Annex A. **Control of the mosquito *Ae. aegypti***

This annex describes the current strategies put in place to limit or eradicate mosquitoes that transmit disease pathogens: chemical control using larvicides and insecticides, biological control based on introduction of other organisms, use of Wolbachia *bacteria in methods for controlling virus transmission through reduction or replacement of the mosquito* Aedes aegypti *population. Research is also conducted on genetic control of* Ae. aegypti. *Then information is given on relevant environmental management aiming to limit its propagation, including integrated control management and the prevention of insecticide resistance.*

Current control strategies

Mosquitoes can be vectors (transmitters) of several infectious diseases to humans and animals and are thus of significant importance to public health. The aim of mosquito control, in general, is to prevent mosquito bites, to maintain mosquito populations at "acceptable" densities, to minimise mosquito-host contact and to reduce the longevity of female mosquitoes (Foster and Walker, 2002).

Vector control is any method to limit or eradicate mosquitoes that transmit disease pathogens. Disease control is the reduction in the incidence, prevalence, morbidity or mortality of an infectious disease to a locally acceptable level or, if possible, its elimination or eradication. In order to be sustainable, a vector control strategy must limit the spread of resistance to insecticides within target mosquito populations.

Aedes aegypti control is generally performed in the context of public health because it is the vector of Zika, dengue, chikungunya and yellow fever, and a number of other diseases. Particularly for Zika, dengue and chikungunya, there are no vaccines, therapeutic treatments or cure. Preventing or reducing Zika, dengue and chikungunya virus transmission depends entirely on control of the mosquito vectors or interruption of human-vector contact (WHO, 2009b). Eradication of *Ae. aegypti* populations may be achievable, but is rarely sustainable, therefore, the present paradigm is to reduce mosquito density below disease transmission threshold levels rather than eliminate entire populations (McCall and Kittayapong, 2006).

Ae. aegypti control largely depends on organised control programmes at the community level administered by ministries of health undertaken together with some self-protection measures. Because *Ae. aegypti* lives in close affinity with humans and human-made ecosystems, it is an ideal candidate for integrated control (utilisation of multiple methods to provide control), which is summarised in the following Table A A.1 and briefly described in the following sections.

Chemicals for mosquito control may only be used in accordance with national legislation and approval of the products. Some of the chemicals mentioned as examples in Table A A.2 might be allowed in some countries but not in others.

Detailed information on the mosquito ecology, dispersal and the distribution of human habitats (see the chapter on Ecology) can be useful to vector control agencies for better targeting populations for suppression. Control programmes can be built on an urban area divided into zones of control along landscape features that are large enough to impede mosquito dispersal. This technique allows for the possibility of local elimination of *Ae. aegypti* mosquitoes, barring or at least minimising re-infestation due to the active transportation of the mosquito. Furthermore, during outbreaks, control agencies can more accurately target areas of higher risk along these same control zones. Understanding the role of landscape features on population dispersal is likely critical to achieving success with any *Ae. aegypti* control strategy.

Chemical control

Immature stages: The control of *Ae. aegypti* larvae and pupae can be effected by treating containers holding water (specifically those that are productive breeding-sites and cannot otherwise be eliminated or managed) with insecticides (larvicides). Larvicides such as diflubenzuron, novaluron pyriproxyfen, fenthion, pirimiphos-methyl, temephos and

spinosad (approved by WHOPES) target the immature mosquitoes living in water before they become biting adults.

Table A A.1. Summary of control tools/strategies available for *Ae. aegypti*

Method	Description	Examples
CHEMICAL CONTROL	Immature stages	Treating containers (breeding-sites) with for e.g. Temephos 1% Sand Granule; biorational larvicides; insect growth regulators (IGR) such as methoprene and pyriproxyfen, spinosad
	Adult in medium/large areas or houses	Aerial treatments, indoor spraying, surface treatments
	Personal protection	Domestic insecticides, repellents (natural or synthetic), insecticide-treated materials and paints
BIOLOGICAL CONTROL	Immature stages and adults (the whole population)	Fish, dragonflies, copepods, *Bti*, *Toxorhynchites*, *Wolbachia*
GENETIC CONTROL (self-limiting)	Immature stages and adults (the whole population)	Self-limiting insects, sterile insect technique, others
GENETIC CONTROL (population replacement)	Forces genes/organism through the whole population	Gene drive systems (i.e. HEGs and CRISPR), *Wolbachia*
ENVIRONMENTAL MANAGEMENT	Modification: permanent transformations in some characteristics to the vector breeding habitats Manipulation: temporal changes (management) to affect the breeding sites (key) behaviour Structural changes in human habitation and human behaviour	Draining/cleaning/recycling/disposal of breeding-sites or potential larval habitats Installation of reliable piped water supply to communities, comprehensive coverage and proper disposal of solid waste collection, filling, draining public spaces Public sensitisation to reduce the availability of breeding sites (source reduction) Installing mosquito screening on windows, doors and other entry points. Using mosquito nets Paints, peridomestic veneering to contribute eliminating natural habitats

Source: Modified from PAHO (1994), *Dengue and Dengue Hemorrhagic Fever in the Americas: Guidelines for Prevention and Control*, PAHO Scientific Publication 548, Pan American Health Organization, Washington, DC, and McCall, P.J. and P. Kittayapong (2006), "Control of dengue vectors: Tools and strategies", in *Report of the Scientific Working Group Meeting on Dengue*, World Health Organization, Geneva, WHO/TDR 2007, pp. 110-119.

The application of larvicides can also be done by ground or aerial treatments. However, the high density of small habitats (< 200 mL) makes it very difficult to treat a reasonable proportion of highly disseminated breeding sites. It has been proposed recently to use auto-dissemination of pyriproxifen by adult females themselves to their breeding sites, after their contamination using dissemination stations (Devine et al., 2009). This approach is very efficient but has a short range of action because of low rates of adult dispersal. It has thus been proposed to release sterile males contaminated with pyriproxifen to contaminate the females through venereal transfer, an approach called the "boosted sterile insect technique" (Bouyer and Lefrançois, 2014). This control method has been successfully demonstrated recently in a field trial against *Ae. albopictus* at

a very small scale (Mains, Brelsfoard and Dobson, 2015), and it is a major research axis to improve larvicidal control at the moment.

Adult: The control of adult vectors with insecticides (adulticides), applied either as residual surface treatments or as space treatments (thermal fogging and ultra-low volume aerosol sprays), is expected to impact mosquito densities, longevity and other transmission parameters. Insecticides from three chemical groups, namely pyrethroids, organophosphates and carbamates, are recommended by WHOPES both for indoor and outdoor spraying (WHO, 2003). The application of adulticides can be done by ground or aerial treatments but has a very short-term and local action.

Indoor residual spraying (IRS) involves the spraying of an insecticide on all the walls inside the house. This is usually done only once or twice a year because the effect is lasting and continues to kill mosquitoes for many months after treatment. Targeted indoor residual spraying involves spraying dark shady areas used by adult *Ae. aegypti* as resting places, such as under beds and tables, inside closets and dark objects such as plastic crates and suitcases. This method uses less pesticide and has been successfully used to protect residences from dengue transmission (Vazquez-Prokopec et al., 2017).

Indoor space-spraying (ISS) involves delivery of an insecticidal fog inside houses. However, space sprays do not leave a residual layer providing long-term control and have found to be ineffective for dengue control (Esu et al., 2010).

Outdoor fogging is the method commonly used in many parts of the world. The insecticide is usually sprayed from vehicles as a cloud of "fog" outside houses, targeting the flying female mosquitoes. Vector populations can be suppressed over large areas by the use of space sprays released from low-flying aircraft, especially where gaining access with ground equipment is difficult and extensive areas must be treated rapidly. It is generally ineffective against *Ae. aegypti* populations that have access to indoor harbourage sites.

Personal protection: *Ae. aegypti* exposure can be avoided with chemical products such as domestic insecticides, repellents (natural or synthetic) and insecticide-treated materials and paints including spatial repellents such as metofluthrin (Ritchie and Devine, 2013).

In general, pyrethroids are the main active ingredients in household aerosol products available to the public. Where indoor biting occurs, household insecticide aerosol products, mosquito coils or other insecticide vaporisers may reduce biting activity (WHO, 2009a).

Numerous insect repellent products are available commercially in a variety of formulations. Some of these products contain active ingredient(s) from botanical origin and some are synthetic organic products, with a vast majority available as sprays. Repellents may be applied to exposed skin or to clothing. Repellents recommended contain DEET (N, N-diethyl-3-methylbenzamide), IR3535 (3-[N-acetyl-N butyl]-aminopropionic acid ethyl ester) or Icaridin (1-piperidinecarboxylic acid, 2-(2-hydroxyethyl)-1 methylpropylester) (WHO, 2009a).

Long-lasting insecticidal netting (LLIN) is factory-produced mosquito netting pre-loaded with synthetic pyrethroid insecticide that is intended to retain its biological activity for at least 20 standard washes under laboratory conditions, and three years of recommended use under field conditions (WHO, 2013). Deployed as bed nets, LLIN potentially can reduce human biting rates and vector longevity at both household and community levels (McCall and Kittayapong, 2006). In Latin America, encouraging results for *Ae. aegypti*

control have also been obtained when LLIN are deployed as window or door screens, curtains or as container covers (Vanlerberghe et al., 2011; Rizzo et al., 2012; Manrique-Saide et al., 2015).

Biological control

Biological control is based on the introduction of organisms that prey upon, parasitise, compete with or otherwise reduce populations of the target species. *Bacillus thuringiensis* var. *israelensis* (*Bti*) is an entomopathogenic bacterium that has demonstrated high efficacy against *Ae. aegypti* larvae and is commercially available in different formulations that can be utilised in a variety of breeding habitats (Lacey, 2007; Boyce et al., 2013). Its strain AM65-52 in a water-dispersible granulated formulation is recommended by WHOPES (2016).

Other biological control agents that have been used for larval control of *Ae. aegypti* include species of larvivorous fish (WHO/EMRO, 2003) e.g. *Poecilia reticulata*, dragonflies (Sebastian et al., 1980, 1990; Venkatesh and Tyagi, 2013) and predatory copepods (Copepoda: Cyclopoidea) (Kay et al., 2012) which have proved effective in operational contexts in specific container habitats, but seldom on a large scale.

Wolbachia *as a biological control method for virus transmission*

Uses of Wolbachia *in control methods*

Wolbachia bacteria can be used to control *Ae. aegypti* and the diseases it spreads in two different ways, population reduction or population replacement:

a) Population reduction: *Ae. aegypti* males infected with *Wolbachia* are released. When the infected males mate with wild females, no offspring are produced, and with such release renewed over a period of time, the mosquito population can be reduced. It is important with this approach that no infected females be released as that could potentially lead to failure of the control programme; the infected females can pass *Wolbachia* onto their offspring, which survive and can spread into the environment. To date, there have been no successful suppression trials using *Wolbachia* for population reduction with *Ae. aegypti*.

b) Population replacement: *Wolbachia* can also be used in a population replacement strategy approach, similar to gene drive systems. In the wild, Wolbachia can spread through a species by a process known as cytoplasmic incompatibility (CI). CI is similar to a gene drive mechanism, which kills any offspring that are not infected with *Wolbachia*, effectively selecting for only offspring that are infected and hence spreading the *Wolbachia* through a population. The following paragraphs detail population replacement strategies being tested in *Wolbachia* and *Ae. aegypti*.

Introducing the *Wolbachia* strain wMelPop into wild populations of *Ae. aegypti* can shorten the adult mosquito lifespan, thereby theoretically reducing but not eliminating the transmission of dengue since it has not fully proven to reduce mosquito longevity shorter to the extrinsic incubation period for dengue virus (DENV). However, high fitness costs have prevented wMelPop from being successfully established in wild populations of *Ae. aegypti* in Australia and Viet Nam (Nguyen et al., 2015).

Two *Wolbachia* strains (wMel and wMelPop-CLA) have shown to confer antiviral properties to *Ae. aegypti* and limit DENV-2 infection in the mosquito by reducing the

virus' ability to disseminate from the midgut (MG) into mosquito saliva and affected mosquito fitness for disease transmission. A major open field trial was conducted in which about 300 000 *Wolbachia* wMel-infected *Ae. aegypti* mosquitoes raised under laboratory conditions were deliberately released in 2011 at 2 locations near Cairns, Australia. The frequency of *Wolbachia*-infected *Ae. aegypti* initially increased to more than 15% in both locations at two-week post-release. After additional releases, frequencies increased to > 60% and reached near fixation levels 5 weeks after releases were terminated, and these high frequencies were maintained through 2017. These observations suggest that *Wolbachia* could potentially become a powerful bio-control agent to suppress DENV transmission by *Ae. aegypti* in endemic areas, though field data demonstrating reduction of DENV transmission has not been shown.

Wolbachia *transfer into* Ae. aegypti *mosquitoes*

Although *Wolbachia* infections are relatively common in mosquitoes (Kittayapong et al., 2000; Ricci et al., 2002) including *Culex pipiens* (Yen and Barr, 1973), *Cx. quinquefasciatus*, *Ae. fluviatilis* (Moreira et al., 2009) and *Ae. albopictus* (Sinkins, Braig and O'Neill, 1995), the main vectors for dengue fever (*Ae. aegypti*) and malaria (*Anopheles* spp.) are not naturally infected by *Wolbachia*. Approaches that use *Wolbachia* for the control of diseases transmitted by uninfected, naive insects rely on the successful establishment of stable *Wolbachia* infections, usually by embryonic microinjection of *Wolbachia*-infected cytoplasm or *Wolbachia* purified from infected insect hosts.

To create stably transinfected lines, embryo injections must target the region near the pole cells in pre-blastoderm embryos in order to incorporate *Wolbachia* into the developing germline and favour the transmission of *Wolbachia* to offspring. Several *Wolbachia* strains have been transferred across sometimes phylogenetically distant insects and, importantly, the phenotypes induced by these strains in their native hosts are generally also expressed in the newly infected hosts. *Wolbachia* transinfection experiments are more likely to be successful when the donor and recipient organisms are closely related.

In line with this, the transfer of wMelPop from its natural host, *Drosophila melanogaster*, into the dengue fever vector *Ae. aegypti* was achieved in the laboratory after *Wolbachia* was first maintained by continuous passage in *Ae. albopictus in vitro* cell culture for almost four years (McMeniman et al., 2008). *Wolbachia* adapted to a mosquito intracellular environment, facilitating transinfection *in vivo*. After microinjection of thousands of *Ae. aegypti* embryos, two stable wMelPop-CLA (cell-line-adapted) lines with maternal transmission rates of approximately 100% were generated (McMeniman et al., 2009). The wMelPop-CLA-infected mosquitoes showed an approximately 50% reduction in adult lifespan, compared with their uninfected counterparts (McMeniman et al., 2009). The halving of adult mosquito lifespan and the high *Wolbachia* maternal transmission rates were also maintained in more genetically diverse outbred mosquitoes and larval nutrition did not affect the life-shortening ability of the wMelPop-CLA strain (Yeap et al., 2010).

The wMelPop-CLA infection is widespread in *Ae. aegypti* tissues, with high bacterial densities in the head (brain and ommatidia), thorax (salivary glands, muscle) and abdomen (fat tissue, reproductive tissues and malpighian tubules) (Moreira et al., 2009). Wide distribution across tissues has been found in other transinfected mosquitoes, such as *Ae. aegypti* infected with the wAlbB strain from *Ae. albopictus* (Bian et al., 2010). By using quantitative PCR and western blot analyses, this strain was also found in reproductive tissues, MG, muscles and heads, in both native *Ae. albopictus* (Dobson

et al., 1999) and the transinfected *Ae. aegypti* (Bian et al., 2010), although the densities are not as high as those found in *Ae. aegypti* infected with wMelPop-CLA.

In addition, there is evidence that *Wolbachia* infection can result in permanent genetic modification of its insect hosts in a process called Lateral gene transfer (LGT). LGT of fragments of the *Wolbachia* genome (total size approximately 1.2 Mb), ranging from 500 base pairs to more than 1 Mb, have been observed in many invertebrates, including beetles (Nikoh et al., 2008), grasshoppers (Funkhouser-Jones, 2015; Toribio-Fernández et al., 2017), wasps (Dunning-Hotopp et al., 2007), fruit flies (Dunning-Hotopp et al., 2007; Klasson et al., 2014; Choi, Bubnell and Aquadro, 2015; Morrow et al., 2015), tsetse flies (Brelsfoard et al., 2014; Nakao et al., 2016), butterflies and moths (Ahmed et al., 2016), kissing bugs (Mesquita et al., 2015), mosquitoes (Klasson et al., 2009; Hou et al., 2014), filarial nematodes (Fenn et al., 2006; Dunning-Hotopp et al., 2007; Keroack et al., 2016) and spiders (Baldo et al., 2008).

Next step

The ability of some *Wolbachia* strains to reduce the lifespan of *Ae. aegypti*, invade mosquito populations through the induction of CI and, in particular, interfere with the replication of a variety of pathogens has distinct implications for disease control. There is some evidence that the *Wolbachia* can spread through a mosquito population as predicted, and the next phase is to prove that this leads to disease reduction.

Genetic control

Many trials have been conducted using classical sterile insect technique (SIT) and self-limiting insects (OX513A transgenic line) (Alphey, 2014). Classical SIT pilot projects have been tested in Indonesia, Malaysia, Mexico, Sri Lanka and Thailand. This technology is based on the mass-rearing production of male mosquitos sterilised under X-rays or by irradiation (Gamma). This technology is very well applied on agricultural pests and other vector species like the tsetse fly (Dicko et al., 2014; Vreysen et al., 2014), and can be very powerful on insect population suppression or even eradication. However, successful population suppression for *Ae. aegypti* using SIT has yet to be demonstrated. In China, *Ae. albopictus*-*Wolbachia* IIT/SIT strategies that use the introduction of infected males (IIT) and sterile females (SIT) are tested to reduce wild populations (Zhang et al., 2016). In Europe, the classical SIT is considered as a biological control technique and exempted from the "GMO" regulation, unlike self-limiting insects (EFSA Panel on Genetically Modified Organisms (GMO), 2013).

Self-limiting insects are engineered with a gene that causes offspring to die before reaching functional adulthood, a species-specific control approach that has been developed for *Ae. aegypti* but which is applicable to a wide range of insects. Released mosquitoes die along with their offspring and therefore do not persist in the environment (Gorman et al., 2016). Additionally, the self-limiting OX513A mosquitoes and their offspring contain a fluorescent marker (DsRed2) that allows identification of OX513A larvae and pupae under laboratory conditions. Deployment of this technology through the release of self-limiting OX513A mosquitoes has achieved effective population suppression of wild *Ae. aegypti* in multiple trials in Brazil, the Cayman Islands and Panama (Harris et al., 2012; Carvalho et al., 2015; Gorman et al., 2016), and has been positively reviewed by regulatory bodies in Brazil, the European Union and the United States.

Environmental management

Environmental management seeks to change the environment in order to prevent or minimise vector propagation and human contact with the vector of pathogen by destroying, altering, removing or recycling non-essential containers that provide larval habitats. Such actions should be the mainstay of vector control and require important efforts for public sensitisation. Three types of environmental management are defined as follows (WHO, 1982; PAHO, 1994; Erlanger, Keiser and Utzinger, 2008; McCall, Lloyd and Nathan, 2009).

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

Environmental manipulation: Temporary changes to vector habitats involving the management of "essential" containers, such as frequent emptying and cleaning by scrubbing of water-storage vessels, flower vases and desert room coolers, cleaning of gutters, sheltering stored tires from rainfall, recycling or proper disposal of discarded containers and tires, management or removal from the vicinity of homes of plants such as ornamental or wild bromeliads that collect water in the leaf axils. There are a great variety of man-made containers in backyards or patios that collect rainwater or that are filled with water by people. Disposing of unused containers, placing useful containers under a roof or protected with tight covers, and frequently changing the water of animal drinking pans and flower pots will greatly reduce the risk of dengue infections. Water storage containers should be kept clean and sealed so mosquitoes cannot use them as aquatic habitats (CDC, 2010).

Changes to human habitation or behaviour: Actions to reduce human-vector contact, such as installing mosquito screening on windows, doors and other entry points, and using mosquito nets while sleeping during daytime.

Integrated control management

Integrated vector management (IVM) is the strategic approach to vector control promoted by the World Health Organization (WHO, 2008) and includes control of the vectors of dengue. Defined as "a rational decision-making process for the optimal use of resources for vector control", IVM considers five key elements in the management process, namely (McCall, Lloyd and Nathan, 2009):

1. *Advocacy, social mobilisation and legislation* – the promotion of the IVM principles in development policies of all relevant agencies, organisations and civil society; the establishment or strengthening of regulatory and legislative controls for public health; and the empowerment of communities.
2. *Collaboration within the health sector and with other sectors* – the consideration of all options for collaboration within and between public and private sectors; planning and decision-making delegated to the lowest possible administrative level; and strengthening communication among policy-makers, managers of programmes for the control of vector-borne diseases, and other key partners.
3. *Integrated approach to disease control* – ensuring the rational use of available resources through the application of a multi-disease control approach; integration of non-chemical and chemical vector control methods; and integration with other disease control measures.

4. *Evidence-based decision-making* – adaptation of strategies and interventions to local vector ecology, epidemiology and resources, guided by operational research and subject to routine monitoring and evaluation.
5. *Capacity-building* – the development of essential infrastructure, financial resources and adequate human resources at national and local levels to manage IVM programmes, based on a situation analysis.

Prevention and management of insecticide resistance

The evolution and spread of resistance to insecticides is a major concern for the control of the dengue vector *Ae. aegypti*. The reliance by most dengue control programmes on just two classes of insecticide (pyrethroids and organophosphates) available for use in public health, poses additional selection pressure on the mosquito vectors (Ranson et al., 2010).

Alterations in the molecular target sites of insecticides, which reduce the binding of insecticides, are the most understood resistance mechanisms. Several mutations in the sodium channel, the target site of DDT and pyrethroid insecticides, have been reported in *Ae. aegypti* (Brengues et al., 2003). Two alternative substitutions at one of the polymorphic sites, residue 1 016, have been linked to pyrethroid resistance and recently, methodologies to detect these mutations (often referred to as kdr mutations) in individual mosquitoes have been reported (Saavedra-Rodríguez et al., 2007; Rajatileka et al., 2008).

Resistance management strategies generally recommend the rotation of chemicals with different modes of action and the use of non-chemical methods of control. The implicit assumption is that resistance to a chemical will disappear from a population once the selection pressure is removed. Effective IVM will be possible only through an important development of available biological control tools, to be combined with insecticide and physical control.

In order to successfully develop and implement any resistance management strategies based on rotations, mosaics, mixtures or combinations, knowledge of the mode of action, chemical properties and residual life of the available insecticide products is crucial. Focusing on surveillance wherever possible is essential in order to react proactively once a regional population manifests a shift in its susceptibility towards synthetic insecticides.

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