



DAPTHOXKIT F MAGNA

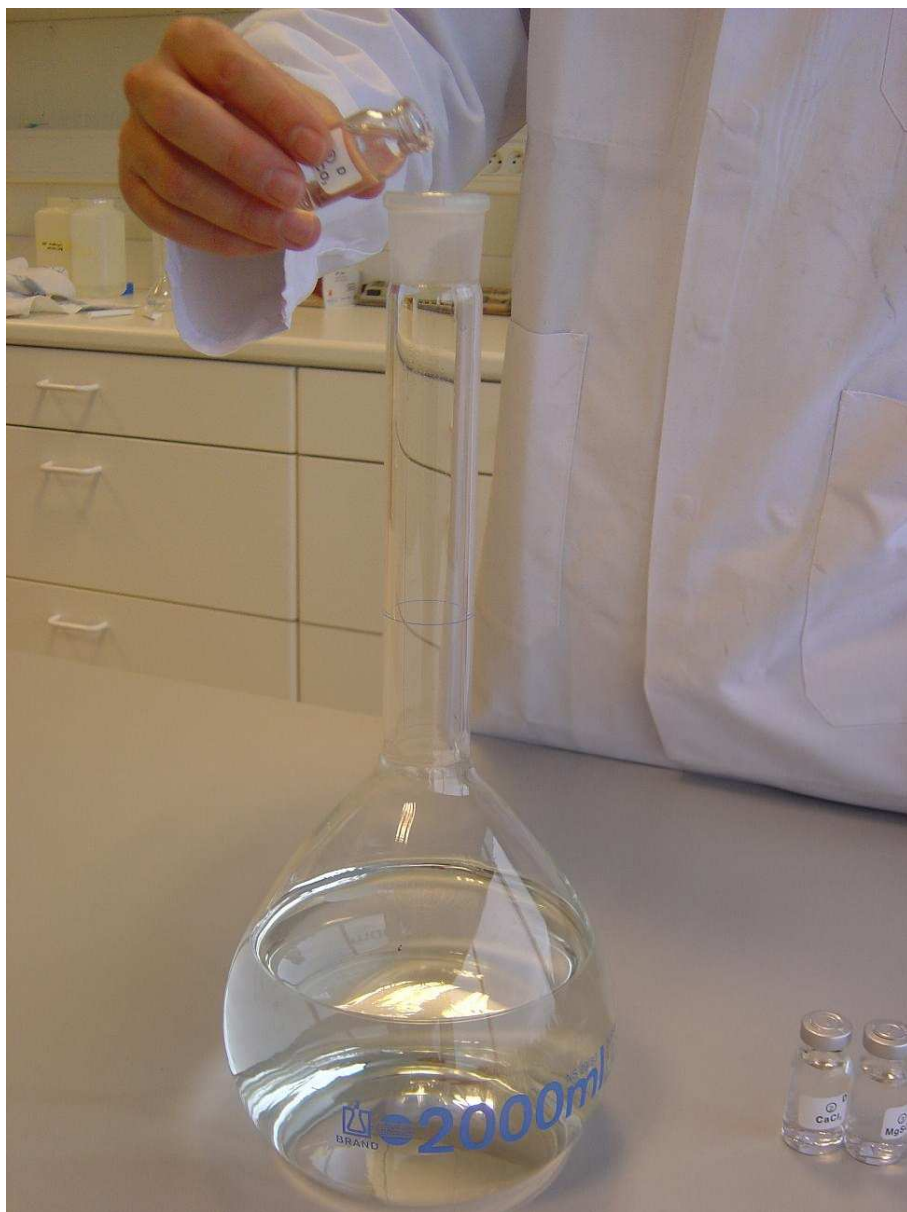
Test procedure



1

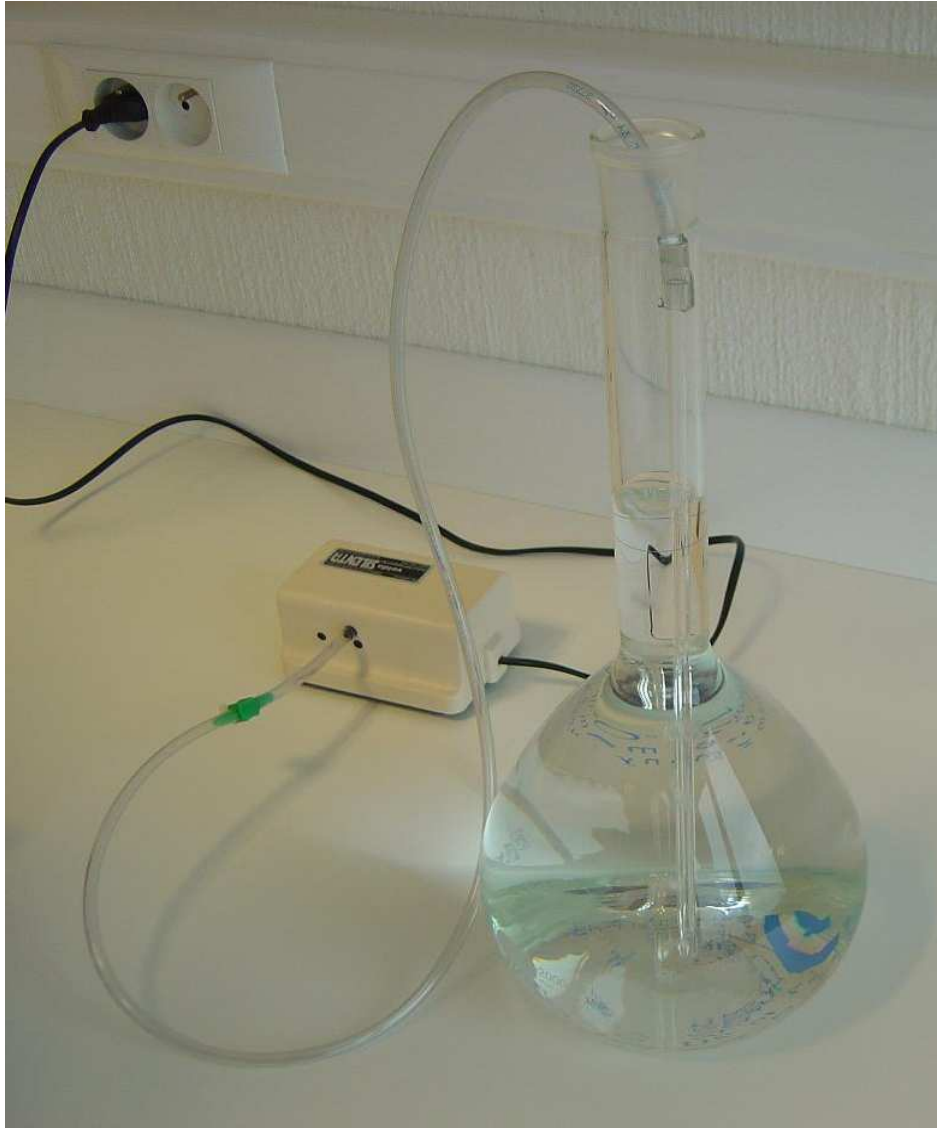
**PREPARATION OF
STANDARD FRESHWATER**

- 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

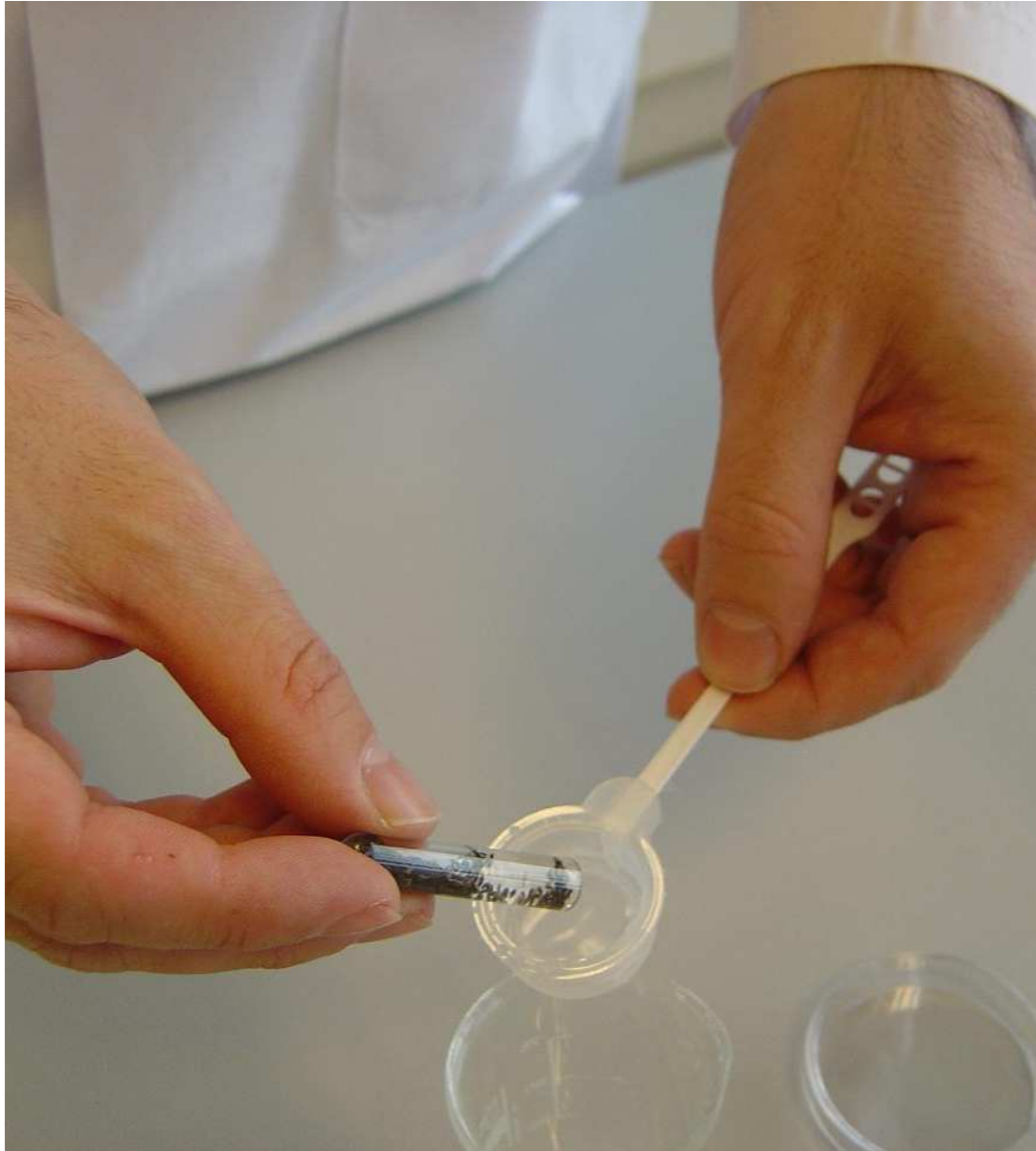
FILL THE FLASK TO THE 2 LITER MARK
AND AERATE FOR AT LEAST 15 MINUTES



4

HATCHING OF THE EPHIPPIA

REMOVE THE
ALUMINIUM FOIL
FROM A TUBE
WITH DAPHNIA
EPHIPPIA



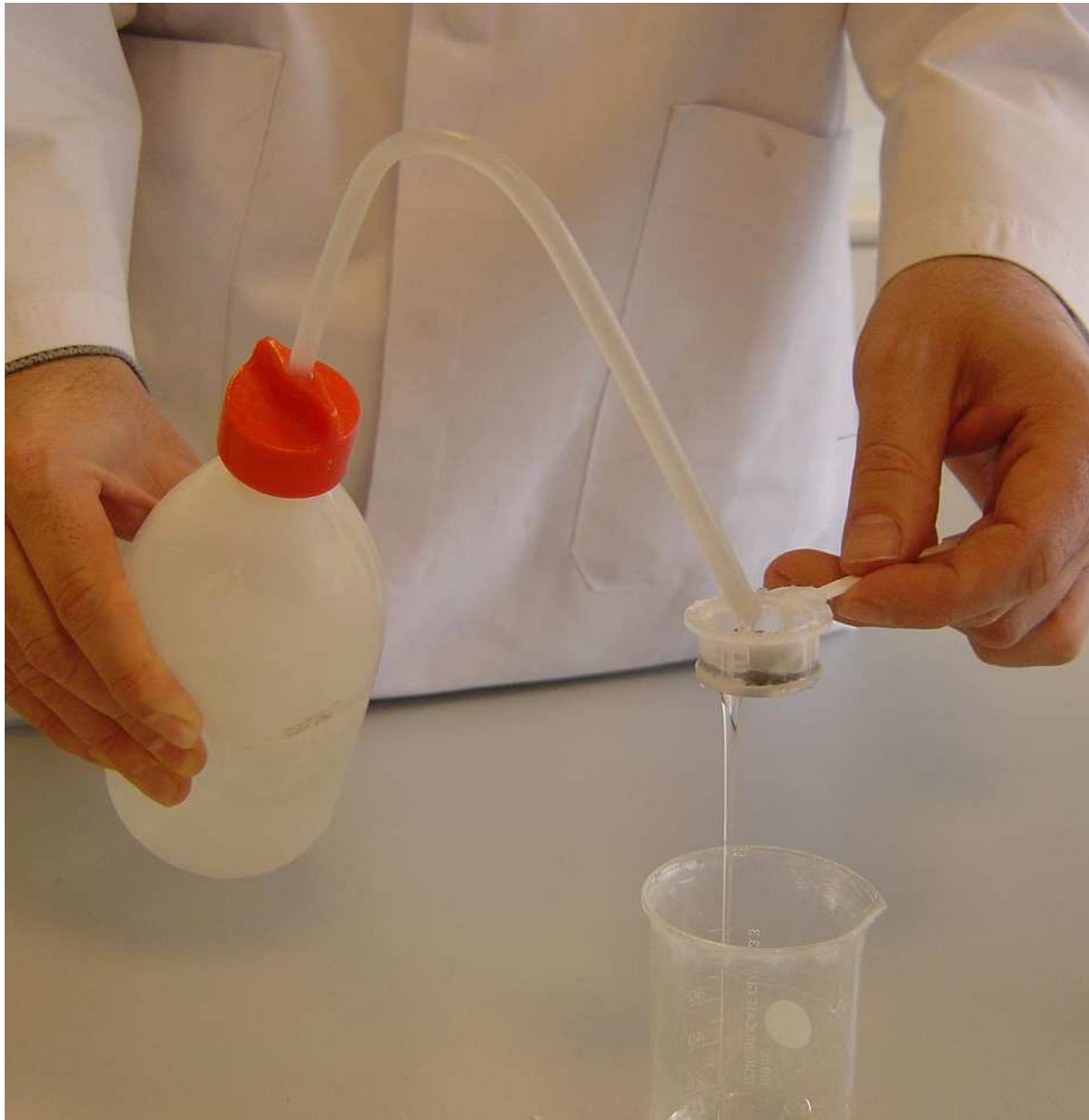
5

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

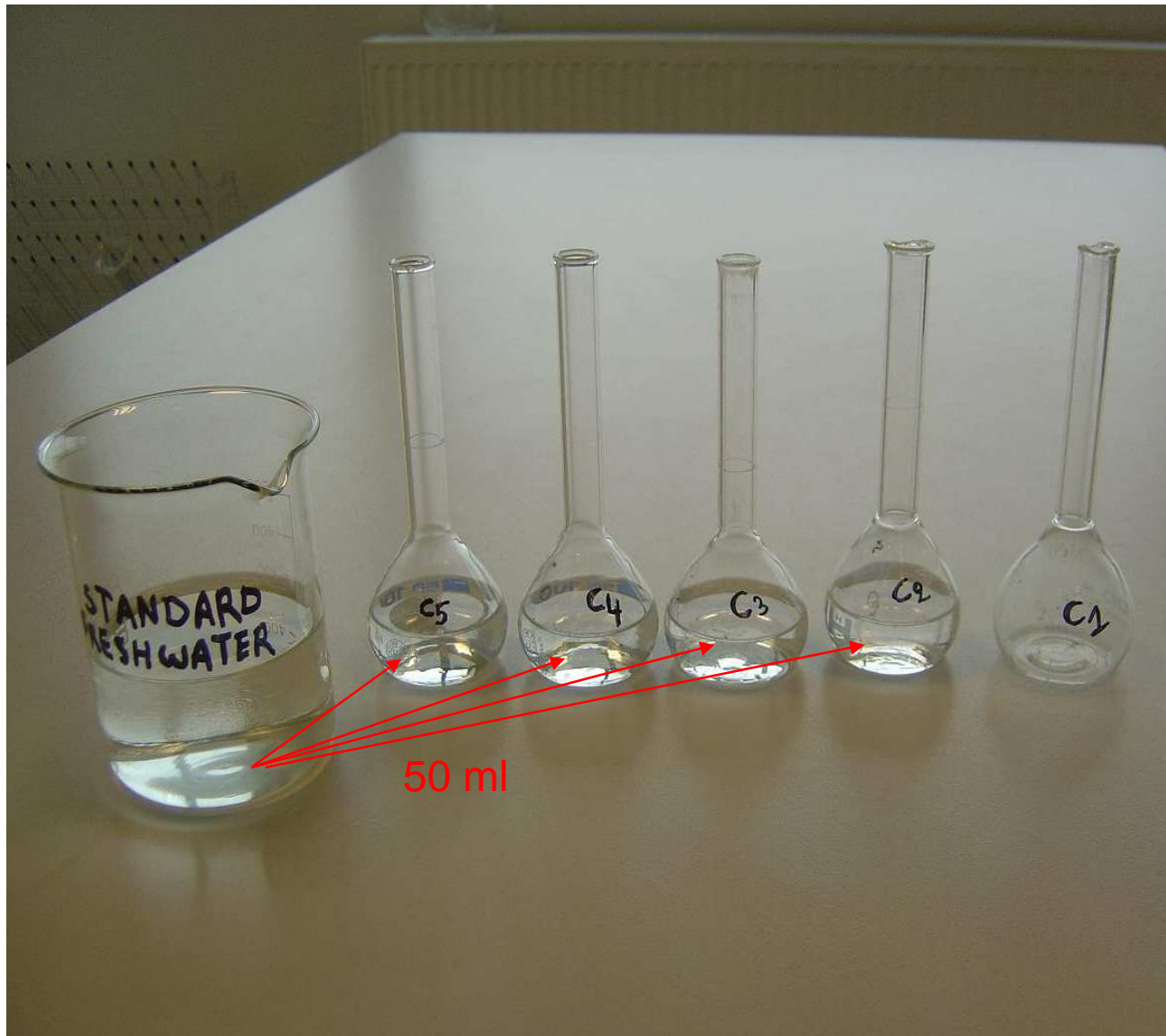
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PREPARATION OF THE TOXICANT DILUTIONS

For example :
TEST ON A EFFLUENT
IN 5 DILUTIONS (C1-C5)
+ ONE CONTROL



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TRANSFER 50 ML
STANDARD FRESHWATER
INTO FLASKS
C2, C3, C4 AND C5



12

FILL FLASK C1
TO THE 100 ML MARK
WITH EFFLUENT



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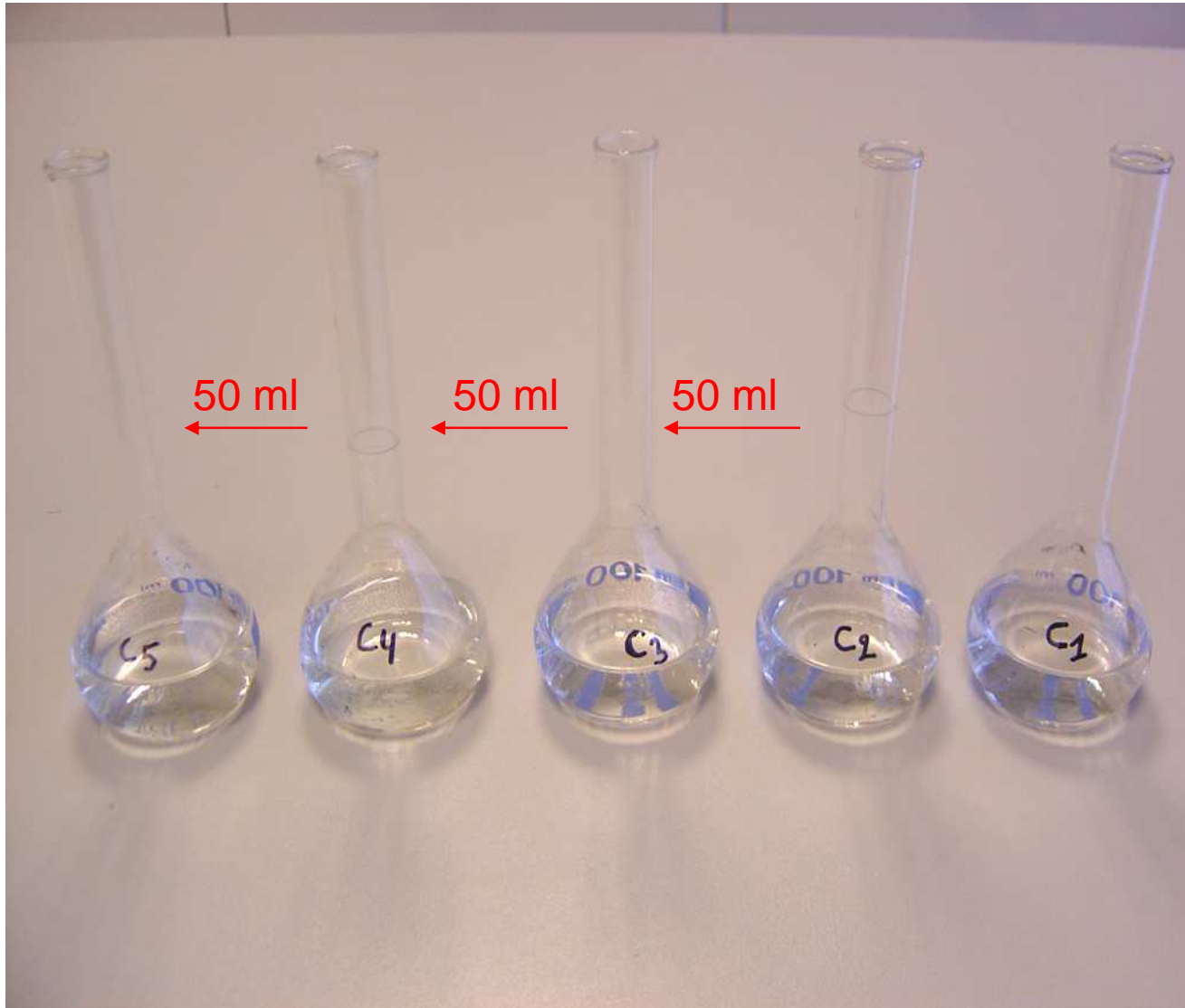
TRANSFER 50 ML EFFLUENT
FROM FLASK C1
INTO A GRADUATED CYLINDER.



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

15



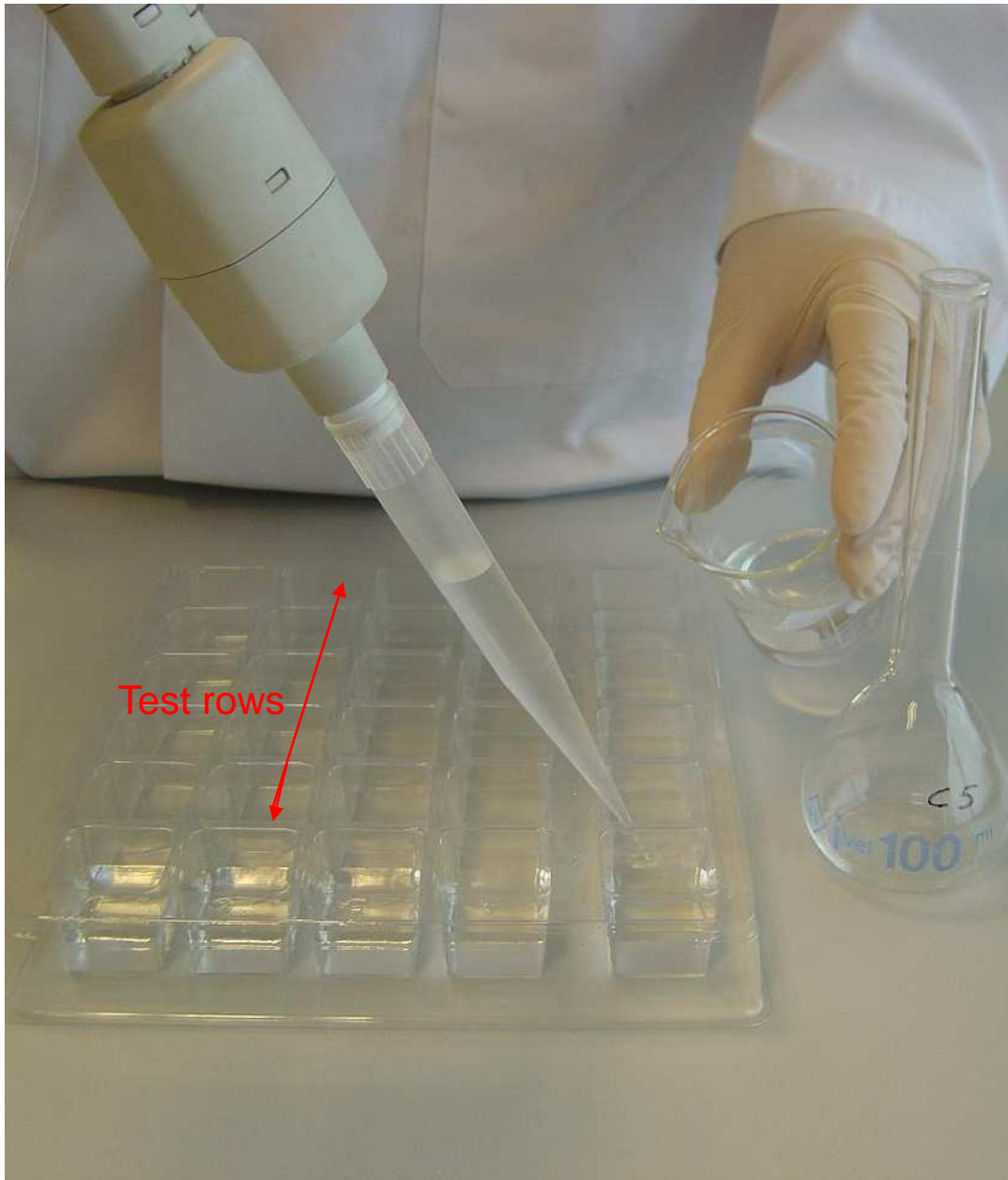
REPEAT THE FORMER
DILUTION PROCEDURE
FOR THE OTHER FLASKS
(i.e. 50 ml from C2 to C3, etc).

16



FILLING OF THE TEST PLATE :

TRANSFER 10 ML
STANDARD FRESHWATER
INTO EACH WELL
OF THE CONTROL ROW



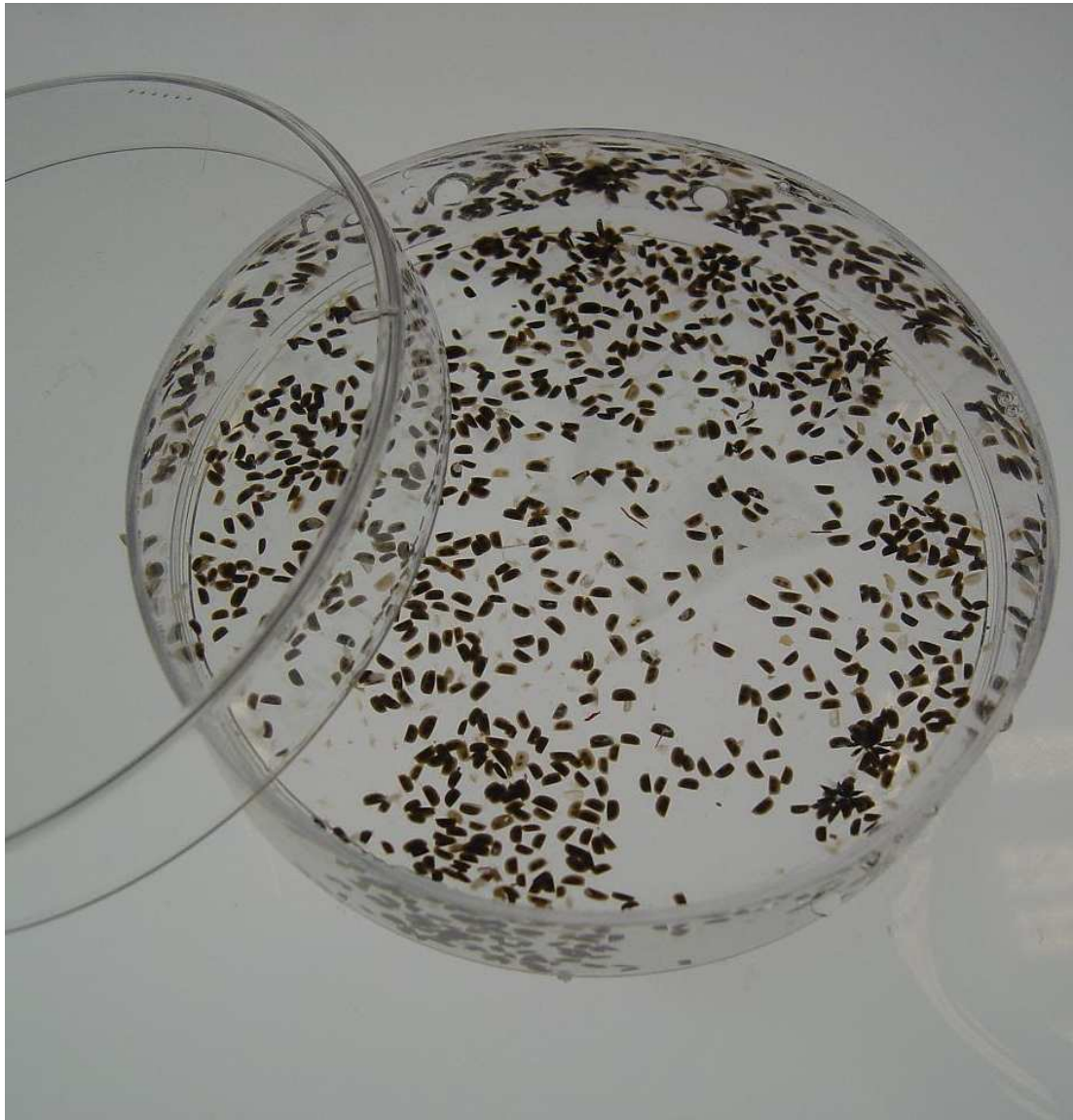
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TRANSFER 10 ML OF THE
RESPECTIVE TOXICANT
CONCENTRATIONS
INTO EACH WELL
OF THE CORRESPONDING ROWS
FROM C5 TO C1



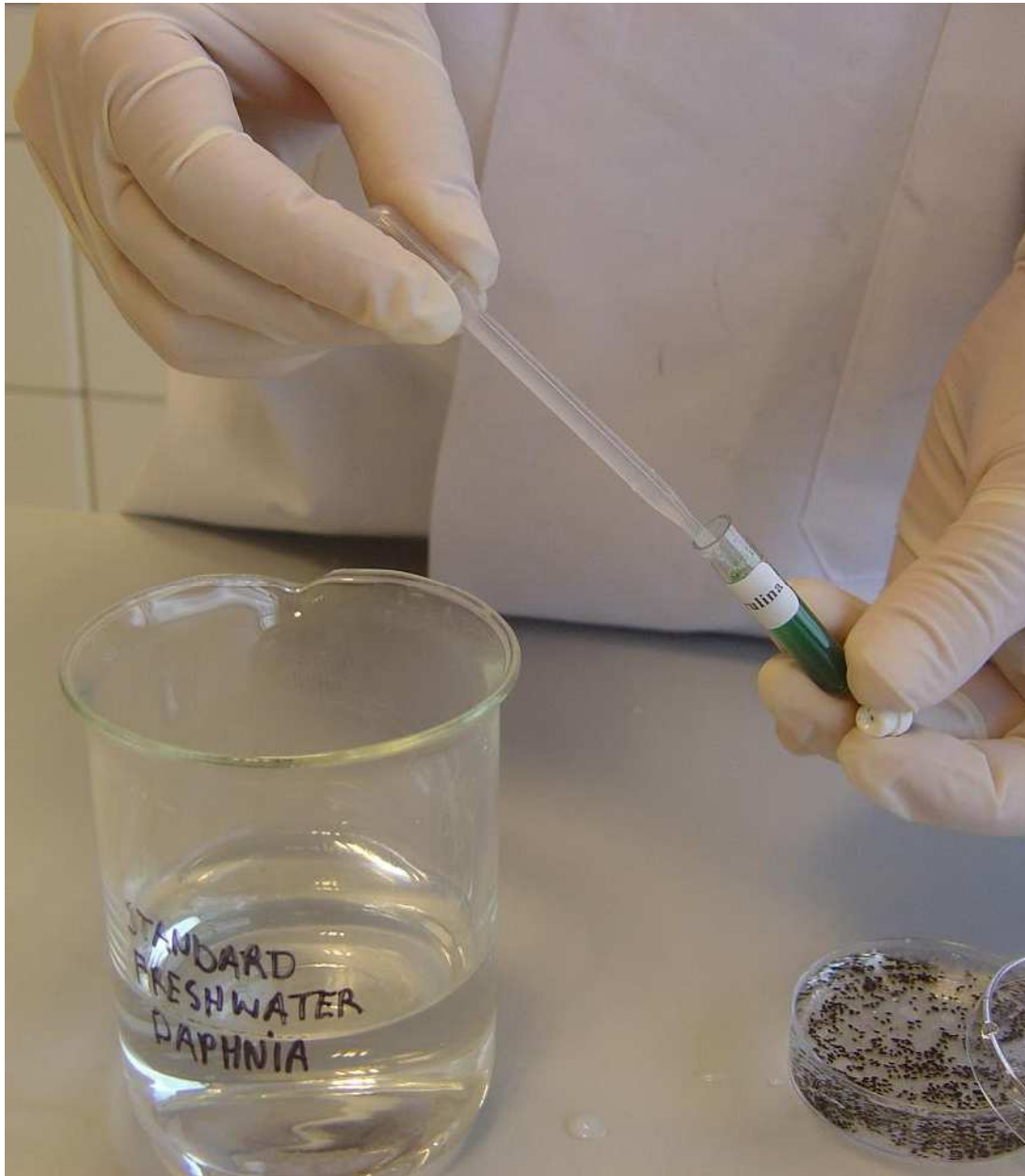
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AFTER 72h TO 80h
INCUBATION
VERIFY THE HATCHING
OF THE DAPHNIA NEONATES



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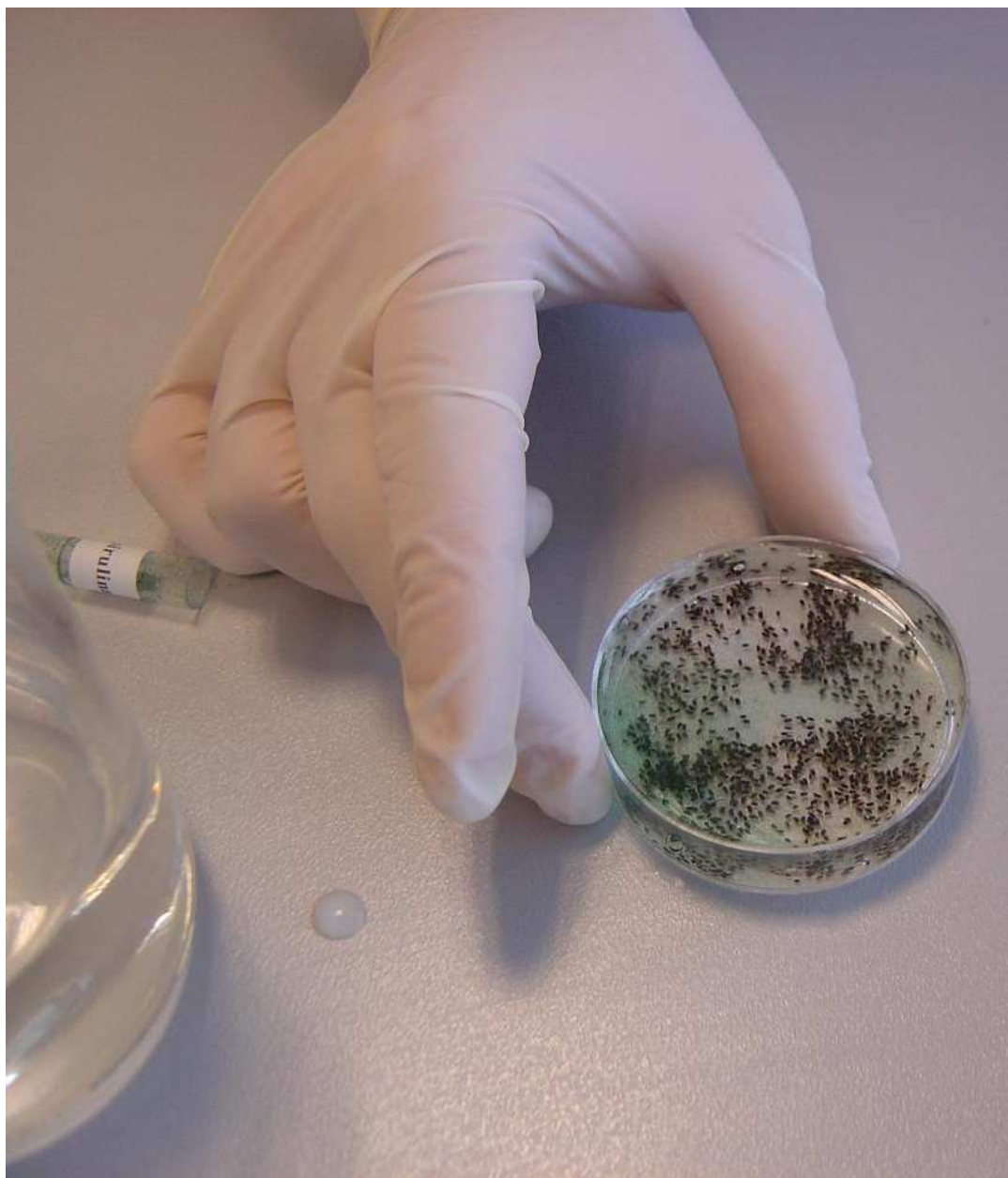
A MINIMUM OF 120 NEONATES
ARE NEEDED TO PERFORM
ONE TEST AND THE NEONATES
SHOULD NOT BE OLDER THAN 24H



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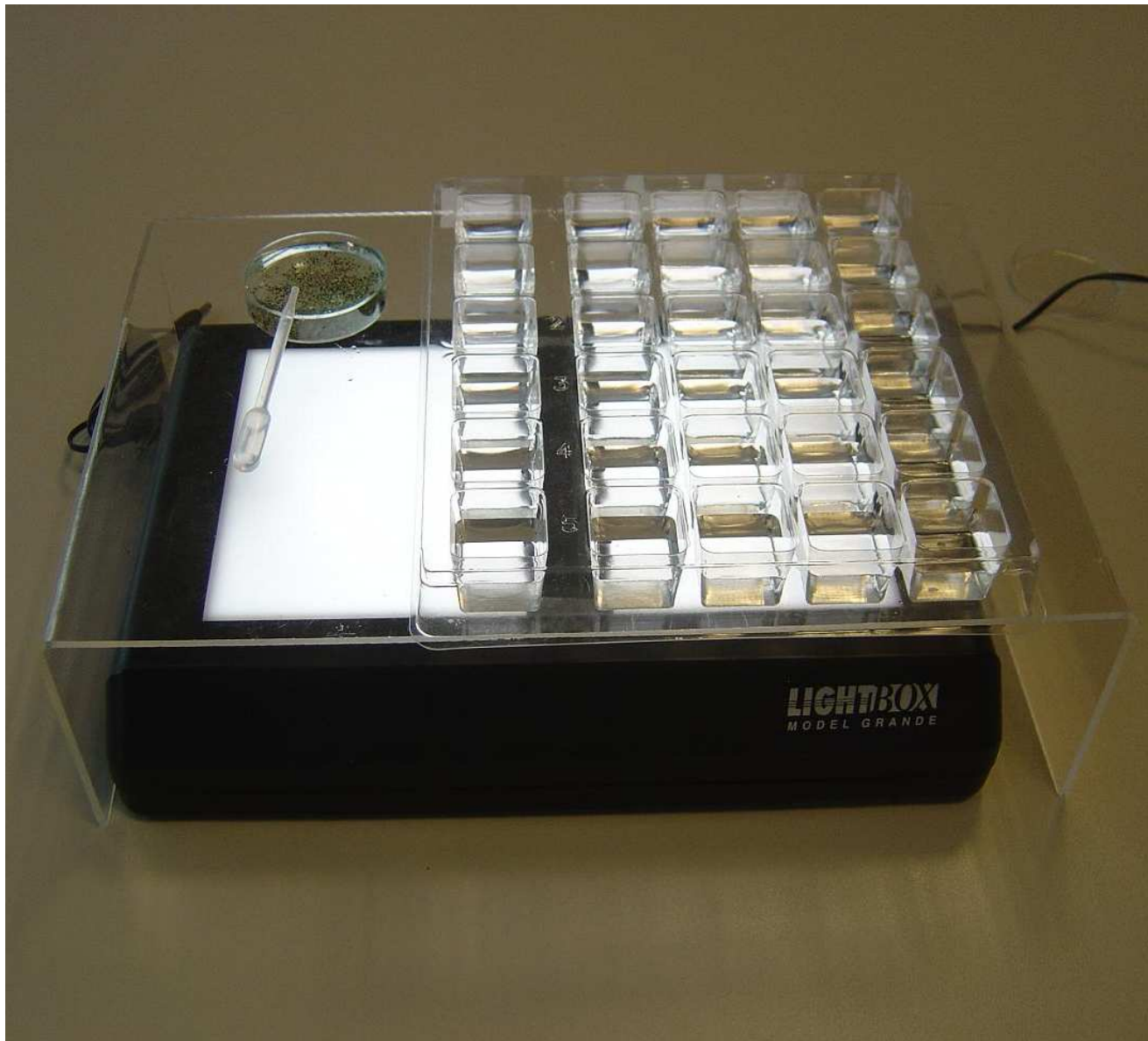
**2h PRE-FEEDING
OF THE TEST ORGANISMS**

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



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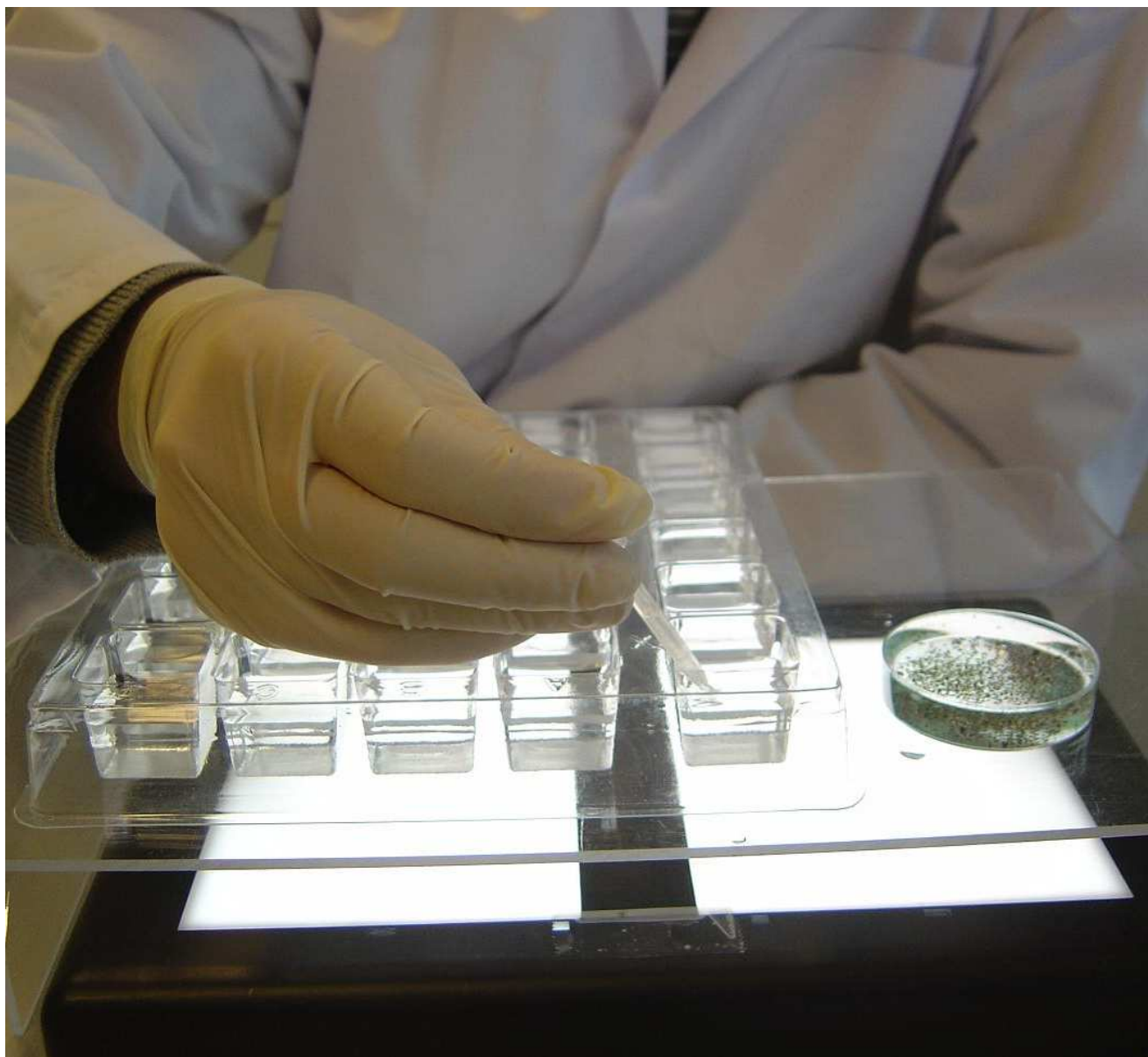
SHAKE THE VIAL
WITH THE SPIRULINA SUSPENSION,
POUR IT IN THE PETRI DISH
WITH THE DAPHNIA NEONATES
AND SWIRL THE PETRI DISH GENTLY



22

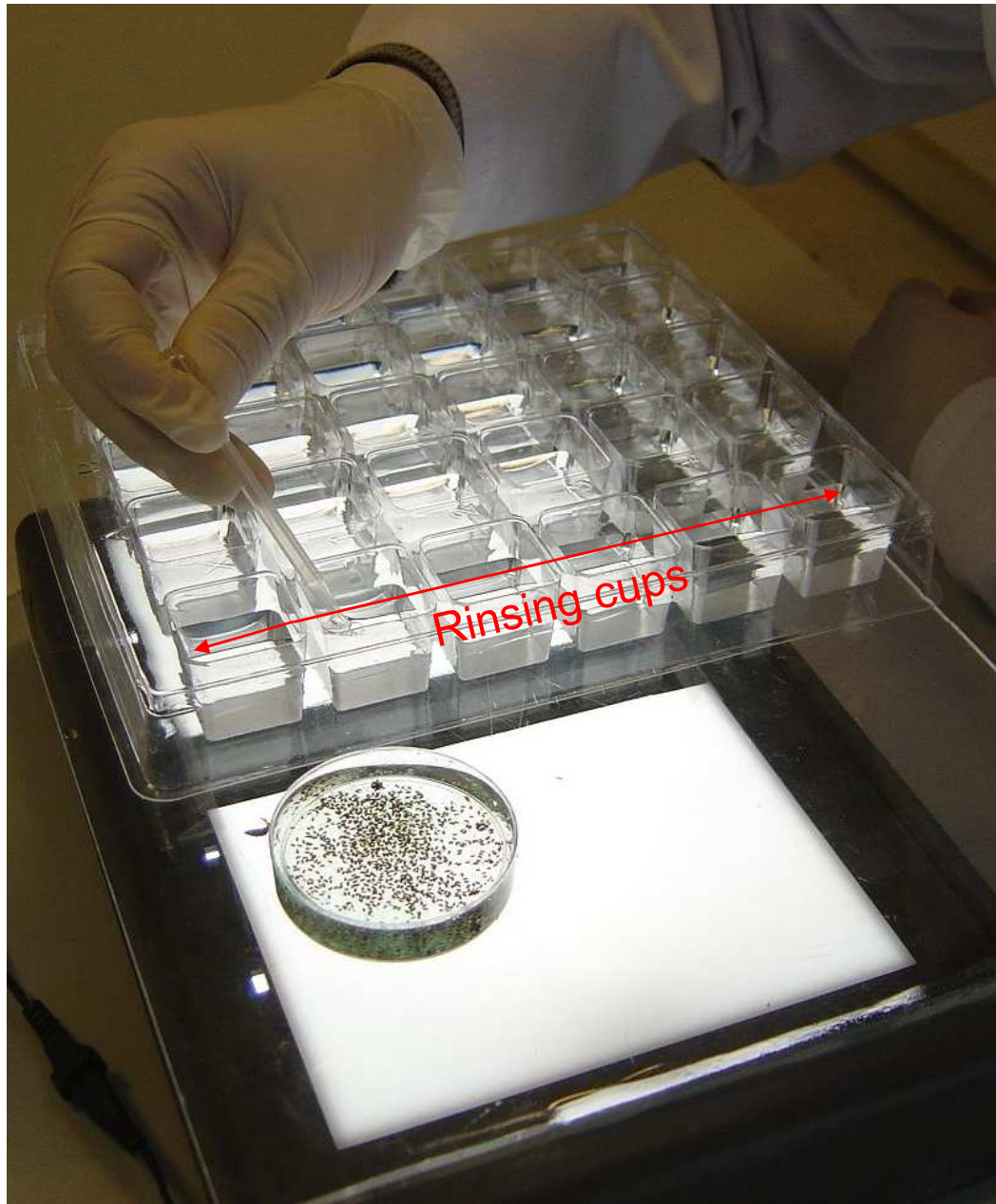
**SET UP
OF THE TRANSFER
OF THE DAPHNIAS
TO THE TEST WELLS**

- MULTIWELL PLATE
- LIGHT BOX WITH
TRANSPARENT STAGE
- MICROPIPETTE



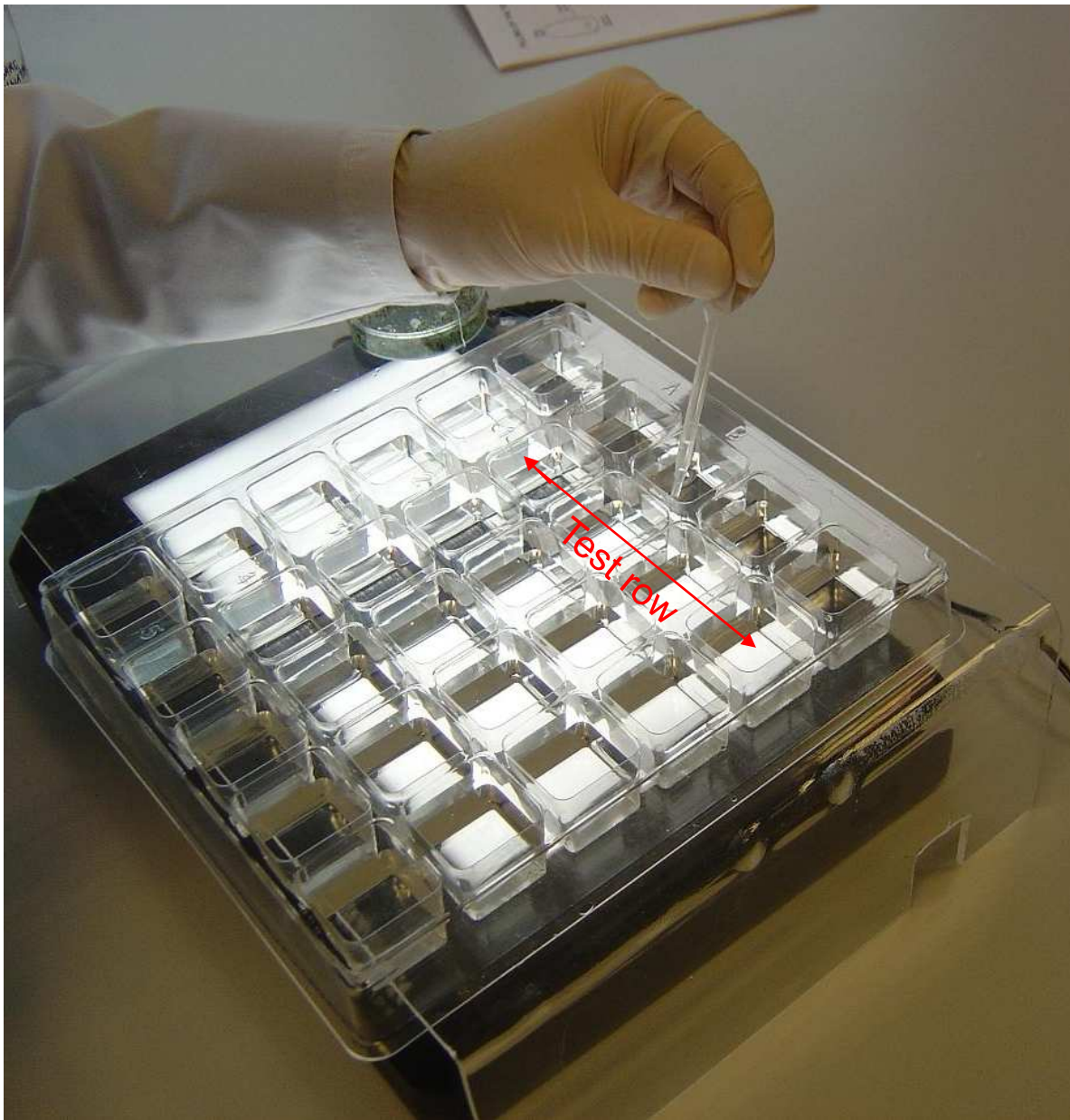
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TRANSFER AT LEAST 20
(actively swimming)
DAPHNIAS INTO
THE RINSING CUP
OF THE CONTROL ROW,



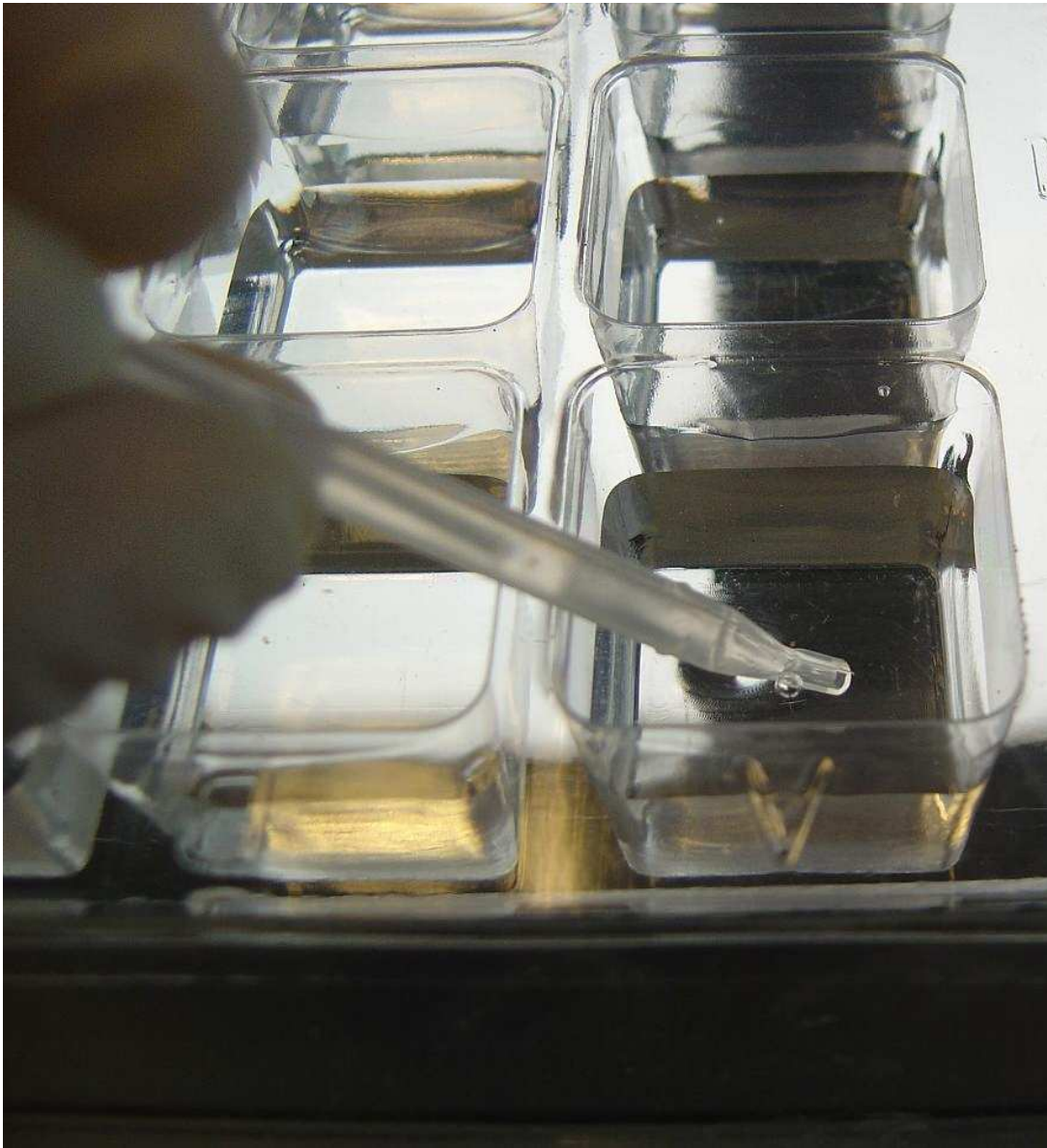
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TRANSFER 20 DAPHNIAS (minimum)
TO ALL THE OTHER RINSING CUPS,
IN ORDER OF INCREASING
CONCENTRATIONS OF TOXICANT



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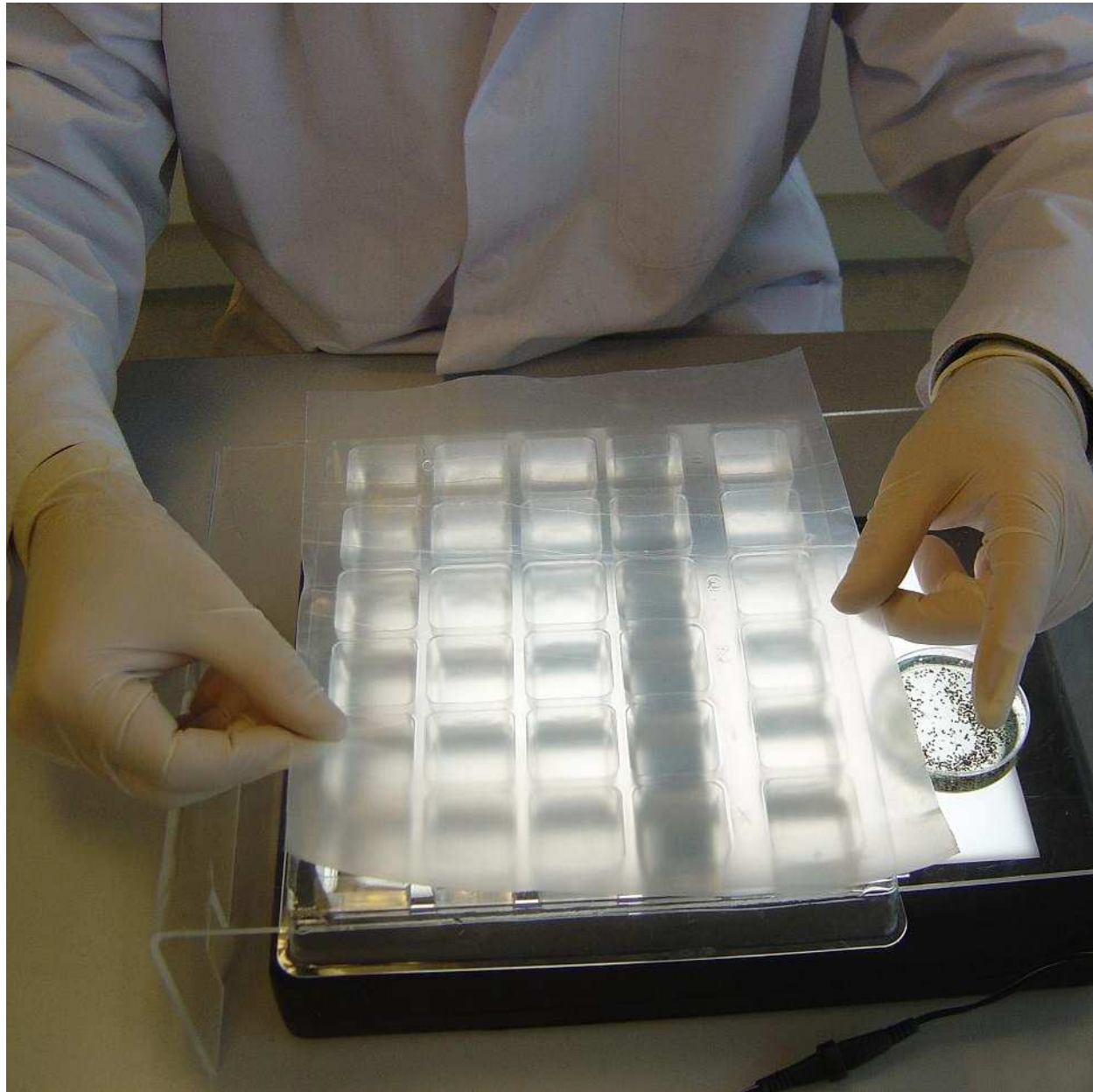
TRANSFER EXACTLY 5
DAPHNIAS FROM EACH
RINSING WELL
INTO THE 4 WELLS
OF THE CORRESPONDING
ROW



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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



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INCUBATION OF THE TEST PLATE

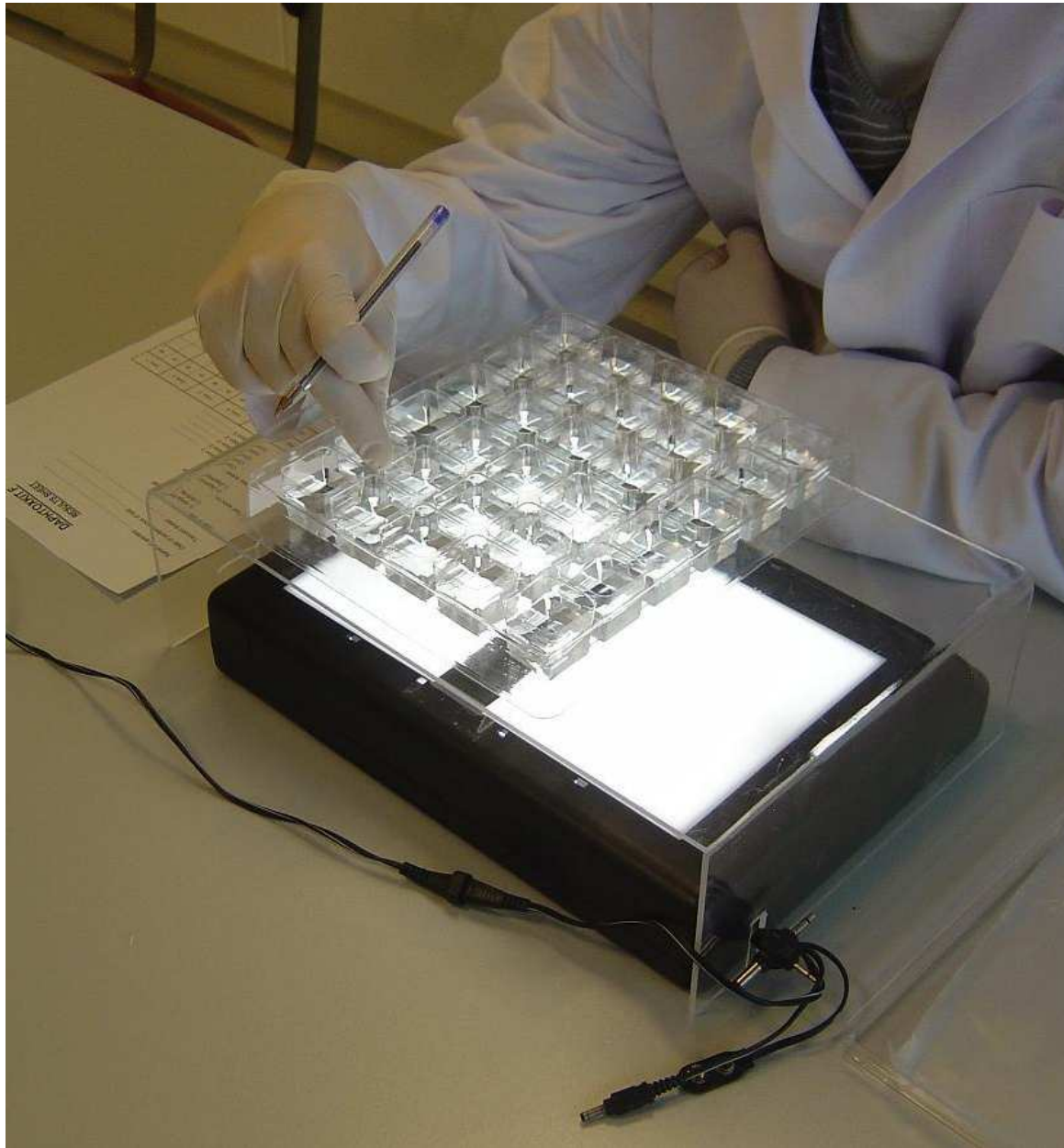
INCUBATE THE MULTIWELL
AT 20 ± 2 °C IN DARKNESS

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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





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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)



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- 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