



OSTRACODTOXKIT F

Test procedure



1

PREPARATION OF STANDARD FRESHWATER

- VOLUMETRIC FLASK (1liter)
- 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



4

HATCHING OF OSTRACOD CYSTS

POUR THE CONTENTS OF ONE VIAL
WITH CYSTS IN THE PETRI DISH



5

TO ENSURE THE TRANSFER OF ALL THE CYSTS, THE VIAL SHOULD BE RINSED TWICE WITH 1 mL STANDARD FRESHWATER



6

INCUBATION OF THE CYSTS

INCUBATE THE PETRI DISH
FOR 48 HOURS AT 25 °C
UNDER CONTINOUS ILLUMINATION
OF MIN. 3 000 – 4 000 LUX



7

**PRE-FEEDING
OF THE TEST ORGANISMS**

TAKE ONE VIAL
WITH SPIRULINA POWDER
AND FILL IT
WITH STANDARD FRESHWATER

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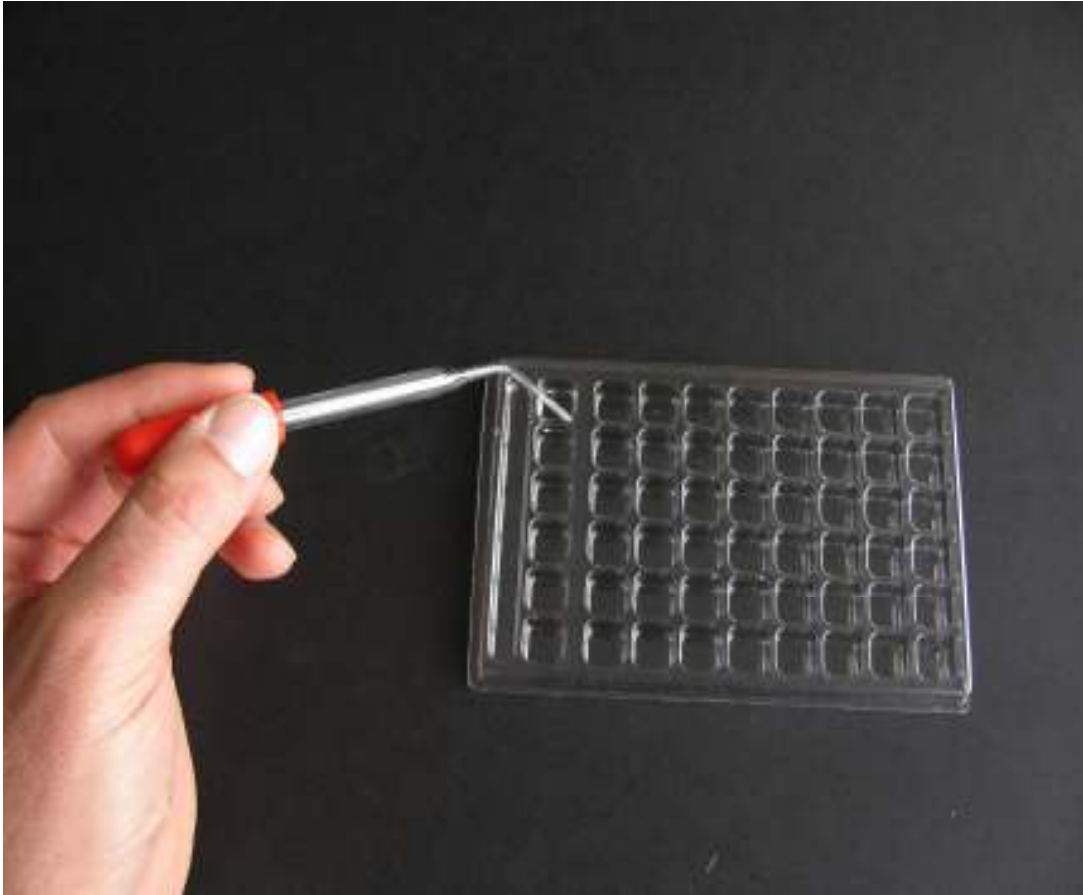
- SHAKE THE VIAL WITH THE SPIRULINA SUSPENSION
- POUR THE CONTENTS IN THE PETRI DISH CONTAINING THE HATCHED OSTRACODS AND SWIRL THE PETRI DISH GENTLY
- ALLOW THE OSTRACODS TO PRE- FEED FOR 4 HOURS



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LENGTH MEASUREMENT OF FRESHLY HATCHED OSTRACODS

PICK UP 10 OSTRACODS FROM THE
HATCHING PETRI DISH
WITH A GLASS MICROPIPETTE



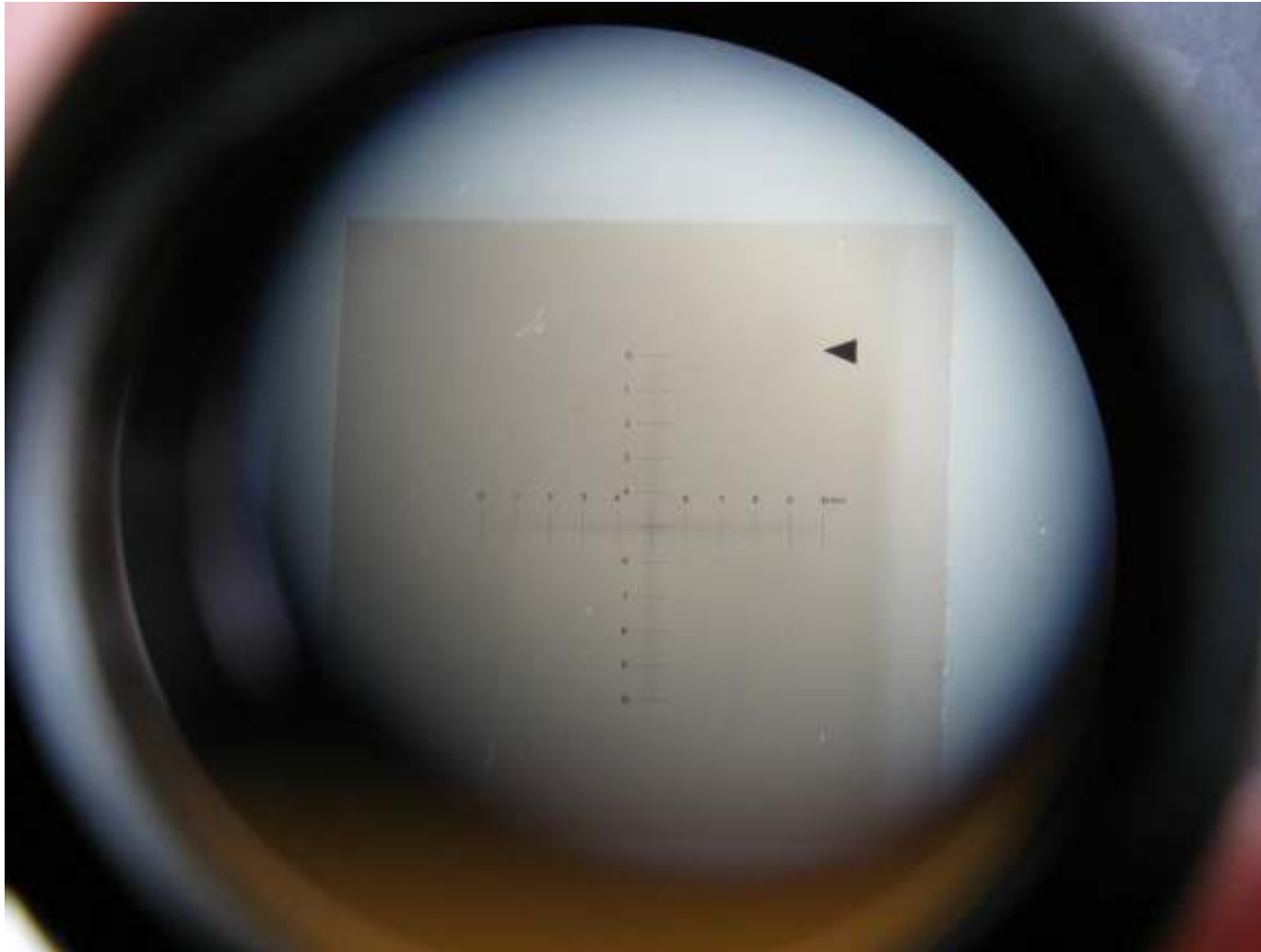
10

TRANSFER THE OSTRACODS
INTO ONE CUP
OF THE MULTIWELL
FOR "LENGTH MEASUREMENTS"



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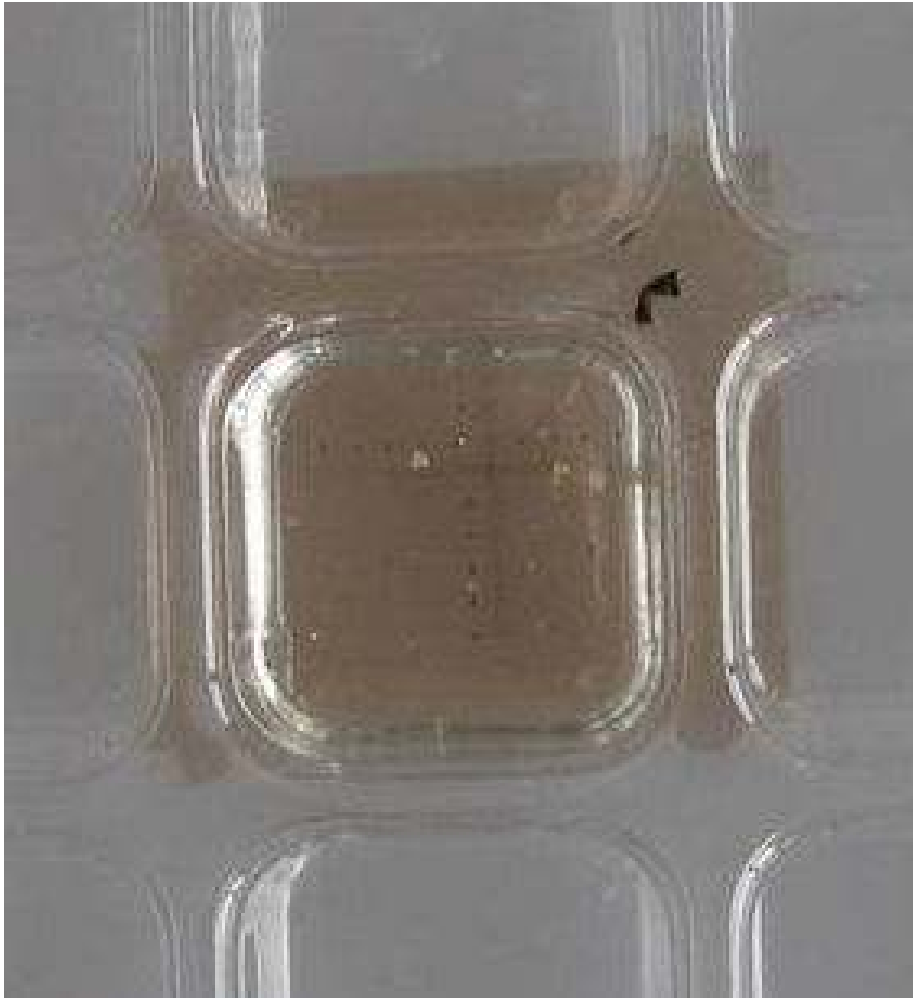
ADD ONE DROP OF LUGOL FIXATIVE
TO THE CUP CONTAINING THE OSTRACODS
AND WAIT UNTIL THE ORGANISMS ARE
COMPLETELY IMMOBILE



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PUT THE MICROMETER SLIP ON THE GLASS STAGE OF THE
DISSECTION MICROSCOPE, IN THE CENTRE OF THE VISUAL FIELD

13



PUT THE MULTIWELL
FOR LENGTH MEASUREMENTS
ON THE STAGE OF THE DISSECTION
MICROSCOPE, AND MEASURE
THE LENGTH OF THE OSTRACODS

N.B : the smallest subdivisions of the
micrometer slip are $50\ \mu\text{m}$

FRESHLY HATCHED OSTRACODS HAVE
A LENGTH OF ABOUT $200\ \mu\text{m}$

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SCORE THE LENGTH
OF THE OSTRACODS
ON THE “RESULTS SHEET”
IN COLUMN “Day 0”





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PREPARATION OF THE ALGAL FOOD SUSPENSION

TAKE ONE TUBE WITH ALGAL BEADS
AND POUR OUT THE STORAGE MEDIUM
TAKING CARE NOT TO LOSE ANY BEAD
DURING THE OPERATION



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ADD 7 mL MATRIX DISSOLVING MEDIUM TO THE TUBE WITH ALGAL BEADS
AND CLOSE THE TUBE WITH THE CAP



17

DE-IMMOBILISATION OF THE ALGAE

SHAKE THE TUBE ON A VORTEX UNTIL THE MATRIX IN WHICH THE ALGAE ARE IMMOBILISED IS TOTALLY DISSOLVED AND THE ALGAE ARE SET FREE



CENTRIFUGE THE TUBE FOR 10 MINUTES
AT 3000 rpm IN A CONVENTIONAL
LAB CENTRIFUGE



CAREFULLY POUR OUT THE
SUPERNATANT FROM THE TUBE

18



ADD 10 mL DISTILLED WATER
TO THE TUBE WITH THE ALGAL PELLETT

19



CAP AND SHAKE THE TUBE
TO RESUSPEND THE ALGAE



20

CENTRIFUGE THE TUBE AGAIN AT 3000 rpm FOR 10 MINUTES
AND POUR OUT THE SUPERNATANT



ADD 10 mL STANDARD FRESHWATER
TO THE TUBE WITH THE ALGAL PELLETT

21



CAP AND SHAKE THE TUBE
TO RESUSPEND THE ALGAE



22

- POUR THE CONCENTRATED ALGAL SUSPENSION INTO A 25 mL VOLUMETRIC FLASK
- ADD STANDARD FRESHWATER TO THE 25 mL MARK
- CAP THE FLASK AND SHAKE TO OBTAIN A HOMOGENOUS ALGAL SUSPENSION



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ADDITION OF SEDIMENT, ALGAL FOOD AND OSTRACODS TO THE TEST PLATES

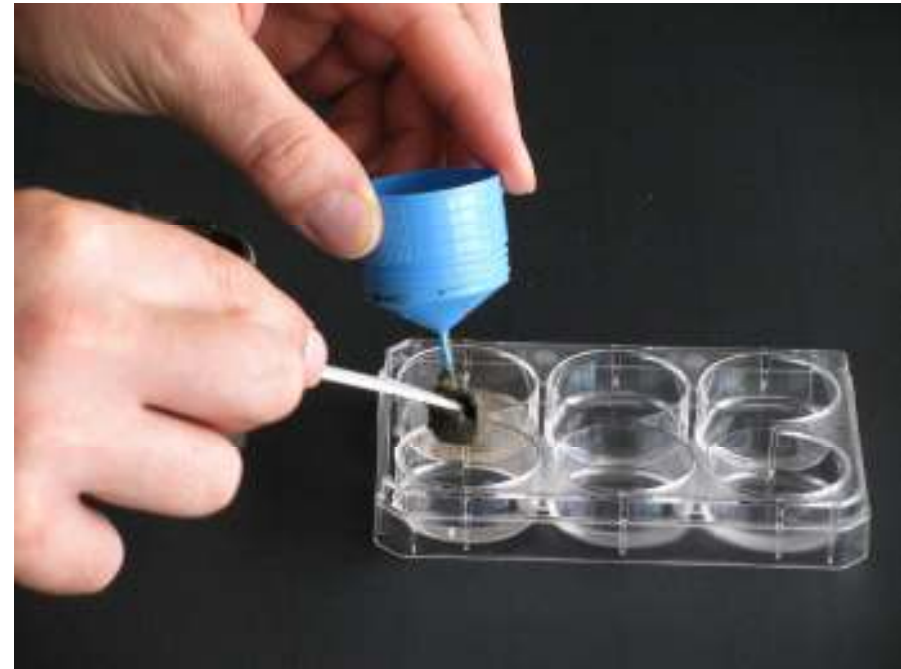
PUT 2 mL STANDARD FRESHWATER
INTO EACH WELL OF TWO TEST
PLATES (multiwell for reference sediment
and multiwell for test sediment)



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TEST PLATE FOR REFERENCE SEDIMENT

- FILL THE SPOON WITH REFERENCE SEDIMENT AND STRIKE OFF THE EXCESS SEDIMENT WITH THE PLASTIC SPATULA (the filled spoon then contains 500 μ l sediment)
- PUT 2 SPOONS (= 1000 μ l) REFERENCE SEDIMENT INTO EACH WELL OF THE TEST PLATE



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TEST PLATE WITH TEST SEDIMENT

- FILL THE SPOON WITH TEST SEDIMENT AND STRIKE OFF THE EXCESS SEDIMENT WITH THE PLASTIC SPATULA (the filled spoon then contains 500 μl sediment)
- PUT 2 SPOONS (= 1000 μl) OF TEST SEDIMENT INTO EACH WELL OF THE TEST PLATE (use the tip of the spatula to perform the transfer)



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- POUR THE ALGAL FOOD SUSPENSION FROM THE 25 mL FLASK INTO A BEAKER
- SHAKE THE BEAKER TO DISTRIBUTE THE ALGAE EVENLY
- PIPET 2 mL ALGAL SUSPENSION INTO EACH WELL OF THE TWO TEST PLATES



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FILL THE LID OF THE HATCHING
PETRI DISH WITH 10 mL STANDARD
FRESHWATER



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TRANSFER WITH THE GLASS
MICROPIPETTE A NUMBER OF
OSTRACOD NEONATES FROM THE
HATCHING PETRI DISH INTO THE LID
OF THIS DISH



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TRANSFER 10 OSTRACODS FROM THE PETRI DISH LID INTO EACH WELL
OF THE TWO TEST PLATES



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INCUBATION OF THE TEST PLATES

- COVER THE TWO TEST PLATES WITH A SHEET OF PARAFILM AND PUT THE LID ON TOP
- INCUBATE THE TEST PLATES AT 25 °C, IN DARKNESS, FOR 6 DAYS



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TEST SCORING – 1. TRANSFER OF THE OSTRACODS INTO A PETRI DISH

WITH THE AID OF THE “LARGE MOUTH” MICROPIPET, SUCK UP PART OF THE SEDIMENT SUSPENSION FROM ONE CUP OF THE TEST PLATE AND TRANSFER IT INTO THE MICROSIEVE



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- GENTLY RINSE THE CONTENTS OF THE MICROSIEVE WITH TAPWATER TO WASH OUT THE FINE SEDIMENT
- PROCEED FURTHER WITH THE STEPWISE TRANSFER OF THE SEDIMENT SUSPENSION TO THE MICROSIEVE AND RINSE EACH TIME THE CONTENTS OF THE MICROSIEVE



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- ADD A FEW mL STANDARD FRESHWATER TO THE CUP
- MIX THE WATER WITH THE REMAINING SEDIMENT AND TRANSFER IT TO THE MICROSIEVE FOR RINSING.
- REPEAT THIS OPERATION UNTIL ALL THE SEDIMENT AND OSTRACODS HAVE BEEN TRANSFERRED INTO THE MICROSIEVE



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- TURN THE MICROSIEVE UPSIDE DOWN AND WASH THE CONTENTS INTO A PETRI DISH, WITH THE AID OF A WASH BOTTLE CONTAINING TAPWATER
- REPEAT THE SEDIMENT TRANSFER AND RINSING OPERATIONS FOR ALL THE CUPS OF THE TWO TEST PLATES



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TEST SCORING – 2. MORTALITY SCORING

PICK UP ALL THE LIVE OSTRACODS FROM THE PETRI DISH WITH A GLASS MICROPIPETTE
AND TRANSFER THEM INTO ONE CUP OF THE “LENGTH MEASUREMENTS” MULTIWELL



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- COUNT THE NUMBER OF LIVE OSTRACODS IN THE CUP
- SUBSTRACT THIS NUMBER FROM 10 (i.e. from the original number of ostracods put in the cup)
- SCORE THE OUTCOME (i.e. the number of dead ostracods) ON THE RESULTS SHEET
- REPEAT THIS OPERATION FOR ALL THE CUPS OF THE TWO TEST PLATES
- CALCULATE AND SCORE THE MEAN % OSTRACOD MORTALITY FOR ALL THE CUPS

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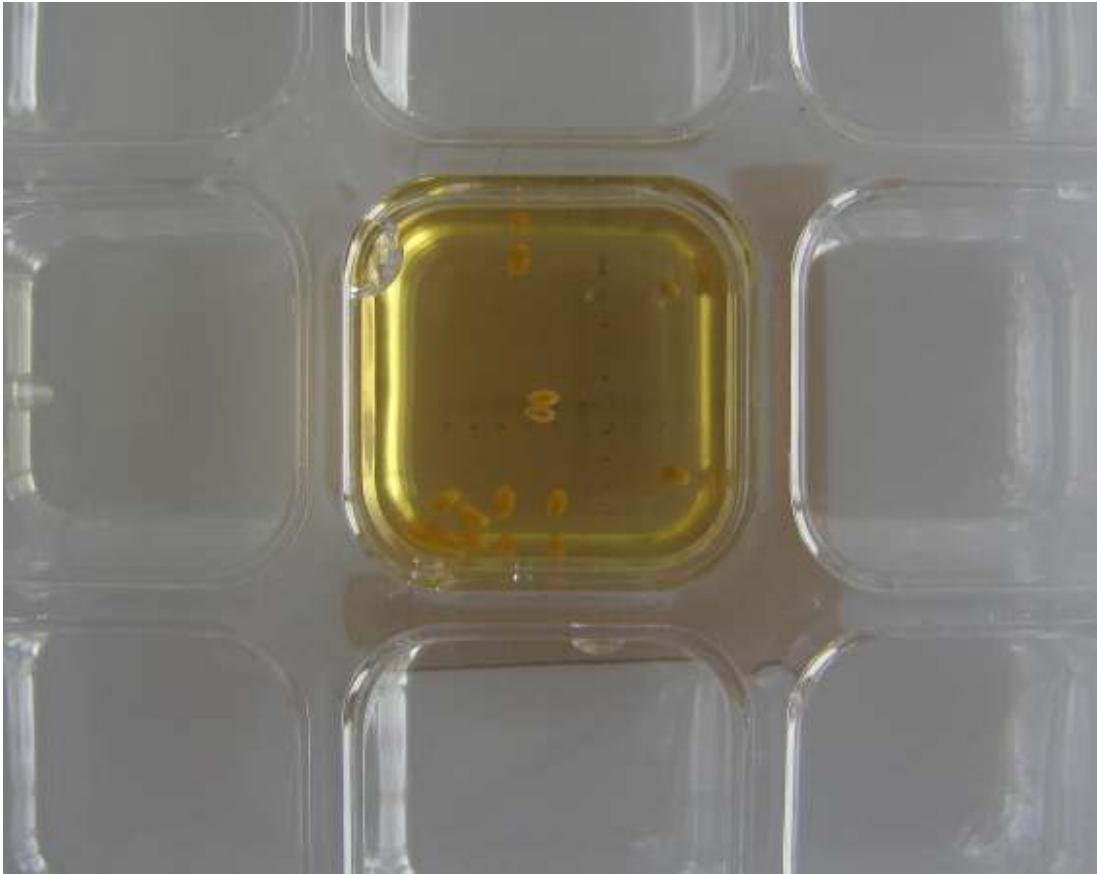
TEST SCORING – 3. LENGTH MEASUREMENT

NB : only to be performed if

the percentage mortality is < 30%

- ADD ONE DROP OF LUGOL FIXATIVE TO EACH CUP OF THE LENGTH MEASUREMENTS MULTIWELL WHICH CONTAIN THE LIVE OSTRACODS FROM THE TWO TEST PLATES





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- WAIT UNTIL THE OSTRACODS ARE IMMOBILE
- MEASURE THE LENGTH OF EACH OSTRACOD FOLLOWING THE PROCEDURE INDICATED IN STEPS 13 & 14
- SCORE THE RESULTS IN THE CORRESPONDING “LENGTH” BOXES OF THE RESULTS SHEET

OSTRACODTOXKIT F

RESULTS SHEET

Mortality (D) and length (μ) of test organisms

Name of operator : TAMINGOL RIK

Date of performance of test : 16/07/2000

Test sediment : SAMPLE 304A

LENGTH	DAY 0	REFERENCE SEDIMENT						TEST SEDIMENT					
		DAY 6						DAY 0					
		Replicate						Replicate					
Test organism	1	2	3	4	5	6	1	2	3	4	5	6	
1	200	1100	1100	1100	1100	1100	1100	160	150	170	160	170	150
2	200	1100	1100	1100	1100	1100	1100	150	150	160	160	160	150
3	200	1100	1000	1000	1000	1000	1100	150	160	160	170	160	160
4	200	1100	1100	1100	1100	1000	1000	150	160	160	160	160	160
5	200	1000	1100	1000	950	1000	1000	150	160	150	150	160	160
6	200	1000	1100	1100	950	950	900	150	160	150	160	160	160
T	200	1000	1100	1100	1100	1100	1100	150	160	150	160	160	160
II	200	300	300	300	350	350	300	500	600	600	600	650	550
III	200	300	300	300	350	300	350	500	M	600	600	650	M
10	200	300	350	M	350	300	M	500	M	550	550	600	M
MORTALITY													
Number of dead ostracods per replicate		0	0	1	0	0	1	0	1	0	0	0	1
Mean % mortality per replicate													
Mean % mortality for all replicates													
Standard deviation of mean % mortality													
Variation coefficient of mean % mortality													
GROWTH INHIBITION													
Mean length of ostracods per replicate													
Mean length for all replicates													
Mean length increment per replicate													
Mean length increment for all replicates													
Mean growth inhibition (%) for all replicates													
Mean % growth inhibition to test sediment													

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- PERFORM THE DATA TREATMENT OF THE RESULTS WITH AN APPROPRIATE PROGRAMME