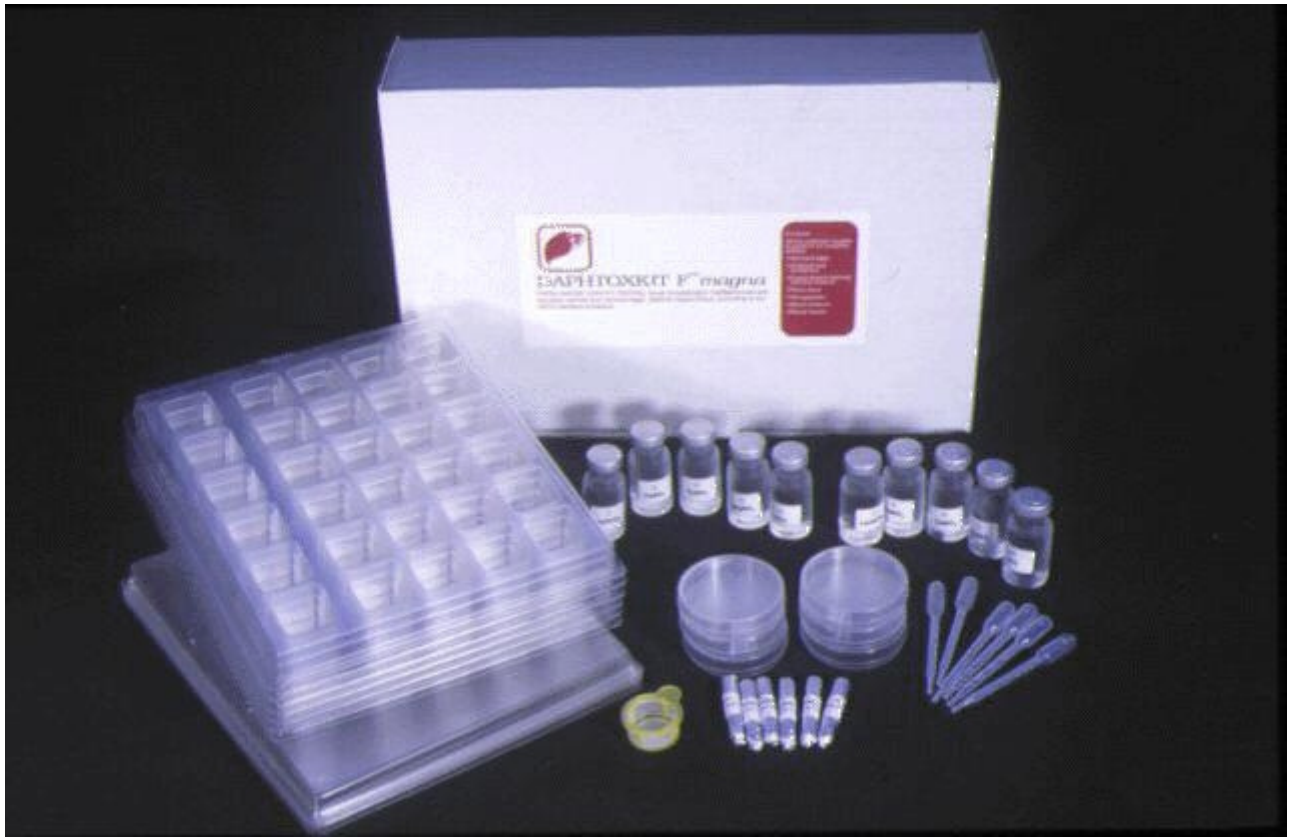


DAPHTOXKIT F MAGNA

Test procedure

1

PREPARATION OF

STANDARD FRESHWATER

- VOLUMETRIC FLASK (2 liter)
- VIALS WITH SOLUTIONS OF CONCENTRATED SALTS
- DISTILLED (or deionized) WATER

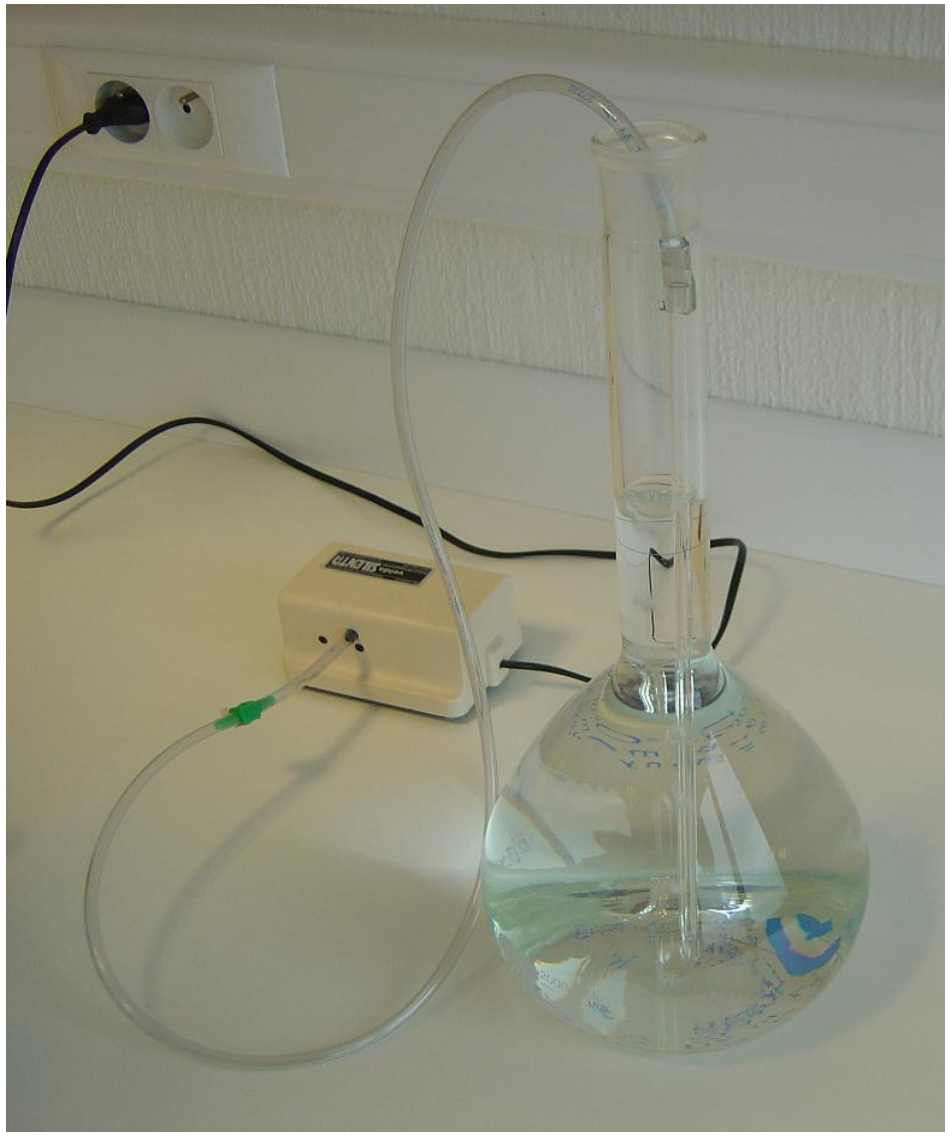
2

POUR THE 4 VIALS

WITH CONCENTRATED SALT SOLUTIONS

IN $\pm$ 1 LITER DISTILLED WATER,

IN THE 2 LITER VOLUMETRIC FLASK



3

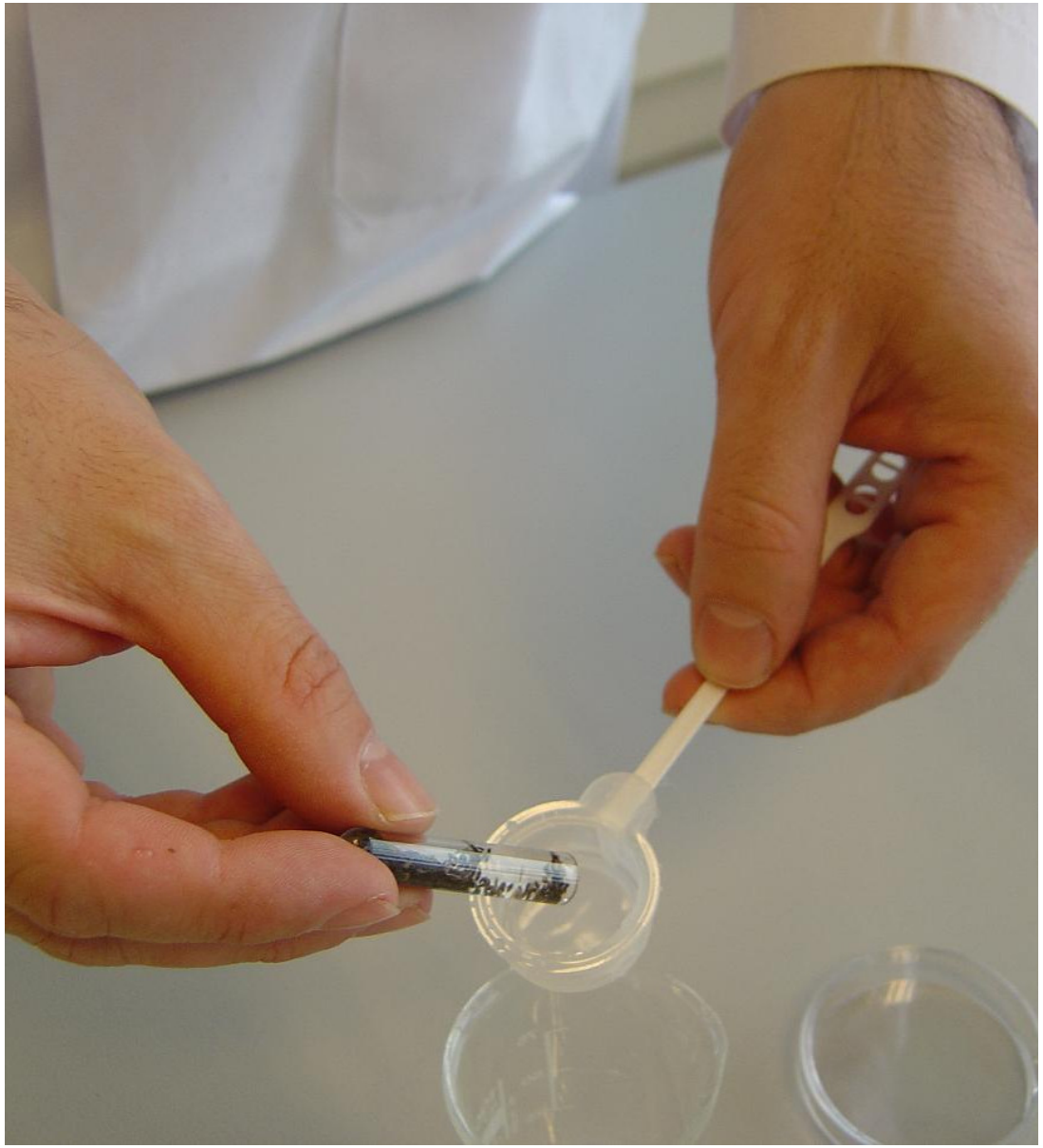
FILL THE FLASK TO THE 2 LITER MARK

AND AERATE FOR AT LEAST 15 MINUTES

4

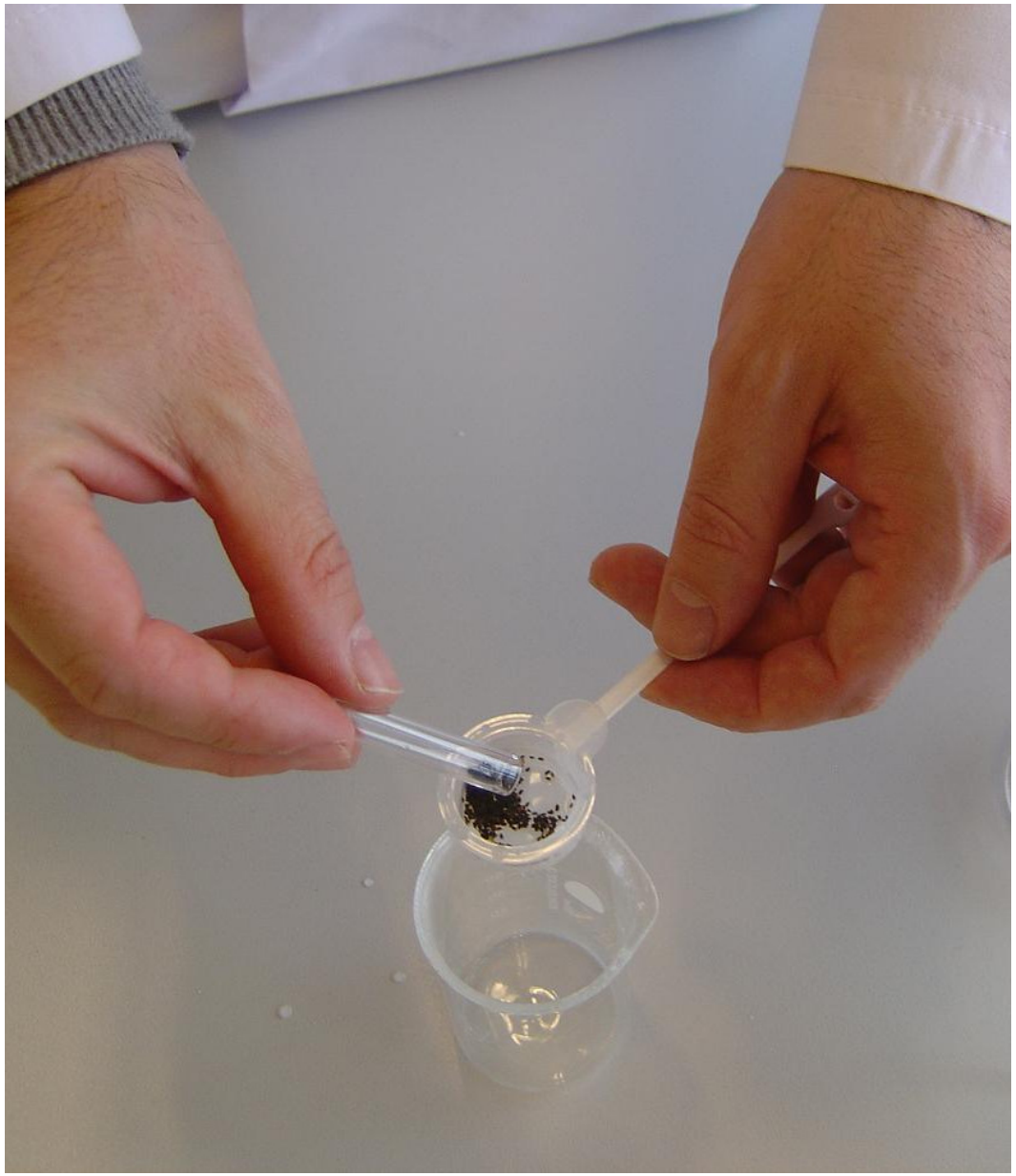
HATCHING OF THE EPHIPPIA

REMOVE THE
ALUMINIUM FOIL
FROM A TUBE
WITH DAPHNIA
EPHIPPIA



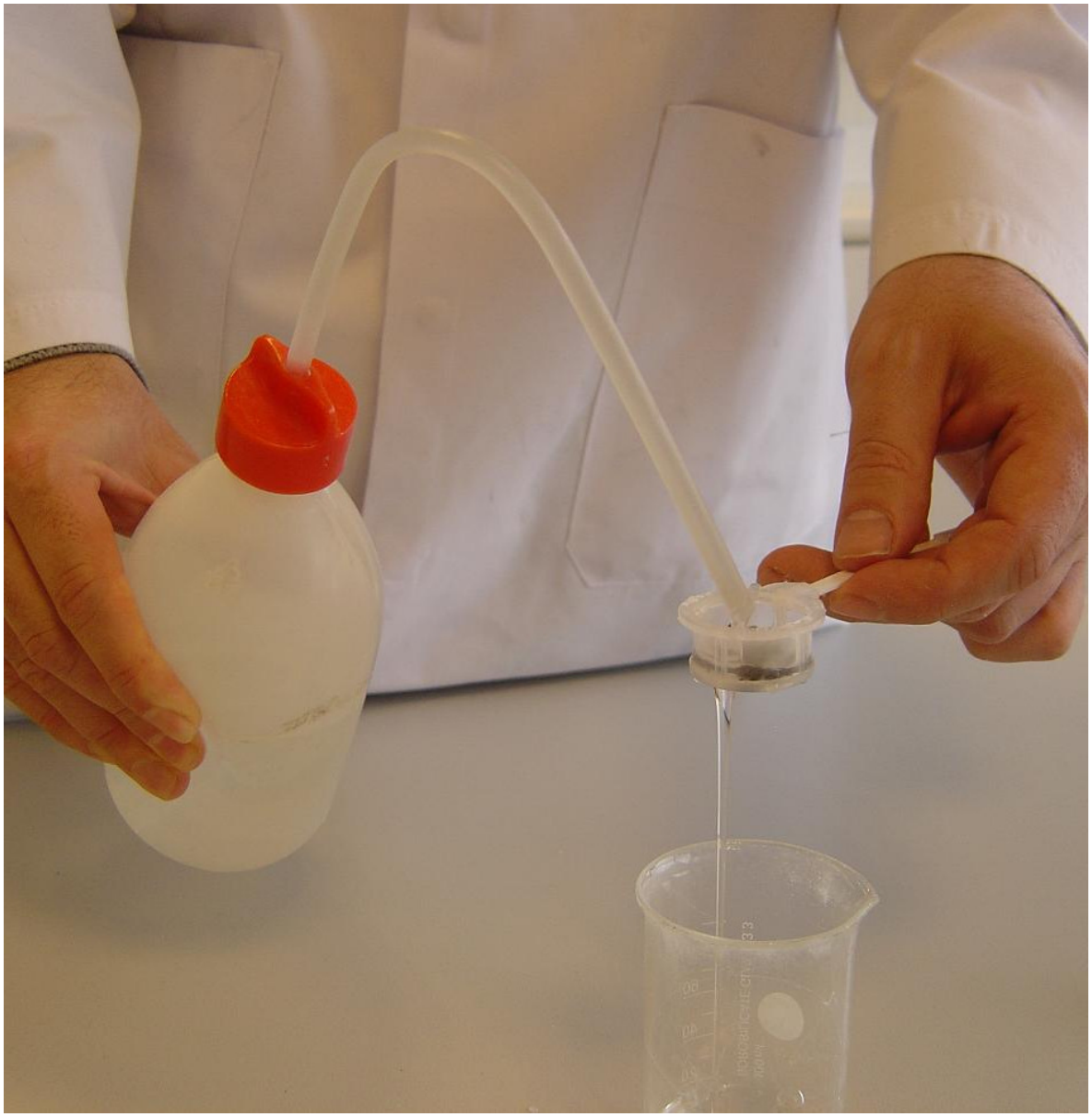
5

POUR THE CONTENTS
OF THE TUBE WITH EPHIPPIA
IN THE MICROSIEVE



6

MAKE SURE THAT
ALL THE EPHIPPIA
ARE TRANSFERRED
INTO THE MICROSIEVE



7

RINSE THE EPHIPPIA
THOROUGHLY
WITH TAP WATER

8

TRANSFER THE EPHIPPIA
INTO THE
HATCHING PETRI DISH
IN STANDARD FRESHWATER

9

INCUBATE THE PETRI DISH

FOR 72h AT 20-22 °C

UNDER CONTINUOUS ILLUMINATION

OF 6 000 LUX

10

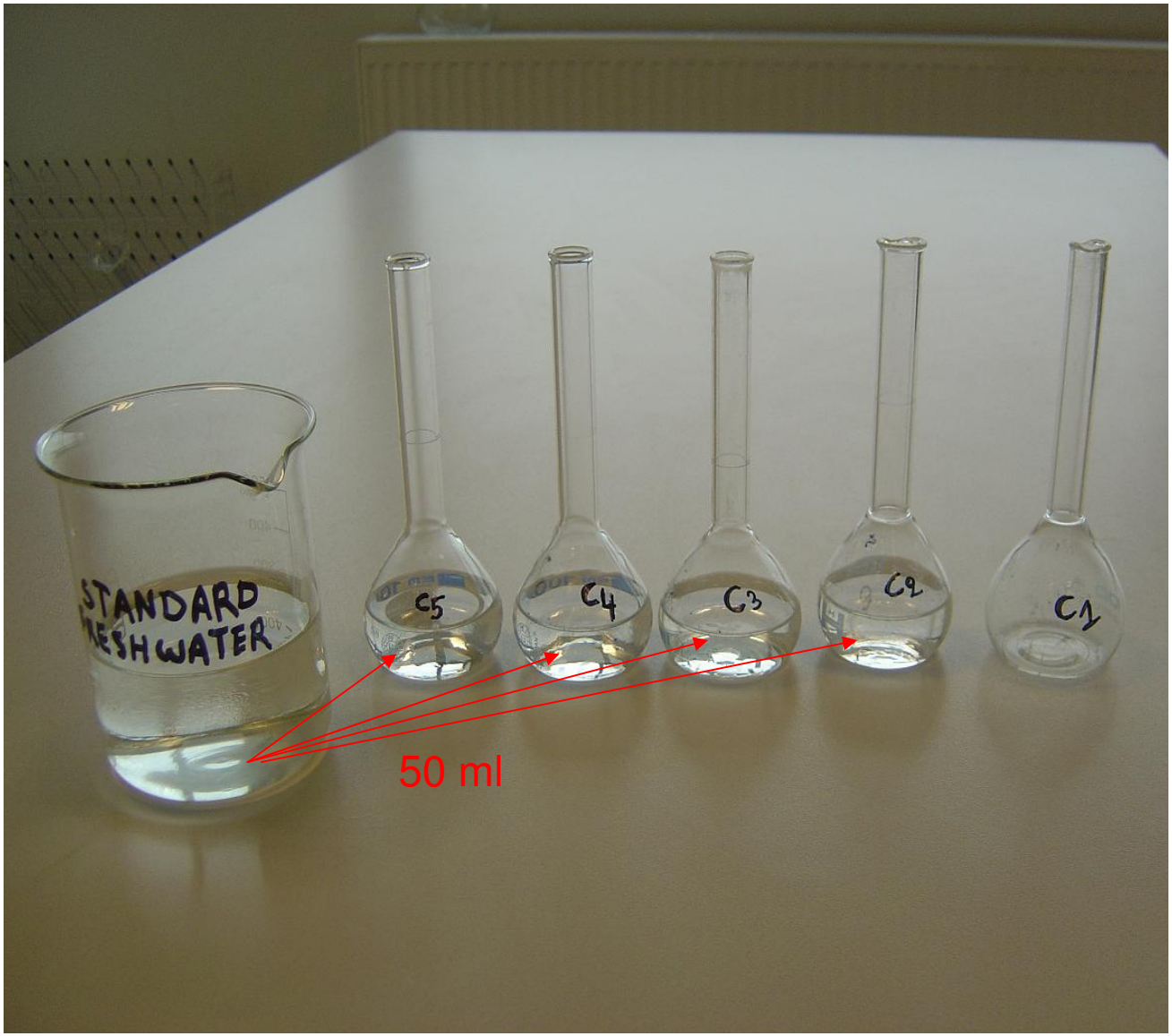
PREPARATION OF THE TOXICANT DILUTIONS

For example :

TEST ON A EFFLUENT

IN 5 DILUTIONS (C1-C5)

+ ONE CONTROL



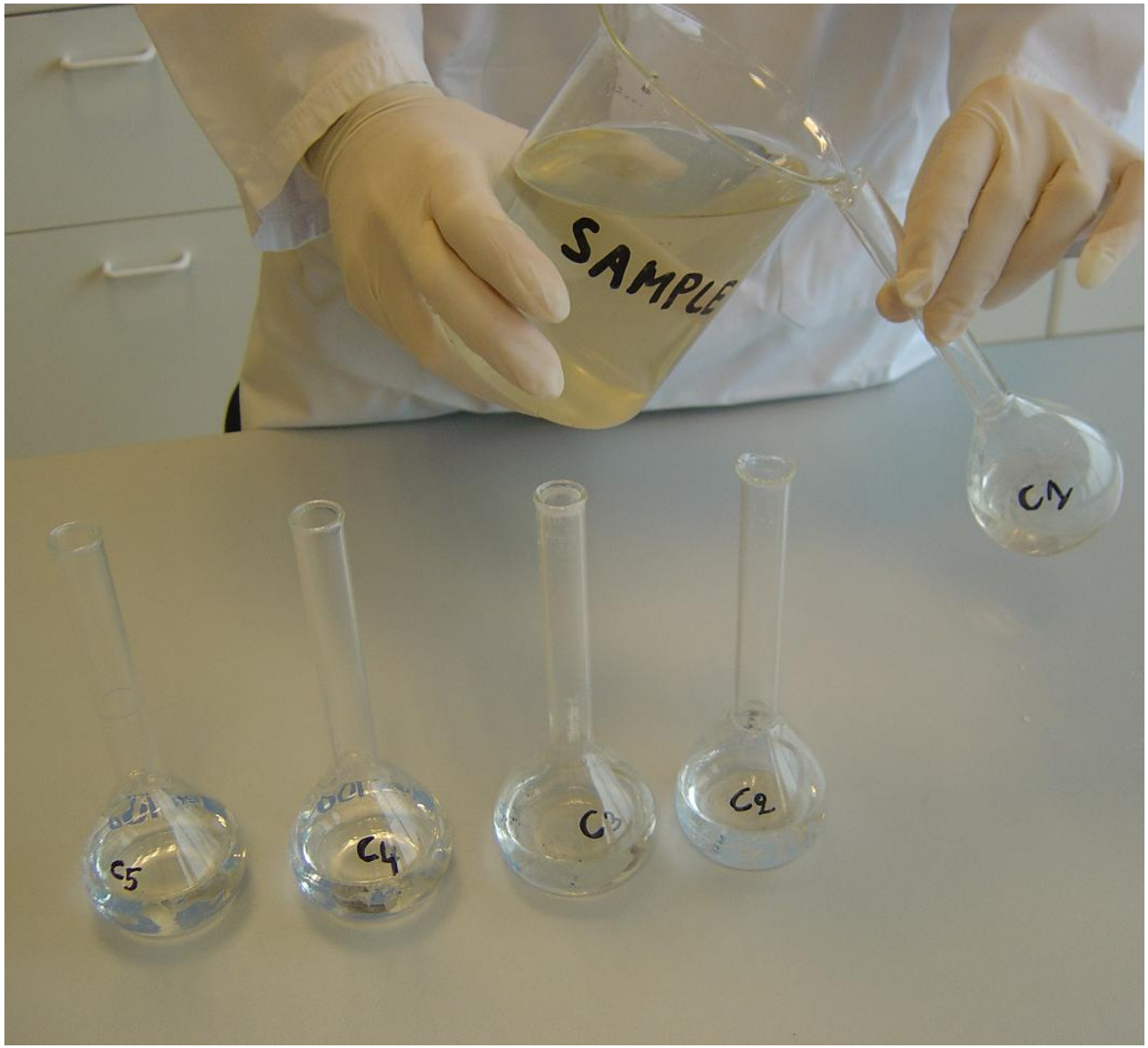
11

TRANSFER 50 ML

STANDARD FRESHWATER

INTO FLASKS

C2, C3, C4 AND C5



12

FILL FLASK C1

TO THE 100 ML MARK

WITH EFFLUENT

13

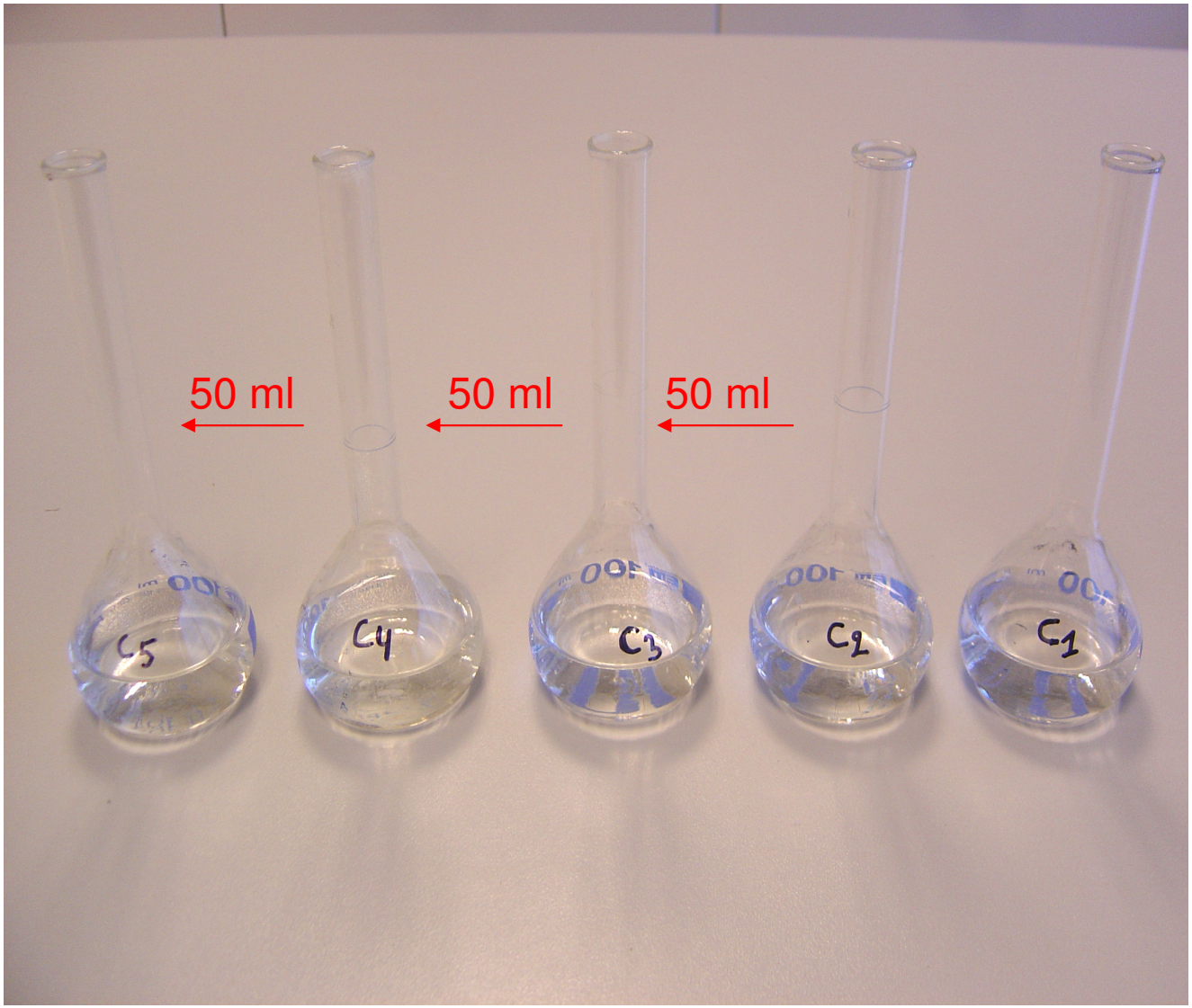
TRANSFER 50 ML EFFLUENT

FROM FLASK C1

INTO A GRADUATED CYLINDER.

14

TRANSFER THE 50 ML EFFLUENT FROM THE GRADUATED CYLINDER TO FLASK C2 AND SHAKE THOROUGHLY



15

REPEAT THE FORMER
DILUTION PROCEDURE
FOR THE OTHER FLASKS
(i.e. 50 ml from C2 to C3, etc).

16

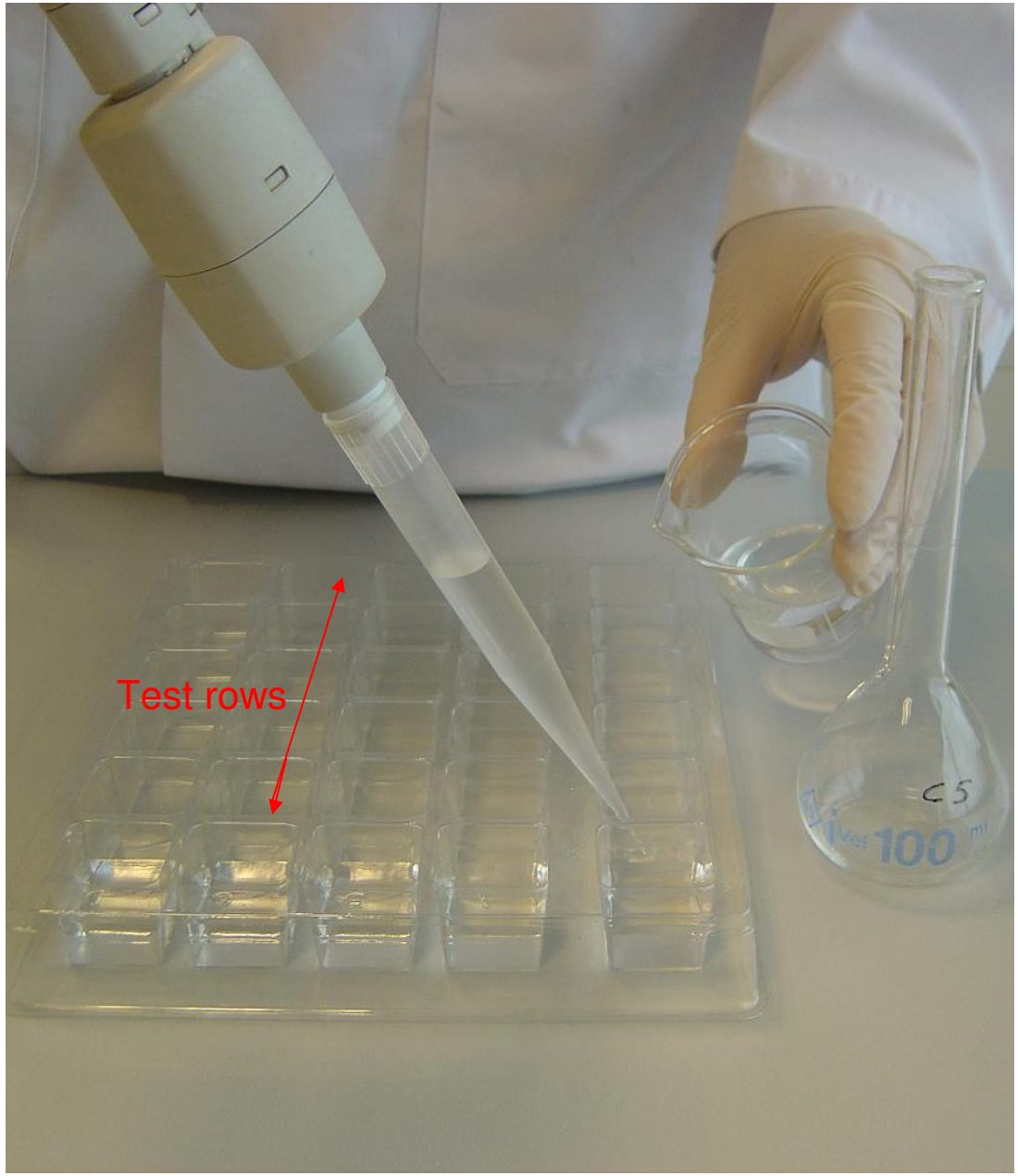
FILLING OF THE TEST PLATE :

TRANSFER 10 ML

STANDARD FRESHWATER

INTO EACH WELL

OF THE CONTROL ROW



17

TRANSFER 10 ML OF THE
RESPECTIVE TOXICANT
CONCENTRATIONS
INTO EACH WELL
OF THE CORRESPONDING ROWS
FROM C5 TO C1

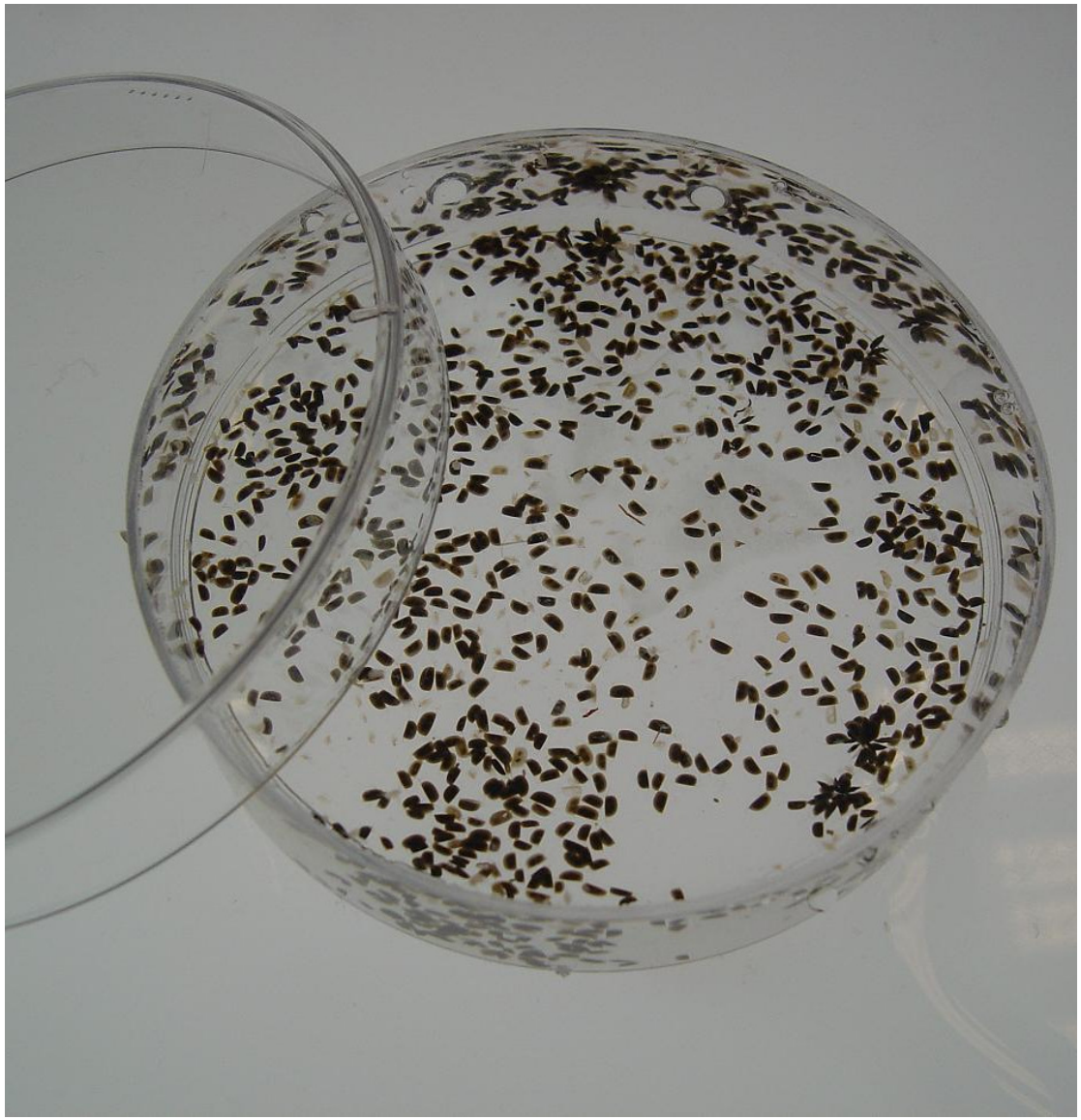
18

AFTER 72h TO 80h

INCUBATION

VERIFY THE HATCHING

OF THE DAPHNIA NEONATES



19

A MINIMUM OF 120 NEONATES
ARE NEEDED TO PERFORM
ONE TEST AND THE NEONATES
SHOULD NOT BE OLDER THAN 24H

20

2h PRE-FEEDING

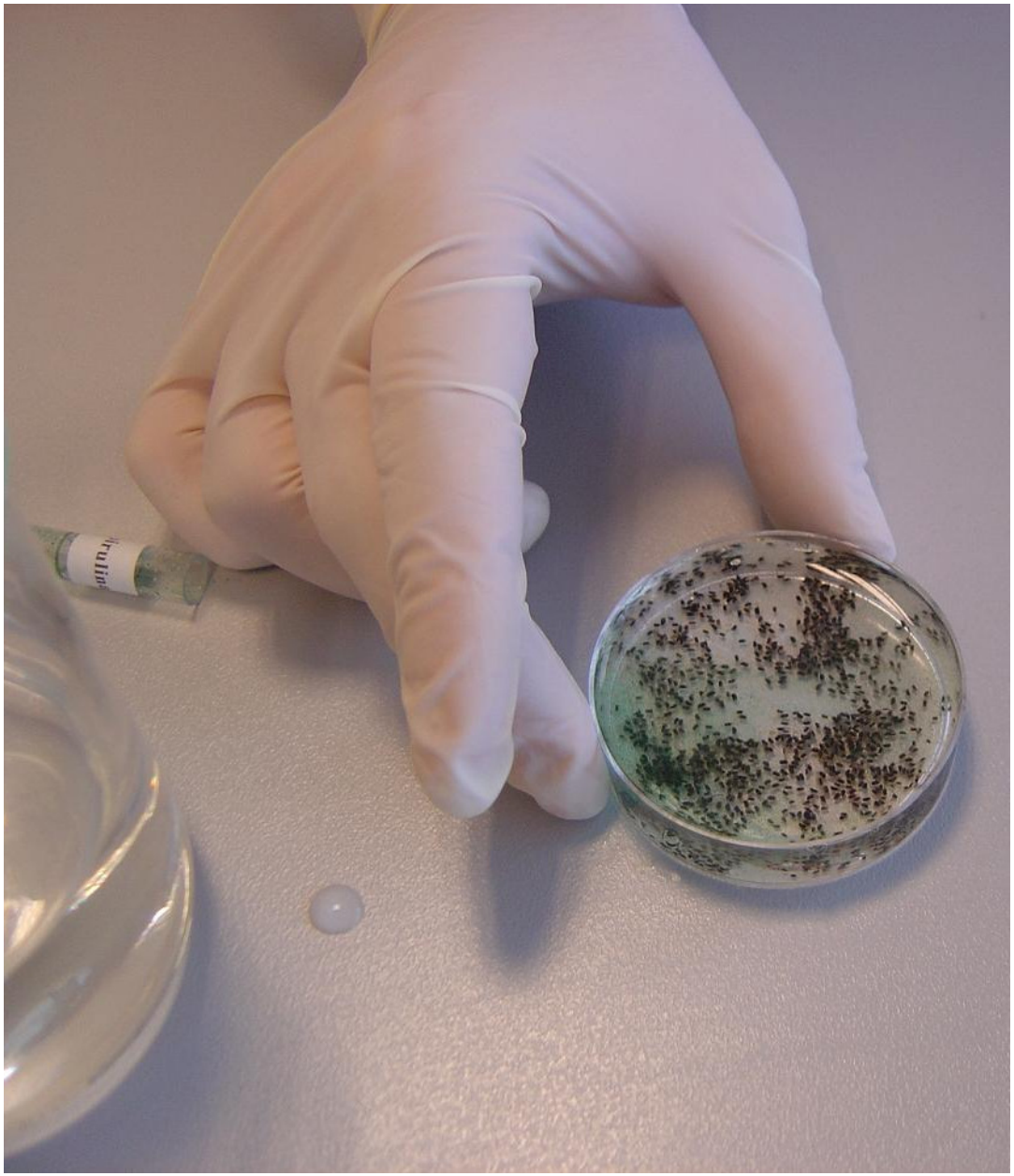
OF THE TEST ORGANISMS

TAKE ONE VIAL

WITH SPIRULINA POWDER

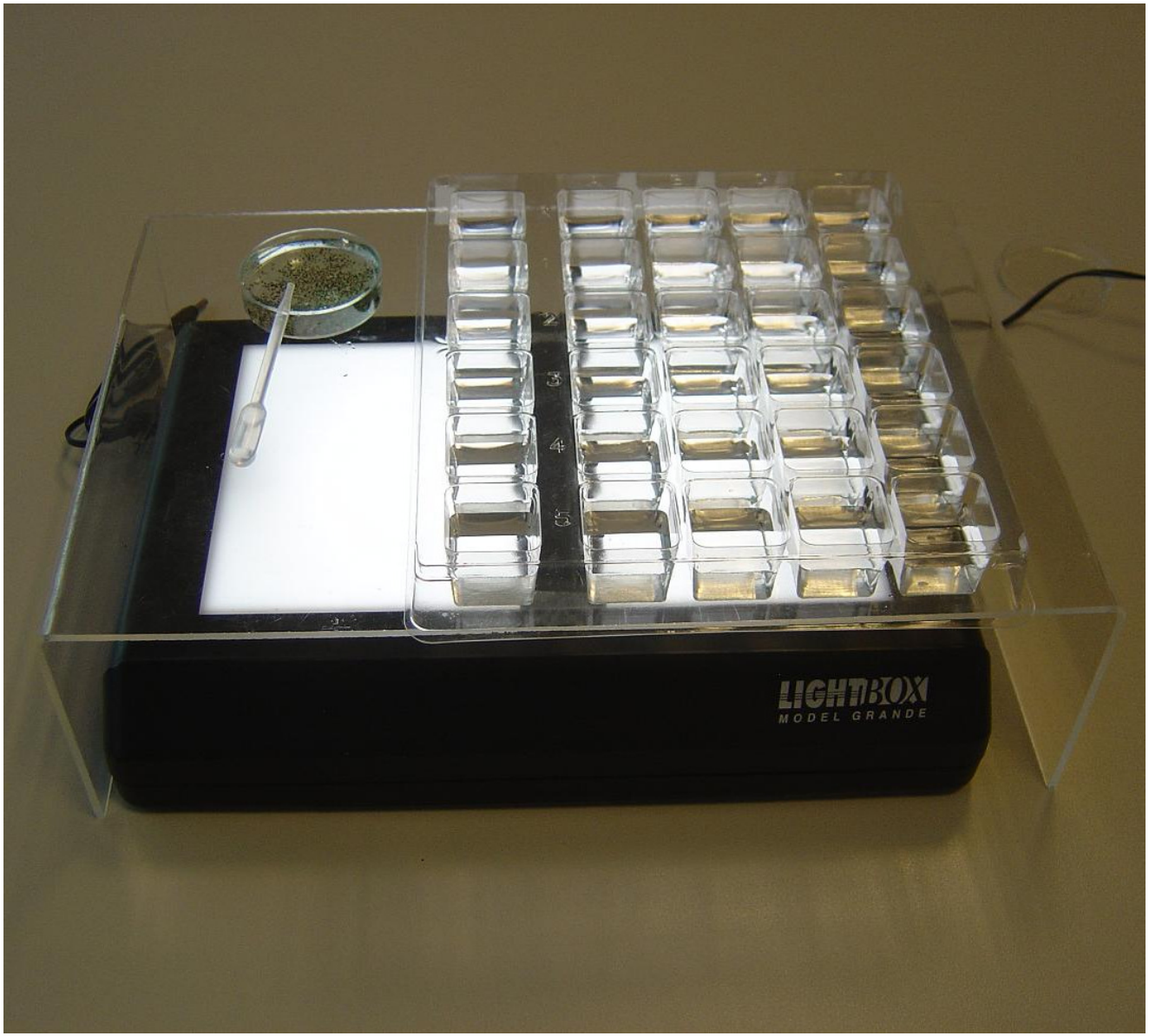
AND FILL IT

WITH STANDARD FRESHWATER



21

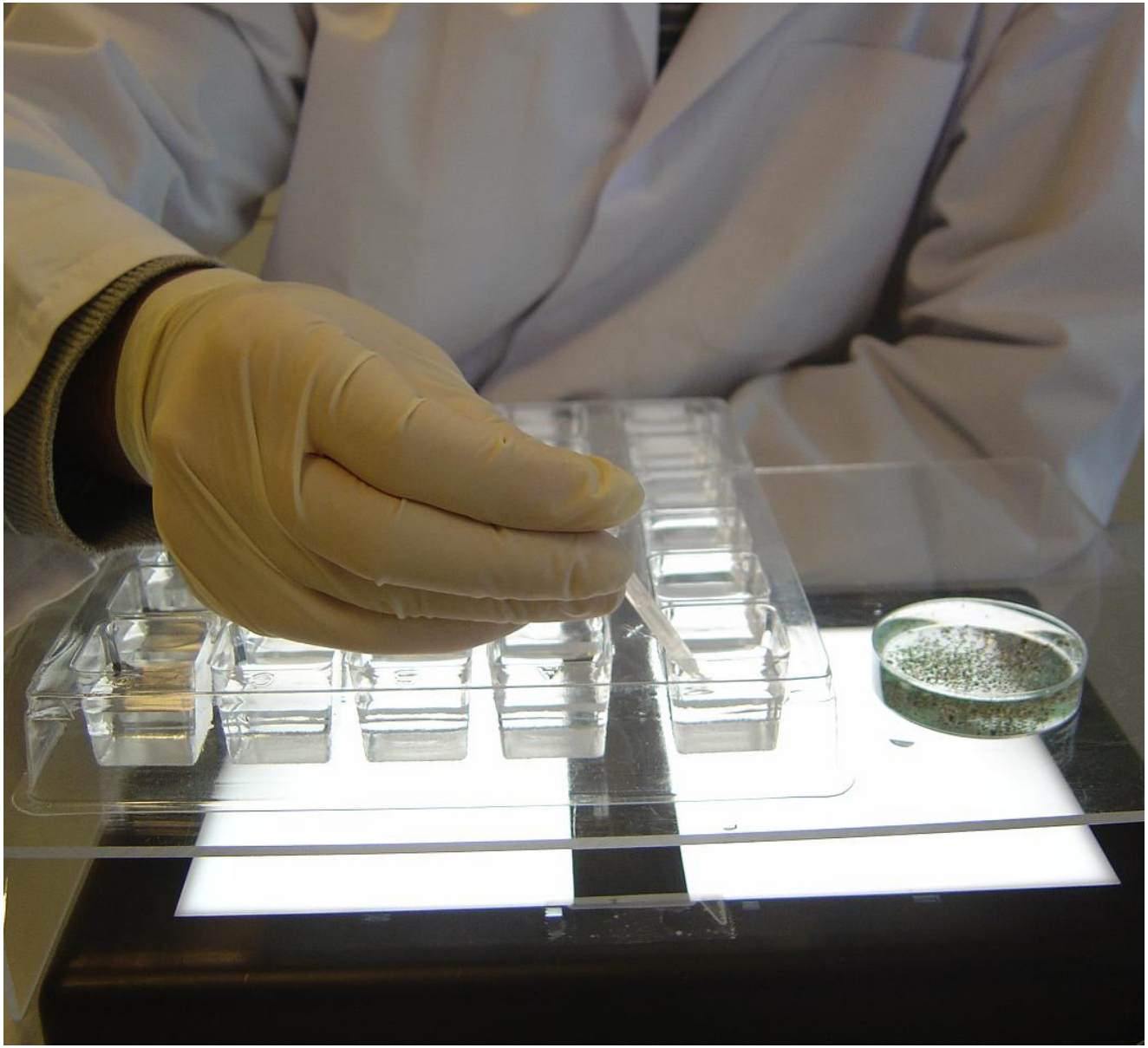
SHAKE THE VIAL
WITH THE SPIRULINA SUSPENSION,
POUR IT IN THE PETRI DISH
WITH THE DAPHNIA NEONATES
AND SWIRL THE PETRI DISH GENTLY



22

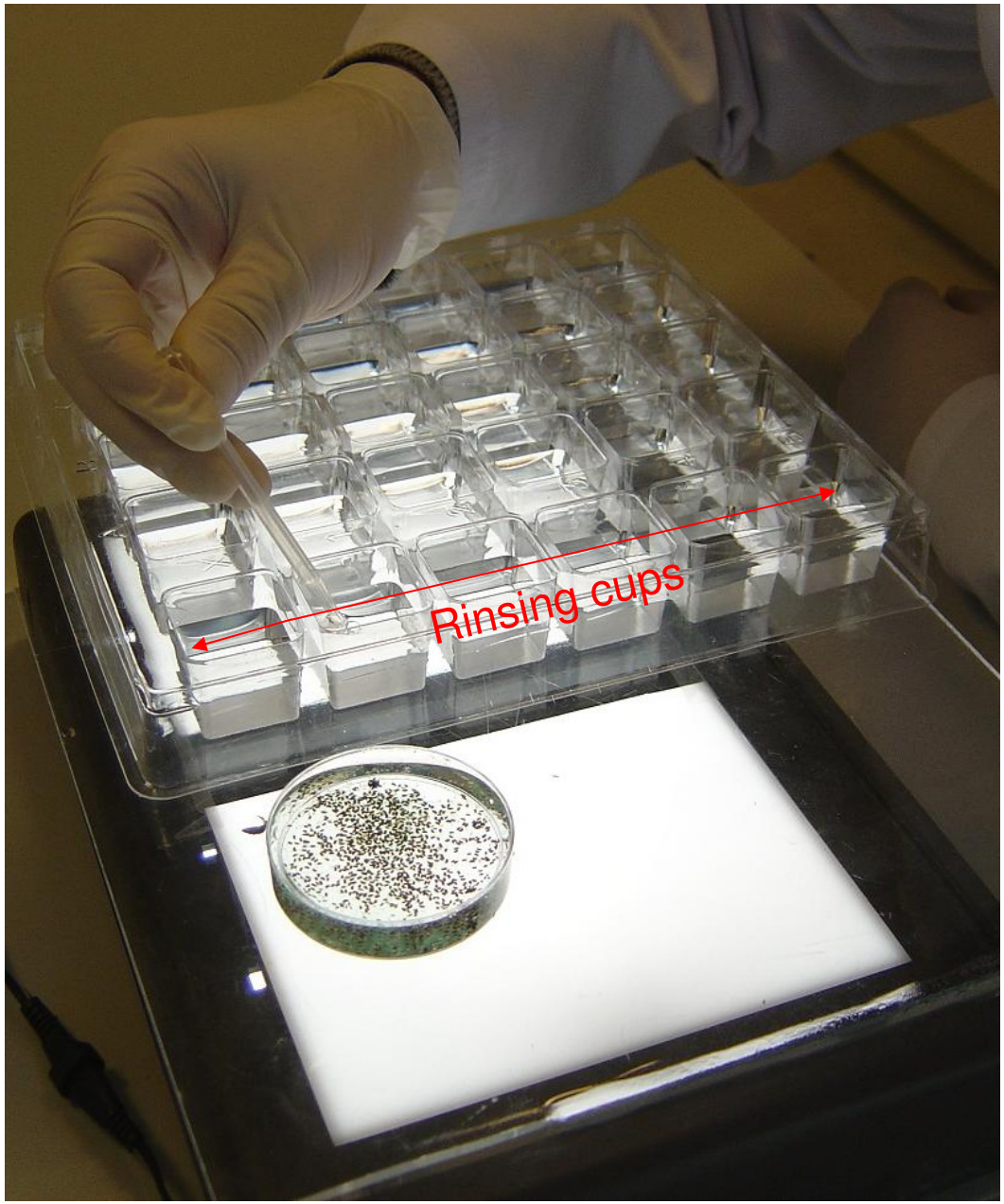
SET UP
OF THE TRANSFER
OF THE DAPHNIAS
TO THE TEST WELLS

- MULTIWELL PLATE
- LIGHT BOX WITH TRANSPARENT STAGE
- MICROPIPETTE



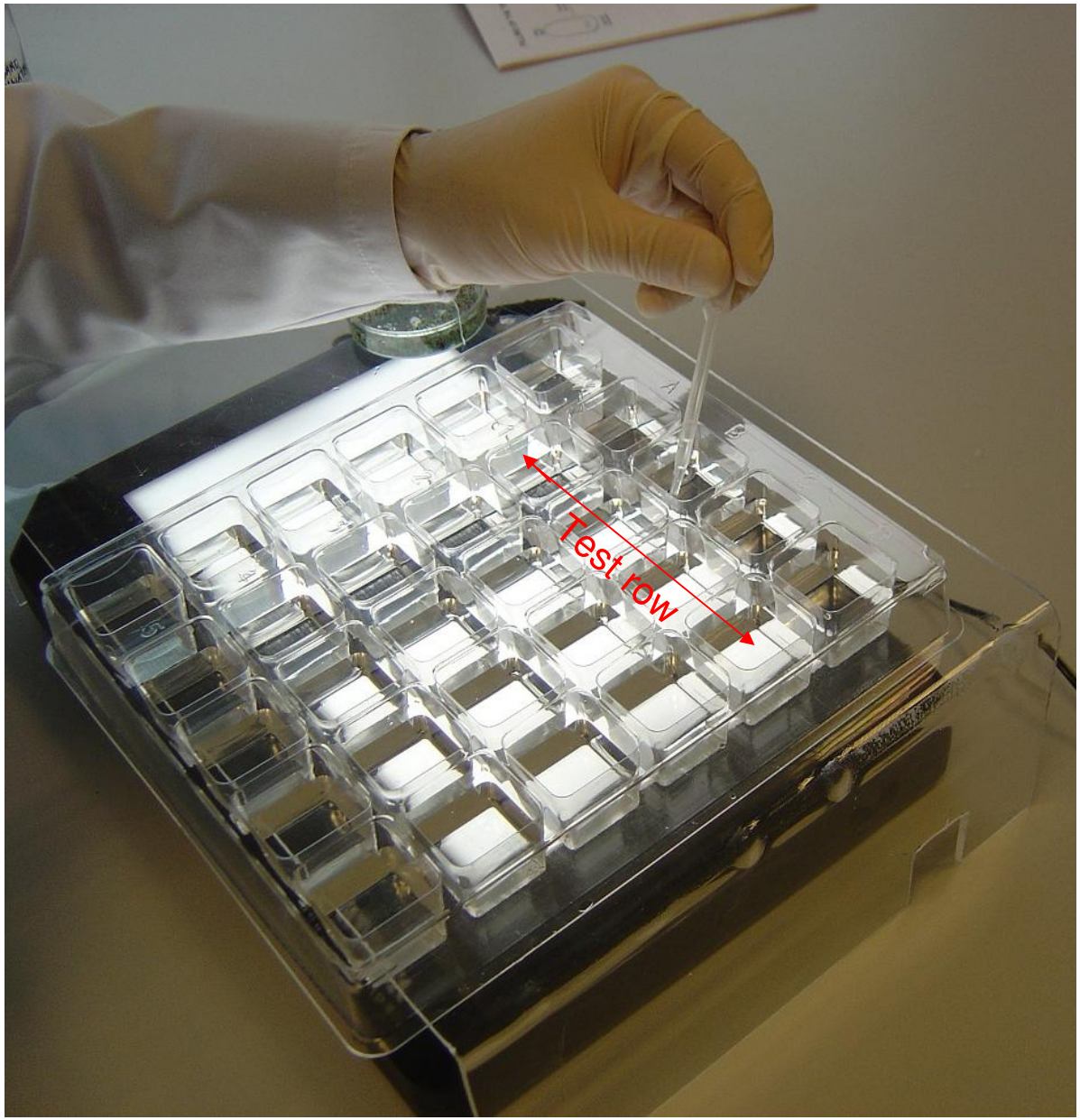
23

TRANSFER AT LEAST 20
(actively swimming)
DAPHNIAS INTO
THE RINSING CUP
OF THE CONTROL ROW,



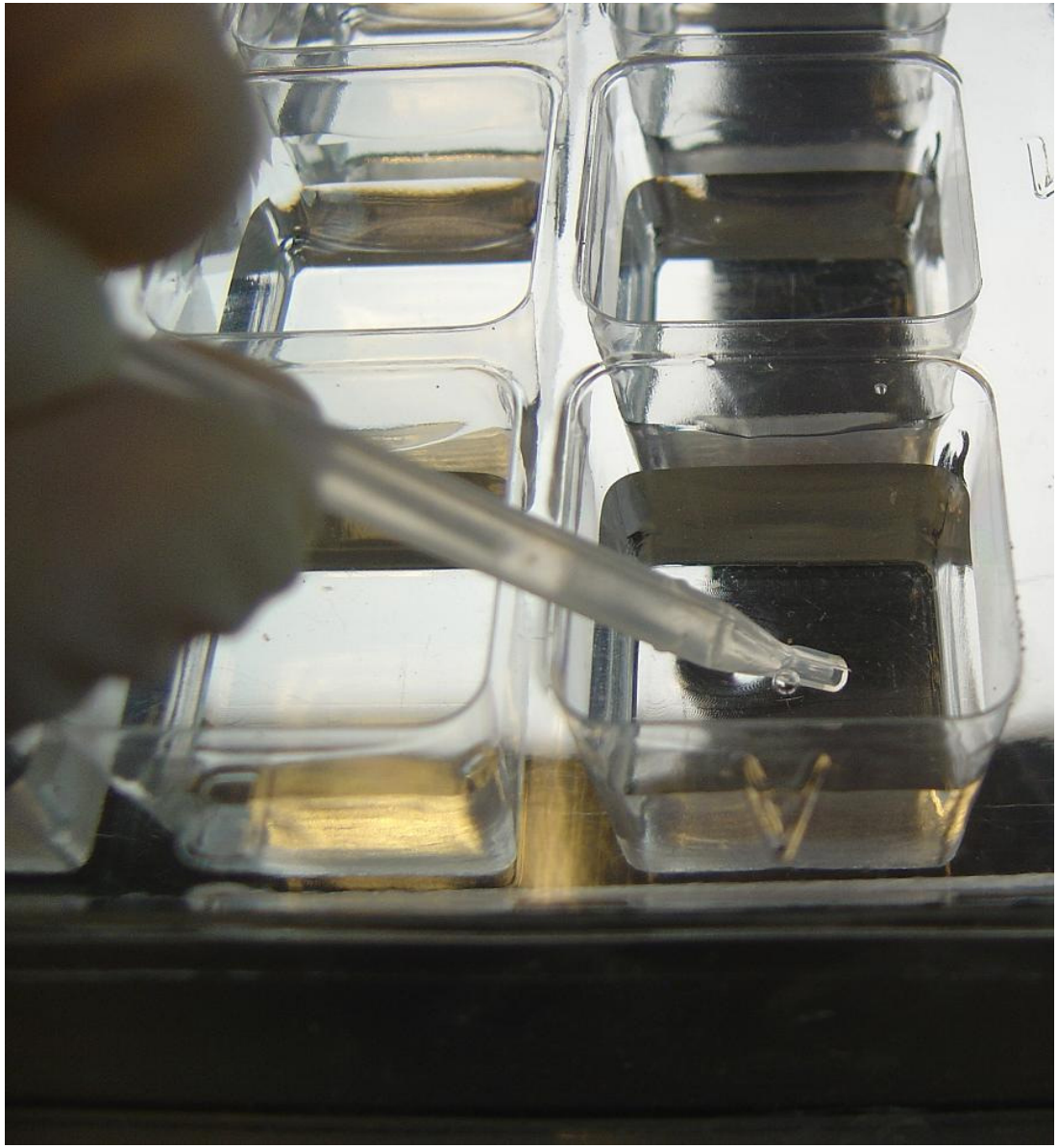
24

TRANSFER 20 DAPHNIAS (minimum)
TO ALL THE OTHER RINSING CUPS,
IN ORDER OF INCREASING
CONCENTRATIONS OF TOXICANT



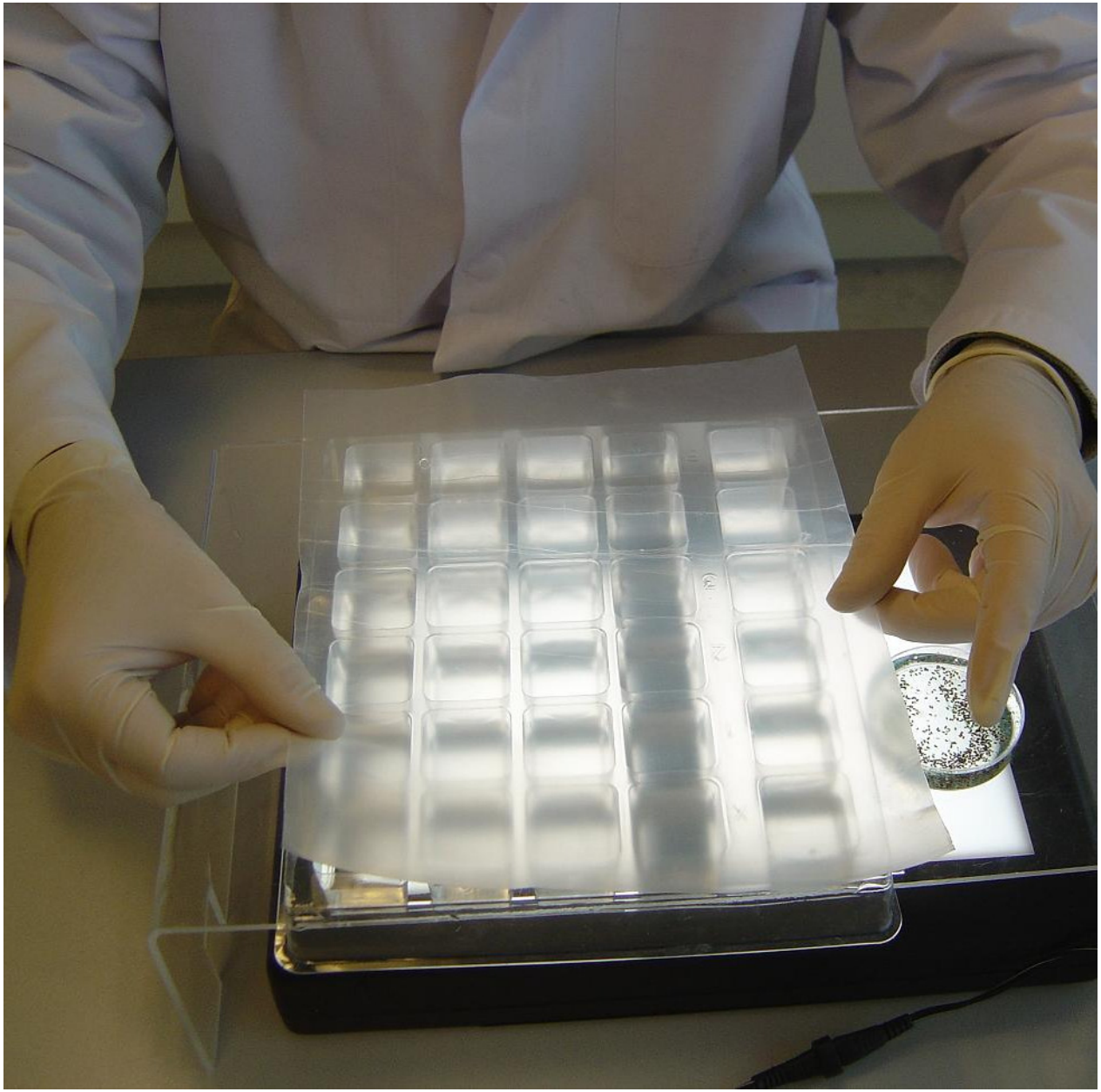
25

TRANSFER <u>EXACTLY 5</u>
DAPHNIAS FROM EACH
RINSING WELL
INTO THE 4 WELLS
OF THE CORRESPONDING
ROW



26

TO AVOID SURFACE FLOATING
OF THE DAPHNIAS
DURING THE TRANSFER,
PUT THE TIP OF THE
MICROPIPETTE IN THE MEDIUM,
AND DO NOT DROP THE ORGANISMS
AT THE SURFACE OF THE MEDIUM



27

PUT A PIECE OF PARAFILM
ON THE MULTIWELL PLATE
AND PUT THE COVER
ON TIGHTLY

28

INCUBATION OF THE TEST PLATE

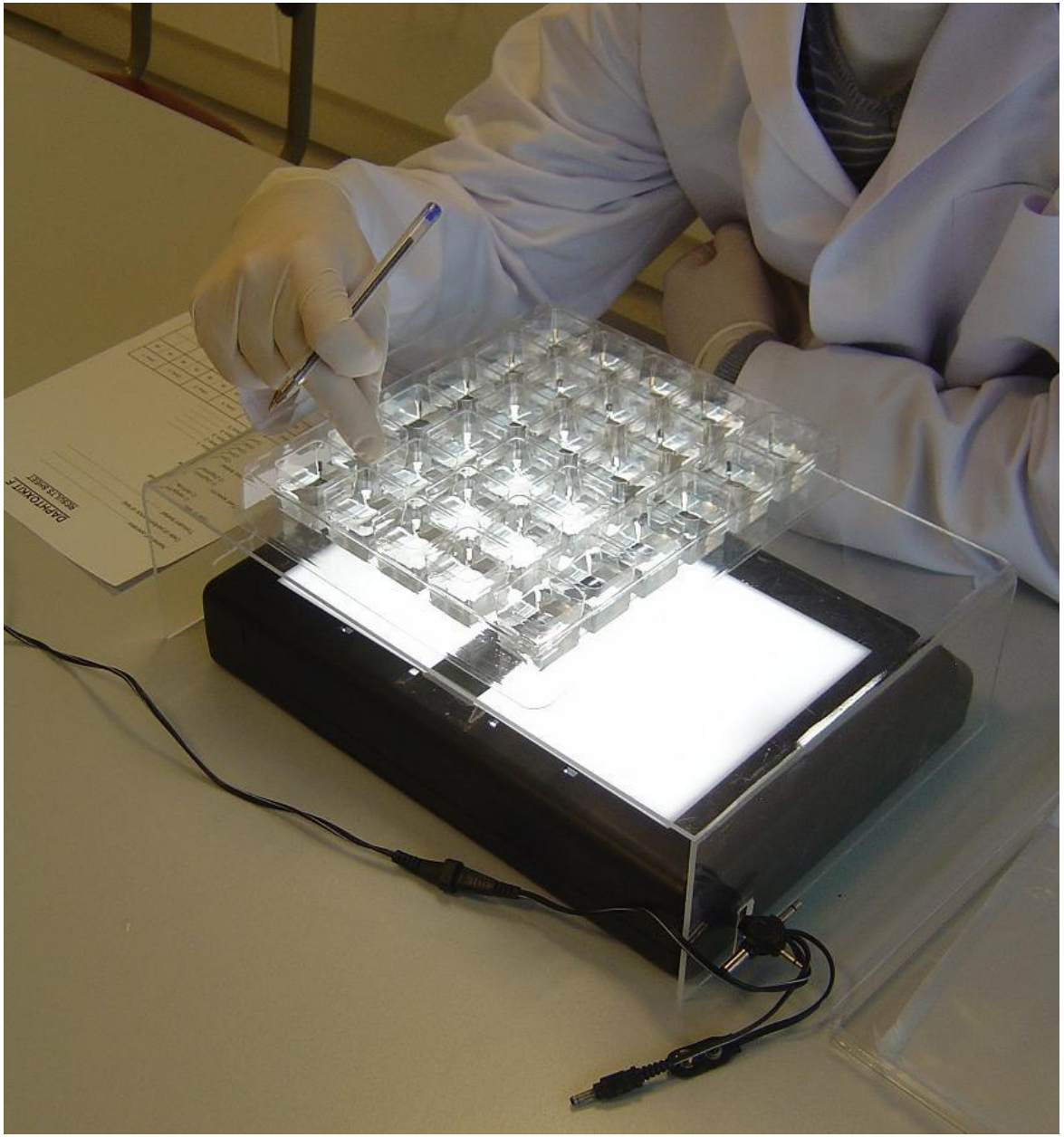
INCUBATE THE MULTIWELL

AT 20 ± 2 °C IN DARKNESS

29

SCORING OF THE RESULTS

AFTER 24h AND 48h INCUBATION
PUT THE MULTIWELL PLATE
ON THE LIGHT TABLE
AND RECORD THE NUMBER
OF DEAD AND
IMMOBILIZED DAPHNIAS



30

DAPHNIAS WHICH ARE NOT
ABLE TO SWIM
AFTER GENTLE AGITATION
OF THE LIQUID FOR 15 SECONDS
SHALL BE CONSIDERED
AS IMMOBILIZED
(even if they can still
move their antennae)

31

- SCORE THE FIGURES
  ON THE RESULTS SHEET.

- CALCULATE THE TOTAL NUMBER
  OF DEAD AND IMMOBILE DAPHNIAS
  FOR EACH TOXICANT
  CONCENTRATION

- CALCULATE THE MEAN EFFECT
  AND THE PERCENTAGE EFFECT