CERIODAPHTOXKIT F

TOXICITY SCREENING TEST

BENCH PROTOCOL

Principle:

The **Ceriodaphtoxkit F** contains all the materials, including the test species ***Ceriodaphnia dubia*** in the form of "dormant eggs (ephippia)", to perform 6 complete acute toxicity tests. The tests make use of the "neonates" which are hatched in about 80 hours from the eggs.

1. Preparation of Standard Freshwater (*EPA formula for moderately hard water*) as hatching and dilution medium:
Fill a 1 liter volumetric flask with approximately 800 ml deionized (or distilled) water and add the contents of one of the 5* vials of concentrated salt solutions, in the sequence 1 to 4 (as indicated on the labels). Add deionized (or distilled) water up to the 1000 ml mark and shake to homogenize the medium.

* *Note that there are 2 vials with $CaSO_4.2H_2O$, both of which must be used !*

2. Storage of the medium:
If the 6 tests are not carried out within a few days after preparation of the medium, store the Standard Freshwater in the refrigerator in darkness. Take care to bring the cooled medium (gradually) back to room temperature prior to use.

3. Hatching of the ephippia:
Hatching of the ephippia should be initiated 80 hours prior to the start of the toxicity test.

Pour the contents of one vial of ephippia into the microsieve and rinse thoroughly with tap water to eliminate all traces of the storage medium. Transfer the ephippia into the hatching petri dish in 15 ml Standard Freshwater (one can also use a 10 cm diameter dish with 50 ml Standard Freshwater), pre-aerated by air bubbling. Cover the petri dish and incubate for 80 hours, at 25 °C, under continuous illumination of min. 6000 lux.

The largest percentage of hatching will occur after about 80h incubation. Since standard testing procedures indicate that the neonates should not be older than 24h at the start of the toxicity test, the young Daphnids must be collected at the latest about 100h after the start of the incubation.

4. Preparation of the toxicant dilution series:
Dilution series of the test compound or effluent should be prepared according to standard procedures. A minimum volume of 5 (preferably even 10) ml shall be prepared for each toxicant dilution.

5. Filling the Test Plate:

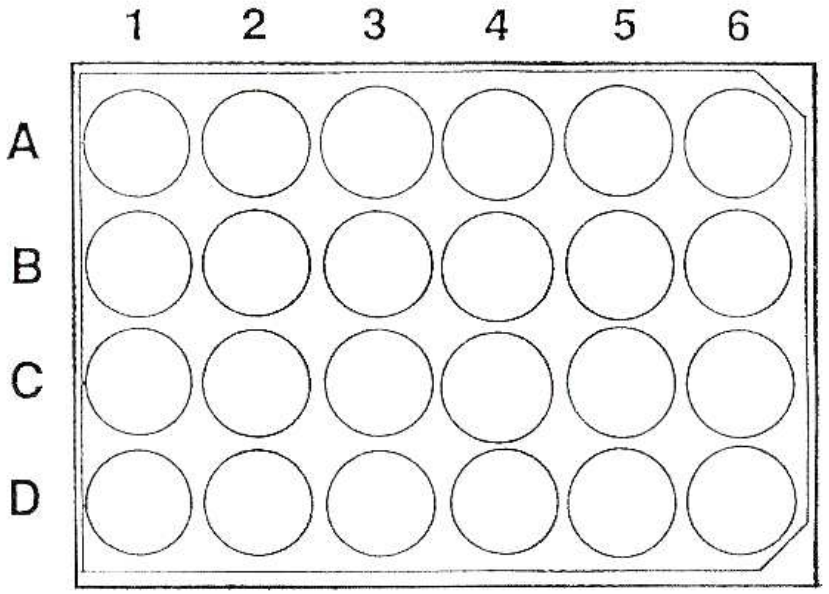

Figure: Multiwell test plate composed of 6 x 4 wells; the 6 wells of row D serve as rinsing wells.

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.

6. Adding the test organisms:
Using a dissection microscope (at magnification 10-12x) or a light table and a magnifying glass (6-8x), transfer approximately 35-40 neonates with a micropipette from the petri dish to each well in row D (rinsing wells*) of the multiwell plate. Subsequently transfer 10 test organisms 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 *Ceriodaphnia*. Repeat this operation for columns 2 to 6.

* *The intermediate passage of the test organisms from the petri dish to the definitive test wells via rinsing wells "washes" the Ceriodaphnia 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 CERIODAPHTOXKIT **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 micropipette.**

Important remark :

SURFACE FLOATING
Daphnids are quite susceptible to being trapped at the surface of the liquid medium in the test wells, by "surface tension" phenomena. "Floating" test organisms may die and hence jeopardize the outcome of the bioassay. In order to avoid "surface floating", it is important, during the transfer of the neonates, to put the tip of the micropipette into the medium and not to drop them onto the surface of the medium in the wells.

7. Incubation of the test plate and scoring of the results:
Put the Parafilm strip and the cover on the multiwell plate and incubate in darkness at 25 °C.
After 24h incubation, put the test plate under the dissection microscope or on the transparent stage of the light table and determine the number of dead test organisms.

Test organisms are considered dead if they do not exhibit any internal or external movement in 10 seconds of observation.

Score the data on the Results Sheet and calculate the % mortality and the 24hLC_{50}, using any standard data processing method.

8. Validity of the test:
Besides all other specific validity conditions prescribed in standard Daphnia bioassay protocols, the number of dead organisms in the controls should not exceed 10%.

9. Reference test:
In order to check the correct execution of the test procedure and the sensitivity of the test animals, it is advised to perform a reference test from time to time.
Quality control tests can e.g. be performed with the reference toxicant potassium dichromate ($K_2Cr_2O_7$), using the following dilution series : 1.8 - 1 - 0.56 - 0.32 - 0.18 mg/l.
The 24h LC_{50} in the quality control should be situated within the limits stipulated in the specification sheet of the Ceriodaphtoxkit.