THAMNOTOXKIT F
Test procedure

1

PREPARATION OF STANDARD FRESHWATER

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

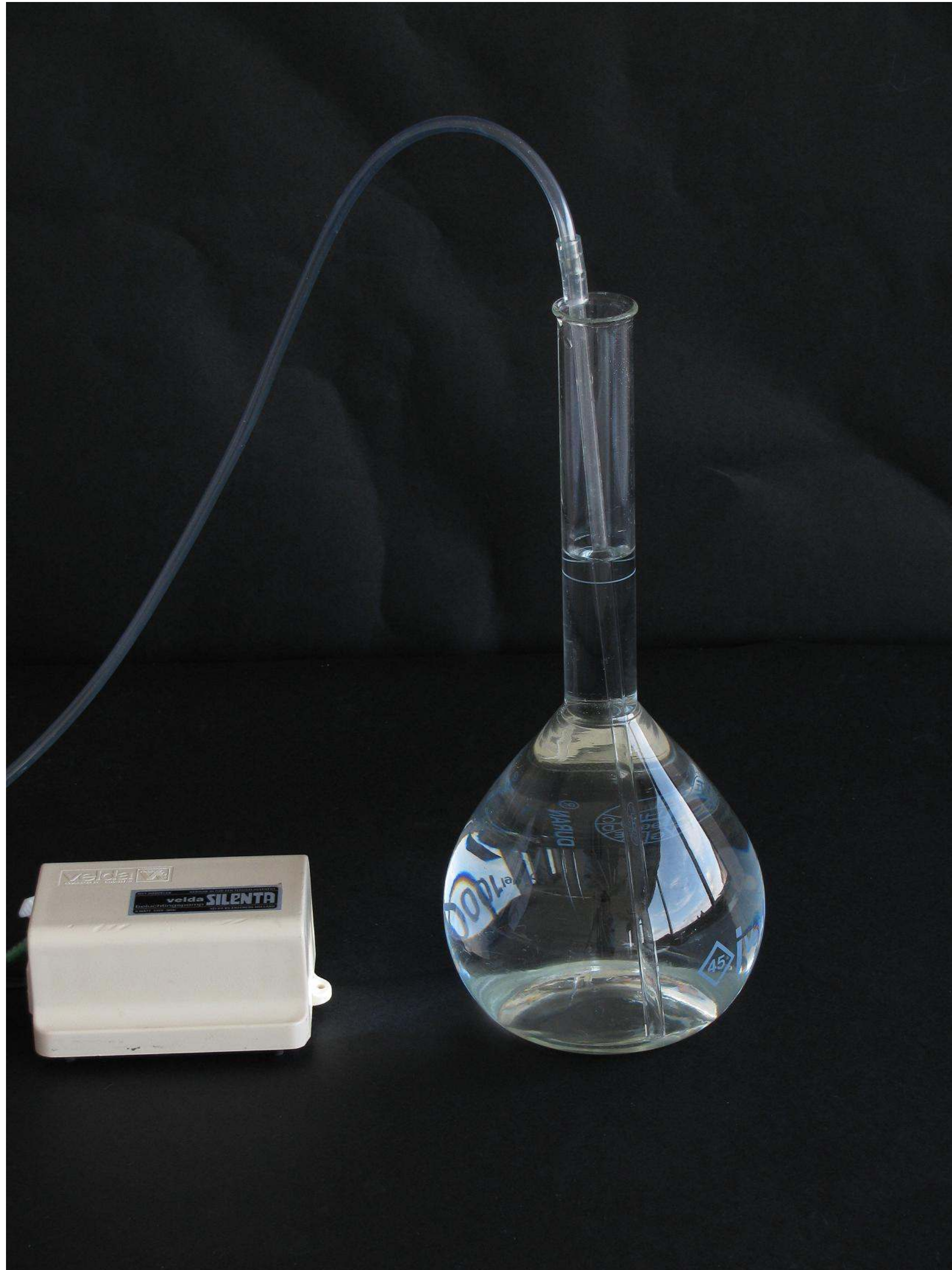
2

POUR THE 5 VIALS

WITH CONCENTRATED SALT SOLUTIONS

IN $\pm$ 800 ML DISTILLED WATER,

IN THE 1 LITER VOLUMETRIC FLASK



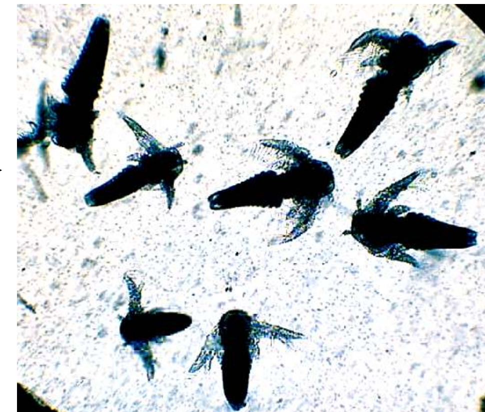
3

- FILL THE FLASK TO THE 1 LITER MARK

- AERATE FOR AT LEAST 15 MINUTES

Tube with
Thamnocephalus platyurus
cysts

Thamnocephalus
platyurus cysts



Thamnocephalus platyurus
larvae

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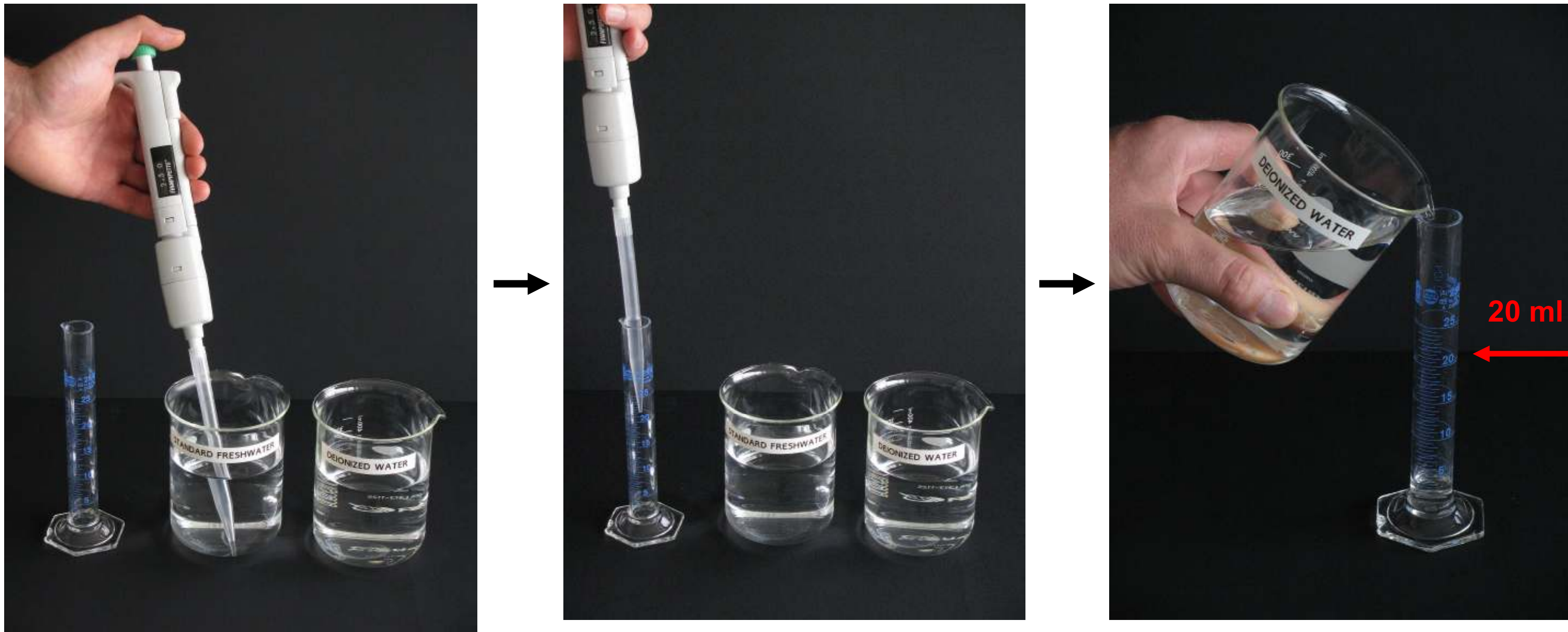
HATCHING OF THE CYSTS

CYST HATCHING SHOULD BE INITIATED 20-22 HOURS PRIOR

TO THE START OFTHE TOXICITY TEST



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1. PREHYDRATION OF THE CYSTS

PREPARE 20 ML “HATCHING MEDIUM” (=DILUTED STANDARD FRESHWATER)

BY PUTTING 2,5 ML STANDARD FRESHWATER IN A GRADUATED 25 ML CYLINDER

AND ADDING DEIONIZED WATER TO THE 20 ML MARK

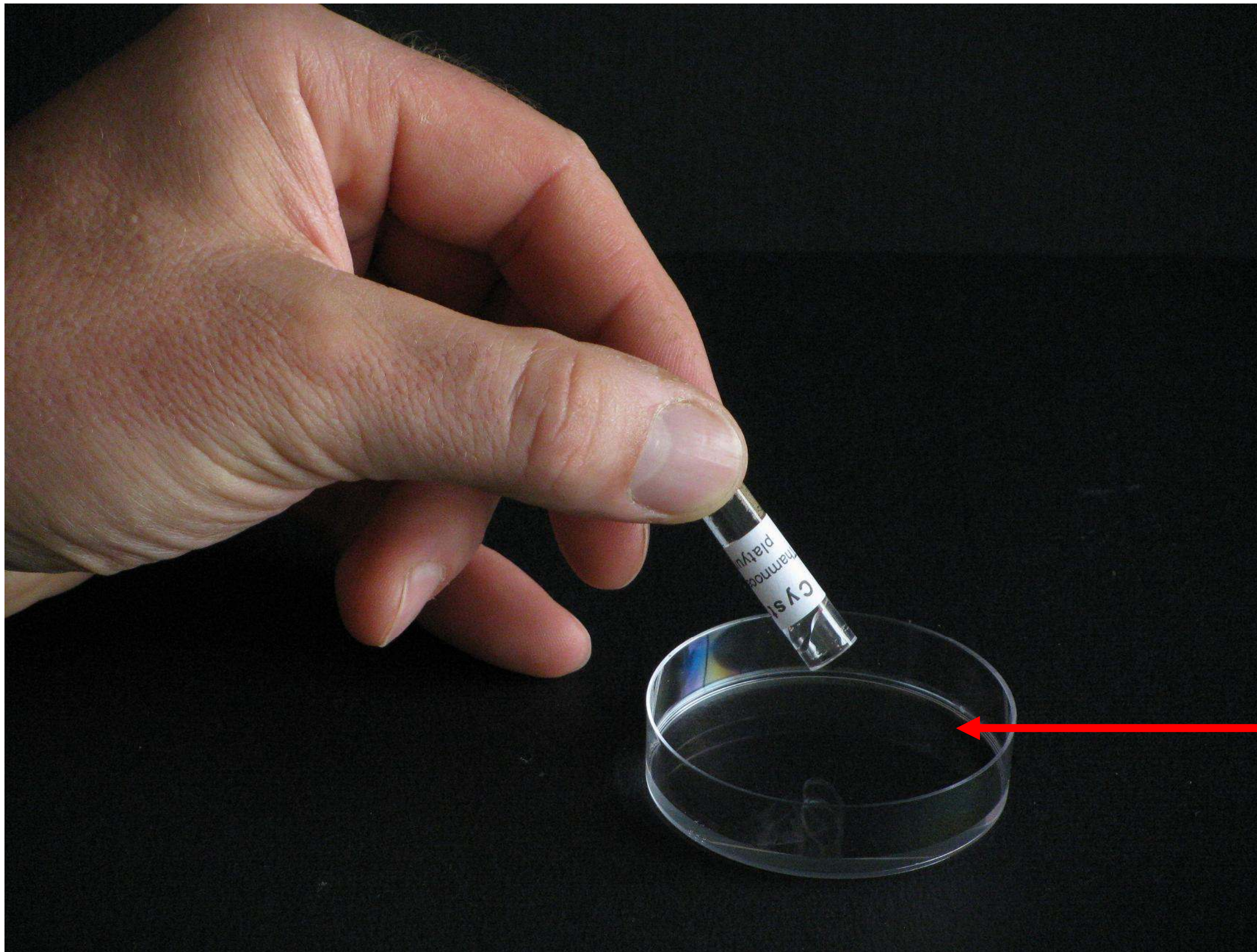
6

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

7

- CLOSE THE TUBE WITH THE STOPPER

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



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2. TRANSFER OF THE PREHYDRATED CYSTS INTO THE HATCHING PETRI DISH

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

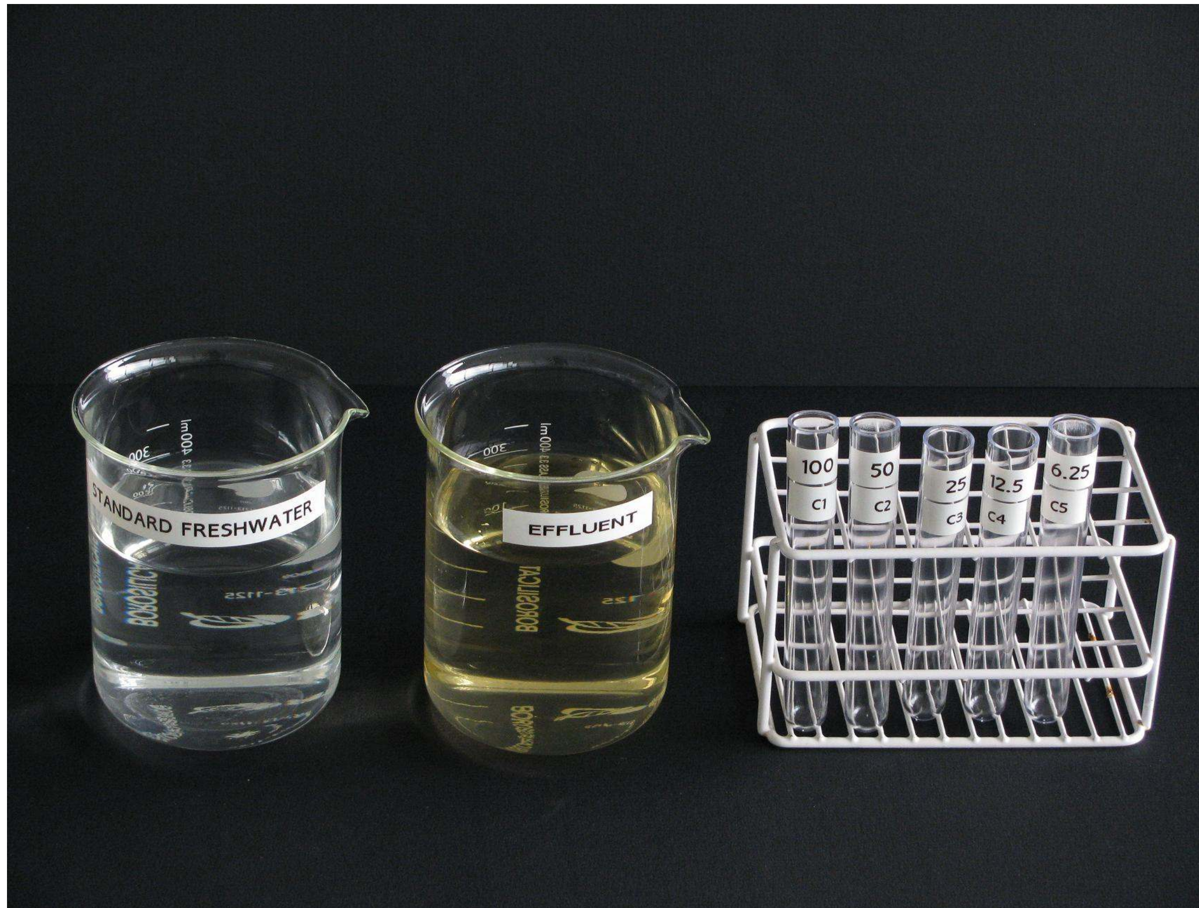
INCUBATION OF THE CYSTS

INCUBATE THE PETRI DISH

FOR 20-22 HOURS AT 25 °C

UNDER CONTINOUS ILLUMINATION

OF MIN. 3 000 – 4 000 LUX



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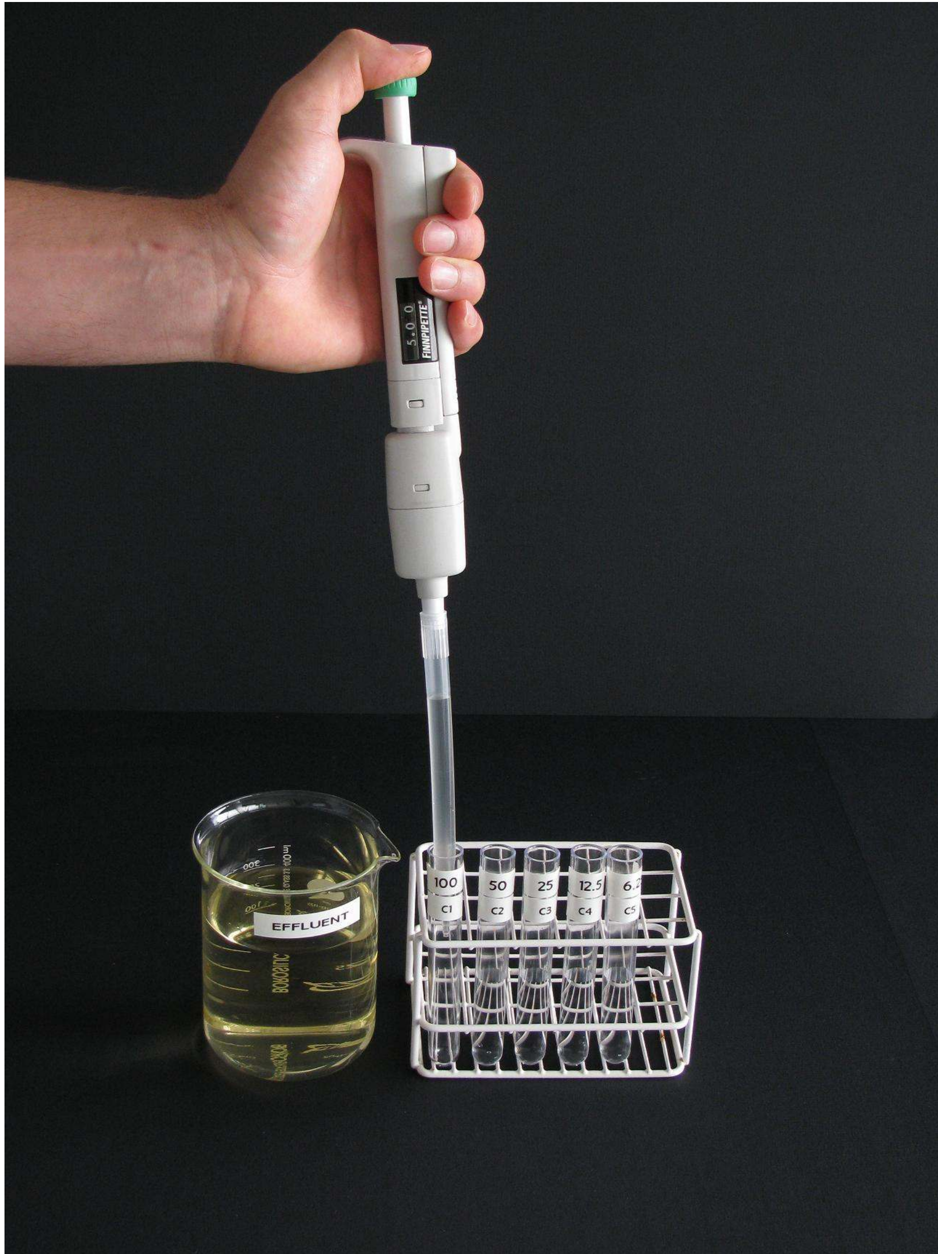
PREPARATION OF THE TOXICANT DILUTIONS (e.g. a test on an effluent)

- 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 5 ML 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 5 ML EFFLUENT TO TUBE C2
- MIX THE CONTENTS OF TUBE C2 (= 50% dilution) WITH THE AID OF THE PIPET

15

- 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)

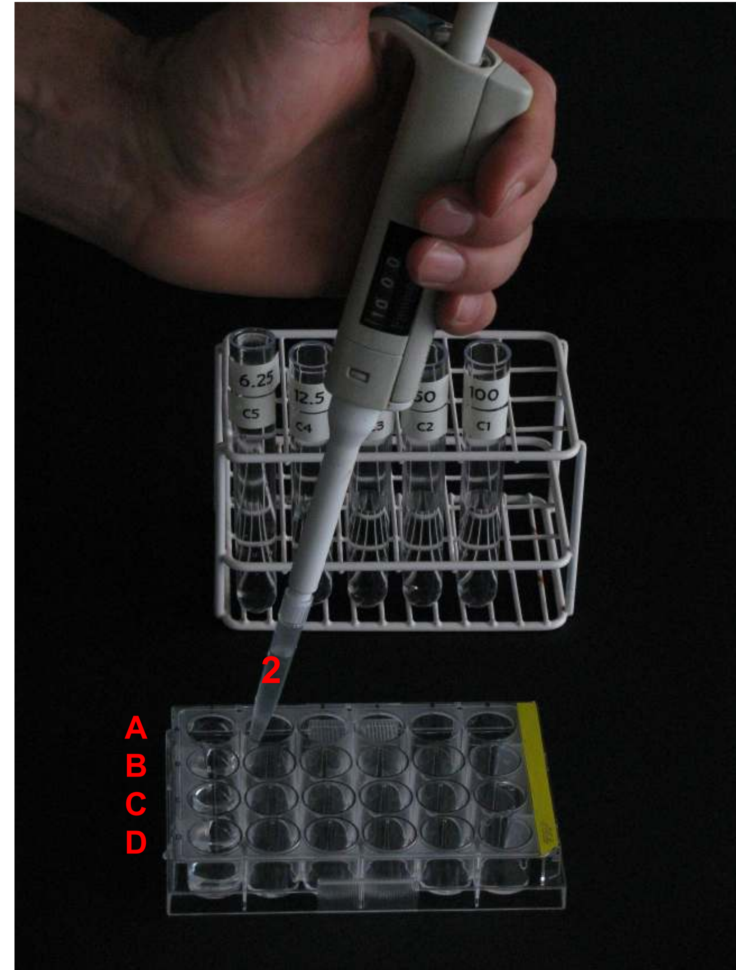
* 5 ML FROM TUBE C4 TO TUBE C5 (= 6,25% dilution)

17

FILLING OF THE TEST PLATE

<u>CONTROLS</u>

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



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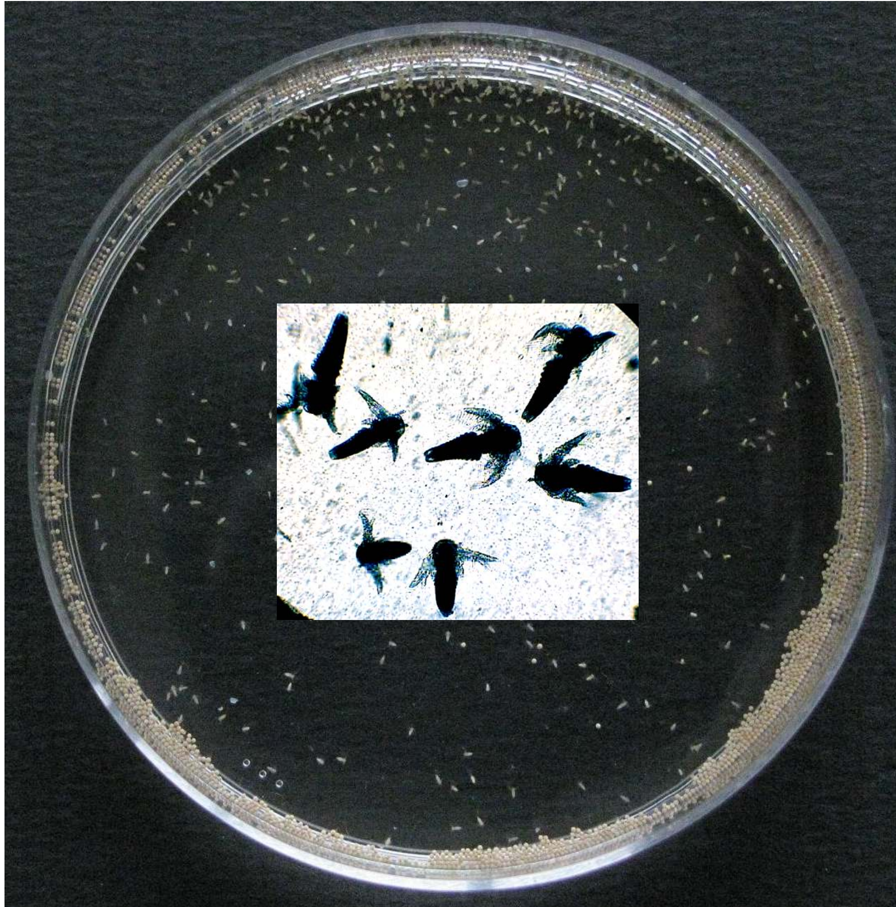
<u>TOXICANT DILUTIONS</u>

TRANSFER 1 ML 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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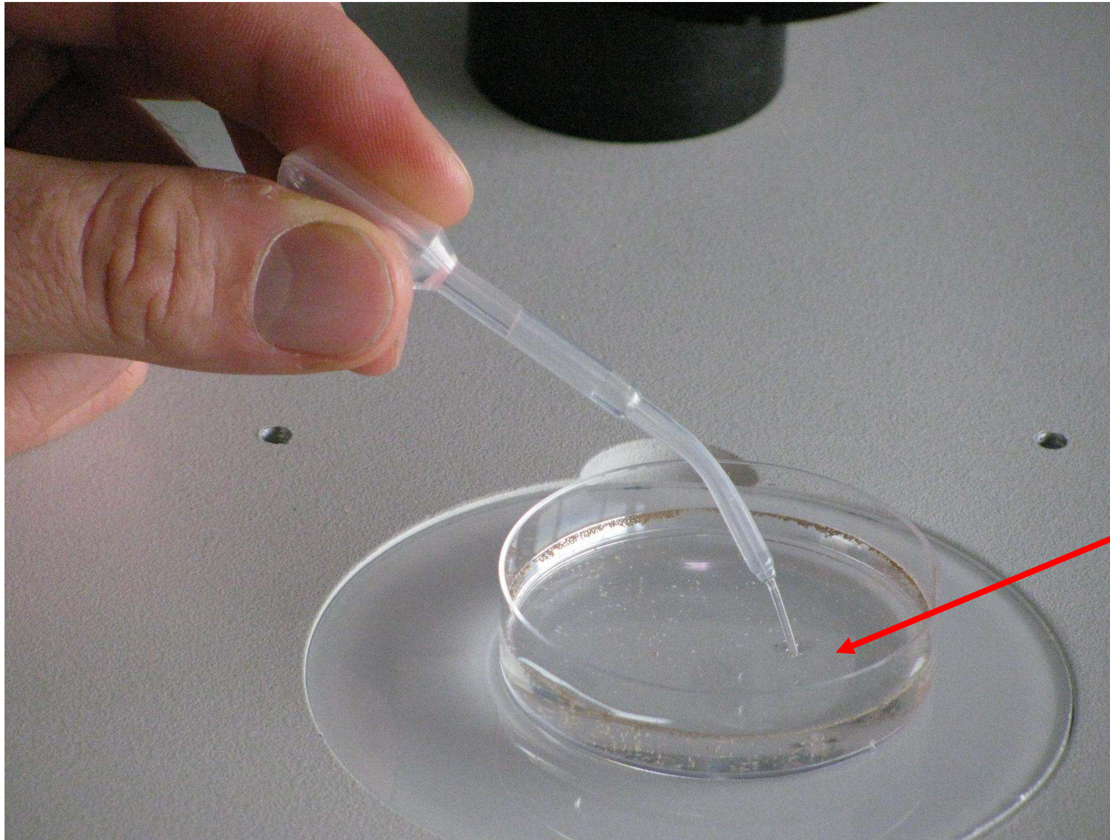
TRANSFER OF THE LARVAE FROM THE HATCHING PETRI DISH TO THE TEST WELLS



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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.

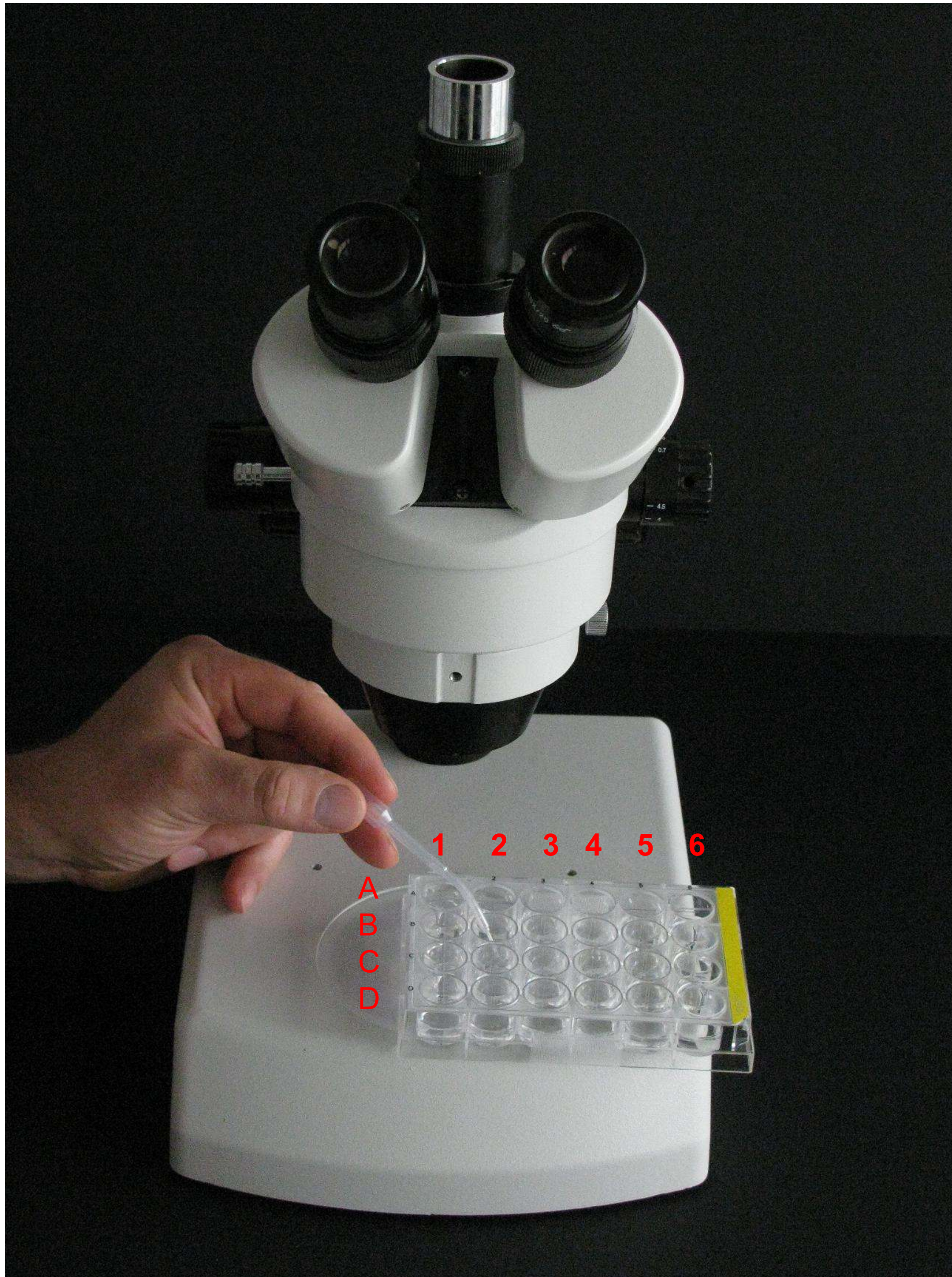
22

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 10 LARVAE FROM RINSING WELL A1 INTO THE 3 OTHER WELLS OF COLUMN 1 (CUPS B1, C1 AND C1 = controls)



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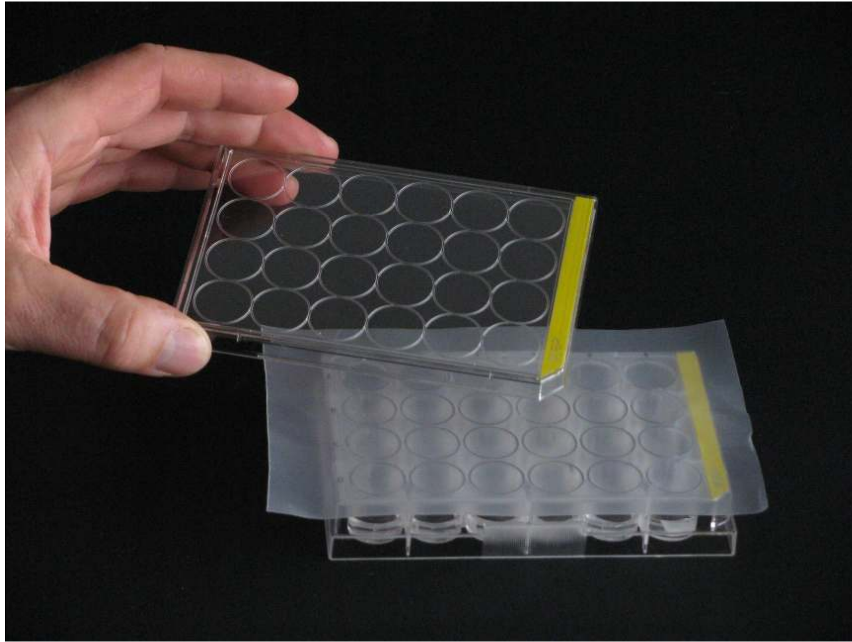
REPEAT THE SAME TRANSFER OF 10 LARVAE FROM RINSING WELLS A2 TO A6 TO THE 3 WELLS OF COLUMNS 2 TO 6

(in this sequence, i.e. from the lowest to the hightest toxicant concentration)

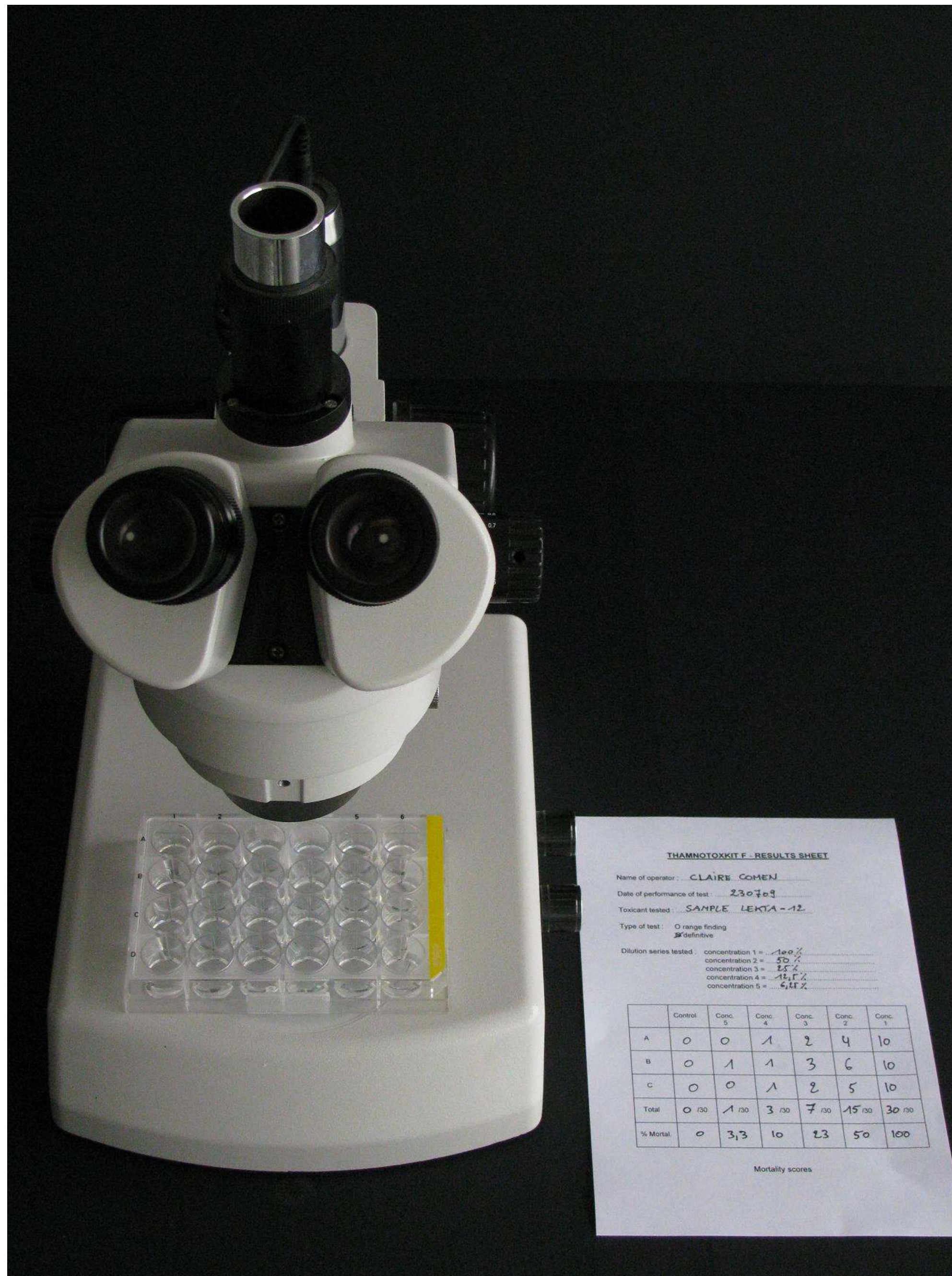


25

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

26

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