Joint FAO/IAEA Programme
Nuclear Techniques in Food and Agriculture

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

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EXECUTIVE SUMMARY

In June 2014, an experts' 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".

As the effectiveness of chemical vector control is decreasing due to mosquitoes developing insecticide resistance, there is an urgent need for alternative innovative mosquito control methods. The objective of the experts' meeting was to review the state of mosquito management (with an emphasis on vectors of 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.

However, soon after the experts' meeting and the publication of the thematic plan, the Zika crisis in late 2015 changed the public perception regarding mosquito- borne diseases, especially Zika which is often associated with phenomena of micro-encephalitis. Moreover, dengue incidence is increasing exponentially and since 2016, there has been no significant progress in the further reduction of global malaria cases. Consequently, more efforts to manage human disease vectors has to be undertaken, as evidenced by a surge in demand from FAO and IAEA Member States to support national campaigns for the control of these vectors.

In response to this demand, the Joint FAO/IAEA Division increased efforts towards the full development and improvement of the SIT package for the area-wide management of mosquitoes to support its Member States. This was done through a significant extrabudgetary contribution in support of research and development activities. Collaborators have also intensified their research and implementation of SIT in pilot projects against human disease vectors.

Therefore, the recent progress made on the development of the SIT package for mosquito vectors and the status of the transfer of the existing technology in view of the continuous requests by the Member States are the main drivers for reviewing the existing Thematic Plan.

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 mosquito species that transmit malaria, dengue, chikungunya, Zika 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 genetic engineering and symbiont-based approaches, this can be useful in the control of mosquito-borne diseases as well as to exploit their complementary potential with 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, enhanced funding support for facilities and personnel must continue be provided 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 a 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 continue scaling up the AW-IVM approach and further development and/or refinement of the SIT components, additional pilot projects should be supported in Member States.
- To develop an inter-regional project on dengue, chikungunya and Zika, 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 continue to be used to review the evidence and to make initial recommendations for their use by Member States.
- To continue providing technical and policy advice on existing or any new technology towards the control of mosquito populations.

1. STATEMENT OF THE PROBLEM

1.1 Diseases transmitted by *Anopheles* mosquitoes

Anopheles mosquitoes are the unique vectors of human *Plasmodium*, the malignant agents of malaria. In some areas of Africa such as in rural Northern Nigeria, *Anopheles* mosquitoes were shown to further contribute to the transmission of lymphatic filariasis together with *Culex* mosquitoes. They were also incriminated in the transmission of O'Nyong-Nyong fever, an Alphavirus closely related to Chikungunya. This disease is endemic to coastal East Africa and provokes rash and fever, but seldomly leads to severe symptoms.

1.1.1 Malaria

Key Facts about Malaria

- There are five species of *Plasmodium* that cause malaria in humans.
- Mortality and morbidity due to malaria are highest in sub-Saharan Africa but the disease is found globally in the tropics and sub-tropics.
- Malaria transmission is maintained by mosquitoes from the genus Anopheles.
- In spite of years of development and research, only one malaria vaccine is under field trials. Preliminary results suggest efficacy in children but no effect in infants.
- Malaria can be effectively managed through prompt diagnosis and treatment with appropriate drugs.
- Vector interventions have been the most effective means of reducing transmission of the parasites.
- Vector control effectiveness is being threatened by the development of insecticide resistance, the dwindling number of useful compounds and changes in vector behaviour.
- Many countries that suffer the highest burden of disease are also those that are least able to diagnose infection, treat the disease, and provide reliable disease reports.
- Past interventions have effectively reduced malaria but are dependent upon sustained national and donor support.

Clinical manifestations

Infection with malaria parasites may result in a wide variety of symptoms, ranging from absent or very mild symptoms to severe disease and even death. Malaria disease can be categorized as uncomplicated or severe (complicated). In general, malaria is a curable disease if diagnosed and treated promptly and correctly. The common symptoms of malaria include fever, sweats, chills, headache, nausea, body aches and general malaise. Other clinical manifestations include enlarged spleen and liver, mild jaundice, weakness and increased respiratory rate.

All the clinical symptoms associated with malaria are caused by the asexual erythrocytic (blood stage) parasites. When the parasite develops in the erythrocyte, numerous known and unknown waste substances and toxic factors accumulate in the infected red blood cell. Invasive merozoites are released *en masse* into the bloodstream when the infected cells lyse. Toxic factors such as glucose phosphate isomerase (GPI) stimulate macrophages and other cells to produce cytokines and other soluble factors, which act to produce fever and rigors and probably influence other severe pathophysiology associated with malaria.

Transmission 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, all with unique biological and ecological characteristics. However, adults of almost all *Anopheles* species are 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 throughout the day. Only *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 become infectious to a naïve host, gametocytes must develop into sporozoites in the mosquito. Adult females must therefore survive long enough for the parasites to complete development and find a susceptible host. Parasite transmission is therefore increased with increased mosquito longevity, and hosts that spend evenings outdoors, sleep in untreated structures, or without a bed net. Host preference is also a strong determinant of vectorial capacity. For example, differences in strength of preference for biting humans between individual species of the *An. gambiae s.l.* and *An. funestus s.l.* species complexes mean that only specific members of these complexes are responsible for transmitting malaria in Africa. In response to the scaling up of insecticide-treated bed nets, some behavioural changes have been documented in major vectors, including shifts in peak biting times and increased outdoor (rather than indoor) feeding. The morphological similarity of species complex members confounds determination of vectors responsible for

transmission. Furthermore, sympatric occurrence of vectors complicates efforts to control disease by elimination of only one member of the complex. Various degrees of reproductive, genetic and ecological differentiation exist between species within the *An. gambiae* complex. These range from complete reproductive isolation in allopatric species, to ecologically-driven assortative mating with geographical and seasonal overlap in species distributions, favouring genetic introgression between closely-related species.

Disease mortality and morbidity, and distribution

According to WHO, there were approximately 219 million cases of malaria and an estimated 435,000 deaths in 2017 (World Malaria Report, 2018). 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. In 2017, the two countries with the highest burden, Nigeria and the Democratic Republic of the Congo, accounted for 36% of global 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 facilities for proper diagnosis and treatment strongly affects disease outcomes since malaria is usually treatable when diagnosed early and treated correctly. Consequently, those living in communities located in remote rural areas with limited health care suffer the most.

Increased prevention and control measures have been effective in reducing the malaria burden in many places. Between 2000 and 2015, prevalence of falciparum malaria halved, and the incidence of clinical disease fell by 40%. There are an estimated 663 million clinical malaria cases that have been averted, with about 75% attributable to effective scaling-up of insecticide-based vector control. Since 2015, little progress in reducing global malaria burden has been made, but a number of countries have now eliminated local transmission.

In 2016, of the 91 countries that declared an indigenous malaria case, malaria cases had fallen by more than 20% (compared with the previous year) in 16 countries, although an increase of a similar magnitude was estimated in 25 countries. In the same year, there were 20 countries that were earmarked for elimination of malaria across South and Central America (6 countries), Africa (6 countries), Middle East (2 countries) and Asia (6 countries). Malaria elimination is used in this context to mean the permanent interruption of local mosquito-borne malaria transmission in a defined geographical area.

The CDC estimates that around 3.4 billion people, coming close to ~50% of the world's population are at risk of malaria, with transmission occurring in 91 countries and territories.

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.

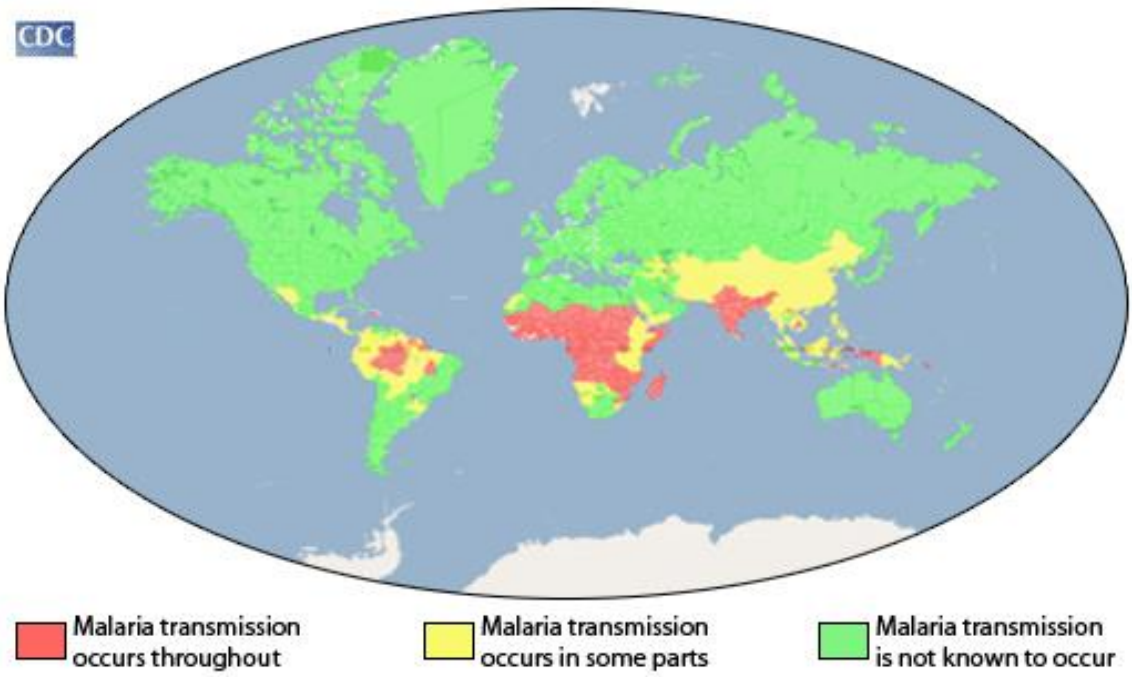

Figure 1. Global distribution of malaria risk. (Source: CDC: https://www.cdc.gov/malaria/malaria_worldwide/impact.html).

The basic ecology of malaria vector species determines both their seasonality and geographical distribution. Detailed maps of the distribution of major vectors are in progress, but due to the lack of sensitive surveillance systems, maps are still less than perfect, and information about the vector occurrence is even more limited than for the disease. Figure 2 highlights the diversity of the malaria vector species and their distribution on a global scale. The identification of potential habitats of the *Anopheles gambiae* complex and *An. funestus* group 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. While most malaria vectors are not considered invasive species, *An. stephensi* has recently invaded parts of East Africa from India, and endemic malaria vectors have now expanded their range to urban areas, representing an increased threat of urban malaria transmission. In some areas a single species is responsible for transmission, whereas in most others, multiple species are involved. The former case provides particularly attractive targets for genetic control efforts that focus on only one species at a time.

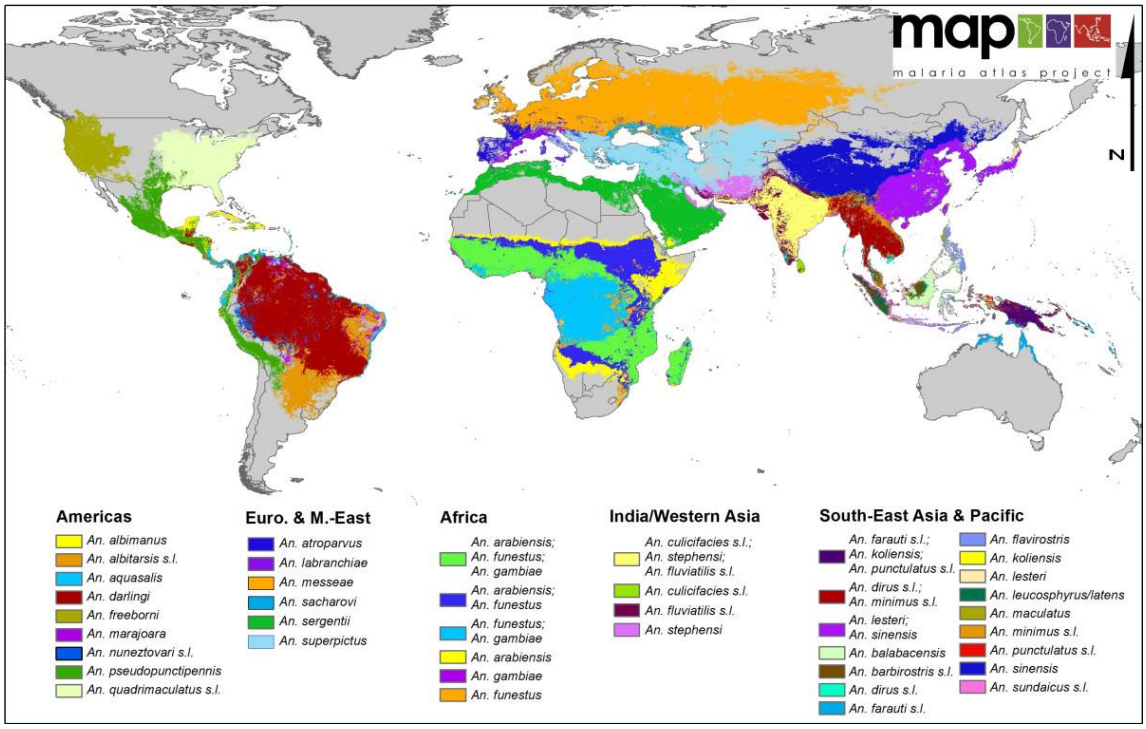

Figure 2: Global map of dominant malaria vectors. Sinka et al. (2012). Parasites and vectors.

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: Characteristics of stable and unstable malaria transmission settings

	Stable malaria transmission	**Unstable malaria transmission**
Immunity in adults	High	Low
Clinical manifestations	Primarily in young children and pregnant women	Affecting all ages
Parasite in population	High prevalence, multiplicity of infection (MOI) in population High levels of asymptomatic carriage of parasites Multiple strains in circulation	Low prevalence of parasite in humans Single strain (clonal infections)
Vectors	Multiple species	Predominantly a single species in these areas
Climate	Favourable for rapid development in mosquito	Not favourable for rapid 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: Modified from http://malaria.wellcome.ac.uk/doc_WTDO23873.html)

1.2 Diseases transmitted by Aedes mosquitoes

Arboviruses are a general term used to describe infections caused by a group of arthropod-borne RNA viruses (arboviruses) transmitted to people through the bite of infected arthropods, such as mosquitoes and ticks. Both *Aedes aegypti* and *Aedes albopictus* are capable of transmitting a suite of arboviruses of significant medical importance, including dengue, chikungunya, Zika and yellow fever. These species have also been found naturally infected with a number of viruses of zoonotic origin in different areas of the world where they interact with wildlife.

Both of these vectors are highly anthropophilic, and presently undergoing a geographic range expansion, which is expected to grow further as climate change and associated changes in human behaviours provide additional suitable habitats for these invasive mosquitoes. This highlights the pressing need for effective tools and strategies to control these vectors, in order to curtail transmission and prevent future spread of arboviruses, especially in densely populated urban metropoles where these vectors thrive.

1.2.1 Major vectors

Aedes aegypti

Aedes aegypti, the "yellow fever mosquito", is the primary vector of dengue. This mosquito is a highly competent vector of a multitude of viruses, and even some parasites. *Aedes aegypti* mosquitoes live in urban habitats and larvae develop mostly in man-made containers. Unlike many 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, unlike malaria, the use of insecticide treated bed nets (ITNs) are ineffective in preventing dengue transmission. Other personal protective measures however can be effective when used routinely. Female *Ae. aegypti* can bite multiple people during each feeding period, making this species a particularly efficient vector of pathogens.

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*.

Aedes albopictus

Aedes albopictus, the "Asian tiger mosquito", is a competent vector of many both flavivirus and alphaviruses. It is normally an outdoor container breeder, but may be found feeding and resting indoors at times. *Ae. albopictus* feeds primarily in humans, but shows opportunistic feeding behaviours when humans are in limited supply. 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.

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.

Distribution of vectors and their pathogens

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 became established in areas of Africa, Europe and the Americas.

Changes in climate and associated human behaviours allow for the geographic range of these vectors to expand. Figure 3 highlights the suitable range of both vectors around the world. With suitable seasonal climates, these areas also represent the potential range expansion of the pathogens that these mosquitoes can transmit.

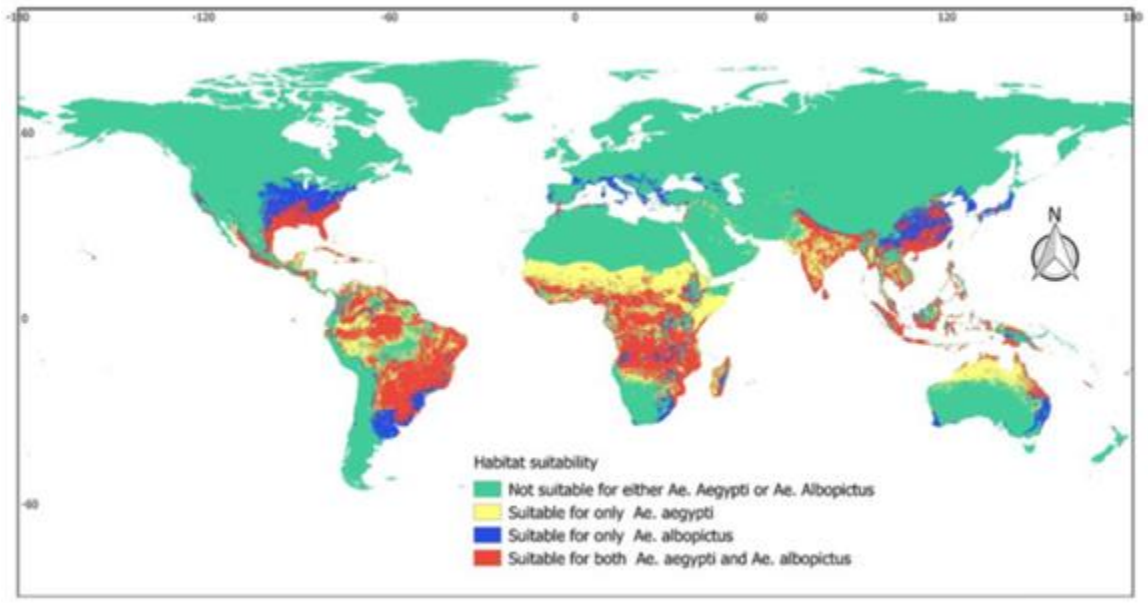

Figure 3. Global predicted habitat suitability for Aedes aegypti and Aedes albopictus (Source: Leta et al IJID 2018 Global risk mapping for major diseases transmitted by *Aedes aegypti and Aedes albopictus*)

1.2.2 Dengue

Key facts about dengue

- Dengue virus is a flavivirus, transmitted between humans by the bite of infected *Aedes* mosquitoes.
- There are four different serotypes of the virus, meaning that an individual may be infected multiple times.
- The infection causes flu-like illness, and occasionally develops into a potentially lethal complication called severe dengue.
- 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 an estimated 390 million dengue virus infection each year, with 96 million being symptomatic. 128 countries are at risk of dengue.
- Dengue is found in tropical and sub-tropical climates worldwide, mostly in urban and semi-urban areas. Local (autochthonous) transmission has been however recently documented in Europe following establishment of *Ae. albopictus.*
- 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%.
- There is a recently licenced vaccine for dengue, however it has limited efficacy, and is only recommended in certain subpopulations, where sero-prevalence for the disease is over 70%.
- The only reliable prevention and control of dengue virus transmission is sustained vector control.

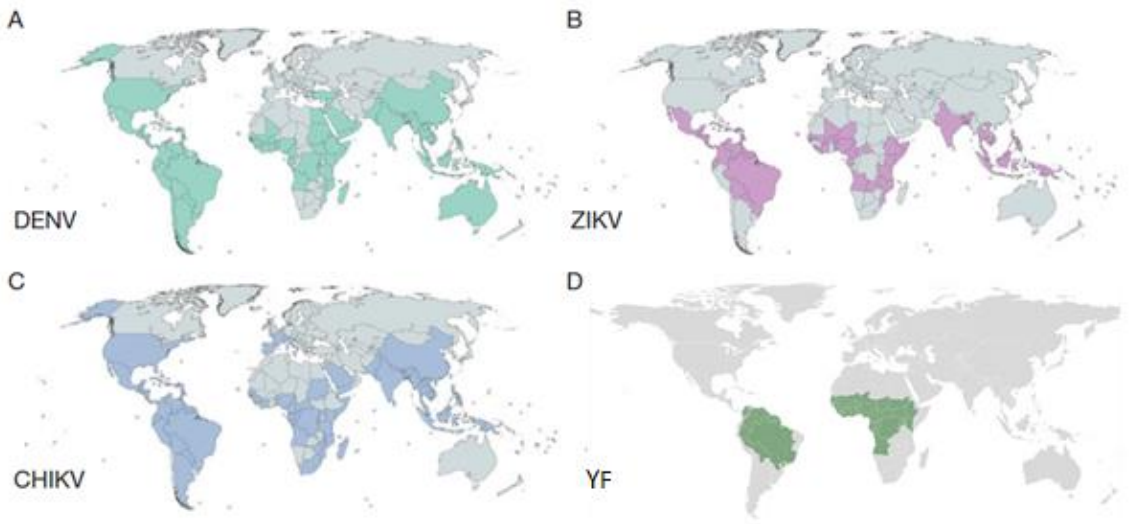

Figure 4. Global distribution of major Aedes-transmitted arboviruses including A) Dengue viruses, B) Zika virus, C) Chikungunya Virus and D) Yellow Fever virus. (Source: Wu et al., 2018; Golding et al., 2015)

Clinical manifestations

Dengue is an acute, self-limited viral disease that manifests with symptoms that may resemble the flu. Patients often have high fever (>40°C), headache, body aches and pains, fatigue, and nausea. With prompt, and appropriate clinical management, most dengue sufferers make a full recovery within one week after symptom onset. However, after the initial phase of the illness appears to be improving, some cases progress to severe dengue. This potentially lethal complication is associated with plasma leakage, respiratory distress, severe bleeding, and intense abdominal pains. Risk factors associated with severe disease include whether a person has experienced dengue before (with one of the other serotypes) and whether they have other comorbidities.

Because of the risk of haemorrhaging in dengue, the management of pain and fever in dengue (and suspected dengue) should be managed with drugs such as acetaminophen and paracetamol. NSAIDS and aspirin should be strictly avoided because of they are blood thinners and therefore challenge later clinical management if the disease progresses to severe dengue and the patient starts to haemorrhage.

The number of people that experience symptoms during dengue virus infection actually make up only a small proportion of the infected population (~20%). It is estimated that the remaining 80% of people infected with the virus have an asymptomatic infection, where any symptoms observed as mild enough not to interrupt one's daily life. The presence of asymptomatic infections can be confirmed through the detection of both virus during the infection, as well as anti-DENV antibodies that develop in response to the infection, as is the case for those with clinical manifestations.

Transmission dynamics

Dengue is a mosquito-borne viral disease that has rapidly spread in all regions of WHO in recent years. Dengue is caused by a virus of the Flaviviridae family and there are 4 distinct, but closely related, serotypes of the virus that cause dengue (DENV-1, DENV-2, DENV-3 and DENV-4). Recovery from infection by one provides lifelong immunity against that particular serotype. However, cross-immunity to the other serotypes after recovery is only partial and temporary. Subsequent infections (secondary infection) by other serotypes increase the risk of developing severe dengue.

Dengue can manifest symptomatically, or asymptomatically. Irrespective of symptom manifestation, virus circulating in the blood can be infectious to mosquitoes, and transmission between humans and mosquitoes can be maintained. In symptomatic people, the highest probability of transmission to mosquitoes occurs around the day of symptom onset, and the 1-2 days following. However, transmission can frequently occur up to the 5th day of illness (depending on serotype). It is unknown how long asymptomatic people are infectious to mosquitoes, because the kinetics of asymptomatic viral infection is not well understood.

Dengue virus is transmitted by female mosquitoes mainly of the species *Aedes aegypti* and *Ae. albopictus*. While *Ae. aegypti* is considered the primary dengue vector across most of the world, *Ae. albopictus* has been solely responsible for transmission in a number of regions, including in China, La Reunion and mainland Europe.

All four dengue virus serotypes can be transmitted by each of the two major vectors. After imbibing an infectious blood meal, the mosquito is able to transmit the virus after a period of 7-10 days. Factors such as ambient temperature and virus concentration in the blood meal (amongst other things) can influence this extrinsic incubation period.

Disease mortality and morbidity, and distribution

Dengue is widespread throughout the tropics, with local variations in risk influenced by rainfall, temperature and unplanned rapid urbanization. Peak dengue season normally corresponds with the rainy season in most tropical regions. Most dengue cases around the world are concentrated in South-East Asia, followed by Latin America.

The incidence of dengue has grown dramatically around the world in recent decades. Before 1970, only nine countries had experienced severe dengue epidemics. There is now the risk of infection of dengue virus in 128 countries, throughout Africa, the Americas, the Eastern Mediterranean, South-east Asia and the Western Pacific regions. Bhatt et al. (2013) estimate that there are around 390 million dengue infections worldwide annually, of which 96 million are symptomatic.

With the geographical expansion of the two major vectors in recent years, autochthonous cases as well as more explosive epidemics of dengue have been documented in previously dengue-free countries, including in mainland Europe and the US. A map of dengue prevalence is shown in Figure 4A.

In 2019, much of the dengue endemic world has suffered from heightened transmission. For example, countries such as Brazil, the Philippines, Bangladesh and Indonesia have seen their highest case numbers ever.

1.2.3 Chikungunya

Key facts about chikungunya

- Chikungunya virus is an alphavirus and is transmitted between humans by infected *Aedes* mosquitoes.
- Chikungunya fever and severe joint pain. Other symptoms include muscle pain, headache, nausea, fatigue and rash.
- Chikungunya is often associated with prolonged morbidity in the form of arthritis and joint pain for several months or years.
- 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.
- There are two vaccines in clinical trial at the moment, but any licensure is still a number of years away.
- Since 2004, chikungunya fever has caused multiple epidemic outbreaks.
- The geographic distribution of the disease has recently expanded, with outbreaks having occurred throughout Africa, Asia and the Indian subcontinent. In recent years, even outbreaks have occurred in Europe (including Italy, France and Spain).

Clinical manifestations

Chikungunya is characterized by an abrupt onset of fever, frequently accompanied by joint pain. The joint pain is often very debilitating; it usually lasts for a few days but may be prolonged for weeks. Hence, the virus can cause acute, subacute or chronic disease. Other common signs and symptoms include muscle pain, headache, nausea, fatigue and rash.

Often symptoms in infected individuals are mild and the infection may go unrecognized or may be misdiagnosed.

The symptoms of chikungunya are also similar to other arboviruses; in areas where there is co-circulation, chikungunya is often misdiagnosed as dengue. Like dengue, most patients recover fully from the infection with CHIKV, but in some cases joint pain may persist for several months, or even years. Chikungunya infection rarely progresses to become life threatening, unless in individuals that have significant co-morbidities.

Also, like dengue, there is no specific treatment for the disease. However, until a differential diagnosis of *not*-dengue is ruled out, the use of aspirin and NSAIDs to treat pain and fever should be avoided because of the risks of haemorrhaging in case the infection is DENV.

Transmission dynamics

CHIKV is transmitted between humans via mosquitoes. Both *Ae. aegypti* and *Ae. albopictus* have been implicated in large outbreaks of chikungunya. When a naïve mosquito feeds upon a viremic person (someone who has virus circulating in their blood), the mosquito can pick up the virus as it ingests the blood. The virus then undergoes a period of replication in the mosquito, before which time it can then be transmitted back to a new, naïve host, when the mosquito next feeds. The virus again begins to replicate in this newly infected person, and amplify to high concentrations. If a mosquito feeds on them during the time they have virus circulating in their blood, the mosquito can pick up the virus, and the transmission cycle begins again.

Within the mosquito, the virus replicates in the mosquito midgut until it disseminates to secondary tissues, including the salivary glands. CHIKV can be transmitted to a new, naïve host more quickly than for other mosquito-borne viruses; laboratory experiments have demonstrated virus can be detected in saliva as little as 2-3 days after the infective blood meal. This suggests that the complete transmission cycle from human to mosquito, and back to humans can occur in well under a week. Once infectious, the mosquito is believed to be capable of transmitting virus for the rest of its life.

Disease mortality and morbidity, and distribution (Figure 4C)

The actual numbers of Chikungunya cases are not reliably reported by many countries, making it difficult to estimate the burden of the disease. However, reporting practices are changing at the national level in line with increasing frequency and magnitudes of outbreaks around the world.

The most recent chikungunya outbreak was in 2016-17. In 2016, PAHO regional office reported almost 350,000 suspected and 150,000 laboratory-confirmed cases. Countries reporting most cases were Brazil, Bolivia and Colombia (with around 300,000 suspected cases between them). Argentina reported the first evidence of autochthonous transmission of chikungunya, following an outbreak of more than 1,000 suspected cases. In Africa, Kenya reported an outbreak of chikungunya resulting in more than 1,700 suspected cases, while in Somalia, the town of Mandera was hard hit, with about 80% of the population affected by chikungunya. Chikungunya cases in India and Bangladesh affected close to 100,000 people. European case reports remained below 500.

In 2017, ECDC reported a total of 10 countries, with 548 cases with chikungunya, of which 84% were confirmed cases. Italy bore more than 50% of the chikungunya burden. As in previous years, Asia and the Americas were the regions most affected by chikungunya. Pakistan was dealing with a persistent outbreak that started the year before, and reported 8,387 cases, while India suffered with 62,000 cases. In the Americas and the Caribbean, there were 185,000 cases; cases in Brazil accounted for >90% of that in the region.

Globally, there are more than 60 countries that have reported autochthonous transmission of Chikungunya virus, from Africa, Asia, Europe and the Americas.

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.

1.2.4 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 annually, causing 30,000 deaths, worldwide, with 90% of those occurring in Africa.
- The virus is endemic in tropical areas of Africa and Latin America, meaning an extremely large population of more than 900 million people are at risk of infection.
- 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.
- Yellow fever is the only arbovirus for which there is an available vaccine. The vaccine is very effective, and safe. A single dose of the vaccine can provide life-long immunity. However, the vaccine differs in availability and its supply across different regions.
- There is no specific curative treatment for yellow fever. Treatment aims to reduce the symptoms for the comfort of the patient.

Clinical manifestations

Once contracted, the yellow fever virus incubates in the body for 3 to 6 days. Many people do not experience symptoms, but when these do occur, the most common are fever, muscle pain with prominent backache, headache, loss of appetite, and nausea or vomiting. In most cases, symptoms disappear after 3 to 4 days.

A small percentage of patients, however, enter a second, more toxic phase within 24 hours of 'recovering' from initial symptoms. High fever returns and several body systems are affected, usually the liver and the kidney. In this phase people are likely to develop jaundice (yellowing of the skin and eyes, hence the name 'yellow fever'), experience abdominal pain with vomiting, and have dark coloured urine. Bleeding can occur from the mouth, nose, eyes or stomach. Half of the patients who enter the toxic phase die within 7-10 days.

Yellow fever is difficult to diagnose, especially during the early stages. More severe cases can be confused with severe malaria, leptospirosis, viral hepatitis (especially fulminant

forms), other haemorrhagic fevers, infection with other flaviviruses (such as dengue haemorrhagic fever), and even poisoning.

Polymerase chain reaction (PCR) testing in blood and urine is the most effective method of detection of virus in early stages of the disease. In later stages, testing to identify antibodies is needed (ELISA and PRNT). There are known challenges in diagnosis of flavivirus infections using serology however, because many of the antibodies produced in response to flaviviruses cross react.

Transmission dynamics

The yellow fever virus is an arbovirus of the flavivirus genus and is closely relate to DENV. *Aedes* mosquitoes are the primary vector, carrying the virus from one host to another, primarily between monkeys, from monkeys to humans, and from person to person. Several different species of *Aedes* and *Haemagogus* mosquitoes transmit the virus, breeding either in domestic environments, in the jungle (wild) or in areas which bridge these two environments (semi-domestic).

There are three types of yellow fever transmission cycle.

1) 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).

2) 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.

3) 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 urban *Aedes* mosquitoes. Infected mosquitoes transmit the virus from person to person.

Cross-border transmission of YFV is limited by international health regulations which allows countries that are yellow fever-free to deny entry to those persons who have recently travelled in yellow fever endemic countries, that do not have evidence of having received the YFV vaccine.

If someone has not received the vaccine, individual protection from infection is achieved as per other mosquito-borne diseases, by avoiding contact with mosquitoes.

Disease mortality and morbidity, and distribution

Yellow fever is endemic in 47 countries (34 in Africa and 13 in South America, Figure 4D). Analysis of African data sources from 2013 estimated the burden of yellow fever in Africa of 84,000–170,000 severe cases and 29,000–60,000 deaths. It is estimated that 90% occur in Africa; the remaining burden is in central areas of South America, in countries such as Ecuador, Peru and Bolivia.

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 the vectors for the virus are abundant. The reason for the lack of YF in Asia has frequently been questioned.

1.2.5 Zika

Key facts about Zika

- ZIKV is a flavivirus that is transmitted primarily by *Ae. aegypti* and *Ae. albopictus*.
- ZIKV causes a mild illness, with symptoms that may include fever, rash, conjunctivitis, muscle and joint pain, malaise or headache. Symptoms typically last for 2–7 days. Most people with ZIKV infection do not develop symptoms.
- ZIKV infection during pregnancy can cause infants to be born with microcephaly and other congenital malformations, known as congenital Zika syndrome. Infection with ZIKV is also associated with other complications of pregnancy including preterm birth and miscarriage.
- An increased risk of neurologic complications is associated with ZIKV infection in adults and children, including Guillain-Barré syndrome, neuropathy and myelitis.
- There is no treatment or cure for ZIKV infection; prevention of infection through reducing contact with mosquitoes is the only effective protective measure.
- There is ongoing research into vaccines for preventing ZIKV transmission, but these are in very early stages of clinical development, and are not likely to be available or licenced in the near future.

Clinical manifestations

Zika virus infection commonly manifests with fever, rash and headache, and a number of symptoms that are similar to chikungunya and dengue. A differential diagnosis between the infections is often challenging for clinicians in areas where the diseases are co-occurring. A few symptoms that aid in the differential diagnosis have been identified though, that are more commonly associated with ZIKV compared to chikungunya or dengue, and include

rash (early in the illness), itchiness, conjunctivitis, polyarthralgia. Laboratory testing of samples by PCR, detecting active viremia are the best method of confirmation.

Several severe, chronic/life-long clinical complications associated with ZIKV infections exist. These include the development of microcephaly in babies born to ZIKV-infected mothers, and the development of neurological issues, associated with Guillain-Barré syndrome.

For managing symptoms associated with the acute infection, clinicians should be cautious with prescribing treatment for the symptoms of Zika syndrome. Until a differential diagnosis of *not-dengue* is clear, aspirin and NSAIDs should be avoided in case the infection is DENV, and the patient is thereby at risk of haemorrhaging if the disease progresses.

Transmission dynamics

ZIKV is a highly transmissible virus. It can be transmitted between humans in multiple ways, including being vectored by mosquitoes, transmitted through bodily fluids during sexual contact between two people, as well as between mother and child, through the placenta. The period of infectiousness in humans varies, and depends on the mode of transmission. For example, virus in the blood can be infectious for <1 week, whereas virus found in urine is infectious for ~3 weeks, and in semen for several months.

The risk of mosquito-borne transmission of ZIKV is therefore relatively short-lived compared to other modes, and this mode of transmission is prevented by eliminating contact with infected mosquitoes.

Given the close relatedness of ZIKV with the four DENV (and other non-DENV flaviviruses), there is *in vitro* evidence of cross reactivity antibodies produced in response with both ZIKV and DENV, with the alternate viruses. Under the premise of antibody-dependent enhancement among flaviviruses, this cross-reactivity of antibodies poses a risk for disease enhancement in individuals with subsequent DENV and ZIKV infections. There has been limited *in vivo* evidence of this to date, we may still be in the period of cross-protection, and not yet cross-reactivity. Continued vigilance will be necessary to understand the *in vivo* risk in more detail.

Disease mortality and morbidity, and distribution

Zika virus is a mosquito-borne flavivirus that was first identified in Uganda in 1947 in monkeys, and later identified in humans in 1952 in Uganda and the United Republic of Tanzania. Outbreaks of Zika virus disease have been recorded in Africa, the Americas, Asia and the Pacific. From the 1960s to 1980s, rare sporadic cases of human infections were found across Africa and Asia, typically accompanied by mild illness.

The first recorded outbreak of Zika virus disease was reported from the Island of Yap (Federated States of Micronesia) in 2007. This was followed by a large outbreak of Zika virus infection in French Polynesia in 2013 and other countries and territories in the Pacific.

In March 2015, numerous reports of an illness associated with rash and fever came to light. This was soon associated with Zika virus infection. Later that year, increased incidence of Guillain-Barré syndrome and microcephaly in babies were also attributed to ZIKV infections. The following year, outbreaks and evidence of transmission soon appeared throughout the Americas, Africa, and other regions of the world.

Reporting of ZIKV was a priority for ~18 months for a number of countries, however when the risks of infection became better understood, the frequency of reporting became less of a priority. As of early 2018, WHO received reports of ZIKV in almost 100 countries/territories (Fig. 4B). The threat of another ZIKV outbreak remains strong, as does the importance of effective vector control to reduce transmission.

1.3 Other vectors, and diseases that they transmit

1.3.1 Culex mosquitoes

There are several arboviruses that are transmitted by *Culex* mosquitoes, including Japanese encephalitis virus (JEV), West Nile Virus (WNV), Saint Louis' Encephalitis virus (SLEV), Western Equine Encephalitis virus (WEEV), Eastern Equine Encephalitis virus (EEEV) and Venezuelan Equine Encephalitis virus (VEEV). *Culex* mosquitoes can also transmit parasites such as *Wuchereria bancrofti*, *Brugia malayi* and *B. timori,* the pathogens that cause the debilitating lymphatic filariasis.

While many of these viruses are not sustained in a pure human-mosquito-human transmission cycle, they are still of medical importance. The reservoir hosts of these pathogens allow for continued re-emergence of the pathogen into the human population, and even when humans are dead-end hosts for transmission, the viruses remain of greater agricultural importance.

The mosquito genus of *Culex* breeds prolifically, and it is considered a pest throughout the world. Control of *Culex* vectors is therefore considered a priority for many countries.

1.3.2. Other Vectors and Pathogens

Other arboviruses that are considered to be emergence threats include Usutu virus, Mayaro virus (MAYV), and Rift Valley fever virus (RVFV). These viruses are transmitted by mosquitoes, some of which may be transmitted through vertical transmission. In this case, pest control methods like SIT may eliminate the reservoir hots as well as the vectors.

Other invasive and medically important arthropods, like sandflies, ticks, bed bugs and kissing bugs, may be potential targets for SIT in the future. Tsetse flies, the vectors of Human and Animal African Trypanosomiasis (HAT/AAT), have been successfully suppressed in the past using SIT, and there are plans for targeting some tsetse populations for medical purposes.

2. CURRENT CONTROL PRACTICES

2.1 Current control methods for *Anopheles* vectors

The goals of malaria vector interventions are two-fold: to provide personal protection by reducing human-vector contact (usually through –Insecticide Treated Nets, ITN) 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).

2.1.1 Bed nets

Bed nets can be divided into conventional nets treated with insecticide (Long Lasting Insecticidal Nets, LLIN) and the recently new generation nets that are treated with a mixture of insecticides or a synergist. 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, and urges adoption of LLINs to replace less durable nets and adoption of new generation nets to target insecticide resistant vector populations. We will use the term LLIN to refer to all bed nets in this document.

Advantages:

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

Disadvantages:

- Nets are mainly treated with pyrethroids, this has resulted in an increase in selection of vector resistance to these insecticides reducing effectiveness of chemical based interventions that uses this class of insecticides.
- Some nets require retreatment or specialised disposal.
- Cost can be prohibitive when individuals are expected to purchase nets themselves.
- Not useful to protect from day-biting mosquitoes.

2.1.2 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. The Global Plan for Insecticide Resistance Management in malaria vectors (GPIRM) recommends rotation of different classes of insecticides including the use of new-generation insecticides to tackle the growing threat of insecticide resistance.

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 insecticides.
- Requires maintenance of equipment, quality assurance, monitoring and evaluation.
- Requires proper storage and disposal of excess pesticide and pesticide contaminated rinse.
- Household compliance and support is essential to make this intervention effective.
- Implementation costs are relatively high.
- Residual effect (remanence) is substrate-dependent a varies across settings.

2.1.3 Larval source management

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 by WHO.

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.

The 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.
- Larviciding formulations are not adapted to surface feeding larval mosquitoes.

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

2.2 Current control methods for *Aedes* vectors

Viruses such as dengue (DENV), chikungunya (CHIKV) and Zika (ZIKAV) are mainly transmitted by *Ae. aegypti* and *Ae. albopictus*. The current global dengue picture is alarming, with a surge in dengue epidemics in several non-endemic countries and worsening dengue situations in many endemic countries. Chikungunya and Zika are pushing up with outbreaks suddenly appearing in several regions. As neither preventive/curative treatment nor licensed vaccine are available, disease prevention relies on vector control or the interruption of contact between humans and vectors. The main goal of a vector control programme is to reduce morbidity and mortality through the interruption of transmission of the virus between mosquito vector and human, and *vice versa.*

2.2.1 Larval control

Ae. aegypti and *Ae. albopictus* larvae mainly develop in artificial containers, either permanent (e.g. water tanks, cement tanks cisterns, bathroom basins, water jars, animal drinking troughs) or temporary (e.g. discarded used tyres, out of use bottles, ant-traps, flower vases, under pots, bamboo pole holders), or natural containers including coconut shells, leaf axils, bamboo fences, tree holes, rock pools and many others. The female skip oviposition behaviour is making these species highly efficient to develop in urban habitats. Because of the high number and cryptic nature of larval sites, their complete removal from the environment (source reduction) results very difficult if not impossible. The use of larvicides products (biological and/or chemical) and the introduction of autochthonous larvivorous fishes or copepods is largely adopted in many settings.

Source reduction

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

This can be done by professional operators or by community members themselves after sensitization campaigns or enforced through inspection of households like in Singapore.

Advantages:

- Removal of larval habitats is a permanent highly cost-effective measure.
- The method is environmentally friendly.
- It might control several mosquito species at once, including *Aedes* and *Culex* mosquitoes.

Disadvantages:

- Cryptic or inaccessible larval habitats poses a challenge to source reduction.
- Skilled and motivated operators are required to achieve a satisfactory effectiveness.
- Active and sustained community participation is required to reduce the cost.

Larvicides

Larvicides are recommended by the WHO for the treatment of permanent or temporary larval habitats or potential larval habitats for Dengue/chikungunya/Zika control. Commonly used larvicides include organophosphates (e.g. temephos), insect growth regulators (pyriproxifen, methoprene, diflubenzuron, etc) and biological products such as *Bacillus thuringiensis israelensis* (Bti), *Lysinibacillus sphaericus* (Ls) or spinosad. 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. diflubenzuron) and are recommended by the WHO to treat domestic water supply including potable water.

Advantages:

- Availability of products with low toxicity for humans and the environment.
- No resistance has been documented against Bti.

Disadvantages:

- Resistance to organophosphates and Ls has been documented.
- High coverage rate and regularity of treatment is required to achieve satisfactory results.
- Biocides application may be expensive.

Control by predators

Releases of indigenous larvivorous fish and copepods have been effective in domestic water containers that are seldom emptied.

Advantages:

- The method is environment-friendly.
- May promote the local economy by stimulating employment.
- Predators are effective against other mosquito species.

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.
- Predator population dynamics is dependent upon prey availability.

2.2.2 Methods for the control of adults

Space spraying

Space spraying is the application of a small quantity of insecticides over a large area and it is used for dengue/chikungunya/Zika prevention and control (WHO, http://www.who.int/denguecontrol/arbo-viral/other_arboviral_chikungunya/en/). The main goal of this method is to promptly 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. Resistance monitoring is essential to assure efficacy of operations.

Advantages:

- Reduction of the adult mosquito vector populations (including infected females) can be achieved quickly.

Disadvantages:

- Increased selection pressure for resistance means it should not be used in areas where insecticide resistance occurs.
- Transient effect as vector populations will rapidly replenish.
- 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, storage and disposal of pesticides, 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

Contrary to malaria vectors that bite during the night, *Aedes* mosquitoes are active at daytime and insecticide-impregnated bed nets are therefore not useful to control them. Insecticide impregnated, or treated, materials (ITMs) can however 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:

- Competition with available natural breeding sites.
- 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 unless deployed at high densities.
- No benefit-cost analyses yet conducted.

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

3. NEW VECTOR CONTROL TECHNOLOGIES

3.1 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 1940s to the 1960s, 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 Vector Management (IVM) emerged about 60 years ago, with the general idea being to combine different control methods to reduce the use of insecticides. IVM has been the dominant paradigm of insect pest control in the last 6 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 among 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 IVM, the AW-IVM 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 IVM approach has generally led to the repeated application of pesticides, while the AW-IVM 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-IVM approach to be effective. Among the difficulties or limitations for the application of the AW-IVM 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-IVM should ideally be environmentally

acceptable, for example avoiding application of pesticides in natural 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-IVM 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-IVM approach has been equated with pest eradication programmes and this has been in opposition to the accepted view of IVM. Fortunately, progress has been achieved in understanding that not only are these two concepts not in opposition but that they are in fact complementary. Depending on specific conditions, AW-IVM can be used to prevent, contain, suppress or eradicate pests. Some examples of successful contemporary applications of the AW-IVM 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, Dominican Republic, Guatemala, Israel, Mexico, Spain and the USA, management of cotton pests in the USA, and suppression of cassava mealy bug in sub-Saharan Africa. Insecticide resistance management strategies also involve an AW-IVM 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 AW-IVM approach that integrates modern and novel control methods, such as the Sterile Insect Technique, Biological Control or the Incompatible Insect Technique.

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

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

2) How are vector populations naturally regulated?

3) How do they survive from season to season?

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

5) What are the natural boundaries of the vector population?

3.2 Sterile Insect Technique

Since the 1950s, populations of several insect pests have been controlled or eradicated through a "birth control" method known as the Sterile Insect Technique (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.

According to the International Standards for Phytosanitary Measures No. 5 Glossary of phytosanitary terms, the sterile insect technique is a 'Method of pest control using area-wide inundative releases of sterile insects to reduce reproduction in a field population of the same species'. Following this definition, some transgenic methods like precision-guided

SIT may be considered as SIT. In this document, the SIT is thus restricted to the use of irradiated sterile males.

Between 1962 and 1983, several experimental pilot studies of the SIT strategy against mosquito species were conducted in different geographical areas, as shown in Table 2.

Table 2. Species of mosquitoes against which SIT was 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

Member states requested the IAEA to resume the work on mosquitoes and develop the technological package for the application of SIT for suppression of vector populations within the AW-IVM approach. Progress and achievements towards this goal are described in section 5 of this document, including several applied pilot field projects (section 5.2.6 and tables 4, 5 & 6).

3.2.1 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.

3.2.2 Integration of nuclear and other techniques

SIT is not a stand-alone technology. To be effective, it should be integrated in a package with non-nuclear techniques (biological, chemical, behavioural) while economic considerations and public education should also be considered to secure support and promote further implementation. 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 neighbours do not join efforts, the pest insects re-invade. Regular insecticide applications are thus required to protect agricultural production and in the long term this results in insecticide resistance. However, when growers in a given area or region coordinate efforts and apply an AW management programme against the total population of the pest species, much lower, or no insecticide inputs are required, and the control achieved will be more effective and durable.

3.2.3 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 (virgin) 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, and chemical control. In this way, effective AW-IVM approaches for the management of some of the world's most important insect pests have been developed.

3.2.4 Applications of the SIT

Considerable advances in the 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 management of a pest or vector:

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 and moths, and economic analyses have shown that SIT applied as part of an AW-IPM 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, Dominican Republic, Japan, Mexico, parts of Patagonia and Peru, and in Southern States of the USA. It has also been used to eradicate the New World Screwworm from North and Central America and tsetse fly species, *Glossina austeni*, from Zanzibar.

Containment and prevention

SIT can be used as a biological barrier to protect pest-free areas that are contiguous to infested ones. Moreover, SIT has been applied as a preventive measure over pest-free areas that have a high risk of invasion to avoid the establishment of pest populations. This approach can also be used in the case of mosquitoes, to prevent their introduction into new areas or the increase of their populations.

3.3 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 uninfected females mate with *Wolbachia*-infected males, while the offspring can successfully develop when laid by an infected female no matter whether she has mated with an infected or uninfected male. Bidirectional CI can also happen when infected females mate with a male carrying a different strain of *Wolbachia*.

One important feature of *Wolbachia* is its ability to induce resistance to a variety of pathogens, including Dengue virus (DENV), Zika virus, Chikungunya virus, and malaria parasites, in its mosquito hosts. In transinfected *An. stephensi*, *w*AlbB infection can confer mosquito resistance to both *P. falciparum* and *P. berghei*. Similarly, in transinfected lines of *Ae. aegypti*, a variety of *Wolbachia* strains, including *w*AlbA, *w*AlbB, *w*MelPop-CLA, and *w*Mel, show a significant inhibition to viral replication, dissemination and transmission potential. The mechanisms of *Wolbachia*-mediated viral interference in mosquito include immune priming and metabolism alterations. For example, *Wolbachia* transinfection boosts host immunity, induces oxidative stress and causes significant metabolic changes, including inhibition of the insulin pathway, promotion of lipolysis, and catabolism of fatty acids.

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 midgut and salivary gland is too low to induce resistance to DENV. This indicates that *Wolbachia* may induce only a local but not a systematic antiviral resistance in mosquitoes. By introducing a novel *Wolbachia* strain (such as *w*Pip 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, Zika, 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, complete CI and strong pathogen interference. Success has also been made to transfer *Wolbachia* from *Ae. albopictus* to *Culex quinquefasciatus*, resulting in transinfected lines that show complete CI when mating with wild-type line. Some of those transinfected lines, including *Ae. aegypti* carrying *w*Mel or *w*AlbB strain and *Ae. albopictus* carrying *w*Pip strain, have been successfully used in field trial for proof of concept studies and even operational implementation for vector control with successful registration as microbial insecticides. The current *Wolbachia*-based approaches include population suppression and replacement, and an integration of population suppression with SIT.

3.3.1 Incompatible Insect Technique (IIT)

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. 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. Recently, IIT has also been successfully used in field trial to control both *Ae. aegypti* and *Ae. albopictus* in US, China and Australia with suppression at different levels. 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 field quality control data to show prevention from risk of population replacement.

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. Novel technologies that combine sensors, algorithms and novel engineering for mass rearing and sex sorting have been recently utilized to address those challenges by Verily's Debug program. *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. Thus, the female contamination rate should be well monitored in both mass rearing facility (before release) and field sites (after release) for risk assessment. Due to this risk, vector competence of a transinfected mosquito line used for IIT should be fully characterized before release into the field to prevent from introducing more susceptible vectors into the field. If population replacement were to happen, males infected with another incompatible strain of *Wolbachia* could be released for further population suppression. Alternatively, IIT can be combined with SIT to avoid the release of fertile females and remove the risk of population replacement.

3.3.2 Integration of IIT with SIT

Production of sufficient male mosquitoes for *Wolbachia*-mediated population suppression to be effective requires the capacity for mass rearing and efficient sex separation. Significant progress has been made in mass mass-rearing and sex separation during the last five years. It is likely that a very low number of females contaminating the released males would hinder the IIT. For example, the existing mechanical sorter, which separates males from females based on their size difference, is used in mass rearing with an optimal 0.3% female contamination rate. However, this issue has recently been resolved by integration of IIT and SIT (IIT-SIT), in which a low radiation dose of X-ray is used to sterilize those residual females, while *Wolbachia*-infected males will induce incompatible matings with wild type females. Because female can be sterilized using a lower dose of irradiation than male, IIT-SIT enable to produce highly competitive males. In addition, the irradiated males can further reduce risk of population replacement when they mate with the infected fertile females in both the field and release containers. 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, IIT-SIT could provide an effective way to produce sterile males with high quality. A pilot field trial has recently successfully completed in two islands in South China, demonstrating proof of concept that IIT-SIT can result in near-elimination of the local primary dengue vector, *A. albopictus*, for over two years. Due to IAEA's experience in the design of irradiator specific for mosquito, IIT-SIT can also provide a practical way to scale up production, as seen in mass rearing facility in Guangzhou where over 10 million males are weekly produced.

3.3.3 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 field trials. 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 or strain combination with a maximum level of pathogen interference but a minimal fitness cost. It is worthy to note that the *Wolbachia* strain used for replacement strategy is not efficient to block dengue transmission in all mosquito populations. There are potential sustainability risks as *Wolbachia*-mosquito association may progressively adapt to each other, reducing the pathogen interference over time. Arboviruses may also evolve, resulting in selecting for more virulent strains with unforeseen economical and health impacts. Lastly, as the released females can cause a biting nuisance which may negatively affect the public acceptance of a programme.

3.4 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 and iv) systems for targeted genetic engineering and transgene stability. However, the public acceptance of transgenic technology, scientific data and the clear communication of it is still difficult and has to be addressed further.

3.4.1 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. aegypti* (strain OX513A). It uses the toxic effect of an overexpressed protein (tTA) to eliminate between 95.8 – 97.4 % of the progeny of transgenic males with wild-type (WT) females at the late larval or pupal stage. Initial releases were conducted in 2009 and 2010 on a small scale on the Cayman Islands, and the results were eventually published in 2011. This trial showed the potential of the transgenic control strategies for mosquitoes in general, and of the OX513A in particular, as it achieved 80% population suppression at an overflooding ratio of 5:1. A larger-scale evaluation of the same strain, OX513A, for more than one year, was done in 2015 in a suburb of Juazeiro, Bahia, Brazil, reducing the local population by 81%. Field trials using OX513A were planned in Florida after the 2016 final decision by the FDA that no significant impacts on the environment are expected. Releases have not been initiated because of public opposition until 2019. OX513A is the only transgenic mosquito line which has been semi-mass-reared for releases of up to 1–1.5 million male mosquitoes per week. However, low male mating competitiveness required high transgenic to wild male

ratio. This strain was also not a sexing strain, meaning that sex separation based on pupal size is very labour-intensive. It is also inefficient in the way that some fertile females are released into nature. The rearing of this transgenic *Ae. aegypti* OX513A RIDL strain also depends on the use of the antibiotic tetracycline in mass-rearing. It may have a negative impact on the mosquitoes' endogenous microbiota that is known to be important in the biology, physiology, and ecology of the host, potentially affecting the rearing efficiency and mating competitiveness of the strain. Another study showed the introgression of OX513A's genetic background into the local population, resulting in hybrids of originally genetically distinct populations with unknown robustness and vectorial capacity.

Another transgenic sterilization technique is the induction of reproductive sterility by the transfer of embryonically lethal transgenes. It was first developed in *D. melanogaster* and later adapted and transferred to important fruit fly pest insects like *C. capitata*, *Anastrepha ludens*, and *A. suspensa*. It is based on the conditional expression of lethal effector molecules controlled by an early embryonic promoter, to cause embryo-specific lethality in the progeny. During mass-rearing, the conditional systems can be switched off by a food supplement, in this case, the antibiotics tetracycline or doxycycline. Such strains, if released into the field with no antibiotics present, will lead to biologically fertile matings, but their progeny will die due to the embryonically active lethal system. This results in the reduction of the wild-type population. Such systems have not been created for any mosquito species so far.

3.4.2 Transgenic methods for sex separation systems

In addition to the sterilization procedure, male-only releases are a prerequisite 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 are being developed.

These comprise:

- *Sorting by sex-specifically expressed fluorescent markers*: sex-specifically marked larvae can be sorted by a sorting machines. One option is the use of sex-specific promoters to express fluorescent marker proteins. This strategy has been pursued for *Aedes* and *Anopheles* species. Sex separation can be achieved by mechanical sorting with the COPAS. Using the *beta2-tubulin (b2-tub*) promoter to express the fluorescent protein allows reliable sorting for marked males from non-marked females in *Ae. aegypti* and *Anopheles stephensi*. Importantly, the mechanical sorting by COPAS does not significantly affect the viability and competitiveness of sorted males.
- *Sorting by linking markers to the male-determining chromosome or locus:* Alternatively, insertions of the fluorescent marker gene on the Y chromosome, or the male-determining locus in mosquitoes with homomorphic sex chromosomes can negate the need for sex-specific promoters. Systems including a recombination sequence such as *attP*, *lox*, or *FRT* for site-specific integration allow for additional modifications on the Y chromosome. Such strains are available for *An. gambiae* and *Ae. aegypti*. Reliability and cost of the sorting machines in terms of mass rearing requirements would have to be considered and further developed.

- *Production of male-only strains by female-to-male conversion or female-lethality*: Strategies are being developed to target the sex determination and dosage compensation pathways to change sex ratios towards the male sex. Sex conversion tools have been identified in fruit flies and can be developed for mosquitoes to enable the sex reversion of females to males. This would double the total number of male progeny per parental female. Only very recently a breakthrough was achieved in three mosquito species. Scientists identified the long-sought M-factor *Nix* in *Ae. aegypti*, the maleness gene *Yob* in *An. gambiae*, and *GUY1* in *Anopheles stephensi* Liston. All three genes are located at the top of the sex determination cascade, and are needed for male development. Autosomal expression of Nix in female mosquito embryos leads to the development of fertile males. Autosomal expression of GUY1 and Yob, in contrast, results in female lethality at the embryonic stage. However, in all three cases, conditional expression (e.g., with the tet-off system) of these mosquito genes are still needed to generate a genetic sexing strain that can be tested for mass-rearing and possible release. The "precision-guided SIT (pgSIT)" approach, recently developed in the model organism *D. melanogaster*, targets several sex-specifically spliced genes using CRISPR/Cas to induce female lethality. pgSIT is not only a sexing system, but combines sexing with male sterility by simultaneously targeting genes involved in spermatogenesis.
- Another strategy, the so called *´X shredder´*, was developed for *Anopheles gambiae* and changes the gamete production of males in such a way that it results in 95-97% male progeny by shredding the X chromosome in X gametes. Such a system could be used as a gene drive system after careful evaluation for impacts on performance of resulting males and concerns about its safe and secure implementation (see also below). Similarly, transgenic lines with a tra2 gene knocked down showed male sex-ratio distortion, possibly due to lethality of m-chromosome-bearing sperm and/or mm zygotes.
- Other conditional 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*.

Transgenic sexing systems will be important tools in the future, though the development of genetic screens and sexing strains through classical genetic approaches should also be pursued in view of the potential regulatory difficulties of transgenic systems (See 4.). 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.

3.4.3 Transgenic methods for population replacement

Gene drives offer additional possibilities to reduce mosquito population or vector capacity. The term gene drive summarizes all-natural mechanisms and genetic engineering technologies that are able to propagate a gene or suite of genes into a population by increasing the probability of inheritance to more than 50%. Gene drives could be developed to drive female lethality into a population, or to convert females into males, both of which could reduce or even crash the (local) wild population. On the other hand, natural

populations could be replaced with transgenic strains that are refractory to pathogen infection. Besides strongly facilitating functional gene studies and genome editing, the CRISPR/Cas technology has also revolutionized and facilitated the field of gene drive research. Recent work resulted in two gene drive systems in *Anopheles*. The first approach in *An. gambiae* targets female fertility by inserting a CRISPR/Cas gene drive construct in three candidate genes conferring recessive female sterility upon disruption. A CRISPR-based gene drive in *An. stephensi* is used to drive multiple anti-plasmodium effector genes into a wildtype population, resulting in more than 99% of positive offspring. While numerous drive systems have been developed in different insect species in the past 5-10 years, a major bottleneck currently is the quick resistance development against the drive mechanism, especially when CRISPR/Cas technology is used, resulting in the eventual loss of the drive. Moreover, self-propagating gene drive strains may face significant regulatory hurdles, in addition to the existing regulations for the release of non-driving GMOs.

3.4.4 Transgenic markers for monitoring

Marking of insects before release is crucial 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 ter death of the insect to allow for reliably distinguishing WT from released insects.

Fluorescent proteins like dsRed or EGFP are suitable markers fulfilling these requirements. This has been shown for the pink bollworm, where DsRed proteins were stable for at least 2 weeks after trapping of specimens as confirmed by fluorescence microscopy and PCR. A similar study with a transgenic strain of *A. suspensa* proved the stability of DsRed fluorescent marker protein over a period of 3 weeks in field traps, monitored by visual inspection and PCR. Studies in *A. ludens* confirmed the marker stability under dry conditions over several months. As the fluorescent protein degrades over time, PCR assays have been developed to verify the DsRed marker molecularly.

Several markers have been developed for mosquito species:

- *Ubiquitous 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. Instead of using suitable promoters to express the markers sex-specifically, transgenic lines with insertions of the marker on the Y chromosome have been created (see 3.4.2).

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 regard. The results from future classical mutagenesis screens could further enhance the development of marking systems for additional pest species.

3.4.5 Systems for genetic engineering and stability

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. Site-specific integration via a genomic *attP* docking site is the most frequently used strategy to date to target the mosquito genome. Several *attP* docking site strains are established for major vector species *Ae. aegypti*, *Ae. albopictus*, *An. gambiae*, and *An*. *stephensi*. Most *attP* docking site strains have been created by transposon-mediated transformation, resulting in random integration with potential negative effects on line quality. In contrast, an *attP* docking site has been placed on the *An*. *gambiae* Y-chromosome using meganuclease-induced homologous repair. Site specific integration via a single *FRT* site in mosquitoes to our knowledge has been reported only in plasmid-based assays, and via single *lox* sites only as part of a two-step RMCE. In contrast, site-specific excision via *lox* sites is commonly used in *Ae. aegypti*. Site-specific cassette exchange systems (RMCE) in mosquitoes has been reported for *Ae. aegypti* using either the Cre/*lox* or the phiC31/*att* system. For *Anopheles gambiae,* the phiC31-RMCE system has been reported.

Lines containing docking/landing sites for site-specific recombination are created by transposon-based methods and, therefore, 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. Moreover, the potential effects of the test design on the outcome should be carefully taken into account. In that respect, a guidance framework for testing genetically modified mosquitoes has been published in 2014 by WHO/TDR and FNIH. It aims to foster quality and consistency among processes for testing and regulating new genetic technologies by proposing standards of efficacy and safety testing comparable to those used for trials of other new public health tools.

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 have been used to modify specific regions of mosquito genomes. Since genome editing using ZFN and TALEN is time-consuming and work intense, the scientific community has mainly switched to the use of CRISPR/Cas for editing. Since 2015, CRISPR/Cas was transferred to *Aedes aegypti* and to different *Anopheles* species including African *An. gambiae, An. coluzii* and *An. funestus*, Indian *An. stephensi* and Asian *An*. *albimanus.* The technology is essential for laboratory research as well as the generation of transgenic insects, but can also be used to elucidate classical genetic markers and beneficial mutations as well as generate non-transgenic approaches in the future (see also 3.5).

3.5 Novel approaches for non-transgenic male-only strains

Recent development in genome-editing and mosquito genetics affords opportunity to generate efficient sexing strains without introducing any foreign genes. Such strains may be subject to a different regulatory process than the transgenic strains. We consider three categories here. The first is what we call a “neoclassical” approach in which a sexing strain is produced by linking a selectable marker to the sex-determining chromosome or locus, following the same process as how sexing strains are generated by classical genetic approaches. For example, a wild-type copy of a gene responsible for the red-eye mutant phenotype could be linked to the male-determining locus so that males will be wild-type and females will be red-eye. This method requires the identification of genes responsible for the selectable mutant phenotype. Alternatively, CRISPR/Cas-mediated gene knockout can be used to generate a mutant with a selectable phenotype. The main innovation here is that the linkage to the sex-determining locus is facilitated by precision knock-in or CRISPR/Cas-induced translocation. The second category involves the use of endogenous mosquito genes to produce genetic sexing strains. For example, by ectopically expressing the male-determining factor Nix in the females, female-to-male conversion is achieved in *Aedes aegypti* and this sex-conversion phenotype is highly penetrant and stably transmitted through males. In *Anopheles stephensi*, expressing an endogenous Y chromosome gene named *Guy1* in females conferred 100% female lethality, due to mis-regulation of dosage compensation in the XX females. The challenge is to make such ectopically expressed genes homozygous by conditional expression (e.g., by the introduction of temperature sensitivity) so male-only progeny can be produced for potential release. The third category starts with a strain in which an X- or m-linked transgene that confers female-lethality is maintained by conditional expression. The transgene is removed from the progeny when the transgenic males mate with wild-type females as all transgenic progeny will be dead females. This method requires a cross with wild-type females, which could be extra work but can be integrated into efforts to introgress with local mosquito populations.

3.6 Other novel technologies

To maintain utility of current core interventions and mitigate insecticide resistance spread in natural vector populations, new tools for malaria vector control are being developed and actively tested in the field. These include housing improvement through physical screening of all potential mosquito entry points and/or use of adulticide coated mesh "eave tubes" under the roofline, attractive toxic sugar baits (ATSB) exploiting natural sugar feeding behaviour in adult mosquitoes and the development of attractive synthetic human blends to lure mosquitoes into odour-baited traps for mass-trapping and/or development of protective trap belts.

Biological control of larval and adult stages with entomopathogenic Ascomycetes fungi of the genus *Beauvaria* and *Metarhizium* has also been actively investigated, providing theoretical support for evolution-proof disease transmission control using late-life-acting compounds that might kill vectors after reproduction but before pathogen transmission. Formulation optimization is a major issue to increase fungal longevity and delivery of killing doses to mosquitoes.

After the original trial conducted in Peru by Devine et al., auto-dissemination of juvenile hormone analogues proved efficient in various field trials to reduce the populations of *Aedes aegypti*, *Aedes albopictus*, *Anopheles arabiensis,* and *Anopheles quadrimaculatus*. The principle is that adult females are contaminated with growth inhibitors (e.g. pyriproxyfen) in attractive auto-dissemination stations that they thereafter transport to their oviposition sites, which get contaminated, preventing the development of larvae into adults. Pilot interventions with pyriproxyfen have given promising results. however, improved dissemination stations and devices, actives and formulations need to be devised to further improve effectiveness of autodissemination and demonstration of public health value still lags beyond. It has also been proposed to use sterile males to operate the dissemination of the biocides (see below).

4. REGULATORY ASPECTS

Once proof-of-principle and contained trials show promising results, permission for field-testing is required. No novel technology 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 base-line 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.

In Europe, irradiated males are not considered as GMOs. The European legal definition of a GMO is 'an organism, with the exception of human beings, in which the genetic material has been altered in a way that does not occur naturally by mating and/or natural recombination'. As sterile insects are not considered as organisms, they are not considered GMOs. Even with residual fertility, irradiated sterile male insects are exempted from GMO regulations when they are obtained by mutagenesis techniques that have conventionally been used in a number of applications, have a long safety record, and do not involve the use of recombinant nucleic acid molecules.

Some countries, such as the USA, typically have not specifically regulated the release of irradiated, sterile insects associated with agricultural, forestry and livestock production through the United States Department of Agriculture's Animal and Plant Health Inspection Service (USDA-APHIS). An argument can be made that irradiated, sterile insects could be deemed biological control agents (or beneficial organisms according to ISPM 3 of the IPPC), which, as pesticides, are exempt from regulation by the United States Environmental Protection Agency (EPA) as long as the EPA determines that regulation by another federal agency (e.g., APHIS) is adequate. The states of California, Florida and Texas will permit release of SIT male mosquitoes as long as the mosquito strains used originated in their respective states.

The current status for delivering permits and authorization to release SIT mosquitoes is country-based according to different regulatory authorities. Consequently, not only the different regulatory authorities are looking at different issues, but also the authorizations are based on very different perspectives. The need for a global approach on this subject will be one of the challenges to the full deployment of this technology if proven efficient.

In this regard and according to ISPM No. 3 "*Guidelines for the export, shipment, import and release of biological control agents and other beneficial organisms*" sterile insects are considered as beneficial organisms and defined as "*An insect that, as a result of a specific treatment, is unable to reproduce*" (IPPC 2005). A thorough risk analyses conducted in 2001, concluded that transboundary shipments of sterile insects pose negligible risk regarding sterilization, handling, packaging and shipment (FAO/IAEA 2017a). The analysis used historical data from 1963 to 2001 of transboundary shipments of sterile flies, period in which approximately 580 billion sterile insects were shipped to more than 20 countries. Since then, and considering other groups of sterile insects, the number has increased to over one trillion without a single incident in terms of risk (https://nucleus.iaea.org/sites/naipc/dirsit/SitePages/World-Wide%20Directory%20of%20SIT%20Facilities%20(DIR-SIT).aspx). The mass rearing and

sterilization of insects for use to suppress and eradicate insects of economic and quarantine importance, is carried out under confined conditions in facilities that follow harmonized best practices including rigorous mass rearing, quality control, packaging and shipping protocols (FAO/IAEA 2017a & b; FAO/IAEA 2016). Given this background, transboundary shipments of sterile insects have not been subjected to stringent regulations by importing countries. In most cases a certificate of origin and an export permit have been sufficient, in others, a simple phytosanitary certificate (or equivalent) with an explanatory note indicating "*sterile insects for use in pest control programmes*" has also been required. This includes countries such as Argentina, Australia, Chile, Croatia, Ethiopia, Germany, Guatemala, Israel, Jordan, Mexico, Morocco, Senegal, Spain, and the USA and insects such as fruit flies, moths, screwworms, tsetse flies and mosquitos. Given the negligible risk involved in transboundary shipments of sterile insects, they should be defined separately and importing authorities should not classify and process the sterile insects in the same way as other non-sterile live insects. In contrast, the regulations for the release of genetically modified organisms, including products created by the new gene editing technology CRISPR, are highly diverse around the globe. In the EU, for example, all GMOs are regulated by the EU directive 2001/18/EC. It states that an organism is characterized as GMO if its genetic material has been altered in a way that could not have occurred naturally by mating or recombination. Thus, conventional mutagenesis techniques like radiation or chemical mutagenesis, that were considered safe in 2001, are exempt from the GMO directive as long as they do not involve recombinant DNA (the mutagenesis exemption). On the other hand, however, also organisms developed by non-transgenic methods can be classified as GMO.

CRISPR/Cas allows the introduction of (point) mutations into the genome that would not be different from mutations that could arise naturally. Such CRISPR mutations, therefore, from a scientific point of view, should not be regarded and regulated differently than the widely accepted mutations created by classical mutagenesis or selective breeding. However, the EU court ruled in July 2018 that all products resulting from genome editing are subject to the EU directive 2001/18/EC and are to be treated as GMO without any exceptions. This decision raises different questions and uncertainty for the application of the new technologies. Interestingly enough, however, from a scientific point of view, it also puts the regulations for the established and safe methods in question. This EU decision is in line with the general approach of EU legislation considering the process used to create a product, not the resulting product itself. Therefore, two products with identical traits developed by different technologies could be regulated differently in the EU.

The United States (USA) essentially takes the opposite approach. There, only the product is evaluated, independent of the method used to create it (Global Legal Research Center 2014). Consequently, the USA does not regulate any products that could as well be the result of traditional mutagenesis or breeding techniques. Similarly, the Australian government ruled in April 2019 that the use of gene-editing techniques in plants, animals and human cell lines that do not introduce new genetic material, will not be regulated. These differential regulations will strongly complicate the application of genetically modified insects in support of the SIT, e.g. in form of sexing strains. While in some cases the differential legislation and regulation already create a difficult for self-limiting approaches like the SIT (where the genetically modified organism would not persist in the environment), it will be a tremendous hurdle for the field application of self-sustaining approaches like gene drives, with the purpose of fixation of the genetically modified trait in the environment and their spread across regions (i.e. across borders of countries with different regulation).

5. ACHIEVEMENTS AND CHALLENGES

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 implemented 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 GSS for *Aedes aegypti*, *Ae. albopictus* and *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.

Technical/Management Constraints and Gaps	**Potential IAEA contributions**
Need for efficient sex separation on a large scale	Testing / validation of sex-sorters developed by other teams and development of a sex-sorter based on pupal size for Aedes, fluorescent strain for Anopheles (COPAS sorter)
Creation of GSS (male-only strains)	Screening and/or induction of selectable markers (morphological, temperature sensitive etc) and Coordinated Research Project (D4.40.03) on "Generic approach for the development of genetic sexing strains for SIT applications".
Insecticide resistance	Reduced pressure for resistance development by using SIT and / or related technologies as alternative control methods.
Unacceptable or unaffordable costs of existing control methods	R & D into new control methods.
Insensitivity, difficulty and cost of vector monitoring methods	Testing/validation of more sensitive and inexpensive trapping methods.
Weak control, diagnosis and treatment infrastructure including insufficient trained staff	Training, capacity building, fellowships, etc.
Reintroduction of disease into disease-free areas	Development of containment strategies 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	Development of modelling 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.	Research focusing on the relationship between vector control and mosquito behaviour.
Heterogeneous results of induced sterility observed with identical irradiation doses between programs	CRP D44004: Mosquito Irradiation, Sterilization and Quality Control just launched

5.1 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, chikungunya and Zika, *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.

5.2 R & D achievements

5.2.1 Mass-rearing

Mass-production is a primary component of any pest or vector control programme that requires the release of large numbers 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 and protocols for *Ae. albopictus*, *Ae. aegypti* and *An. arabiensis*. Three guidelines are already available on the IAEA website and one more will be released soon.

Mass-rearing adult 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 from outside the cage, were developed and validated at the IPCL. The structure of the adult mass-rearing cage for mosquitoes was 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 and a device for blood feeding. These preliminary mass-rearing cages have been validated but costs were high. The cage technology was transferred to Italy, Brazil, China, Mauritius and Sudan for testing under local conditions. More recently, a cheaper design for an adult mass-rearing cage (~€200 versus more than ~€2000) has been tested for *Ae. aegypti*, with good results in comparison to previous reference cage:

- no difference in egg production per female
- significantly higher hatch rate of eggs
- no difference in survival

The new cage (same design) was validated for *Ae. albopictus* as well.

The technical drawings of the mass-rearing cage prototype are freely available online as supporting information files (S1-S15 Figs) to the published article and on the website of the FAO/IAEA joint Division under a Creative Commons 4.0 Attribution International license. Based on the IAEA reference design, Moscamed (IAEA Collaborative Center in Brazil) has developed its own mass-rearing cage (70 x 98 x 24 cm) for *Ae. aegypti* using local supplies. (Figure 5) This cage can hold from 11.500 to 22.000 insects depending on the defined density resting surface. The Center has also designed a movable trolley that can hold 10 mass-rearing cages. Other countries are also developing their own mass-rearing cages.

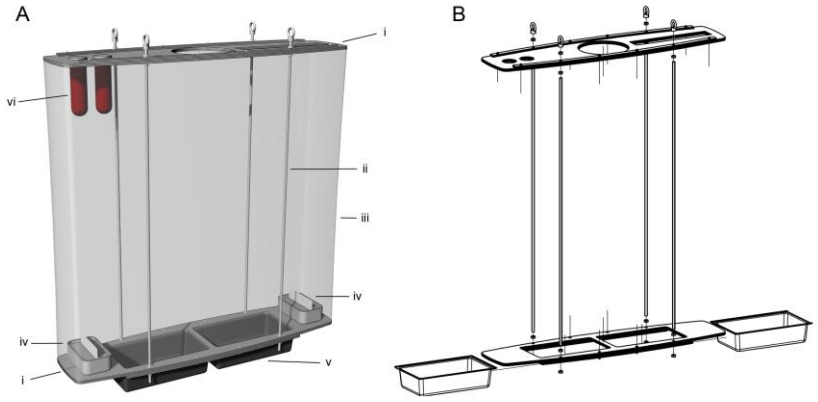

Figure 5 . The 3D design of the mass-rearing cage prototype (A) and structure (B). Plexiglass laser cut plates (i), four metal rods (ii), mesh netting (iii), sugar feeding container (iv), two containers for pupae and egg collection (v); mesh socks blood feeders (vi). The cage has a volume of 162 litres, with overall dimension of 900 (L) × 900 (H) × 200 (W) mm.

Larval rearing systems

The tray for large scale larval rearing is made of thermoformed plastic and has outside dimension of 60 × 100 × 3.5 cm to contain 6 litres of water. The Figure 6, top panel, shows the final prototype which was tested for its suitability for rearing larvae of *An. arabiensis*, *Ae. albopictus* and *Ae. aegypti.* A density of 4,000 L_1 of *An. arabiensis,* or up to 18,000 L_1 of *Ae. albopictus* or *Ae. aegypti* can be reared in one tray, depending on the feeding schedule, strain, and temperature. In optimal conditions, it is expected to have male pupal recollection

of around 75% and 85% for *An. arabiensis* and *Ae. albopictus,* respectively. The IPCL also designed a new larval tray tiltable rack with a reduced number of trays (n=30) in comparison with the previous version and made of aluminium. It has the same production efficiency and is three times cheaper than the reference stainless-steel rack.

Figure 6: Aluminium rack and tray

The tray/rack system has been transferred to Argentina, Brazil, Chile, China, Cuba, Italy, Jamaica, Malaysia, Mauritius, Mexico, Panama, Peru, Singapore, South Africa, Philippines, Thailand and Sudan for testing under local conditions.

A new version of the IAEA rack with 100 smaller trays and a medial wall for tilting was also derived from this design in China and is presently under testing at the IPCL.

An activity related to the rearing of *Aedes* mosquitoes was implemented and consisted of the testing of a larval counter for L1 larvae. This is an important activity, as determining the exact number of larvae to seed in the trays is crucial to optimize mass-rearing conditions. The larval counter showed to have good accuracy, good precision (repeatability and reproducibility of measurements) and no negative impact on larval survival, pupation success, adult emergence and production. The machine has likewise been tested with *An. arabiensis*.

Larval diet
A standard artificial larval diet named "IAEA diet" suitable for *Anopheles* and *Aedes* mosquito species has been developed, comprising ingredients (tuna meal, bovine liver powder, brewer's yeast and vitamin mix) that contain the necessary nutrients for larval development and adult fitness. The diet and SOPs for its proper use have been transferred to Benin, Burkina Faso, China, Indonesia, La Réunion, Malaysia, Pakistan, South Africa, Sri Lanka, Sudan, Thailand, 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. polynesiensis*.

Several research institutes in France, French Polynesia, Italy, Mauritius, Trinidad & Tobago, the UK, and the US are testing or routinely using this larval diet. However, the bovine liver powder ingredient is very expensive and its widespread availability is not always guaranteed. Therefore, to achieve a sustainable and affordable production, cheaper diet ingredients based on insect meals (mainly black soldier fly) were developed and validated for *Anopheles* and *Aedes,* resulting in major economic savings of approximately 80% compared to the standard diet.

5.2.2 Sex separation
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 increase the risk of transmitting human pathogens. The IPCL has started developing genetic sexing strains (GSS) based on classical genetic approaches. This is a challenging task given that at this point there are no many available genetic markers which could be used for the construction of a classical GSS similar to the one currently in use in *Ceratitis capitata* 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, *C. capitata* 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, and particularly the temperature-sensitive lethal (*tsl*), 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. In the last few years, it was possible to isolate a white larva body colour with a white / pink adult eye colour in *An. arabiensis* and to establish a line. Preliminary data suggest that this is due to an X-linked genetic locus, and further studies are needed to see whether this marker can be used for the development of an *An. arabiensis* GSS. In the frame

of the CRP on "Exploring genetic molecular, mechanical and behavioural methods of sex separation in mosquitoes", a *tsl* strain of *An. arabiensis* was established in a collaborating laboratory (Cyrille Ndo), which could potentially be used for the development of a GSS, but so far there is no color or gender selectable marker linked to it. 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 may provide a temporary solution for eliminating female *An. arabiensis* until a new GSS is developed.

In parallel, efforts were concentrated in characterizing mutant lines of *Ae. aegypti* and *Ae. albopictus* carrying morphological markers such as eye-colour. We used the red-eye and the white-eye color markers to establish GSS in *Ae. aegypti*. The red-eye GSS was shown to be a better strain exhibiting recombination rates of about 2-3%. An inversion (Inv35) was isolated via irradiation experiments and it was shown to reduce recombination by 10x thus resulting in a very stable GSS. The red-eye GSS, with or without inversion, was introgressed into different genomic backgrounds, all of which exhibiting the features of the genetic system and the highly reduced recombination rates. Interestingly, if the pupal size marker is combined with the red-eye marker and the Inv35, this would result to only 0.003% female contamination. The red-eye GSS with and without the inversion were successfully used in laboratory cage population suppression experiments. A red-eye GSS was also developed for *Ae. albopictus* but exhibited much higher recombination rates, about 18-20%. The larval body-colour mutants are being characterized.

In the case of *An. gambiae* and *An. arabiensis*, a system based on transgenic fluorescent markers/ COPAS sorter has been proposed (see section 3.4.2).

Other sex separation methods

Mechanical approaches to separate the sexes exist for *Aedes* mosquitoes using sieving or sorting methods. The sexual dimorphism of *Aedes* pupae allows efficient separation and (semi)automated sex sorters based on sexual dimorphism are being developed/tested at IPCL. Also, the phenomenon of protandy has been explored in *Ae. albopictus* in a collaborating laboratory (Romeo Bellini) in the frame of the CRP on "Exploring genetic molecular, mechanical and behavioural methods of sex separation in mosquitoes".

An automated sex-sorter prototype, using high-speed camera and laser and exploiting the sexual dimorphism at the pupal stage, was also tested with various strains of *Ae. aegypti*, *Ae. albopictus* and *Ae. polynesiensis* with excellent results for all *Aedes* species (but not for *An. arabiensis*): more than 80% male recovery with <0.1% female contamination under laboratory small-scale rearing conditions. A phase II of this sorter would be to separate females (instead of killing) from males using as selectable markers the pupal size and the eye colour.

A new automatic sex-sorter, based on the original Fay-Morlan glass plate sorter, has automated the sorting plates, reducing the time spent and the efficiency of the process. With a capacity to separate 3.4 million male pupae in one day by one person, this sorter is used in mosquito mass rearing facility in Guangzhou, China where over 10 million sterile *Ae. albopictus* males are weekly produced. This sorter was developed by Wolbaki company and it is presently under testing at IPCL.

5.2.3 Irradiation

Guidelines for the irradiation of pupae are available for sterilisation of *An. arabiensis, Ae. aegypti* and *Ae. albopictus* with gamma and X-ray irradiators. Dose-response curves were also established for these three species at both adult and pupal stages and demonstrated that when using a Co60 source with a dose rate of around 80 Gy/min, > 99% sterility is reached at 90 Gy for *Ae. aegypti*, 65 Gy for *Ae. albopictus* and 110 Gy for *An. arabiensis*. Lower doses are required to achieve the same level of sterility when lower dose-rate irradiators were used.

Several factors that could affect dose response during pupae irradiation were also assessed such as pupal age, pupal size, geographic origin of the strain, handling methods, and atmospheric conditions during the irradiation. Both pupal age, handling methods and hypoxic conditions significantly affect dose response in pupae and thus the resulting induced sterility, whereas pupal size and geographic origin of the mosquitoes did not have significant effects. Density dependent effects were also observed when increasing pupa numbers for mass irradiation, however these can likely be attributed to varying oxygen levels surrounding the pupae. Several assessments of female radiosensitivity in all three-species showed that female pupae are significantly more sensitive than male pupae.

A procedure to irradiate chilled adult *Ae. aegypti* has been developed and assessed at a large scale (50,000 males / batch) with good preliminary results both on quality and induced sterility. A dose-response curve has been established for pupae and adults of *Ae. aegypti, Ae. albopictus* and *An. arabiensis*. Generally, adults were slightly more radiosensitive than pupae aged 40h and older, although not significantly.

The effects of dose-rate independent of energy has been assessed using Gammacell220 irradiator with a Co60 source. The dose rate has a complex interaction with dose, and a significant effect on the dose-response in pupae has been observed. This partially explains the differences observed in dose-response in mosquitoes following irradiation in gamma-ray irradiators compared to X-ray irradiators. The effects of energy independent of dose-rate still needs further assessment to fully assess the differences and potential advantages of the two radiation sources.

Despite their high costs, self-contained gamma-ray irradiators are recommended as they have demonstrated a high degree of reliability in fruit fly, screwworm and tsetse AW-IPM programme for over 60 years. More recently efforts to find alternative, reliable and more affordable irradiation devices for insect irradiation, such as X-ray machines, are ongoing.

5.2.4 Handling, transport and release

In AW-IVM programmes that includes an SIT component, a large number of male mosquitoes are produced in mass-rearing facilities. Before being released into the environment, they need to be transported to the release site and this operation often requires a release facility to be established where males are fed and then released.

The best stages for transport, both for *Aedes* and *Anopheles*, are eggs and adults.

The suitable conditions for transport of adult sterile male mosquitoes including compaction and temperature have been studied. Transport of non-compacted adults at 20°C has been tested with success within the RER 5022 project to ship *Ae. albopictus* sterile males from Italy to Albania, to Greece and to Montenegro. Shipment of non-compacted adult *Ae. aegypti* and *Psorophora columbiae* within 24 hours in the U.S. has proven successful. However, non-compacted adults take too much space which is not affordable in case of large numbers need to be delivered. Mass-transport systems in insulated boxes are presently tested at IPCL with a target temperature window of 10-12°C. To avoid damage and loss of quality due to compaction, the mosquitoes must be set in layers of 3 cm maximum. The time of transportation is also an important factor with mortality of males increasing above 24 hrs.

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.

Ground release in urban areas are time consuming and do not assure adequate coverage of the private properties. Drones with embedded release machines allowing to release mosquitoes have been developed and tested for *Ae. aegypti* in Brazil and Mexico, and for *Ae. albopictus* in China. They are presently being refined to reduce their weight and increase the security of releases over urban areas.

Ovitraps and BGS traps have been tested in Brazil, China, Italy, La Reunion, Mauritius, Mexico, Spain for *Ae. aegypti* and *Ae. albopictus* monitoring in the context of SIT programs with good results. The development of more efficient and reliable field monitoring tools and methods serving the management of the programs, in particular more efficient male trapping devices for a better evaluation of the effects of a suppression programme is also ongoing.

Guidance for testing traps against Aedes have been recently published by WHO

5.2.5 Process and product quality control

5.2.5.1 Process QC

Several production parameters (e.g. hatch rate, emergence rate, development time, fertility) can be monitored routinely to ensure the quality along the production process for sterile males. Additionally, an adult-index was validated and used as a quick proxy to estimate the mosquito survival rates in mass-rearing settings. Standardization of QC protocols within sterile male production chain is needed.

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.

5.2.5.2 Product QC

Laboratory testing

Many parameters (e.g. survival, survival under starvation, mating propensity, mating capacity, mating competitiveness, flight ability) can be measured to assess the quality of the product in experimental conditions and achieve comparisons between production and release sites. A new QC test based on flight ability has been developed and validated for both *Aedes* and *Anopheles* mosquitoes against other standard parameters including the survival rate and the mating propensity. It needs further refinements and validation to measure how well it can predict the quality of the sterile males.

Semi-field testing

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 effectiveness 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 for *An. arabiensis* from Sudan, *An. coluzzii* 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. Semi-field tests were also performed to assess the mating compatibility of mosquito strains from differing geographic origins, and the competitiveness of the different males, using stable isotopes to follow the matings of each type of male with a local cohort of females. Mating compatibility assessments have been completed for *An. arabiensis* strains from Africa, *Ae. albopictus* strains form Europe, and *Ae. aegypti* strains from South America to evaluate the feasibility of importing sterile males from adjacent countries.

Field testing

Some QC parameters must be measured at regular intervals in the field (survival, recapture rate, dispersal, sexual competitiveness) and there is a strong need for better defining these parameters and standard protocols to measure them.

A new CRP on irradiation and QC in mosquitoes has just been launched (see below) to foster international collaboration on this topic.

5.2.6 Pilot trials

Pilot trials to evaluate SIT and IIT-SIT efficacy are currently underway in multiple countries. Tables 4, 5 and 6 below list the currently ongoing pilot projects with an SIT component against *Ae. aegypti*, *Ae. albopictus* and *An. arabiensis* with a short synthetic description.

Table 4. List of ongoing sterile insect technique (SIT) pilot projects against Ae. aegypti

Country	City	Approach	Size of release area	Inhabitants in the release area	Av. release density	Av. production	Current status	Integration with other tactics	Results
Brazil	Recife (PE) Carnaiba (BA)	SIT	56 ha	18,3k residents	5-10k males/ha/week (anticipated)		BLDC, mass rearing and irradiation capacity, suppression prior release	autodissemination traps	
Cuba	La Habana	SIT	15 ha				BLDC, insectary and irradiation capacity		
Malaysia	Melaka state	SIT	4 ha	16k residents			BLDC, Obtained National Institute of Health, Ministry of Health Grant to conduct pilot field testing on classical SIT with Medical Research Ethics Committee (MREC) approval	Insecticide fogging before the release	
Mexico	Tapachula	SIT	24 ha	697 residents	6k males/ha/week	191k males/week	BLDC. In 2018, 11 weeks of continuous releases comparing aerial and ground. In August 2019, releases restarted.	AW-IVM, door to door, biocontrol	25% reduction in hatch rate; 50% reduction in egg density; 75% suppression in adult density
Mexico	Merida	IIT / SIT	46 ha	1241 residents	>2k males/ha/week	1,000k males/week	Sustained releases started in 2019, mass rearing facility with irradiation capacity; new construction ongoing to expand the mass rearing facility		
Singapore	Nee Soon East (Singapore)	IIT / SIT	5+5 ha	31k residents			BLDC, operational research, insectary with irradiation capacity, communication campaign, MRR, sustained releases in 2016-2019	door to door	reduction in hatch rate ~35% (phase 1); ~70% (phase 2); ~90% (phase 3)
Thailand	Bangkok	IIT / SIT	~5 ha		5k males/ha/week	25k males/week	BLDC, 6 months of sustained releases	door to door	reduction in hatch rate 25-35%
US	Captiva island (Lee County, FL)	SIT	230 ha	379 residents	To be defined	to be defined	BLDC, operational research, , insectary with irradiation capacity, communication campaign department, MRR	Insecticide (adult and larvae), entomological surveillancens	

Table 5. List of ongoing sterile insect technique (SIT) pilot projects against Ae. albopictus

Country	City	Approach	Size of release area	Inhabitants in the release area	Av. release density	Av. production	Current status	Integration with other tactics	Results
China	Guangzhou	IIT / SIT	25+15 ha	1865 +350 residents	>100k males/ha/week	10,000k males/week	Field pilot completed. BLDC, operational research, development of tools, mass rearing capacity, communication, authorizations, sustained releases 2015-2017		reduction in hatch rate >95%
France	Reunion Island	SIT	32 ha		3k males/ha/week	100k males/week	BLDC, operational research, communication, authorizations, insectary with irradiation capacity, MRR	Deltamethrin ULV spraying +	Start Q1-2020
Germany	Heidelberg & Freiburg	SIT (elimination)	4 ha (2016) 10 ha (2018)		3k males/ha/week	30k males/week reared in Italy)	BLDC, sustained releases in 2016-2019	Bti treatment of larval breeding sites	reduction in hatch rate 15% (2016); 45% (2017)
Greece	Vavrona (Athens)	SIT	5+5 ha		3k males/ha/week	30k males/week reared in Italy)	BLDC, communication campaign, sustained releases in 2018 and 2019	door to door	reduction in hatch rate ~75% (2018 and 2019)
Italy	- Caselline, Boschi, Budrio, Santamonica - Bologna	SIT	-16-45 ha (4 villages) - 25 ha (Bologna)		0.9-1.6k males/ha/week (villages) 0.6-2.1k males/ha/week (Bologna)	50-100k males/week	Field pilot completed in 2013 (Upscaling field trials ongoing in Bologna	door to door	reduction in hatch rate: 18-68% in small villages; 32% (Bologna)
Mauritius	Panchvati	SIT	3 ha		20k males/ha/week	60k males/week	BLDC, small insectary, irradiation capacity, 9 months of releases in 2018	larviciding Bti fogging before releases	reduction in hatch rate ~35%
Spain	Valencia	SIT	44 ha (Polinya) + 35 ha (Vilavella)	2.5k residents (Polinya)	2k males/ha/week (2018)	180k males/week	BLDC, rearing and irradiation capacity. Sustained releases in 2018 and 2019	larviciding with Bti in public areas	reduction in hatch rate >30% (2018)

Table 6. List of ongoing sterile insect technique (SIT) pilot projects against Anopheles arabiensis *mosquitoes.*

Country	City	Approach	Size of release area	Inhabitants in the release area	Av. release density	Av. production	Current status	Integration with other tactics	Results
South Africa	KwaZulu / Natal	SIT	5 ha		5k males/ha/week (anticipated)	30k males/week reared in Johannesburg	BLDC, operational research, insectary with irradiation capacity, communication campaign, MRR	IRS and winter larviciding	

5.2.7 Phased conditional approach

As with plant and livestock pests, the implementation of the SIT for vector control is challenging, management intensive and a phased conditional approach is therefore recommended to minimize the risks of failure. A phased-conditional approach was developed by FAO/IAEA, including four phases from preparatory activities to operational deployment, with some milestones highlighted that include go/no-go criteria (Figure 7).

Phase 0 is a pre-intervention phase where stakeholder commitment is secured.

Phase I includes the collection of all relevant baseline data that are required to develop an appropriate intervention strategy against target mosquito populations.

Phase II includes all necessary activities for a successful small-scale field trial.

Phase III includes the necessary activities to upscale the intervention.

Phase IV corresponds to the area-wide deployment of the intervention (including the release of sterile moths) that is guided by an adaptive management approach.

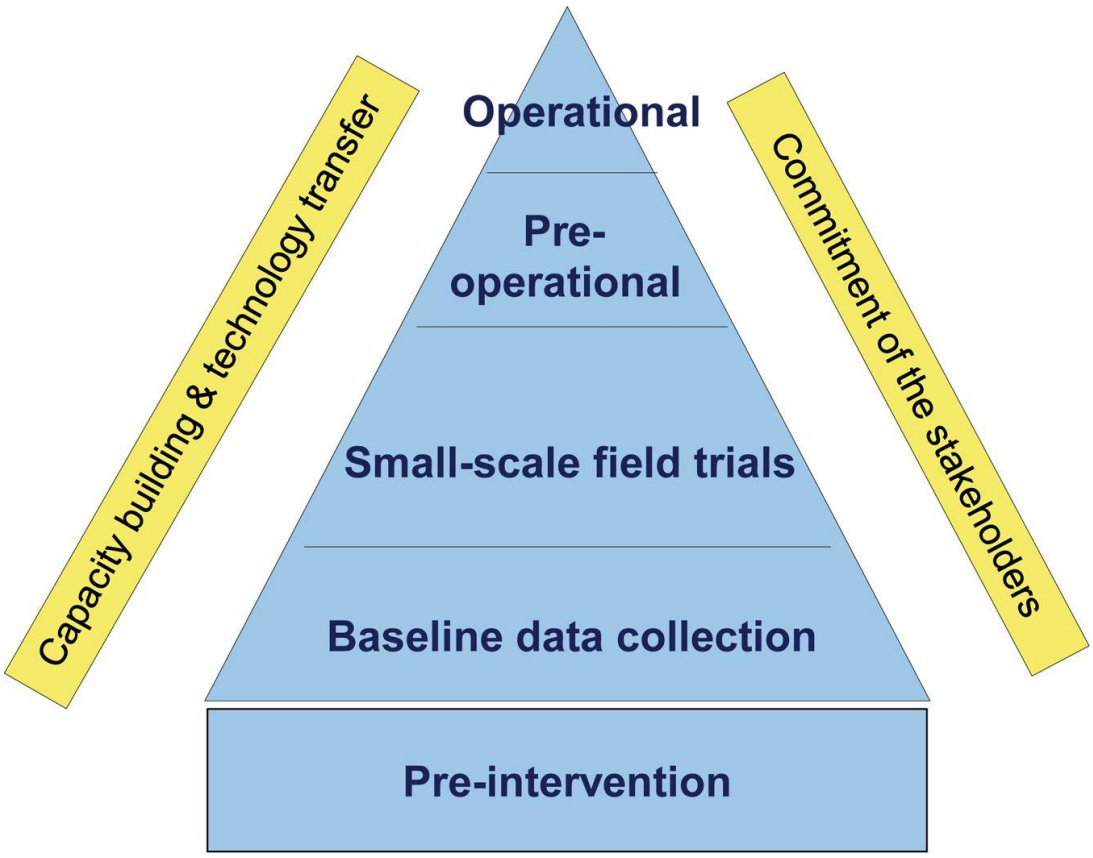

Figure 7. The general outline of the proposed phased-conditional approach.

5.3 CRPs and TCPs

5.3.1 Past, Current and Future Coordinated Research Projects (CRPs)

Three CRPs on human disease vectors have been so far conducted, two are ongoing and the other one will be initiated in 2020 (Table 7). Those are chronologically presented below with the objectives and achievements listed.

Table 7: Coordinated Research Projects (CRPs) on human disease vectors finalized, ongoing and planned to be initiated in 2020.

Project Number	Finalized CRP
G3.40.01	Development of Standardised Mass-Rearing Systems for Male Mosquitoes (2005-2011)
G3.40.02	Biology of Male Mosquitoes in Relation to Genetic Control Programmes (2008-2013)
D4.40.01	Exploring Genetic, Molecular, Mechanical and Behavioural Methods of Sex Separation in Mosquitoes (2013-2018)
	Ongoing CRPs
D4.40.02	Mosquito Handling, Transport, Release and Male Trapping Methods (2015-2020)
D4.40.03	Generic Approach for the Development of Genetic Sexing Strains for SIT Applications (2019-2024)
	New CRP
D4.40.04	Mosquito Radiation, Sterilization and Quality Control (2020-2025)

CRP D4.40.01 on "Development of Standardised Mass Rearing Systems for Male An. arabiensis Mosquitoes" (completed: 2005-2011)

During 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: 2008-2013)

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" (completed: 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 main results of this CRP included: (a) the development of GSS by using irradiation and classical genetic approaches in both *Aedes* and *Anopheles* species; (b) molecular approaches were exploited for sexing in both *Aedes* and *Anopheles* species including the use of Y-linked fluorescent markers and (c) mechanical, behavioural, developmental and symbiont-based approaches were also exploited for sex separation in mosquitoes including the development of a laser sorting system based on pupal size dimorphism for *Aedes* species (https://parasitesandvectors.biomedcentral.com/articles/supplements/volume-11-supplement-2).

CRP D4.40.02 on "Mosquito Handling, Transport, Release and Male Trapping Methods" (ongoing: 2015-2020)

This CRP was launched in 2015 with the following specific research objectives: (a) to explore approaches to perform the necessary handling and transport of irradiated, sex-separated male mosquitoes to the site of release, with minimal impact on survival and quality of released insects, including consideration of pre-release nutritional conditions; (b) to explore approaches to releasing sterile male mosquitoes in a controlled, traceable and documented manner over a large area, with the ability to target specific areas, ensuring low mortality and high quality in released insects, and (c) to explore different monitoring

systems for surveillance of the target population of an AW-IPM programme with an SIT component, and to follow the performance of released males and the efficacy of population suppression. More than twenty participants, from Australia, Brazil, Burkina Faso, China, France, French Polynesia, Germany, Indonesia, Italy, Mauritius, Mexico, Philippines, Senegal, South Africa, Spain, Sweden, Thailand, United Kingdom, United States of America have attended the research coordination meetings held in Vienna (2015), Valencia (2017) and Joazeiro (2018).

CRP D4.40.03 on "Generic Approach for the Development of Genetic Sexing Strains for SIT Applications" (ongoing: 2019-2024)

The First Research Coordination Meeting of the Joint FAO/IAEA Coordination Research Project "Generic approach for the development of genetic sexing strains for SIT applications" was held in Vienna International Centre, Vienna, Austria from 7-11 October 2019. The meeting was attended by 22 scientists from Argentina, Australia, Cameroon, Canada, China, Czech Republic, France, Germany, Greece, Guatemala, Israel, Italy, Mexico, Switzerland, Thailand, United Kingdom and United States of America. In addition, ten observers from Germany, Greece, Israel, Italy, Netherlands, Thailand and United States of America attended this meeting. The main objective of this CRP is to develop and evaluate potential generic approaches for the construction of genetic sexing strains (GSS) to be used for SIT applications, as part of AW-IPM programs, to control populations of agricultural pests and human disease vectors. The expected results of this CRP are: (a) the isolation of selectable markers to be used for generic strategies for the construction of GSS; (b) the development of generic approaches for the construction of GSS for SIT targeted agricultural pests and human disease vectors and (c) the evaluation at small-scale of the GSS strains developed with the generic approaches (for both agricultural pests and human disease vectors).

CRP D4.40.04 on "Mosquito Radiation, Sterilization and Quality Control" (new: 2020-2025)

The main objective of this CRP is the development and evaluation of irradiation and quality control procedures to be used for sterile insect technique (SIT) applications, as part of area-wide integrated pest management (AW-IPM) programmes, to control populations of mosquitoes, vectors of human diseases. as specific objectives: (1) Understand the factors that affect sterilization by irradiation and downstream performance of the sterile male mosquitoes; (2) Design and validate irradiation and dosimetry protocols for large numbers of mosquitoes, that are appropriate for operational programmes (3) Develop and validate standard product Quality Control procedures for sterile male mosquitoes.

5.3.2 Current support to Technical Cooperation Projects

Eleven human disease vectors mosquito Technical Cooperation projects are ongoing (7 national, 3 regional and 1 interregional) (Table 8). The new projects to be initiated in 2020 are listed on the Table 9.

Table 8: IAEA Technical Cooperation Projects on human disease vectors that are ongoing in 2019.

Country	Project Number	Ongoing National Projects
Brazil	BRA5060	Using the Sterile Insect Technique to Evaluate a Local Strain in the Control of *Aedes aegypti*
Cuba	CUB5021	Demonstrating the Feasibility of the Sterile Insect Technique in the Control of Vectors and Pests
Mexico	MEX5031	Using the Sterile Insect Technique to Control Dengue Vectors
Philippines	PHI5033	Building Capacity in Using the Sterile Insect Technique against Dengue and Chikungunya Vectors
South Africa	SAF5014	Assessing the Sterile Insect Technique for Malaria Mosquitos in a South African Setting, Phase II
Sri Lanka	SRL5047	Establishing a National Centre for Research, Training and Services in Medical and Molecular Entomology for Vector-borne Disease Control
Sudan	SUD5038	Implementing the Sterile Insect Technique for Integrated Control of *Anopheles arabiensis*, Phase II
		Ongoing Regional Projects
Regional Asia	RAS5082	Managing and Controlling *Aedes* Vector Populations Using the Sterile Insect Technique
Regional Europe	RER5022	Establishing Genetic Control Programmes for *Aedes* Invasive Mosquitoes
Regional Latin America	RLA5074	Strengthening Regional Capacity in Latin America and the Caribbean for Integrated Vector Management Approaches with a Sterile Insect Technique Component, to Control *Aedes* Mosquitoes as Vectors of Human Pathogens, particularly Zika Virus
		Ongoing Interregional Project
Interregional	INT5155	Sharing Knowledge on the Sterile Insect and Related Techniques for the Integrated Area-wide Management of Insect Pests and Human Disease Vectors

Table 9: IAEA Technical Cooperation Projects on human disease vectors that are approved to be initiated in 2020.

		New National Projects to Start in 2020
Brazil	BRA5061	Using the Sterile Insect Technique to Apply a Local Strain in the Control of *Aedes aegypti* (Phase II)
Ecuador	ECU5032	Building Capacity for Mass Rearing, Sterilization and Pilot Release of *Aedes aegypti* and *Philornis downsi* Males
Jamaica	JAM5014	Establishing a Self-Contained Gamma Irradiation Facility for the Introduction of Sterile Insect Technique and Experimental Mutagenesis and Diagnostic Technologies
Mauritius	MAR5026	Sustaining the Suppression of *Aedes albopictus* in a Rural Area with Possible Extension to An Urban Dengue-Prone Locality through Integrated Vector Management Strategy
Mexico	MEX5032	Scaling Up the Sterile Insect Technique to Control Dengue Vectors
South Africa	SAF5017	Assessing the Sterile Insect Technique for Malaria Mosquitoes — Phase III
Turkey	TUR5026	Conducting a Pilot Program on Integrated Management of *Aedes aegypti* Including Sterile Insect Technique
		New Regional Projects to Start in 2020
Regional Europe	RER5026	Enhancing the Capacity to Integrate Sterile Insect Technique in the Effective Management of Invasive Aedes Mosquitoes
Regional Latin America	RLA5083	Enhancing Capacity for the Use of the Sterile Insect Technique as a Component of Mosquito Control Programmes

6. POTENTIAL FUTURE ROLE OF THE IAEA AND THE JOINT FAO/IAEA DIVISION

6.1 Targeting *Anopheles* and *Aedes* species to control malaria and arboviruses 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 international levels.

Outbreaks of Zika virus disease have been recorded in Africa, the Americas, Asia and the Pacific. From the 1960s to 1980s, rare sporadic cases of human infections were found across Africa and Asia, typically accompanied by mild illness. The first recorded outbreak of Zika virus disease was reported from the Island of Yap (Federated States of Micronesia) in 2007 followed by a large outbreak of Zika virus infection in French Polynesia in 2013 and other countries and territories in the Pacific. Brazil reported a large outbreak of Zika virus infection that was later associated with microcephaly. More outbreaks occurred throughout the Americas, Africa, and other regions of the world (https://www.who.int/news-room/fact-sheets/detail/zika-virus).

6.2 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 plays an important role in developing the technology

and supporting the implementation of AW-IVM programmes. Because the SIT is not a stand-alone technique and its effectivity increases inversely with the vector population density, it must be integrated with other control tactics capable of reducing the vector population in scenarios of high vector densities, as is very often the case in areas under epidemiological risks.

For *Aedes* species, the main suppression tools that are currently available are source reduction and auto-dissemination of juvenile hormones.

Although there is currently not strong evidence that ULV spraying of biological larvicides (Bt, Spinosad) can suppress *Aedes* populations, it would be interesting to investigate if they could be used in integration with SIT since they represent a synergistic combination.

Sterile males can also be used as carriers of biopesticides to control the wild vector population. This approach has been named boosted SIT and, although it has not yet been validated in the field, it has the potential to be cost-effectively integrated with the SIT. Different toxic agents can be carried by the sterile males such as juvenile hormone analogues, entomological fungi and densoviruses. In the case of juvenile hormone analogues like pyroproxifen, the sterile males can transfer it to the wild females during mating but also to the larval breeding sites since it has been observed that males are also caught in sticky ovitraps. An advantage of using juvenile hormone analogues as biopesticides is that it is innocuous to the carriers, as opposed to entomological fungi and densovirus.

6.3 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 environment-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/Zika 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 8).

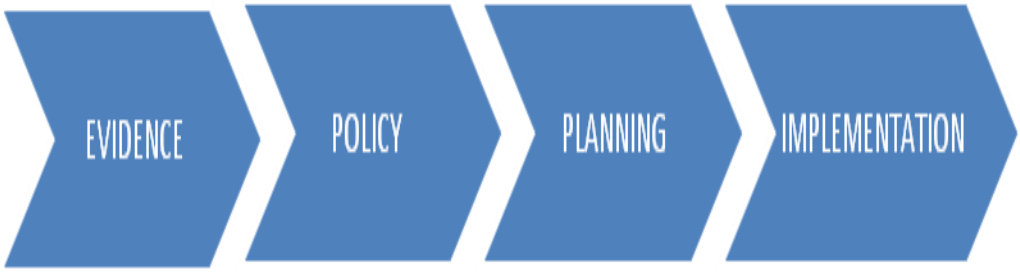

Figure 8. Framework for translating evidence into policy and implementation.

6.4 R&D Priorities to Address Bottlenecks

6.4.1 Development of genetic 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 the number of residual females released together with the sterile males. 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 with an SIT component shows the capacity to sustainably suppress the mosquito population below the epidemiological threshold. Currently, there are no mosquito sexing systems available that are efficient enough in terms of sexing efficacy (residual female contamination and male recovery), robustness and costs to be applied in mass rearing facilities. As it is necessary to reduce the number of residual females further, and at the same time improve the recovery rate of males, GSSs should be developed either through classical genetics and/or genetic engineering approaches to be able to reliably eliminate females before any mosquito release.

Selectable markers useful for the creation of GSSs

As mentioned above, several potential markers are available for *Aedes* species. Two of them (*red-eye* and *white-eye*) have been used for the construction of GSS in *Aedes aegypti* which, with the currently available tools, allows the sex separation at the pupal stage. Similar GSS need to be developed for *Aedes albopictus* including inversions which would allow the drastic reduction of recombination between the selectable marker and the M locus. Efforts also need to be undertaken to investigate whether the red-eye marker can be used for sex separation at earlier developmental stages and / or in combination with the pupal size marker (see below). Also, the larval body colour mutants need to be characterized in respect to their inheritance pattern and biological quality before they can be considered as selectable markers for GSS. In addition, there is an urgent need for the isolation of selectable markers which would allow sex separation at early developmental stages, ideally at the embryonic stage, such as temperature sensitive lethal (*tsl*) mutations, egg melanization, etc.

For *Anopheles arabiensis*, there is an urgent need to isolate and characterize morphological markers which could be used as selectable markers in combination with the *tsl*. This would allow the development of a GSS, similar to the one widely for SIT applications against *Ceratitis capitata*. An alternative approach would be to exploit the use of fluorescent strains which can be sorted by COPAS and the possibility of producing non-transgenic males by crossing males and females originating from two different transgenic strains.

Generic approaches

Despite tangible benefits coming from the use of genetic sexing strains, a 'generic' approach for their development and transfer to other species is not available. The possibility and feasibility of developing such an approach is of major importance, including the investigation of the cross-species transferability of each system, because gene functions may not be conserved between species. For example, it may be possible to transfer sex determination based GSS components among tephritid species but not to mosquitoes. In most cases however, these 'generic' approaches to the development of GSS would reduce

research and development time and costs, allowing SIT programs to be more readily developed and implemented. Research efforts will focus on the gene discovery related to key traits, such as eye colour, larval/pupal body colour, temperature sensitive lethality, slow development, which would then allow the linkage of wild type alleles to the male determining regions of SIT target species such as *Aedes aegypti*, *Aedes albopictus* and *Anopheles arabiensis* (neoclassical approach - see 3.5). Efforts should also be made with collaborators to exploit alternative strategies to produce non-transgenic males for releases by: (a) the ectopic expression of Nix in the *Aedes* females resulting in a sex-conversion phenotype; (b) expressing the endogenous Yob in *An. arabiensis* females resulting in 100% lethality and (c) producing a strain in which an X- or m-linked transgene that confers conditional female-lethality and removing the transgene from the progeny by crossing the transgenic males with wild-type females.

6.4.2 Development and/or improvement of other sex-separation methods

Two methods are currently available to eliminate females from release material without transgenic strains or a GSS: pupal size dimorphism in *Aedes*. An automatic sex-sorter based on pupal size has recently been developed by Wolbaki and is presently tested at the IPCL.

Ivermectin feeding of adult females via the blood has been proposed for *Anopheles*. The use of ivermectin is however not cost-effective since the mosquitoes must be kept at the insectarium for at least one week. Moreover, the risks of contamination are important. These methods still require improvement and additional methods should be explored and further developed, especially for *Anopheles*.

Various companies announced other automatic sex-sorters based on AI and morphological differences in adults (Senecio, Verily). However, no data is published to date.

As mentioned above, an automated sex-sorter prototype, using high-speed camera and laser and exploiting the sexual dimorphism at the pupal stage, has been developed and validated for *Aedes* species. A phase II of this sorter would be to separate females (instead of killing) from males using as selectable markers the pupal size and the eye colour.

6.4.3 Improve mass-rearing systems

In recent years, significant progress has been made to improve and reduce the cost of the mass-rearing of mosquitoes. Nonetheless, further improvement is required to produce the equipment at even 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. Further studies on blood feeding and oviposition optimization to minimize escapees are needed to improve the recent developed tools. More automatization will be needed to reduce workload and increase efficiency. Schematic plans should also be developed for different mosquito species and scales of production. An optimal larval feeding regime with low cost diet ingredients and automated feeding system are needed for optimal male pupae recovery at one-time tilting. In addition, the water quality (water hardness/conductivity) needs to be assessed and considered when mass rearing mosquitoes.

6.4.4 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 slightly between strains of the same species, due to both

internal factors (such as natural genetic variation) and/or external factors (such as varying rearing, handling, and irradiation device, tools and methods). Therefore, it is necessary to determine the dose response curves for each SIT target species and facility. 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 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*, *Ae. albopictus* and *Ae. aegypti*, for pupal and adult stages, and for males and females.

Many significant biological and physical factors affecting dose-response during pupae irradiation have been identified, but more research is needed to evaluate these factors for adult irradiation. Density dependent and temperature dependent effects are also expected for both life stages and need to be investigated. The possible advantages of irradiation of pupae or adults in hypoxia, or anoxia (for example in Nitrogen atmosphere) still need further attention to assess possible methods for improving sterile male quality. Dose-rate effects independent of energy have been shown to significantly alter cellular responses and thus induce sterility in pupae. The interaction between dose-rate and dose has been proven complex and needs better understanding in order to assess any potential benefits leading to improved adult quality. Energy independent of dose-rate also needs further attention to assess the possible advantages of a given radiation source over another. These topics are covered by the CRP entitled "Mosquito radiation, sterilization and quality control" (2020-2025).

In case of pupa irradiation, a complementary process to be able to apply the desired dose at a specific pupa age, it is possible to correlate the darkening process during adult development inside the pupa (metamorphosis), with age (in hours), under constant rearing conditions. And using this information, it is possible to develop a camera-based system using a machine learning algorithm to determine and select pupae at certain and appropriate age interval for irradiation, as a way to automate the pupa selection process for irradiation.

6.4.5 Development of marking systems for monitoring

Markers, whether phenotypic/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 markers, such as the use of fluorescent proteins, are available for field use to easily recognize and detect the fate of released material through molecular technologies.

Recently, a method for mass-marking of chilled adult mosquitoes based on fluorescent dyes has been developed at IPCL. It was successfully tested recently in Brazil; however, more validation trials are needed in the field.

Another method has been proposed which is based on feeding males with a sugar solution containing rhodamine B allows to monitor the sperm, which is also marked, in mated females. It also needs validation in the field to see if it allows measuring the competitiveness directly (without the need to measure hatch rates), knowing that the rhodamine is progressively eliminated so that only part of the males is marked after 4-5 days, as demonstrated at IPCL recently.

6.4.6 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 need to be combined with aerial release systems so that repackaging is not necessary before release. Transport, handling and release methods have been developed at the IPCL 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.

A first drone release system has been validated in the field and is currently being improved to fit with urban settings. Mass-transport systems for chilled sterile males have been developed and are under refinement.

6.4.7 Mating behaviour and interspecific competition studies that affect AW-IVM with an SIT component

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.

Stable isotopes can be useful tools to study the mating behaviour and the fate of sperm in mosquito mating studies. Two groups of males such as sterile and fertile males, or males of different strains can be marked with stable isotopes such as C^{13} and N^{15}, and following a period of mating, the presence (or absence) of these isotopes can be detected in female spermathecae. Previous studies have indicated that virgin female mosquitoes can accept sperm from 2 males if the two matings occurs within a 20 minutes time frame, but sperm transferred thereafter is not used for egg fertilization. This information provides important insight in *Aedes* mosquito mating behaviour which will assist in the improvement of sterile male release strategies.

Stable isotopes are also useful in competitiveness studies in which affects of variables other than sterility can be ascertained. Two groups of fertile (or sterile) males can be marked and added to virgin females to compete directly in one field cage (or in the field) without giving one treatment group an added disadvantage of being sterilized. Similar protocols can also be used to assess mating compatibility between two strains, and occurrence of assortative mating.

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.

Satyrization experiments were conducted at the IPCL to investigate the hetero-specific impact of sterile males when target areas include both *Ae. albopictus* and *Ae. aegypti.* The study demonstrated a resistance to satyrization behaviour in *Ae. aegypti* from La Réunion island against either sympatric and allopatric *Ae. albopictus* species with a very low level of cross-mating. Therefore, the release of sterile male *Ae. albopictus* may not suppress *Ae. aegypti* populations in La Réunion island if an overflooding ratios leads to similar results.

6.4.8 Exploit symbionts and/or supplements 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, behaviour and evolution. In fruit flies, it has been shown that gut-associated bacteria can be given as probiotics to improve pupal and adult productivity, the mating behaviour and performance of irradiated males. Moreover, some compounds derived from plants species have been shown to enhance the mating competitiveness in fruit flies. This is an area which should be exploited for the improvement of mosquito mass rearing and sterile male performance for SIT applications via the characterization of the mosquito associated microbiota including the isolation of cultivable microbial species. In parallel, stimulants, vitamins, semiochemicals etc. could also be considered towards the same goal.

6.4.9 Monitoring tools and protocols

In the case of *Aedes* species, ovitrapping is the most frequent 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.

However, several new adult traps including BG-traps and ovi-sticky traps have been developed and standardized recently. MRR procedures have been validated in Albania, Brazil, Mauritius and Italy recently.

A guideline on MRR to evaluate survival, dispersal and competitiveness of sterile male mosquitoes is under finalization. However, it will be necessary to standardize existing protocols in various environmental settings.

Moreover, monitoring the epidemiological impact will necessitate some adaptation of the current protocols proposed by WHO VCAG which are not compatible with area-wide principles (contamination of arms through female immigration).

6.4.9 Cost-efficacy evaluation of SIT programmes

A tool allowing to estimate the unit cost of production of sterile males at operational scale has been developed and is available to Member States to estimate production costs in different scenarios. This tool can be used to design mass-rearing facilities but also cost-effectiveness analysis.

A first analysis of cost-effectiveness of SIT within an IVM package including larviposition and door to door was commissioned in Italy recently. This evaluation indicated a clear superiority, in terms of cost-effectiveness, of SIT compared to door-to-door (DtD) treatments as integrative strategy to the conventional *Ae. albopictus* control measures adopted in the study area. These results will need to be confirmed in different settings and other countries.

Finally, the cost of the IIT-SIT trial in China was estimated to 54-172 USD/ha/week. However, accounting for various cost reductions related to improvements of the technology that are ongoing, the overall predicted costs can be reduced to 108-163 USD/ha/year for an intervention period of ten years including a two-year period suppression.

6.4.10 Scaling up and sustainability of AW-IVM programmes with an SIT component after successful pilot trials

When a pilot trial is planned, effort should be initiated to sustain the program such that the experienced key team members, equipment and facility can be maintained after a successful trial. Sustaining the program is also important in term of cost-effectiveness as maintaining suppression after knockdown of a population costs significantly less than suppressing a population at a high density. The cost of a SIT program will be low if it is measured in the long term when the program is sustained. The most important factor to affect this sustainability is to have fund secured without a gap. Public support from the government will be a typical fund source. However, there may be a difference between expectations from government and reality of the program's ability in implementation of SIT for disease control at the stage of completion of a pilot trial. For example, implementation will require for much larger scale in both mass-rearing and release with cost-effectiveness that is comparable with the existing technology. Thus, having fund support for further scale-up after pilot trial is essential to sustain the program. In addition to public fund agents, the private sector can be other fund source to sustain the program when mosquito control is needed but there is no disease transmission. A public–private partnership is also a potential mode to run the program to address the need of disease control and reduction of nuisance biting.

6.4.11 Integration of SIT with other suppression techniques

Given the low effectiveness of SIT to control high densities of pests, the suppression of the wild mosquito population densities is critical before the start of the sustained release of sterile males. The cost-effective integration of SIT with other suppression techniques that are effective at high mosquito densities is needed for a sustainable AW-IVM programme with a SIT component.

Examples of suppression tools that can be integrated with the SIT include the use of larvicides, source reduction by destruction of larval habitats, auto-dissemination of juvenile hormones.

We can also mention the use of sterile males as carriers of biopesticides, an approach that has been named boosted SIT, to increase the robustness of the SIT, regardless of the level of the wild mosquito density. Sterile males can be used to carry and transmit different biopesticides such as entomopathogenic fungi, densoviruses and juvenile hormone analogues, such as pyriproxyfen. The advantage of using pyriproxyfen as biopesticide to control small container breeding mosquitoes are: (i) it is not toxic to adults, (ii) it can be transferred from the sterile males horizontally to the females during the matings but also directly to the larval breeding sites and (iii) low concentrations in the water of breeding sites (as low as 1ppb) can prevent the development of immature stages. In addition, if a high percentage of contamination of larval breeding sites is achieved, the control of the population will be less impacted by the reinvasion of gravid females from the non-target area, making the control strategy less dependent on the requirement of a good isolation of the release area to comply with the area-wide approach. From the logistics and operation points of view, boosted SIT can be cost-effectively integrated with SIT.

Although statistical models have been published suggesting a high potential for the control populations of small container breeding mosquitoes, boosted SIT is currently under evaluation at the laboratory level for mosquitoes. There are some examples of operational programmes using sterile insects as carriers of biopesticides such as the Moscamed programme in Chiapas (Mexico) where sterile males of medflies infected with the entomopathogenic fungus *Beauveria bassiana* have successfully controlled hotspots of wild medflies against which the release of sterile males at conventional densities was not sufficient. For mosquitoes, this approach still needs to be validated in the field.

Another example of IVM is the combined SIT-IIT strategy that presents several advantages in comparison to pure SIT, the only drawback being a more complex regulatory framework (see 3.3.2).

6.4.12 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 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).

Recently, a new flight test has been developed and validated at the IPCL and its integration in future standards for mosquitoes is ongoing.

Also, a new CRP including QC has been proposed for the cycle 2020-2025 and will allow to gather a network of collaborators to contribute to develop such a standard.

Protocols for assessing mating competitiveness in semi-field cages are being improved and standardized in the aim to produce more indicative results with less variation between technical repeats. Protocols for assessing sterile male competitiveness in the field have been formulated and implemented in MRR studies and SIT pilot trials.

Quality assessments in terms of life history traits, production parameters and mating studies exist, however standards still need to be agreed upon and set. Currently, the following parameters are used for indications of quality: Hatch rates, pupation rates, emergence rates, body size (wing lengths), longevity (under normal- and under stress conditions), insemination rates, fecundity, flight ability, and mating competitiveness.

6.4.13 Develop and/or refine guidelines and standard operating procedures for each component of the SIT package

Several guidelines are already available on the FAO-IAEA website:

1) Guidelines for standardised mass rearing of Anopheles mosquitoes V1.0
2) Guidelines for routine colony maintenance of Aedes mosquito species V1.0
3) Guidelines for colonisation of Aedes mosquito species V1.0
4) Spreadsheet for Designing Aedes Mosquito Mass-Rearing and Release Facilities V1.0

The following guidelines are planned to be developed:

1) Guidance Framework for Testing the Sterile Insect Technique (SIT) as a Vector Control Tool against Aedes-Borne Diseases
2) the transfer of *Ae. aegypti* red-eye GSS into local genomic background
3) the rearing and QC analysis of *Ae. aegypti* GSS and related lines under small scale rearing conditions / filtering
4) the introgression of the inversion (Inv35) into *Ae. aegypti* GSS to enhance their genetic stability
5) the isolation and genetic analysis of mutations
6) the genotyping of wild type, mutant and GSS lines
7) mass-rearing of *Aedes* mosquitoes
8) mark-release-recapture procedures for *Aedes* mosquitoes
9) small-scale irradiation of mosquito pupae in SIT programs
10) Irradiation procedures for routine irradiation of chilled adult Aedes spp.
11) Sex-sorting of irradiated mosquitoes
12) Handling and release of irradiated mosquitoes
13) Design of pilot trials
14) Phased-conditional approach

7. PRIORITIES FOR CAPACITY BUILDING AND OTHER NEEDS

7.1 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.

7.2 Networking and sharing expertise among Member States

The IAEA has addressed this need through Coordinated Research Projects during the last 15 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.

7.3 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. A global, standardized training package covering all aspects of the SIT for mosquitoes has been initiated and needs further development as new technological advancements become available.

7.4 Research infrastructure needs to be improved

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.

7.5 Peaceful Uses Initiative

In addition to on-going IAEA-TC projects, the IAEA Peaceful Uses Initiative (PUI) and other sources should be explored even further for potential extra-budgetary support in support of IPCL activities in the peaceful uses of nuclear technology. In the past few years the Insect Pest control received substantial support through the PUI and other mechanisms, thanks to the pledge of Japan, United Kingdom and USA.

8. 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 the IAEA collaborating centers MOSCAMED in Brazil and CAA in Italy as well as with long standing collaborators at INSP-CRISP, Chiapas Mexico and TRAGSA in Spain.

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 part of the IAEA and WHO collaboration, a recent call was put out by the Special Programme for Research on Tropical Diseases (TDR/WHO) for public health partners to test the SIT technology as a component of AW-IVM against mosquitoes and carry out epidemiological evaluations. Three multi-country proposals targeting main disease-transmitting mosquito vectors *Ae. aegypti* and *Ae. albopictus* will be selected for two-year pilot projects.

Finally, IAEA and WHO also signed a Memorandum of Understanding in July 2019 to intensify research and development on the use of SIT as a component of AW-IVM to fight disease-transmitting mosquito vectors.

9. Recommendations to the IAEA

General Recommendations

- Malaria, dengue, chikungunya, Zika 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 for prevention and control. Fortunately, only a handful of mosquito species are responsible for much of the transmission. Conventional methods which are largely based on insecticides are neither effective nor sustainable. In addition, there is major concern about the evolution of insecticide resistance and the impact of insecticides on the ecosystems and human health. The above directs the Agency efforts to further 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 continue investing in supporting the control of the mosquito vector species which transmit these diseases through increased funding of the development of the SIT and other related genetic and environment-friendly methods. Pilot population suppression projects and operational programmes should be supported and applied following an Area-Wide Integrated Vector Management approach.

- The Agency is currently supporting R&D efforts on *Anopheles arabiensis* (and other important malaria vectors), *Aedes aegypti* and *Aedes albopictus* that are highly significant targets for vector and disease control.

  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 technology transfer to Member States.

  We therefore recommend the Agency continues and expands these R&D and technology transfer activities in response to increased requests by Member States.

- Besides SIT, there are also other recent technologies available for the control of mosquito-borne diseases such as genetic engineering, symbiont-based approaches or specific transfer of biocides by sterile males.

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

- Recent progress of the IPCL includes the development of genetic sexing strains, mass-rearing, sex-separation, sterilization, handling, release methods and quality control which was largely achieved due to extrabudgetary contributions.

  We therefore recommend that the IPCL continues these activities for their further improvement and dissemination into Member States; to accomplish this and maintain the momentum will require secured long-term support in human and associated resources.

- Nuclear technology is an important tool for the development and application of SIT. 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 has been strengthened by 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 capacity building and transfer of technology to Member States for the control of mosquitoes via an AW-IVM approach with a SIT component.

- Dissemination and outreach activities, including IT platforms and social media, 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 the area of dissemination, outreach and facilitation of 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, WHO and other Agencies, continues to seek strategic partnerships and mobilization of funds to support AW-IVM approaches with an SIT component for mosquitoes.

10. SPECIFIC RECOMMENDATIONS

10.1 Technical aspects

The efficient mass rearing and sex sorting of mosquitoes is a key component of the SIT package. In the currently ongoing SIT projects against *Aedes* mosquitoes, sex sorting procedures rely on exploiting the sex size dimorphism of pupae and on protandry.

We therefore recommend:

a. *to develop a colony management strategy to avoid selection over generations of detrimental characters in the mass rearing strain as a result of the currently common practice of selecting the males with faster development and smaller pupal size for the releases and the rest of the males for the rearing colony.*

b. *To assess the robustness of the sex sorting systems developed for Aedes albopictus in China under continued high load operation in programmes targeting Aedes aegypti and to continue working on the development of sex sorting systems for Anopheles species.*

c. *to investigate the possible impact of the water quality, such as hardness, on the performance of larval rearing including variations in synchronization of larval development, pupal size or pupae recovery rate*

Irradiation is an integral component of the SIT, and understanding of its associated effects on the organism are needed. Furthermore, effective and efficient irradiation systems are required. Although gamma-ray irradiators have been shown to be reliable and effective, their high initial cost and issues related to security raises the need for alternative systems.

We therefore recommend:

a. *the thorough assessment of the impact of irradiation on mosquito-associated microbiota in an effort to ensure the overall biological quality including mating performance of the sterile males*

b. *to provide to suppliers of irradiators, evaluation-based recommendations for the adaptation and optimization of alternative irradiation systems for the purpose of insect (mosquito) sterilization*

The success of SIT relies on the ability of sterile males to outcompete wild males for mating which depends both on the ratio of sterile to wild males and their competitiveness that are conditioned by release rates and frequency as handling processes from production to release.

We therefore recommend:

a. *Defining the optimal wild / sterile male release ratios*

b. *Development and validation of packaging, handling and aerial releases of sterile males*

Crosses of mosquito strains of the same species carrying different genetic backgrounds leads to hybrids with potentially altered fitness, insecticide susceptibility and vectorial capacity.

We therefore recommend to the Agency to strongly encourage, for the mass rearing and release of mosquitoes with less than 100% sterility, the use of strains with the local genetic background. If this is not possible or desirable, then the effects of the hybrid crossing on fitness, insecticide susceptibility and vectorial capacity should be addressed before release.

There is a need to exchange of information and collaboration between the various pilot projects and/or operational programmes. The IAEA and other Agencies can contribute for the compilation and distribution of the information in a harmonized way that can serve all players.

We therefore recommend that collaborations on defining the levels of population suppression required for suppression or blocking disease transmission; correlation between entomological and epidemiological indicators

10.2 Pilot Projects and Operational Programmes

Current SIT developments allow the implementation of pilot projects and operational programmes. However, for successful implementation, the IAEA should assess the technical, economic, social and ecological feasibility and requirements, and should provide expert advice. These pilot projects and operational programmes 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 capacity building including a standardized package on mosquito SIT.*
- *Development of a regional Africa project on mosquito vectors.*
- *Support of national pilot projects and operational programmes 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.*
- *Provide technical assistance to establish mass-rearing facilities for mosquitoes to support of SIT and other related approaches.*
- *Establishment of regional training and collaborating centres 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 and operational programmes.*
- *Prioritize demand-driven research to solving problems arise during the implementation of pilot projects and operational programmes.*
- *Contribute to sustainability schemes including business models and collaboration between the public and private sectors*
- *collaborate with appropriate regulatory authorities and shipping companies to develop protocols for shipping of sterile males*
- *Continuous assessment towards improvement of cost-effectiveness and cost-benefit analysis for the implementation of mosquito SIT applications on a large scale.*

10.3 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, Zika 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.*

The WHO and the IAEA have recently established a MoU officiating their close collaboration on the control of vectors and vector-borne diseases. This facilitates harmonization and alignment of joint activities (such as joint guidance on mosquito SIT), establishment of a platform for capacity development as well as the implementation of entomological and epidemiological trials.

> *We therefore recommend that the two Agencies continue their close collaboration and joint activities on training, expert advice, support of national, regional and interregional projects, improvement of technology, guidelines and SOP development, and encouragement of public engagement and awareness*.

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

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

Significant progress has been achieved on all the components of the mosquito SIT package including continuous refinement and improvement suggesting an urgent need for harmonization and prompt dissemination to the Member States.

> *We therefore recommend that the Agency develops and regularly updates guidelines based on the different components of the SIT package taking into consideration all recent technological advancements*

11. SELECTED REFERENCES

Aik, Joel, Zhi Wei Neo, Jayanthi Rajarethinam, Kaiyun Chio, Wing Mun Lam, and Lee-Ching Ng. "The effectiveness of inspections on reported mosquito larval habitats in households: A case-control study." PLoS neglected tropical diseases 13, no. 6 (2019): e0007492. Berghammer, A. J., M. Klingler and E. A. Wimmer, 1999 A universal marker for transgenic insects. Nature 402: 370-371.

Bernardini, F., R. Galizi, M. Menichelli, P. A. Papathanos, V. Dritsou et al., 2014 Site-specific genetic engineering of the *Anopheles gambiae* Y chromosome. Proceedings of the National Academy of Science U S A 111: 7600-7605.

Bourtzis, K., R. S. Lees, J. Hendrichs, and M. J. B. Vreysen. 2016. More than one rabbit out of the hat: radiation, transgenic and symbiont-based approaches for sustainable management of mosquito and tsetse fly populations. Acta Tropica 157: 115–130.

Catteruccia, F., J. P. Benton and A. Crisanti, 2005 An *Anopheles* transgenic sexing strain for vector control. Nature Biotechnology 23: 1414-1417.

Carvalho, D. O., A. R. McKemey, L. Garziera, R. Lacroix, C. A. Donnelly, L. Alphey, A. Malavasi, and M. L. Capurro. 2015. Suppression of a field population of *Aedes aegypti* in Brazil by sustained release of transgenic male mosquitoes. PLOS Neglected Tropical Diseases 9(7): e0003864.

Culbert, N.J., Balestrino, F., Dor, A., Herranz, G.S., Yamada, H., Wallner, T. and Bouyer, J., 2018. A rapid quality control test to foster the development of genetic control in mosquitoes. Scientific reports, 8(1), p.16179.

Hae-Na Chung, Stacy D. Rodriguez, Kristina K. Gonzales, Julia Vulcan, Joel J. Cordova, Soumi Mitra, Christopher G. Adams, Nathan Moses-Gonzales, Nicole Tam, Joshua W. Cluck, Geoffrey M. Attardo, and Immo A. Hansen. 2018. Toward implementation of mosquito sterile insect technique: The effect of storage conditions on survival of male *Aedes aegypti* mosquitoes (Diptera: Culicidae) during transport. J Insect Sci. 18(6).

Facchinelli, L., L. Valerio, J. M. Ramsey, F. Gould, R. K. Walsh et al., 2013 Field cage studies and progressive evaluation of genetically-engineered mosquitoes. PLoS Neglected Tropical Diseases 7: e2001.

FAO/IAEA/USDA Quality Control Manual 2014: http://www-naweb.iaea.org/nafa/ipc/public/ipc-mass-reared-tephritid.html

Franz, G., Robinson, A. S. 2011. Molecular technologies to improve the effectiveness of the sterile insect technique Genetica 139(1): 1-5

Fu, G., K. C. Condon, M. J. Epton, P. Gong, L. Jin et al., 2007 Female-specific insect lethality engineered using alternative splicing. Nature Biotechnology 25: 353-357.

Galizi, R., L. A. Doyle, M. Menichelli, F. Bernardini, A. Deredec et al., 2014 A synthetic sex ratio distortion system for the control of the human malaria mosquito. Nature Communications 5: 3977.

Gantz VM, Jasinskiene N, Tatarenkova O, Fazekas A, Macias VM, Bier E, James AA. Highly efficient Cas9-mediated gene drive for population modification of the malaria vector mosquito *Anopheles stephensi*. Proc Natl Acad Sci USA 2015; 112(49):E6736-43.

Garziera, L., M. C. Pedrosa, F. A. de Souza, M. Gómez, M. B. Moreira, J. F. Virginio, M. L. Capurro, and D. O. Carvalho. 2017. Effect of interruption of over-flooding releases of transgenic mosquitoes over wild population of *Aedes aegypti:* two case studies in Brazil. Entomologia Experimentalis et Applicata 164: 327–339.

Halasa, Yara A.; Shepard, Donald S.; Wittenberg, Eve; et al. 2012. Willingness to pay for an area-wide integrated pest management to control the Asian tiger mosquito in New Jersey. Journal of the American Mosquito Control Association. 28 (3): 225-236

Häcker I, Harrell II RA, Eichner G, Pilitt KL, O'Brochta DA, Handler AM, Schetelig MF. Cre/lox-recombinase-mediated cassette exchange for reversible site-specific genomic targeting of the disease vector, Aedes aegypti. Sci Rep 2017; 7:43883.

Hammond A, Galizi R, Kyrou K, Simoni A, Siniscalchi C, Katsanos D *et al*. A CRISPR-Cas9 gene drive system targeting female reproduction in the malaria mosquito vector *Anopheles gambiae*. Nat Biotechnol 2016; 34(1):78-83.

Handler, A. M., and R. A. Harrell, 2001a Polyubiquitin-regulated DsRed marker for transgenic insects. Biotechniques 31: 820, 824-828.

Handler, A. M., and R. A. Harrell, 2001b Transformation of the Caribbean fruit fly, *Anastrepha suspensa*, with a *piggyBac* vector marked with polyubiquitin-regulated GFP. Insect Biochemistry and Molecular Biology 31: 199-205.

Harris, A. F., A. R. McKemey, D. Nimmo, Z. Curtis, I. Black et al., 2012 Successful suppression of a field mosquito population by sustained release of engineered male mosquitoes. Nature Biotechnology 30: 828-830.

Harris, A. F., D. Nimmo, A. R. McKemey, N. Kelly, S. Scaife et al., 2011 Field performance of engineered male mosquitoes. Nature Biotechnology 29: 1034-1037.

Hendrichs, J., A. S. Robinson, J. P. Cayol and W. Enkerlin, 2002 Medfly areawide sterile insect technique programmes for prevention, suppression or eradication: the importance of mating behavior studies. Florida Entomologist 85: 1-13.

Horn, C., and E. A. Wimmer. 2003. A transgene-based, embryo-specific lethality system for insect pest management. Nature Biotechnology 21: 64–70.

Kistler KE, Vosshall LB, Matthews BJ. Genome engineering with CRISPR-Cas9 in the mosquito *Aedes aegypti*. Cell Rep 2015; 11(1):51-60.

Klassen, W. 2005. Area-wide integrated pest management and the sterile insect technique. In Dyck, VA; Hendrichs, J; Robinson, AS (Eds). Sterile Insect Technique: Principles and Practice in Area-Wide Integrated Pest Management. Springer, Amsterdam pp: 39-68

Kokoza, V., A. Ahmed, E. A. Wimmer and A. S. Raikhel, 2001 Efficient transformation of the yellow fever mosquito *Aedes aegypti* using the *piggyBac* transposable element vector pBac[3xP3-EGFPafm]. Insect Biochemistry and Molecular Biology 31: 1137-1143.

Lacroix, R., A. R. McKemey, N. Raduan, L. Kwee Wee, W. Hong Ming et al., 2012 Open field release of genetically engineered sterile male *Aedes aegypti* in Malaysia. PLoS One 7: e42771.

Lees R.S., Gilles J.R.L., Hendrichs J., VreysenM.J.B. and Bourtzis K. 2015. Back to the future: The Sterile Insect Technique against mosquito disease vectors. Current Opinion in Insect Science 10: 156-162.

Li, M., et al., 2017 Germline Cas9 expression yields highly efficient genome engineering in a major worldwide disease vector *Aedes aegypti*. Proceedings of the National Academy of Sciences 114(49): E10540.

Lutrat C., Giesbrecht D., Marois E., Whyard S., Baldet T. and Bouyer J. 2019. "Sex Sorting for Pest Control: It's Raining Men!." Trends in Parasitology.

Lux, S. A., J. C. Vilardi, P. Liedo, K. Gaggl, G. E. Calcagno et al., 2002 Effects of irradiation on courtship behavior of medfly (Diptera: Tephritidae) mass reared for Sterile Insect Technique. Florida Entomologist 85: 102-112.

Marinotti, O., N. Jasinskiene, A. Fazekas, S. Scaife, G. Fu et al., 2013 Development of a population suppression strain of the human malaria vector mosquito, *Anopheles stephensi*. Malaria Journal 12: 142.

Marois, E., C. Scali, J. Soichot, C. Kappler, E. A. Levashina et al., 2012 High-throughput sorting of mosquito larvae for laboratory studies and for future vector control interventions. Malaria Journal 11: 302.

Novelo-Rincon, L. F., Montoya, P., Hernandez-Ortiz, V., Liedo, P., & Toledo, J. (2009). Mating performance of sterile Mexican fruit fly *Anastrepha ludens* (Diptera: Tephritidae) males treated with *Beauveria bassiana* (Bals.) Vuill. Journal of Applied Entomology, 133, 702_710. doi:10.1111/j.1439-0418.2009.01427.x

O'Brochta, D. A., N. Sethuraman, R. Wilson, R. H. Hice, A. C. Pinkerton et al., 2003 Gene vector and transposable element behavior in mosquitoes. Journal of Experimental Biology 206: 3823-3834.

Papathanos, P.A., Bourtzis K., Tripet F., Bossin H. et al. 2018. A perspective on the need and current status of efficient sex separation methods for mosquito genetic control. Parasites & Vectors 11 (Suppl 2):654.

Phuc, H. K., M. H. Andreasen, R. S. Burton, C. Vass, M. J. Epton et al., 2007 Late-acting dominant lethal genetic systems and mosquito control. BMC Biology 5: 11.

Pinkerton, A. C., K. Michel, D. A. O'Brochta and P. W. Atkinson, 2000 Green fluorescent protein as a genetic marker in transgenic *Aedes aegypti*. Insect Molecular Biology 9: 1-10.

Qi Y., Wu Y., Saunders T.R., Chen X., Mao C., Biedler J.K. and Tu Z., 2019. Guy1, a Y-linked embryonic signal, regulates dosage compensation in *Anopheles stephensi* by increasing X gene expression. eLife.

Salvador Flores, Sergio Campos, Antonio Villaseñor, Alvaro Valle, Walther Enkerlin, Jorge Toledo, Pablo Liedo & Pablo Montoya, 2013 Sterile males of *Ceratitis capitata* (Diptera: Tephritidae) as disseminators of *Beauveria bassiana conidia* for IPM strategies. Biocontrol Science and Technology, 23:10, 1186-1198.

Schetelig, M. F., and A. M. Handler, 2012a Strategy for enhanced transgenic strain development for embryonic conditional lethality in *Anastrepha suspensa*. Proceedings of the National Academy of Sciences USA 109(24): 9348-53.

Schetelig, M. F., and A. M. Handler, 2012b A transgenic embryonic sexing system for *Anastrepha suspensa* (Diptera: Tephritidae). Insect Biochemistry and Molecular Biology 42(10): 790-5.

Schetelig, M. F., and A. M. Handler, 2013 Y-linked markers for improved population control of the tephritid fruit fly pest, *Anastrepha suspensa*. Advances in Biochemical Engineering and Biotechnology 136: 123-133.

Schetelig, M. F., A. Milano, G. Saccone and A. M. Handler, 2012 Male only progeny in *Anastrepha suspensa* by RNAi-induced sex reversion of chromosomal females. Insect Biochemistry and Molecular Biology 42: 51-57.

Schetelig, M. F., F. Scolari, A. M. Handler, S. Kittelmann, G. Gasperi et al., 2009 Site-specific recombination for the modification of transgenic strains of the Mediterranean fruit fly *Ceratitis capitata*. Proceedings of the National Academy of Sciences, USA 106: 18171-18176.

Schetelig, M. F., A. Targovska, J. S. Meza, K. Bourtzis, and A. M. Handler. 2016. Tetracycline-suppressible female lethality and sterility in the Mexican fruit fly, *Anastrepha ludens*. Insect Molecular Biology 25: 500–508.

Scolari, F., M. F. Schetelig, S. Bertin, A. R. Malacrida, G. Gasperi et al., 2008 Fluorescent sperm marking to improve the fight against the pest insect *Ceratitis capitata* (Wiedemann; Diptera: Tephritidae). New Biotechnology 25: 76-84.

Smith, R. C., M. F. Walter, R. H. Hice, D. A. O'Brochta and P. W. Atkinson, 2007 Testis-specific expression of the *beta2 tubulin* promoter of *Aedes aegypti* and its application as a genetic sex-separation marker. Insect Molecular Biology 16: 61-71.

Strecker, J., et al. (2019). "RNA-guided DNA insertion with CRISPR-associated transposases." Science 365(6448): 48.

Toledo J, Campos SE, Flores S, Liedo P, Barrera JF, Villaseñor A, Montoya P, 2007. Horizontal transmission of Beauveria bassiana in the mexfly, *Anastrepha ludens* (Diptera: Tephritidae), under semi-natural conditions. Journal of Economic Entomology 100: 291-297.

Vargas, R. I., Pinero, J. C., Mau, R. F. L., et al. 2010. Area-wide suppression of the Mediterranean fruit fly, *Ceratitis capitata*, and the Oriental fruit fly, Bactrocera dorsalis, in Kamuela, Hawaii Journal of Insect Science 10 Article Number: 135

Vreysen, M. J. B., Seck, M. T., Sall, B., et al. 2013. Tsetse flies: Their biology and control using area-wide integrated pest management approaches. Journal of Invertebrate Pathology. 112 Supplement: 1: S15-S25

WHO (2013) World Malaria Report 2013, pp: 284. (http://www.who.int/malaria/publications/world_malaria_report_2013)

WHO (2013). Larval Source Management: a supplementary measure for malaria vector control An Operational Manual, pp: 128.

WHO (2018). Efficacy-testing of traps for control of *Aedes spp*. Mosquito vectors. http://eprints.gla.ac.uk/173660/1/173660.pdf

Yu, R., Leung, P.S. 2011. Estimating the economic benefits of area-wide pest management: an extended framework with transport cost. Annals of Regional Science. 46(2): 455-468.

Zheng, Xiaoying, Dongjing Zhang, Yongjun Li, Cui Yang, Yu Wu, Xiao Liang, Yongkang Liang et al. "Incompatible and sterile insect techniques combined eliminate mosquitoes." Nature 572, no. 7767 (2019): 56-61.

12. 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 their 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 ● Potential toxicity to residents
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 ● Prevents man-vector contact	● Only effective in places where mosquitoes are still susceptible to the insecticide used ● Can be a potential larval habitat if not properly maintained ● Highly labour intensive

	● Simple to prepare ● Large number of eggs are trapped and removed from the environment	● 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

Table S4. List of Participants

Name	Organization	Email
BELLINI, Romeo	Centro Agricoltura Ambiente, Italy	rbellini@caa.it
HÄCKER, Irina	Justus-Liebig-University Gießen, Department of Insect Biotechnology in Plant Protection, Germany	irina.haecker@agrar.uni-giessen.de
CARRINGTON, Lauren	World Health Organization (WHO) Department of Control of Neglected Tropical Diseases (CDS/NTD), Switzerland	carringtonl@who.int
GÓMEZ PACHECO, Maylen	Biofabrica Moscamed Brasil (BMB), Brazil	maylen@moscamed.org.br
LIEDO FERNANDEZ, Jose Pablo	Departamento de Entomología El Colegio de la Frontera Sur (ECOSUR), Tapachula, Chiapas, Mexico	pliedo@ecosur.mx
LINTHICUM, Kenneth	Center for Medical, Agricultural & Veterinary Entomology USDA-ARS, Gainesville, FL 32608, USA	kenneth.linthicum@ars.usda.gov
MUNHENGA, Givemore	National Health Laboratory Service National Institute for Communicable Diseases NICD, Johannesburg, South Africa	gmunhenga@yahoo.com
SIMARD, Frederic	Maladies Infectieuses et Vecteurs: Ecologie, Génétique, Evolution et Contrôle IRD, Montpellier Cedex 5, France	frederic.simard@ird.fr
SCHETELIG, Marc Florian	Justus-Liebig-University Gießen, Department of Insect Biotechnology in Plant Protection, Germany	Marc.Schetelig@agrar.uni-giessen.de
XI, Zhiyong	Department of Microbiology and Molecular Genetics & SYSU-MSU Joint Center of Vector Control for Tropical Diseases Michigan State University East Lansing, MI 48824, USA	xizy@msu.edu
CARDOSO PEREIRA, Rui	IAEA, NAFA, SH-IPC	R.Cardoso-Pereira@iaea.org
ARGILES HERRERO, Rafael	IAEA, NAFA-IPC	R.Argiles-Herrero@iaea.org
BOURTZIS, Kostas	IAEA, NAFA-IPC	K.Bourtzis@iaea.org
BOUYER, Jeremy	IAEA, NAFA-IPC	J.Bouyer@iaea.org
YAMADA, Hanano	IAEA, NAFA-IPC	H.Yamada@iaea.org

de OLIVEIRA CARVALHO, Danilo	IAEA, NAFA-IPC	D.Carvalho@iaea.org
MAIGA, Hamidou	IAEA, NAFA-IPC	H.Maiga@iaea.org
MAMAI, Wadaka	IAEA, NAFA-IPC	W.Mamai@iaea.org