THAMNOTOXKIT F

FRESHWATER TOXICITY SCREENING TEST

BENCH PROTOCOL

Principle :

The ***Thamnocephalus* toxicity test-kit** contains all the materials to perform standardized, simple and cost-effective bioassays for screening toxicity in freshwater. Using Instar II-III larvae of the fairy shrimp *Thamnocephalus platyurus* hatched from cysts, an acute toxicity test is executed in 24 hours. Each THAMNOTOXKIT F provides for 6 complete tests (range-finding or definitive 24h-LC_{50}), or 5 bioassays and 1 quality control test with a reference toxicant.

1. Preparing Standard Freshwater :

Fill a 1 liter volumetric flask with approximately 800 ml deionized water and add the contents of the five* vials of concentrated salt solutions, in the sequence 1 to 4 as indicated on the flasks. Add deionized water up to the 1000 ml mark and shake to homogenize the medium.
** Note that there are 2 vials with CaSO4, both of which must be used!*

2. Storing the medium :

The 1 liter solution of Standard Freshwater suffices for the 6 bioassays of each Toxkit. If all 6 tests are not carried out within a few days after preparation of the medium, store the Standard Freshwater in the refrigerator in darkness. In the latter case, the contents should preferably be distributed between several flasks, for separate use. Take care to bring the cooled medium (gradually) back to room temperature prior to use.

3. Hatching the *Thamnocephalus* cysts :

3.1. Preparation of the hatching medium

The hatching medium is prepared by transfering 2.5 ml Standard Freshwater into a vial and adding 17.5 ml deionized water (i.e. dilution 1:8).

3.2. Hatching of the cysts

The hatching of the *Thamnocephalus* cyst should be initiated 24 hours before the start of the toxicity test.

Cyst prehydration

Open a tube with cysts and fill it with hatching medium (approx. 1 ml). Close the tube and shake it at regular intervals for approx. 30 minutes.

3.3. Transfer of prehydrated cysts into the hatching petri dish

Put 10 ml hatching medium into a small petri dish and empty the contents of the vial with prehydrated cysts into this petri dish; make sure most of the cysts are transferred by rinsing the tube with hatching medium. Swirl the petri dish gently to distribute the cysts evenly. Cover the hatching petri dish and incubate **at 25°C for 20-22 hours**, under continuous illumination (light source of 3000-4000 lux).

4. Preparing the Toxicant Dilution Series :

Prepare a dilution series of the test compound or effluent according to standard methods (e.g. USEPA, 1985).

5. Filling the Test Plate :

The bioassay is conducted in a disposable multiwell test plate with 24 (6 x 4) test wells. The wells are labelled as columns 1 to 6 across, and rows A to D down (see figure).
The distribution of the test solutions should always be carried out starting from the control (column 1, left) towards the highest concentration (column 6, right). To fill the control column, add 1 ml Standard Freshwater to the four wells of column 1. Repeat this procedure for the other columns with the respective toxicant concentrations, progressing from low to high concentrations in columns 2 to 6.

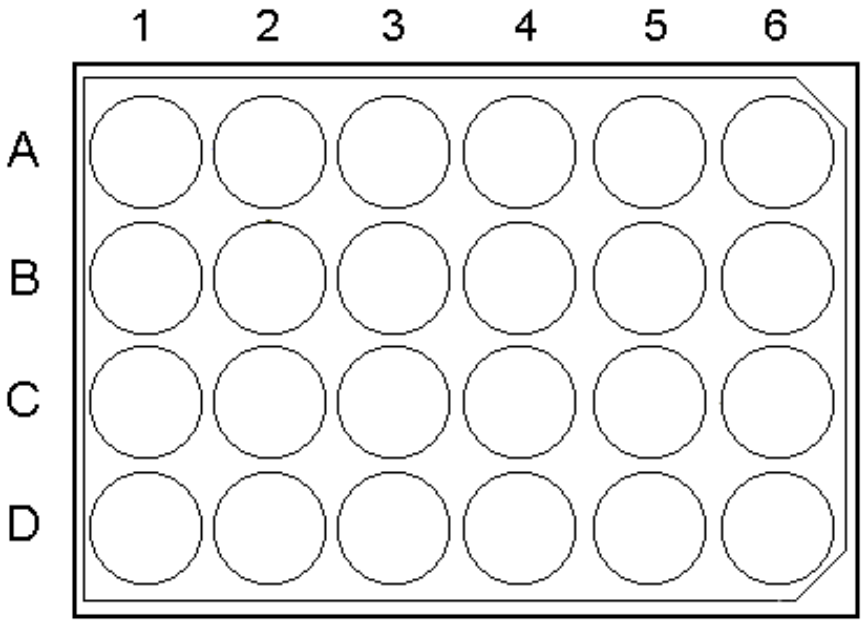

Figure: Multiwell test plate composed of 6 x 4 wells; the 6 wells of row D serve as rinsing wells.

6. Adding the larvae :

Using a dissection microscope at magnification 10-12x, transfer approximately 50 instar II-III larvae with a micropipet from the transfer petri dish to each well in row D (rinsing wells*) of the multiwell plate. Subsequently transfer 10 larvae from the rinsing well of column 1 to the three wells of this column. Take care, during this operation, to minimize the transfer of medium along with the larvae. Repeat this operation for columns 2 to 6.

* *The intermediate passage of the fairy shrimp larvae from the petri dish to the definitive test wells via rinsing wells "washes" the larvae in the appropriate test solution before they enter the actual test well, thus minimizing dilution of the test solution during transfer.*

The test design of the THAMNOTOXKIT **is based on one control and five toxicant concentrations, each with 3 replicates of 10 animals. Each bioassay shall be performed in a new multiwell with a new micropipet.**

7. Incubating the Test Plate and Scoring the Results :

Put a strip of Parafilm on the test plate, cover it and incubate at 25°C in darkness. **After 24 hours**, count the dead* larvae in each test well and fill out the results sheet.
Calculate the % mortality** and, for the definitive tests, the 24h-LC_{50} using any standard method (e.g. USEPA, 1985).

* *Larvae are considered dead if they do not exhibit any internal or external movement in 10 seconds of observation.*
** *For the THAMNOTOXKIT test to be valid, control mortality should not exceed 10%*

8. Reference test

It is recommended that every 5 to 10 assays, a quality control test be carried out to check proper adherence to the test protocol, as well as the test sensitivity.

Such a quality control test can e.g. be performed with the reference chemical potassium dichromate ($K_2Cr_2O_7$).
When performing this quality control test, the 24h LC_{50} should be within the 95% confidence limits stipulated in the specification sheet.

Preparation of stock solution and dilution series of the reference chemical
Add 100 mg of potassium dichromate to 100 ml of deionized water to make a 1000 ppm stock solution. Make a dilution series 0.32 - 0.18 - 0.10 - 0.056 - 0.032 mg/l for the quality control test.