

Standard Operating Procedures for impregnation of filter papers

1. PURPOSE

To provide guidance to technical staff on impregnation of filter papers with insecticides and synergists. This SOP provides instructions on the impregnation of filter papers with insecticides for use in WHO tube test to determine insecticide resistance for flying insects such as mosquitoes and sandflies.

2. MATERIALS / EQUIPMENT

- a. Paper cutter for cutting filter paper to the appropriate size
- b. Pencil for marking filter papers
- c. Balance (precision 0.01 mg), spatulas and weighing boats for weighing insecticide (aluminium micro weigh dish)
- d. Appropriate volume pipettes and tips
- e. Glass bottles, caps and labels
- f. Fume hood for all insecticide work
- g. Paper support rack for impregnation of filter papers
- h. Drying line and pegs for drying filter papers in the fume hood (optional).
- i. Refrigerator for storing impregnated filter papers
- j. Waste disposal bins as necessary
- k. Permanent marker
- l. Paper roll (optional)
- m. Whatman No. 1 filter paper catalogue number 1001 917.
- n. Acetone, analytical grade or purer (cf.§5.Table of supplies)
- o. Carrier oils:

Class group	
PY and PBO	Dow Corning 556 cosmetic grade fluid (silicone oil)
OP/C	Olive oil
Note: refer cf.§5.Table of supplies	

- p. Aluminium foil to keep filter paper dry and/or to wrap the filter paper for storage

3. HEALTH, SAFETY and ENVIRONMENT PROTECTION

- a. Refer to Material Safety Data Sheets (MSDS) for chemical hazard information for each chemical used.
- b. Refer to Control of Substances Hazardous to Health (COSHH) Assessment for each chemical used.
- c. All staff working in laboratories must have received appropriate laboratory instruction training.
- d. All staff using this procedure must be trained in safe operation of chemical fume hoods.

- e. Appropriate personal protective equipment must be worn at all times when handling insecticides, including, laboratory coat, gloves, safety glasses and a face mask when weighing out chemicals.
- f. Dispose of all waste materials quickly and appropriately. Chemically hazardous waste materials should be disposed of according to national regulations.

4. PROCEDURE

4.1. Calculation for making solution for paper impregnation

- a. Depending on insecticides to be used: choose appropriate carrier oil for the preparation of solvent solution according to Tables 1 and 2.

Table 1 - Insecticide and oil required to make up papers

Insecticide class (Group I)	Insecticide	Oil required/ Solvent
Organophosphates	Malathion	Olive oil/ Acetone
	Pirimiphos-methyl	Acetone*
Carbamates	Bendiocarb	Olive oil/ Acetone
Pyrethroids	Permethrin	Silicone oil / Acetone
	Deltamethrin	Silicone oil / Acetone
	Alpha-cypermethrin	Silicone oil / Acetone
Organophosphates (OP) Control	N/A	Olive oil/ Acetone
Carbamates (C) Control	N/A	Acetone
Pyrethroids (PY) Control	N/A	Silicone oil/ Acetone

* No oil should be used as per manufacturer instructions.

Table 2 - Synergist and oil required to make up papers

Synergist	Oil required
Piperonyl Butoxide (PBO)	Silicone oil / Acetone

- b. Take note of the degree of purity of insecticide to be used for the impregnation. This information is essential for the calculation of the quantity of the active ingredient (AI).
- c. Choose the concentration of insecticide necessary for the impregnation. Use Excel file: WHO Calculation for impregnation to prepare the stock solution (higher concentration) and dilute it for final concentration for impregnation of papers (see example Table 3).
- d. Complete the calculation (see example Table 3).

Note¹: Oil allows the production of a stable, thin and homogeneous layer of the active ingredient on the paper and prevents crystallization of active ingredients that are solid at room temperature. **The concentrations are generally expressed as the percentage % of active ingredient per unit volume of oil (except for Pirimiphos-methyl) on the filter-paper (the acetone being volatile).**

For insecticides intended to be mixed with silicon oil, papers are impregnated with 3.6 mg/cm² of the carrier, i.e. 648 mg/paper or **0.66 ml/paper for silicon oil** (considering that silicon oil has a density of 0.98). A filter-paper impregnated at 1% then contains 6.61 mg of technical insecticide, or 367.3 mg/m².

For insecticides intended to be mixed with olive oil, papers are impregnated with 3.6 mg/cm² of the carrier, i.e. 648 mg/paper or **0.71 ml/paper for olive oil** (considering that olive oil has a density of 0.91). A filter-paper impregnated at 1% then contains 7.12 mg of technical insecticide, or 395.6 mg/m².

Final concentration (% m/v)	Carrier	Mass of carrier (mg)	Density (g/cm ³)	Volume of carrier (ml) for 1 paper	Volume of acetone (ml) for 1 paper	Mass of <u>pure</u> (mg) insecticide for 1 paper	Concentration per surface area (mg/m ²)
1	Silicon oil	648.00	0.98	0.66	1,34	6.61	367.3
1	Olive oil	648.00	0.91	0.71	1.29	7.12	395.6

Note²: If serial dilution is needed to achieve the desired concentration, consider the example as follows – If the stock is 95% and required concentration is 0.001%, best practice would be to prepare 1% stock solution, dilute to 0.05% and from this to the final 0.001% dilution. Remember not to dilute directly from 1% to 0.001%.

Note³: Small quantities of AIs for example below 10mg (**0.01g**) are extremely difficult to weigh out accurately on the micro balance. A preferred method is to weigh approximate quantity (slightly above the required amount) and adjust the weight to the volume of the solvent (oil and acetone).

Table 3 - Example of Excel file: WHO calculation for impregnation

Impregnation of papers using pyrethroids or PBO

Use silicone oil (DC 556 - Dow Corning)

variable



Density of oil	0,98
Insecticide name	Deltamethrin
Batch number	F032640

Date	24-Sep-2020
Operator	Stéphane

Concentration AI (mg/m ²)	91,84
Concentration AI (%) of the stock solution (S)	0,25
Number of paper	4
Degree of purity (%)	99,6

Active Ingredient to weigh (mg)	6,64
Total volume of acetone / silicone oil (+ AI) (ml)	8

Actual weight (mg)	8,1
Total adjusted volume (ml)	9,8

Solution	Final concentration (%)	Final Volume (ml)	Initial Volume (ml)	Initial concentration (%)	Volume of Ac/silic. to complete (ml)
S	0,25	9,8			
S1	0,025	10	1	0,25	9
S2			#DIV/0!		#DIV/0!
S3			#DIV/0!		#DIV/0!
S4			#DIV/0!		#DIV/0!
S5			#DIV/0!		#DIV/0!
S6			#DIV/0!		#DIV/0!
S7			#DIV/0!		#DIV/0!
S8			#DIV/0!		#DIV/0!
S9			#DIV/0!		#DIV/0!
S10			#DIV/0!		#DIV/0!

4.2. Preparation of solutions for impregnation

Wearing the appropriate PPE in the lab, check the cleaning log for decontamination of the fume hood and the area to be used for testing and keep ready all the equipment and consumables required. This includes:

- Glass bottles of appropriate size
- Pipettes of appropriate volume and pipette tips
- Spatula
- Chemicals/active ingredient
- Balance (precision 0.01 mg)
- Acetone
- Carrier oil (Excel file:WHO Calculation for impregnation)
- Graduated glass container for example beakers or flask labelled for acetone and oil
- Nitrile or latex gloves of different sizes (place in reach of fume hood)
- 12 cm x 15 cm Whatman No. 1 filter paper catalogue number 1001 917
- Paper holding rack/s (one for each compound being used)
- Marker pen
- Label with pencil (better resistance to acetone solvent)
- String for line and pegs to hold papers/ or aluminium foil
- Holding rack

- a. Hang the string in the hood for drying papers. Always use new clean string and pegs for each paper preparation or arrange aluminium foils on the surface where to place papers to keep them dry.
- b. Ensure that the details for equipment and consumables required for preparation of papers are captured in recording sheet.
- c. Labelling the necessary bottles for stock and final solutions with the name of AI and concentration, the date, the type of solvent and the name of operator.

Weighing out the active ingredient

- d. In the fume hood, prepare the stock solution of oil/ solvent in glass bottles. Prepare a stock solution of solvent (oil + acetone) of the amount needed for all dilutions (for papers with PY and PBO to prepare 200 mL of solvent, mix 66 mL of silicone oil and 134 mL acetone and for paper with OP/C to prepare 200 mL of solvent, mix 71 mL of olive oil and 129 mL of acetone).
- e. Calibrate the balance with doors closed.
- f. Put the aluminium micro weigh dish or piece of aluminium foil on the balance (for a small quantity of AI) or the stock solution bottle (for a large quantity of AI) and adjust/ tare the balance.
- g. Use a pipette if active ingredient (AI) is a liquid or a spatula if AI is a powder, carefully weigh out the amount required.
- h. Put the amount of AI in the weighing container (foil or bottle), close the doors to check the weight. If required, add more quantity of AI to reach desired the approximate quantity.
- i. Press button on the printer of the balance, then press the print sign on the balance to obtain a date, time and amount weighed. Print out a copy and attach to the record sheet or if printing facility is not available with the balance, make a note in the lab book. Recording the real weight of AI in the calculation table and it adjust the volume of stock solution (MS).
- j. Put the aluminium micro weigh dish with the AI in glass bottle to prepare stock solution.
- k. Pipette the required amount of solvent into the stock bottle containing the active ingredient and vortex/ shake until fully homogenized. Repeat f-k if there is more than one concentration of AI or chemical required.
- l. Prepare the dilution solutions according to the calculation table.

NOTE: Always ensure to use separate gloves for different active ingredients and NEVER handle paperwork with contaminated gloves.

Always refer to protocol for specific amounts of active required per paper!

4.3. Preparation of paper

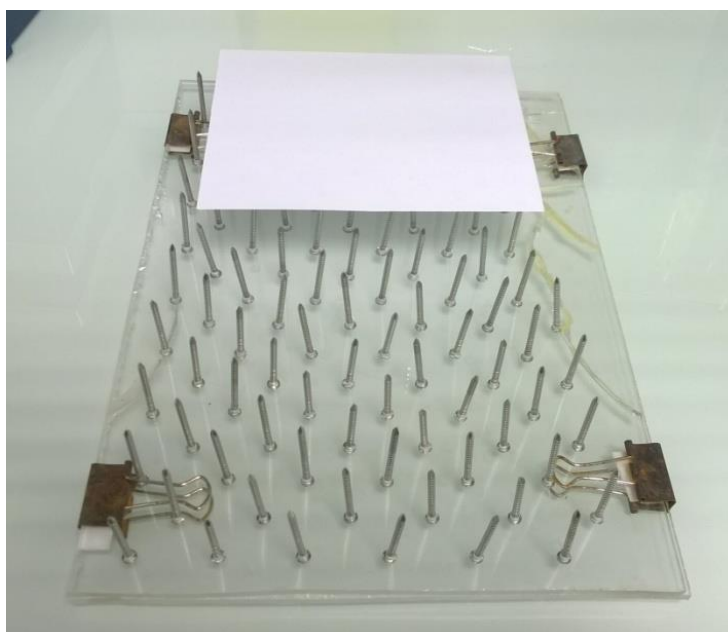
- a. Using the paper cutter, cut 12 x 15 cm pieces Whatman No.1 filter paper taking care to make sufficient number of pieces required.

NOTE: Take care when using the paper cutter to keep fingers and hands away from the sharp edges.

- b. Stamp each paper with name of insecticide and % concentration at bottom center along the 12 cm edge. For control papers stamp class group (namely, PY Control, OP Control, C Control)
- c. The papers are now ready to be impregnated.

4.4. Impregnation of paper

- a. Put on the gloves and place papers on holding rack with the stamp/ label side down (Picture 1).



Picture 1 - Filter paper ready to be impregnated on the holding rack

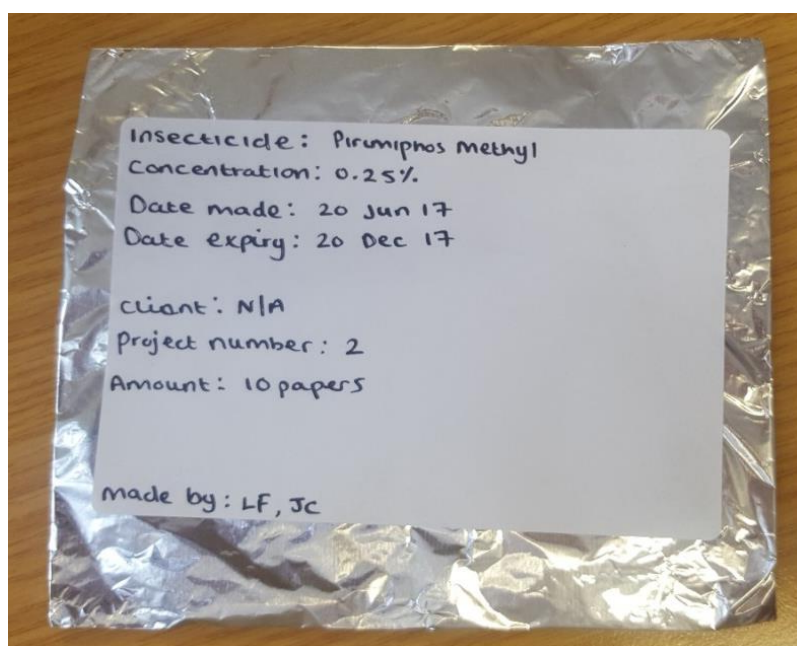
- b. Always impregnate the control papers first using the same solvent as for the insecticide to be used for the impregnation.
- c. Shake the solution just before use. For example vortex for 10 seconds just before impregnation of paper, even if the same solution will be used for more than one paper, the solution is vortexed before each pipetting.
- d. **2 mL is the standard amount required per paper**, so take 2 mL with glass pipette and slowly draw up the homogeneous stock into the tip. Close the flask.
- e. Impregnate papers with the insecticide on the opposite of the stamp (in actual use roll the paper keeping the stamp visible and readable from outside).
- f. Impregnate the paper with 2 mL solution by moving the pipette tip keeping equal distance between the columns/rows. To ensure even impregnation of the paper surface, do not treat the same column/row treated before. Do not touch the paper with fingers while treating it. Ensure each subsequently treated paper does not over the previously treated paper.

NOTE: Because acetone evaporates rapidly it is crucial the lid on should be replaced between pipetting/ impregnation of papers.

- g. If the 2 mL amount is exhausted before reaching end of the paper, discard this paper.
- h. If papers are to be impregnated with serial concentrations, always begin with lowest to the highest concentration, using the same gloves and pipettes.
- i. Always change gloves and pipette between handling of papers with a different insecticide.
- j. Leave the treated paper on aluminium foil at room temperature under dark condition overnight to dry or hang the treated papers from the top left hand 12 cm corner using a peg and the line that was set up earlier. Ensure each paper does not overlap/ touch other treated paper. Put Control papers to dry in separate place.
- k. Do not leave the filter papers to dry for a longer period, several insecticide chemicals deteriorate rapidly on exposure to daylight.

NOTE: Impregnated papers will be dry up from one night to 24h in the dark before the test or wrapped in aluminium foil and store in the fridge.

- l. The following day, wearing appropriate PPE, wrap 2/ or 4 treated papers of the same concentration (impregnated face against impregnated face, the note by the pencil should be visible) in one aluminium foil and wrap each concentrations as the same way. At each wrap write with a permanent pencil: insecticide, concentration, date of impregnation and number of papers. Still begin by the control papers followed by the lowest to the highest concentration. Wrap in group as one test the 10-12/ or 6 concentrations together in a hermetic plastic bag.
- m. Ensure gloves are changed between different compounds and always wrap lower to higher concentrations of the same compound.
- n. Store papers at 6 ± 2 °C until used or shipped in a clearly labelled zip lock bag/s that are labelled with name of insecticide, concentrations (from... to...), date of preparation, date of expiry or number use, client or project details, number of papers and initials of staff who carried out the procedure (see Picture 2 below).



Picture 2 - Completed, foil wrapped and Labelled Insecticide papers

3. CLEANING

Apply the following method as feasible:

- Clean the interiors of the fume hood and wipe down balance and sharps bin with followed by 70% Isopropanol wipe. Ensure all paper holding racks are left to soak in for appropriate time for decontamination.
- Decontamination: soaking overnight in the decontaminant 20% of an alkaline solution (TFD4 or Decon90) if the equipment is in direct contact with the insecticide and 10% solution for the equipment used for the handling. The following day, rinse three times with tap water and dry at room temperature.
- The benches and fixed equipment are decontaminated with ethanol.

NOTE: Decontaminant solution at 20 and 10% should be change at least once per month, or more if necessary.

4. REFERENCED DOCUMENTS

GUIDELINES FOR TESTING MOSQUITO ADULTICIDES FOR INDOOR RESIDUAL
SPRAYING AND TREATMENT OF MOSQUITO NETS.

WHO/CDS/NTD/WHOPES/GCDPP/2006.

5. TABLE OF SUPPLIES

To complete with your supplies and suppliers (for example)

Supply	Name of supplier	Address	Reference
Acetone for HPLC, $\geq 99.9\%$	Sigma Aldrich	3050 Spruce Street, Saint Louis, MO 63103, USA	439126
Dow Corning(R) 556 Cosmetic Grade Fluid	Dow Corning Corporation	South Saginaw Road Midland Michigan 48686	000000000001010476
Olive oil	Sigma Aldrich Chimie S.a.r.l	L'Isle D'Abeau Chesnes F-38297 St. Quentin Fallavier, France	75343
TFD4 (Alkaline liquid detergent)	Franklab	3 Avenue des Frênes 78180 Montigny le Bretonneux, France	
Decon 90	Decon Laboratories Limited	Conway Street, Hove, East Sussex, BN3 3LY, England	00188082