The Insecticide Resistance Action Committee

Prevention and Management of Insecticide Resistance in Vectors of Public Health Importance

December 2014

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Foreword

• Insecticides remain the mainstay of many tropical disease control programmes and insecticide resistance can have a major impact on our ability to control these diseases. The need to protect and extend the useful life of current, and future insecticides is therefore critical. For this reason, resistance management must be given a high priority in all vector control programmes.

• This guide has been designed to provide the reader with the knowledge and tools required to implement insecticide resistance management in vector management programmes.

The Insecticide Resistance Action Committee (IRAC) is a specialist technical group of the industry association CropLife International and provides a coordinated industry response to prevent or delay the development of resistance in insect pests. For more information on IRAC and resistance management please visit the IRAC website at www.irac-online.org.
What is Resistance?

Resistance to insecticides may be defined as ‘a heritable change in the sensitivity of a pest population that is reflected in the repeated failure of a product to achieve the expected level of control when used according to the label recommendation for that pest species’ (IRAC).

The use of insecticides as such does not create resistance but it can develop through the over-use or misuse of an insecticide against a pest species. Resistance develops when naturally occurring genetic variation allows a small proportion of the population to resist and survive the effects of the insecticide. If this advantage is maintained by continually using the same insecticide, the resistant insects will reproduce and the genetic changes that confer resistance are transferred from parents to offspring so that eventually they become numerous within the population.

Major factors that influence resistance development

- Application frequency
- Dosage
- Persistence of effect
- Rate of reproduction
- Population isolation
Possible Scenario for Resistance Development in a Mosquito Population

1. Resistance rare

Exposure to insecticide

Survivors reproduce

Resistance development

2. Resistance increasing

Further exposure to the same insecticide

Survivors reproduce

3. Resistance common

Further exposure to the same insecticide

Survivors reproduce

4. Majority of population resistant

Key: Resistant Susceptible
Insecticides can be classified by Mode of Action (MoA) based on their site of action. Different insecticides can have the same target site within the insect. Insecticides from the same chemical class, e.g. pyrethroids, will have the same MoA. There may be many different commercial products based on insecticides from the same chemical class.

IRAC has produced a comprehensive classification of all commercially available insecticides, allowing products with the same MoA to be readily identified. There is little value in switching from one product to another with the same MoA on the grounds of Insecticide Resistance Management (IRM). All products from a MoA class will expose the insects to selection pressure for the same resistance mechanisms.

Therefore, when selecting products for IRM, it is vital to identify which MoA class a given product comes from. Rotating from one pyrethroid to another simply exposes the mosquito population to the same MoA and has no value in resistance management.

The IRAC MoA Classification allocates each insecticide to a numbered group based on their target site. Chemical sub-groups are identified with a letter, for example, pyrethroids are given the IRAC MoA classification 3A.

For further information see pages 9 and 10, or visit www.irac-online.org.
Various mechanisms enable insects to resist the action of insecticides:

- **Metabolic resistance**
- **Target-site resistance**
- **Reduced penetration**
- **Behavioural resistance**

The mechanisms expressed may resist more than one insecticide - *Cross-resistance*

An insect may express more than one resistance mechanism – **Multiple resistance**

Major mechanisms conferring resistance to important classes of insecticides in adult mosquitoes.

**Biochemical mechanism of resistance**

<table>
<thead>
<tr>
<th></th>
<th>Metabolic</th>
<th>Target-site</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Estarases</td>
<td>Monoxygenases</td>
</tr>
<tr>
<td>Pyrethrins</td>
<td>![Circle size]</td>
<td>![Circle size]</td>
</tr>
<tr>
<td>DDT</td>
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<tr>
<td>Carbamates</td>
<td>![Circle size]</td>
<td>![Circle size]</td>
</tr>
<tr>
<td>Organophosphates</td>
<td>![Circle size]</td>
<td>![Circle size]</td>
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</tbody>
</table>

*Circle size reflects the relative impact of the mechanism on resistance*
Insecticide Resistance Management (IRM) should be undertaken as part of an Integrated Vector Management (IVM) programme that includes use of both insecticidal and non-insecticidal interventions.

There are several approaches to Insecticide Resistance Management, but all rely on minimising the selection pressure for resistance development by limiting the extended exposure of a mosquito population to insecticides that have the same mode of action:

**Rotation** – This strategy is based on the rotation over time of two or more insecticide classes with different Modes of Action (MoA). This approach assumes that if resistance to each insecticide is rare, then multiple resistance will be extremely rare.
Mixtures – A mixture is a single formulation containing two or more insecticides, or different insecticide formulations being applied in the same spray tank, or an LN* or ITM* treated with two or more insecticides with different MoA. It can also include the combination of an LN or ITM with an IRS* application in the same dwelling. This approach assumes that if a mosquito survives one insecticidal MoA, it will be killed by the other, and that if resistance to one is rare, resistance to both will be extremely rare.

Fine-scale mosaic - Spatially separated applications of different MoA insecticides against the same mosquito population. e.g. using two different MoA insecticides in different dwellings within the same village. Mosquitoes are therefore likely to come into contact with a second insecticide during their lifetime, if they survive exposure to the first. This reduces the selection pressure for both insecticides.

* LN (Long lasting insecticide treated Net), ITM (Insecticide Treated Material), IRS (Indoor Residual Spray)
## Mode of Action Classes Available for Vector Control – Adults

### Nerve and Muscle Targets
Most current insecticides act on nerve/muscle targets and are generally fast acting.

**Group 1 Acetylcholinesterase (AChE) inhibitors**
- 1A Carbamates, 1B Organophosphates

**Group 3 Sodium channel modulators**
- 3A Pyrethrins, Pyrethroids, 3B DDT

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<table>
<thead>
<tr>
<th>MoA</th>
<th>Class</th>
<th>Insecticide or Product</th>
<th>IRS</th>
<th>ITN</th>
<th>LN</th>
</tr>
</thead>
<tbody>
<tr>
<td>1A</td>
<td>Carbamate</td>
<td>Bendiocarb, Propoxur</td>
<td>✓</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>1B</td>
<td>Organophosphate</td>
<td>Malathion, Fenitrothion, Pirimiphos-methyl</td>
<td>✓</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>3A</td>
<td>Pyrethroid</td>
<td>Alphacypermethrin</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Deltamethrin</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Permethrin</td>
<td>X</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Etofenprox</td>
<td>✓</td>
<td>✓</td>
<td>X</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Lambdacyhalothrin</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Bifenthrin</td>
<td>✓</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cyfluthrin</td>
<td>✓</td>
<td>X</td>
<td>✓</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Deltamethrin + PBO</td>
<td>X</td>
<td>X</td>
<td>✓</td>
</tr>
<tr>
<td>3B</td>
<td>Organochlorine</td>
<td>DDT</td>
<td>✓</td>
<td>X</td>
<td>X</td>
</tr>
</tbody>
</table>

* Indicates Full WHOPES approval as an LN (NB: Those without * indicates Interim approval only.)

‡ Indicates approval as long lasting net treatment
Mode of Action Classes Available for Vector Control – Larvae

**Nerve and Muscle Targets**
Act on nerve/muscle targets and are generally fast acting.
*Group 1* - Acetylcholinesterase (AChE) inhibitors - 1B Organophosphates
*Group 5* - Nicotinic acetylcholine receptor (nAChR) allosteric activators – Spinosyns

**Growth and Development Targets**
Insect development is controlled by juvenile hormone and ecdysone. Insecticides that disrupt this process are generally slow to moderately slow acting.
*Group 7* - 7A Juvenile hormone mimics, 7C Pyriproxyfen
*Group 15* - Inhibitors of chitin biosynthesis Type 0 – Benzoylureas

**Midgut**
Derived from bacteria, these toxins need to be ingested and disrupt the insect midgut membranes

*Group 11* - Microbial disruptors insect midgut membranes
*B. thuringiensis var. israeliensis*, *B. sphaericus*.

<table>
<thead>
<tr>
<th>MoA</th>
<th>Class</th>
<th>Insecticide or Product</th>
</tr>
</thead>
<tbody>
<tr>
<td>1B</td>
<td>Organophosphate</td>
<td>Temephos, Chlorpyrifos, Pirimiphos-methyl, Fenthion</td>
</tr>
<tr>
<td>5</td>
<td>Spinosyns</td>
<td>Spinosad</td>
</tr>
<tr>
<td>7A</td>
<td>Juvenile Hormone Mimics</td>
<td>Methoprene, Hydroprene</td>
</tr>
<tr>
<td>7C</td>
<td>Pyriproxyfen</td>
<td>Pyriproxyfen</td>
</tr>
<tr>
<td>15</td>
<td>Benzoylureas</td>
<td>Diflubenzuron, Novaluron</td>
</tr>
<tr>
<td>11</td>
<td>Bacterial Larvicide</td>
<td><em>Bt var. israeliensis</em> and <em>Bacillus sphaericus</em></td>
</tr>
</tbody>
</table>
The monitoring of insecticide susceptibility in vector control programmes has three main objectives:

- **Baseline data collection:** Conducted prior to the start of a control programme in order to provide baseline data to inform planning and insecticide choice.
- **Monitoring of susceptibility over time:** To evaluate the proportion of susceptible mosquitoes in populations over time, comparing it with the pre-intervention baseline. Hence the impact of the control strategy on the proportion of susceptible individuals in the mosquito population can be evaluated.
- **Detection of resistance:** To detect resistant individuals when they are at a low frequency in the population so that resistance management can be effectively introduced.

*Monitoring based on the Discriminating Dose DD ( = 2x LC₉₉) of an insecticide:* Resistance may go unnoticed for a long time providing the LC₉⁹ is not affected. However, an increase in the number of heterozygous resistant individuals would cause a shift in the LC₅₀.

*Detecting resistance at an early stage based on LC₅₀ and Resistance Factor:* Looking at the complete dose mortality data including the LC₅₀ will enable detection of a shift in vector susceptibility, an early sign of resistance before reduced insecticide efficacy in the field occurs.

*Calculating the Resistance Factor based on LC₅₀:* Provides a susceptibility comparison of a vector population over time, or to compare between strains.

\[
\text{Resistance Factor (RF)} = \frac{\text{LC}_{50} \text{ Resistant Population}}{\text{LC}_{50} \text{ Susceptible Population}}
\]

*NB: LC₅₀ (LC₉₉) is the concentration of insecticide required to kill 50% (99%) of the test mosquitoes*
There are various bioassay, biochemical and molecular methods that can be used to test and monitor resistance development. These can be used together to maximize outputs from monitoring in a region.

**WHO Test Kit - Adult mosquitoes**
The principle of this test is to expose mosquitoes for a given time in a specially designed plastic tube lined with a filter paper treated with a standard concentration of insecticide. The dose rate on the paper (diagnostic concentration) is 2x the lethal dose estimated to kill 100% of mosquitoes of a susceptible strain. Mosquitoes are generally exposed to the treated papers for one hour, mosquito mortality is assessed after 24 hours. This approach has been designed to avoid spurious reports of resistance in the field where none may exist. The kit and papers can be purchased with full instructions on their use. Supplier details can be found at: www.who.int/whopes/resistance/en/
**WHO Test Kit - Adult mosquitoes - Interpreting results**

The 24 hour mortality is expressed as a percentage. If the mortality in the control groups is over 5% but less than 20%, a correction of mortality is made by applying Abbot’s formula.

\[
\frac{100 \times (\% \text{ test mortality} - \% \text{ control mortality})}{100 - \% \text{ control mortality}}
\]

If the control mortality is $\geq 20\%$ the test results are discarded and the test will need to be repeated. The average mortality obtained at the same concentration is calculated in at least three replicates.

Results are interpreted as follows:

- **98 – 100% mortality**  
  Susceptible population

- **90 – 97% mortality**  
  Resistant individuals within the population suspected, but verification/confirmation required  
  If at least two additional tests consistently show mortality below 98%, then resistance is confirmed

- **<90% mortality**  
  Confirmation of the existence of resistant genes in the test population

For further information please refer to:  
“Test procedures for insecticide resistance monitoring in malaria vector mosquitoes”, WHO April 2013,  
http://www.who.int/malaria/publications/atoz/9789241505154/en/
**Monitoring Methods (Mosquito Larvae) – WHO Test Kit**

**WHO Test Kit – Larvicides (Chemical):**
This methodology aims to determine resistance in mosquito larvae based on diagnostic concentrations developed from dose response lines against susceptible species. The test assesses the resistance to the insecticide used, but can also be used to determine if cross-resistance is present.

The technique requires the testing of 3rd and 4th instar larvae collected from the wild. A wide range of concentrations is used to start with, so that an approximate dose response can be calculated. Then a narrower range of 4-5 concentrations yielding 10% and 95% mortality in 24 hour or 48 hours are used to determine LC$_{50}$ and LC$_{90}$ values.

**WHO Test Kit – Larvicides (Insect Growth Regulators)**
Different tests are conducted with IGRs as mortality may be slower or not take place until the pupal stage. Therefore, mortality is assessed every other day or every third day until the completion of adult emergence. The result is expressed in terms of the percentage of larvae that do not develop into successfully emerging adults, or adult emergence inhibition.

**Bacterial larvicides**
Larvicides such as Bti* or Bs* may be tested in the laboratory to determine resistance with the same methodology as for chemical larvicides, except in the preparation of stock solution.

Full details of the tests be found at: www.who.int/whopes/guidelines/en/

* Bti: Bacillus thuringiensis var. Israelensis, Bs: Bacillus sphaericus.
**Monitoring Methods (Adult Mosquitoes) – CDC Bottle Assay**

**CDC Bottle Test Kit - Adult Mosquitoes:**
The bottle bioassay method is a tactical surveillance tool for detecting and characterising changes in susceptibility to insecticides in vector populations. The bioassay uses 250 ml glass bottles. The internal surfaces of the bottle are coated with the desired insecticide diluted in acetone or ethanol. Once the solvent has evaporated, between 10 and 20 adult mosquitoes are aspirated into the bottles and sealed using the lid. Assessments of knockdown or mortality are made at 10 minute intervals. Knockdown or mortality is then plotted against time. Changes in the slope of this graph over time are indicative of changes in the susceptibility of the mosquito population.

An appropriate diagnostic dose should be calculated at the start of the monitoring programme using an insecticide rate range study. To guide this, the following doses are suggested: cyano-pyrethroids, e.g. deltamethrin = 25 µg/bottle and non-cyano-pyrethroids, e.g. permethrin = 43 µg/bottle.

For further details on this method see: [http://www.cdc.gov/parasites/education_training/lab/bottlebioassay.html](http://www.cdc.gov/parasites/education_training/lab/bottlebioassay.html). The CDC will furnish, at no cost, premeasured amounts of WHOPES approved IRS and LLIN insecticides, sufficient to conduct approximately 100 bottle assays for each insecticide. Recipient is responsible for approval to import these insecticides into their country. Contact Dr. William Brogdon (wgb1@cdc.gov) for additional information or to request shipment of insecticides.
There are some challenges associated with bioassays that may complicate interpretation of the results. For example; when field collected mosquitoes are used, often not enough adults can be found, and those found will be of mixed age and blood fed status. They may also have had prior exposure to insecticides. All of which may effect their susceptibility. If larvae are collected or F1 adults are used, access to a laboratory or insectary is required, and the results may not be fully representative of the local mosquito population.

Test methods based on biochemical or molecular assays are also available for resistance monitoring. These assays detect the presence of a particular resistance mechanism or gene and, for some, are able to identify genotypes (heterozygous or homozygous for resistance). However, specialist equipment is needed for these techniques. While advances in these technologies continue, their potential as true field assays has not yet been fully realized.

Preparation of Enzyme-linked immunosorbent assay (ELISA); a biochemical technique that can detect the presence of malarial antibodies or antigens from human blood sera, and the presence of resistance alleles from mosquitoes.
Simplified diagram indicating possible steps in a resistance monitoring programme

- Field collection (of blood fed adults / larvae)
- Species identification (using keys)
- Rear to F1 generation and/or larvae to adults
- CDC bottle bioassay with synergists
- WHO or CDC susceptibility tests
- Phenotyped samples packaged and couriered to laboratory
- Species complex / Molecular form PCR
- kdr RT-PCR
- Sporozoite rate, indicative of impact of resistance on epidemiology
- Detoxification gene upregulation (Microarray/ SNP detection)

Field, Local laboratory, Laboratory with molecular capability
If Resistance is Suspected:
The first question to ask is, why is resistance suspected? There may be several reasons:
• Decreased susceptibility detected during monitoring
• Complaints from local users
• Disease transmission rates increasing
• Vectors seen in large numbers in treated areas and evidence of breeding

In many cases, control failure might be due to reasons other than insecticide resistance and therefore, the suspicions of resistance must be confirmed using assays. A survey of the area should be made and mosquitoes collected and tested. If resistance is confirmed then the survey should be expanded so that the extent of the problem can be assessed.

If Resistance is Confirmed:
There are several questions to consider before any action is decided:
• How widespread is the resistance?
• Which species are resistant?
• What is the proportion of resistant individuals in the population, and what is its impact on the control programme?
• What is the resistance mechanism(s) involved and the level of resistance in the target species?
• What is the source of insecticide pressure?

See the following flowcharts for more information:
Why do you suspect resistance?
Complaints from population? Disease rates increasing? Large numbers of vectors around after intervention?

Check for Resistance

Resistence indicated? YES

Adulticides: WHO cylinder test or Bottle bioassays. Larvicides: WHO larvicide resistance monitoring kits

Resistance indicated? NO

Don’t Panic! How widespread is resistance? Conduct survey to check. If resistance is not causing failure of product then you may be able to continue using it but monitor any further changes.

Further tests using biochemical or molecular methodologies, where labs are available to do this, can provide detailed information on resistance.

Failure may be due to other factors involving application techniques

Were manufacturers label recommendations followed exactly? e.g.
• Correct dilution rate?
• Correct application rate?
• Correct application equipment?
• Product within date?
• Product stored correctly?
• Was a good quality WHOPES recommended product used?

YES

Go to Page 20 & 21 for details

Re-apply appropriate product at correct rates and monitor effect

NO

Go to Page 22 for details
**Bednets – LN`s**

**Training**
- Have the population been educated how to use and care for their nets correctly?

**Application**
- Are the nets being used?
- Is coverage of house and villages complete?
- Are the nets being washed as recommended? e.g. enough but not too often.
- Do nets need replacing due to age or excessive damage?

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**Indoor Residual Spray**

**Training:**
- Are all staff correctly trained?
- Has the population been instructed in what to do following an IRS application?
- Have spray team been trained in correct wall spraying technique?
- Refer to W.H.O. IRS Manual:  
  http://www.who.int/whopes/equipment/en/

**Equipment:**
- Are the sprayers and nozzles in good condition e.g. able to maintain correct pressure no leaking hoses, unworn fan nozzles.
- Has equipment been calibrated and checked for the correct flow rate?
- Has the correct spray pressure been maintained throughout the spraying?

**Application**
- Was the correct insecticide dose used and thoroughly mixed before spraying?
- Were all houses sprayed or only partial coverage achieved?
- Has the deposit been painted over/cleaned off etc.
- Were applications made to coincide with transmission season?

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The product does not work but bioassays show no resistance - check application techniques
The product does not work but bioassays show no resistance - check application techniques

Space sprays:

*Training:*
  - Have staff been properly trained in space spraying methodology?
  - Refer to Space Spray Application manual: http://www.who.int/whopes/equipment/en/

*Equipment:*
  - Is the equipment properly maintained?
  - Has the machine been calibrated correctly e.g. flow rate, droplet size?

*Application:*
  - Are applications made at the correct volume and dose rate per unit area? (Note: for ULV outdoor spraying the flow rate and vehicle speed must be correct to achieve the required dose/ha)
  - Was application made at the right time of day for insect activity?
  - Was the frequency of spraying correct?
  - Were meteorological conditions correct e.g. wind speed <15kph and inversion characteristics considered if spraying outdoors?
  - Was the area surveyed properly and area calculated to ensure the correct dose rates?

Larvicides:

*Training:*
  - Have staff been fully trained in larvicide surveying and application methodology

*Equipment:*
  - If using liquids, are the sprayers calibrated and flow rates determined relative to the area and volume of water to be treated?
  - If using granules, has the weight/unit area been calculated correctly?

*Application:*
  - Was the water depth considered when calculating the application rate?
  - Is the frequency of application correct? (Regular monitoring of larval breeding sites is essential and re-application when new larvae re-appear).
  - Delayed larval mortality may occur using an IGR - check pupal emergence rate.
If resistance is confirmed:

- Notify WHO & Regional Authorities
- Inform the manufacturer, they can often advise
- Identify likely cause of resistance e.g. has the same insecticide type been used locally for agriculture?
- If the existing class of insecticide is resisted but others are not, switch class (but check first that there is no cross resistance)
- If all adulticide classes are resisted, switch to larviciding where alternative MoA insecticides are available
- Undertake a continuous monitoring program
- Develop remedial programmes in conjunction with National Authorities, WHO and the manufacturer
- Read IRAC Prevention and Management of Insecticide Resistance in Vectors of Public Health Importance, for detailed advice (www.irac-online.org)
Key Considerations in Resistance Management - Summary

• Prevention of resistance is much better than trying to resolve the problem once resistance has developed. Insect susceptibility and effective products are both non-renewable valuable economic resources which should be preserved.

• Resistance management strategies are most effective when developed before control programmes are started.

• It is essential that the delivery of insecticide to the target insect is correct. This includes dose, application timing and technique.

• Insecticidal interventions should be part of a wider integrated vector management programme.

• If resistance occurs take immediate steps to contain it and reduce the selection pressure produced by the insecticide.

• Failure to successfully manage resistance has well documented financial implications and failure to implement an IRM programme on financial grounds is a false economy that will lead to increased costs in the future.

Hypothetical vector control programme cost with or without resistance management
Mosquito eggs are laid on or near water. When the conditions are suitable, the eggs hatch releasing the larvae into the water.

The females require a meal of blood before their eggs can develop, and the cycle starts again. Under optimal conditions a female can lay between 30 and 150 eggs, every 2 – 3 days.

The larvae filter feed on organic matter, returning to the water surface to obtain oxygen.

After a number of larval moult s, the larvae become pupa, from which the adults emerge onto the water surface.
Behavior:

- Eggs laid in rafts on water surface
- Larvae tolerate water with a high organic matter content
- Larvae have siphons and rest at an angle to the water surface
- Adults hold their body parallel to the surface they are resting on
- Adults feed in the evening/at night or early morning when it is dark

*Culex* is a vector of the following diseases:

- West Nile Virus
- Japanese encephalitis
- St. Louis encephalitis
- Western equine encephalitis
- Japanese encephalitis
- Ross River virus
- Murray Valley encephalitis
- Rift Valley fever
- Filariasis
**Behaviour:**

- Lay eggs singly often on dry or damp surfaces. A proportion of the total eggs will hatch each time the area is flooded.
- Larvae are found in containers, leaf axils or tree holes containing water.
- Larvae have a short breathing tube, rest at an angle to the water surface and swim in an s-shaped or vermiform pattern.
- Adults hold their body parallel to the surface they are resting on.
- Adults feed during the daylight hours.

**Aedes is a vector of the following diseases:**

- Yellow Fever
- Dengue Fever
- LaCrosse
- Chikungunya virus
- Filariasis
- St Louis encephalitis
Behaviours:

- Eggs are laid singly on the water surface. Eggs have small floats attached to the sides.
- Larvae require relatively clean water.
- Larvae lack siphon and rest parallel to the water surface.
- Adults rest at an angle to the surface, proboscis and body in the same straight line.
- Adults generally feed during the night or early morning.

*Anopheles* is a vector of the following diseases:

- Malaria
- Filariasis
- O’nyong-nyong fever
- Western equine encephalitis
- Dog heartworm
Further Reading:
Prevention and Management of Insecticide Resistance in Vectors of Public Health Importance. IRAC 2011: www.irac-online.org
WHO Pesticide Evaluation Scheme: www.who.int/whopes/en/
Centers for Disease Control and Prevention: www.cdc.gov

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