Toxicity Test:
14-day Study"**

• Performance of the test

If a vehicle is used in the preparation of the stock solution of the test substance, it is necessary to run, in addition to the control group, a control group of fish exposed to the highest concentration of the vehicle used in the test.

In the flow-through test, the concentration of the substance in the test solution may be determined at the beginning of the test; in the semi-static test at the beginning, immediately prior to the first renewal of the test solution and at the termination of the test. Appropriate procedures other than analysis for giving evidence that adequate concentrations of the test substance have been maintained can also be used.

Conditions of exposure

Duration:	Normally 14 days, but can be extended by one or two weeks.
Tanks:	Of suitable capacity in relation to the recommended loading.
Loading:	For semi-static tests maximum loading of 1.0 g fish/litre is recommended; for flow-through systems higher loading can be acceptable.
Number of animals:	At least 10 for each concentration and control.
Test concentrations:	The test concentrations chosen must permit the determination both of the threshold levels for the lethal and other observable effects and of the NOEC value. Concentrations of the substance in excess of 100 mg/l need not be tested if a threshold level has not been reached up to this concentration.
Water:	Drinking water supply (dechlorinated if necessary), good quality natural water or reconstituted water (see Test Guideline 203). Waters with a total hardness of between 50 and 250 mg of CaCO ₃ per litre, and with a pH 6.0 to 8.5 are preferable.

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The reagents used for the preparation of the dilution water should be of analytical grade and the deionised or distilled water should be of conductivity equal to or less than $10 \mu\text{Scm}^{-1}$.

- Light:** 12 to 16 hours photoperiod daily.
- Temperature:** Appropriate to the species (see Test Guideline 203) constant within $\pm 2^\circ\text{C}$.
- Oxygen concentration:** Not less than 60 per cent of the maximum air saturation value throughout the test.
- Feeding:** Either several times daily (the quantity of feed administered should not exceed the amount ingested immediately by the fish) or daily (the quantity of food being kept constant - e.g. 2 per cent dry weight related to the initial fish weight).
- Cleaning:** Inside surfaces of the test tank in the flow-through test must be cleaned if necessary and the remaining excrement removed, at least twice weekly; in the semi-static test the test tank is replaced by a clean one each time the water is changed.

Observations

Observed effects are defined as follows:

Lethal effects: a fish is presumed to be dead if no respiratory movement and no reaction to a slight mechanical stimulus can be detected.

Effects other than lethal effects: these include all effects observed on the appearance, size and behaviour of the fish that make them clearly distinguishable from the control animals, e.g. different swimming behaviour, different reaction to external stimuli, changes in appearance of the fish, reduction or cessation of food intake, changes in length or body weight.

The fish are inspected at least once a day for mortality. Dead fish are removed when observed and mortalities are recorded.

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It is desirable that daily records be kept of all observed effects, but a minimum of three observation sessions per week must be conducted.

Measurements of pH, dissolved oxygen and temperature must be carried out at least twice a week.

Representative samples of the test population should be weighed and measured before the test starts. All survivors should be weighed and measured at the termination of the test. Fish should not be weighed or measured during the test as unnecessary handling may lead to damage and/or mortality.

3. DATA AND REPORTING

- Interpretation of results

If it is observed that the stability or homogeneity of the test solutions cannot be maintained, care should be taken in the interpretation of the results and note made that these may not be reproducible.

- Test report

The test report should include the following information:

Test substance: chemical identification data.

Test organisms: scientific name, strain, size, supplier, any pretreatment, etc.

Test conditions:

- test procedure used (e.g. semi-static or flow-through, aeration, fish loading, etc.)
- water quality characteristics (treatment, including dechlorination, dissolved oxygen concentration, pH, hardness, temperature, any other information available)
- dissolved oxygen concentration, pH values, temperature and total hardness of the test solutions at each of the recommended observation times

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- methods of preparation of stock and test solutions
- concentrations used
- information on the maintenance of the concentration of the test substance in the test solutions
- number of fish at each test concentration

Values from the fish acute toxicity test

Results:

- observed effects at each concentration for each observation time in tabular form
- concentrations that produce lethal or other effects can be presented graphically with respect to time
- Threshold level of lethal effect
- Threshold level of observed effects
- NOEC
- cumulative mortality at each concentration and for each recommended observation time if possible
- mortality in the controls
- behavioural observation of the fish
- incidents in the course of the test which might have influenced the results
- any deviation from the Test Guideline

4. LITERATURE

1. D.M.M. Adema, in *Degradability, Ecotoxicity and Bioaccumulation*, Chapter 5, Government Publishing Office, The Hague (1980).
2. R. Bathe, *Arch. Toxicol. Suppl. 2*, 417-423 (1979).

