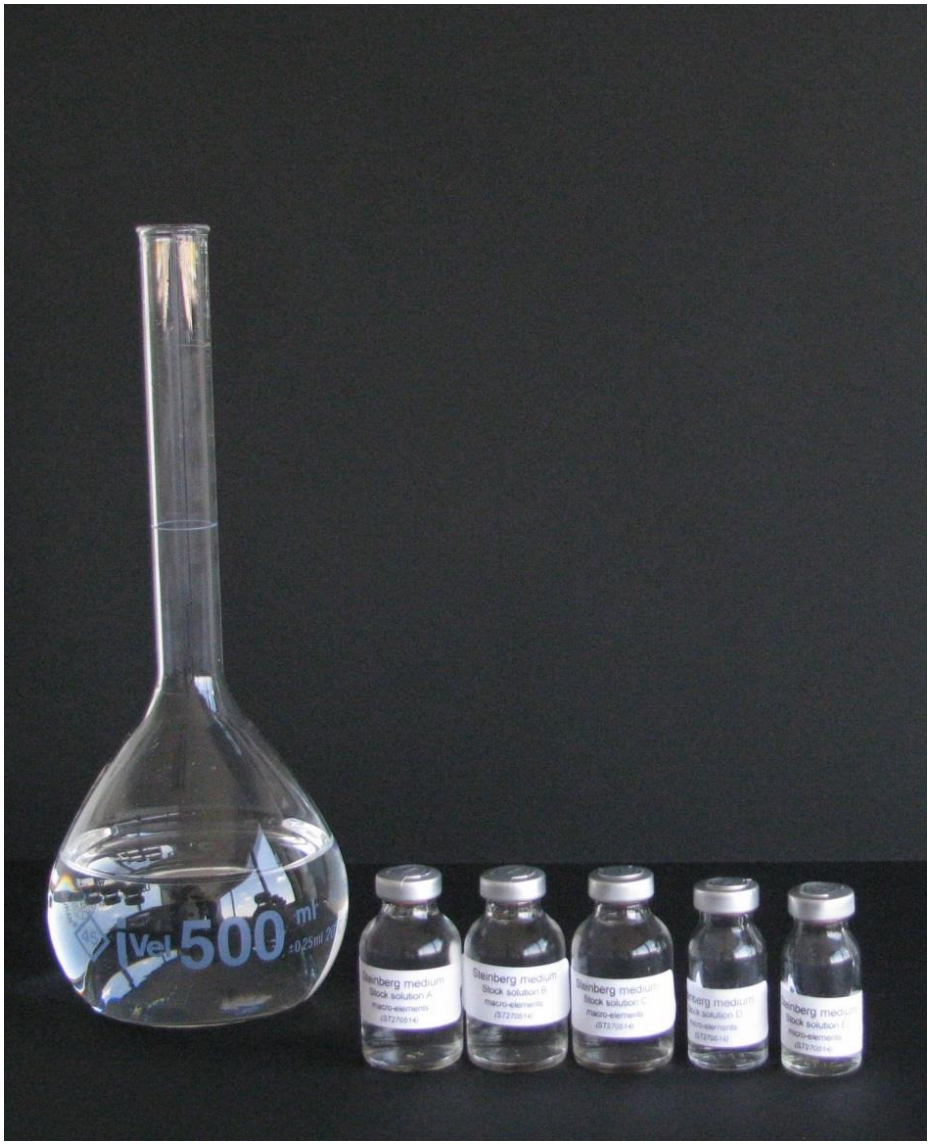
SPIRODELA DUCKWEED TOXKIT

Test procedure



1

PREPARATION OF DUCKWEED GROWTH AND TEST DILUTION MEDIUM

- VOLUMETRIC FLASK (500 ml)
- VIALS WITH CONCENTRATED STEINBERG MEDIUM SOLUTIONS
- PURE WATER (deionized or distilled)

2

TRANSFER 10 ml FROM STOCK SOLUTIONS A, B AND C WITH A PIPET IN ± 300 ml PURE WATER IN THE 500 ml VOLUMETRIC FLASK

3

TRANSFER 0,5 ml OF NUTRIENT

STOCK VIALS D AND E INTO

THE VOLUMETRIC FLASK

4

FILL THE FLASK UP TO THE
500 ml MARK WITH PURE WATER

STOPPER THE FLASK AND SHAKE
THOROUGHLY TO HOMOGENIZE THE
MEDIUM

5

GERMINATION OF THE *SPIRODELA POLYRHIZA* TURIONS

TAKE A TUBE WITH *SPIRODELA* TURIONS AND SHAKE IT SLIGHTLY TO RESUSPEND THE TURIONS

6

- POUR THE CONTENTS OF THE TUBE WITH TURIONS IN THE MICROSIEVE
- MAKE SURE THAT ALL THE TURIONS ARE TRANSFERRED

7

RINSE THE TURIONS

THOROUGHLY

WITH PURE WATER

8

TURN THE SIEVE UPSIDE DOWN AND FLUSH THE TURIONS INTO THE PETRI DISH WITH STEINBERG MEDIUM

N.B. The final volume of Steinberg medium in the petri dish shall be 30 ml

9

A

B

INCUBATE THE PETRI DISH FOR 72h AT 25 °C
UNDER CONTINOUS “TOP” ILLUMINATION
OF 6 000 LUX

A : an incubator provided with illumination

B : an incubator with a LED Illumination Unit

10

PREPARATION OF THE TOXICANT DILUTIONS

For example :

TEST ON A EFFLUENT

IN 5 DILUTIONS (C1-C5)

100% - 50% - 25% - 12.5% - 6.25%

11

ADDITION OF CONCENTRATED STEINBERG MEDIUM SOLUTIONS TO THE EFFLUENT

- TRANSFER ABOUT 80 ml EFFLUENT IN A 100 ml VOLUMETRIC FLASK

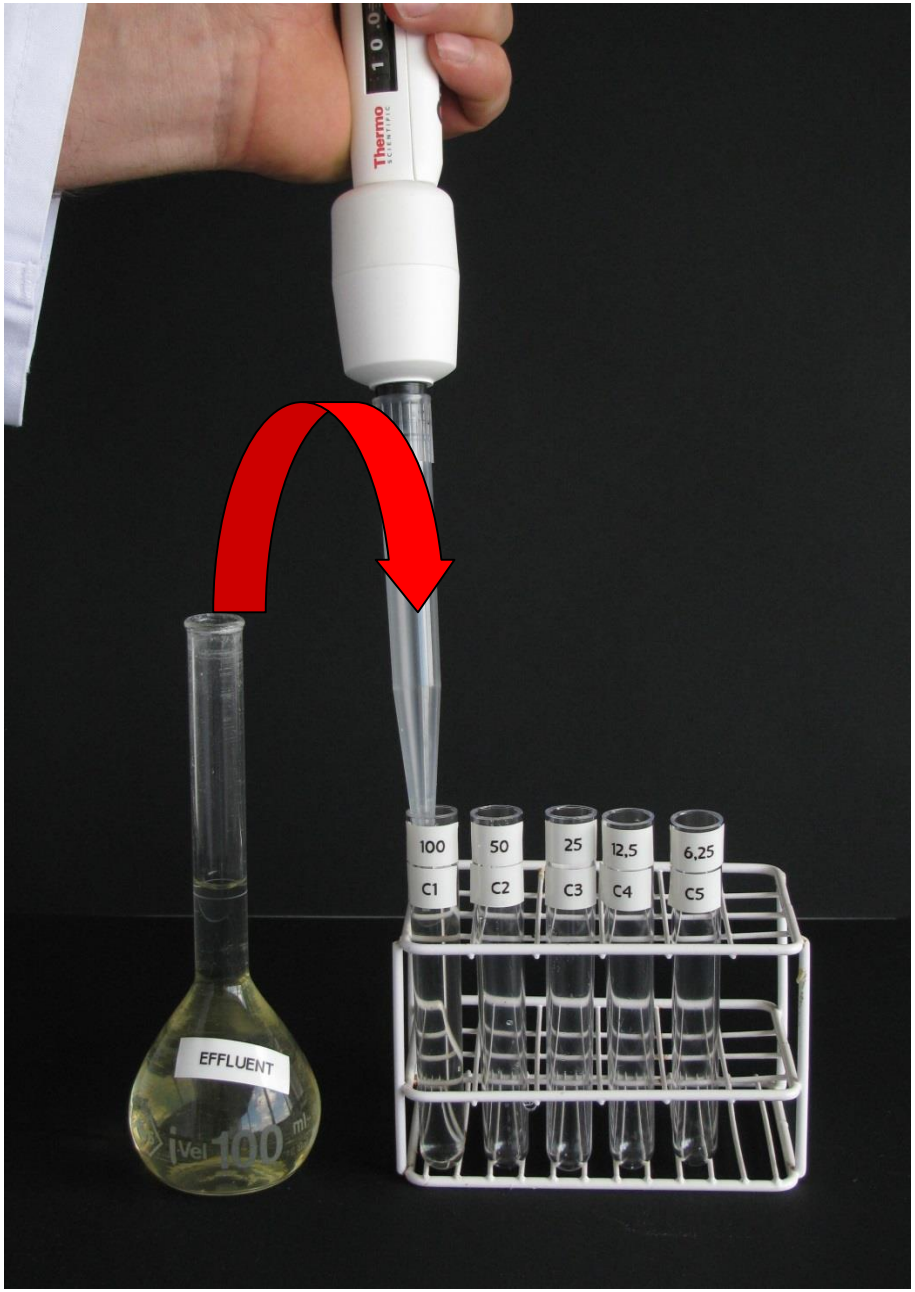
- ADD 2 ml NUTRIENT STOCK SOLUTION OF VIALS A, B , C

12

ADD 100 µl OF NUTRIENT STOCK SOLUTIONS D AND E
TO THE VOLUMETRIC FLASK

13

- FILL THE FLASK UP TO THE 100 ml MARK WITH EFFLUENT
- STOPPER THE FLASK AND SHAKE TO MIX THE CONTENTS



14

PREPARATION OF THE EFFLUENT DILUTIONS

- TAKE FIVE 20 ml TUBES AND LABEL THEM C1 TO C5
- ADD 20 ml OF THE TREATED EFFLUENT TO TEST TUBE C1

15

ADD 10 ml STEINBERG MEDIUM TO THE TEST TUBES C2, C3, C4 AND C5

16

- TRANSFER 10 ml EFFLUENT FROM TUBE C1 TO TUBE C2
- CAP AND SHAKE THE TEST TUBE

17

- TRANSFER 10 ml TEST DILUTION FROM TUBE C2 TO C3
- CAP AND SHAKE THE TEST TUBE.
- REPEAT THIS PROCEDURE FOR THE NEXT DILUTIONS

18

FILLING OF THE TEST PLATE

WITH THE TOXICANT DILUTIONS

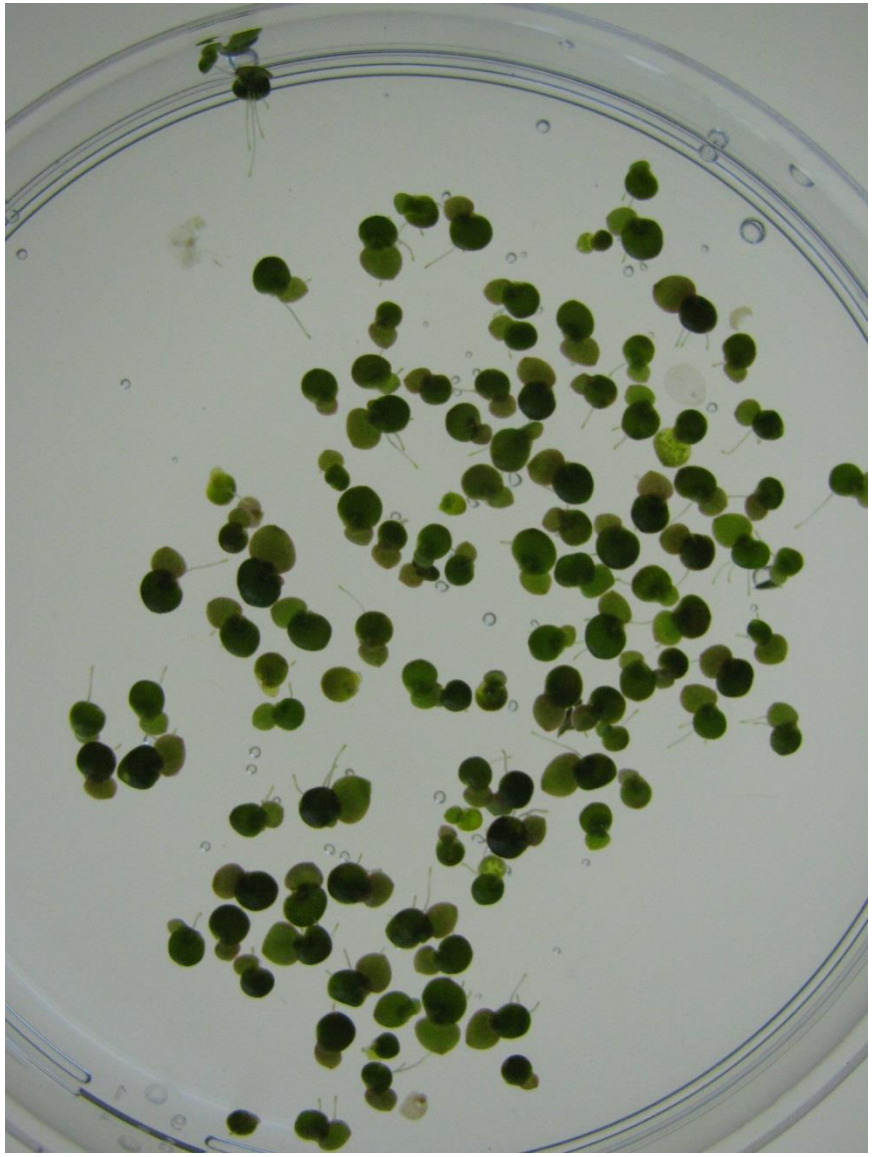
TRANSFER 1 ml STEINBERG MEDIUM

IN THE 8 CUPS OF ROW A (= Control row)

19

- PUT 1 ml OF THE TUBE C5 IN THE 8 CUPS OF ROW B.

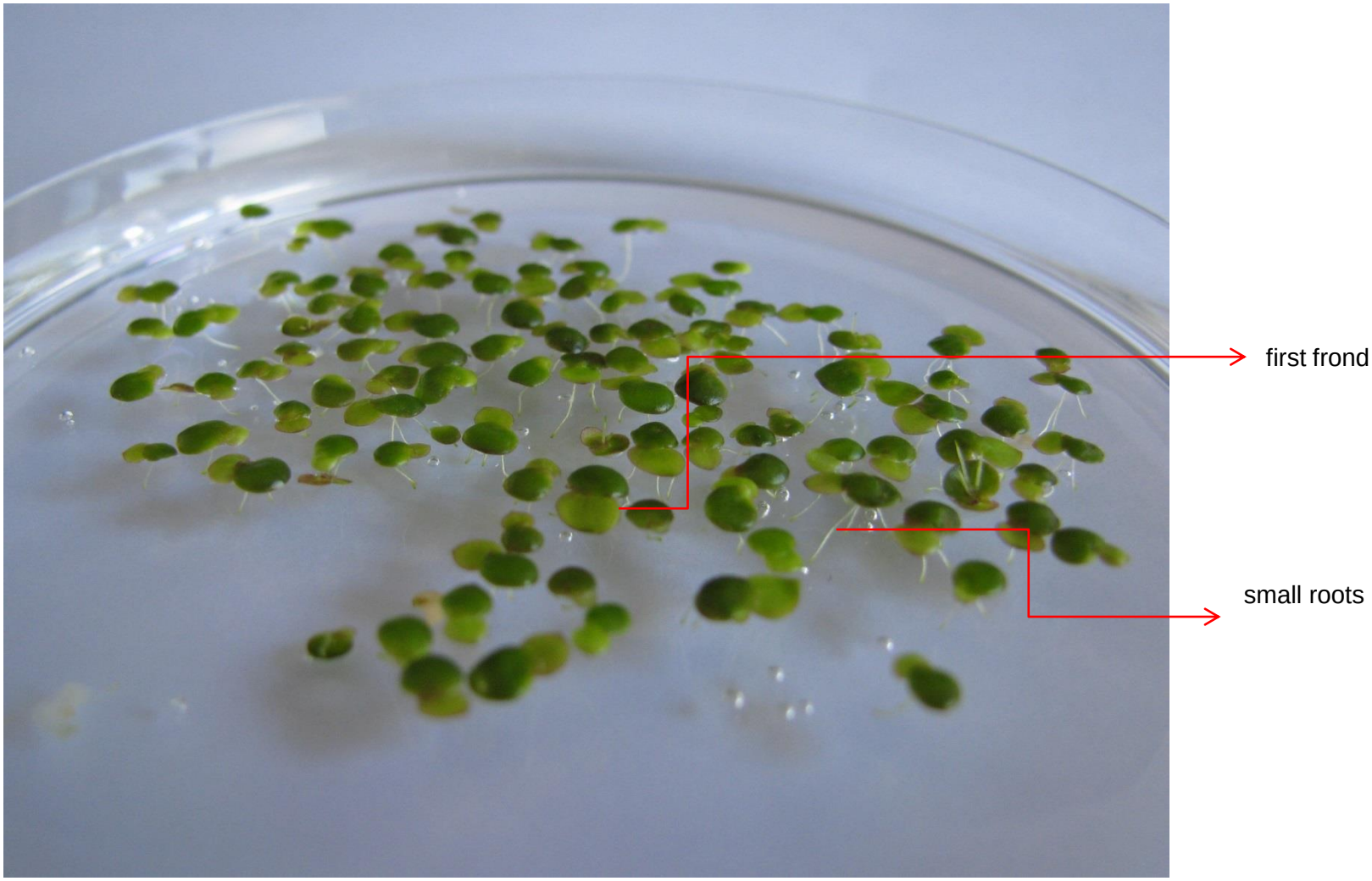
- REPEAT THIS PROCEDURE WITH THE TUBES C4, C3, C2 AND C1 FOR THE 8 CUPS IN THE ROWS C, D, E AND F RESPECTIVELY



20

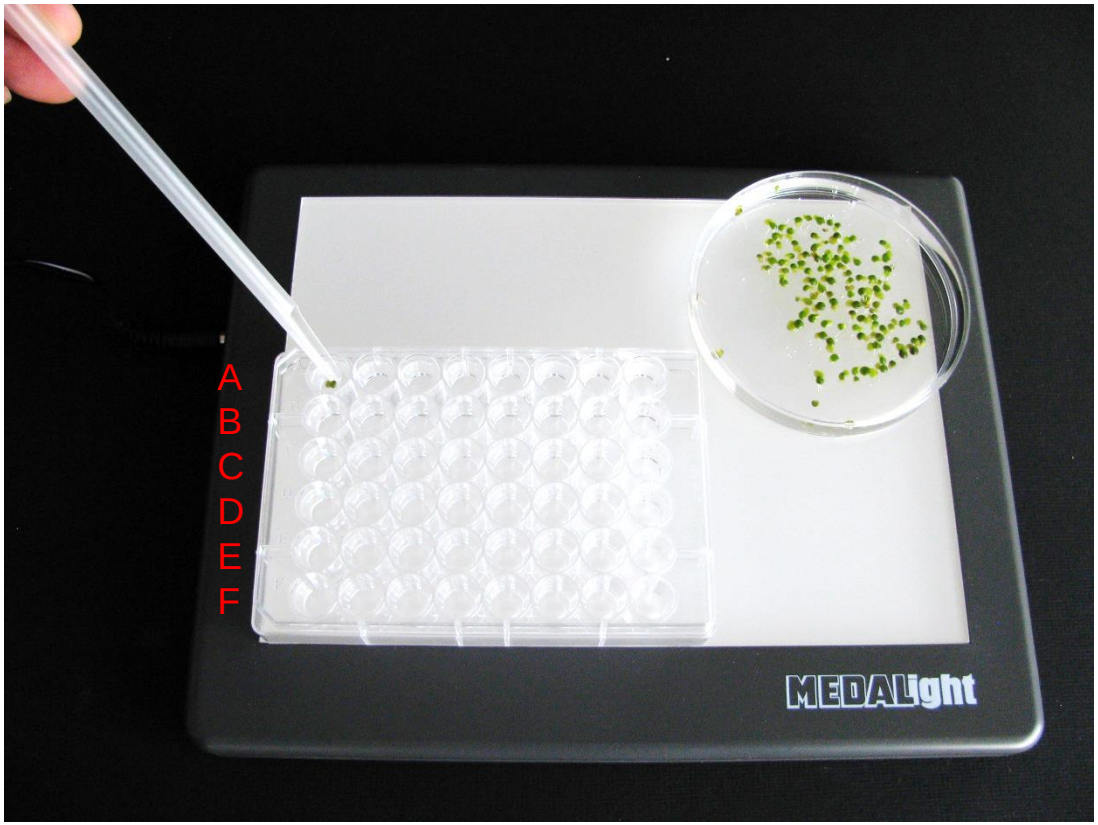
TRANSFER OF THE GERMINATED TURIONS IN THE TEST CUPS

21



GERMINATED TURIONS CAN EASILY BE RECOGNIZED BY THE PRESENCE

OF A SMALL FIRST FROND (on one side of the turion) WITH SMALL ROOTS



- WITH THE AID OF THE SPATULA, TRANSFER "**ONE**" GERMINATED TURION
  INTO EACH CUP OF THE CONTROL ROW (= ROW A)
- REPEAT THIS PROCEDURE WITH THE OTHER ROWS, STARTING WITH ROW B DOWN TO ROW F

N.B. The transfers must be made "at random", i.e. not starting with the turions which have the largest first fronds !

23

TAKING OF A PHOTO OF THE MULTIWELL AT THE START OF THE TOXICITY TEST

TAKE A PHOTO OF THE MULTIWELL WITH THE GERMINATED TURIONS AT THE START OF THE 3 DAYS TEST (= t0h), AND TRANSFER THE PHOTO TO A COMPUTER FILE

N.B. To obtain a photo with the best contrast between the turions and their first frond it is advised to put the multiwell on a light table

24

MULTIWELL PLATE WITH THE GERMINATED TURIONS AND THEIR SMALL FIRST FRONDS AT t0h

A

B

25

INCUBATION OF THE TEST PLATE

- PUT THE COVER ON THE MULTIWELL PLATE.
- INCUBATE THE TEST PLATE FOR 72h AT 25 °C UNDER CONTIONOUS “TOP” ILLUMINATION OF 6 000 LUX

A : an incubator provided with illumination

B : an incubator with a LED Illumination Unit

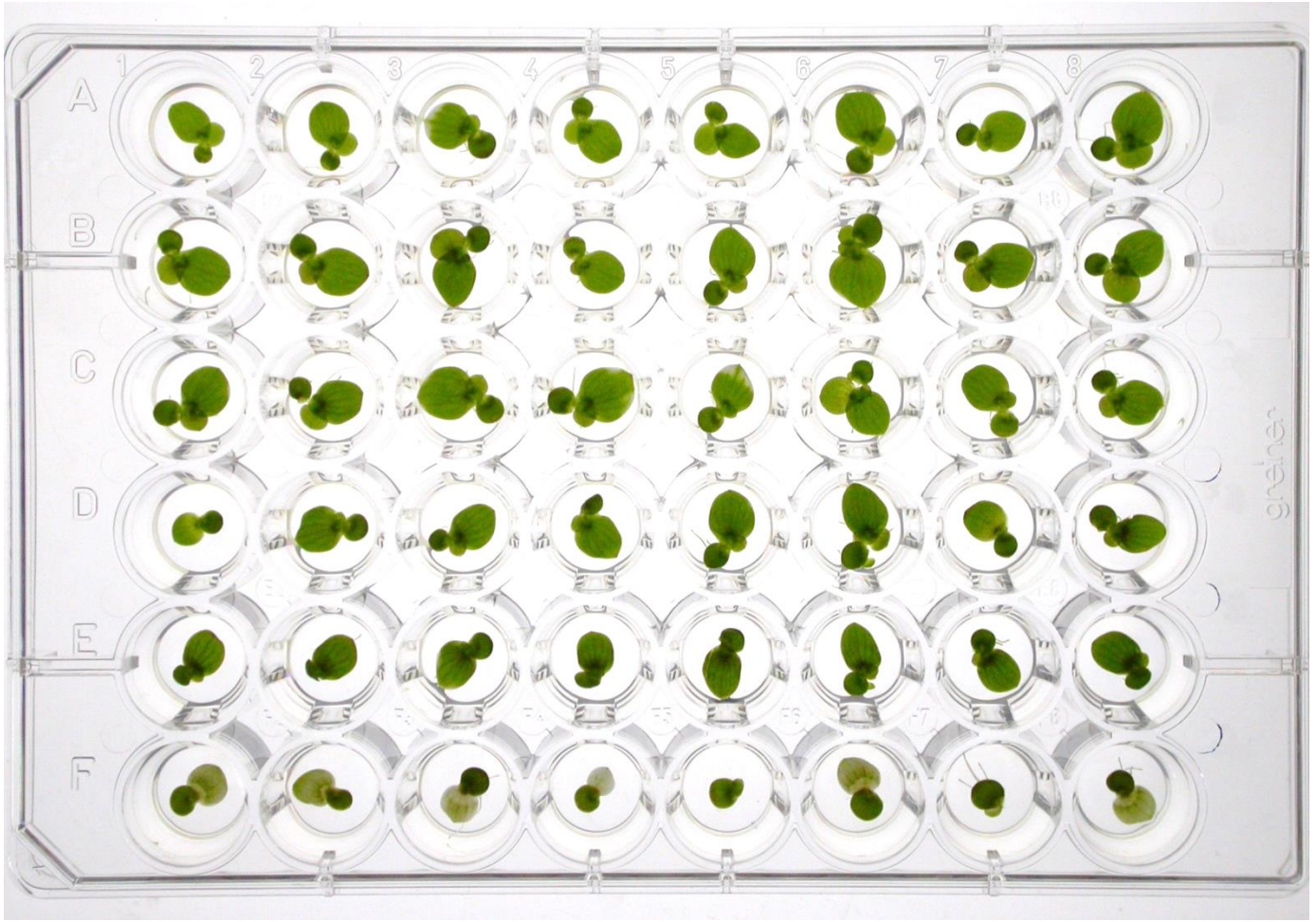
26

TAKING OF A PHOTO OF THE MULTIWELL AT THE END OF THE TOXICITY TEST

TAKE AGAIN A PHOTO OF THE MULTIWELL WITH THE GROWN FRONDS AT THE END OF THE 3 DAYS TEST, (= t72h) AND TRANSFER THE PHOTO TO A COMPUTER FILE

N.B. To obtain a photo with the best contrast between the turions and the fronds, it is advised to put the multiwell on a light table

27



MULTIWELL PLATE WITH THE GROWN FIRST FRONDS
TAKEN AFTER 3 DAYS INCUBATION (t72h)

28

MEASUREMENT OF THE AREA OF THE FIRST FRONDS

AREA MEASUREMENTS OF THE SMALL FRONDS OF THE GERMINATED TURIONS ARE MADE IN EACH CUP OF THE MULTIWELL AT THE START OF THE TOXICITY TEST (t0h) AND A SECOND TIME AT THE END OF THE 3 DAYS TOXICITY TEST (t72h) ON THE GROWN FIRST FRONDS.

THE AREA MEASUREMENTS ARE MADE ON THE 2 SAVED PHOTOS OF THE MULTIWELL, WITH THE AID OF AN APPROPRIATE “IMAGE ANALYSIS” PROGRAM (e,g,.“IMAGE J”).

N.B. Only the area of the “first frond” (= the largest frond of each turion) shall be measured !

29

DATA TREATMENT

THE GROWTH OF THE DUCKWEED IS CALCULATED BY SUBSTRACTING THE “INITIAL” SIZE OF THE FIRST FROND (t0h area) FROM THE “FINAL” SIZE OF THIS FROND (t72h area) , IN THE CONTROL AND IN THE DIFFERENT TOXICANT CONCENTRATIONS.

THE PERCENTAGE GROWTH INHIBITION OF THE DUCKWEEDS IN THE RESPECTIVE TOXICANT CONCENTRATIONS CAN THEN BE CALCULATED, FOLLOWED BY THE EVALUATION OF THE 72h EC50.

A data treatment program has been worked out by MicroBioTests Inc.
and can be obtained on request.