IAEA
International Atomic Energy Agency
Atoms for Peace

Meeting

Thematic Plan for the Development and Application of the Sterile Insect Technique (SIT) and Related Genetic and Biological Control Methods for Disease Transmitting Mosquitoes

INT0089 – Developing Human Resources and Supporting Nuclear Technology

16th-20th June 2014, IAEA, Vienna

INDEX

EXECUTIVE SUMMARY

Under IAEA TC project INT0089 - Developing Human Resources and Supporting Nuclear Technology, an expert meeting was convened to develop a "Thematic Plan for the Development and Application of the Sterile Insect Technique (SIT) and Related Genetic and Biological Control Methods for Disease Transmitting Mosquitoes". This meeting was held at the Vienna International Center at IAEA Headquarters from 16 to 20 June 2014 with the participation of the World Health Organization (WHO) and other experts.

The objective was to review the state of mosquito management (with an emphasis on malaria, dengue, chikungunya and yellow fever) and to provide guidance on the opportunities and research gaps in fields related to the SIT and other potential tactics and strategies to control vector-borne diseases, including policy issues.

The burden of mosquito transmitted diseases remains enormous, with the incidence of dengue and other diseases growing dramatically around the world in recent decades, while the effectiveness of chemical vector control is decreasing as mosquitoes develop insecticide resistance. Therefore, the experts agreed that there is an urgent need for innovative mosquito control methods. The experts identified several technologies, including the SIT, which could be complementary to the commonly used control tactics for malaria, dengue, chikungunya and yellow fever.

During the meeting current control methods and recent developments in SIT for vector-borne disease control strategies were reviewed for application in an Area-Wide Integrated Vector Management (AW-IVM) approach. Special consideration was given to the advantages, disadvantages, constraints, gaps and challenges of implementing AW-IVM approaches.

Research and development needs were identified, including requirements for developing and fine-tuning components of the SIT and other genetic control methods. Subsequently, scientific evidence on the feasibility of AW-IVM can then be used for policy making, planning and implementation. AW-IVM approaches, which can include the SIT or other genetic methods, were encouraged as these are not only environmentally friendly and more sustainable, but are aligned with the IAEA and WHO policies and goals.

The discussions during this meeting resulted in production of this Thematic Plan, a comprehensive document, which states: 1) the magnitude of the problem of mosquito-borne diseases; 2) general trends of application of control tactics addressing specific methods for *Anopheles* and *Aedes* species; 3) R&D needs to further develop the SIT and other strategies for vector control in an AW-IVM approach; 4) recent developments within the IAEA and ongoing projects and collaborations; 5) identification of knowledge gaps and potential future role of the IAEA and the Joint FAO/IAEA Division; and 6) recommendations for policy makers with respect to planning and implementation. Based on this review a number of recommendations were proposed.

General recommendations to the IAEA

- To invest in supporting the control of the mosquito species which transmit malaria, dengue, chikungunya and yellow fever through continued funding of the further development of the SIT and other related genetic and environment-friendly methods. Control projects should be developed and applied following an Area-Wide Integrated Vector Management approach.
- To continue the assistance in developing and implementing effective interventions using SIT and other related species-specific technologies.
- To continue R&D and technology transfer activities related to the SIT package.
- To continue the support of R&D on transgenic and symbiont-based approaches, this can be useful in the control of mosquito-borne diseases as well as to exploit their complementary potential with classical SIT.
- To continue developing the SIT package for mosquito management at the Insect Pest Control Laboratory (IPCL) of the FAO/IAEA Agriculture and Biotechnology Laboratories e.g. mass-rearing technology, sex-separation and sterilization methods that should be further refined and disseminated to Member States. However, to accomplish the aforementioned in an effective and timely manner, and to provide adequate technical support to technology transfer under technical cooperation projects, funding support for facilities and personnel must be enhanced significantly. Current personnel (one professional and one technician) are clearly insufficient to address the increasing demand and future needs related to mosquito control.
- To develop efficient, environment-friendly and economically affordable irradiation-induced sterility methods for SIT.
- To continue providing technology transfer and capacity building support to Member States for the management of mosquitoes using an AW-IVM approach with an SIT component.
- To support dissemination and outreach activities, including novel IT platforms, as a way to expand AW-IVM projects with an SIT component against mosquito species, and facilitate their transfer to Member States.
- To continue to seek strategic partnerships and funds mobilization to support AW-IVM approaches with an SIT component in cooperation with Member States.

Pilot Projects

- To gradually scale up the AW-IVM approach and further develop the SIT components, pilot projects should be supported in Member States.
- To develop an inter-regional project on dengue and chikungunya and regional projects on malaria and dengue especially in Africa.
- To support specific national projects that aim at integrating the SIT and other related approaches, including technical advice to establish mass rearing facilities, sterilization methods and related technologies.

Translation into Policy

- To incorporate the AW-IVM approach into public health policies within a holistic approach. The existing policy setting mechanism within WHO should be used to review the evidence and to make initial recommendations for their use by Member States.
- To establish a MoU or Practical Arrangement between WHO and IAEA to facilitate harmonization and alignment of joint activities.
- To continue providing technical and policy advice on existing or any new technology towards the control of mosquito populations.

STATEMENT OF THE PROBLEM

Malaria

Key Facts about Malaria

- Malaria transmission depends on *Anopheles* mosquitoes.
- No effective malaria vaccine exists in spite of years of development.
- Mortality and morbidity due to malaria are highest in sub-Saharan Africa but the disease is found globally in the tropics and sub-tropics.
- Vector interventions have been the most effective means of reducing transmission.
- Malaria can usually be effectively treated with prompt diagnosis and appropriate drugs.
- Vector control effectiveness is threatened by the development of insecticide resistance and the dwindling number of useful compounds.
- Many countries that suffer the highest burden of disease are also those that are least able to diagnose and treat the disease and provide to reliable disease reports.
- Past interventions have effectively reduced malaria but are dependent upon sustained national and donor support.

Disease mortality and morbidity

According to WHO, in 2012 there were approximately 207 million cases of malaria and an estimated 627,000 deaths (World Malaria Report, 2013). While malaria is generally a tropical and sub-tropical disease, the disease burden is heavily concentrated in sub-Saharan Africa where about 90% of malaria deaths occur. The two countries with the highest burden, the Democratic Republic of the Congo and Nigeria, account for approximately 40% of malaria deaths.

Young children, pregnant women, those living with HIV or affected by humanitarian emergencies or natural disasters are the most vulnerable to the disease. Furthermore, non-immune travellers entering endemic areas are also at risk. The availability of health care for proper diagnosis and treatment strongly affects disease outcomes since malaria is usually treatable when diagnosed and treated correctly. Consequently, those living in communities that are located in remote rural areas with limited health facilities suffer the most.

Increased prevention and control measures have been effective in reducing the malaria burden in many places. Since 2000, malaria mortality rates have fallen by 42% globally and by 49% in the WHO defined African Region. Eleven of the 97 countries with ongoing malaria transmission are classified as being in pre-elimination phase, and eight countries in the elimination phase (WHO, World Malaria Report 2013). Malaria elimination is used in this context to mean the permanent interruption of local mosquito-borne malaria transmission in a defined geographical area. An additional seven countries are in the "prevention of re-introduction" phase. In spite of this progress, malaria transmission still places about 3.4 billion people at risk.

Transmission settings and dynamics

Five *Plasmodium* species cause malaria in humans, with *P. falciparum* and *P. vivax* being the two most common. Of all malaria species, *P. falciparum* is the most dangerous and it is

associated with the highest rates of case complications and mortality. This form of malaria is common in most countries in sub-Saharan Africa.

Human malaria is transmitted when the sporozoite stage of *Plasmodium* parasites are present in the saliva of biting female *Anopheles* mosquitoes. Sixty species of *Anopheles* are known to transmit malaria, and each has its own unique biological and ecological characteristics. However, *Anopheles* adults are generally active between dusk and dawn which is when they seek blood, mates, sugar and oviposition sites. Before dawn, they must find resting sites in shady, cool humid places where they remain during the day. No mosquito species beside *Anopheles* can transmit human *Plasmodium* parasites, and there are no animal reservoirs of the major *Plasmodia*, so transmission is dependent upon the interactions of mosquitoes and humans. This specific relationship produces certain vulnerabilities that might provide opportunities to interrupt transmission.

In order for parasites to develop from gametocytes to sporozoites in the mosquito and to infect another person, adult females must survive long enough for the parasites to complete development and find a susceptible host. Therefore, parasitic transmission is generally more intense when mosquitoes have a long lifespan and hosts are accessible e.g. sleeping without bednets or outdoors in the evening. Host preference is also a strong determinant of vector capacity. For example, differences in strength of preference for biting humans between member species of the *An. gambiae s.l.* and *An. funestus s.l.* species complexes mean that only some members of these complexes are responsible for transmitting malaria in Africa. The morphological similarity of species complex members confounds determination of whether efficient vectors are present and complicates efforts to control disease by elimination of only one member of the complex.

Distribution of malaria

As shown in Figure 1, Malaria is distributed throughout the humid tropics and subtropics. The quantitative estimates of disease burden developed by WHO are admittedly uncertain due to the inadequacy of diagnosis and reporting mechanisms available in places where the public health infrastructure is the weakest; often these are the countries with the highest burden of disease.

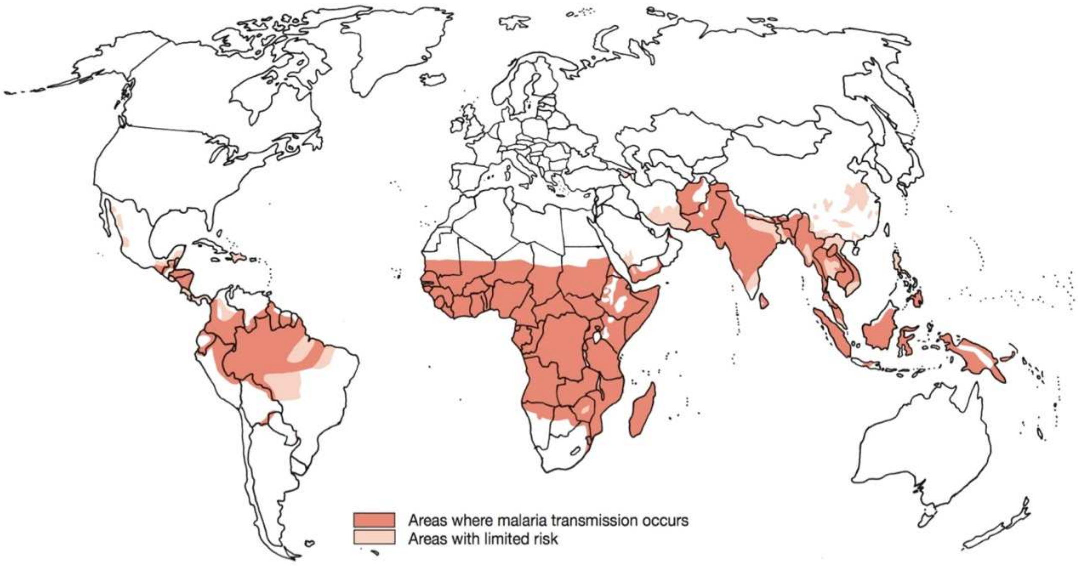


Figure 1. Global distribution of malaria. (Source: Int. Assoc. for Med. Assist. to Travellers. http://www.iamat.org/pdf/world_malaria_risk_chart.pdf)

Distribution of malaria disease vectors

The basic ecology of malaria vector species determines both their seasonality and geographical distribution. Detailed maps of the distribution of major vectors are desirable, but due to the lack of sensitive surveillance systems, information about their occurrence is even more limited than for the disease. Attempts have been made to use models to predict the global distribution of the dominant malaria vector species. Figure 2 is such an attempt, highlighting the diversity of the malaria vector species and their distribution on a global scale. The identification of potential habitats of the *Anopheles gambiae* and *funestus* complexes is of particular importance in the context of African malaria and the preponderance of mortality and morbidity as these species are responsible for most transmission in that region. As shown in Figure 2, in some areas a single species is responsible for transmission, whereas in others, a number of species contribute. The former case provides particularly attractive targets for genetic control efforts that focus on only one species at a time.

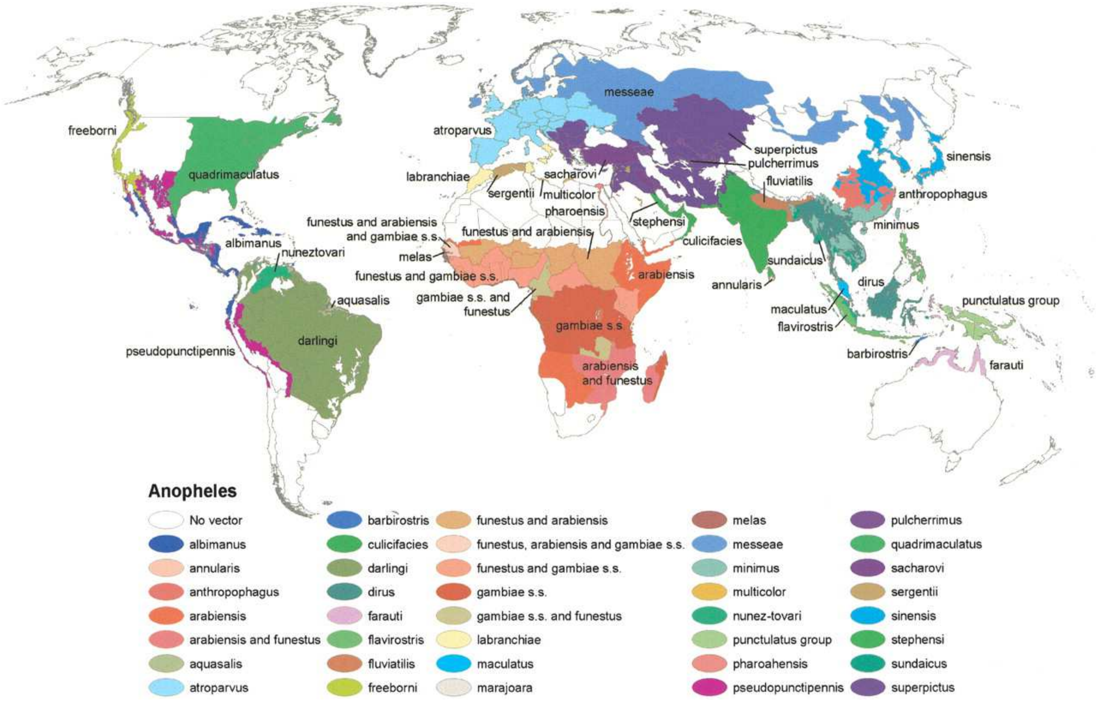


Figure 2. Distribution of major malaria vector mosquitoes worldwide. (Source: Kiszewski, A., Mellinger, A., Spielman, A., 2004. A global index representing the stability of malaria transmission. The American journal of Tropical Medicine and Hygiene 70, 486–498.)

Significant progress in controlling these vectors could be made should the technology for detecting them be enhanced. Current species distribution maps could be improved by overlaying vector behaviour data (biting preference, time and location) and vector capacity, both of which are critical determinants of malaria transmission and choices of effective control. However, a significant investment is required to develop the necessary capacity to obtain such detailed information and therefore it is unlikely that it will be developed in the near future. A range of factors determine whether malaria transmission is stable or unstable (Table 1) which are dependent on the specific location and combination of mosquito species and *Plasmodium* strain.

Table 1. The extremes of malaria transmission dynamics: stable vs. unstable. Each extreme is distinguished by parameters including the most common parasites, level of human immunity and seasonality.

	Stable malaria transmission	**Unstable malaria transmission**
Immunity in adults	High	Low
Clinical manifestations	Primarily in young children and pregnant women	Affecting all ages
Local mosquito species	Efficient vectors	Inefficient vectors
Main parasite	*Plasmodium falciparum*	Usually *Plasmodium vivax*
Climate	Favourable for rapid	Not favourable for rapid development in mosquito

	development in mosquito	
Level of transmission	Moderate to very high	Low (high when epidemic)
Seasonal changes in incidence	Not very pronounced - possibly short dry season	Pronounced
Fluctuations in incidence	Not marked - related to seasons	Very marked
Epidemics	Unlikely in the indigenous population	Likely when climatic conditions suitable

(Source: http://malaria.wellcome.ac.uk/doc_WTDO23873.html)

Dengue

Key facts about dengue

- Dengue is a mosquito-borne viral infection.
- The infection causes flu-like illness, and occasionally develops into a potentially lethal complication called severe dengue.
- The global incidence of dengue has grown dramatically in recent decades.
- About half of the world's population is now at risk.
- Dengue is found in tropical and sub-tropical climates worldwide, mostly in urban and semi-urban areas.
- Severe dengue is a leading cause of serious illness and death among children in some Asian and Latin American countries and for people with underlying conditions and comorbidities.
- There is no specific treatment for dengue or severe dengue, but early detection and access to proper medical care lowers fatality rates to below 1%.
- Dengue prevention and control solely depends on effective vector control measures.

The Global Burden of Dengue

The incidence of dengue has grown dramatically around the world in recent decades. As shown in Figure 3, over 3 billion people – 40% of the world's population – are now at risk from contracting dengue. At present, WHO predicts that there are around 390 million dengue infections worldwide. An estimated 500,000 people with severe dengue require hospitalization each year, a large proportion of which are children. About 2.5% of the affected people die.

Before 1970, only nine countries had experienced severe dengue epidemics. The disease is now endemic in more than 100 countries in Africa, the Americas, the Eastern Mediterranean, South-east Asia and the Western Pacific. The American, South-east Asia and the Western Pacific regions are the most seriously affected.

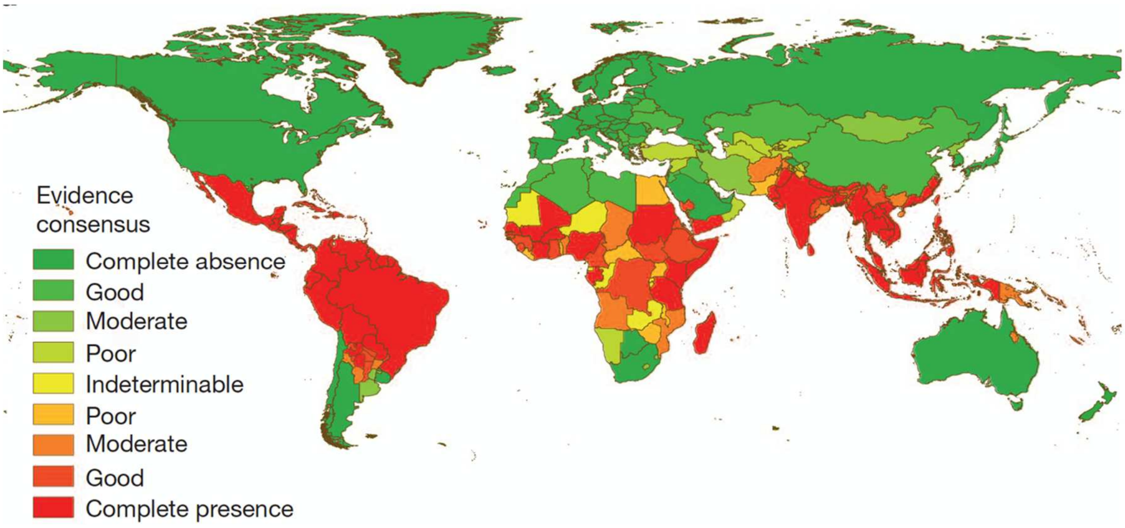


Figure 3. Global evidence consensus, risk and burden of dengue in 2010. National and subnational evidence consensus on complete absence (green) through to complete presence (red) of dengue. (Source: Bhatt, S. *et al*. 2013. The global distribution and burden of dengue. Nature, 496:504-507.)

Not only is the number of cases increasing as the disease spreads to new areas, but previously unseen explosive outbreaks are occurring. The threat of an outbreak of dengue now exists in Europe. Local transmission of dengue was reported for the first time in France and Croatia in 2010 and imported cases were detected in three other European countries. In 2012, an outbreak of dengue on Madeira Islands of Portugal resulted in over 2000 cases and imported cases were detected in 10 other European countries apart from mainland Portugal. Also of particular concern, in 2013, cases occurred in Florida (USA) and Yunnan province (China), and dengue continues to affect several North- and Central-American countries, notably Honduras, Costa Rica and Mexico. In Asia, Singapore has reported an increase in cases after a lapse of several years and outbreaks have also been reported in Laos. In 2014, the Cook Islands, Malaysia, Fiji and Vanuatu experienced an increase in the number of dengue type 3 (DENV3), which affected these Pacific Island countries again after an absence of over 10 years.

<u>Transmission</u>

Aedes aegypti, the "yellow fever mosquito," is the primary vector of dengue. The virus is transmitted to humans through the bites of female *Aedes* mosquitoes who have previously bitten a dengue patient and become infected. After an incubation period of 4–10 days, an infected mosquito is capable of transmitting the virus for the rest of its life. Patients who are already infected with the dengue virus can transmit the infection for an average of 4–5 days and a maximum of 12 after their first symptoms appear.

Aedes aegypti mosquitoes live in urban habitats and larvae develop mostly in man-made containers. Unlike other mosquitoes, *Ae. aegypti* is a daytime feeder: its peak biting periods are early in the morning and in the evening before dusk. This means that personal protection measures such as insecticide treated bed nets (ITNs) are ineffective in preventing dengue transmission. Female *Ae. aegypti* can bite multiple people during each feeding period.

Aedes albopictus, the "Asian tiger mosquito," is the secondary dengue vector in Asia. It has spread to America and Europe largely through the international trade of used tyres, a habitat in which larva and dry eggs can survive, and other goods, e.g. lucky bamboo. *Ae. albopictus* is highly adaptive, and diapausing forms can survive in cooler, temperate regions, for example in Europe. Its invasiveness is due also to its tolerance to temperatures below freezing, hibernation behaviour, and the ability to shelter in microhabitats.

Chikungunya

Key facts about chikungunya

- Chikungunya is a viral disease transmitted to humans by infected mosquitoes. It causes fever and severe joint pain. Other symptoms include muscle pain, headache, nausea, fatigue and rash.
- The disease shares some clinical signs with dengue, and can be misdiagnosed in areas where dengue is common.
- There is no cure for the disease. Treatment is focused on relieving the symptoms.
- The proximity of mosquito breeding sites to human habitation is a significant risk factor for chikungunya.
- Since 2004, chikungunya fever has reached epidemic proportions, and causes considerable painful morbidity.
- The disease occurs in Africa, Asia and the Indian subcontinent. In recent decades mosquito vectors of chikungunya have spread to Europe and the Americas. In 2007, disease transmission was reported for the first time in a localized outbreak in north-eastern Italy.

<u>Burden, disease outbreaks and transmission</u>

As shown in Figure 4, chikungunya occurs in Africa, Asia, the Indian subcontinent, and to a lesser extent in the Americas. In recent decades, there have been outbreaks of the disease in countries that have never recorded cases before

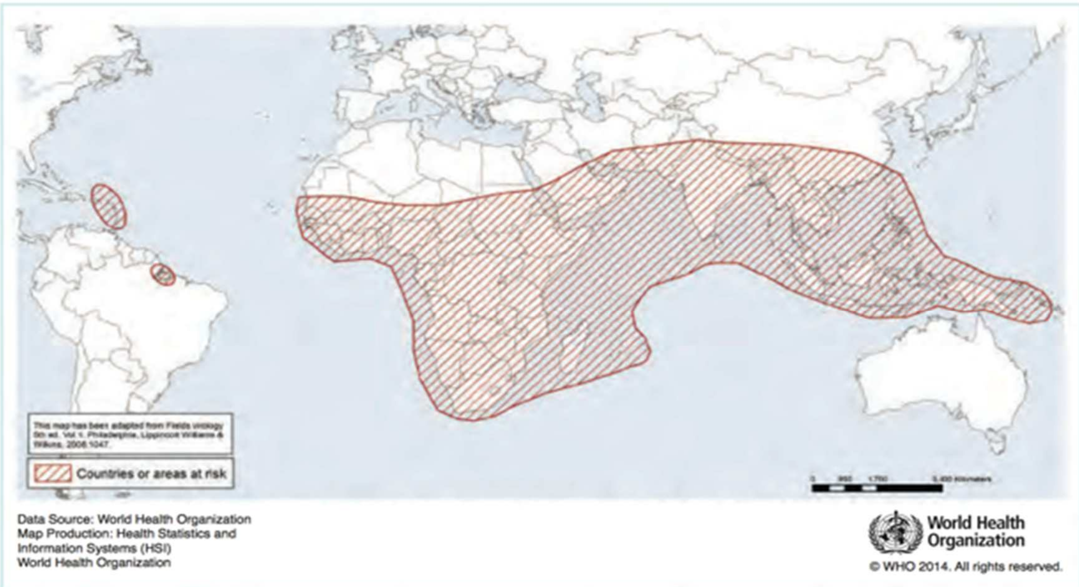
In 2005–2006, a major outbreak in Réunion Island, a French territory in the Indian Ocean, affected 266,000 of the 775,000 inhabitants, around one-third of the population. The outbreak spread to several countries in the WHO South-East Asia Region, including India, where 1,400,000 cases were reported in 2006 when the epidemic was at its peak. Since 2005, India, Indonesia, Thailand, Maldives and Myanmar have reported over 1.9 million cases. In Asia and the Indian Ocean Region, the main vectors of chikungunya are *Ae. albopictus* and *Ae. aegypti*.

In 2007, disease transmission was reported for the first time in Europe with a localized outbreak in north-eastern Italy, which confirmed that mosquito-borne outbreaks by *Ae. albopictus* are plausible in Europe. There were 197 cases recorded. In comparison, a large number of imported cases in Europe were associated with the Indian Ocean outbreak of 2006

In the WHO Region of the Americas, the first cases of local transmission of chikungunya were detected in Saint Martin. In December 2013, France reported two laboratory-confirmed autochthonous (native) cases of chikungunya in the French part of the Caribbean island. Since then, local transmission has been confirmed in the Dutch part of Saint Martin

[St. Maarten], Anguilla, British Virgin Islands, Dominica, French Guiana, Guadeloupe, Martinique and St. Barthelemy. Aruba reported only imported cases. This is the first documented outbreak of chikungunya with autochthonous transmission in the Americas but since then transmission has spread to affect Dominican Republic and Haiti, where the attack index is high (30-50%). There are many imported cases reported in Venezuela and Brazil, and more than 48,000 cases have been reported in Latin America and the Caribbean since 2013 (CAPHA, 2014).

In Africa, a larger range of *Aedes* species transmit the virus as do *Culex annulirostris, Mansonia uniformis* and some *Anopheles* species. In this region, human infections have been at relatively low levels for a number of years, however in 1999-2000 there was a large outbreak in the Democratic Republic of Congo and in 2007 another outbreak affected Gabon.



WHO Map from International Travel and Health showing chikungunya, countries or areas at risk [11]

Figure 4. Global risk of chikungunya (Source: A Global Brief on Vector Borne Diseases, WHO, http://apps.who.int/iris/bitstream/10665/111008/1/WHO_DCO_WHD_2014.1_eng.pdf)

Additional information about arbovirus vectors

Both *Ae. aegypti* and *Ae. albopictus* have been implicated in large outbreaks of chikungunya. Whereas *Ae. aegypti* is confined to the tropics and sub-tropics, *Ae. albopictus* also occurs in

temperate and even cold temperate regions. In recent decades, *Ae. albopictus* has spread from Asia and become established in areas of Africa, Europe and the Americas.

Ae. albopictus thrives in a wider range of water-filled larval sites than *Ae. aegypti*, including coconut husks, cocoa pods, bamboo stumps, tree holes and rock pools, in addition to artificial containers such as vehicle tyres and saucers beneath plant pots. This diversity of habitats explains the abundance of *Ae. albopictus* in rural as well as peri-urban areas and shady city parks.

Ae. aegypti is more closely associated with human habitation and uses indoor breeding sites, including flower vases, water storage vessels and concrete water tanks in bathrooms, as well as the same artificial outdoor habitats as *Ae. albopictus*.

In Africa several other mosquito vectors have been implicated in disease transmission, including species of the *Ae. furcifer-taylori* group and *Ae. luteocephalus*. There is evidence that some animals, including non-primates, rodents, birds and small mammals may act as reservoirs.

Yellow Fever

Key facts about yellow fever

- Yellow fever is an acute viral haemorrhagic disease transmitted by infected mosquitoes. The "yellow" in the name refers to the jaundice that affects some patients.
- Up to 50% of severely affected persons will die from yellow fever without treatment.
- There are an estimated 200,000 cases of yellow fever, causing 30,000 deaths, worldwide each year, with 90% of those occurring in Africa.
- The virus is endemic in tropical areas of Africa and Latin America, which have a combined population of over 900 million people.
- The number of yellow fever cases has increased over the past two decades due to declining population immunity to infection, deforestation, urbanization, population movements and climate change.
- There is no specific curative treatment for yellow fever. Treatment aims to reduce the symptoms for the comfort of the patient.
- Vaccination is the most important preventive measure against yellow fever. The vaccine is safe, affordable and highly effective, and a single dose of yellow fever vaccine is sufficient to confer sustained immunity and life-long protection against yellow fever disease without the need for a booster dose. The vaccine provides effective immunity within 30 days for 99% of persons vaccinated.

<u>Transmission</u>

The yellow fever virus is an arbovirus of the *flavivirus* genus. The mosquito is the primary vector, carrying the virus from one host to another, primarily between monkeys, from monkeys to humans, and from person to person. There are three types of transmission cycle.

Sylvatic (or jungle) yellow fever: In tropical rainforests, monkeys are infected with yellow fever by mosquitoes that breed in the wild, and in turn infect further mosquitoes, which may bite humans entering the forest, resulting in occasional cases of yellow fever. The majority of infections occur in young men working in the forest (e.g. for logging).

Intermediate yellow fever: In humid or semi-humid parts of Africa, small-scale epidemics occur when semi-domestic mosquitoes, breeding in the wild and around households, infect both monkeys and humans. Increased contact between people and infected mosquitoes leads to transmission, often in multiple villages in an area simultaneously. This is the most common type of outbreak in Africa, and can become a more severe epidemic if the infection is carried into an area populated with both domestic mosquitoes and unvaccinated people.

Urban yellow fever: Large epidemics occur when infected people introduce the virus into densely populated areas with a high number of non-immune people and *Aedes* mosquitoes. Infected mosquitoes transmit the virus from person to person.

Several different species of *Aedes* and *Haemogogus* mosquitoes transmit the virus, breeding either around houses (domestic), in the jungle (wild) or in both habitats (semi-domestic).

Populations at risk

In forty-four countries in Africa and Latin America, with a combined population of over 900 million, yellow fever is endemic. In Africa alone, an estimated 508 million people are at risk of contracting the disease. The remaining populations at risk are in 13 countries in Latin America, especially Bolivia, Brazil, Colombia, Ecuador and Peru. Figures 5 and 6 show the major areas at risk from yellow fever in Africa and South America, respectively.

According to WHO estimates from the early 1990s, 200,000 cases of yellow fever - and 30,000 deaths - are expected globally each year, of which 90% will occur in Africa. A recent analysis of African data sources, due to be published later this year, estimates similar figures, but with a slightly lower burden of yellow fever in Africa for the year 2013 (84,000–170,000 severe cases and 29,000–60,000 deaths).

Small numbers of imported cases occur in countries otherwise free of yellow fever. Although the disease has never been reported in Asia, the region is at risk because the conditions required for transmission are present locally, and prior to the 19th Century outbreaks of yellow fever were reported in North America (including Charleston, New Orleans, New York and Philadelphia) and Europe (England, France, Ireland, Italy, Portugal and Spain).

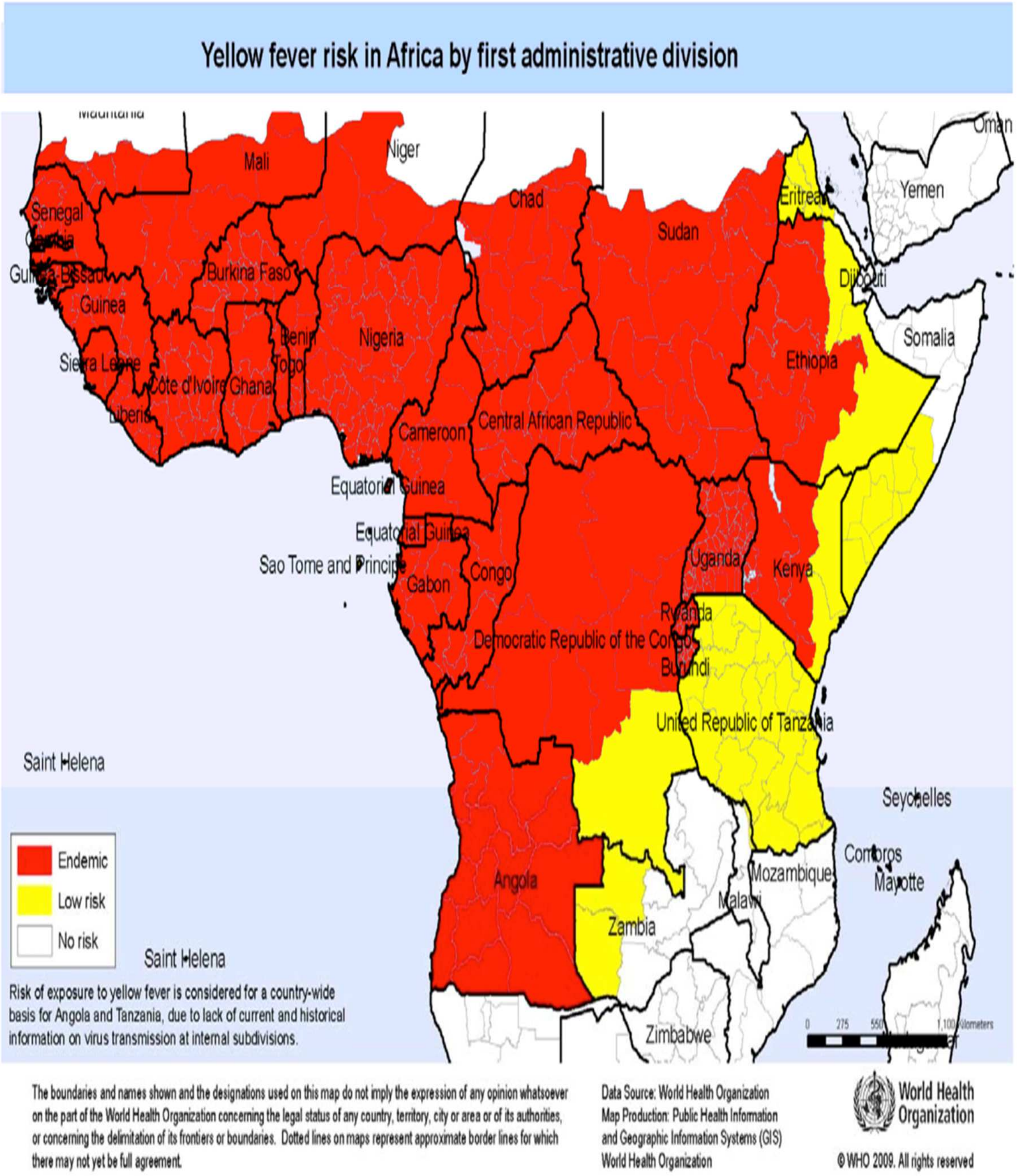


Figure 5. Areas at-risk of yellow fever in Africa. (Source: Background for the Consultation on Yellow Fever and International Travel, 2010: Informal Working Group on Geographic Risk of Yellow Fever (WG), a subgroup formed from the World Health Organization (WHO) Consultation on Yellow Fever (YF) and International Travel)

Figure 6. Areas at-risk of yellow fever in South America (Source: Background for the Consultation on Yellow Fever and International Travel, 2010: Informal Working Group on Geographic Risk of Yellow Fever (WG), a subgroup formed from the World Health Organization (WHO) Consultation on Yellow Fever (YF) and International Travel)

EXISTING MOSQUITO CONTROL PRACTICES

Current control methods for malaria vectors

The goals of malaria vector interventions are two-fold: to provide personal protection by reducing human-vector contact (usually through Long Lasting Insecticidal Nets - LLIN) and to lower the intensity of malaria transmission at the community level by reducing the average lifespan of the local mosquito population (through indoor residual spraying, IRS).

Bed nets

Bed nets can be divided into conventional nets treated with an insecticide, and LLIN. They are a core intervention, effective on the individual level, protecting the person sleeping under the net and on the community level, as the insecticide's effect is felt over a larger area. WHO recommends universal coverage of at-risk populations with ITNs (Insecticide-Treated Nets), and urges adoption of LLINs to replace less durable nets. We will therefore use the term LLIN to refer to all bednets in this document.

Advantages:

- Barrier provided against human-vector contact.
- Increased vector mortality and reduced transmission.
- Numerous designs available to suit different house structures.

Disadvantages:

- Nets can only be treated with pyrethroids, so potential vector resistance to these insecticides reduces ITNs effectiveness.
- Some nets require retreatment or specialised disposal.
- Cost can be prohibitive when individuals are expected to purchase nets themselves.

Indoor Residual Spraying - IRS

IRS is the second core intervention. It involves the application of residual insecticides to the inner surfaces of dwellings, targeting *Anopheles* mosquitoes that rest on walls after having taken a blood meal. IRS programmes can rapidly reduce local malaria prevalence and mortality, provided that most houses and animal shelters in targeted communities are sprayed. WHO recommends the spraying of at least 80% (and ideally 100%) of houses and other structures in the targeted area in any round of spraying. Insecticide resistance can be managed by the rotation of different classes of insecticides.

Advantages:

- Aims to reduce vector populations and disease transmission.
- High coverage of an area can be achieved.

Disadvantages:

- Labour intensive since it requires retreatment at least once a season and skilled trained staff to apply the insecticides.
- Requires maintenance of equipment, quality assurance, monitoring and evaluation.

- Household compliance and support is essential to make this intervention effective.
- Implementation costs are relatively high.

Larval control

In specific settings and circumstances, the core interventions of LLINs and IRS may be supplemented by larval source management, which includes four subcategories: vector habitat modification, habitat manipulation, larviciding and biological control. Currently, WHO recommends 10 compounds and formulations for mosquito larval control. Detailed guidance on larval source management is available in '*Larval source management – a supplementary measure for malaria vector control. An operational manual*', released in 2013.

The most widely used larval source management approach involves the regular application of a biological or chemical insecticide to water bodies to reduce the number of mosquito larvae and pupae. These interventions can be useful in urban and peri-urban areas. However, they are unlikely to be effective in most areas of rural Africa as larval sites are generally innumerable, shifting and widely dispersed.

WHO recommends larviciding only in settings where larval sites are *few, fixed and findable*, that is where sites are easy to identify, map and treat. WHO and its partners should continue working with endemic countries that choose to use larviciding to ensure that such programmes are implemented and monitored appropriately.

Advantages:

- Reduced vector abundance.
- When done appropriately, this method can contribute to insecticide resistance management.
- Overall improvement of human environment.

Disadvantages:

- This method may be expensive and its impact on vector abundance is difficult to monitor and evaluate.
- Larviciding may not be applicable in certain larval sites, such as small puddles, hoof prints etc.

Further details on the core interventions towards the prevention and control of malaria can be found in the following links:

- http://www.who.int/whopes
- http://www.who.int/malaria

Current control methods for prevention of dengue and chikungunya

Dengue (DENV) and chikungunya virus (CHIKV) are mainly transmitted by *Ae. aegypti* and *Ae. albopictus*. The current global dengue picture is dire, with a surge in dengue epidemics in several non-endemic countries and worsening dengue situations in endemic countries. In parallel, chikungunya is slowly expanding its global footprints. It has become a serious concern in places where vectors are present in large numbers due to its ability to cause a widespread epidemic in a short period of time. As neither curative treatment nor licensed vaccine are available, disease prevention relies on vector control or the interruption of contact between human and vectors. The main goal of a control programme is to reduce

morbidity and mortality through the interruption of transmission of the virus between mosquito vector and human, and *vice versa.*

Larval control

Ae. aegypti and *Ae. albopictus* larvae develop in artificial containers, either permanent (e.g. water tanks, cement tanks cisterns, bathroom basins, water jars, animal drinking troughs) or temporary (e.g. used tyres, discarded bottles, ant-traps, flower vases, bamboo pole holders), or natural containers including coconut shells, leaf axils, bamboo fences and many others. Because of the nature of these larval sites, the removal of larval sites from the environment (source reduction), use of larvicides (biological and chemical) and release of larvivorous fish and copepods may all be effective control measures.

Source reduction
Ae. aegypti and *Ae. albopictus* larval sites may be altered by temporary manipulation, permanent modification, or physically removed, in order to prevent or minimize the propagation of these vectors.

Advantages:

- Removal of larval habitats and thus reduced vector populations.
- Cost-effectiveness.
- The methods are environmentally friendly.

Disadvantages:

- Presence of cryptic or inaccessible larval habitats poses a challenge to source reduction.
- Without active community participation, this strategy can be expensive and difficult to implement.

Larvicides
Larvicides are recommended by the WHO for the treatment of permanent or temporary larval habitats or potential larval habitats for dengue/chikungunya control. Commonly used larvicides include organophosphates (e.g. temephos), insect growth regulators (pyriproxifen, methoprene, etc) and biological products such as *Bacillus thuringiensis israelensis* (Bti), *Bacillus sphaericus* (BS) or spinosads. These larvicides come in several forms depending on their intended usage/mode of delivery: granular, briquettes, droplets, ice-cubes etc. Some of these larvicides (e.g. Bti, temephos) have been recommended by the WHO to treat domestic water supply including potable water.

Advantages:

- Reduced vector population in situations where source reduction is difficult to achieve.
- No resistance has been documented against Bti.

Disadvantages:

- Resistance to organophosphates and BS has been documented.
- Biocides may be expensive for large-scale operational use while alternative larvicides are available but awaiting regulatory approval.

Control by predators
Releases of larvivorous fish and copepods have been effective in domestic containers that are seldom emptied.

Advantages:

- No requirement for chemicals, therefore constitutes an "environment-friendly" alternative to insecticides.
- Promotes the local economy by stimulating employment.

Disadvantages:

- The predators can be costly and labour intensive to mass-produce for large-scale operational use.
- Communities may dislike the idea of putting live organisms into their domestic containers.
- These organisms may also carry pathogens of public and veterinary importance.
- There is only limited evidence from Vietnam to show the effectiveness of copepods to reduce vector density.

Methods for control of adults

Space spraying
Space spraying is the application of small quantity of insecticides over a large area and it is used for dengue/chikungunya prevention and control (WHO, http://www.who.int/denguecontrol/arbo-viral/other_arboviral_chikungunya/en/). The goal of this method is to break the chain of transmission by reducing the number of infected mosquitoes. Commonly used insecticides are pyrethroids and organophosphates, most commonly applied using thermal fogging or ultra-low volume (ULV) spraying.

Advantages:

- Reduction of mosquito populations (including infected adults) can be achieved quickly.

Disadvantages:

- Increased selection pressure for resistance means it should not be used in areas where insecticide resistance occurs.
- Negative effects on the environment and non-target organisms possible.
- Poor community support for this type of programme can limit its effectiveness.
- Expensive, requiring proper maintenance of equipment, monitoring and evaluation.
- No evidence that this method is effective for indoor-resting mosquitoes.
- Harmful effect on people suffering from respiratory illnesses.

Insecticide-impregnated materials
Insecticide impregnated, or treated, materials (ITMs) can be used to provide personal protection (e.g. on clothing), reduce indoor mosquito populations or prevent mosquitoes from coming indoors (using screens or curtains). Pyrethroids are commonly used for the impregnation of curtains and screens.

Advantages:

- Prevented entry and reduced indoor vector populations.
- Use of clothing impregnated with pyrethroids may also prevent man-vector contact or kill mosquitoes that alight on these materials.

Disadvantages:

- The insecticide used for treatment of these materials is similar to those used for space spraying, which may further increase the chance of mosquitoes developing resistance.
- There is limited evidence to show impact of ITMs against *Aedes*-borne diseases.
- Limited duration of efficacy and durability of materials.

Lethal / sticky traps

Lethal and/or sticky ovitraps exploit the oviposition behaviour of *Ae. aegypti* and *Ae. albopictus*. Lethal ovitraps usually incorporate insecticide on the oviposition substrate, killing any mosquitoes which alight on it. In contrast, sticky or gravid ovitraps incorporate a non-repellent sticky lining inside the wall and mosquitoes are trapped when they land on the sticky surface. Pyrethroids are the chemical of choice for use in lethal ovitraps.

Advantages:

- Simplicity and specificity for container breeding mosquitoes
- Reduction of man/vector contact.
- Mosquitoes caught in these traps can be used for xenomonitoring.
- By killing or trapping female mosquitoes, large numbers of eggs are potentially removed from the environment.
- Several designs available for different situations.

Disadvantages:

- Lethal ovitraps are only effective in places where mosquitoes are still susceptible to the insecticide used.
- Sticky linings used for the sticky/gravid ovitraps can be very expensive for large scale operational use.
- Traps can be potential larval habitats if not properly maintained.
- Limited evidence available to show the effectiveness of these traps in reducing vector density and transmission intensity.
- No benefit-cost analyses yet conducted.

Further details on dengue and chikungunya prevention and control methods can be found at the following link: http://www.who.int/topics/dengue/en .

NEW VECTOR CONTROL TECHNOLOGIES

Need for an area-wide integrated vector management (AW-IVM) approach

Since ancient times, humankind has suffered the effects of insects and other arthropods that compete for our food and fiber or transmit diseases. Various methods or strategies to suppress insect populations and/or reduce damage they cause have been developed and used, and the history of pest control can be divided into the pre- and post-insecticide eras. From the 1940 to 1960, pest control focused on the use of chemical pesticides; during this time, relatively cheap and effective products were available. The negative effects on the environment, on beneficial organisms, the accumulation of toxic waste, and the emergence of resistance and of secondary pests were phenomena that were initially not given any notice. The abuse of pesticides in some cases has caused irreparable damage to nature and even the loss of human lives. The exclusive reliance on pesticides has resulted in the search of more effective chemicals with less negative impacts on human health and the environment but at a much increased cost.

That was how the concept of Integrated Pest Management (IPM) emerged about 50 years ago, with the general idea being to combine different control methods to reduce the use of insecticides. IPM has been the dominant paradigm of insect pest control in the last 5 decades. Although some satisfactory results have been achieved, damage caused by insect pests remains very high and both resistance to the insecticides used and secondary pests have emerged, posing the question of whether we should continue doing the same thing or seek more efficient and sustainable alternatives. Pest management over large areas is not a new concept. This approach was used before the era of pesticides to address the most important pests (e.g. Bubonic plague, locusts, livestock ticks, etc.), or for the application of biological control. However, it was not until the early 1990's when Edward F. Knipling, World Food Prize, emphasized its importance and potential.

The “Area-Wide” concept (AW) has a close relationship with the ecological concept of "metapopulations", composed of local populations with some degree of communication or migration between them. The AW idea is to manage the total population or metapopulation of a pest, rather than limit control actions to areas where the pest causes damage. Unlike traditional IPM, the AW-IPM approach requires coordinated actions at an ecosystem level in a preventive way rather than a reactive strategy when the pest populations reach damaging or economically unacceptable thresholds. In practice, the traditional IPM approach has generally led to the repeated application of pesticides, while the AW-IPM approach looks to reduce or avoid their use.

The application of environment-friendly control methods, such as the SIT, Augmentative Biological Control, Incompatibility Insect Technique or the use of symbiotic organisms for suppression or replacement, require an AW-IPM approach to be effective. Among the difficulties or limitations for the application of the AW-IPM approach, two requirements stand out: 1) a greater understanding of the biology and ecology of the pest species, particularly its population dynamics in time and space; and 2) major community organization and engagement due to the complex social dynamics essential for application. In any case, control methods considered for AW-IPM should ideally be environmentally acceptable, for example avoiding applyication of pesticides in protected areas or human settlements. This second constraint requires an assessment of direct and indirect socio-economic costs and benefits in the short and long term. If the AW-IPM approach is found to

be feasible and worthwhile, research on public information strategies to facilitate its implementation will be the next step.

In some cases, the AW-IPM approach has been equated with pest eradication programmes and this has been in opposition to the accepted view of IPM. Fortunately, progress has been in understanding that not only are these two concepts not in opposition but that they are in fact complementary. Depending on specific conditions, AW-IPM can be used to prevent, contain, suppress or eradicate pests. Some examples of successful contemporary applications of the AW-IPM approach are the eradication of the new world screwworm from North and Central America and Libya, eradication of tsetse fly *Glossina austeni* from Zanzibar, Tanzania, eradication of Khapra beetle from Northern Mexico and South-Western USA, fruit fly prevention, suppression or eradication programmes in Argentina, Chile, Guatemala, Israel, Mexico, Spain and the USA, management of cotton pests in the USA, and suppression of cassava mealy bug in sub-Saharan Africa. Resistance management strategies also involve an AW-IPM approach.

The impact of diseases caused by mosquitoes and the current reliance on pesticides for vector control require the integration of new and more sustainable control methods into those currently in use. A more successful vector control strategy will likely involve an Area-Wide Integrated Vector Management (AW-IVM) approach that integrates modern and novel control methods, such as the Sterile Insect Technique, Biological Control or the Incompatibility Insect Technique.

An AW-IVM approach can only be effective when the following questions are addressed:

1) Where are individuals of the pest or vector species located when they are not attacking or causing damage?

2) How are pests or vector populations naturally regulated?

3) How do they survive from season to season?

4) What are the populations' abilities to grow and spread?

Sustainable 'green' methods – their potential and restrictions

<u>Sterile Insect Technique</u>

Since the 1950s, populations of several insect pests have been controlled or eradicated through a "birth control" method known as the SIT. It involves the colonization and mass rearing of the target pest species, sterilization, and their subsequent release into the field in over-flooding ratios to control wild insect populations. The principle is that the released sterile males will seek out and mate with wild females and these crosses will produce no offspring, thereby causing a reduction in the natural pest population. The validity of this method has been demonstrated for several insect pests of agricultural and veterinary importance including fruit flies, moths, screwworms and tsetse flies.

Several experimental pilot studies of the SIT strategy against mosquito species have been conducted in different geographical areas, as shown in Table 2.

Table 2. Species of mosquitoes against which SIT has been tested.

Species	Area	Period
Aedes aegypti	Florida	1962
	New Delhi, India	1972-75
Anopheles albimanus	El Salvador	1970-80
Anopheles gambiae	Burkina Faso (Haute-Volta)	1969-70
Anopheles quadrimaculatus	Florida	1959-62
Culex quinquefasciatus	New Delhi, India	1962-74
	Rangoon, Birmania (Burma)	1966-71
	Sea Horse Key, Florida	1970
	Montpellier, France	1972
Culex tarsalis	California	1977-83
Culex tritaeniorhynchus	Lahore, Pakistan	1972-79

Need for nuclear technology

Sterilization is usually accomplished by exposing insects to a specific dose of radiation emitted by radioisotopes (Cobalt-60 or Cesium-137) or X-rays. Of the alternative approaches, chemosterilants carry a high risk for environmental contamination and pose serious health concerns, and linear accelerators have not shown sufficient applicability or reliability in consistently achieving the desired level of sterility.

Nuclear technology not only has a comparative advantage in sterilizing mass reared insects, but is, at present, the only reliable technology available for this purpose. As every single insect used in SIT activities must be sterilized, irradiation is a central and indispensable part of the whole process. Radiation causes dominant lethal mutations which occur randomly and development of resistance is therefore not possible.

Integration of nuclear and non-nuclear techniques

SIT is not a stand-alone technology. To be effective, it should be integrated in a package with non-nuclear techniques (biological, chemical, behavioral) while economic considerations and public education should also be considered. Ideally, SIT should be part of an Area-Wide Integrated Pest (or Vector) Management Approach in which the total population of a pest or a disease vector in a region is managed. Indeed, several studies focusing on agricultural pests have clearly shown that uncoordinated field-by-field action, such as the sporadic or isolated use of insecticides by individual farmers on a small segment of the pest population, is only a temporary control measure. Insects move, often over considerable distances, and as long as the farmer's neighbors do not join efforts, the pest insects re-invade; regular

insecticide applications are thus required to protect the agricultural produce and in the long term this results in insecticide resistance. However, when growers in a given area or region co-ordinate efforts and apply an area-wide control programme against the total population of the pest species, much lower insecticide inputs will be required and the control achieved will be more effective.

Attributes of the SIT

SIT has specific attributes which make it a unique insect pest management tool:

- Species-specificity: unlike non-selective insecticide-based control, SIT represents a genetic control method which induces sterility and thereby controls pest populations in a species-specific manner. Unlike other biological control methods for which many cases of adverse impacts on non-target organisms have been reported, no such case is known for the SIT.

- Inverse density-dependency: unlike most control methods, SIT has the unique attribute of increased efficiency with decreasing target population density. SIT is the only environment-friendly technology available with the ability to eradicate insect pests if applied consistently on an area-wide basis: the sterile males have the ability to find the last wild females across the whole target area.

- Compatibility for integration: SIT is compatible, and can therefore be effectively integrated, with other control methods including biological control with parasitoids, predators, and insect pathogens. In this way, effective IPM approaches for the management of some of the world's most important insect agricultural pests have been developed.

Applications of the SIT

Considerable advances in development of SIT have resulted in major applications of this technology against tephritid fruit flies and other major pest insects, which have a significant economic importance. There are several ways to employ SIT for the population control of a pest:

Suppression

To avoid devastating fruit losses, intensive insecticide treatments are routinely required to control major agricultural pests such as fruit flies, moths, etc. with the accompanying damage to non-target beneficial organisms, disruption of biologically based controls of other orchard pests, insecticide residues on produce and general contamination of the environment. As a result of its species-specificity, SIT can be effectively used to replace insecticides in controlling some of these pests. Pilot tests and operational programmes have demonstrated the effectiveness of SIT in controlling fruit flies, moths, etc. and economic analyses have shown that SIT applied as part of an integrated approach is competitive with conventional methods. The development of genetic sexing strains enhances the ease of application and effectiveness of SIT for suppression purposes. Routine use of sterile insects for pest control has allowed the commercialization of SIT for some fruit fly and moth pests.

Also, SIT for pre-harvest control, applied as part of a system-wide approach in combination with a post-harvest treatment, can be used to create internationally recognized pest free or low prevalence areas and overcome trade barriers to agricultural produce.

Eradication

As a result of its inverse density dependence, application of the SIT on an area-wide basis and with adequate quarantine support, has been used to eradicate fruit fly pests successfully in Chile, Japan, Mexico, parts of Patagonia and Peru, and in Southern States of the USA.

Containment and prevention

SIT can be used as a biological barrier to protect pest-free fruit production areas that are contiguous to infested ones. Moreover, SIT can be applied as a preventive measure over pest-free areas that have a high risk of invasion to avoid the establishment of pest populations.

Transgenic approaches

In addition to classical genetic approaches for SIT enhancement, where rearrangements or breakage of endogenous DNA are used to create a desired effect, for example sex-specific conditional lethality, transgenic approaches are being exploited. Transgenic insects are herein defined as insects whose genetic material has been altered in a heritable way through the techniques of genetic modification, all of which allow for the combination and/or introduction of foreign genetic material into host insect genomes in a way that does not occur naturally by mating and/or natural recombination.

Developments and scientific activities in the area of transgenic insects indicate that future transgenic strains may include traits related to: i) sterilization of mosquitoes resistant or too susceptible to irradiation ii) efficient production of male-only mosquito populations for release, iii) marking of released insects for improved monitoring, iv) systems for targeted genetic engineering and transgene stability. However, the regulation of transgenic technology still needs to be addressed further.

Transgenic methods to mimic the sterilization process
The sterilization by irradiation is an important step during the production of insects for SIT releases. However, mass-rearing, transport and radiation might reduce the performance of male insects, and this needs to be evaluated before large scale applications take place. Similarly, transgenic sterilization methods, once developed, need to be evaluated for their impact on fitness.

One such ´sterilization system´ developed for mosquitoes is the RIDL system for *Ae. aegpyti*. This uses the deadly effect of an overexpressed protein (tTA) to kill between 95.8 – 97.4 % of progeny of transgenic males with WT females at the late larval or pupal stage. Initial releases were conducted in 2009 on a small scale on the Cayman Islands, and the results were eventually published in 2011. A larger scale evaluation of the same strain, OX513A, is ongoing in Brazil and will hopefully answer several questions about the use of transgenic technology in general. Transgenic sterilization technologies will be most valuable in countries where the release of transgenic mosquitoes is approved and for species where radiation induced sterility comes with a fitness load higher than that of transgenic technologies.

Transgenic methods for sexing separation systems

In addition to the sterilization procedure, male-only releases are a pre-requisite for any operational mosquito programme. The release of disease-transmitting females has to be avoided or at least kept to a minimum in regions were disease density is low.

Despite the pressing need, there are currently no sexing systems available to be used on an operational scale. Several promising technologies have been developed for tephritids and other insects and should either be transferred to mosquitoes or further developed and evaluated.

These comprise:

- Sorting of fluorescent embryos: sex-specifically marked embryos or larvae can be sorted by a COPAS sorting machine. Reliability and cost of the machines in terms of mass rearing requirements have to be considered and further developed.
- Sex reversion / sex distortion strategies: sex reversion tools have been identified in fruit flies and could be developed for mosquitoes to enable the sex reversion of females to males. This would double the total amount of male progeny produced.
- Another strategy, so called ´sex distortion´, was developed for *Anopheles gambiae* and changes the gamete production of males in such a way that results in 95-97% male progeny. Such a system could be used as a gene drive system after careful evaluations for impacts on performance of resulting males.
- Female lethality systems: two different systems of female-specific lethality were developed for fruit flies and could be developed further for mosquitoes. A system lethal for females in late larval/pupal stage, called ´female-specific RIDL´ (fsRIDL), has been developed in *Ae. aegypti*. Secondly, a transgenic embryonic sexing system (TESS) is able to eliminate female fruit flies early during embryogenesis. Due to the early death of female embryos in TESS strains, there is a cost reduction in mass rearing. A release of fsRIDL or TESS males is feasible even without sterilization.

Transgenic sexing systems will be important tools in the future, though the development of genetic screens and sexing strains through classical genetic approaches should be pursued in view of the regulatory difficulties of transgenic systems. Both technologies can benefit from each other; knowledge acquired through genetic screens like EMS screens could lead to new developments of transgenic strains, and both approaches share a lot of technical requirements such as release and surveillance strategies.

<u>Transgenic markers for monitoring</u>

Marking of insects before release is important to calculate the efficiency of a release programme by re-trapping insects and being able to distinguish WT from released insects. Markers have to be non-transferable to WT females during mating and stable in the traps after death of the insect to allow for reliable interpretation.

Several markers have been developed for mosquito species:

- *Random markers*:
  Different promoters have been used to express fluorescent proteins in mosquitoes. The actin 5C, *PUb* and *UbL40* promoters are able to promote strong expression in different tissues. Expression patterns are dependent on the integration position of the markers into the mosquito genome.

- *Tissue specific markers*:
  The 3xP3 promoter was first developed in 1999 and used in *Ae. aegypti* in 2001. 3xP3 driven expression is clearly detectable in the eyes and the marker was successfully used in several other species. The sperm-specific *beta2-tubulin* promoter was used to drive the expression of fluorescent protein specifically in testes of *Anopheles stephensi, An. gambiae,* and *Ae.*

aegypti. Sperm markers can be used not only for field detection of released males, but also for identifying the mating status of WT females and genotype of her mate.

All transgenic strains include some kind of marker to visualize the integration of DNA during the transformation process. Therefore most strains could be directly tested and evaluated for field use, in this regards. The results from future classical mutagenesis screens could further enhance the development of marking systems for additional pest species.

<u>Systems for targeted genetic engineering and transgene stability</u>

Current technologies for genetic manipulation of insects include transposable elements. At least four different systems have been developed and successfully used over a wide variety of insect species, including mosquitoes, for which *piggyBac*, *Hermes* and *Mos/mariner* integrations have been used to produce stable transgenic lines.

While transposable elements are active across different species, there are some restrictions in the way they can be used. Insertion of transposable elements into the target genome is random and thus subject to genomic position effects, which modify transgene expression, and to insertional mutagenesis. Moreover, the carrying capacity of transposable elements is limited. Therefore, once a suitable integration site is identified and an insertion made into it, it would be desirable to use this as a landing site for further manipulations.

Site-specific transgene integration can be achieved using systems such as FLP-*FRT*, CRE-*lox* or phiC31 integrase, which have all been successfully applied in several fruit fly species. As yet, for mosquitoes only *attP* integration has worked reliably, in *Ae. aegypti*, *An. stephensi* and *An. gambiae*. With the CRE-*lox* system excisions but no integrations could be achieved, and the FLP-*FRT* system has failed to work so far.

Lines containing landing sites need to be evaluated for any impact of insertions on their performance to select the best strains for downstream applications. Parameters that should be evaluated as being important for demands of a mass rearing and release programme are: fecundity, fertility, larval viability, larva-to-pupa development time, pupal sex ratio, adult longevity, and mating competitiveness of transgenic individuals with their wild type counterparts. Up to now, the design of such studies has not been harmonized between research institutes, and a standardised quality control protocol. for tests of robustness and competitiveness should be developed to enable comparisons between different strains.

The subsequent targeting of evaluated landing site lines via transgene integration systems would be desirable for different applications including:

- Allowing a true comparison of transgenic systems when inserted at the same genomic location
- Transgene stabilization, which is highly dependent on the species and system used. For example in *Ae. aegypti*, *pBac* transposable elements seem to be immobilized and *Mos/mariner* remobilization is very inefficient. In contrast, *pBac* remobilization in *An. stephensi* is achievable.

In addition to site-specific integration systems, site-specific targeting systems like zinc finger nucleases (ZFNs), TALEN or CRISPR could be used to modify specific regions of mosquito genomes. These technologies are currently being evaluated in a few species and laboratories but need to be further explored. Once successfully tested, they could help to develop new pest control systems, though to achieve this goal greater knowledge about the biology of each target mosquito is required.

Regulation of transgenic technologies

Once proof-of-principal and contained trials show promising results, permission for field-testing is required. No scientific advances can be implemented in field trials without obtaining the approval of government authorities, and the local population should be informed. These processes are not trivial and may involve large efforts over an extended period of time. Therefore, the information-gathering and regulatory application process should be started early, with efforts undertaken jointly by all stakeholders. This is especially true for transgenic technologies but also for other new technologies.

Wolbachia-based strategies (population suppression and replacement)

Wolbachia spp. are intracellular Alphaproteobacteria closely related to Rickettsia. Maternally inherited *Wolbachia* infections occur in more than 65% of all insect species and approximately 28% of the mosquito species investigated. Through the phenomenon of cytoplasmic incompatibility (CI), *Wolbachia* can induce early embryo death when an uninfected female mates with a *Wolbachia*-infected male, while the offspring can successfully develop when laid by an infected female no matter whether she has mated with an infected or uninfected male.

One important feature of *Wolbachia* is its ability to induce resistance to a variety of pathogens, including dengue virus (DENV) and malaria parasites, in its mosquito hosts. In transinfected *An. stephensi*, wAlbB infection can confer mosquito resistance to both *P. falciparum* and *P. berghei*. Similarly in transinfected lines of *Ae. aegypti*, all the three different strains of *Wolbachia* - wAlbB, wMelPop-CLA, and wMel, show a significant inhibition to replication and dissemination of DENV resulting in either complete or partial block of viral transmission. Recent studies further show that *Wolbachia* induces production of reactive-oxygen species (ROS) which then activates Toll-pathway to induce expression of antiviral effectors.

Native *Wolbachia* can also confer resistance to DENV and other pathogens in a *Drosophila* host. This resistance appears to be induced by the non-immune related mechanisms because the immune genes tested do not show differential expression in response to *Wolbachia* infection. Inhibition of dengue virus replication was also observed in cell lines, with the extent of inhibition being related to bacterial density. There is a strong negative linear correlation between the genome copy of *Wolbachia* and DENV, with a dengue infection completely removed when *Wolbachia* density reaches a certain threshold. Although *Ae. albopictus* naturally carries the *Wolbachia* infection in reproductive tissues, its density in key somatic tissues such as mid-gut and salivary gland is too low to induce resistance to DENV. This indicates that *Wolbachia* can induce only a local but not a systematic antiviral resistance in mosquitoes. By introducing a novel *Wolbachia* strain (such as wPip from *Culex pipiens*) into *Ae. albopictus*, *Wolbachia*-mediated pathogen interference can be induced and *Ae. albopictus* can become refractory to DENV.

Significant progress has been made in developing *Wolbachia* to control mosquito-borne diseases, including dengue, malaria and lymphatic filariasis. Different *Wolbachia* strains have been transferred into three major disease vectors, *Ae. aegypti, Ae. albopictus* and *An. stephensi*, resulting in 100% maternal transmission and both complete CI and strong pathogen interference. These lines could thus be used in field trial for proof of concept studies and even operational implementation for vector control. The current *Wolbachia*-based approaches include population suppression and replacement, and an integration of these different methods.

Wolbachia-based population suppression

Wolbachia-based population suppression refers to a control strategy in which mating of released males incompatible with native females results in a decrease in the females' reproduction and eventually, if males are released in sufficient numbers over a sufficient period, elimination or a local eradication of the mosquito population could be achieved. This strategy is also called the Incompatible Insect Technique (IIT). Previously this approach has been successfully used to eradicate a *Cx. pipiens* population in a village in Burma after release of CI-inducing males for 12 weeks. Recently, releases of *Wolbachia*-infected male *Ae. polynesiensis* to induce incompatible mating were used to supplement the current control approaches for lymphatic filariasis in the South Pacific. Further field studies are needed for additional proof of concept and to better define some essential parameters that will determine overall success. These parameters include ratio of released males vs. wild males.

For future large-scale experiments, hurdles that need to be overcome include mass rearing, sex separation and quality control. Only when the capacity to produce sufficient number of males has been developed, will *Wolbachia*-based population suppression be deployable for area-wide implementation. *Wolbachia*-infected males are usually able to compete with wild males to mate with wild females. In some cases, *Wolbachia*-infected males even have a fitness advantage compared to wild males. On the other hand, special attention has to be paid to the risk of unintended population replacement caused by accidental release of *Wolbachia*-infected females alongside released males, which would lead to a loss of efficacy in population suppression. If this were to happen, males infected with another incompatible strain of *Wolbachia* could be released in order to resume population suppression. Alternatively, a combination of IIT with SIT might be considered in order to avoid the release of fertile females and remove the risk of population replacement.

Wolbachia-based population replacement

When *Wolbachia*-infected females are released, *Wolbachia* can spread quickly into a population resulting in fixation or population replacement since the infected females have reproductive advantage over uninfected ones. Such *Wolbachia*-mediated population replacement has been observed to occur naturally in *Drosophila simulans*, and demonstrated in *Ae. aegypti* through both laboratory cage studies and a recent field trial. In both experiments, an initial female release threshold had to be reached in order to enable *Wolbachia* to invade populations. *Wolbachia*-infected *Ae. aegypti* collected from the field sites three years after release still maintained a strong pathogen interference, serving as proof of concept for this approach. Future studies need to collect epidemiological data in addition to entomological data to measure the impact on dengue transmission. In addition, a trade-off was discovered between pathogen blocking efficacy and fitness costs in *Wolbachia*-infected females. It is critical to generate a mosquito line carrying a *Wolbachia* strain with a high level of pathogen interference but a minimal fitness cost. Lastly, as the released females can cause a biting nuisance which may negatively affect the public acceptance of a programme, future studies should reduce the number of released females and optimize the release method to minimize the nuisance caused.

Integration of population suppression with population replacement

In this strategy, mosquito populations would be suppressed to a very low level via release of males carrying CI-inducing *Wolbachia*, followed by replacement through female release. After population replacement, males carrying a different strain of *Wolbachia* will be released to further suppress the mosquito population, resulting in less biting even by

pathogen-resistant mosquitoes. It is assumed that the integration of the two methods would provide an additional advantage to application of the *Wolbachia*-based strategy for mosquito-borne disease control. This could lead to a reduction in both mosquito biting and vector competence, which are the two key parameters determining vectorial capacity. With population replacement alone, it is likely that pathogens would develop resistance to *Wolbachia*-mediated interference in mosquitoes. In addition, release of a sufficient number of *Wolbachia*-infected females to reach the initial female infection threshold for population replacement may cause difficulty in public acceptance due to the biting nuisance of females. Population suppression alone requires a long-term and continuous investment in the programme, in order to maintain suppression, which may not be feasible under certain situations. This integrated method could generate a more practical and sustainable effect in vector-borne disease control than one method alone, though proof of concept tests of this approach are on-going.

Integration of *Wolbachia*-mediated population suppression with traditional SIT

Production of sufficient male mosquitoes for *Wolbachia*-mediated population suppression to be effective requires the capacity for mass rearing and efficient sex separation. While progress has been made in mass rearing, sex separation is still a bottleneck. It is likely that a very low number of females contaminating the released males would hinder the IIT, which makes high efficacy of sex separation extremely important. However, this issue may be resolved by using a low radiation dose to sterilize any remaining females, while *Wolbachia*-infected males will induce incompatible matings with the wild type females. In addition, the low irradiation dose will contribute to the male sterility at a low fitness cost, if any. Furthermore, this integrated method mitigates the concern that the accidental release of sterile females may enhance disease transmission because *Wolbachia*-infected females carry a pathogen interference phenotype. Thus, integration of the SIT with *Wolbachia*–based population suppression and pathogen interference could provide an effective way to facilitate mosquito and disease control, and is currently being tested for proof of concept.

Paratransgenic approaches (gut bacteria to prevent pathogen infection)

One aspect that has not been considered in depth until recently is the potential role that insect microbiota may play in insect reproduction, fitness and their ability to transmit pathogens. The ability to maximize insect performance by manipulation of microbiota would be highly beneficial for SIT programmes.

The life cycle of most insect-vectored pathogens starts in the insect's gut. In most cases, parasite numbers in this compartment are at their lowest point, making this the most vulnerable stage (bottleneck) of the pathogen's cycle in the insect. Importantly, insects harbor microbiota composed of well-defined bacteria genera that share the same insect compartment (the midgut lumen) with the most vulnerable stages of the pathogens they transmit. This proximity between microbiota and pathogens suggests a potential strategy to control transmission, namely the engineering of resident bacteria to secrete anti-pathogen molecules – also known as paratransgenesis. Alternatively, the insect midgut could be populated with bacteria that naturally inhibit pathogen development. In proof-of-principle, a mosquito-borne bacteria (*Pantoea agglomerans*) has been engineered to secrete a variety of anti-*Plasmodium* molecules, resulting in a dramatic inhibition of vectorial competence. In another proof-of-principle set of experiments, an *Enterobacter* bacterium that strongly inhibits the development of *Plasmodium* in anopheline mosquitoes has been identified.

While these initial findings are encouraging, a major challenge for field implementation of this strategy is to develop a means to spread the inhibitory bacteria into mosquito

populations in the field. This remains a high priority for future research. One possible mechanism is to use bacteria that are vertically transmitted such as *Asaia*. However, in addition to vertical transmission, the bacteria should have a selective advantage over existing insect bacteria to allow their spread into insect populations. Moreover, issues such as transgene stability, pathogen resistance to the effector molecules, potential harm of the bacteria to humans and the environment, and toxicity of the effector molecules also need to be evaluated. Finally, as this strategy involves the release of genetically-modified organisms in nature, regulatory issues and acceptance by the local population also need to be considered.

CURRENT TECHNICAL AND MANAGEMENT CONSTRAINTS AND GAPS FOR SUSTAINABLE MOSQUITO CONTROL AND AREAS WHERE THE IAEA CAN CONTRIBUTE

The major constraint for sustainable mosquito control programmes that include an SIT component for both *Anopheles* and *Aedes* mosquitoes remains the lack of genetic sexing strains (GSS), to produce only males and avoid female release. The IPCL is implementing a Coordinated Research Project (D4.40.01) on "Exploring Genetic, Molecular, Mechanical and Behavioural Methods of Sex Separation in Mosquitoes" (2013-2018) and is directly involved in the screening of morphological markers to develop a new GSS for *An. arabiensis*. Other technical and management constraints and gaps are listed in Table 3.

Table 3. Current technical/management constraints and needs for sustainable mosquito control and areas where the IAEA can contribute.

<table>
<tr><th>Technical/Management Constraints and Gaps</th><th>Potential IAEA contributions</th></tr>
<tr><td>Need for efficient sex separation on a large scale</td><td>…</td></tr>
<tr><td>Creation of a GSS(male-only strain)</td><td>Screening of morphological markers and Coordinated Research Project (D4.40.01) on "Exploring Genetic, Molecular, Mechanical and Behavioural Methods of Sex Separation in Mosquitoes".</td></tr>
<tr><td>Insecticide resistance</td><td>Reduced pressure for resistance development by using SIT and / or related technologies as alternative control methods.</td></tr>
<tr><td>Unacceptable or unaffordable costs of existing control methods</td><td>R & D into new control methods.</td></tr>
<tr><td>Insensitivity, difficulty and cost of vector monitoring methods</td><td>Development of more sensitive and inexpensive trapping methods.</td></tr>
<tr><td>Weak control, diagnosis and treatment infrastructure including insufficient trained staff</td><td>Training, capacity building, fellowships, etc.</td></tr>
<tr><td>Existence of animal reservoirs for some pathogens</td><td rowspan="2">R & D regarding mitigating technologies such as paratransgenesis and Wolbachia.</td></tr>
<tr><td>Pathogenic agent changes in host range that diminish disease control effectiveness</td></tr>
</table>

Reintroduction of disease into disease-free areas	Development of prophylactic release methods to create barriers against reintroduction of infected mosquitoes.
Changes in population dynamics of non-target vectors in response to control measures of the target species	CRPs on modeling mosquito population dynamics in the context of genetic control.
Inadequate capacity to implement Monitoring & Evaluation (M & E) of control effectiveness standards at the national level	Facilitate partnerships between UN and national agencies, e.g. WHO/country MoH in the context of M & E for vector control.
Inability to detect early and respond to outbreaks effectively	
Difficult logistics of implementing control	Advocacy and awareness promotion related to vector-borne diseases.
Unsustainable political support at the national and/or local level	Continued advocacy for vector control support and implementation of AW-IVM. Community education on vector control.
Inadequacy of technical entomological capacity	Training in entomological techniques including species identification and monitoring, and implementation of AW-IVM.
Vector behaviour that makes interventions ineffective or are subject to selection in response to control measures.	CRPs focusing on the relationship between vector control and mosquito behavior.

Current Role of the IAEA and the Joint FAO/IAEA Division

In response to the GC resolution (56)/RES/12, the Insect Pest Control Laboratory (IPCL) of the Joint FAO/IAEA Division initiated a research programme towards the development of the SIT package for disease-transmitting mosquitoes, i.e. the malaria vector *An. arabiensis* and the vectors for dengue and chikungunya, *Ae. aegypti* and *Ae. albopictus.* This programme's aim was to develop protocols for mass rearing, sexing systems to separate males from females, irradiation-induced sterility, quality control and assessment of the field competitiveness, transport / shipment to the field as well as for the release and the monitoring of sterile mosquitoes. The achievements to date are summarized here.

R & D achievements at the FAO/IAEA Insect Pest Control Laboratory (IPCL)

<u>Mass-rearing</u>

Mass production is an important component of any pest or vector control programme that requires the release of large number of insects. As part of efforts to develop an area-wide programme involving SIT for the control of mosquitoes, the IPCL has developed mass-production tools for *Ae. albopictus*, *Ae. aegypti* and *An. arabiensis*.

Mass-rearing cages

Adult mass-rearing cages that allow mass production and easy collection of *An. arabiensis, Ae. aegypti* or *Ae. albopictus* eggs, cage cleaning, blood feeding, and sugar delivery were developed and validated at the IPCL. The structure of the adult mass-rearing cage for mosquitoes is similar to the Mediterranean fruit fly cage; nevertheless, specific features for mosquitoes have been included, such as a water reservoir at the bottom of the cage for oviposition. Preliminary results indicated that the *An. arabiensis* cage filled with 20,000 individuals produced in total 300,000 eggs during the first gonotrophic cycle (10 days of operation). For *Ae. albopictus*, the mean number of eggs produced per female in the mass rearing cage was lower than in the small cages (30 x 30 x 30 cm).

Figure 7: Example of the Ae. aegypti and Ae. Albopictus mass-rearing cage

Further studies are required to improve the productivity of the available system; he cage technology has been transferred to Italy, Brazil, China, Mauritius and Sudan for testing under local conditions.

Larval rearing systems

The trays for large scale larval rearing are made of thermoformed plastic and has outside dimension of 60 × 100 × 3.5 cm and can contain 6 litres of water. Figure 8, top panel, shows the final prototype which was tested for its suitability for rearing larvae of *An. arabiensis* and *Ae. albopictus* and is being tested now for *Ae. aegypti.* 4,000 L_1 of *An. arabiensis* and 20,000 L_1 of *Ae. albopictus* can be reared in one tray, depending on the larval density, feeding schedule and temperature. In optimal conditions, it is expected to have a pupal recollection of around 75% and 85% before sexing for *An. arabiensis* and *Ae. albopictus,* respectively. The IPCL also designed a movable and tiltable rack prototype that can hold 50 trays with a footprint of less than $1m^2$. The tray/rack system has been transferred to Italy, Brazil, China, Mauritius and Sudan for testing under local conditions.

Figure 8: Rack and tray system

Larval diet

A universal larval diet suitable for *Anopheles* and *Aedes* mosquitoes has been developed, comprising ingredients that are widely and readily available. The diet and SOPs for its proper use have been transferred to South Africa, Sudan, China, Indonesia, Malaysia, Pakistan, Sri Lanka, Thailand, La Réunion, Benin, Burkina Faso, and the UK. Preliminary tests showed good results not only for *An. arabiensis* and *Ae. albopictus* but also for other important mosquito pest species such as *An. funestus*, *An. gambiae*, *An. melas*, *Ae. aegypti*

and *Ae. polynesienis*. Several research institutes in France, French Polynesia, Italy, Mauritius, Trinidad & Tobago, the UK, and the US are now testing or routinely using this larval diet.

Larva-pupa separation

A larva-pupa separator designed and developed at the IPCL to mechanically separate *Anopheles* mosquitoes based on developmental stage of was transferred to Sudan and South Africa and is being evaluated under local conditions. This system, that allows the efficient separation of a 30,000 larvae-pupae mixture in only two minutes, will benefit all MS working with malaria vector species including South Africa, Mauritius, and La Reunion. This technology is not yet available for *Aedes* species.

Production standardization

Studies are being pursued to standardize all steps of the mosquito production process from egg quantification to adult male production. The aim is to provide SOPs to the Member States that will optimize the rearing methods whilst minimizing the factors that could impact negatively the target quality and quantity of sterile males produced and therefore the efficacy of a mosquito control programme with an SIT component. Efforts are ongoing to make the manufacturing and operation of the automated equipment more cost effective.

Genetic sexing strains (GSS)

The main challenge to be addressed in the coming years remains the need to achieve easy and safe elimination of females on a mass-rearing scale. The development of an efficient sexing system is a prerequisite for any mosquito SIT-based population control programme since the accidental release of females could be potentially risky for the transmission of human pathogens. The IPCL has started developing a genetic sexing strain (GSS) based on classical genetic approaches. This is a challenging task given that at this point there are no available genetic markers which could be used for the construction of a classical GSS similar to the one currently in use in medfly SIT projects.

Typically, a GSS consists of at least two principal components: (a) a selectable marker which is necessary for sex separation or female killing, ideally at the embryonic stage and (b) a Y-autosome translocation, T(Y;A), which is required to link the inheritance of this marker to sex. For example, the Mediterranean fruit fly VIENNA-8 GSS currently in use in operational SIT projects is based on two elements, the white pupa (*wp*) and temperature-sensitive lethal (*tsl*); the tsl marker allows the elimination of females through the incubation of eggs at 34°C for 24 h. Such markers are still lacking for mosquitoes and would be extremely helpful for the development of a GSS via classical genetic approaches.

A GSS of *An. arabiensis* that requires a dieldrin treatment to kill female mosquitoes has been available for several years and its potential use for field releases was assessed. The strain exhibited key limitations, namely low natural productivity (due to multiple translocations), and the fact that male adults, after being treated with dieldrin as eggs, were found to contain insecticide residues, which is not acceptable for an environment-friendly approach like SIT. Efforts are now ongoing to identify markers and develop new GSSs for *An. arabiensis*. Human resources and funding limitations have so far not allowed the IPCL to embark on similar efforts to develop a GSS for *Ae. aegypti* and *Ae. albopictus*.

Nevertheless, for an imminent pilot suppression trial a chemical method of separating the sexes was tested for *An. arabiensis*. Adding ivermectin to the blood meal offered to female mosquitoes achieved complete elimination of females from a laboratory population. This method will provide a temporary solution for eliminating female *An. arabiensis* until a new GSS has been developed. Mechanical approaches to separate the sexes exist for *Aedes*

mosquitoes using sieving or sorting methods. The sexual dimorphism of *Aedes* pupae allows efficient separation but the productivity is low and risk of female contamination of resulting males relatively high.

In view of the urgency to develop GSSs as prerequisites for application of the SIT against mosquitoes, a new CRP has been initiated on "*Exploring Mechanical, Molecular, Behavioural or Genetic Methods of Sex Separation in Mosquitoes*". The first Research Coordination Meeting (RCM) was held in Vienna, Austria in October 2013. During the meeting, 23 participants from Africa, Asia, Europe, South/Central America, and the USA discussed the issue and agreed on plans for future work.

Irradiation
SOPs for irradiation procedures are available for sterilisation of *An. arabiensis* and *Ae. albopictus* with gamma and X-ray irradiators. Dose-response curves were established for these two species at both adult and pupal exposures and demonstrated that full sterility is reached at 40 Gy for *Ae. albopictus* and 100 Gy for *An. arabiensis*. Sterilisation of male mosquitoes is less harmful when done at the adult stage but more difficult from a practical perspective, so it is recommended to sterilize mosquitoES at the pupal stage with a dose close to that required for at least 95% sterility, which allows a higher competitiveness at the adult stage than a higher, fully sterilising dose.

Despite their high costs, self-contained gamma-ray irradiators are recommended as they are reliable machines that have been used for more than 30 years in fruit fly, screwworm and tsetse AW-IPM programmes. Efforts are being made to find a reliable company that could provide more affordable but equally reliable solutions, for example X-ray irradiators.

<u>Quality control, mating competitiveness and transport</u>

Biological performance characteristics
Regarding Quality Control (QC), life-history parameters of the mosquito species of interest have been estimated under controlled environments and with provision of adequate larval and adult food. Survival and duration of each developmental stage, sex ratio, adult emergence rate, female fecundity and longevity are known, and efforts are being made to produce a QC manual for the mass-rearing process.

The insect greenhouse at the IPCL in Seibersdorf that simulates field conditions has been used for behavioural studies. This important tool offers a good surrogate for the natural environment to look at mosquito biology, including assessing sterile male competitiveness, swarming, mating compatibility and dispersal. The greenhouse has been used to test the effect of various sterile to wild male ratios and the age of the sterile males on their mating competitiveness and impact on a population's egg production and hatch rate. These preliminary data, obtained *An. arabiensis* from Sudan, *An. gambiae* from Burkina Faso and *Ae. albopictus* from China and Italy, have provided information which can be used to improve the production process and thus competitiveness of sterile males, which is a crucial factor for the success of an SIT programme.

Transport/shipment to the field
During an AW-IPM programme that includes an SIT component, a large number of mosquitoes are produced in a mass-rearing facility. Before being released into the wild, they need to be transported to the release site and this operation often requires an emergence facility to be established where adult emerge, are fed and then released. Even if an operational programme does not yet exist for mosquitoes, there is an urgent need to

develop tools and procedures for this stage of a programme. So far, in the context of the pilot suppression trial in Sudan, a modest transport box was developed to transfer adult *An. arabiensis* males from the laboratory in Khartoum to the field site in Merowe (300km away). Additional efforts need to be made with the support of external institutions and extra-budgetary funds.

Release and monitoring
There is also an urgent need to develop release tools and methods for AW-IVM programmes with an SIT component against mosquitoes. Discussions between IPCL and external collaborators working on release machines, chilling methods and release using small airplanes or gyrocopters, have started but require additional human and financial resources and / or external collaborations.

Another challenge that needs to be addressed in the coming years is the development of better field surveillance tools to be able to monitor the presence and distribution of wild and sterile mosquitoes, in particular more efficient male trapping devices for a better evaluation of the effects of a suppression programme.

CRPs and Contracts

<u>Past and Current Coordinated Research Projects (CRPs)</u>

CRP G3.40.01 on "Development of Standardised Mass Rearing Systems for Male An. arabiensis Mosquitoes" (completed)
In March 2011, the final Research Coordination Meeting (RCM) of the CRP G3.40.01 on "Development of Standardized Mass Rearing Systems for Male *An. arabiensis* Mosquitoes" was held in St Clotilde, La Reunion, France and representatives of nine Member States attended the meeting. During the five years of the CRP, significant progress was made with the development and validation of new mass-rearing tools for mosquitoes. A tray-rack system was developed for *An. arabiensis* and tested for *Ae. albopictus*. A novel device, called the larval pupal separator, was developed to separate larvae and pupae of *An. arabiensis*. The new equipment was validated and shown capable of separating a larvae-pupae mixture of one million individuals in one hour. An affordable and well-performing larval diet is now available and is contributing to the establishment and up-scaling of new colonies in the laboratory. New mass-rearing procedures were likewise developed.

CRP G3.40.02 on "Biology of Male Mosquitoes In Relation To Genetic Control Programmes" (completed)
In March 2013, the final RCM of CRP G3.40.02 on the "Biology of Male Mosquitoes in Relation to Genetic Control Programmes" was held in Juazeiro, Bahia, Brazil, at the Juazeiro MOSCAMED insect rearing and release facility. From 2008 to 2013, twenty international experts (14 research contracts and 6 research agreements) from 16 different countries participated in this research group and contributed to the development of a better knowledge of adult male mosquito biology which included factors that affect the ability of males to attract, court, and inseminate females in the field as well as specific biological and behavioural determinants that contribute to male mosquito sexual competitiveness. The research results can be summarized as follows: (1) Optimal rearing conditions (larval & adult diet), and the resource acquisition/allocation determined for several mosquito species; (2) Protocols for male competitiveness studies established; (3) Temporal and spatial characteristics of mating encounter sites of some *Aedine* and *Anopheline* mosquitoes determined; (4) Copulation / insemination systems and patterns of female remating

determined; and (5) Compound involved in male swarming identified. The main results and achievements of this CRP were published in a special issue of the peer-reviewed journal ACTA TROPICA (http://www.sciencedirect.com/science/journal/0001706X/132/supp/S). The IPCL involvement during this CRP was to: maintain different mosquito species and colonies; study the effect of nutrients, larval food on sexual competitiveness; study male biology and sexual behaviour in a controlled environment; develop standardized protocols for male mating competitiveness assays; support the networking and collaborations between researchers; host scientists to conduct their research linked to the CRP; and host and/or organize RCMs.

CRP D4.40.01 on "Exploring Genetic, Molecular, Mechanical and Behavioural Methods of Sex Separation in Mosquitoes" (2013-2018)

Unlike agricultural pests where the release of both sexes is primarily of economic concern, in mosquitoes it is an essential prerequisite to release only males since females are blood feeders and can transmit disease. The first RCM of the CRP on "Exploring Mechanical, Molecular, Behavioural or Genetic Methods of Sex Separation in Mosquitoes" was held in September 2013 in Vienna, Austria and was attended by 23 participants from Africa, Asia, South/Central America, Europe, and the USA. During the first two days of the meeting, participants presented their ongoing and future research. Subsequently, participants were divided into three groups according to their approaches for the development of a new GSS: (1) classical genetics; (2) molecular genetics and (3) mechanical, behavioural and developmental approaches. The 19 participants (9 contracts and 10 agreements) from 14 different countries discussed and agreed on the future work plan for each participant; furthermore, collaborations were established. Finally, the date and location for the second RCM were discussed and tentatively set for the Moscamed mosquito facility in Juazeiro, Brazil from 2–6 March 2015. The IPCL involvement during this new CRP will consist of maintaining different mosquito species and colonies; screening for new selectable markers for the development of a classical GSS for *An. arabiensis* and providing assistance to the researchers of the group to assess the competitiveness of the new GSS strains as they are developed.

Current support to Technical Cooperation Projects

1. Technical support is being provided to 5 national technical cooperation (TC) projects in Mauritius, Pakistan, South Africa, Sri Lanka and Sudan (MAR5019, PAK5049, SAF5013, SRL5044 and SUD5034) and one regional TC project in the Indian Ocean region (RAF5065, including Madagascar, Mauritius and Seychelles, as well as La Réunion, France). All six projects focus mainly on capacity building, however, studies to determine the feasibility of applying the SIT are being undertaken in South Africa and Sudan. Furthermore, since the GC (56), fellows from China, Madagascar, Seychelles, South Africa, Sri Lanka and Sudan received training at the IPCL on mosquito rearing and related activities under the Agency's TC programme. Topics included mass-rearing of mosquitoes, the use of the GSSs, the use of the new larval diet, mating studies, radiation biology and quality control procedures. For the biennium 2016-2017, several concept notes for national and regional TC projects have been received from Member States.

2. In response to Member States' requests related to the recent geographical spread of dengue/chikungunya, two new regional technical cooperation (TC) projects were launched in 2014: one in the Asia/Pacific region (RAS5066 including China, Indonesia, Malaysia, Pakistan, Philippines, Sri Lanka and Thailand) and another in the Indian Ocean region (RAF5072, including Madagascar, Mauritius, Seychelles and La Reunion, France). These two

projects focus on networking, sharing of expertise and capacity building. Staff of participating countries will be trained at the IPCL on mosquito rearing and related activities under the Agency's TC programme. Mosquito surveillance has been already initiated in pilot sites in most of these countries.

3. Technical support continues, through TC project SUD5034, to an initiative in Sudan that aims to assess the feasibility of integrating the SIT into an AW-IVM approach against *An. arabiensis*. The project is also supported by the Islamic Development Bank (IDB) through a US$ 4.8 million soft loan to the Government of Sudan. Sudanese representatives have visited the Agency on several occasions and Agency staff have continued to provide technical support on-site. During various meetings, Agency staff have recommended that the project should adhere to a "phased conditional approach" and initially focus on conducting a pilot trial (3-4 years) in a small representative area along the Nile to assess the feasibility of the integrated use of the SIT on mosquitoes in Sudan. The main activities have focused in the past on testing the diet, the collection of baseline data on larval breeding sites, and the development of a model that predicts temporal and spatial fluctuations in adult populations in the target area, research conducted in collaboration with the Institut National de Recherche en Informatique et en Automatique (INRIA) in France. In 2013 - 2014, field studies carried out in a 20 km^2 pilot site aimed to improve male trapping systems to be able to measure the efficacy of future releases; they confirmed the participation of laboratory-reared sterilized male mosquitoes in swarms. Following the purchase of 3 vehicles by the IDB, mosquito surveillance has started in the 20 km^2 pilot site and releases of sterile males were initiated in an initial 100 Ha area. In addition, a group of 4 experts provided assistance to TMRI staff and a Sudanese engineering company to design a plan for an IDB-funded mosquito mass-rearing facility that will be constructed in Khartoum.

Potential Future Role of the IAEA and the Joint FAO/IAEA Division

Targeting *Anopheles* and *Aedes* species to control malaria, dengue, chikungunya is a high priority

Malaria is still the main vector borne disease in Africa with millions of cases yearly and mortality caused by *Plasmodium falciparum* exacerbated by drug resistance and issues related to poverty. South East Asia faces a similar situation. In Latin America and the Caribbean, where many countries have achieved elimination of the disease, vectors are still present and therefore surveillance and control efforts need to be sustained.

Dengue is a challenge for many Member States because of the increasing number of cases and case fatalities, which often represents the main cause of morbidity and mortality related to infectious disease and is highly influenced by other health determinants. Additionally, traditional control methods for *Ae. aegypti*, the main vector of the disease, are becoming increasingly inefficient and non-cost effective.

The disease caused by chikungunya virus (CHIKV) has been known of since the second half of the 20th century due to outbreaks in Africa, South East Asia and India. In the current century, some European countries have also reported outbreaks, probably attributable to climate change expanding the host species' range. In all cases, *Aedes* species were involved in the transmission, with *Ae. albopictus* considered the main vector. Since 2013, Latin America and the Caribbean region are reporting outbreaks in places where programmes for vector control and outbreak response are relatively weak. The French Caribbean, Dominican Republic, Haiti and Guyana have reported indigenous transmission. There have

also been imported cases reported by other countries, thus it is expected that chikungunya and its vectors will be a challenge to control at both the local and the international level.

Development of AW-IVM including SIT

Current reliance on pesticides and other conventional methods for control of dengue and malaria mosquito vectors is not sustainable and represents a high-risk situation. All the threats discussed above could be addressed by AW-IVM approaches that in some situations may include an SIT component. Therefore all pest control methods, such as the SIT and other genetic and environmentally friendly strategies should be considered potentially useful. The Joint FAO/IAEA Division could play an important role in developing the technology and supporting the implementation of AW-IVM programmes.

Translating evidence into policies

The control of mosquito-borne diseases (crucially malaria, dengue, chikungunya, yellow fever) is currently faced with a number of challenges. These include reliance on the use of pesticides and associated development and spread of vector resistance to insecticides, and changes in the behaviour of vectors to avoid coming into contact with interventions. To address these challenges, new tools such as SIT and other genetic and environmentally-friendly control strategies are urgently needed to complement current strategies. As the FAO/IAEA and partners continue to develop and/or refine these tools for malaria and dengue/chikungunya control, there is a need to fast-track the process of translating this evidence into policy for rapid uptake by Member States. The WHO has a mechanism to review and propose initial recommendations on the use of new tools for vector control; the results of these studies will be essential to inform policy makers. Eventually, these results will be used for planning and implementation of projects, in line with the UN Sustainable Development Goals, post 2015.

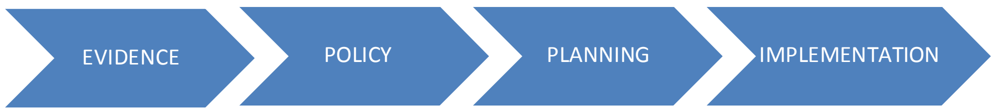


Figure 9. Framework for translating evidence into policy and implementation.

R&D Priorities to Address Bottlenecks

Develop standard quality control protocols for mosquitoes

Mosquito strains that are intended to be used in genetic control programmes are being created in numerous laboratories using different technological platforms (classical genetics, transgenic, symbiont-based) and their number is expected to increase in the near future. Regardless of their origin, it will be useful to develop Standard Quality Control Protocols to evaluate strains' performance in a comparative way. Comparisons of parameters such as development rate, size, mating competitiveness and capacity, dispersal capacity and field

longevity might be considered important factors. Two different phases should be identified for evaluation protocols: (a) male quality comparison in the case of new strains to be evaluated for their performance and (b) male quality control in the case of mass rearing and routine production. Presently, there are no standards for such evaluation / comparison; however, the Joint FAO/IAEA has all the necessary knowledge and expertise to develop such standards in a way analogous to the standards developed for fruit flies (FAO/IAEA/USDA Quality Control Manual 2014: http://www-naweb.iaea.org/nafa/ipc/public/ipc-mass-reared-tephritid.html).

Identification of selectable markers useful for the creation of GSSs

A non-transgenic GSS requires a selectable marker. In contrast to a screenable marker such as the medfly "white pupa," a selectable marker such as a temperature sensitive lethal allows *en masse* selection of individuals that carry one of the alleles when exposed to treatments that might include temperature extremes or various chemicals. Insecticide resistance markers are an obvious choice, but given the possibility that the use of such might present hazards for insects or leave harmful residues, other selectable markers should be identified.

Development of sexing strains in *Aedes* and *Anopheles* species

In the case of SIT application for the suppression of mosquito populations, where the adult female is responsible for spreading the disease-causing pathogen, there is an imperative to make available highly accurate sexing systems in order to reduce to a minimum -ideally to zero- the number of residual females released together with the sterile males. It must be noted that to implement a replacement strategy this is not the case. Differences may exist in the acceptable residual presence of females between disease endemic countries (DEC) where the number should be close to zero and non-DEC, where the acceptable number might be higher, provided that the AW-IVM shows the capacity to sustainably suppress the mosquito population below the epidemiological threshold. Currently, there are no efficient mosquito sexing systems available to be applied in mass rearing facilities. In *Ae. albopictus* the mechanical sexing of pupae by exploiting the sex dimorphism allows recovery of only 20-25 % of the reared males with a residual presence of female pupae of about 1%. If it becomes necessary to reduce the number of residual females further, the recovery rate of males would decrease. Hence, the low performance of mechanical separation strongly affects the productivity of mass rearing and increases the costs of sterile male production. GSSs should be developed either through classical genetics and/or transgenic approaches to be able to reliably eliminate females before any mosquito release.

Develop sex-separation methods that do not require genetically altered strains

Two methods are currently available to eliminate females from release material without transgenic strains or a GSS: pupal size dimorphism in *Aedes* and ivermectin feeding of adult females via the blood in *Anopheles*. These methods still require improvement and additional methods should be explored and further developed.

Development of markers for monitoring

Markers, whether phenotypical/genetic or transgenic, are needed to follow released mosquitoes after release and calculate the efficacy/efficiency of AW-IVM programmes. Through mutagenesis screens, new visible markers should be isolated for easy discrimination of released from wild mosquitoes. In addition, heritable fluorescent markers

should be developed for field use in order to easily recognize and detect the fate of released material through molecular technologies if needed.

Improve large-scale mass rearing systems

In recent years, significant progress has been made to improve the mass-rearing of mosquitoes. Nonetheless, further improvement is required to produce the equipment at a lower cost, minimize the labour required for assembly and cleaning, and ensure that the equipment is suitable to produce high quality males, measured by key biological characteristics. Schematic plans should also be developed for different mosquito species and scales of production.

Refine irradiation procedures for target mosquito species

Irradiation is the standard means to sterilize insects. The response to irradiation dose is species-specific and may also vary between strains of the same species. Therefore, it is necessary to determine the dose response curves for each SIT target species. There is a natural trade-off between sterility level and performance. The optimal dose is selected to produce the highest sterility level without compromising the performance of the sterile insects, and thus the highest capacity to induce sterility in the local population. The dose response curve is now well developed for *An. arabiensis* and *Ae. albopictus* while it still needs to be defined for other species such as *Ae. aegypti*.

Mosquito handling, transport and release

All mosquito suppression programmes would require transport by road or air of large numbers of pupae or adults, which will eventually need to be packed efficiently without compromising their performance. Transporting equipment might need to be combined with aerial release systems so that repackaging is not necessary before release. Promoting design, construction and testing of such equipment would fill an essential requirement that is not likely to be developed in research laboratories. The Agency's existing network of collaborators who utilize such technology would be a strong starting point for creating and validating this technology. Release methods must also be developed considering both pupae and adults, species specific requirements (e.g. dispersal capacity of the released males) and characteristics of the target area (e.g. urban or rural), with the aim of guaranteeing the best possible efficacy of the released sterile males.

Basic ecology and behaviours that affect AW-IVM

Male mating competitiveness (capacity to induce sterility) is a fundamental aspect in successful application of the SIT. Experience with a number of insect species has shown that knowledge and understanding of the specific mating system and the elements that determine mating success are required to adequately assess the mating competitiveness of mass-reared and sterilized insects. It is also needed to determine the optimum mass-rearing protocols. In the case of mosquitoes, it is essential to adequately compare the mating behaviour of wild and mass-reared sterile insects and to assess the compatibility between strains from different geographic origins and/or different genetic backgrounds.

Interspecific competition has been an argument against the use of species-specific control methods. In theory, it could be expected that when one species is suppressed, a competitor species could replace it. This has not been tested empirically, though there are reports that invasion phenomena occurred where *Ae. albopictus* took over *Ae. aegypti* niches and/or co-exist. Research on this subject is desirable.

Exploit symbionts to improve mass rearing and sterile male performance for SIT applications

In many animal systems, including humans, gut-associated microbiota has been shown to play a major role in the biology, ecology and physiology of its hosts including nutrition, immunity and behaviour. In fruit flies, it has been shown that gut-associated bacteria can be given as probiotics to improve the mating behaviour and performance of irradiated males. This is an area which should be exploited for the improvement of mosquito mass rearing and sterile male performance for SIT applications. In parallel, stimulants, vitamins, semiochemicals etc. could also be considered towards the same goal.

Combine SIT and IIT in the absence of efficient sexing systems

Females released along with the sterile males after irradiation have the potential to transmit disease, and can compromise the SIT's efficacy. Because *Wolbachia* can induce pathogen interference in mosquitoes and reduce their vector competence, such concerns should be resolved if those radiation-treated females carry *Wolbachia* transinfections. An additional advantage to the use *Wolbachia*-infected mosquitoes for SIT is reducing the radiation dose required for treatment, thus increasing the male mating performance and resulting in better control efficacy. Developing a strategic partnership between the IAEA and groups working on the IIT to further exploit the potential of combining SIT and *Wolbachia*-based approaches would be advantageous.

Evaluating the efficacy of release programmes

Reliable methods to evaluate both the induced sterility rate and the level of population density suppression in the target population of must be developed to assess the efficacy ofAW-IVM programmes . In the case of *Aedes* species, ovitrapping is the method currently used, which involves collecting eggs and hatching them in the lab with a standard protocol. Useful methods for *Anopheles* species should be investigated and developed.

Priorities for Capacity Building and Other Needs

IPCL personnel for mosquito research should be increased

The mosquito laboratory of the IPCL is an important independent laboratory for new scientific developments and the focal point of international groups developing mosquito control strategies. Current personnel are not sufficient to address the future needs of mosquito control and should therefore be expanded at the professional but also the technical personnel level.

Networking and sharing expertise among Member States

The IAEA has addressed this need through Coordinated Research Projects during the last 10 years and more recently through regional TC projects. Additional efforts should be made and financial support given for the organization of technical panels and workshops on high priority topics.

Training of Member State staff

The IAEA has already supported capacity building in some Member States. For those States that will assess the feasibility of an AW-IVM approach with an SIT component, the IAEA may facilitate onsite training courses or develop, in alliance with other Agencies of the UN or the Member States parties themselves, other models for the education of permanent staff on new technologies in the field of vector borne diseases control.

Research infrastructure needs to be increased

The IAEA could support better research infrastructure and conditions in the Member States by supporting the improvement of medical entomology laboratories through national or regional projects.

Peaceful Uses Initiative

In addition to on-going IAEA-TC projects, the IAEA Peaceful Uses Initiative (PUI) and other sources should be explored for potential extra-budgetary support in support of IPCL activities in the peaceful uses of nuclear technology. Additional funds are now required in several projects where support for pilot suppression trials in the field is requested for proof of principle.

Partnerships and Collaborations

During our discussions, strategic collaborators and partnerships were identified and foreseen for the next decade. The World Health Organization (WHO), the Pan American Health Organization (PAHO) and other existing R&D networks, such as the Eco-Health Network consisting of 17 Asian countries, are primary candidates for the diffusion and the sharing of knowledge on the new approaches to control mosquito-borne diseases.

From a more technical point of view, it is crucial to develop interactions with stakeholders that have been involved in AW-IPM programmes including SIT implementation against other pests. Reinforced partnerships are foreseen with MOSCAMED in Brazil, TRAGSA in Spain, and a new collaboration will be initiated with the INSP-CRISP, Mexico, Chiapas.

The establishment of strategic collaborations with institutes and research centers working on combining SIT with other genetic and environment-friendly control approaches should also be encouraged.

As shown in Table 4, the IPCL mosquito group is a very unique group in view of its role and its on-going partnerships and collaborations (n = 37) throughout the world.

Table 4. On-going IAEA partnerships and collaborations

Institute Name	Country/ Region	Disease/ Vector	Collaboration Type	Approach

IRD-CRVOI	La Reunion (France) / Africa	*Ae. albopictus* / Chikungunya	Partnership / Training center	Classical SIT
Institut Regional de la Santé publique	Benin / Africa	*Anopheles* species / Malaria	Partnership	Classical SIT
Institut de Recherche en Sciences de la Santé	Burkina Faso / Africa	*Anopheles* species / Malaria	Partnership	Classical SIT
Tropical Medicine Research Institute	Sudan / Africa	*An. arabiensis* / Malaria	Partnership, TC SUD5034	Classical SIT
Vector Control Reference Unit National Institute for Communicable Diseases	South Africa / Africa	*An. arabiensis* / Malaria	Partnership, TC SAF5013	Classical SIT
Vector Biology And Control Division; Ministry Of Health And Quality Of Life	Mauritius / Africa	*Ae. albopictus* / Dengue, Chikungunya	Partnership, TC MAR5019 TC RAF5072	Classical SIT
Ministry Of Health And Social Services	Seychelles / Africa	*Ae. albopictus* / Dengue, Chikungunya	Partnership, TC RAF5072	Classical SIT
National Malaria Control Program (NMCP), Ministry Of Public Health Service Of Vector Control	Madagascar / Africa	*Ae. albopictus* / Dengue, Chikungunya	Partnership, TC RAF5072	Classical SIT
IRD La Reunion / CRVOI	La Reunion (France)/ Africa	*Ae. albopictus* / Chikungunya	CRP	GSS - Classical genetics
OCEAC	Cameroun / Africa	*An. arabiensis* / Malaria	CRP	GSS - Classical genetics
Vector Control Reference Unit National Institute for Communicable Diseases	South Africa / Africa	*An. arabiensis* / Malaria	CRP	Genetic sexing and insecticide resistance
Institut Louis Malardé	French Polynesia / Asia-Pacific	*Ae. Aegypti, Ae. polynesiensis* / Dengue, Filariasis	Partnership, CRP on GSS- Mechanical method	IIT
Anti-Malaria Campaign University Of Kelaniya, Faculty Of Medicine	Sri Lanka / Asia-Pacific	*Ae. Aegypti, Ae. albopictus* / Dengue, Chikungunya	Partnership, TC RAS5066	Classical SIT
Nuclear Institute For Food And Agriculture & Pakistan Atomic Energy Commission	Pakistan / Asia-Pacific	*Ae. Aegypti, Ae. albopictus* / Dengue, Chikungunya	Partnership, TC RAS5067	Classical SIT

Faculty of Science Mahidol University National Institute Of Health Department Of Medical Sciences Thailand Institue Of Nuclaear Technology	Thailand / Asia-Pacific	*Ae. Aegypti, Ae. albopictus* / Dengue, Chikungunya	Partnership, TC RAS5068	Classical SIT
Philippine Nuclear Research Institute	Philippines / Asia-Pacific	*Ae. Aegypti, Ae. albopictus* / Dengue, Chikungunya	Partnership, TC RAS5069	Classical SIT
Center For The Application Of Isotope And Radiation Technology	Indonesia / Asia-Pacific	*Ae. Aegypti, Ae. albopictus* / Dengue, Chikungunya	Partnership, TC RAS5070	Classical SIT
Institute For Medical Research	Malaysia/Asia-Pacific	*Ae. Aegypti, Ae. albopictus*/Dengue, Chikungunya	Partnership, TC RAS5071	Classical SIT
NIFA	Pakistan / Asia	*Ae. albopictus* / Dengue, Chikungunya	CRP	GSS- Mechanical method
University of Kelaniya	Sri Lanka / Asia-Pacific	*Ae. albopictus* / Dengue, Chikungunya	CRP	GSS- Mechanical method
Sun Yat-sen University-Michigan State University Joint Center of Vector Control for Tropical diseases; Guangzhou Center For Disease Control And Prevention	China / Asia-Pacific	*Ae. albopictus* / Dengue, Chikungunya	Partnership, TC RAS5072	Combination SIT and IIT
Moscamed	Brazil / Latin America	*Ae. aegypti* / Dengue	Partnership / Training center	Classical and transgenic SIT
Universidade de Sao Paulo Instituto de Ciencias Biomedicas	Brazil / Latin America	*Ae. aegypti* / Dengue	CRP	GM
Virginia Tech	USA / Latin America	*Anopheles* species / Malaria	CRP	GSS - Molecular approach
Harvard School of Public Health	USA / Latin America	*Anopheles* species / Malaria	CRP	GSS - Molecular approach
University of Maryland	USA / Latin America	*Anopheles* species / Malaria	CRP	GSS - Molecular approach
Instituto de Medicina Tropical "Pedro Kourí"	Cuba / Latin America	*Ae. aegypti* / Dengue	CRP	GSS - Classical genetics

CAA-IAEA Collaborating Center	Italy/Europe	*Ae. albopictus* / Chikungunya	Partnership / Training center	Classical SIT
TRAGSA	Spain / Europe	*Ae. albopictus* / Dengue, Chikungunya	Partnership / Training center	Classical SIT
Fraunhofer-Institute for Molecular Biology and Applied Ecology (IME)	Germany / Europe	Ae. aegypti / Dengue	CRP	GSS - Molecular approach
Polo d'Innovazione di Genomica, Genetica e Biologia	Italy / Europe	*Anopheles* species / Malaria	CRP	GSS - Molecular approach
Centro Agricoltura Ambiente "G.Nicoli"	Italy / Europe	*Ae. albopictus* / Dengue, Chikungunya	CRP	GSS- Mechanical method
Dipartimento di Biologia e Biotecnologie Universita degli Studi di Pavia	Italy / Europe	*Ae. albopictus* / Dengue, Chikungunya	CRP	GSS - Molecular approach
ENEA	Italy / Europe	*Ae. albopictus* / Dengue, Chikungunya	CRP	GSS- Mechanical method
TRAGSA	Spain / Europe	*Ae. albopictus* / Dengue, Chikungunya	CRP	GSS- Mechanical method
The Pirbright Institute	UK / Europe	*Anopheles* and *Aedes* species / Malaria and Dengue	CRP	GSS - Molecular approach
Imperial College London	UK / Europe	*Anopheles* species / Malaria	CRP	GSS - Molecular approach

Future Opportunities for Application of AW-IVM

Integrate paratransgenesis in SIT programmes

Paratransgenesis refers to the use of mosquito midgut bacteria engineered to secrete anti-*Plasmodium* proteins, to inhibit the spread of malaria. Initial laboratory experiments show great potential to cause strong inhibition (up to 98%) of parasite development. While technical and regulatory issues are still to be resolved, this approach has promising possibilities. An important aspect of the paratransgenesis strategy is that it is compatible with, and can complement, existing control approaches such as SIT, insecticide-impregnated bednets and indoor residual spraying. For example, if implementation of SIT in a given target area successfully reduces the vector population, paratransgenesis could be employed to dramatically reduce the vectorial competence of the remaining mosquitoes. Another example would be if malaria is completely eliminated in a given target area by reducing the vectorial competence of the existing vector, paratransgenic mosquitoes refractory to the pathogen would prevent the re-introduction of the disease due, for example, to the migration of infected people into the area. In the future, paratransgenesis could effectively

complement implementation of technologies (e.g. SIT) developed by the IAEA. Finally, there is evidence that suggests that certain bacteria can be sexually transmitted from male to female mosquitoes during copulation. Thus, the effectiveness of SIT could be enhanced by releasing sterile male mosquitoes that carry engineered bacteria. This would render the field females with which the SIT males mate not only sterile but also unable to transmit the parasite.

Sterile males as carriers of pathogens or insecticides for auto-dissemination

In addition to inducing sterility, sterile males can be used as carriers of pathogens or insecticides to be horizontally transmitted into wild populations. This can be achieved naturally or through the use of adhesive powders. The advantage of this approach, known as auto-dissemination, is that the killing agent is directly targeted and the amount of product released into the environment is substantially reduced without reducing the impact on the target organism. The approach has been successfully tested with sterile fruit flies carrying spores of entomopathogenic fungi and transmitting this to wild flies through intra and intersexual interactions. The above mentioned approach might be a future application of the SIT for the control of mosquito vectors.

RECOMMENDATIONS TO IAEA

General Recommendations

- Malaria, dengue, chikungunya and yellow fever have astounding global effects on human mortality and morbidity. Except for yellow fever for which an effective vaccine exists, vector interventions are the most effective means of control. Fortunately, a handful of mosquito species are responsible for much of the transmission. The above directs the Agency efforts to develop SIT and related approaches against specific species that are responsible for most of the mortality and morbidity; the Agency is in a pivotal position to contribute toward reduction of these global scourges.

  We therefore recommend that the Agency should invest in supporting the control of the mosquito vector species which transmit these diseases through continuous funding of the development of the SIT and other related genetic and environment-friendly methods. Control projects should be supported and applied following an Area-Wide Integrated Vector Management approach.

- The Agency should continue to emphasize control of members of the *Anopheles gambiae* complex (and other important malaria vectors), *Aedes aegypti* and *Aedes albopictus*. These few species alone are highly-significant targets.

  We therefore recommend that the Agency continues assisting in developing effective interventions using SIT and other related species-specific technologies.

- The Agency has a unique role unmatched by any other institution in developing methods, evaluation and standards for producing, releasing and monitoring insects used in SIT and related technology programmes.

  We therefore recommend the Agency continues these R&D and technology transfer activities.

- In addition to classical SIT, there are other technological platforms available which might be useful for the control of mosquito -borne diseases based on transgenic and symbiont-based approaches.

We therefore recommend the Agency continues the support of R&D in these areas and exploit their potential integration with classical SIT.

- The previous accomplishments of the IPCL in developing mass-rearing technology, sex-separation and sterilization methods should be further refined and disseminated into Member States.

  We therefore recommend that the IPCL continues these activities; however, to accomplish this, in an effective and timely manner, there must be an increase in support for personnel and facilities.

- Nuclear technology is an important tool for the development and application of SIT against mosquito species. However, significant challenges still exist in respect to the shipment of gamma cells to end-users or the efficient and robust use of X-rays.

  We therefore recommend the Agency investigates efficient, environment-friendly and economically affordable irradiation-induced sterility methods for SIT.

- The network of collaborating projects should be strengthened through training in the use of technology and its application for pilot and eventually large scale projects.

  We therefore recommend that the Agency continues providing support in the transfer of technology and capacity building to Member States for the control of mosquitoes via an AW-IVM approach with an SIT component.

- Dissemination and outreach activities, including novel IT platforms are required, to support and expand AW-IVM projects with an SIT component against mosquito species.

  We therefore recommend that the Agency provides support in this area and facilitates their transfer to Member States.

- Control of mosquitoes and mosquito-borne diseases require significant mobilization of resources.

  We therefore recommend that the Agency, in cooperation with Member States, continues to seek strategic partnerships

and mobilization of funds to support AW-IVM approaches with an SIT component for mosquitoes.

SPECIFIC RECOMMENDATIONS

Pilot Projects

Current SIT developments allow the implementation of pilot projects. To implement successful pilot projects, however, the IAEA should assess the economic, social and ecological feasibility and requirements and should provide technical expert advice. These pilot projects will serve to scale up and further develop the AW-IVM, including the SIT, and will contribute to analyze the feasibility of its use under a wide range of conditions. We therefore recommend:

- ➢ *Development of an inter-regional project on dengue and chikungunya.*
- ➢ *Development of a regional Africa project on malaria.*
- ➢ *Support of national pilot projects in countries with the ability to work on integrating the SIT and other related approaches. These should be specific for each target vector and disease.*
- ➢ *Prioritize support to these countries on the proof of principle of SIT and other related approaches for each disease and vector combination.*
- ➢ *Provide technical assistance to establish mass rearing facilities for mosquitoes to support of SIT and other related approaches.*
- ➢ *Develop regional training and collaborating centers for SIT and other related approaches against mosquitoes.*
- ➢ *Establish links and collaborate with regional networks to inform about the SIT and develop more pilot projects.*
- ➢ *Conduct a benefit-cost analysis for the implementation of mosquito SIT applications on a large scale.*

Translation into policy

Results from research and development and evidence from practical field applications from the past 10 years indicate that AW-IVM, including the SIT, is a feasible additional tool to deal with malaria, dengue, chikungunya, and yellow fever diseases.

We therefore recommend that AW-IVM should be incorporated into public health policies within a holistic approach. The existing WHO policy setting mechanism should be used to review the evidence and make initial recommendations to Member States.

Harmonization between the WHO and the IAEA will be required to build capacity through training, expert advice, support of national, regional and interregional projects, improvement of the technology, standards development, and encouragement of public engagement and awareness.

We therefore recommend that the two Agencies establish a MoU or Practical Arrangement to facilitate harmonization and alignment of joint activities.

It is important to provide evidence-based support on the use of other available genetic control strategies, in addition to the classical SIT, (e.g. transgenic or symbiont-based approaches).

We therefore recommend that the Agency should continue providing technical and policy advice on existing or any new technology towards the control of mosquito populations.

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ANNEXES

Table S1. Advantages and disadvantages/limitations of current malaria prevention and control methods directed against vectors in the adult and immature stages.

Methods	Advantages	Disadvantages/Limitations
	Adult stage[a]	
Insecticide-treated mosquito nets	• Barrier against human-vector contact • Enhances vector mortality and reduce transmission • Numerous designs available to suit house structures	• Nets can only be treated with pyrethroids • Insecticide resistance limits its effectiveness
Indoor residual spraying	• Effective in reducing vector population and transmission • Can be used to manage insecticide resistance by the rotation of different classes of insecticides	• The method is labour intensive • Appropriate training is needed • Require maintenance of equipment, quality assurance, monitoring and evaluation. • Household compliance and support is essential
	Immature stages[a]	
Larviciding	• Reduces vector abundance • If done appropriately, this method can contribute to insecticide resistance management. • Overall improvement of human environment.	• This method may be expensive • Impact on vector abundance is difficult to monitor and evaluate • Not applicable in certain environments such as nature of breeding sites (e.g. small puddles, hoof prints)

[a]Core interventions towards the prevention and control of malaria are directed against the adult stage while larviciding is considered as supplementary interventions

Table S2. Advantages and disadvantages/limitations of current dengue and chikungunya prevention and control methods directed against vectors in the immature stages.

Methods	Advantages	Disadvantages/Limitations
Larval sites management	• Effectively removed larval habitats reduces vector population • Cost-effective • Environmentally safe	• Cryptic or inaccessible larval habitats pose a challenge to source reduction • Without active community engagement, this strategy can be expensive and difficult to implement
Larvicides	• Reduce vector population when source reduction is difficult to achieve/implement • No resistance has been reported for Bti	• Resistance to organophophates and Biocides have been documented • Biocides may be expensive for large scale operations • Alternative larvicides are available but awaiting regulatory approval
Release of larvivorous fish and copepods	• Don't require the need for chemical intervention • Eco-friendly alternative method to insecticides • Promotes local economy by stimulating employment	• Can be costly and labour intensive to mass rear for large-scale operational use • Community may dislike the idea of putting live organisms into their domestic containers. • These organisms may carry pathogens of public and veterinary importance • There is limited evidence in Vietnam to show the effectiveness of copepods to reduce vector density

Table S3. Advantages and disadvantages/limitations of current dengue and chikungunya prevention and control methods directed against vectors in the adult stages.

Methods	Advantages	Disadvantages/Limitations
Space spraying	• Reduction of mosquito population (including infective adults) in the short term, thus breaking chains of transmission or reducing the intensity of transmission • Using resistance studies to better manage resistance • Political support	• Can increase selection pressure for resistance • Cannot not be used in areas where insecticide resistance occurs • Negative effect of space spraying on the environment and non-target organisms • Poor community support for this type of programme limits its effectiveness • Expensive and requires proper maintenance, monitoring and evaluation • No evidence that this method is effective for indoor mosquitoes • Harmful effect to person suffering from respiratory illnesses
Insecticide impregnated materials	• Prevent entry and reduce indoor vector populations • Use of uniforms impregnated with pyrethroids may also prevent man-vector contact or kill mosquitoes that alight on these materials	• The insecticide used in the treatment of these materials is similar to those used for space spraying, thus may increase the chance of mosquitoes developing resistance • There is limited evidence to show impact of ITMs against *Aedes*-borne diseases • Duration of efficacy and durability of materials
Lethal ovitraps	• Can be used for xenomonitoring, surveillance and control • Target-specific against container inhabiting mosquitoes	• Only effective in places where mosquitoes are still susceptible to the insecticide used • Can be a potential larval habitat if not properly

	• Prevents man-vector contact • Simple to prepare • Large number of eggs are trapped and removed from the environment	maintained • Highly labour intensive • Limited evidence to show the effectiveness of these traps to reduce vector density or transmission intensity • Absence of cost-benefit analysis
Sticky/gravid ovitraps	• Can be used for xenomonitoring, surveillance and control • Target-specific against container inhabiting mosquitoes. • Immatures collected can be used for insecticide resistance studies • Prevents man-vector contact • Simple to prepare • Many designs are available	• The sticky lining can be very expensive for large scale operational use • Can be a potential larval habitat if not properly maintained • Highly labour intensive • Limited evidence to show the effectiveness of these traps to reduce vector density or transmission intensity • Absence of cost-benefit analysis

Agenda

Monday 16th of June 2014 **Room: M0E13**		
Time	Session	Presenter
08:00 – 09:00	Registration with passport at Gate 1 to obtain ground passes	
09:00 – 09:30	Welcome remarks	Daud Mohamad, DDG-NA Ana Raffo, DIR-TCPC and Acting DDG-TC
09:30 – 09:45	IAEA Technical Cooperation Programme Overview	Oscar Acuña, Section Head (1)TCAP
09:45 – 10:00	FAO/IAEA support to the development and application of the Sterile Insect Technique	Jorge Hendrichs, Section Head NAFA-IPCS
10:00 – 10:30	Mosquito team activities – Development of an SIT package for disease transmitting mosquitoes.	Jeremie Gilles, IAEA-IPCL
Coffee break		
10:45 – 11:15	Sterile Insect Technique successful applications in Mexico: Implications for mosquitoes control	Jose Pablo Liedo Fernandez, Mexico
11:15 – 11:45	Developments and needs for mosquito control	Marc Florian Schetelig, Germany
11:45 – 12:15	Current *Aedes aegypti* control tactics – their advantages and limitations	Dave Chadee, Trinidad and Tobago
12:15 – 12:45	Global policy updates on malaria vector control - where are we with the insect sterile technique?	Abraham Mnzava, World Health Organization (WHO)
Lunch Break		
14:00 – 14:30	Advances in *Aedes albopictus* mass breeding and quality control for SIT application	Romeo Bellini, Italy
14:30 – 15:00	Surveillance and control of dengue and chikungunya in Thailand	Pattamaporn Kittayapong, Thailand

15:00 – 15:30	*Wolbachia*-mediated pathogen interference and cytoplasmic incompatibility in mosquitoes and their use for vector-borne disease control	Zhiyong Xi, China
Coffee break		
15:45 – 16:15	Target Malaria: Controlling mosquito vectors of malaria with engineered endonucleases	Mark Benedict, USA
16:15– 16:45	Fighting malaria with engineered symbiotic bacteria from vector mosquitoes	Marcelo Jacobs-Lorena, Brazil
16:45– 17:15	Threaten of insecticide resistance on current vector control strategies in Burkina Faso: alternatives for wide integrated resistant vectors' management.	Rock K. Dabire, Burkina Faso
17:15– 17:45	*Aedes* surveillance and control in Singapore Government	Cheong Huat TAN, Singapore
17:45– 18:15	Mosquito nuisance and control in Sweden	Jan O. Lundström, Sweden
19:00 – 21:00	Social event	

Tuesday 17th of June 2014 **Room: M0E13**		
Time	Group Discussion Session	Chairperson / Rapporteur
09:00 – 10:30	Analysis of background situation and general trends	Marcelo Jacobs-Lorena, Brazil / Mark Benedict, USA
Coffee break		
11:00 – 12:30	Analysis of background situation analysis and general trends (cont.)	Marcelo Jacobs-Lorena, Brazil / Mark Benedict, USA
Lunch Break		

14:00 – 15:30	Current technical/management constraints and gaps for sustainable mosquito control and areas where the IAEA can contribute	Dave Chadee, Trinidad and Tobago / Pattamaporn Kittayapong, Thailand
Coffee break		
16:00 – 17:30	Current and potential future role of the IAEA Mosquito Programme and long-term prospects of AW-IVM programmes with SIT/genetic control components	Kostas Bourtzis, IAEA-IPCL / Romeo Bellini, Italy

Wednesday 18th of June 2014 **Room: M0E13**		
Time	Group Discussion Session	Chairperson /Rapporteur
09:00 – 10:30	Priorities in terms of R&D needs to address bottlenecks	Jeremie Gilles, IAEA-IPCL / Marc Schetelig, Germany
Coffee break		
11:00 – 12:30	Priorities in terms of capacity building, technology transfer and other needs	Mark Benedict, USA / Marcelo Jacobs-Lorena, Brazil
Lunch Break		
14:00 – 15:30	Future Opportunities of application of AW-IVM	Pattamaporn Kittayapong, Thailand / Roch Dabire, Burkina Faso
Coffee break		
16:00 – 17:30	Potential IAEA partners and collaborating countries and institutions	Romeo Bellini, Italy / Zhiyong Xi, China

Thursday 19th of June 2014 **Room: M0E13, (and M0E18, M0E19)**		
Time	Drafting Session	Chairperson /Rapporteur

09:00 – 10:30	Drafting in Subgroups	
Coffee break		
11:00 – 12:30	Drafting in Subgroups (cont.)	
Lunch Break		
14:00 – 15:30	Drafting in Subgroups (cont.)	
16:00 – 17:30	Group Discussion: Recommendations to IAEA and activities and resources required	Jose Pablo Liedo Fernandez, Mexico / Cheong Huat Tan, Singapore

Friday 20th of June 2014 **Room: M0E13**		
Time	Group Review Session	Chairperson
09:00 – 10:30	Group review of the Thematic Plan Report and Presentation	Kostas Bourtzis
Coffee break		
11:00 – 12:30	Group review of the Thematic Plan Report and Presentation (cont.)	Kostas Bourtzis
Lunch Break		
14:00 – 15:30	Presentation to TC and NA officials and formal closing	Marc Schetelig, Germany
Coffee break		
16:00 – 17:30	Group review and finalizing the Thematic Plan Report based on feedback received	Marc Schetelig, Germany

Abstracts of Experts' Presentations

Sterile Insect Technique successful applications in Mexico and implications for mosquito control

Jose Pablo Liedo Fernandez, Mexico

The Sterile Insect Technique (SIT) is an environmental friendly pest control method. The idea was developed and proposed by Knipling and colleagues and first applied to eradicate the Screwworm, *Cochliomyia hominivorax,* from the island of Curazao in 1955. Since then, it has been developed and used against important insect pests in different parts of the world.

In Mexico, it was first used against the Screwworm soon after the success in Curazao, Florida and the South USA. In a period of 17 years this pest was eradicated from Mexico and right now there is a containment barrier in Panama. In the late 1970's it was decided to use the SIT to prevent the introduction of the Mediterranean fruit fly, *Ceratits capitata* into Mexican territory. A joint USA, Guatemala and Mexico programme was established and this programme has been able to stop the northward spread of the pest and keep free Mexico and the US. Finally, in the 1990´s it was decided to use the SIT for the control of native fruit flies. Today over half of the national territory is considered free of these fruit flies and the sterile flies are produced to protect areas of low prevalence.

A common trait of these programmes is the Integrated Pest Management (IPM) area-wide approach. The SIT requires this area-wide approach and is important to understand that for successful applications is necessary to have low densities of the target population. In many cases it is necessary to first suppress the wild populations, or take advantage of naturally low populations.

This IPM area-wide approach, incorporating the SIT, could represent a sustainable alternative for the control of mosquito vectors of human diseases. This is particularly important in urban and/or suburban locations where dengue fever is an important health problem, there is also the thread of chikungunya, and current control alternatives rely heavily on the use of insecticides. The positive experience integrating the SIT against cattle and agricultural pest could serve as the basis for the development of IPM area-wide SIT mosquito control programs to protect sustainably urban areas.

Developments and needs for mosquito control

Marc F. Schetelig, Germany

Environment friendly sterile insect technique (SIT) is being applied effectively as a component of area-wide integrated pest management (AW-IPM) for agricultural pests since the 1960s. Lately, the development of several genetic technologies opened up the possibility to use SIT in several additional pest species. In particular, highly efficient sexing systems and directed transformation systems for increased flexibility will enable the generation, evaluation, and use of SIT against several fruit fly species. As an example, transgenic embryonic sexing strains developed for *Ceratitis capitata* and *Anastrepha suspensa* had 100% male-only progeny, with female death limited primarily to embryogenesis. In large-scale tests, more than 30,000 eggs from two strains resulted in 100% male-only progeny. In contrast, the development of SIT for mosquitoes is still challenging in the areas of markers, sexing, and mass rearing for several key mosquito species.

Here, several systems developed for agricultural pests will be presented and a transfer to mosquitoes will be discussed. First focus will be on the isolation of highly specific and effective cell death genes for controlled lethality systems. Second, the possibility of sexing systems for mosquitoes, genetic or

transgenic, will be discussed. The elimination of human disease transmitting females before release is important for the efficiency as well as the security of mosquito SIT programmes. Therefore, sexing systems will be the key to start an SIT for several mosquito species and improve mass rearing for these species. Third, the possibility to combine several advantageous traits at evaluated genomic positions will be discussed as a tool to combine and compare different systems in a scientific way. Such technologies will help to evaluate the benefits but also the downsides of a variety of mosquito strains for vector control.

Current *Aedes aegypti* control tactics – their advantages and limitations

Dave D. Chadee, Trinidad y Tobago

Dengue fever has emerged as the main cause of hospitalization of children in South East Asia and is the main arbovirus disease reported in the Americas. The global epidemiology pattern is described and reviewed in the context of traditional vector control measures. The reasons for failure of traditional vector control measures are described and discussed, highlighting the need for community support as well as political will and the application of suitable control strategies. Based on the evidence provided, there is need for the adoption of traditional and alternative vector control strategies. With the application of these combined strategies a more effective management programme can be developed which can exert a more significant impact on vector populations thereby reducing morbidity and mortality rates and reducing the impact of future outbreaks of dengue at the local, regional and global levels.

Global policy updates on malaria vector control - where are we with the insect sterile technique?

Abraham Mnzava,World Health Organization (WHO)

Since 2000, a tremendous expansion in the financing and coverage of malaria control programmes has been mirrored by a wide-scale reduction in malaria incidence and mortality, reversing a decades-long trend of neglect and inaction that led to the deaths of tens of millions of people, most of them young children in Africa. Worldwide, between 2000 and 2012, estimated malaria mortality rates fell by 45% in all age groups and by 51% in children under 5 years of age. On the African continent, the estimated reductions were slightly greater: 49% and 54% respectively.

In terms of policy, for a very long time, there has been no functional policy-setting mechanism for malaria vector control. It was for this reason that WHO established two committees to address this problem. The first was the Vector control Advisory Group (VCAG) jointly run by the Global Malaria Programme (GMP) and the Neglected Tropical Diseases (NTD) departments which makes recommendations on new forms/tools/technologies on vector control.

The other committee – Technical Expert Group (VCTEG) is tasked with reviewing and providing guidance on the implementation of malaria vector control - including issues related to programme management. Outcomes of the recent VCTEG recommendations include sustaining universal coverage with LLINs (including estimation of access); capacity building in entomology and vector control; estimating the longevity of LLINs under field conditions; management of old LLINs; and justification for combining IRS and LLINs.

The gains recorded in the past decade as a result of scaling up vector control interventions with the use of LLINs and IRS are very fragile. In addition to unstable resources, maintaining the effectiveness of these tools is threatened by the development and spread of insecticide resistance as well as the increased tendency of vectors to bite in places and times where IRS and LLINs are not practical. To address both threats requires development and deployment of new tools. One of such tools is the Insect Sterile Technique currently pursued by the International Atomic Energy Agency (IAEA) with their partners. It is therefore recommended that when IAEA would have collected sufficient evidence on the contribution of this technique to malaria control, they would need to submit their claim to the VCAG for appropriate policy recommendation by WHO. The following is the link on how VCAG operates and how potential innovators submit their dossiers for review by WHO - http://www.who.int/neglected_diseases/vector_ecology/VCAG/en/

Advances in *Aedes albopictus* mass breeding and quality control for SIT application

Romeo Bellini, Italy

The SIT strategy requires the mass production of the target species itself and the release of males only. Studies have been conducted at the CAA to develop the mass rearing technology and to evaluate the feasibility of the SIT approach against *Aedes albopictus*, the most important invasive mosquito species in the world.

The egg production phase was investigated by testing several cage prototypes according to adult densities and protocols designed to reduce labor requirements.

The larvae/pupae productivity per lab space unit has been progressively increased by testing the interaction between larval diet components, diet doses, larval density, water depth and temperature in the tray, tray shape, up to 2, 3, 4 larvae/ml.

In non-GM strains the sex separation still remains an open issue as the output provided by the sieving method performed on the pupal stage in the water allow the recovery of about the 20% of the reared males to keep residual females at very low level.

Quality control of reared males is conducted in the lab by testing male flight ability, male mating ability and male longevity, while mating competitiveness studies are conducted from 2006 in semi-field condition using simple large enclosures (8 x 5 x 2.8 m).

In our semi-field module the competitiveness index (CI) shows high variability levels between replications making the model not very sensitive in strains comparison. The CI value for the best performing radio-sterilized males resulted 0.96. Extrapolation of semi-field results to real field situation remains questionable.

We also conducted field releases of radio-sterilized males to investigate possible release methods, planning the release timing and evaluating the impact of sterile males on the local population.

Surveillance and control of dengue and chikungunya in Thailand

Pattamaporn Kittayapong, Thailand

Dengue and Chikungunya are important vector-borne diseases worldwide. The outbreak of dengue has occurred in Thailand since 1958. In the past 5 years, the total dengue cases varied from 56,651 to the highest of 150,934 in 2013 with the case fatality rate per 100,000 populations of 0.05-0.13. The major epidemic of Chikungunya occurred throughout Thailand in 2009 in which 52,057 cases, without case

fatality, were reported. The major vectors of dengue and Chikungunya in Thailand are *Aedes aegypti* and *Ae. albopictus* respectively.

Aedes vector surveillance is routinely conducted throughout the country by public health office of each province using health volunteers. Ovitrap has been used as a tool to estimate the mean number of egg laid in research and immature surveillance has routinely been conducted in practice in order to calculate larval and pupal indices. Surveillance of adult vectors was initiated by research using several techniques, i.e., portable vacuum aspirator, BG sentinel trap, and sticky ovitrap.

Dengue vector control in Thailand depends mostly on the use of larvicides, i.e., temephos and Bti, applied to household breeding containers. Chemical fogging to kill adult mosquitoes is conducted routinely especially within 100 meters around reported dengue case houses. Other preventive measures are health education and public awareness campaign operated by each provincial health offices. Control of Chikungunya vectors has been focused on pyrethroid space spraying in combination with source reduction and health education especially when there was disease outbreak. Nation-wide prevention and control of dengue and Chikungunya through vector control was not effective in Thailand. Innovative methodologies, i.e., insecticide-treated materials (ITM) and integrated vector management (IVM), combined with community participation have been proposed and emphasized. So far classical SIT trial to reduce dengue and Chikungunya mosquito vectors has never been conducted in Thailand despite the feasibility of technical support for sterilization and the potential field site operation.

Wolbachia-mediated pathogen interference and cytoplasmic incompatibility in mosquitoes and their use for vector-borne disease control

Zhiyong Xi, China

Diseases transmitted by blood-feeding arthropod vectors, such as mosquito-borne malaria and dengue fever, cause 1.5 million human deaths every year. The insufficiency of currently available strategies, including vaccines, drugs, and pesticides, has led to an increase in vector-borne diseases. The endosymbiotic bacterium Wolbachia is widely recognized for its potential as a vehicle to introduce disease-resistance traits into mosquitoes, making them refractory to the human pathogens they currently transmit. This is due to its ability to induce both a reproductive abnormality known as cytoplasmic incompatibility and a resistance to human pathogens, including malaria and dengue virus, in mosquitoes. Two Wolbachia-based control strategies are currently under development and tested in the field trial. Population suppression, or Incompatible Insect Technique (IIT), is a strategy in which Wolbachia-infected males are released to induce sterile matings, resulting in suppression or even area-wide eradication of target populations. Alternatively, Wolbachia-based population replacement emphasizes to modify the mosquito populations through female release such that they will no long to be hospitable to human pathogens, resulting in permanent crash of disease transmission. In order to implement these two strategies, we had developed a highly efficient approach to transfer Wolbachia into target disease vectors and force Wolbachia to form symbiosis with the novel hosts. We discovered that Wolbachia conferred resistance to both dengue and malaria on the mosquitoes which used to be primary disease vectors and highly compatible to those pathogens, and this pathogen interference was determined by Wolbachia-mediated production of reactive oxygen species (ROS). By seeding the Wolbachia-infected mosquitoes into wild populations, Wolbachia successfully invaded into laboratory populations within seven generations. With the regulation approval in China, we now are preparing for a field release to test how population suppression and replacement can be integrated to reach a rapid and sustainable control of disease transmission.

Target Malaria: Controlling mosquito vectors of malaria with engineered endonucleases

Mark Q. Benedict, Italy

Achieving sustainable and inexpensive approaches for controlling mosquitoes that transmit malaria is perplexing in disease-endemic developing countries. While bednets and indoor-residual spraying have reduced transmission, additional technology that is affordable and durable is needed to complement these. The Target Malaria project aims to develop inexpensive technology for suppressing *Anopheles gambiae* mosquitoes using homing- and other endonucleases. Several hurdles lie between the proof-of-concept and implementation in the field including mating competitiveness, fitness, acceptability, regulatory approval and demonstration of effectiveness against genetically diverse vector mosquito populations.

A facility has been established in Perugia, Italy, specifically for the purpose of testing performance of transgenic mosquitoes in larger enclosures than are typical for insectaries that create proof-of-concept mosquitoes for control. Such testing is a vital intermediate step in moving mosquitoes from the bench to the field. These activities are being conducted in a research environment that is transitioning to Good Laboratory Practices that will produce data that can satisfy regulatory review.

Much uncertainty exists regarding conditions that simulate the most salient factors to measure in the laboratory that also reflect field performance. Toward this end however, we have demonstrated that conditions can be created in which *A. gambiae* males will swarm. We believe this augments observations of mating behavior that occurs in the absence of swarming and provides a more realistic test of performance than when swarming does not occur. I will describe these activities and other efforts to develop supporting information needed to address risk and effectiveness assessments.

Fighting Malaria With Engineered Symbiotic Bacteria From Vector Mosquitoes

Sibao Wang and Marcelo Jacobs-Lorena, Brazil

The unbearable burden of malaria demands the urgent development of novel approaches to fight this deadly disease. Technical advances in vector biology have allowed the development of a new strategy to combat malaria, by genetically modifying the mosquito to reduce its vectorial competence. However, one crucial unresolved aspect of this approach is how to introduce transgenes into wild mosquito populations ("genetic drive"). Several strategies have been proposed, but a number of technical hurdles have yet to be overcome.

We are exploring an alternative approach based on the fact that the mosquito, as all higher organisms, carries a microbiota in its midgut. Rather than genetically modifying mosquitoes, our strategy is to genetically modify symbiotic bacteria for delivering anti-malarial effector molecules into the mosquito midgut (paratransgenesis). It is in the midgut that the most vulnerable stages of *Plasmodium* development in the mosquito take place. We have shown that the bacterium *Pantoae agglomerans* engineered to express and secrete anti-*Plasmodium* effector molecules strongly inhibits *Plasmodium* development in the mosquito by up to 98%.

Recently, we have identified another mosquito symbiotic bacterium of the genus *Serratia* that is

transmitted both vertically from female to larval progeny and horizontally from male to female during mating. These transmission properties suggest that it should be possible to introduce recombinant *Serratia* into mosquito populations in the field for the purpose of malaria control. This approach is 'low-tech' and does not involve the introduction of a new species of bacteria, since *Serratia* is a natural component of the African mosquito microbiota.

Transgenesis, paratransgenesis and other malaria-fighting tools are complementary approaches that ideally can be used simultaneously for maximum effectiveness.

Threaten of insecticide resistance on current vector control strategies in Burkina Faso: alternatives for wide integrated resistant vectors' management.

Roch K. Dabire, Burkina Faso

Pending

Mosquito nuisance and control in Sweden

Jan O. Lundström, Sweden

Mosquitoes and mosquito nuisance are important topics in Sweden. In the last 14 years it is becoming evident that mosquitoes are causing massive nuisance affecting humans and society in a very negative way. This has prompted the government to allow mosquito larvicidal activities using Bti in a National Park, in several Nature Reserves, and in a large number on Natura 2000 sites. The main nuisance species is *Aedes sticticus*, an aggressive and day-active mosquito that actively disperse 5-15 km in a few days, thus causing nuisance within a very large area around the temporary flooded wetlands that are used as larval habitats. In the floodplains of River Dalälven up to 77,000 mosquitoes (mainly Aedes sticticus) per night is collected in CDC-traps, compared to the maximum of 2500 per trap and night collected in areas with only snow pool species. The nuisance caused by these mosquitoes are forcing kids to spend the summer indoor, people are running from the house to the car because of the mosquitoes, families that like to leave the area cannot sell their properties or houses, sheep and cattle are losing weight, dogs refuse to go out and do their daily activities, etc.

Large scale floodwater mosquito control, using areal application of Vectobac G (with Bti as active ingredient), was commenced in 2002 and have continued since. Although no negative environmental effects on non-target organisms are documented, concern is still raised about the long-term environmental effects of using Bti. Therefore, it is interesting to look into other alternative methods of mosquito control, especially methods that might have even less environmental impact than Bti. The Sterile Insect Technique is used against other insects including mosquitoes, and might be possible to adapt for large scale control also of floodwater mosquito species such as *Aedes sticticus*, *Aedes vexans* and *Aedes caspius*. The transmission of Sindbis virus and *Francisella tularensis holarctica* by floodwater mosquitoes in Sweden is further reasons for controlling these mosquito species with efficient, sustainable and environmentally safe methods.

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