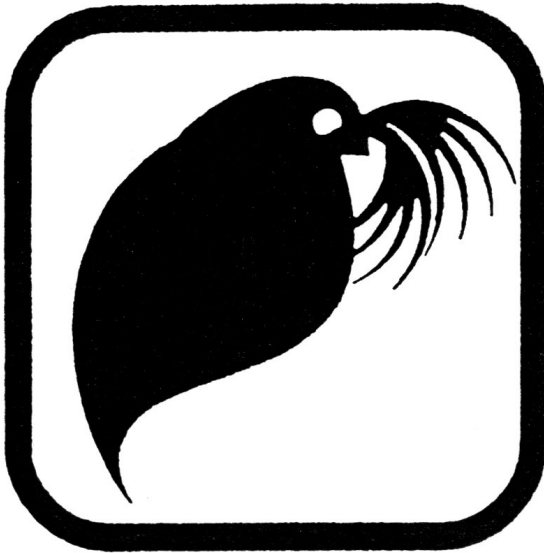


# DAPHTOXKIT F *MAGNA*

Crustacean Toxicity Screening Test  
for Freshwater



STANDARD OPERATIONAL  
PROCEDURE

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# INTRODUCTION TO THE DAPHTOXKITS

## **Origin :**

The screening bioassays with the daphnid species *Daphnia magna* and *Ceriodaphnia dubia* have been developed by the research teams of Prof. Dr. G. Persoone at the Laboratory for Biological Research in Aquatic Pollution (LABRAP\*) at the Ghent University in Belgium.

\* *The Laboratory has been renamed Laboratory for Environmental Toxicology and Aquatic Ecology (LETAE)*

## **Scope :**

TOXKITS are microbiotests containing all the materials (including the test organisms) necessary to perform simple, rapid, sensitive and reproducible toxicity tests at low cost. Toxkit tests are particularly suited for routine toxicity testing of chemicals and wastes released in aquatic as well as in terrestrial environments.

## **Advantages of Toxkit tests :**

The major advantage of Toxkit microbiotests, in comparison to conventional bioassays, is that the test organisms are incorporated in the kits in a "dormant" or "immobilized" form, from which they can be activated "on demand", prior to performance of the toxicity test.

*This eliminates the need for continuous recruitment and/or stock culturing of test organisms and hence the major practical burden and cost factor.*

*All Toxkits tests have been "miniaturized" into practical and user friendly microbiotests which can be performed with conventional lab materials and equipment, on little bench space .*

## **Biological characteristics of the Daphtoxkits :**

The Daphtoxkits use the dormant eggs (ephippia) of the crustaceans *Daphnia magna* or *Ceriodaphnia dubia*, which are used worldwide for toxicity testing. These eggs are protected by a chitinous capsule called ephippium, and can be stored for long periods of time without losing their viability. When the ephippia are placed in specific environmental conditions and triggers, the eggs develop in about 3 days of time into neonates which can then be used immediately for the toxicity tests.

**Principle of the Daphtoxkits :**

24h to 48h EC<sub>50</sub> (or LC<sub>50</sub>) bioassays are performed in disposable multiwell test plates departing from neonates, uniform in size and in age, hatched from ephippia.

**Test methodology :**

Daphtoxkit tests are performed in accordance with test procedures prescribed by national and international organizations (e.g. OECD, ISO, EEC, USEPA, ASTM).

**Features :**

Each Daphtoxkit contains all the (disposable) materials to perform 6 complete bioassays (range finding or definitive 24-48h EC<sub>50</sub>). The only equipment needed is an incubator or a temperature controlled room at 20-25°C, a small light table or a dissection microscope, and conventional laboratory glassware.

**Sensitivity :**

Comparative research in several laboratories in different countries has shown that the sensitivity of the bioassays performed with Daphtoxkits is similar to that of the conventional toxicity tests carried out with neonates from stock cultures.

**Precision :**

Since Daphtoxkits contain standard test materials (and biomaterials), the repeatability of these microbiotests is very high.

**Shelf life :**

The ephippia must be stored in darkness, at 5 °C (+/- 2 °C) to keep their viability. The hatching success of the ephippia kept in the former conditions is guaranteed for several months as indicated on the expiry date label on each kit.

**Representativity :**

Like rotifers and copepods, cladocerans are ecologically very important members of freshwater aquatic communities. Daphnia's are the most commonly used crustacean test species for determination of the effects of xenobiotics on primary consumers in freshwater aquatic ecosystems.

## CONTENTS OF THE DAPHTOXKIT F MAGNA

### Vials with ehippia :

Six 1 ml plastic tubes covered by aluminium foil, containing ehippia of *Daphnia magna*, to be stored in a refrigerator at 5 °C (+/- 2 °C) until use. The number of neonates obtained from each vial suffices for one full toxicity test.

### Concentrated salt solutions :

Two sets of 4 small glass bottles, each containing a concentrated solution of one salt, to make up 2 x 2 litres Standard Freshwater (ISO medium, formula according to ISO 6341) with deionized or distilled water, for preparation of the hatching and toxicant dilution medium.

#### Composition :

vial 1 : NaHCO<sub>3</sub> (129.5 mg - dissolved in 2 l. = 64.75 mg/l)

vial 2 : CaCl<sub>2</sub>.2H<sub>2</sub>O (588 mg - dissolved in 2 l. = 294 mg/l)

vial 3 : MgSO<sub>4</sub>.7H<sub>2</sub>O (246.5 mg - dissolved in 2 l. = 123.25 mg/l)

vial 4 : KCl (11.5 mg - dissolved in 2 l. = 5.75 mg/l)

### Petri dishes :

Six polystyrene petri dishes of 5 cm diameter, for the hatching of the ehippia.

### Multiwell test plates :

Six polycarbonate test plates composed of 6 rinsing wells and 24 wells for the toxicant dilutions.

### Vials with Spirulina powder :

Six 1 ml plastic tubes containing a small amount of Spirulina powder for "pre-feeding" the test organisms prior to the toxicity test.

### Parafilm strips :

Six Parafilm strips for sealing the multiwell plates to minimize evaporation during the incubation period.

### Micropipettes :

Six polyethylene micropipettes for transfer of the test organisms.

### Microsieve :

A small sieve with 100 µ mesh, for rinsing of the ehippia.

Standard Operational Procedure manual :

A detailed brochure with all instructions for performance of range finding and/or definitive assays on pure chemicals or wastes.

Bench protocol :

An abbreviated version of the Standard Operational Procedure manual.

Results sheets :

Six sheets for scoring the results and calculation of the mean effect percentages.

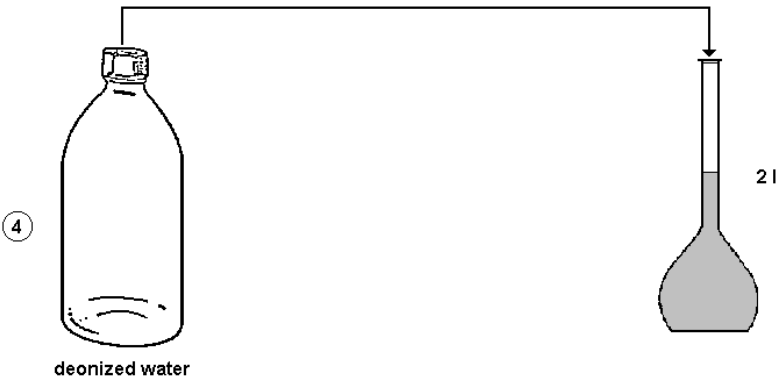
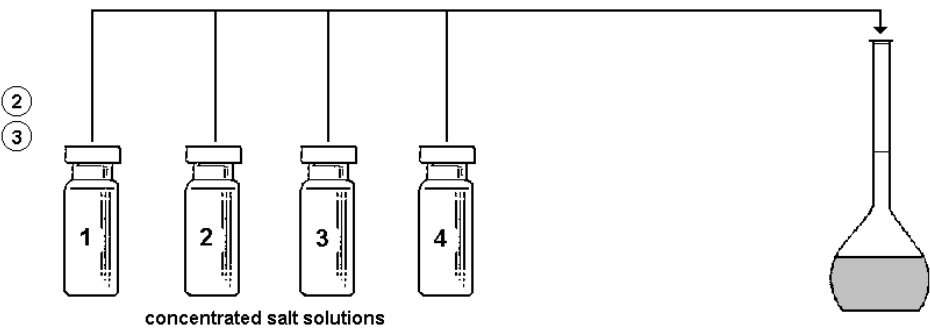
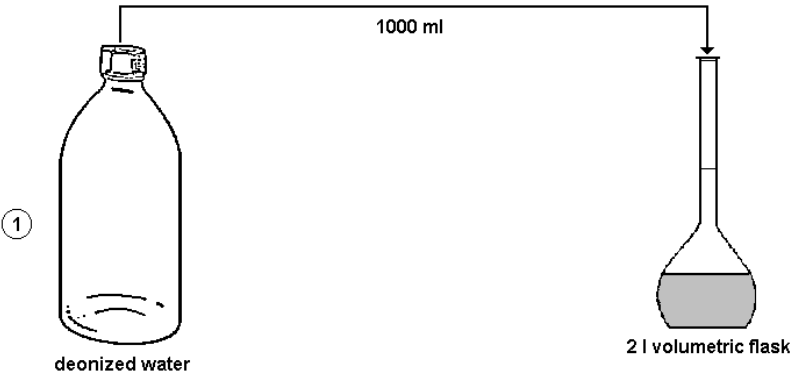
Specification sheet :

A sheet indicating the batch number and shelf life of the ehippia, the batch number of the concentrated salt solutions, the expiry date of the Daphtoxkit and the 24-48h EC<sub>50</sub> values for the reference chemical potassium dichromate.

*All the non-biological materials provided in the Daphtoxkit F magna are made of inert, non-toxic products.*

*These materials are disposable and should only be used once.*

# PREPARATION OF STANDARD FRESHWATER



## 1. PREPARATION OF STANDARD FRESHWATER

General remark : The solutions described hereunder are prepared with distilled water or deionized water. To avoid repetition, only the wording "distilled water" will be used further on.

*The **Daphtoxkit F magna** contains 2 sets of 4 vials with concentrated salt solutions to prepare 2 x 2 litres of a "reconstituted" natural freshwater, according to the formula (see page 4) recommended by the International Standardization Organization (ISO) for the acute toxicity test with *Daphnia magna* (ISO 6341).*

The Standard Freshwater will be used as hatching medium for the ephippia and as dilution medium for preparation of the toxicant dilution series.

### Procedure for the preparation of 2 litres Standard Freshwater :

(see figure)

1. Fill a 2000 ml volumetric flask with approximately one liter distilled water.
2. Uncap vial labelled number 1 ( $\text{NaHCO}_3$ ), and pour the contents in the flask.
3. Repeat this operation for the 3 other vials : vial 2 ( $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ ), vial 3 ( $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ ) and vial 4 (KCl), respecting this sequence.
4. Add distilled water up to the 2000 ml mark; stopper the flask and shake to homogenize the medium.

*N.B. a "double strength" Freshwater can be prepared in a 1 litre volumetric flask, with subsequent dilution by half at the time of using the medium.*

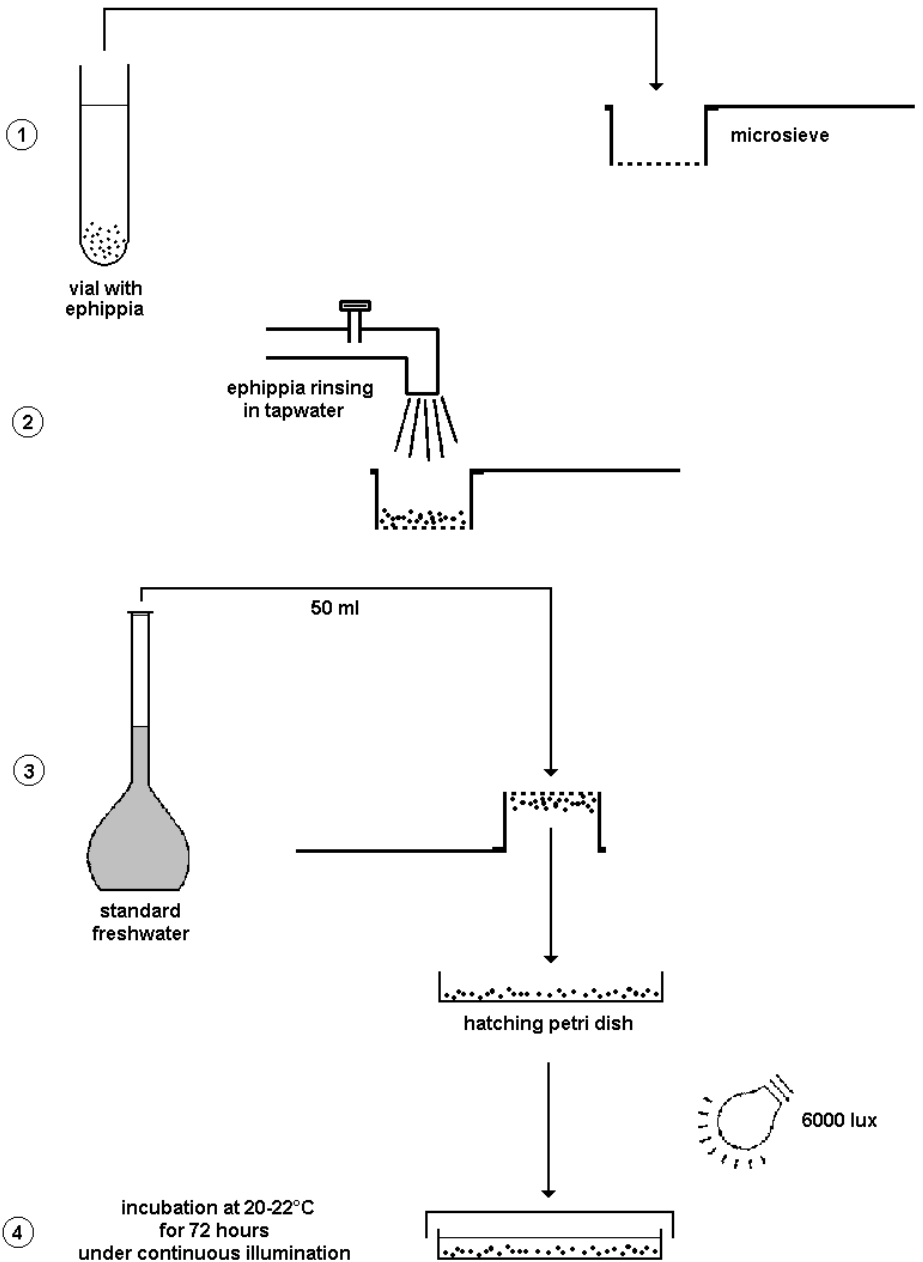
## 2. STORAGE OF THE HATCHING AND DILUTION MEDIUM

*Two litres Standard Freshwater suffice for performance of 3 complete *Daphnia magna* bioassays.*

*If the 3 tests are not carried out within a few days after preparation of the Standard Freshwater, the medium should be stored in the refrigerator in darkness. Take care to bring the cooled medium (gradually) back to room temperature prior to use.*



# HATCHING OF THE EPHIPPIA



### 3. PRE-AERATION OF THE STANDARD FRESHWATER

*The Standard Freshwater must be aerated for at least 15 minutes prior to use it for the hatching of the dormant eggs and for the preparation of the toxicant dilutions. Pre-aeration can be performed very easily by air bubbling through a tube connected to an aquarium air pump.*

### 4. HATCHING OF THE EPHIPPIA

Hatching of the ephippia must be initiated 3 days prior to the start of the toxicity test (see box "Important considerations with regard to ephippia hatching").

**Procedure** (see figure) :

1. Pour the contents of one vial with ephippia into the microsieve; make sure that all the ephippia are transferred.
2. Rinse the ephippia thoroughly with tap water to eliminate all traces of the storage medium.
3. Transfer the ephippia into the hatching petri dish in 15 ml pre-aerated Standard Freshwater (one can also use a 10 cm diameter petri dish with 50 ml pre-aerated Standard Freshwater).
4. Cover the hatching petri dish and incubate for 72h, at 20-22°C under continuous illumination of min. 6000 lux (light intensity at the top of the petri dish).

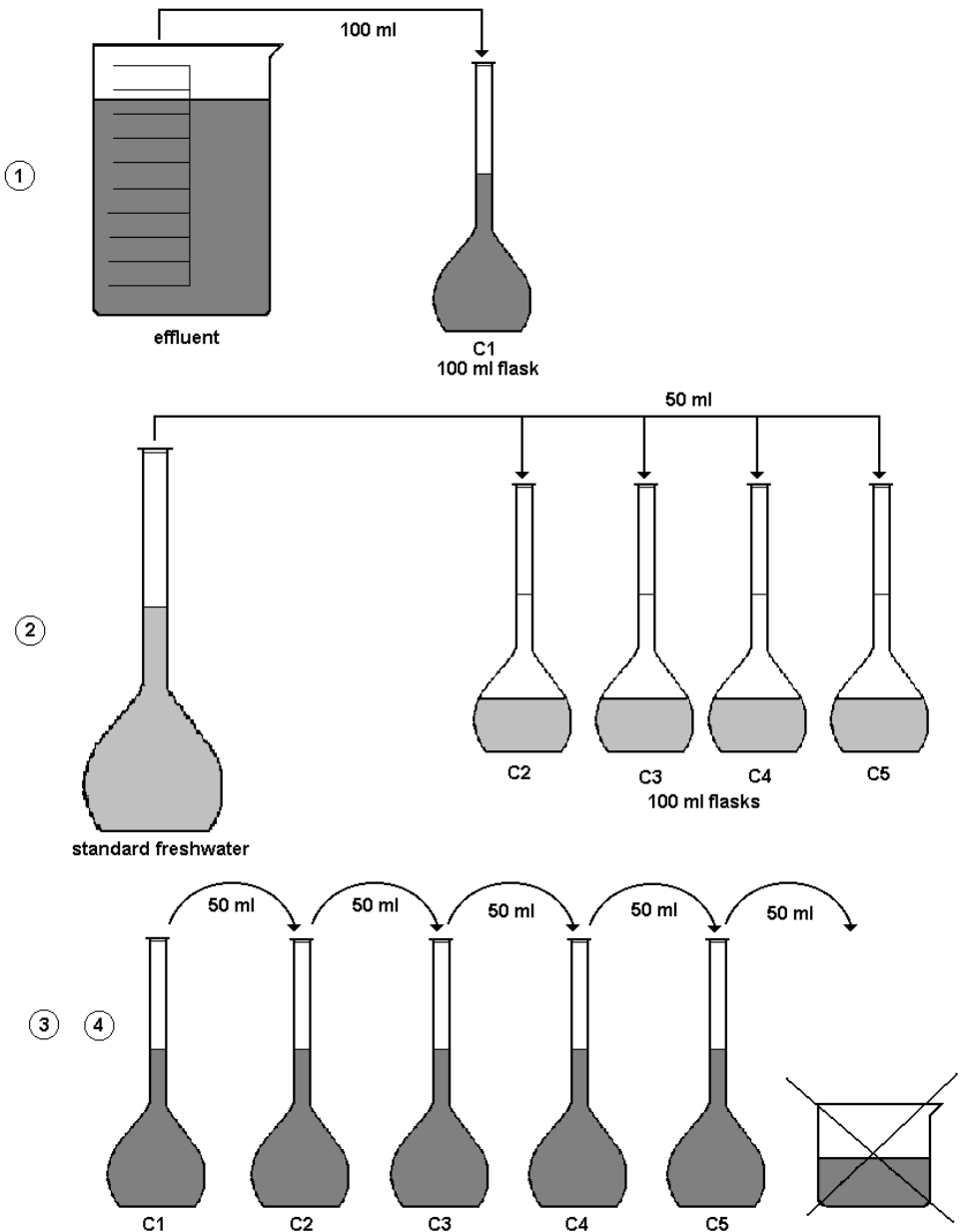
#### IMPORTANT CONSIDERATIONS WITH REGARD TO EPHIPPIA HATCHING

The embryonic development of *Daphnia magna* eggs takes about 3 days in optimal conditions. Under the illumination and temperature conditions indicated above (i.e. 6.000 lux and 20-22°C) the first neonates may even appear before 72h incubation, but the largest hatching will occur between 72h and 80h of incubation.

Taking into account that a minimum of 120 neonates are needed to perform one complete test and that the neonates should not be older than 24h at the start of the toxicity test ***the organisms must be collected at the latest 90h after the start of the incubation.***

# I - TEST ON EFFLUENTS

## preparation of 1 : 1 dilution series



## 5. PREPARATION OF THE TOXICANT DILUTIONS

### 5.1. TESTS ON EFFLUENTS

General remark : *The procedure described below for the testing of effluents (or liquid wastes) can also be applied to any other type of contaminated liquids such as e.g. surface waters, ground waters, sediment pore waters, soil or waste leachates etc.*

#### Preparation of toxicant dilutions

*A dilution series (100% - 50% - 25% - 12.5% and 6.25%) of the effluent sample is prepared by serial 1:1 dilution with Standard Freshwater.*

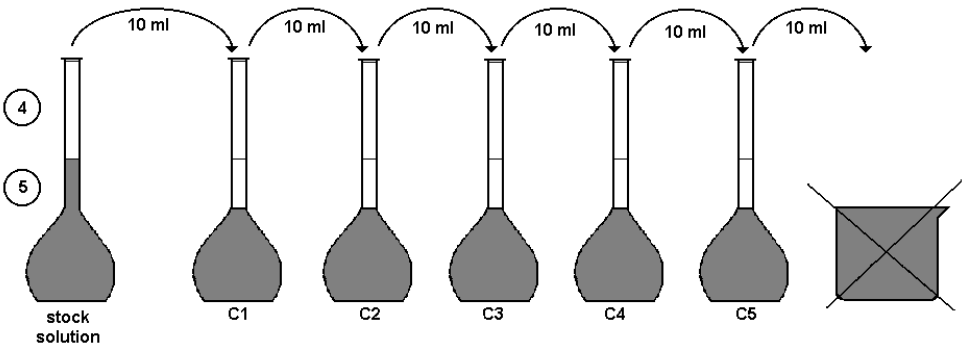
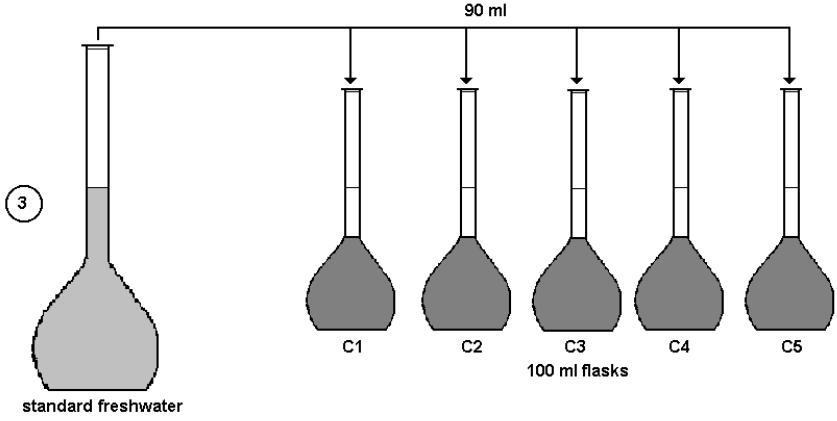
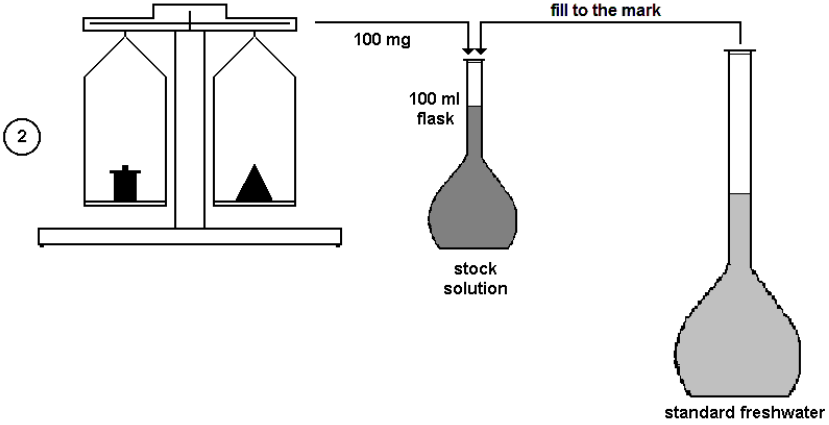
1. Take five 100 ml calibrated flasks and label them from C1 to C5.  
C1 is the non-diluted effluent and C5 the highest dilution (see Table 1)
2. Fill flask C1 to the 100 ml mark with effluent.
3. Put 50 ml Standard Freshwater into flasks C2, C3, C4, and C5.
4. Transfer 50 ml from flask C1 into C2. Stopper and shake flask C2 thoroughly.
5. Repeat this procedure (step 3) for the next dilutions i.e. 50 ml from C2 to C3; 50 ml from C3 to C4 and 50 ml from C4 to C5
6. Proceed to section 7 : **Filling of the Test Plate.**

**Table 1 : Dilution series of the effluent**

Flask	Effluent concentration (in %)
C1	100
C2	50
C3	25
C4	12.5
C5	6.25

# II - TEST ON CHEMICALS

## range finding test



## **5.2. TESTS ON CHEMICAL COMPOUNDS**

If the approximate toxicity (order of magnitude) of the chemical compound to *Daphnia magna* is known, a **definitive test** can be performed immediately. If no such information is available, two consecutive assays must be performed :

- a) a **range finding test** to determine the 0-100% tolerance range of the *Daphnids* to the toxicant,
- b) a **definitive test** to determine the 50% effect concentration ( $EC_{50}$ )

### **A. RANGE FINDING TEST**

A dilution series 1:10 must be prepared. An example is given below for a concentration series ranging from 100 mg/l down to 0.01 mg/l.

**Procedure** (see figure) :

1. Take six 100 ml calibrated flasks and label them as follows : Stock solution - C1 - C2 - C3 - C4 - C5 (see Table 2).
2. Weigh 100 mg of the test chemical on an analytical balance.
3. Transfer the chemical to the Stock solution flask, fill the flask to the 100 ml mark with Standard Freshwater (to make up a 1 g/l concentration). Stopper the flask and shake to dissolve the chemical.
4. Put 90 ml Standard Freshwater into all the other flasks.
5. Transfer 10 ml Stock solution into flask C1 to make up the 100 mg/l concentration. Stopper and shake thoroughly to homogenize the contents.
6. Repeat the operation indicated in step 5 for flasks C2 to C5, i.e. : - 10 ml from C1 into C2 (10mg/l); 10 ml from C2 into C3 (1mg/l); 10 ml from C3 into C4 (0.1mg/l) and 10 ml from C4 into C5 (0.01mg/l)
7. Proceed to section 7 : **Filling of the Test Plate.**

**Table 2 : Dilution series of the chemical compound**

Flask	Chemical concentration (mg/l)
Stock solution	1000
C1	100
C2	10
C3	1
C4	0.1
C5	0.01

**B. DEFINITIVE TEST**

*The dilution series to be prepared spans the range of the lowest concentration producing 100 % effect and the highest one producing less than 10 % effect in the range finding test.*

*As shown in Table 3, this range can span one order of magnitude (case A) or two orders of magnitude (case B).*

The new concentration range to be tested will again be called C1-C5.

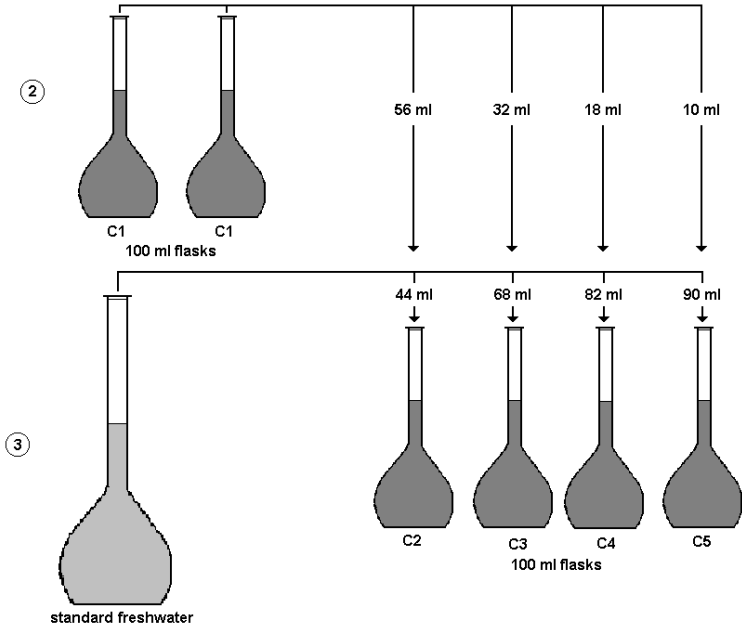
**Table 3 : Schematic presentation of the 100-0% effect concentration range determined in the range finding test**

<b>Case A</b>	<p style="text-align: center;">           100      100      0      0            - - - * - - - * - - - - - * - - - * - - -                                             C1      C5         </p>
<b>Case B</b>	<p style="text-align: center;">           100      100                                   0      0            - - - * - - - * - - - - - * - - - * - - - - - *                                             C1     C5         </p>

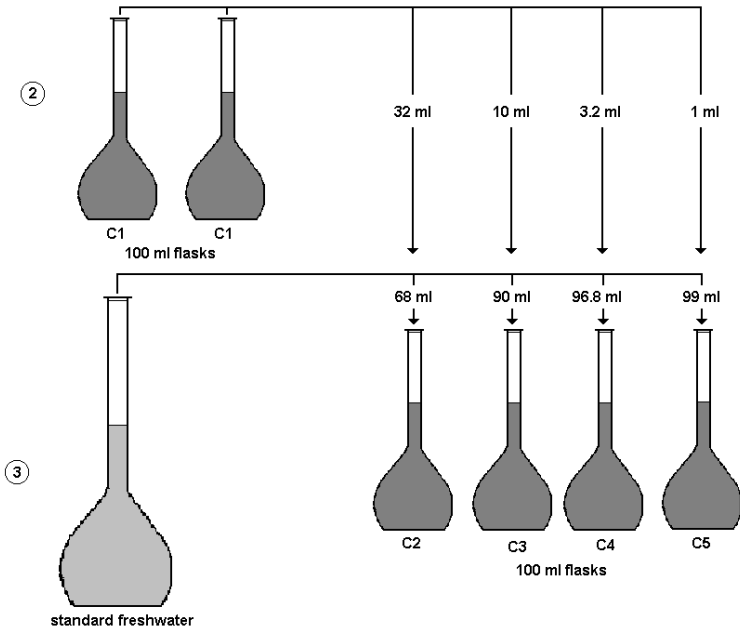
## II - TEST ON CHEMICALS

### definitive test

a) C1-C5 spans one order of magnitude



b) C1-C5 spans two orders of magnitude





## Case A. C1-C5 spans one order of magnitude

**Procedure** (see Figure) :

1. Take six 100 ml calibrated flasks and label them C1 - C1 - C2 - C3 - C4 - C5.  
*NB: C1 (two flasks) is the lowest concentration that produced 100 % effect, and C5 the highest concentration that gave less than 10% effect in the range finding test.*
2. Make up the two C1 flasks with 100 ml toxicant concentration C1, according to the instructions given for the range finding test.
3. Transfer the following volumes of toxicant solution from C1 into the other flasks (see Table 4) :
  - 56 ml to flask C2; 32 ml to flask C3; 18 ml to flask C4 and 10 ml to flask C5*N.B. This transfer will empty one of the two C1 flasks and leave 84 ml in the second one.*
4. Add Standard Freshwater to the 100 ml mark in flasks C2 to C5.
5. Starting from the toxicant concentration in flask C1, calculate the actual concentration of toxicant in each flask (*these figures will be needed for the EC<sub>50</sub> calculation*).

**Table 4 : Dilution series C1-C5**

Flask	One order of magnitude		Two orders of magnitude	
	C1 (ml)	Dilution medium (ml)	C1 (ml)	Dilution medium (ml)
C1	100	0	100	0
C2	56	44	32	68
C3	32	68	10	90
C4	18	82	3.2	96.8
C5	10	90	1	99

$$C1 = \dots\dots\dots\text{mg/l}$$

$$C2 = 0.56 \times C1 = \dots\dots\dots\text{mg/l}$$

$$C3 = 0.32 \times C1 = \dots\dots\dots\text{mg/l}$$

$$C4 = 0.18 \times C1 = \dots\dots\dots\text{mg/l}$$

$$C5 = 0.10 \times C1 = \dots\dots\dots\text{mg/l}$$

6. Proceed to section 7 : **Filling of the Test Plate.**

**Case B. C1-C5 spans two orders of magnitude.**

**Procedure** (see Figure)

1. Take six 100 ml calibrated flasks and label them C1 - C1 - C2 - C3 - C4 - C5.  
*N.B. : C1 (two flasks) is the lowest concentration that produced 100 % effect, and C5 the highest concentration that gave less than 10% effect in the range finding test.*
2. Make up the two C1 flasks with 100 ml toxicant concentration C1, according to the instructions given for the range finding test.
3. Transfer the following volumes of toxicant solution from C1 into the other flasks (see Table 4) :
  - 32 ml to flask C2; 10 ml to flask C3; 3.2 ml to flask C4 and 1 ml to flask C5
4. Add Standard Freshwater to the 100 ml mark in flasks C2 to C5.
5. Starting from the toxicant concentration in flask C1, calculate the actual concentration of toxicant in each flask (*these figures will be needed for the EC<sub>50</sub> calculation*).

$$C1 = \dots\dots\dots\text{mg/l}$$

$$C2 = 0.32 \times C1 = \dots\dots\dots\text{mg/l}$$

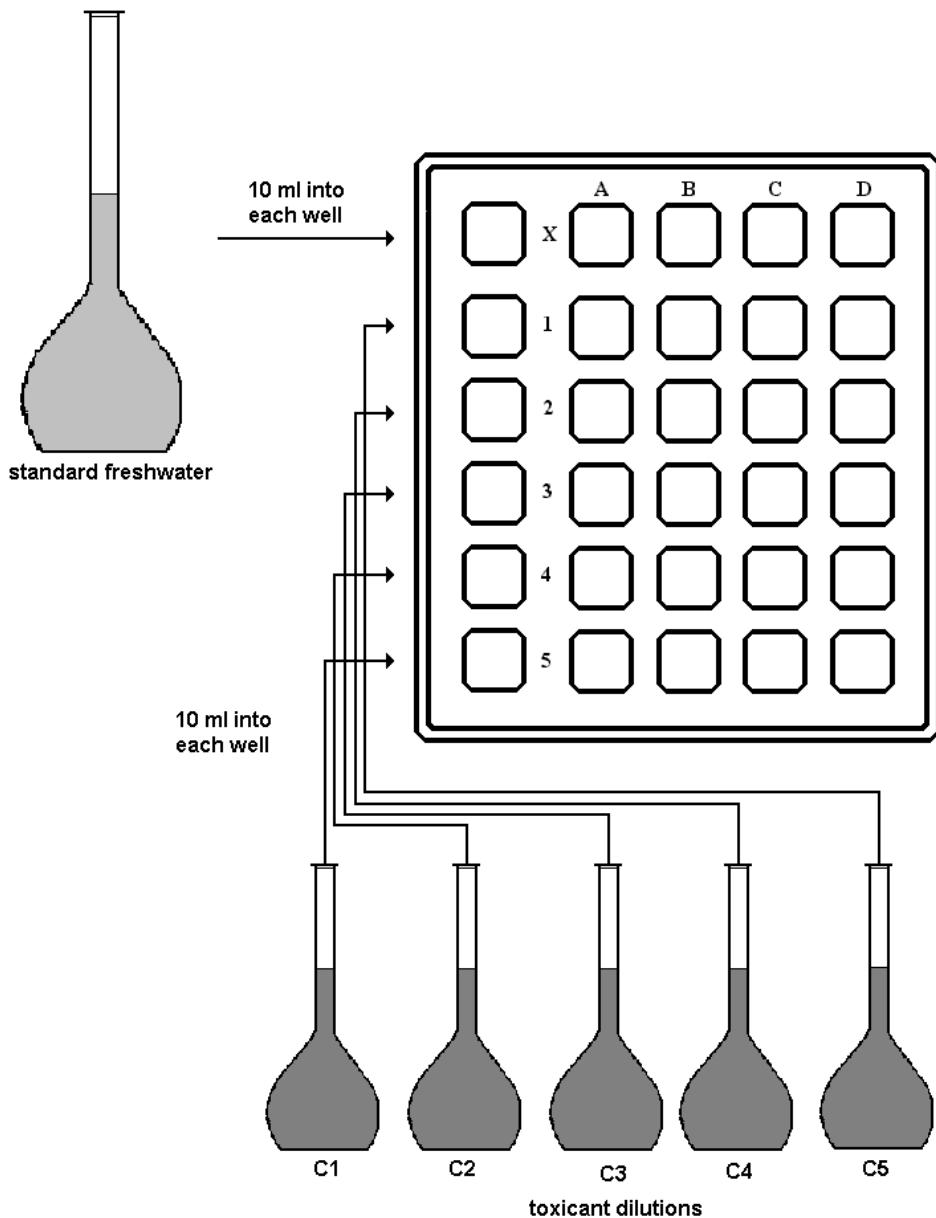
$$C3 = 0.10 \times C1 = \dots\dots\dots\text{mg/l}$$

$$C4 = 0.03 \times C1 = \dots\dots\dots\text{mg/l}$$

$$C5 = 0.01 \times C1 = \dots\dots\dots\text{mg/l}$$

6. Proceed to section 7 : **Filling of the Test Plate.**

# FILLING OF THE TEST PLATE



## 6. PRE-FEEDING OF THE TEST ORGANISMS

*The neonates used in “conventional” Daphnia tests are born in the stock cultures, from which they can take up food until they are collected for the assays. This food uptake provides them with an “energetic reserve” and precludes mortality by starvation (which would bias the test results) during the subsequent 48 h test exposure during which the organisms are not fed.*

*In order to also provide the neonates hatched from the ephippia with food prior to the test, a 2h “pre-feeding” is applied with a suspension of Spirulina microalgae.*

### **Procedure :**

1. Take one vial with Spirulina powder and fill it with Standard Freshwater.
2. Shake the vial thoroughly to homogenize the contents.  
*N.B. Vortex shaking is advised to obtain a very homogenous suspension of the Spirulina particles.*
3. Two hours prior to collecting the neonates for the test, pour the algal suspension into the hatching petri dish and swirl the contents gently to distribute the food evenly.

## 7. FILLING OF THE TEST PLATE

### **General remarks :**

*For a statistically acceptable evaluation of the effects, each test concentration as well as the control have to be assayed in 4 replicates.*

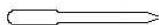
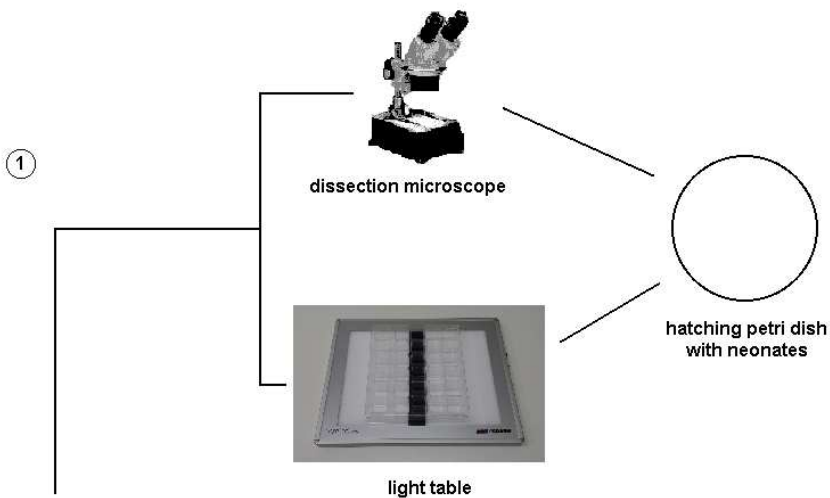
*Each multiwell plate is provided with 4 test wells for the controls and 4 test wells for each toxicant concentration (see configuration of the multiwell plate).*

*Additionally, Daphtoxkit test plates are provided on the left side with a column of “rinsing wells”. These rinsing wells serve to prevent dilution of the toxicant in the multiwell cups during the transfer of the test organisms from the hatching petri dish to the test plate.*

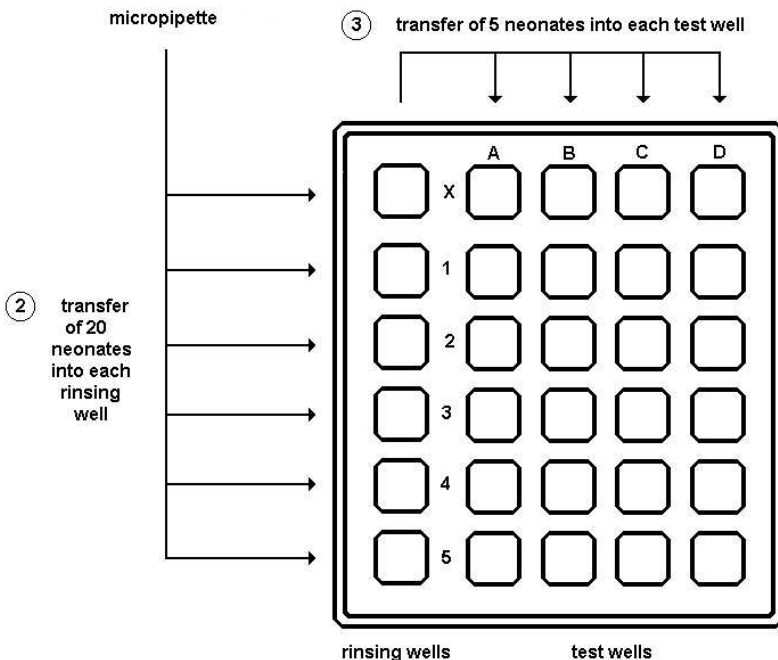
*The test wells in each column are labelled A, B, C and D and the rows are labelled X (controls), 1, 2, 3, 4 and 5 for the five toxicant dilutions.*

*All the wells of each row have to be filled with one toxicant dilution (or with the dilution medium for the control row).*

# TRANSFER OF THE NEONATES TO THE TEST WELLS



micropipette



**Procedure** (see figure) :

Transfer 10 ml dilution water into each well of the control row and 10 ml of the respective toxicant concentrations into each well of the corresponding rows, in the sequence of increasing toxicant concentrations.

## 8. TRANSFER OF THE NEONATES TO THE TEST WELLS

*The transfer of the Daphnia neonates into the test wells is performed at the aid of a **micropipette**.*

*Because of the small size of the young born Daphnids this transfer is usually carried out under a **dissection microscope** at low magnification (e.g. 10X). One can also use a light table (see Figure). The use of a strip of black paper considerably enhances the contrast between the test organisms and the white background of the light table, and substantially facilitates the visual observation of the test organisms.*

### LIGHT TABLE

For a better contrast between the test organisms and the white background of the light table, one can use a strip of black paper placed in the middle of the viewing surface.



- Transfer of the Daphnids to the multiwell plate is accomplished in two steps :*
- transfer of the neonates from the petri dish into the rinsing wells of the multiwell plate (first column to the left).*
  - transfer of the neonates from the rinsing wells to the 4 test wells of the same rows.*

**Procedure** (see figure) :

- Put the Petri dish with the pre-fed neonates on the stage of a dissection microscope or on the transparent stage of the light table with dark light strip.
- Transfer at least 20 (actively swimming) neonates into each rinsing cup in the sequence : row X (control), row 1, row 2, row 3, row 4 and row 5 (*i.e. in order of increasing concentrations of toxicant*).  
Try to carry over as little as possible liquid from the petri dish to the wells during this transfer and rinse the micropipette thoroughly after each transfer.
- Put the multiwell plate on the stage of the dissection microscope or on the transparent stage of the light table and transfer exactly 5 neonates from each rinsing well into the 4 wells of each row.  
*This transfer shall also be performed in the sequence of increasing test concentrations.*  
N.B. Count the neonates as they exit the micropipette to be sure of the transfer of exactly 5 test organisms per well.

**Important remark**

**SURFACE FLOATING OF TEST ORGANISMS**

*Daphnids are quite susceptible to being trapped at the surface of the liquid medium in the test wells, by the phenomenon of "surface tension".*

*Once "floating", some test organisms may not be able to free themselves from the surface and may die.*

*In order to avoid "surface floating" which can seriously jeopardize the outcome of the bioassays, it is of utmost importance, during the transfer of the neonates into the test wells, to put the tip of the micropipette in the medium, and not to drop the organisms onto the surface of the medium.*

## 9. INCUBATION OF THE TEST PLATE

### Procedure :

1. Put the Parafilm strip on the multiwell plate and put the cover on tightly.
2. Put the multiwell in the incubator at 20°C, in darkness.

## 10. SCORING OF THE RESULTS

### Procedure :

1. After 24h and 48h incubation, put the multiwell plate under the dissection microscope or on the stage of the light table.
2. Record the number of dead and immobilized\* neonates, versus that of the actively swimming test organisms in each well.

\* *The neonates which are not able to swim after gentle agitation of the liquid for 15 seconds shall be considered to be immobilized, even if they can still move their antennae.*

3. Score the figures on the "Results Sheet".
4. Calculate the total the number of dead and immobile neonates for each toxicant concentration and calculate the mean and the % effect.

## 11. VALIDITY OF THE TEST

*Next to all other specific validity conditions prescribed in standard Daphnia bioassay protocols (e.g. with regard to pH and oxygen concentration) a major condition is that the number of dead + immobile organisms should not exceed 10 % in the controls.*



# DAPHTOXKIT F

## RESULTS SHEET

Name of operator : .....

Date of performance of test : .....

Toxicant tested : .....

Type of test :

range finding test

definitive test

Test species : *Daphnia magna* - batch : .....

Dilution series tested :

Concentration 1 : .....

Concentration 2 : .....

Concentration 3 : .....

Concentration 4 : .....

Concentration 5 : .....

	Control		Conc. 5		Conc. 4		Conc. 3		Conc. 2		Conc. 1	
	24	48	24	48	24	48	24	48	24	48	24	48
Exposure (h)												
A												
B												
C												
D												
Total	/20	/20	/20	/20	/20	/20	/20	/20	/20	/20	/20	/20
% Effect												

Effect scores

24h EC<sub>50</sub> : .....

48h EC<sub>50</sub> : .....

## 12. ESTIMATION OF THE EC<sub>50</sub>

*There are many procedures for calculating 50% effect thresholds. A data treatment program to calculate the 24h and the 48h EC<sub>50</sub> for the Daphtoxkit microbiotest is available on demand from MicroBioTests Inc.*

## 13. 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 from time to time a reference test.*

*Such a quality control test can e.g. be performed with the reference toxicant potassium dichromate (K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub>)*

### **Procedure :**

A dilution series ranging from 3.2 mg/l to 0.32 mg/l has to be prepared following the guidelines given in section **B.2. Definitive test - A. C1-C5 spans one order of magnitude.**

1. Take seven 100 ml calibrated flasks. Label the first flask as "stock 1", the second flask as "stock 2" and the others as C1 - C1 - C2 - C3 - C4 - C5.
2. Weigh 100 mg potassium dichromate on an analytical balance, transfer it into the "stock 1" flask and fill to the mark with deionized water.
3. Transfer 1 ml "stock 1" solution into "stock 2" flask and fill the latter flask to the mark to make up a 10 mg/l toxicant concentration.
4. Transfer the following volumes of toxicant solution from "stock 2" into the other 100 ml flasks :
  - 32 ml to flask C1 (3.2 mg/l)
  - 18 ml to flask C2 (1.8 mg/l)
  - 10 ml to flask C3 (1 mg/l)
  - 5.6 ml to flask C4 (0.56 mg/l)
  - 3.2 ml to flask C5 (0.32 mg/l)
5. Add Standard Freshwater to the mark, stopper the flasks and shake to homogenize the solutions.

6. Fill the multiwell with the toxicant solutions as indicated in section 7. **Filling of the Test Plate.**

From the data obtained in the quality control test, the 24h EC<sub>50</sub> has to be calculated, the value of which should be situated within the limits stipulated in the specification sheet of the Daphtokit.

# LIST OF TOXKIT MICROBIOTESTS

## Tests for freshwater and soils

- PROTOXKIT F** : 24h reproduction inhibition test based on the ciliate protozoan *Tetrahymena thermophila*. This assay is under consideration as an OECD Guideline.
- ROTOXKIT F** : 24h mortality test, based on the rotifer *Brachionus calyciflorus*. This assay adheres to ASTM Standard Guide E1440-91.
- ROTOXKIT F short chronic** : 48h reproduction inhibition test based on the rotifer *Brachionus calyciflorus*. This assay adheres to ISO norm 20666 and AFNOR norm T90-377.
- THAMNOTOXKIT F** : 24h mortality test, based on the anostracan crustacean *Thamnocephalus platyurus*. This assay adheres to ISO norm 14380.
- DAPHTOXKIT F magna** : 24h-48h mobility inhibition test, based on the cladoceran crustacean *Daphnia magna*. This assay adheres to ISO norm 6341 and OECD Guideline 202.
- CERIODAPHTOXKIT F** : 24h mortality test, based on the cladoceran crustacean *Ceriodaphnia dubia*. This assay is in current practice in the USA as an EPA Method.
- OSTRACODTOXKIT F** : 6 days chronic mortality and growth inhibition test with the ostracod crustacean *Heterocypris incongruens*. This assay adheres to ISO norm 14370.
- RAPIDTOXKIT F** : 30-60 min particle ingestion inhibition test based on the anostracan crustacean *Thamnocephalus platyurus*. This assay adheres to ISO norm 14380.
- ALGALTOXKIT F** : 72h growth inhibition test, based on the green alga *Selenastrum capricornutum* (presently named *Pseudokirchneriella subcapitata*). This assay adheres to ISO norm 8692 and OECD Guideline 201.
- PHYTOTOXKIT** : 3 days germination and root growth inhibition test with seeds of 3 higher plants.
- PHYTOTESTKIT** : A short germination and root/shoot growth inhibition microbiotest for determination of the direct effect of chemicals on higher plants.
- SPIRODELA DUCKWEED TOXKIT** : 72h growth inhibition test with the duckweed species *Spirodela polyrhiza*.

## Tests for estuarine/marine environments

- ROTOXKIT M** : 24h mortality test based on the rotifer *Brachionus plicatilis*. This assay adheres to ASTM Standard Guide E1440-91.
- ARTOXKIT M** : 24h mortality test based on the anostracan crustacean *Artemia salina* (renamed *Artemia franciscana*). This assay adheres to ASTM Standard Guide E1440-91.
- MARINE ALGALTOXKIT** : 72h growth inhibition test based on the marine diatom *Phaeodactylum tricorutum*. This test adheres to ISO norm 10253.

MANUFACTURED BY :



Kleimoer 15  
9030 Mariakerke (Gent)  
Belgium  
[www.microbiotests.be](http://www.microbiotests.be)