Progress on the OECD Consensus Document on the Biology of *Aedes aegypti* (Linn)

Dr. B.K. Tyagi
Centre for Research in Medical Entomology (ICMR)
Madurai 625 002, TN, India
Email: abktyagi@gmail.com
An Introduction to the Expert Workshop for Developing the OECD Consensus Document on the Biology of Mosquito *Aedes aegypti*

(Mexico City, Mexico; May 7 - 9, 2014)
Workshop was attended by:

- **LEADS**: Dr. Andrew Roberts, representing Dr. McClean Morven (CERA, ILSI Research Foundation)
- **CO-LEADS**: Dr. Marcus Vinicius Segurado Coelho and OECD’s Dr. Bertrand Dagalier,
- **THE LOCAL ORGANIZER**: Dr. Sol Ortiz Garci,
- **THE REPRESENTATIVE FROM USA-FDA**: Dr. Brinda Dass
- **PARTICIPANTS**: A total of 19 participants from following countries attended the workshop: Mexico (11), Brazil (2), USA (2), UK (2), France (1) and India (1).
Main objectives of the workshop

1. Share updated information and expertise on the biology of mosquito, *Ae. aegypti*;
2. Identify elements to be included in the future OECD Consensus Document;
3. Review the draft outline of the document and initiate the drafting of some sub-sections; and
4. Agree on the next steps for finalizing a first full draft of the document, to be circulated for comments and examination by the Working Group on Harmonization of Regulatory Oversight in Biotechnology at its next plenary meeting.
Organization Aspects of the Workshop

Day 1

• Introduction to OECD Biosafety Consensus Documents
• Overview presentation on the Draft Operational Plan and the Outline
• General overview on each proposed section of the Draft Outline (20’ – 40’, followed by 15’ of discussion)
Organization Aspects of the Workshop

Day 2

• General overview on each proposed section of the Draft Outline (cont.)

• Future issues relevant to risk assessment

• Development of each sections and sub-sections based on the previous day discussion (sketch of detailed information and experts envolved on this task)
Organization Aspects of the Workshop

Day 3

• Development of each section and sub-section (continuing)

• General Discussion - Revisiting the complete outline proposal, elements drafted and aspects for completion

• Reviewing the Operational Plan – Expert Group report

• Next steps
### Timeline for the preparation of OECD document on Aedes aegypti

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<th>Deadline</th>
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<td>by 30 June 2014</td>
<td>Workshop report circulated to WG-HROB</td>
<td>OECD Secretariat and co-leaders</td>
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<td>by 31 July 2014</td>
<td>Experts nominated to join the Ad hoc expert group for drafting the document. Inputs by new experts</td>
<td>Interested delegations (countries and</td>
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<td>identified (completing the list agreed during the Workshop, as relevant)</td>
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<td>Contact OECD Secretariat and subgroup</td>
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<td>coordinators</td>
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<td>by 15 September 2014</td>
<td>Seven pre-draft sections sent to co-leads</td>
<td>Coordinators and their teams</td>
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<td>by 31 October 2014</td>
<td>Check and technical improvements</td>
<td>Co-leaders, coordinators and experts</td>
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<td>Collation in a single full pre-draft</td>
<td>Co-leaders</td>
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<td>by 30 November 2014</td>
<td>Internal review by the Ad Hoc group of the full pre-draft</td>
<td>Ad Hoc group experts</td>
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<td>by 10 December 2014</td>
<td>Full initial draft sent to OECD Secretariat</td>
<td>Co-leaders</td>
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<td>by 24 December 2014</td>
<td>First draft circulated to WG-HROB</td>
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<td>by 28 February 2015</td>
<td>Comments on the first draft by WG-HROB delegates</td>
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<td>By 6 March 2015</td>
<td>Synthesis documents/comments compendium, prepared as relevant, for circulation to WG-HROB</td>
<td>Co-leaders</td>
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<td>By 6 March 2015</td>
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<td>20-22 April 2015</td>
<td>WG-HROB meeting discuss the first draft with comments received. Agreement on follow-up</td>
<td>WG-HROB plenary meeting</td>
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<td>1.1 Classification and nomenclature of <em>Aedes aegypti</em></td>
<td>Dr. Juan Guillermo Bond Compean, Dr. Ildefonso Fernandez Salas, Dr. Mauricio Casas Martinez, Dr. B.K. Tyagi</td>
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2.2 Life table analysis, under natural and artificial conditions  
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Dr. Mauricio Casas Martinez  
Dr. Maria de Lourdes Munoz Moreno  
Dr. Margareth Lara Capurro | Yet to be circulated |
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| APPENDIX I. REARING, MANAGEMENT OF CONTROL PRACTICES | 1. Current strategies for vector reduction or suppression  
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Dr. Ildefonso Fernandez Salas  
Dr. Hussein Sanchez Arroyo  
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Dr. Margareth Lara Capurro | Yet to be circulated |
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Aedes aegypti (Linnaeus) : Taxonomy, systematics and distribution

Introduction

Aedes aegypti (Linnaeus), popularly known as The Yellow Fever Mosquito, is also the principal vector for the deadly dengue and debilitating chikungunya infections. Originating in Africa, it was most likely brought to the new world on ships used for European exploration and colonization (1).

As the common name suggests, Ae. aegypti is the primary vector of yellow fever, a disease that is prevalent in tropical South America and Africa, and often emerges in temperate regions during summer months. During the Spanish-American War, U.S. troops suffered more casualties from yellow fever and dengue transmitted by Ae. aegypti than from enemy fire (2).

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Dr. Juan Guillermo Bond Compean, Dr. Ildefonso Salas, Dr. Mauricio Casas Martinez, Dr. B.K. Tyagi
Aedes aegypti is naturally competing with Aedes albopictus (Skuse), theian Tiger Mosquito. Since the introduction of the Asian tiger mosquito in merica, by way of Texas, the population of Ae. aegypti in Florida has declined dramatically, but still thrives in urban areas of South Florida.

Aedes albopictus larvae out-compete Ae. aegypti larvae for food, and develop at a faster rate (Barrera, 1996). Some research also suggests there is a hybridization of the two species in zones where they overlap, producing sterile offspring (3)
*Aedes aegypti* is a container-inhabiting mosquito; often breeding in unused flowerpots, phytotelmata of variety, spare tires, untreated swimming pools, and drainage ditches. The mosquito thrives in urbanized areas, in close contact with people making them an exceptionally successful vector.

*Aedes aegypti* are extremely common in areas lacking piped water systems, and depend greatly on stored water for breeding sites. Male and female adults feed on nectar of plants; however, females bloodfeed primarily on humans in order to produce eggs, and are active in the daytime. Eggs have the ability to survive desiccation for long periods of time, allowing eggs to be easily spread to new locations.

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Synonymy

*Culex aegypti* Linnaeus 1762
*Culex excitans* Walker 1848
*Culex taeniatu* Weidemann 1828
*Aedes aegypti* Mattingly, Stone, and Knight 1962

Distribution

*Aedes aegypti* is ubiquitous in distribution prodigiously prevalent in tropical region, and spreads to more temperate regions during the summer months. Originating in Africa, *Ae. aegypti* is now present globally in tropical and sub-tropical regions.
• *Aedes aegypti* is approximately 4 to 7 mm long. It can be distinguished from the Asian Tiger Mosquito with a slight difference in size and thorax patterns.

• *Aedes aegypti* adults have white scales on the dorsal (top) surface of the thorax that form the shape of a violin or lyre, while adult *Ae. albopictus* have a white stripe down the middle of the top of the thorax.

• Each tarsal segment of the hind legs possesses white basal bands, forming what appear to be stripes. The abdomen is generally dark brown to black, but also may possess white scales (4).
• Females are larger than males, and can be distinguished by small palps tipped with silver or white scales. Males have plumose antennae, whereas females have sparse short hairs.
• When viewed under a microscope, male mouthparts are modified for nectar feeding, and female mouthparts are modified for blood feeding.
• The proboscis of both sexes is dark, and the clypeus (segment above the proboscis) has two clusters of white scales. The tip of the abdomen comes to a point, which is characteristic of all *Aedes* specie(5).
**Eggs:**

- After taking a complete blood meal, females produce on average 100 to 200 eggs per batch; however, the number of eggs produced is dependent on the size of the bloodmeal.

- Females can produce up to five batches of eggs during a lifetime.

- A smaller bloodmeal produces fewer egg. Eggs are laid on damp surfaces in areas likely to temporarily flood, such as tree holes and man-made containers, and are laid singly, rather than in a mass. Not all the eggs are laid at once, but can be spread out over hours or days, depending on the availability of suitable substrates.

- Most often, eggs will be placed at varying distances above the water line, and a female will not lay the entire clutch at a single site, but rather spread out the eggs over two or more sites.
• Eggs of *Ae. aegypti* are long, smooth, ovoid shaped, and approximately one millimeter long.

• When first laid, eggs appear white but within minutes turn a shiny black. In warm climates, such as the tropics, eggs may develop in as little as two days, whereas in cooler temperate climates, development can take up to a week.

• *Aedes aegypti* eggs can survive desiccation for months and hatch once submerged in water, making the control of *Ae. aegypti* difficult (2).
Larvae:

• Mosquito larvae are often called "wrigglers" or "wigglers," because they appear to wiggle sporadically in the water when disturbed. Larval Ae. aegypti breathe oxygen through a posteriorly located siphon, which is held above the water surface while the rest of the body hangs vertically. Most Aedes larvae can be distinguished from other genera by the unaided eye by their short siphon.

• Larvae feed on organic particulate matter in the water, such as algae and other microscopic organisms. Most of the larval stage of Ae. aegypti is spent at the water's surface, although they will swim to the bottom of the container if disturbed or when feeding.
• Larvae are often found around the home in puddles, tires, or within any object holding water. Larval development is temperature dependent. The larvae pass through four instars, spending a short amount of time in the first three, and up to three days in the fourth instar. Fourth instar larvae are approximately 8 mm long.

• Males develop faster than females, so males generally pupate earlier. If temperatures are cool, *Ae. aegypti* can remain in the larval stage for months so long as the water supply is sufficient.
Pupae:

• After the fourth instar, Ae. aegypti enter the pupal stage.
• Mosquito pupae are different from many other holometabolous insects in that the pupae are mobile and respond to stimuli.
• Pupae, also called "tumblers," do not feed and take approximately two days to develop.
• Adults emerge by ingesting air to expand the abdomen thus splitting open the pupal case and emerge head first.
Adults:
- *Aedes aegypti* is a holometabolous insect, meaning that it goes through a complete metamorphosis with an egg, larva, pupa, and adult stage.
- The adult life span can range from two weeks to a month depending on environmental conditions.
- *Aedes aegypti* comes in three polytypic forms: domestic, sylvan, and peridomestic. The domestic form breeds in urban habitat, often around or inside houses. The sylvan form is a more rural form, and breeds in tree holes, generally in forests, and the peridomestic form thrives in environmentally modified areas such as coconut groves and farms.
1. CURRENT CONTROL STRATEGIES:

The aims of mosquito control, in general, are to prevent mosquito bites, to maintain mosquito populations at “acceptable” densities, minimize mosquito-host contact, and reduce the longevity of female mosquitoes; primarily, because their nuisance and/or their public health importance, since mosquitoes can be vectors (transmitters) of infectious diseases to humans and animals.

The last situation incorporates the concept of vector and disease control. Vector control is any method to limit or eradicate mosquitoes that transmit disease pathogens. Disease control is the reduction in the incidence, prevalence, morbidity or mortality of an infectious disease to a locally acceptable level or if possible, to elimination or eradication.

*Aedes aegypti* control is generally performed on the context of public health because it is the vector of dengue, chikungunya and yellow fever, and a number of other diseases. Particularly for dengue and chikungunya, there are no vaccines, therapeutic treatments or cure. Preventing or reducing dengue and chikungunya virus transmission depends entirely on control of the mosquito vectors or interruption of human–vector contact. Eradication of *Ae. aegypti* populations may be achievable, but rarely sustainable; therefore, the present aim is to control, to below certain threshold levels, rather than eliminate vector populations.

*Aedes aegypti* control nowadays, largely depends on organized control programs at the community level within ministries of health with some community participation, and some and self-protection measures; and because *Aedes aegypti* lives in close affinity with humans and human-made ecosystems, is an ideal candidate for integrated control (a number of methods to provide control) which are summarized in the following table and briefly described in the following sections.

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CHEMICAL CONTROL

Immature stages: The control of *Aedes aegypti* larvae and pupae can be done treating containers with water (breeding-sites) with insecticides (larvicides); specifically those that are productive and cannot otherwise be eliminated or managed. Larval control targets the immature mosquitoes living in water before they become biting adults. Larvicides approved by WHOPES are: *Bacillus thuringiensis* israelensis, strain AM65-52, Diflubenzuron, Novaluron Pyriproxyfen, Fenthion, Pirimiphos-methyl, Temephos and Spinosad. The application of larvicides can be done by ground or aerial treatments.

Adult: The control of adult vectors with insecticides (adulticides), applied either as residual surface treatments or as space treatments (thermal fogging and ultra low volume aerosol sprays), is expected to impact on mosquito densities, longevity and other transmission parameters. MoH does most adult control and with insecticides – from three chemical groups: pyrethroids, organophosphates and carbamates – recommended by WHOPES both for indoor and outdoor spraying or those accepted by local authorities in each country. The application of adulticides can be done by ground or aerial treatments.

Indoor residual spraying (IRS) involves the spraying of an insecticide on all the walls and ceilings inside the house. This is usually done only once or twice a year because its effect should last, so that it continues to kill mosquitoes for many months after treatment.

Indoor Space-Spraying (ISS) when the fog is delivered inside houses, and should leave insecticide residue on the surfaces inside the home, thus providing some sustained effect against adult mosquitoes in the days or weeks following treatment.

Outdoor fogging is the method commonly used in many parts of the world: the insecticide is sprayed usually from vehicles as a cloud of ‘fog’ outside houses and targeting the flying female mosquitoes. Vector populations can be suppressed over large areas by the use of space sprays released from low-flying aircraft, especially where access with ground equipment is difficult and extensive areas must be treated rapidly.

http://www.who.int/whopes/Insecticides_IRS_Malaria_09.pdf
**Personal protection:** *Aedes aegypti* exposure can be avoided with chemical products such as domestic insecticides, repellents (natural or synthetic), insecticide treated materials and paints.

Insecticide aerosol spray is a major product the public has been using. In general, pyrethroids are the main active ingredients in these household aerosol products. Where indoor biting occurs, household insecticide aerosol products, mosquito coils or other insecticide vaporizers may also reduce biting activity³.

Numerous insect repellent products are available commercially in a variety of formulations. Some of these products contain active ingredient(s) that are botanical and some are synthetic organic products, with a vast majority available as sprays. Repellents may be applied to exposed skin or to clothing. Repellents recommended contain DEET (N, N-diethyl- 3-methylbenzamide), IR3535 (3-[N-acetyl-N-
butyl]-aminopropionic acid ethyl ester) or Icaridin (1-piperidinecarboxylic acid, 2-(2-hydroxyethyl)-1-methylpropylester)³.

Long-lasting insecticidal netting (LLIS) is factory-produced mosquito netting pre-loaded with synthetic pyrethroid insecticide that is intended to retain its biological activity for at least 20 standard washes under laboratory conditions and 3 years of recommended use under field conditions. Deployed as bednets, LLIS potentially can impact by reducing human biting rates, vector longevity, at both household and community levels. Encouraging results have also been shown when LLIS are deployed as window or door screens, curtains or as container covers for *Aedes aegypti* control in Latin America.,,

**BIOLOGICAL CONTROL**

Biological control is based on the introduction of organisms that prey upon, parasitize, compete with or otherwise reduce populations of the target species. Biological control has been used for larval control of *Aedes aegypti*; and only certain species of larvivorous fish i.e *Poecilia reticulata* and predatory copepods (Copepoda: Cyclopoidea) have proved effective in operational contexts in specific container habitats, and even then seldom on a large scale.


ENVIRONMENTAL MANAGEMENT

Environmental management seeks to change the environment in order to prevent or minimize vector propagation and human contact with the vector-pathogen by destroying, altering, removing or recycling non-essential containers that provide larval habitats. Such actions should be the mainstay of dengue vector control. Three types of environmental management are defined,


Pan American Health Organization (1994) *Dengue and dengue hemorrhagic fever in the Americas: guidelines for prevention and control*. PAHO (Scientific publication no. 548), Washington DC,


Environmental modification – long-lasting physical transformations to reduce vector larval habitats, such as installation of reliable piped water supply to communities, including household connections.

Environmental manipulation – temporary changes to vector habitats involving the management of “essential” containers, such as frequent emptying and cleaning by scrubbing of water-storage vessels, flower vases and desert room coolers; cleaning of gutters; sheltering stored tires from rainfall; recycling or proper disposal of discarded containers and tires; management or removal from the vicinity of homes of plants such as ornamental or wild bromeliads that collect water in the leaf axils.

Changes to human habitation or behavior – actions to reduce human–vector contact, such as installing mosquito screening on windows, doors and other entry points, and using mosquito nets while sleeping during daytime.
1.1 Integrated control management (WHO IVM framework)

Integrated vector management (IVM) is the strategic approach to vector control promoted by WHO and includes control of the vectors of dengue. Defined as “a rational decision-making process for the optimal use of resources for vector control”, IVM considers five key elements in the management process, namely:

- **Advocacy, social mobilization and legislation** – the promotion of these principles in development policies of all relevant agencies, organizations and civil society; the establishment or strengthening of regulatory and legislative controls for public health; and the empowerment of communities;

- **Collaboration within the health sector and with other sectors** – the consideration of all options for collaboration within and between public and private sectors; planning and decision-making delegated to the lowest possible administrative level; and strengthening communication among policy-makers, managers of programmes for the control of vector-borne diseases, and other key partners;

- **Integrated approach to disease control** – ensuring the rational use of available resources through the application of a multi-disease control approach; integration of non-chemical and chemical vector control methods; and integration with other disease control measures;

- **Evidence-based decision-making** – adaptation of strategies and interventions to local vector ecology, epidemiology and resources, guided by operational research and subject to routine monitoring and evaluation;

- **Capacity-building** – the development of essential infrastructure, financial resources and adequate human resources at national and local levels to manage IVM programmes, based on a situation analysis.

Pan American Health Organization (1994) *Dengue and dengue hemorrhagic fever in the Americas: guidelines for prevention and control.* PAHO (Scientific publication no. 548), Washington DC,


1.2 Prevention and management of insecticide resistance

The evolution and spread of resistance to insecticides is a major concern for the control of the dengue vector *Aedes aegypti*. The reliance by most dengue control programs on just two (pyrethroids and organophosphates) classes of insecticide available for use in public health, poses additional selection pressure on the mosquito vectors.

Alterations in the molecular target sites of insecticides, which reduce the binding of insecticides, are the most understood resistance mechanisms. Several mutations in the sodium channel, the target site of DDT and pyrethroid insecticides, have been reported in *Ae. aegypti*. Two alternative substitutions at one of the polymorphic sites, residue 1016, have been linked to pyrethroid resistance and recently, methodologies to detect these mutations (often referred to as kdr mutations) in individual mosquitoes have been reported.

Resistance management strategies generally recommend the rotation of chemicals with different modes of action and the use of non-chemical methods of control. The implicit assumption is that resistance to a chemical will disappear from a population once the selection pressure is removed.


Thank You