THAMNOTOXKIT F
Test procedure

1

PREPARATION OF STANDARD FRESHWATER

- VOLUMETRIC FLASK (1 LITER)
- 5 VIALS WITH SOLUTIONS OF CONCENTRATED SALTS
- PURE (distilled or deionized) WATER

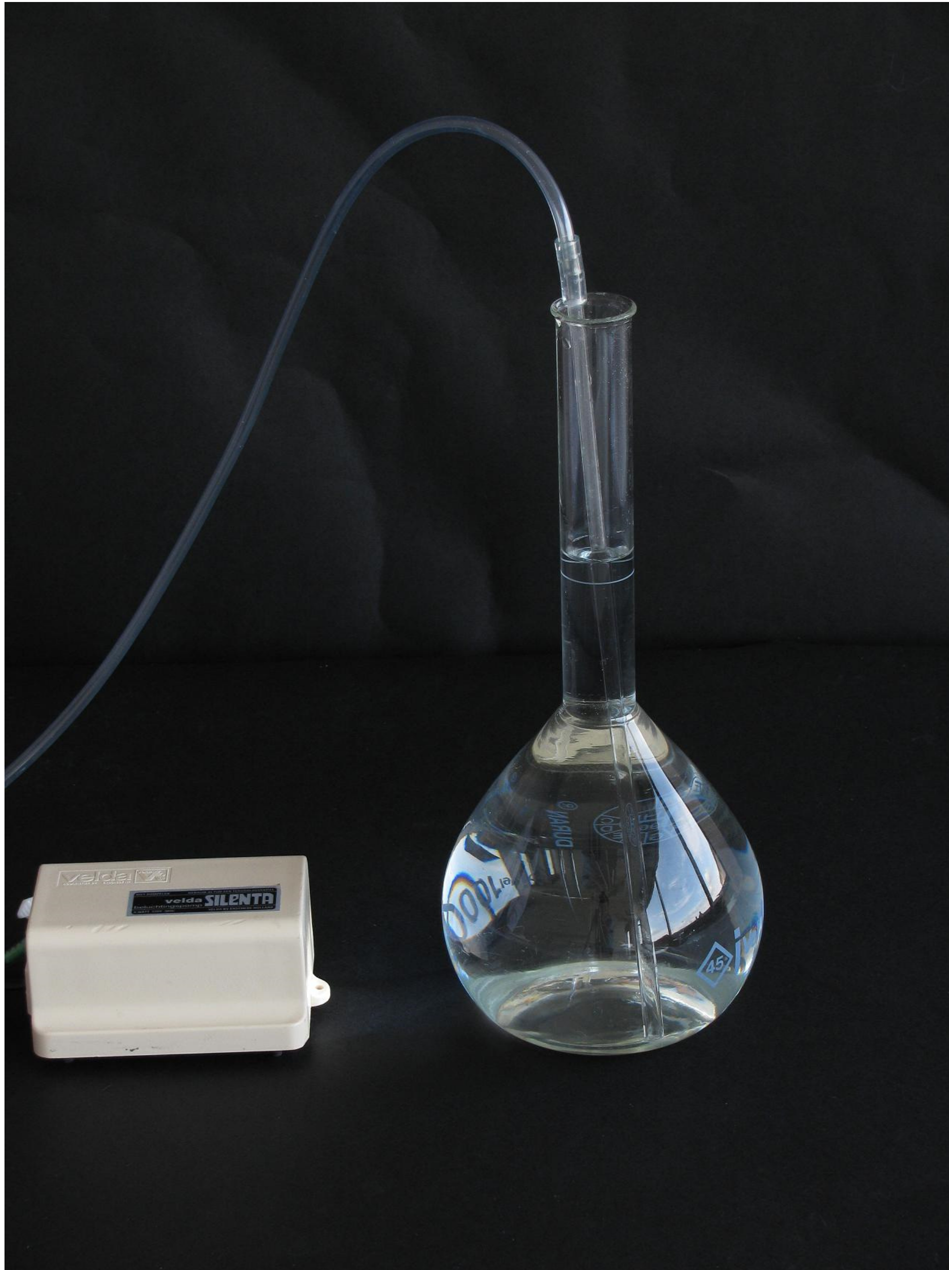
2

POUR THE 5 VIALS

WITH CONCENTRATED SALT SOLUTIONS

IN ± 800 ml PURE WATER,

IN THE 1 LITER VOLUMETRIC FLASK



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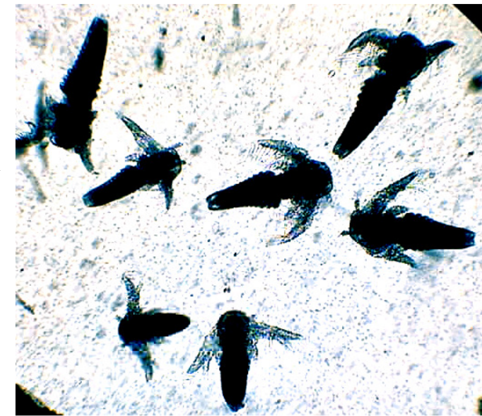
- FILL THE FLASK TO THE 1 LITER MARK WITH PURE WATER

- AERATE FOR AT LEAST 15 MINUTES

Tube with
Thamnocephalus platyurus
cysts

Thamnocephalus platyurus cysts



Thamnocephalus platyurus larvae

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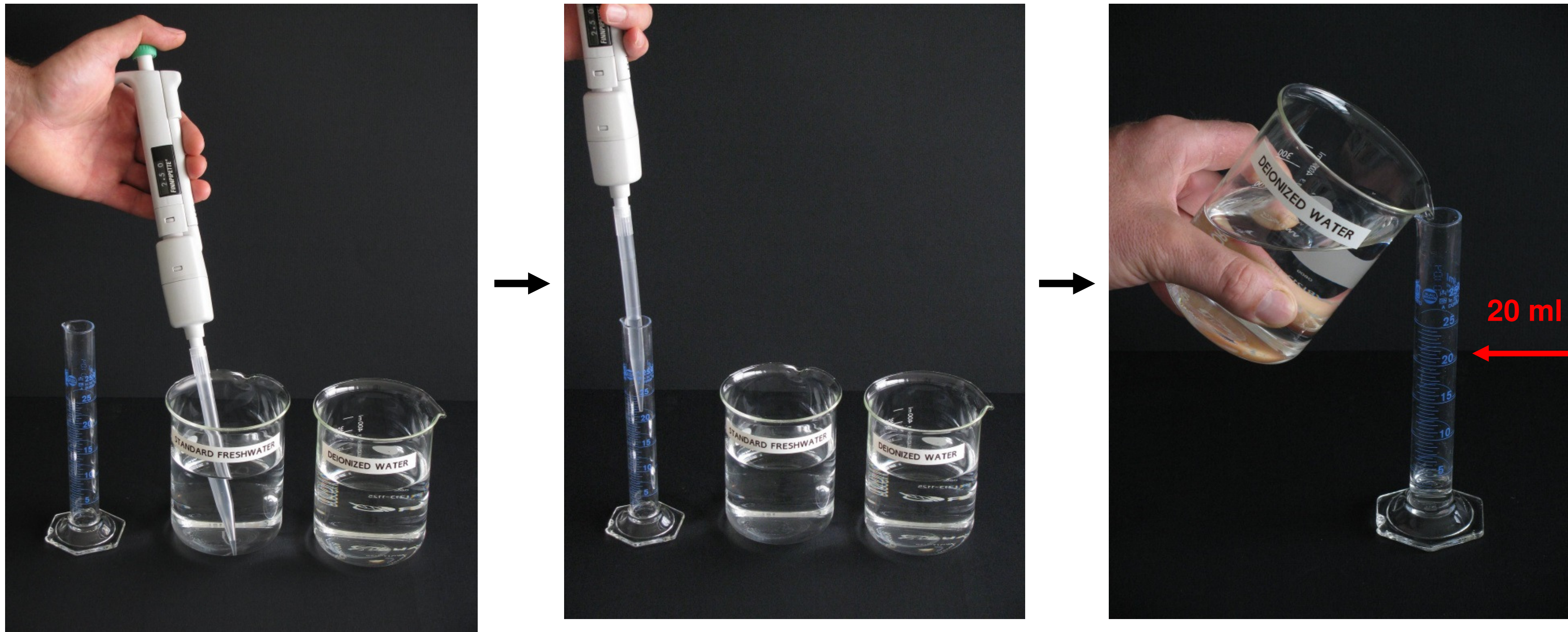
HATCHING OF THE CYSTS

CYST HATCHING SHOULD BE INITIATED **<u>20-22</u>** HOURS PRIOR

TO THE START OFTHE TOXICITY TEST



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1. <u>PREHYDRATION OF THE CYSTS</u>

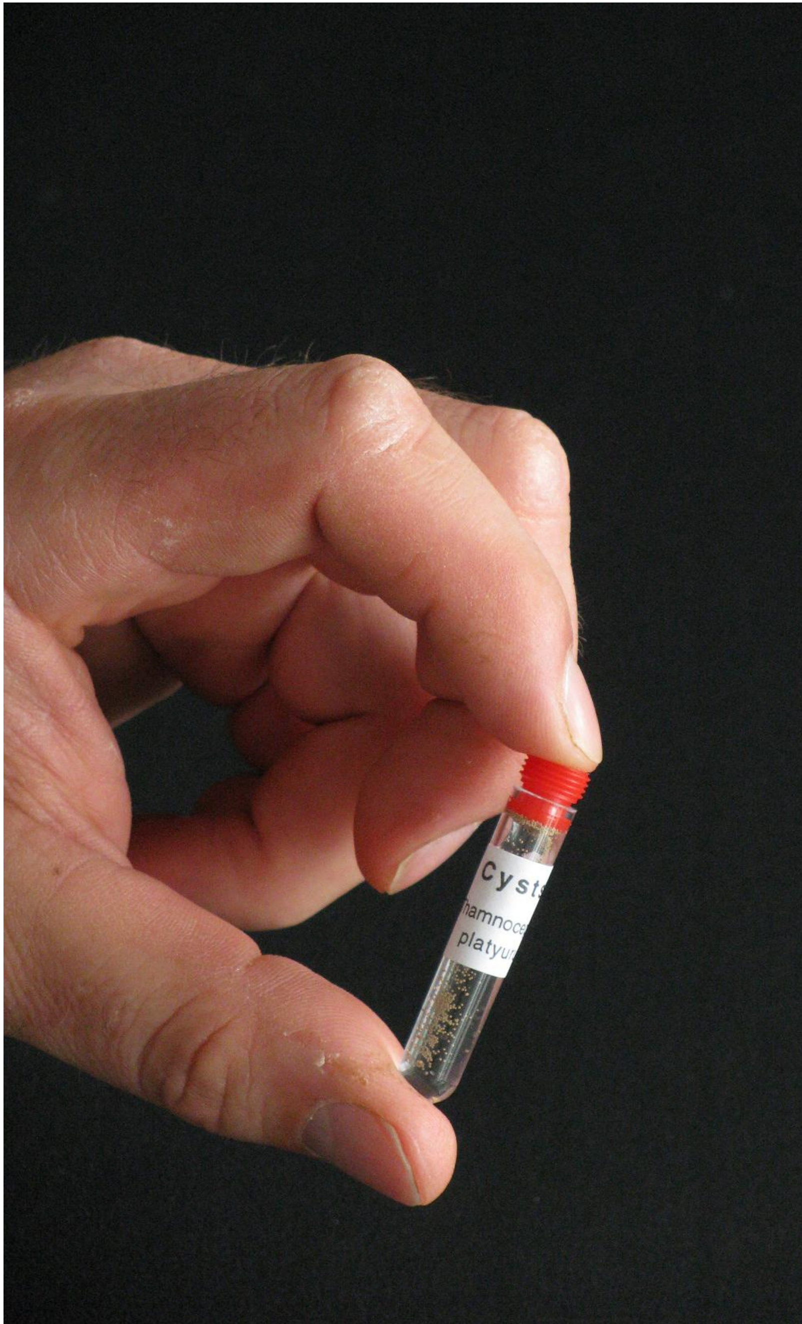
PREPARE **<u>20 ml</u>** "HATCHING MEDIUM" (=DILUTED STANDARD FRESHWATER)

BY PUTTING **<u>2,5 ml</u>** STANDARD FRESHWATER IN A GRADUATED 25 ml CYLINDER

AND ADDING PURE WATER TO THE **<u>20 ml</u>** MARK

6

OPEN A TUBE WITH CYSTS AND
FILL IT WITH HATCHING MEDIUM
(approximately 1 ml)



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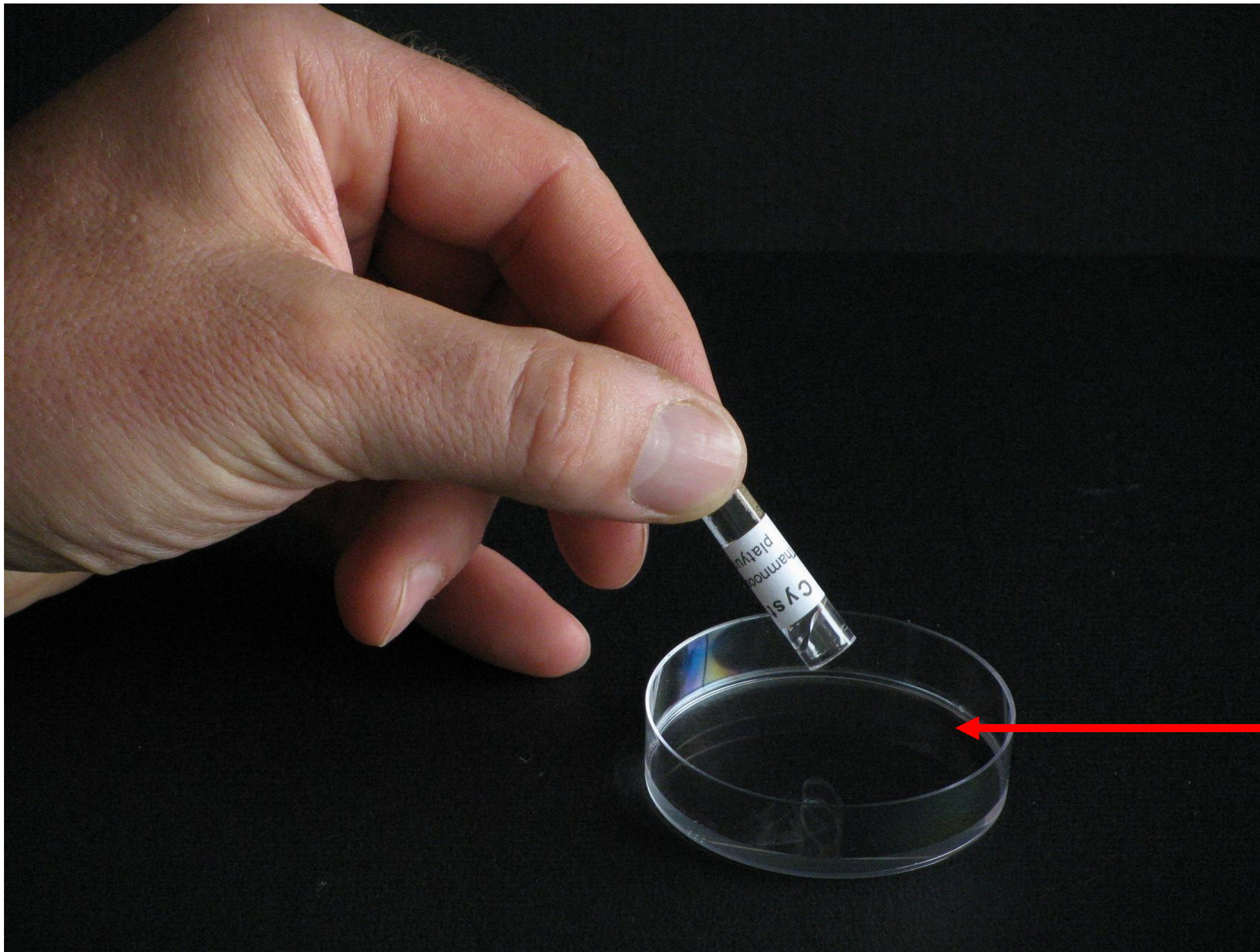
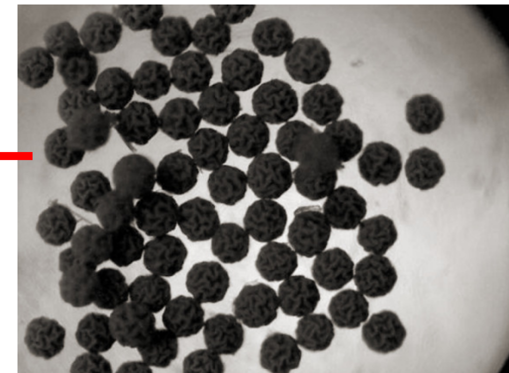
- CLOSE THE TUBE WITH THE STOPPER

- SHAKE THE TUBE AT REGULAR INTERVALS
  DURING A 30 MINUTES PERIOD





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2. <u>TRANSFER OF THE PREHYDRATED CYSTS INTO THE HATCHING PETRI DISH</u>

EMPTY THE CONTENTS OF THE VIAL WITH PREHYDRATED CYSTS INTO A PETRI DISH

9

- MAKE SURE THAT ALL THE CYSTS ARE TRANSFERRED BY RINSING THE TUBE WITH HATCHING MEDIUM
- ADD **10 ml** HATCHING MEDIUM TO THE PETRI DISH AND SWIRL GENTLY TO DISTRIBUTE THE CYSTS EVENLY

10

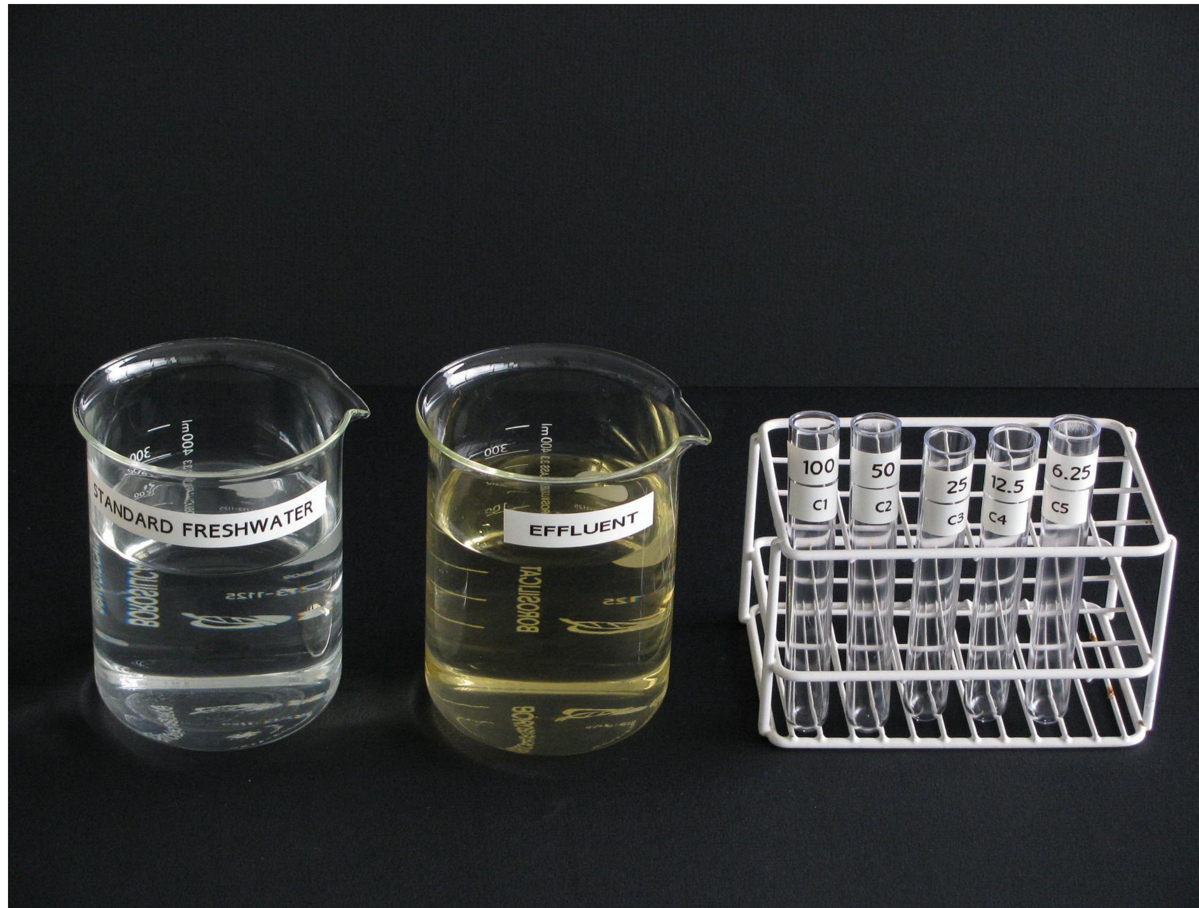
INCUBATION OF THE CYSTS

INCUBATE THE PETRI DISH

FOR **<u>20-22 HOURS AT 25 °C</u>**

UNDER **<u>CONTINOUS ILLUMINATION</u>**

OF MIN. **<u>3 000 – 4 000 LUX</u>**



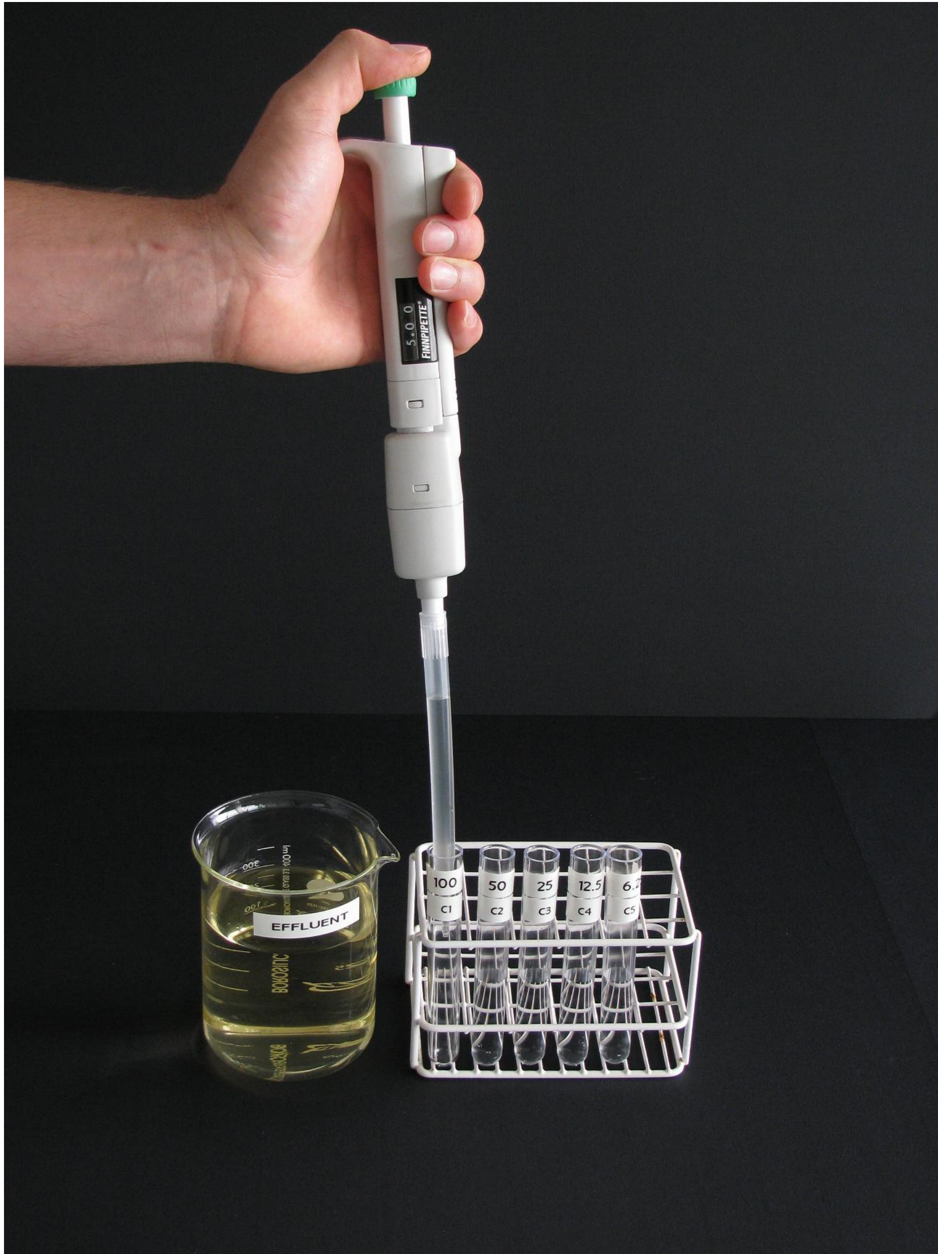
PREPARATION OF THE TOXICANT DILUTIONS (e.g. a test on an effluent)

- TAKE **5 TUBES** OF 10-15 ml CONTENTS AND LABEL THEM

C1 (100), **C2** (50), **C3** (25), **C4** (12.5), AND **C5** (6.25)

12

- ADD **<u>5 ml</u>** STANDARD FRESHWATER TO TUBES C2, C3, C4 AND C5



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ADD **5 ml** EFFLUENT SAMPLE TO

TUBE C1 (= 100% sample)

14

- ADD **<u>5 ml</u>** EFFLUENT TO TUBE C2

- MIX THE CONTENTS OF TUBE C2 (= 50% dilution) WITH THE AID OF THE PIPET

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- TRANSFER **5 ml** FROM TUBE C2 TO TUBE C3
- MIX THE CONTENTS OF TUBE C3 (= 25% dilution) WITH THE AID OF THE PIPET

16

REPEAT THE SAME PROCEDURE FOR THE NEXT DILUTIONS :

* **5 ml** FROM TUBE C3 TO TUBE C4 (= 12,5% dilution)

* **5 ml** FROM TUBE C4 TO TUBE C5 (= 6,25% dilution)

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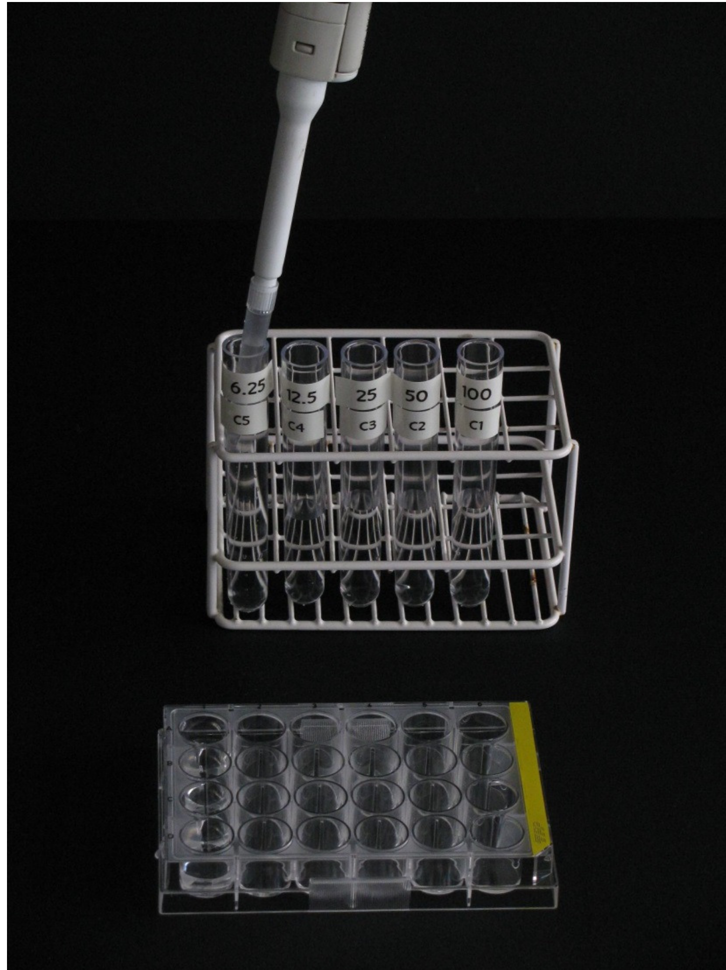
FILLING OF THE TEST PLATE

CONTROLS

ADD **1 ml** STANDARD FRESHWATER TO EACH WELL OF COLUMN 1 (WELLS A1, B1, C1, D1)



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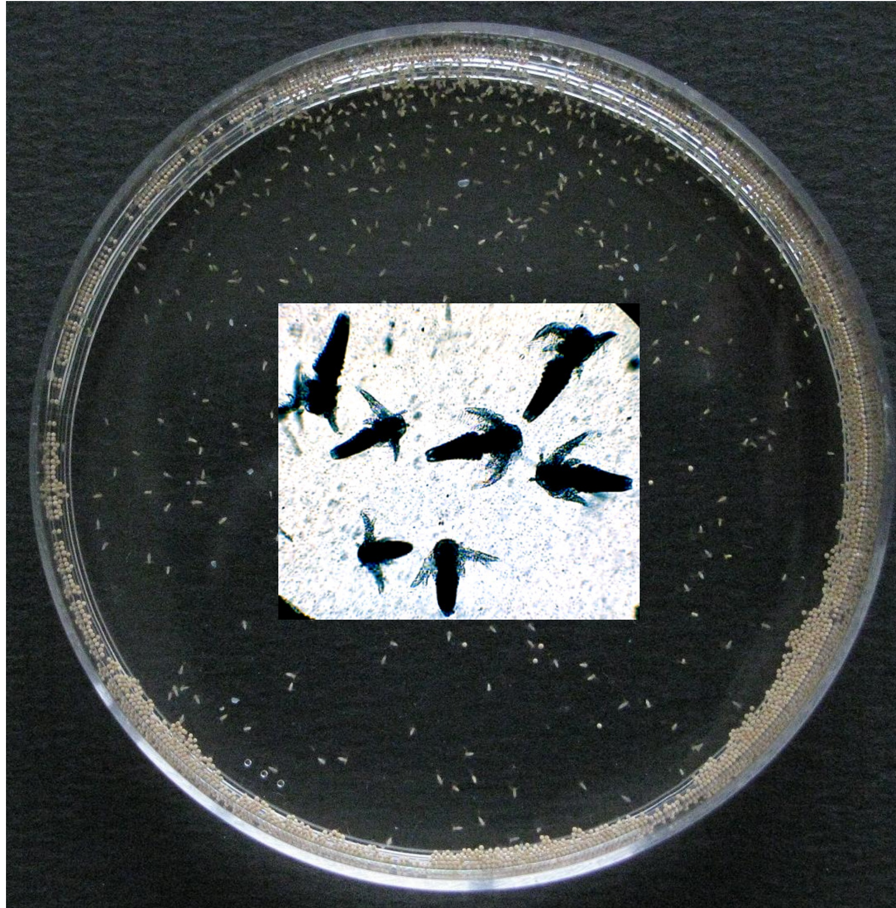
<u>TOXICANT DILUTIONS</u>

TRANSFER **<u>1 ml</u>** OF TEST TUBE 5 TO EACH WELL IN COLUMN 2 (WELLS A2, B2, C2, D2)

19

REPEAT THIS PROCEDURE WITH TEST TUBES 4, 3, 2 AND 1 TO FILL THE WELLS OF COLUMNS 3, 4, 5 AND 6 RESPECTIVELY



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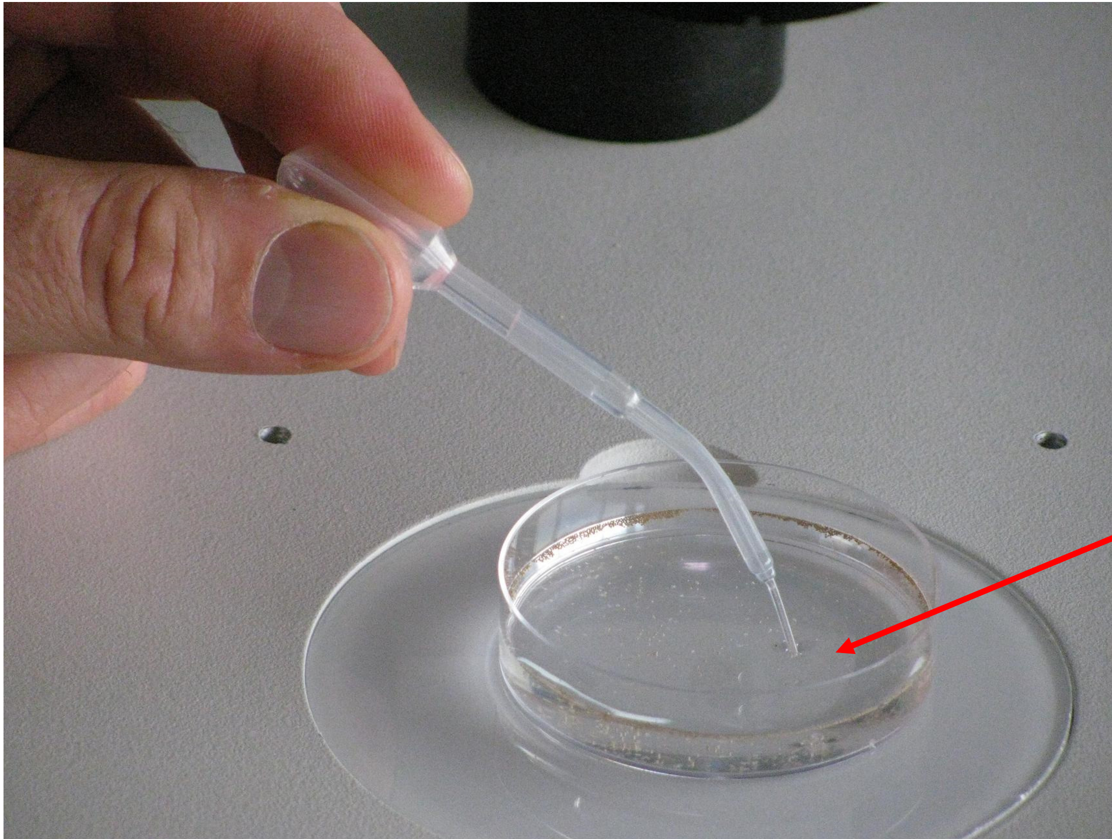
TRANSFER OF THE LARVAE FROM THE HATCHING PETRI DISH TO THE TEST WELLS



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- PUT THE HATCHING PETRI DISH ON THE STAGE OF THE DISSECTION MICROSCOPE
- TAKE THE MICROPIPETTE LIKE A PENCIL WITH THE INDEX FINGER AND THE THUMB TO EXERT CONTROLLED PRESSURE ON THE BULB.
- SQUEEZE THE BULB GENTLY TO PROVIDE ADEQUATE SUCTION FOR PICKING UP LARVAE.

22

TRANSFER APPROXIMATELY **50 LARVAE** FROM THE **PETRI DISH** TO EACH **RINSING WELL** IN THE FOLLOWING SEQUENCE: A1 (control), A2, A3, A4, A5 AND A6 (= increasing concentrations of toxicant)

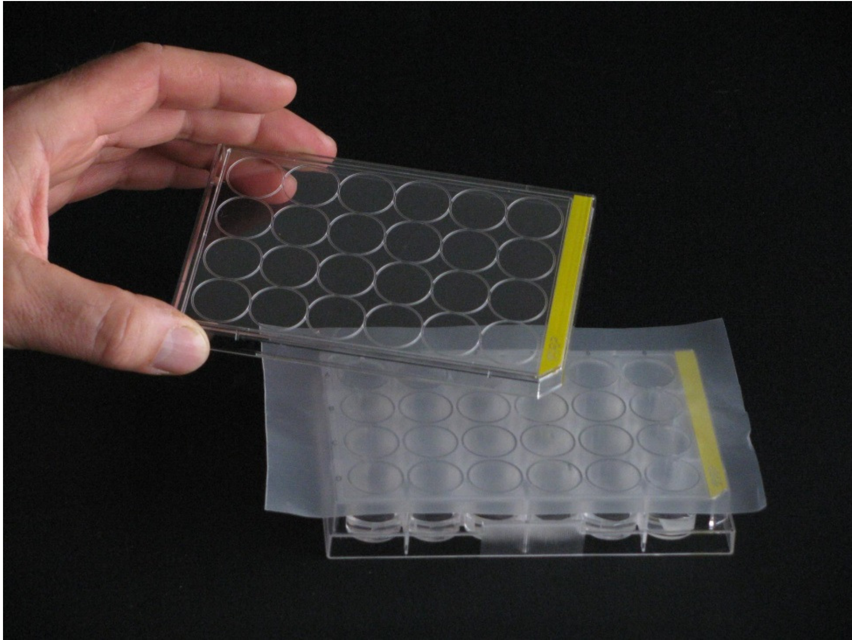
23

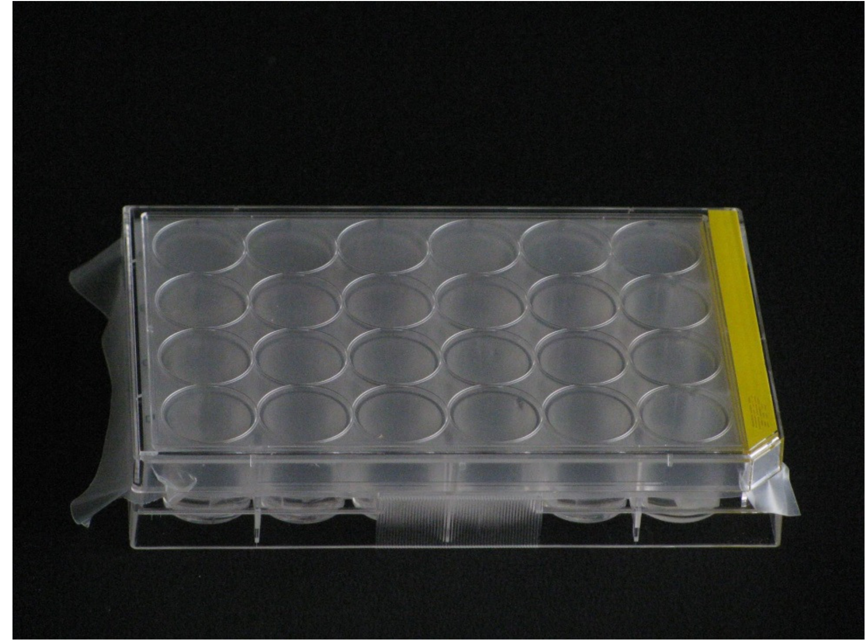
- PUT THE MULTIWELL PLATE ON THE STAGE OF THE DISSECTION MICROSCOPE

- TRANSFER **<u>10 LARVAE</u>** FROM RINSING WELL A1 INTO THE 3 OTHER WELLS OF COLUMN 1 (CUPS B1, C1 AND D1 = controls)

24

REPEAT THE SAME TRANSFER OF **<u>10 LARVAE</u>** FROM RINSING WELLS A2 TO A6 TO THE 3 WELLS OF COLUMNS 2 TO 6 (in the sequence from the lowest to the hightest toxicant concentration)





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PUT THE PARAFILM STRIP ON TOP OF THE MULTIWELL PLATE AND PUT THE COVER ON TOP

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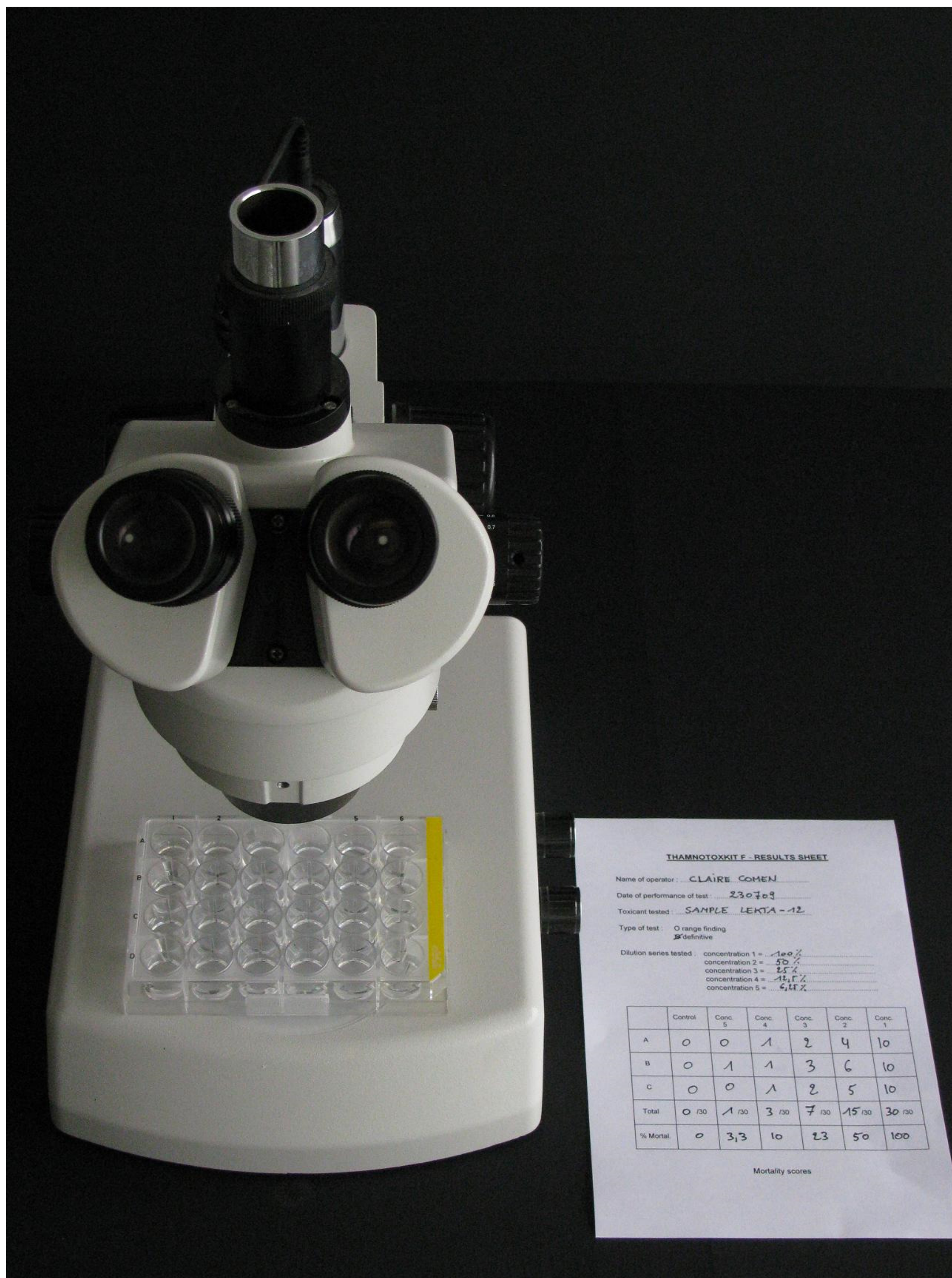
PUT THE MULTIWELL PLATE IN THE INCUBATOR **AT 25 °C, IN DARKNESS, FOR 24 HOURS**



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SCORING OF THE RESULTS

- PUT THE MULTIWELL PLATE ON THE STAGE OF THE DISSECTION MICROSCOPE

- CHECK THE WELLS OF ROWS B, C AND D AND COUNT THE NUMBER OF DEAD LARVAE IN EACH CUP

- SCORE THE MORTALITY DATA ON THE "RESULTS SHEET"

- CALCULATE THE 24h LC50 WITH AN APPROPRIATE PROGRAM