New WHO procedures and insecticide discriminating concentrations for monitoring resistance in adult mosquito vectors

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Neglected Tropical Diseases
WHO multi-center study to establish new DCs and procedures

Study duration
• 2017 – 2021
• 23 participating laboratories in five WHO regions

Key outcomes
• Built capacity of laboratories on test methods & procedures
• Established and validated 17 new insecticide DCs for Aedes spp.
• Established and validated 13 new DCs for Anopheles spp.
• Developed and validated the new standard bottle bioassay method, “the WHO bottle bioassay”,
• Developed the first version of the SOPs
• Created a database of bioassay records of concentration–response tests for > 400 000 mosquitoes including source of variations in test outcomes under different test conditions
• Identified and recommended measures to improve laboratory testing procedures and to guide the selection of DCs of insecticides commonly used for vector control.
• Identified knowledge gaps for future work.

https://apps.who.int/iris/handle/10665/352616
New DCs for WHO tube test

**Anopheles**

<table>
<thead>
<tr>
<th>Insecticide</th>
<th>Species</th>
<th>Discriminating concentration</th>
<th>Carrier oil or solvent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alpha-cypermethrin</td>
<td><em>An. albimanus</em> and <em>An. stephensi</em></td>
<td>0.30%</td>
<td>Silicone oil</td>
</tr>
<tr>
<td></td>
<td><em>An. funestus</em>, <em>An. minimus</em> and <em>An. gambiae</em></td>
<td>0.05%</td>
<td></td>
</tr>
<tr>
<td>Pirimiphos-methyl⁶</td>
<td><em>An. albimanus</em>, <em>An. stephensi</em>, <em>An. minimus</em> and <em>An. funestus</em></td>
<td>100 mg/m³</td>
<td>Acetone alone</td>
</tr>
<tr>
<td></td>
<td><em>An. gambiae</em></td>
<td>170 mg/m³</td>
<td></td>
</tr>
</tbody>
</table>

**Aedes**

- Species specific discriminating concentrations (DCs)
- New DCs for *Aedes* replace all previous tentative DCs
- For pirimiphos-methyl, programs and partners are advised to validate results obtained with the former tentative DC against the new DC

<table>
<thead>
<tr>
<th>Insecticides</th>
<th>Species</th>
<th>Discriminating concentration</th>
<th>Carrier oil or solvent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alpha-cypermethrin</td>
<td><em>Ae. aegypti</em></td>
<td>0.05%</td>
<td>Silicone oil</td>
</tr>
<tr>
<td></td>
<td><em>Ae. albopictus</em></td>
<td>0.08%</td>
<td></td>
</tr>
<tr>
<td>Bendiocarb</td>
<td><em>Ae. aegypti</em> and <em>Ae. albopictus</em></td>
<td>0.20%</td>
<td>Olive oil</td>
</tr>
<tr>
<td>Chlorpyrifos-ethyl</td>
<td><em>Ae. aegypti</em> and <em>Ae. albopictus</em></td>
<td>1%</td>
<td>Olive oil</td>
</tr>
<tr>
<td>Permethrin (40:60³)</td>
<td><em>Ae. aegypti</em> and <em>Ae. albopictus</em></td>
<td>0.40%</td>
<td>Silicone oil</td>
</tr>
<tr>
<td>Deltamethrin</td>
<td><em>Ae. aegypti</em> and <em>Ae. albopictus</em></td>
<td>0.03%</td>
<td>Silicone oil</td>
</tr>
<tr>
<td>Lambda-cyhalothrin</td>
<td><em>Ae. aegypti</em></td>
<td>0.05%</td>
<td>Silicone oil</td>
</tr>
<tr>
<td></td>
<td><em>Ae. albopictus</em></td>
<td>0.08%</td>
<td></td>
</tr>
<tr>
<td>Malathion</td>
<td><em>Ae. aegypti</em></td>
<td>1.50%</td>
<td>Olive oil</td>
</tr>
<tr>
<td></td>
<td><em>Ae. albopictus</em></td>
<td>5%</td>
<td></td>
</tr>
<tr>
<td>Pirimiphos-methyl⁶</td>
<td><em>Ae. aegypti</em> and <em>Ae. albopictus</em></td>
<td>60 mg/m³</td>
<td>Acetone alone</td>
</tr>
</tbody>
</table>
The new WHO bottle bioassay

- Developed for insecticides that cannot be impregnated on filter papers: transfluthrin, prallethrin, metofluthrin, clothianidin, flupyradifurone, chlorfenapyr, pyriproxyfen

- Similar to the CDC bottle bioassay but with endpoints aligned with those of WHO tube tests:

  **Exposure period (1 h):**
  Introduce 25 mosquitoes into each treatment and

  **Holding period (24 h or 72 h):**
  Transfer mosquitoes into the holding paper cups. Provide them access to a water-sugar solution. Read mortality at the end of the 24 h (or 72 h for chlorfenapyr)
**Aedes**

<table>
<thead>
<tr>
<th>Insecticide</th>
<th>Species</th>
<th>Discriminating concentration (µg/bottle)</th>
<th>Solvent and surfactant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clothianidin</td>
<td><em>Ae. aegypti</em></td>
<td>20</td>
<td>Acetone + MERO 1500 ppm</td>
</tr>
<tr>
<td></td>
<td><em>Ae. albopictus</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Flupyradifurone</td>
<td><em>Ae. aegypti</em> and <em>Ae. albopictus</em></td>
<td>80</td>
<td>Acetone + MERO 1500 ppm</td>
</tr>
<tr>
<td>Metofluthrin</td>
<td><em>Ae. aegypti</em> and <em>Ae. albopictus</em></td>
<td>1</td>
<td>Acetone</td>
</tr>
<tr>
<td>Prallethrin</td>
<td><em>Ae. aegypti</em> and <em>Ae. albopictus</em></td>
<td>30</td>
<td>Acetone</td>
</tr>
<tr>
<td>Transfluthrin</td>
<td><em>Ae. aegypti</em> and <em>Ae. albopictus</em></td>
<td>3</td>
<td>Acetone</td>
</tr>
</tbody>
</table>

**Anopheles**

<table>
<thead>
<tr>
<th>Insecticide</th>
<th>Species</th>
<th>Discriminating concentration (µg/bottle)</th>
<th>Bottle drying time (h)</th>
<th>Recording/holding time (h)</th>
<th>Solvent/surfactant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clothianidin</td>
<td><em>An. albimanus</em></td>
<td>10</td>
<td>24</td>
<td>24</td>
<td>Acetone + MERO 200 ppm</td>
</tr>
<tr>
<td></td>
<td><em>An. stephensi</em></td>
<td>10</td>
<td>24</td>
<td>24</td>
<td>Acetone + MERO 800 ppm</td>
</tr>
<tr>
<td></td>
<td><em>An. funestus</em> and <em>An. gambiae</em></td>
<td>4</td>
<td>24</td>
<td>24</td>
<td>Acetone + MERO 800 ppm</td>
</tr>
<tr>
<td></td>
<td><em>An. minimus</em></td>
<td>6</td>
<td>24</td>
<td>24</td>
<td>Acetone + MERO 800 ppm</td>
</tr>
<tr>
<td>Flupyradifurone</td>
<td><em>An. albimanus</em></td>
<td>500</td>
<td>24</td>
<td>24</td>
<td>Acetone + MERO 200 ppm</td>
</tr>
<tr>
<td></td>
<td><em>An. stephensi</em> and <em>An. gambiae</em></td>
<td>60</td>
<td>24</td>
<td>24</td>
<td>Acetone + MERO 800 ppm</td>
</tr>
<tr>
<td></td>
<td><em>An. funestus</em> and <em>An. minimus</em></td>
<td>100</td>
<td>24</td>
<td>24</td>
<td>Acetone</td>
</tr>
<tr>
<td>Transfluthrin</td>
<td><em>An. albimanus</em>, <em>An. stephensi</em>, <em>An. funestus</em> and <em>An. gambiae</em></td>
<td>2</td>
<td>24</td>
<td>24</td>
<td>Acetone</td>
</tr>
<tr>
<td>Chlorfenapyr</td>
<td><em>An. gambiae</em>, <em>An. stephensi</em>, <em>An. funestus</em> and <em>An. albimanus</em></td>
<td>100</td>
<td>24</td>
<td>72</td>
<td>Acetone</td>
</tr>
<tr>
<td>Pyriproxyfen²</td>
<td><em>An. gambiae</em>, <em>An. stephensi</em> and <em>An. funestus</em></td>
<td>100</td>
<td>2</td>
<td>7 d²</td>
<td>Acetone</td>
</tr>
</tbody>
</table>

**MERO** (surfactant) was used to allow for adequate coating of bottles and prevent crystallization of technical materials (insecticide active ingredients)
**Automated spread sheets for preparing stock solutions**

**Available at:** [https://cdn.who.int/media/docs/default-source/ntds/vector-ecology-management/calculation-tables-paper-impregnation-bottles-17jan2022-locked.xlsx?sfvrsn=83a556a4_7](https://cdn.who.int/media/docs/default-source/ntds/vector-ecology-management/calculation-tables-paper-impregnation-bottles-17jan2022-locked.xlsx?sfvrsn=83a556a4_7)

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### Calculation for weight of insecticide Al for coating glass bottles with or without a surfactant

<table>
<thead>
<tr>
<th>Insecticide class group</th>
<th>Surfactant</th>
<th>Insecticide</th>
<th>Targeted concentration of Al in the bottles (µg/g bottle)</th>
<th>No. of bottles to be coated</th>
<th>Amount of surfactant per bottle (ppm or µg)</th>
<th>Total weight of surfactant needed (mg)</th>
<th>Density of surfactant</th>
<th>Volume of surfactant (mL)</th>
<th>Volume of acetone (mL)</th>
<th>Total volume of coating solution (mL)</th>
<th>Amount of Al to weigh for coating bottles (g)</th>
<th>Purity of insecticide Al (%)</th>
<th>Adjusted amount of Al to weigh (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td>a</td>
<td>b</td>
<td>e = (b x c)/1000</td>
<td>0.900</td>
<td>0.001</td>
<td>1.00</td>
<td>1.00</td>
<td>j = (a x b) / 10^6</td>
<td>0.000000</td>
<td>99.2</td>
<td>0.000000</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td>1</td>
<td>800</td>
<td>0.8</td>
<td>0.900</td>
<td>0.001</td>
<td>1.00</td>
<td>1.00</td>
<td>0.000000</td>
<td>99.8</td>
<td>0.000000</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>None</td>
<td>None</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1.00</td>
<td>1.00</td>
<td>0.000000</td>
</tr>
</tbody>
</table>

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**Notes:**
- **MERO**: 81% rapeseed oil methyl ester
- **Operating Manual**: [Link](https://www.who.int/malaria/)
- **A1-Calculation with oil**
- **A2-Calculation without oil**
- **A3-Stock solution paper impregnation**
- **B1-Calculation for bottles**
- **B2-Stock solution bottles**

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**Global Malaria Programme & Neglected Tropical Diseases**
WHO bottle bioassay with Chlorfenapyr

PROCEDURE

- Test to be conducted strictly at: 27 °C ± 2 °C
- Susceptible colony mosquitoes to be tested in parallel

CONFIRMING RESISTANCE

Three bioassays with same mosquito population for the same site and at different time points are needed to confirm resistance. Resistance is confirmed when:

- the mortality of test mosquitoes 72 h post-exposure is <90% in all 3 tests
- the mortality in the susceptible laboratory colony, tested in parallel to the wild mosquitoes, is ≥98% in all 3 tests

Global Malaria Programme & Neglected Tropical Diseases
**First part** of the test is a WHO bottle bioassay but with:
- Blood fed mosquitoes
- Mortality measured at 24h, 48h and 72h

**Second part** of the test is new:
- Chambering mosquitoes individually
- Counting the numbers that lay eggs

End point: oviposition inhibition

The process is done with mosquitoes from field and a susceptible colony in parallel
Ordering test kits and impregnated papers

The catalogue of the Universiti Sains Malaysia (USM) is being updated

Control of Neglected Tropical Diseases

Form and catalogue available at: https://www.who.int/teams/control-of-neglected-tropical-diseases/vector-ecology-and-management/vector-control/insecticide-resistance
New insecticide resistance monitoring manual

- **Replaces** the former ‘Test procedures for insecticide resistance monitoring in malaria vector mosquitoes’
- **Integrates** *Anopheles*, *Aedes* and *Culex* spp.
- **Includes:**
  - 27 DCs for *Anopheles* (13 of them new) and 13 DCs for *Aedes*
  - Description of the WHO bottle bioassay and special requirements and processes for chlorfenapyr, pyriproxyfen
  - Prioritizing resistance tests amid limited resources and mosquitoes
- **Provides new guidance on:**
  - Testing a representative sample of mosquitoes
  - Data management and reporting
  - Using resistance data for decision making

Soon online!
New Standard Operating Procedures (SOPs)

Provide step by step instruction to conduct all WHO susceptibility bioassay. A tool for field technicians that are running the bioassays.

Available at:
WHO IRIS or https://www.who.int/teams/global-malaria-programme/prevention/vect or-control/insecticide-resistance

* For research & product development purposes
Update DHIS2 modules for resistance monitoring

Include:

- New insecticide DCs (enhanced option set)
- New data elements for bioassay with chlorfenapyr and pyriproxyfen
- New data elements to enter data per replicate (tube or bottle bioassay)
- New data elements to record temperature during bioassay
- New data elements to capture bottle coating and filter paper impregnation date
- Demo at: https://extranet.who.int/dhis2-ento-vc
New GMP webpage on insecticide resistance in malaria vectors

Bringing all resources together to improve accessibility:

**Insecticide resistance**

Widespread and increasing insecticide resistance poses a threat to effective malaria vector control. Failure to mitigate and manage insecticide resistance is likely to result in an increased burden of disease, potentially reversing some of the substantial gains made in controlling malaria over the last decade.

To help countries monitor and manage this threat, WHO provides:

- overarching key principles to shape resistance management strategies in the Global plan for insecticide resistance management in malaria vectors (GPRM),
- a framework to develop national plans for monitoring and the management of insecticide resistance in malaria vectors,
- test procedures to monitor insecticide resistance in malaria vector, including SOPs for each specific procedure, and guidance on how to use these data to guide programmatic decisions,
- an annual update on the status of insecticide resistance in the annual World malaria report,
- DHIS2-based digital tools to collect and analyse insecticide resistance monitoring data,
- a global database that contains insecticide resistance monitoring data collected worldwide since 1973,
- an interactive online data visualization platform Malaria Threats Map, to explore the global status of insecticide resistance, including its intensity, molecular mechanisms and PBO’s ability to restore susceptibility to pyrethroids.

Through the Vector Control Advisory Group (VCAQ), WHO oversees the evaluation of new tools aiming to target insecticide resistance vectors and, through the Guideline Development Group, the development of WHO recommendations on these tools once they have demonstrated public health value.

**Resources and tools**

- Global database on insecticide resistance in malaria vectors
- DHIS2 data collection and collation tools
- Malaria Threats Map

**Standard operating procedures on insecticide resistance**

**Link:**
https://www.who.int/teams/global-malaria-programme/prevention/vector-control/insecticide-resistance
Updated NTD page on insecticide resistance

Control of Neglected Tropical Diseases

Insecticide resistance

To be replaced by new manual soon

Insecticide resistance

27 MARCH 2022
Determining discriminating concentrations of insecticides for monitoring resistance...

WHO conducted a multi-centre study in 2017–2018 involving 23 laboratories throughout the world to establish and validate discriminating concentrations...

Download Read More

31 May 2018
Test procedures for insecticide resistance monitoring in malaria...

Download Read More

8 September 1998
Insecticide resistance monitoring

Download Read More

Order test kits and supplies

- Test lot order form (updated 1 September 2021)
- Catalogue for test kits (updated 1 September 2021)

Discriminating concentrations

- Discriminating concentrations of insecticides for adult mosquitoes

Standard Operating Procedures

4 MARCH 2022
Standard operating procedure for impregnation of filter papers for testing insecticide...

This SOP describes the process for impregnating filter papers with insecticides and synergists to be used in WHO tube tests for testing insecticide susceptibility...

Download Read More

4 March 2022
Standard operating procedure for testing insecticide susceptibility...

Download Read More
Acknowledgements

- **Staff from the 23 laboratories that participated in the WHO multi-centre study for establishing new DCs and SOPs:** London School of Hygiene & Tropical Medicine (Uk), Fiocruz (Brazil), Institut de Recherche en Sciences de la Santé (Burkina Faso), Organisation de coordination et de coopération pour la lutte contre les grandes endémies en Afrique Centrale (Cameroon), National Institute for Communicable Disease Control and Prevention (China), Instituto Nacional de Salud (Colombia), University of Colombia, Institut de Recherche pour le Développement (France), Malaria Research and Training Centre (Mali), (Indian Council of Medical Research–Vector Control Research Centre, Universiti Sains Malaysia, Universidad Autonoma de Nuevo Leon (Mexico), National Institute of Health (Peru), Environmental Health Institute, (Singapore), National Institute for Communicable Diseases (South Africa), Swiss Tropical and Public Health Institute, (Kasetsart University of Agriculture (Thailand), Mahidol University (Thailand), Liverpool School of Tropical Medicine (UK), Kilimanjaro Christian Medical University College (Tanzania), Centers for Disease Control and Prevention (U.S.A)

- **Experts and partners participating in WHO consultations on the results of the study**
- **Experts contributing to the development of the new WHO insecticide resistance manual and SOPs**
- **Bill & Melinda Gates Foundation for financial support**

The full list of names is provided in the [multi-center study report](#) and the new WHO manual for resistance monitoring.
Thank you for your attention

Moving towards better-informed decisions