List of agreed standardized endpoints for the assessment of the effectiveness of vector control approaches.

Project milestone

ML 2.4.1

PE13 – RESEARCH NODE2 "Arthropod Vectors and Vector-Borne Diseases"

WP2.4 Development and validation of vector eco-friendly control approaches

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ML 2.4.1. List of agreed standardized endpoints for the assessment of the effectiveness of vector control approaches.

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INTRODUCTION

To facilitate the implementation of ecologically sustainable mosquito control measures in Italy, it is crucial to establish a more robust evidentiary foundation concerning their efficacy and efficiency. Presently, global efforts to demonstrate the public health significance and effectiveness of vector control interventions predominantly rely on epidemiological endpoints. However, the applicability of such an approach within the Italian context is difficult due to the sporadic and epidemic nature of disease outbreaks in our country, rendering the planning and execution of trials with epidemiological endpoints arduous. Given that arthropod vectors drive disease transmission, a feasible alternative may lie in the utilization of entomological endpoints, which can serve to assess the impact of vector control interventions on the containment of vector-borne diseases [Van Hul, 2021]. To comprehensively assess the effectiveness of vector control interventions via entomological endpoints, additional field studies are highly required.
Following an exhaustive review of the existing literature, we have identified specific entomological endpoints suitable for evaluating the effectiveness of mosquito vector control

interventions within the Italian context. This process has yielded a well-defined and standardized list of selected endpoints, potentially suitable for the vector control activities proposed in work package 2.4 of the PNRR INF-ACT project. These chosen endpoints will undergo field validation during the second and third years of the PNRR INF-ACT project and may be updated or implemented by the involved researchers.
The interventions that will be developed/tested in the frame of the work package 2.4 of the PNRR INF-ACT project are hereafter described and classified into three main categories: autocides interventions, adulticides interventions, and bio-larvicides interventions.

AUTOCIDES INTERVENTIONS

1: Sterile insect technique

The Sterile Insect Technique (SIT) represents an eco-friendly approach to managing insect pest populations [Knipling, 1959]. This method involves the large-scale production and sterilization of a targeted pest species using ionizing radiation, such as gamma rays or X-rays. Subsequently, these sterilized insects are systematically released by air over predefined regions. Within these regions, the sterile males engage in copulation with wild females, leading to the production of no viable offspring and a consequent reduction in the pest population. The SIT ranks among the most environmentally benign strategies for insect pest control. It relies on the irradiation-induced sterilization of mass-reared insects, preserving their sexual competitiveness while rendering them incapable of reproduction. Importantly, the SIT does not involve the introduction of transgenic elements, thereby excluding genetic engineering processes. The SIT, often described as autocidal control, specifically targets the reproductive cycle of the pest species, ensuring a species-specific impact. Unlike classical biological control, the SIT refrains from introducing non-native species into ecosystems.

2: Incompatible insect technique

The Incompatible Insect Technique (IIT) operates on a foundational principle akin to that of the Sterile Insect Technique (SIT). IIT, however, employs the release of fertile males with a "sterilizing" capacity, and its efficacy hinges upon the phenomenon of *Wolbachia*-induced cytoplasmic incompatibility (CI). In this technique, adult males undergo a *Wolbachia* endosymbiont transformation, depleting them of their natural Wolbachia strains and equipping them with a *Wolbachia* strain distinct from that naturally occurring in the target mosquito populations. *Wolbachia* is a maternally inherited endosymbiont commonly found in various insect species, including several mosquito species. The mating process between *Wolbachia*-infected males and wild females, either devoid of *Wolbachia* or carrying a disparate *Wolbachia* strain, culminates in embryonic lethality [Sinkins, 2004]. Therefore, sterility is induced in the native wild female population, which will decline over the generations.

ADULTICIDES INTERVENTIONS

1: Entomopathogenic fungi

Many studies conducted over the last decade have provided solid evidence that entomopathogenic fungi (EPFs) can represent a next-generation tool to manage mosquito and tick populations as a significant complement/substitute for conventional chemical pesticides [Cafarchia et al., 2022]. At present, EPFs such as *Beauveria bassiana* (*Bb*) and *Metarhizium anisopliae* cover an important position for their easy delivery, as well as their ability to infect both soft- and hard-bodied arthropods by direct penetration of the host cuticle [Cafarchia et al., 2022]. The effectiveness of EPFs against mosquitoes or ticks has been investigated under laboratory conditions resulting in encouraging results with high levels of mortality, even if the Lethal Time (LT) was higher than those registered by using conventional pesticides [Cafarchia et al., 2022]. However, under natural conditions the whole infection process by EPF is linked to abiotic factors (i.e., temperature, soil acidity; Humidity; the solar UVA, and UVB) that affect the host–fungus interactions, thus requiring a well-efficacious delivering system. Recently, new eco-friendly formulations and delivery methods based on biomaterials that mimic the natural characteristics of target insect habitats or breeding sites were proposed as a strategy to deliver EPFs [Friuli et al., 2022 A; Friuli et al., 2022 B]. These systems are based on biomaterial-based hydrogels, which are biocompatible, and their parameters such as water content, and rheological and mechanical properties can be easily adjusted and adequate to the target arthropod species. At the moment, laboratory and semi-field studies were built and designed against *Aedes albopictus*. In particular, the viability of *Bb* in hydrogels based on alginate or cellulose with proven oviposition attraction capability for tiger mosquitoes [Friuli et al., 2022 A; Friuli et al., 2022 B] and the *in vitro* effect of *Bb* as in conidial aqueous suspension (CIS) or in biocompatible hydrogels (HBBs) against *Ae. albopictus* eggs were evaluated. The preliminary results show that the combination of *Bb* with cellulose hydrogel is effective against *Ae. albopictus* eggs, while the simple suspension in water of *Bb* did not show any efficacy. The usage of natural hydrogels in combination with *Bb* represents a promising tool to be used in alternative to chemical compounds currently used for the control of *Ae. albopictus*. Future studies have been designed to evaluate the efficacy of natural hydrogels in combination with *Bb* in semi-field conditions. In addition, the study could be extended to other arthropod vectors such as sandflies.

BIO-LARVICIDES INTERVENTIONS

1: Formulations based on *Bacillus thuringiensis israelensis* (*Bti*)

Bio-larvicides are effective and eco-compatible tools for mosquito control. In this context, formulations based on *Bacillus thuringiensis israelensis* (*Bti*) are widely used in domestic

environments and in municipal pest control programs. However, since *Bti* has low residual activity and requires repeated applications, the development of products that provide prolonged delivery and efficacy of *Bti* is highly desirable. To this purpose, we developed MosQuito raft, a novel type of hydrogel-based structure, suitable to incorporate bio-larvicides [Piazzoni et al., 2022; Pitton et al., 2023]. The goal was to generate floating structures for the release of bio-insecticides, with the following characteristics: eco-compatibility; attractiveness for mosquito larvae; and a protective action on *Bti*, with prolonged delivery of this bio-insecticide. The efficacy of MosQuito raft was tested on larvae from both native [*Culex pipiens;* Negri et al., in revision] and alien mosquitoes [*Aedes albopictus* and *Aedes koreicus;* Pitton et al., 2023], and its eco-compatibility was validated on two non-target model organisms.

2: Formulations based on essential oils

Several essential oils have larvicidal activity against mosquitoes of medical importance mainly Aedes and Culex so that they have been widely tested as control agents for vectors of important diseases, such as dengue, chikungunya, and Zika as alternative to synthetic chemical insecticides. In the past decades and up to now larval chemical control consists of application of inorganic and synthetic compounds applied to reduce or eliminate the mosquito populations. However, such compounds have disadvantages such as high cost, selection of resistant mosquitoes, and toxicity to humans, non-target organisms and environment. Due to those problems, researchers have been working on safer alternatives to control this vector [Benelli et al., 2018]. From the perspective of developing products to be used in mosquito larvae control for reducing the risks of epidemics, the use of plants with insecticidal and larvicidal activity is a promising alternative [Pavela, 2015]. Among these alternatives there are essential oils, complex mixtures, characterized by strong aromas and presence of several secondary metabolites, mainly monoterpenes, sesquiterpenes, and phenylpropanoid [Pavela, 2015]. These mixtures act mainly on the defense mechanism, protecting the plant from attack by predators and pathogenic microorganisms, as well as attracting pollinators. Compounds present in volatile oils can vary widely from species to species and even vary considerably from the same species. Application of essential oils is limited due to their volatility, strong odor, insolubility in water, and low physicochemical stability. Thus, to formulate essential oils, they must be encapsulated or form aqueous emulsions to spread satisfactorily at the site of application of the larvicidal product. Among the main formulations for this purpose are nano/microemulsions and micro-encapsulations using cyclodextrins [Lima et al., 2016]. Very limited in number, interesting studies on essential oils used as repellents against sand flies [Kimutai et al., 2017] lead to exploit also this way of study to collect more data of efficacy in contrasting the spread of Leishmaniasis by such new measures.

3: Formulations based on double-stranded RNA molecules

In the last decade, the RNA interference (RNAi) pathway was exploited to silence essential genes in pest insects, leading to toxic/lethal effects, and paving the way for the development of RNAi-based bioinsecticides for the control of mosquito vectors [Niu et al., 2023; Lopez et al., 2019]. The work plan of the WP2.4 includes the bioinformatic identification of specific target genes (e.g., essential metabolic or developmental genes, genes antagonist of arbovirus infection in the mosquito, etc.) and their *in vitro* functional analysis via dsRNA silencing (soaking and or transfection) in suitable cell cultures (i.e., *Ae. albopictus* C6/36). The most promising candidates will be selected for *in vivo* assays. Different dsRNA delivery techniques will be explored: i) adult feeding, ii) cuticle permeation, iii) larval feeding (at different larval stages); iv) novel non-transgenic delivery technologies (e.g., polymer or liposomal nanoparticle) to avoid the use of genetically modified microorganisms (bacteria, yeasts) producing dsRNA in the target organs and/or developmental stages [Silver et al., 2021; Christiaens et al., 2020].

ENTOMOLOGICAL ENDPOINTS

Entomological endpoints refer to entomological outcome variables, concerning the arthropod vectors, that should be reached to consider a control intervention as successful. In this guideline, according to the control interventions that will be tested within the tasks planned for the work package 2.4 of the INF-ACT project, we selected the following two entomological endpoints:

Induced sterility

The ratio of sterility observed in the vector population measured as the percentage of non-hatching eggs or the percentage of sterile females between treated and control areas.

Reduction in vector density

Significant reduction in the vector population between treated and control areas.

For both the selected endpoints we set up a threshold of 50% to consider the vector control intervention as successful. The entomological endpoints could be measured using the following indicators, classified as short-term and long-term, and schematized in Table 1.

SHORT-TERM INDICATORS

We defined as short-term the following indicators that could be applied within the first four weeks from the start of the control intervention.

Egg Sterility Index (ESI)
Statistically significant induced sterility rate between treated and control areas (measured as the percentage of hatching eggs).

Female Sterility Index (FSI)
Statistically significant induced sterility rate between adult females collected in treated and control areas (measured as the percentage of females able to lay fertile eggs).

Adult female density (AFD)
Statistically significant reduction in adult female density between treated and control areas. Adult female density could be measured by standard trapping using sticky traps, electric traps or human landing catches.

Immature abundance (IA)
Post-treatment immature abundance (all stages) should be monitored on day 2 and then weekly until the density of fourth instar larvae in the treated habitats reaches a level comparable to that in the control. The efficacy and residual activity of the larvicide at different dosages are determined from the post-treatment counts of live larvae and pupae in treated and control sites compared with the pretreatment counts or the control.

Adult emergence (AE)
Adult emergence can be monitored directly in the field by floating sentinel emergence traps in treated and untreated habitats, by pupal isolation, or by sampling and counting pupal skins. Adult emergence may also be assessed by collecting pupae and bringing them to the laboratory with the water from the respective habitats. When monitored directly in the field, the pre-treatment and post-treatment data on adult emergence in treated and untreated habitats are analyzed for adult Emergence inhibition (Annex D).

Adult density (AD)
Statistically significant reduction in adult density between treated and control. Adult density could be measured by standard trapping using sticky traps, electric traps or human landing catches.

Effect on non-target organisms (NTO)
Observations on the non-target biota cohabiting with mosquito larvae, especially predators (Larvivorous fish, snails, polychaetas, shrimps, crayfish, crabs, mayfly naiads, copepods, dragonfly naiads, coleopterans, and heteropterans, ostracods, and amphipods are some of the non-target organisms that coexist with mosquito fauna).

Cellular density (CD)

Cellular Density (CD) is a measure of the proportion of live, healthy cells within a population. Cell viability assay will be used to determine the overall health status of cells, and it will be necessary to measure cell survival following dsRNA treatment.

LONG-TERM INDICATORS

We defined as long-term the following indicators that could be applied after the first four weeks from the start of the control intervention and up to the end of the intervention.

Egg density (ED)
The average number of eggs collected using standardized ovitraps between treated and control areas.

Adult density (AD)
Statistically significant reduction in adult density between treated and control.

Effect on non-target organisms (NTO)
Observations on the non-target biota cohabiting with mosquito larvae, especially predators (Larvivorous fish, snails, polychaetas, shrimps, crayfish, crabs, mayfly naiads, copepods, dragonfly naiads, coleopterans, and heteropterans, ostracods, and amphipods are some of the non-target organisms that coexist with mosquito fauna).

TABLE 1

Control intervention		Sterile Insect Technique (SIT) Incompatible Insect Technique (IIT)				Bio-larvicides		Entomopathogenic fungi		RNAi-based bioinsecticide	
Endpoints		*>50% induced sterility*		*>50% reduction in density*		*>50% reduction in density*		*>50% reduction in density*		*>50% reduction in density*	
		STI	LTI	STI	LTI	STI	LTI	STI	LTI	STI	LTI
Laboratory	eggs	ESI			ED						
	larvae					IA	NTO			IA	IA
	pupae					IA	NTO				
	adults				AD	AE	AD	AD/NTO	AD/NTO		AD
	female	FSI		AFD							
	male										
	cultured cells									CD	
Semi-field	eggs	ESI			ED						
	larvae					IA	NTO			IA	IA
	pupae					IA	NTO				
	adults				AD	AE	AD	AD/NTO	AD/NTO		AD
	female	FSI		AFD							
	male										
	cultured cells										
Field	eggs	ESI			ED						
	larvae										
	pupae										
	adults				AD						
	female	FSI		AFD							
	male										
	cultured cells										

Legend	
STI	Short-term indicator
LTI	Long-term indicator
ESI	Egg Sterility Index
FSI	Female Sterility Index
AFD	Adult female density
AD	Adult density
AE	Adult emergence
ED	Egg density
IA	Immature Abundance
NTO	Effect on non-target organisms

Table 1. Summary of the indicators for outcome evaluation according to the type of control intervention and of the selected endpoints. In addition, for each control intervention, the indicators are classified according to the type of test to be performed (laboratory test, semi-field test, or field trial).

ANNEX LIST

Attached to this document, we provide a list of publications that have been utilized in the selection of the entomological endpoints described. In Annexes B, C, and D, all of which consist of guidelines published by the World Health Organization, the reader will find further information regarding international reference standards for the application of the indicators suggested in this document as the most appropriate for evaluating the effectiveness of innovative vector control systems in the Italian context.

The increased incidence of arboviruses and the resistance developed by mosquitoes to conventional control methods provide the impetus for further studies aimed at the discovery of new control interventions. For this reason, the World Health Organization (WHO) published the "Guidelines for Laboratory and Field Testing of Mosquito Larvicides" in 2005, the "Guidelines on the efficacy-testing of traps for control of Aedes spp. mosquito vectors" in 2018 and the "Guidance Framework for Testing the Sterile Insect Technique as a Vector Control Tool against Aedes-Borne Diseases" in 2020, to standardize testing procedures for novel larvicides, trapping instruments and autocides control methos such as the SIT.

ANNEX A
EFSA systematic review on entomological endpoints. "A systematic review to understand the value of entomological endpoints for assessing the efficacy of vector control interventions". Nick Van Hul, Marieta Braks, Wim Van Bortel. EFSA supporting publication 2021:EN-6954. 37 pp. doi:10.2903/sp.efsa.2021.EN-6954

ANNEX B
WHO guidance Framework for Testing the Sterile Insect Technique as a Vector Control Tool against Aedes-Borne Diseases. © World Health Organization, 2020.

ANNEX C
WHO guidelines on the efficacy-testing of traps for control of Aedes spp. mosquito vectors. © World Health Organization, 2018.

ANNEX D
WHO guidelines for Laboratory and Field Testing of Mosquito Larvicides. © World Health Organization, 2005.

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EXTERNAL SCIENTIFIC REPORT

VectorNet European Network for Medical and Veterinary Entomology

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A systematic review to understand the value of entomological endpoints for assessing the efficacy of vector control interventions

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Abstract

To guide implementation of vector control interventions in Europe, a stronger evidence base of their efficacy and effectiveness is needed. Currently, epidemiological endpoints are used to demonstrate the public health value of a vector control intervention. This systematic literature review aimed to help assess whether entomological endpoints (such as mosquito abundance, infection rates, inoculation rates, parity rate as proxy for longevity, or others) can be used on their own as evidence of efficacy of vector control interventions against vector-borne diseases. We searched electronic bibliographic databases (The Cochrane Library, CAB Abstracts, MEDLINE and Web of Science) for intervention trials where vector control interventions were evaluated and extracted epidemiological and entomological effect size estimates. The selection process resulted in 31 studies (extracted from 35 publications) for which both types of endpoints were available. The final database included studies on malaria (n=16), dengue (n=9), leishmaniasis (n=5) and tick-borne diseases (n=1). Epidemiological and entomological effect sizes often pointed in the same direction (i.e. both favouring intervention or favouring control). However, based on the statistical inference (whether the effect size estimate is significantly different from no-effect) of the results, we observed some disagreement between endpoints, though we rarely saw complete disagreement in effect estimates. This review illustrates the complex relation between entomological and epidemiological endpoints. Based on this review, it is concluded that evaluating interventions on entomological endpoints only is insufficient to understand their potential epidemiological impact. To better asses the value of entomological endpoints for the assessment of efficacy of vector control intervention, there is a need for studies to be powered for both epidemiological and entomological endpoints.

Key words: systematic literature review, vector control, entomological endpoint, epidemiological endpoint, intervention trials

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Summary

To guide implementation of vector control interventions in Europe, a stronger evidence base of their efficacy and effectiveness is needed. Currently, epidemiological endpoints are used to demonstrate the public and veterinary health value of a vector control intervention. To obtain potentially significant effect of interventions on epidemiological endpoints in Europe, high sample sizes would be needed for most studies on vector-borne diseases due to the low transmission rate. Even when cases of local transmission are more frequent, such as for West Nile virus infection and some veterinary vector-borne diseases (bluetongue, Schmallenberg or canine leishmaniasis), outbreaks are often of an epidemic nature, making trials with epidemiological endpoints hard to plan and execute. As the arthropod vector drives the transmission, it is expected that entomological endpoints can be used to appraise the impact of vector control interventions on control vector-borne diseases. Yet, different factors may influence the effect measure of a vector control intervention so that the relationship between the entomological and epidemiological endpoints might be obscured.

This systematic literature review assessed whether effects on entomological endpoints (such as the change in mosquito abundance, infection rates, inoculation rates, parity rate as proxy for longevity, or others) can be used on their own as evidence of efficacy or effectiveness of vector control interventions to control disease. We searched electronic bibliographic databases (The Cochrane Library, CAB Abstracts, MEDLINE and Web of Science) for intervention trials where vector control interventions were evaluated and extracted the effect size estimates of the epidemiological and entomological endpoints.

In total, 1345 publications were retrieved resulting in 1119 publications after removal of duplicates. Of these, 103 papers were selected for full text screening. Some studies did not report both types of endpoints in the same publication, but reported their data in separate publications subsequently called "sister articles". Hence, before the full text screening, we searched for "sister articles" to ensure that both types of endpoints could be extracted. The selection process resulted in 31 studies (extracted from 35 publications) for which both types of endpoints were available. The final database of included studies comprised four disease categories: malaria (n=16), dengue (n=9), leishmaniasis (n=5) and tick-borne diseases (n=1). Twenty one of the 31 studies were published after 2010 and the included studies assessed a range of interventions or combination of interventions in various epidemiological settings.

Various entomological endpoints were used, including density-related endpoints such as adult female mosquito density, adult host-seeking density, adult indoor resting density and larval density, sporozoite and parity rate and entomological inoculation rate (EIR). In malaria trials a large variety of entomological endpoints were used compared to leishmaniasis or dengue trials. The latter two focused more often on adult and larval density estimates, respectively.

The results show that for entomological endpoint, we often observe large confidence intervals. Further, the analysis indicated that entomological effect estimates often pointed in the same direction as epidemiological effect estimates. Yet, based on statistical inference (whether an effect estimate is significantly different from no effect, or not and whether it favours control or intervention) we observed some disagreements within studies between results based on either type of endpoints. Yet, only in rare instances did we find complete disagreement between entomological and epidemiological results. Based on this review, evaluating interventions with only entomological endpoints seems to be insufficient to understand their potential epidemiological impact.

A limitation of this review is that it compared intervention effects on entomological and epidemiological endpoints from studies that were not specifically designed to evaluate whether entomological endpoints can be used as a proxy for epidemiological endpoints when assessing efficacy of vector control interventions. Further, the included studies covered different diseases, in a variety of settings, evaluating various interventions and using different endpoints resulting in a heterogenous data set. Hence, this review only gives a first appraisal of possible correlation between entomological and epidemiological endpoints. To better asses the value of entomological endpoints for the assessment of efficacy of vector control interventions against disease, there is a need for studies to be powered for both epidemiological and entomological endpoints.

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1. Introduction

1.1. Background and Terms of Reference as provided by the requestor

This contract was awarded by EFSA to: VectorNet (VectorNext consortium)

Contractor: VectorNet (VectorNext consortium)

Contract number and title: VECTORNET SPECIFIC CONTRACT No 01/EFSA implementing framework contract NO ECDC/2019/020

1.2. Interpretation of the Terms of Reference

In the fight against vector-borne diseases, vector control is one of the first lines of defence. Because of this, there have been many studies conducted on both the development of new vector control measures and on the effectiveness of existing measures. According to WHO "*Phase III studies should be designed around epidemiological endpoints to demonstrate the public health value of the intervention. Entomological outcomes cannot be used on their own for this purpose, although they can be combined with epidemiological outcomes to evaluate a claimed entomological effect*" (World Health Organization, 2017). For vector-borne diseases in Europe, however, in order for epidemiological endpoints to provide significant results, this would require very large and expensive studies for most vector-borne diseases, because of the low number of locally transmitted reported cases in humans.

To guide implementation of vector control interventions in Europe, a stronger evidence base of their efficacy and effectiveness is needed. As the arthropod vector drives the transmission, it is expected that entomological endpoints can be used to appraise the effect of vector control interventions on the infections in the population because of a causal link between entomological endpoints and epidemiological ones. Yet, different factors, such as the immune status of the human population or a non-linear relationship between the entomological and epidemiological endpoints (Smith and McKenzie, 2004; Smith et al., 2010) may influence the degree to which effect estimates on entomological endpoints can be used to infer effect estimates on disease. Further, as trials are designed to power effects on primary endpoints, which are often epidemiological, it is expected that entomological endpoints are not incorporated in the power calculations. Due to this, the sample size may not provide adequate statistical power to assess the efficacy of vector control intervention on entomological endpoints. For example, in many mosquito-borne diseases, mosquito infection rates are low, even during high transmission seasons, and to obtain statistically significant changes in mosquito infection rates due to the intervention may require very large sample sizes. From the perspective of vector control intervention in Europe, we ask the following question: can entomological endpoints (i.e. mosquito abundance, infection rates, inoculation rates, parity rate as proxy for longevity, or others) be used on their own as evidence of efficacy of vector control interventions against disease? To answer this research question, we conducted a systematic literature review.

2. Data and Methodologies

2.1. Data

2.1.1. Inclusion criteria

Population

We included studies on vector control intervention trials against human and veterinary vector-borne diseases, measuring both entomological and epidemiological endpoints. This did not mean both types of endpoints had to be reported in the same publication. Due to the scarcity of cluster randomised controlled trials (cRCT) or other studies of sufficiently high quality in which both entomological and epidemiological endpoints were measured, studies were not limited to specific settings, regions, age classes or populations.

Intervention

Only interventions targeting vectors, for which efficacy or effectiveness was investigated at population level, were considered. Interventions directly targeting the pathogen (e.g. vaccination) or evaluated at the level of isolated individual humans or animals (e.g. topical repellents as personal protection) were not included. Studies in which personal intervention measures were implemented on a large scale (e.g. repellent-impregnated clothing, topical repellents on a cohort) were included.

Interventions of vector control included in this review were (not limited to): long-lasting insecticidal nets (LLIN), indoor residual spraying (IRS), insecticide-treated nets (ITNs), repellent-impregnated clothing, reservoir culling, house improvements, traps, biological control, environmental management, dipping, thermal fogging, ultra-low volume spraying, space spraying and pesticides (See Annex A for details).

Comparator

Existing measures, placebo measures or no-vector control measures were regarded as acceptable control groups. Placebo measures (e.g. non-insecticide treated bed nets) can have some effect on the entomological and epidemiological endpoints. Studies with this type of placebo often incorporate an alternative control group without vector control measures; these were also included. Some studies used ITN or LLIN as vector control measure to evaluate the synergy of these measures with other interventions; these studies were also included.

Study design

Acceptable study designs included: cluster randomised controlled trial, randomised cross-over study, step-wedge design, controlled before-and-after study, controlled time series or controlled interrupted time series, case-control, cohort or cross-sectional study. Initially studies, which only report entomological or epidemiological endpoints, were included because different entomological or epidemiological endpoints can be reported in the same publication or in separate publications. If eventually only one of the two types of endpoints was found, the study was excluded.

We focused on the following intervention trials: Phase III efficacy studies and Phase IV effectiveness studies. Phase I and II studies, as defined by (Wilson et al., 2015), were excluded.

In addition, the following study designs were excluded: non-randomised controlled trials or non-randomised controlled time series; studies without a control group or using a historical control group; studies under artificial conditions (e.g. lab conditions); studies on individual control measures; case studies; studies on individual-level protection; non-vector-borne disease; studies on disease treatment; studies of interventions without a direct effect on a vector; awareness studies; questionnaire-based studies; feasibility studies.

2.1.2. Search strategy

Search terms

The search strategy included terms of three categories: vector control interventions, disease/pathogens and study design. Despite the probable insufficient indexation of the study design in most databases, we decided to keep this category of search terms. This allowed us to retrieve a considerable amount of studies whilst keeping it manageable. Study design search terms were used in tandem with study design filters if present in databases.

In epidemiological trials, epidemiological endpoints commonly refer to incidence or prevalence of a given disease or symptoms of said disease. In this review, we focused not only on epidemiological endpoints, but also on entomological endpoints. Entomological endpoints refer to entomological outcome variables concerning the arthropod vectors and refer for example to vector density or vector infection.

It is possible that some studies that have both epidemiological endpoints and entomological endpoints reported their finding in separate articles (i.e. in an epidemiological journal and an entomological journal, respectively). It was of utmost importance to find these linked articles. While screening the complete texts of the journals only reporting epidemiological endpoints, we looked for the "sister article" reporting the entomological data or vice versa.

We chose not to include vector species names in our search strategies, because studies did not need to mention the vector species to be included. For example, if a study was performed that measured both entomological and epidemiological endpoints, and the authors decided to publish both endpoints separately, we would still include the study. For this we would want to retrieve at least one of the two publications in order to find the associated second paper at a later stage. The reason we initially only focused on epidemiological search terms (diseases/pathogens) and not entomological search terms (vector species) was that, in epidemiological publications, study methodology is often better indexed. Besides this, if a study includes both epidemiological and entomological data, the diseases or pathogen is more often mentioned in the title and abstract than the vector species is.

Databases searched

The following electronic bibliographic databases were searched:

- The Cochrane Library
- CAB Abstracts
- MEDLINE
- Web of Science

2.2. Methodologies

Study acquisition

After searches were performed in respective databases, all retrieved publications were pooled in an EndNote™ (Clarivate) library. Duplicates were removed using the EndNote tool. After this, the results were manually screened for duplicates.

Selection process

Study selection was performed in parallel by two independent reviewers. Discrepancies were resolved by consensus. If a consensus was not reached, a third independent researcher decided. Selection was performed using an online tool (Covidence systematic review software, Veritas Health Innovation, Melbourne, Australia; available at www.covidence.org).

After the screening of abstract and title, the remaining studies were screened for the presence of both epidemiological and entomological endpoints. If one of the two types of endpoints was not present in a given publication, the full text was screened in search of an associated publication containing the other type of endpoints. Besides this, the trial registration code was used to search for mentioned endpoints. Publications not containing both endpoint types and not having associated publications containing said endpoint types, were excluded. After this, the full text was screened using the inclusion criteria.

Assessment of risk of bias

For the assessment of the risk of bias, we based our domains of bias on the Cochrane "Risk of Bias" tool (Higgins et al., 2011). We expanded on this with a domain to take into account the statistical adjustments for clustered randomised trials and a domain to evaluate entomological sampling quality. Furthermore, we divided the domains "blinding of outcome assessment" into an epidemiological and entomological domain. The same was done for "incomplete outcome data" and "selective reporting".

Data extraction and analysis

Data was extracted according to the fields shown in Annex B. If a study reported more than three entomological or epidemiological endpoints, the primary endpoints were extracted, followed by the secondary ones in order of reporting.

In order to evaluate entomological endpoints, we generated forest plots to visually compare effect sizes as measured by entomological endpoints with those of epidemiological endpoints. Because of the substantial variation in endpoints present in the studies and the fact that some studies report multiple entomological and/or epidemiological endpoints, we chose one entomological and one epidemiological endpoint within every disease (with the exception of malaria for which we generated two plots) for which we would generate a forest plot. For each disease, the endpoints for which the most epidemiological-entomological pairs could be formed were chosen to be included in the forest plots. This was done to compare as much studies as possible, despite only including one entomological and one epidemiological endpoint per intervention arm in comparison with control. Scatterplot versions of data represented in forest plots are provided for an alternative visual representation.

Studies could only be included in the plots if endpoints were expressed in ratios (e.g. odds ratios (ORs), relative risk or risk ratios (RRs) or relative risk reduction (RRRs)) or if raw data was provided. RRRs were transformed to RRs by taking the compliment (formula: RR = 1 − RRR). OR were transformed to RR using the formula proposed by Zhang and Yu (Zhang and Yu, 1998). The RR was calculated for studies for which the raw data were available but no RR was reported. These estimates were calculated from the data provided in the publications without adjustment (e.g. for confounding factors), and confidence intervals may not reflect true uncertainty.

3. Results

3.1. Database searches and text screening

In total, 1345 publications were retrieved. Removing duplicates in EndNote resulted in 1129 publications. These publications were then uploaded into Covidence (www.covidece.org), the software used to perform the selection process, quality assessment and data extraction. Covidence found another 10 duplicates, leaving 1119 publications. Of the resulting 1119 publications, some reported different data from the same study. These "sister articles" were linked in Covidence because they represent a single study. Thus, the resulting 1119 publications made up 1112 studies for screening on title and abstract. After screening of title and abstract, 97 studies (100 publications) were left. These proceeded to the full text screening. Before the full text screening, we searched additional "sister articles" of studies reporting only epidemiological or entomological endpoints to ensure that both types of endpoints could be extracted. The selection process resulted in 31 studies, reported in 35 publications, for which both types of endpoints were available (Figure 1, Table 1).

Figure 1: Overview of the selection and screening process of the publications.

Table 1: Overview of the selected publications after full text screening with indication of the disease, intervention and type of endpoint reported in the publication.

Disease	Publication*	Intervention(s) **	Epi EP Yes/No	Ento EP Yes/No	Reference
Dengue	Kroeger A, et al. Bmj-. 2006;332(7552):1247-50A.	IT curtains; larviciding + water container covers	Yes	Yes	(Kroeger et al., 2006)
	Lenhart A, et al. Trop Med Int Health. 2008;13(1):56-67.	LLIN	Yes	Yes	(Lenhart et al., 2008)
	Degener CM, et al. J Med Entomol. 2014;51(2):408-20.	Mass trapping	Yes	Yes	(Degener et al., 2014)
	Degener CM, et al. Mem Inst Oswaldo Cruz. 2015;110(4):517-27.	Mass trapping	Yes	Yes	(Degener et al., 2015)
	Kittayapong P, et al. PLoS Negl Trop Dis. 2017;11(1):e0005197.	IT school uniforms	Yes	Yes	(Kittayapong et al., 2017)
	Andersson N, et al. Bmj. 2015;351:h3267.	Community mobilisation	Yes	Yes	(Andersson et al., 2015)
	Toledo ME, et al. PLoS Negl Trop Dis. 2017;11(11).	IT curtains; IRS	Yes	Yes	(Toledo et al., 2017)
	Lenhart A, et al. PLoS Negl Trop Dis. 2020;14(4):e0008097.	IT curtains	Yes	Yes	(Lenhart et al., 2020)

	Newton-Sánchez OA, et al. Int J Public Health. 2020;65(3):249-55.	Community mobilisation	Yes	Yes	(Newton-Sánchez et al., 2020)
Leishmaniasis	Kroeger A, et al. Bmj. 2002;325(7368):810-3.	IT curtains	Yes	Yes	(Kroeger et al., 2002)
	Picado A, et al. PLoS Negl Trop Dis. 2010;4(1):e587.	LLIN	No	Yes	(Picado et al., 2010a)
	Picado A, et al. Bmj. 2010;341:c6760.		Yes	No	(Picado et al., 2010b)
	Gunay F, et al. J Vector Ecol. 2014;39(2):395-405.	LLIN	Yes	Yes	(Gunay et al., 2014)
	Faraj C, et al. Am J Trop Med Hyg. 2016;94(3):679-85.	LLIN; IRS	Yes	Yes	(Faraj et al., 2016)
	Courtenay O, et al. PLoS Negl Trop Dis. 2019;13(10):e0007767.	Synthetic sex-aggregation pheromone + insecticide; Impregnated dog collar	Yes	Yes	(Courtenay et al., 2019)
Malaria	Beach RF, et al. Am J Trop Med Hyg. 1993;49(3):290-300.	ITN; IT curtains	Yes	Yes	(Beach et al., 1993)
	Mbogo CNM, et al. Med. Vet. Entomol. 1996;10(3):251-9.	ITN	No	Yes	(Mbogo et al., 1996)
	Nevill CG, et al. Trop. Med & Int. Health. 1996;1(2):139-46.		Yes	No	(Nevill et al., 1996)
	Curtis CF, et al Trop. Med & Int. Health. 1998;3(8):619-31.	ITN; IRS	Yes	Yes	(Curtis et al., 1998)
	Kroeger A, et al. Trans R Soc Trop Med Hyg. 1999;93(6):565-70.	ITN	Yes	Yes	(Kroeger et al., 1999)
	Rowland M, et al. Lancet. 2001;357(9271):1837-41.	Sponging cattle with deltamethrin	Yes	Yes	(Rowland et al., 2001)
	West PA, et al. PLoS Med. 2014;11(4).	ITN + IRS	Yes	Yes	(West et al., 2014)
	Majambere S, et al. Am. J. Trop. Med. Hyg. 2010;82(2):176-84.	Larviciding	Yes	Yes	(Majambere et al., 2010)
	Corbel V, et al. Lancet Infect. Dis. 2012;12(8):617-26.	Universal LLIN; targeted LLIN + IRS; IT plastic sheeting	Yes	Yes	(Corbel et al., 2012)
	Smithuis FM, et al. Malar J. 2013;12:363.	ITN	Yes	No	(Smithuis et al., 2013a)
	Smithuis FM, et al. Malar J. 2013;12:364.		No	Yes	(Smithuis et al., 2013b)
	Pinder M, et al. Lancet. 2015;385(9976):1436-46.	LLIN + IRS	Yes	Yes	(Pinder et al., 2015)
	Bousema T, et al. Plos Medicine. 2016;13(4).	Larvicide + LLIN + IRS	Yes	Yes	(Bousema et al., 2016)
	Homan T, et al. Lancet. 2016;388(10050):1193-201.	Mass trapping	Yes	Yes	(Homan et al., 2016)
	Protopopoff N, et al. Lancet. 2018;391(10130):1577-88.	LLIN-PBO nets; IRS	Yes	Yes	(Protopopoff et al., 2018)

	Tiono AB, et al. Lancet. 2018;392(10147):569-80.	LLIN	Yes	Yes	(Tiono et al., 2018)
	Kenea O, et al. Malar J. 2019;18.	LLIN + IRS; LLIN; IRS	No	Yes	(Kenea et al., 2019)
	Loha E, et al. Malar J. 2019;18(1):141.		Yes	No	(Loha et al., 2019)
	Syafruddin D, et al. Am. J. Trop. Med Hyg. 2020;103(1):344-58.	Spatial repellent	Yes	Yes	(Syafruddin et al., 2020)
Tick-borne diseases ***	Hinckley AF, et al. J Infect Dis. 2016;214(2):182-8.	Barrier treatment	Yes	Yes	(Hinckley et al., 2016)

Note. *Publications of the same study were linked in Covidence before the data extraction. **LLIN: Long-lasting Insecticidal Nets; IT: Insecticide Treated; IRS: Indoor Residual Spraying; PBO: Piperonyl butoxide; Epi EP: epidemiological endpoint present; Ento EP: entomological endpoint present. *** No specific tick-borne disease is reported in the reference, only the incidence of tick-borne diseases in general in humans.

3.2. Characteristics of the included studies

The final database of included studies was comprised of four disease categories: malaria (n=16), dengue (n=9), leishmaniasis (n=5) and tick-borne diseases (n=1) (Table 1). Twenty one of the 31 studies were published after 2010. The publications before the year 2000 were all on malaria (Figure 2). The included studies assessed a range of interventions or combination of interventions (Figure 3). Here, we provide a short summary of the intervention categories used to represent the interventions.

Long lasting insecticidal nets (LLIN). LLIN are bed nets which have been factory treated to include insecticidal compounds. LLIN are made of material into which insecticide is incorporated or bound around the fibres of the net, making the nets stay insecticidal for a longer period (up to three years). Examples of these are the Olyset Net® (Sumitomo Chemicals) and PermaNet® (Vestergaard Frandsen). In settings with high insecticide resistance, LLIN are often treated with an additional synergist targeting identified resistance mechanisms. LLIN can differ in the way they are distributed. When LLIN are distributed to a target population (e.g. pregnant women) this is called targeted LLIN or TLLIN. When LLIN are distributed to everyone this is called universal LLIN or ULLIN.

Insecticide-treated nets (ITN). ITN are bed nets dipped in a mixture of water and insecticide (pyrethroids). Due to the superficial impregnation achieved by this process, ITN lose their insecticidal properties at a faster rate than LLIN (often only lasted for 6 months).

Indoor residual spraying (IRS). IRS is the process in which insecticide is sprayed on inside walls of houses and animal sheds. Vectors resting indoors will come in contact with the insecticide on the wall and will be killed.

Insecticide-treated material (other than nets). Curtains, most often impregnated in the same way as ITN or LLIN, are a way to improve the protection of living quarters against vectors. It does so by forming a barrier around windows and doors. Also, insecticide impregnated school uniforms or insecticide-treated screens on stables belong to this category.

Mass trapping. All interventions in which adult vectors are captured on a large scale using traps fall under mass trapping. This can be done using a variety of traps provided with various lures such as light, kairomones or pheromones. Common example of trapping units are BG Sentinel® traps.

Community interventions. Community-centred interventions or community mobilisations include a range of interventions that are organised by specialised organisations, but are implemented by the members of the local communities. The process of community mobilisation often starts by approaching community leaders to ask permission and engage them in discussions of baseline evidence. With the permission of community leaders, facilitators are appointed to form acting groups of community members. These groups will then discuss which interventions and activities will be implemented inside

their respective community. The aim of this intervention is to raise awareness among the local population and build sustainable vector control capacity.

Animal protection. The protection of animals aims to reduce vector populations by reducing the contact with potential hosts in a given area. Besides the protection of animals, the intervention may reduce the presence of zoonotic diseases in the protected animal population, reducing the risk of transmission to humans. In the case of livestock, the protection from vectors has the possibility to increase yield. Examples of animal protection are insecticide impregnated dog collars or sponging of insecticide on livestock.

Larvicide. In mosquito control the application of larvicide to surface water in a given area aims to reduce the number of larvae in larval habitats. An example of a larvicide is *Bacillus thuringiensis* subspecies *israelensis* (Bti).

Spatial repellent. The aim of spatial repellents is to keep vectors from biting humans or livestock. An example is the use of metofluthrin-treated coils which can be burned to release their active substance.

Table 2 gives the list of entomological and epidemiological endpoints and their definition used in the included studies. Figures 4 and 5 provide an overview of the frequency of the endpoints found in the included studies.

Table 2: Overview of entomological and epidemiological endpoints in the included studies. For the four diseases, dengue, malaria, leishmaniasis and tick- borne diseases

Type	Outcome variables	Explanation	Diseases
Entomological	Breteau index	Used in dengue studies, this index measures the number of water containers containing *Aedes* larvae or pupae per 100 houses inspected.	Dengue
	Biting rate	Average number of vector bites received by a host in a unit time, specified according to host and mosquito species (for mosquitoes often measured by human landing collection).	Dengue, leishmaniasis, malaria
	Blood-fed females	The proportion of captured female vectors that are engorged with blood.	Malaria
	Container index	Percentage of water-holding containers infested with *Aedes* mosquito larvae or pupae.	Dengue
	Entomological inoculation rate (EIR)	Number of infective vector bites received per person in a given unit of time, in a human population.	Malaria
	House index	Percentage of houses infested with *Aedes* mosquito larvae and/or pupae.	Dengue
	Larval density	A measure of mosquito larval abundance in specific habitats. This can be calculated by taking the average number of mosquito larva found when scooping a given volume of water from a given habitat.	Dengue
	Parity rate	The female mosquitoes that have taken a blood meal and have laid their eggs at least once are parous. The parity rate reflects the proportion of parous females from the total number of females.	Malaria
	Pupa index	Number of *Aedes* pupae found in water containers per 100 houses inspected.	Dengue
	Pupae-per-person index	The total number of pupae found divided by the total population of the inspected households.	Dengue

	Sporozoite rate	Number of examined mosquitoes infected with sporozoites divided by the total number of mosquitoes examined.	Malaria
	Ticks found crawling	The number of ticks that are found crawling on the body of the host.	Tick-borne disease
	Vector density	Number of vectors (adults) caught during a given time, specifying the method of collection. This includes indoor and outdoor resting density.	Dengue, leishmaniasis, malaria
Epidemiological	Anaemia	A condition wherein a person's red blood cells drop below a given threshold.	Malaria
	Haemoglobin concentration	The concentration of haemoglobin in sampled individuals.	Malaria
	Incidence	Number of newly diagnosed cases during a defined period in a specified population.	Dengue, leishmaniasis, malaria, tick-borne diseases
	Mortality	A measure of the number of deaths in a particular population.	Malaria
	Parasitaemia as measure of parasite load	Measure of the number of parasites present in the host. Expressed as number of parasites per given volume of blood. Sometimes expressed as a rate of individuals above a given threshold.	Malaria
	Prevalence	Proportion of a specified population with a given infection at one time.	Malaria
	Seroconversion	Development of specific antibodies as a result of infection. The seroconversion rate is the proportion of seroconverted individuals among sampled individuals.	Dengue, malaria
	Splenomegaly	Number of individuals with enlarged spleens out of the number of examined individuals.	Malaria

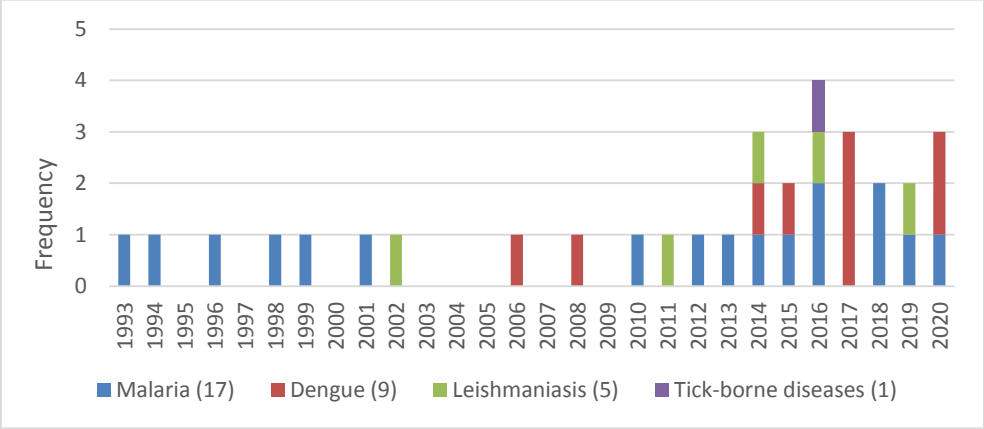

Figure 2: The number of publications included in the review per year of publication. Bars are colour-coded to represent the disease of interest for every publication included: malaria = blue; dengue = red; leishmaniasis = green; tick-borne diseases = purple (this study referred to tick-borne diseases in general without specifying which diseases).

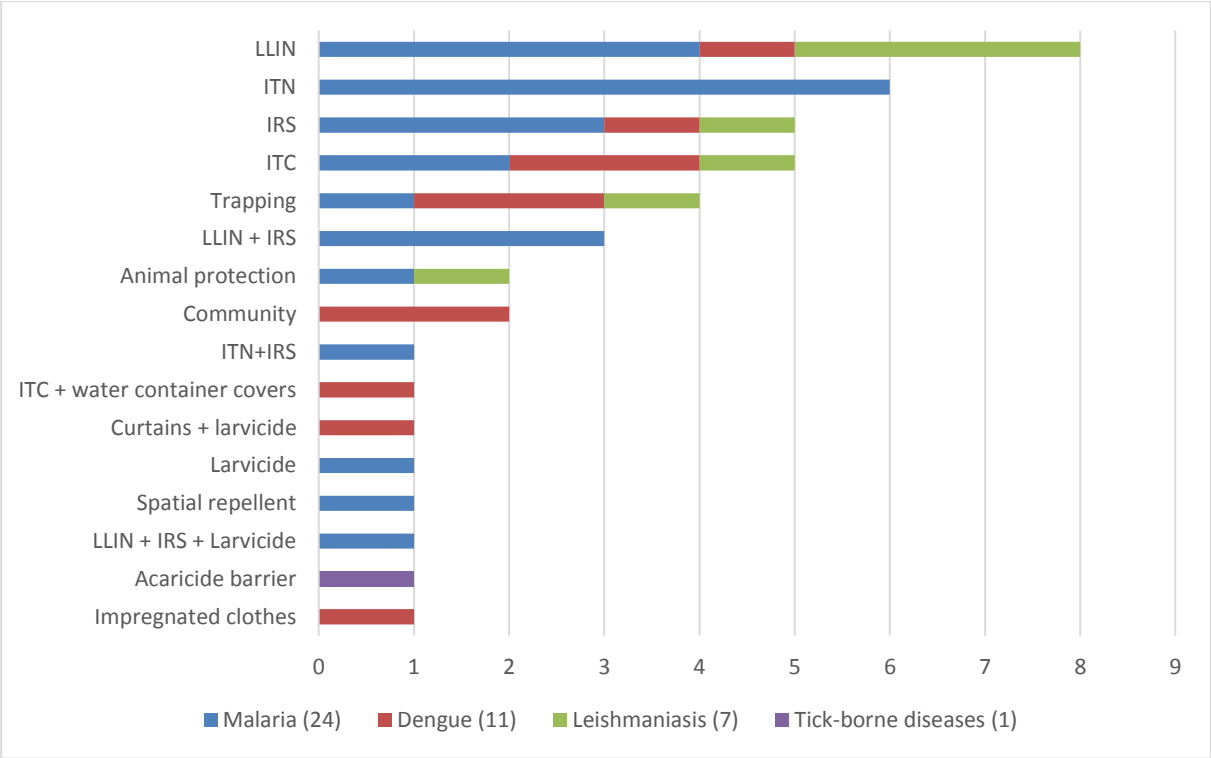

Figure 3: The number of interventions in included studies. Bars are colour-coded to represent the disease of interest for every publication included: malaria = blue; dengue = red; leishmaniasis = green; tick-borne diseases = purple. Number in brackets are the sum of interventions per disease. Abbreviations: LLIN = long-lasting insecticidal nets; ITC = insecticide treated curtains; ITN = insecticide-treated nets; IRS = indoor residual spraying.

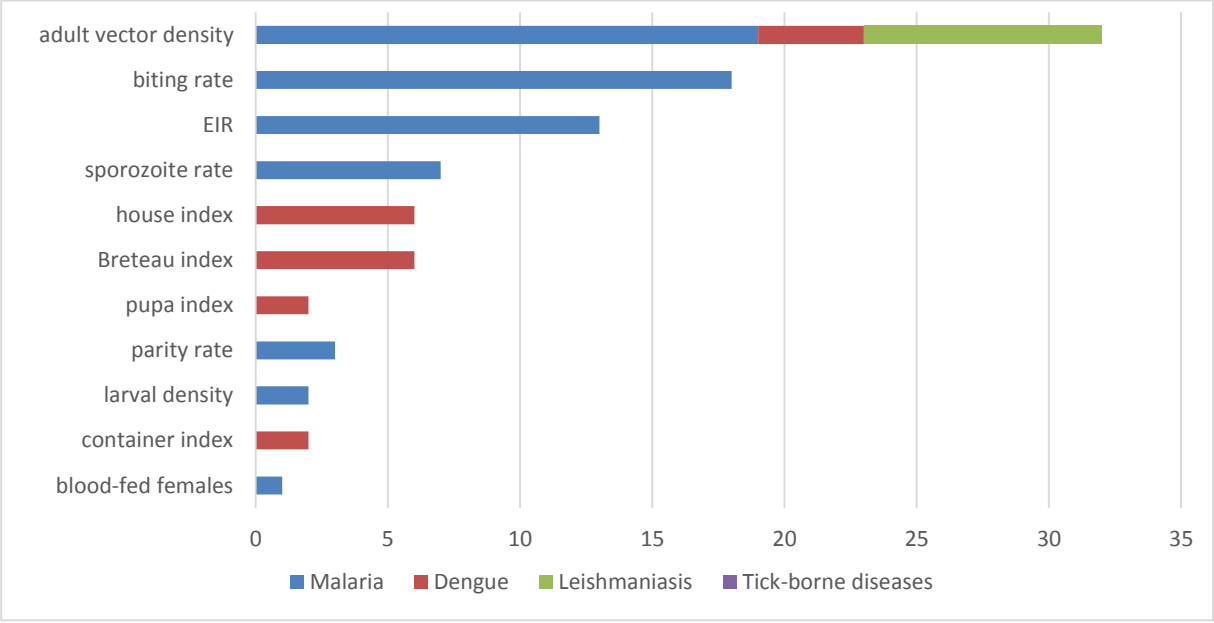

Figure 4: Bar graph showing the frequency of the different entomological endpoints found in the included studies. Bars are colour-coded to represent the different diseases included: malaria = blue; dengue = red; leishmaniasis = green; tick-borne diseases = purple.

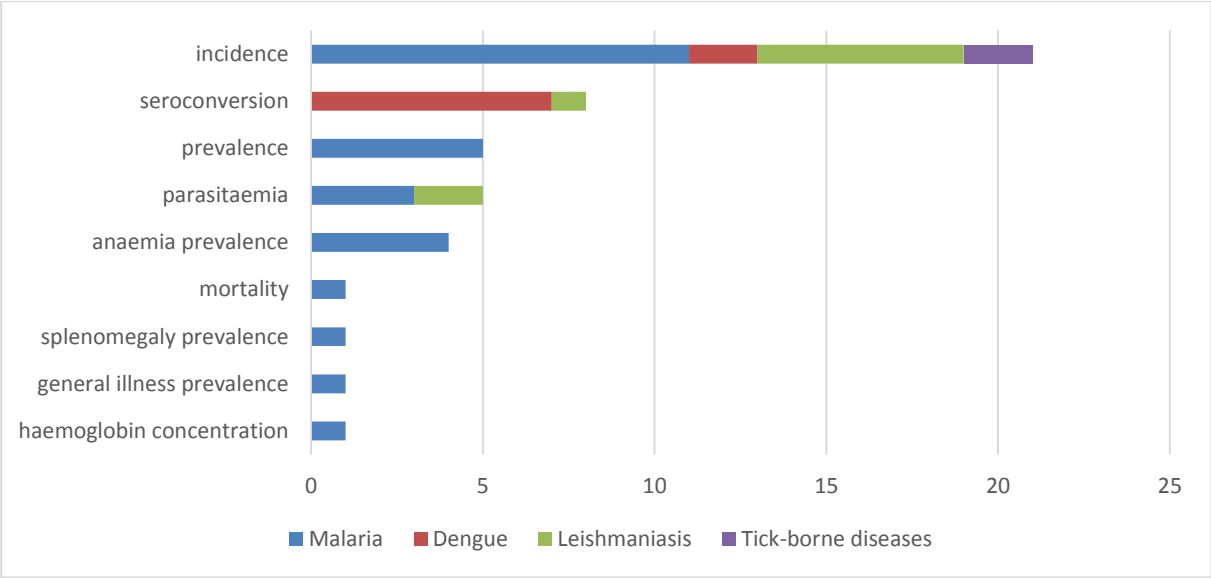

Figure 5: Bar graph with the frequency of the different epidemiological endpoints found in the included studies. Bars are colour-coded to represent the different diseases included: malaria = blue; dengue = red; leishmaniasis = green; tick-borne diseases = purple.

3.3. Quality of studies

Allocation

Allocation of controls or intervention groups were, in general, well performed and well reported. In some studies, allocation was only described as "random", without further explanation. The random sequence generation bias of these studies was scored as unclear. Only two studies did not allocate clusters randomly to intervention or control arms and were therefore scored as at high risk of bias (Beach et al., 1993; Gunay et al., 2014) (Figures 6 and 7).

The risk of bias due to concealment of allocation or the lack thereof was more often high compared to the bias due to random allocation. This was mostly due to the participation of local inhabitants in the intervention, e.g. community mobilisation trials.

Blinding

Due to the nature of most interventions, blinding of participants and personnel is not always possible. Only three studies were blinded for participants, personnel and assessors, using either placebo/untreated interventions in the control group (Hinckley et al., 2016; Syafruddin et al., 2020) or compared a combination of interventions to a standard intervention (Protopopoff et al., 2018) (Figures 6 and 7).

Blinding of assessment was slightly more common than blinding of participants and personnel. Assessment was often done in a laboratory where assessors are more easily blinded to the study arms. Despite this, blinding of outcome assessors was rarely explicitly mentioned. Risk of bias assessment was therefore often unsure.

Incomplete outcome data and selective reporting

Though exclusion of participants and attrition are often found in the studies, these events are in most cases well reported. In many cases, however, these were not of substantial amounts. Only one study (Degener et al., 2014) had both high risk of incomplete epidemiological and entomological outcome

data with epidemiological participation dropping below an average of 50% coverage and performing less than 95% of planned entomological samplings.

There was only one study which had a high risk of selection bias on entomological endpoints (Syafruddin et al., 2020). This was due to not specifying the entomological endpoints to be measured in the trial registration. Publications on trials with an a priori trial registration and which specified what endpoints were to be measured had a low probability of reporting bias.

Standardised sampling method

We added the standardised sampling method domain to account for the variety of sampling methods in entomological surveillance, with classic methods such as human landing catches (HLC), which rely on the capabilities of the human landing catcher. Other methods, such as the use of mechanical or electrical catching tools such as sticky traps, CDC light traps or BG sentinel® traps, induce less operational bias due to the fact that no human operator is directly involved in the catching. Slightly more than half of all studies were at low risk of bias regarding the entomological sampling method.

Analysis accounting for clusters

Most publications accounted for possible effects of clustering.

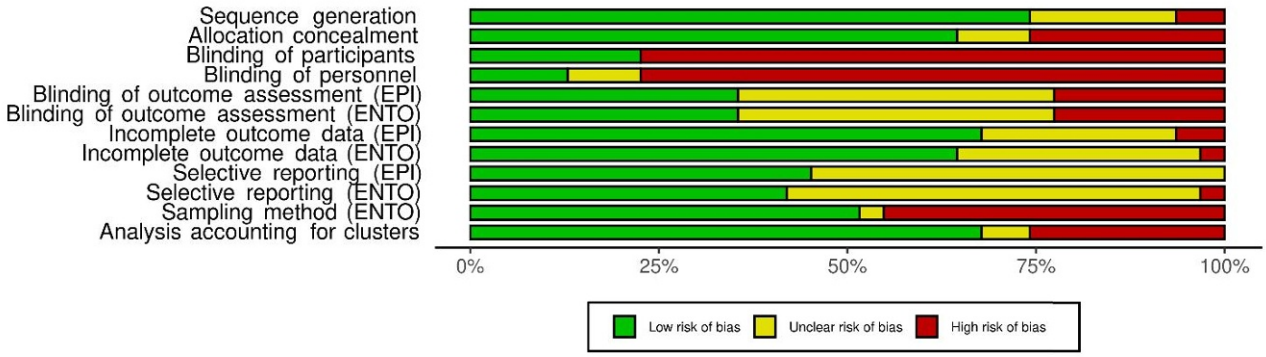

Figure 6: Overall overview of the quality assessment of the included studies. Abbreviations: EPI = epidemiological endpoints; ENTO = entomological endpoints.

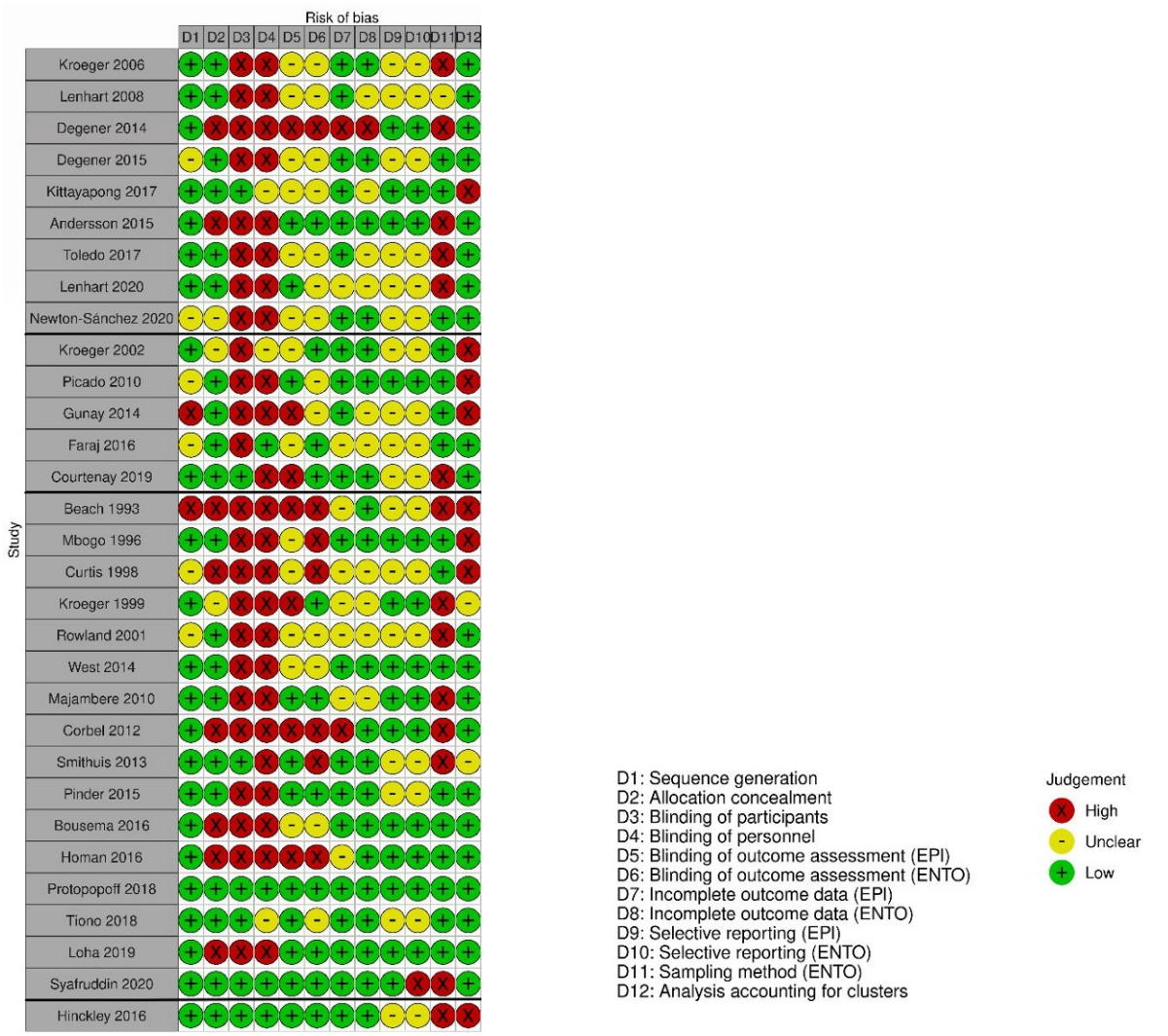

Figure 7: Overview of the quality assessment per included study. Abbreviations: EPI = epidemiological endpoints; ENTO = entomological endpoints.

3.4. Evaluation of epidemiological and entomological endpoints of the selected studies

Nine studies assessed both entomological and epidemiological endpoints of a variety of **dengue** interventions. These interventions included mass trapping, the use of LLIN, impregnated clothing and curtains, and community engagement campaigns. In total, 11 sets of entomological and epidemiological endpoints (corresponding to 11 interventions) could be compared. Of the 11 sets of endpoints, five showed both significant entomological and epidemiological effects. Two of these showed contradictory effects, both showed a significant effect on larval indexes favouring control (i.e. an increased house index in the intervention group) and an effect on clinical dengue case incidence favouring intervention (Lenhart et al., 2008; Toledo et al., 2017). In the remaining four studies, entomological and epidemiological endpoints concurred (Table 3), indicating an effect favouring the intervention (Lenhart et al., 2008; Andersson et al., 2015; Lenhart et al., 2020; Newton-Sánchez et al., 2020).

The entomological endpoints in the dengue intervention studies included adult *Aedes* densities, larval indices such as house, Breteau, container, or pupa index. In only three of the nine studies on dengue, adult vector density was measured. The remaining six studies used larval indices for the calculation of

entomological effects. We found that in some of these studies, epidemiological and entomological endpoints were not in agreement. For example, the fact that larval indices were measured while interventions targeted adults could have contributed to the discrepancies observed between entomological and epidemiological effects. Further, the high seasonal variation, focal nature and outbreak-prone nature of the disease resulted in some studies in higher epidemiological outcomes in the intervention compared to the (non-intervention) control areas (Lenhart et al., 2008; Toledo et al., 2017), thus favouring control. The study where both the entomological and epidemiological endpoints showed an impact was the study of Andersson et al. (Andersson et al., 2015) evaluating the impact on community mobilisation on dengue. This study was exceptionally large with 18 838 enrolled households over two countries (Mexico and Nicaragua). The intervention consisted of a pesticide-free community mobilisation and measured house index, container index, and Breteau index as entomological endpoints. Epidemiological endpoints were serological confirmed recent dengue infection incidence and self-reported dengue incidence.

Five studies on **leishmaniasis** were included in the final selection resulting in seven interventions for which entomological and epidemiological endpoints were available. For five of these interventions, corresponding studies found both significant epidemiological and entomological effects. All studies but one (reported in two publications; (Picado et al., 2010a; Picado et al., 2010b)) had, at least for one intervention, significant epidemiological and entomological effects (Kroeger et al., 2002; Gunay et al., 2014; Faraj et al., 2016; Courtenay et al., 2019). In one case (Picado et al., 2010a), vector density was measured using light traps. The results showed a significant decrease in density of *Phlebotomus argentipes* and an insignificant increase in density of *Phlebotomus papatasi*. The different effect on the entomological endpoints can be an indication of the different effects of interventions (Table 3) on different leishmaniasis vectors. Entomological endpoints were in all cases directly related to vector density (indoor sandfly abundance, overall sandfly abundance*, P. argentipes* density and *P. papatasi* density). Epidemiological endpoints were seroconversion incidence, parasite detection, parasite load and leishmaniasis incidence.

In total, 16 studies were included evaluating interventions to control **malaria**. Only four studies on malaria had both significant epidemiological and entomological effect sizes. One of these (Homan et al., 2016) showed a mixed effect, with only one significant positive entomological effect (i.e. significant decrease in *Anopheles funestus* density) and two significant epidemiological effects, a positive (on parasite prevalence) and negative effect (i.e. significant increase in prevalence of all reported illness in previous two weeks). The remaining three studies showed significant effects favouring intervention in both effect categories (i.e. entomological and epidemiological) (Beach et al., 1993; Mbogo et al., 1996; Rowland et al., 2001; Tiono et al., 2018).

The entomological endpoints used in malaria trials were: larva-positive breeding sites, blood-fed ratio, vector density, entomological inoculation rate (EIR), sporozoite rate, parity rate and bites per person per night. Epidemiological endpoints were clinical malaria incidence, *Plasmodium* incidence, *Plasmodium* prevalence, parasitaemia (>=2,500/mm^3) and anaemia prevalence. Effects on density related endpoints such as adult female mosquito density, adult host-seeking density, adult indoor resting density and larval density generally seemed to be more often significant compared to other effects on indices such as sporozoite and parity rate.

Additionally, there was one leishmaniasis study that, beside leishmaniasis related endpoints, also measured *Anopheles* density and malaria incidence, which were both significantly reduced by the intervention (Picado et al., 2010a).

Only one study on tick-borne diseases in humans was retrieved (Hinckley et al., 2016). The epidemiological outcome of this study was the self-reported incidence of all tick-borne diseases, mostly expecting Lyme disease. The entomological endpoint was the difference in the number of crawling ticks. This study yielded no significant results.

Table 3: Overview of the significance levels of the entomological and epidemiological endpoints per intervention. The effect of each endpoint was recoded into three categories: (1) a significant impact of the intervention with a decrease of the endpoint (green); (2) no significant effect on the endpoint (blue); and (3) a significant increase of the endpoint indicator i.e. higher values were measured in the intervention zone (orange). Endpoints for which no significance levels were available are indicated in yellow. Significance was for all studies defined as p-value <0.05. Details can be found in Annex B.

Disease/ pathogen	Study ID	Intervention	Entomological endpoint			Epidemiological endpoint		
			1	2	3	1	2	3
dengue	Kroeger 2006	ITC + larvicide	Breteau index	house index	pupae per person	seroconversion		
		ITC + water jar covers	Breteau index	house index	pupae per person	seroconversion		
	Lenhart 2008	LLIN	Breteau index	house index	container index	seroconversion		
	Degener 2014	mass trapping	female *aeg.* density			seroconversion		
	Andersson 2015	community mobilisation	house index	container index	Breteau index	seroconversion	dengue incidence	
	Degener 2015	mass trapping	*aeg.* density			seroconversion		
	Kittayapong 2017	IT school uniforms	mosquito density			seroconversion		
	Toledo 2017	ITC	pupa index	house index		dengue incidence		
		IRS	pupa index	house index		dengue incidence		
	Lenhart 2020	ITC	adult female *Aedes* index	Breteau index	pupae per person	seroconversion		
	Newton-Sánchez 2020	community mobilisation	Breteau index			dengue incidence		
leishmaniasis	Kroeger 2002	ITC	indoor sandfly abundance			leishmania incidence		
	Picado 2010	LLIN	*P. argentipes* density	*P. papatasi* density	*An.* density	seroconversion	leishmania incidence	malaria incidence
	Gunay 2014	LLIN	sandfly density			leishmaniasis incidence		
	Faraj 2016	LLIN + standard of care environmental measures	sandfly density			leishmaniasis incidence		
		IRS	sandfly density			leishmaniasis incidence		
	Courtenay 2019	mass trapping	indoor female *Lu. longipalpus* density			seroconversion	parasite detection	parasite load
		IT dog collar	indoor female *Lu. longipalpus* density			seroconversion	parasite detection	parasite load
malaria	Beach 1993	ITN	bites per night	sporozoite rate	EIR	*Pf* incidence	*Pf* parasitaemia	
		ITC	bites per night	sporozoite rate	EIR	*Pf* incidence	*Pf* parasitaemia	
	Mbogo 1996	ITN	blood-fed	indoor An. density	EIR	1 – 59 months mortality	1 – 4 years mortality	*Pf* incidence
	Curtis 1998	ITN	bites per unprotected person per night	EIR	bites per night	malaria incidence		
		IRS	bites per unprotected person per night	EIR	bites per night	malaria incidence		
	Kroeger 1999	ITN (50% coverage)	Bites per night			malaria incidence		

Disease/ pathogen	Study ID	Intervention	Entomological endpoint			Epidemiological endpoint		
			1	2	3	1	2	3
		ITN (31 – 16% coverage)	bites per night			malaria incidence		
		ITN (<16% coverage)	bites per night			malaria incidence		
	Rowland 2001	IT Animal	*An. stephensi* density	*An. culicifacies* density	*An. subpictus* density	*Pf* incidence	*Pv* incidence	
	Majambere 2010	larvicide	larva density	EIR	sporozoite rates	malaria incidence	malaria incidence	
	Corbel 2012	LLIN	bites per night	EIR		*Pf* incidence		
		LLIN+ IRS	bites per night	EIR		*Pf* incidence		
		LLIN + IT plastic sheeting	bites per night	EIR		*Pf* incidence		
	Smithuis 2013	ITN	bites per night	bites per night		*Pf* prevalence	*Pv* prevalence	splenomegaly prevalence
	West 2014	ITN + IRS	*An. gambiae* per house per night	sporozoite rate	EIR	*Pf* prevalence	anaemia prevalence	haemoglobin
	Pinder 2015	LLIN + IRS	*An. gambiae* density	EIR		malaria incidence	malaria incidence	malaria incidence
	Bousema 2016	larvicide	female *An.* density hotspot	female *An.* density evaluation zone	larva density	parasite prevalence evaluation zone	parasite prevalence inside hotspot	
	Homan 2016	mass trapping	*An.* density	*An. funestus* density	*An. gambiae* density	malaria prevalence	parasite prevalence	all illness prevalence
	Protopopoff 2018	LLIN	*An.* density	sporozoite rate	EIR	malaria prevalence	anaemia prevalence	
		IRS	*An.* density	sporozoite rate	EIR	malaria prevalence	anaemia prevalence	
	Tiono 2018	LLIN	parity rate	sporozoites rate	EIR	malaria incidence	anaemia prevalence	
	Loha 2019	LLIN + IRS	indoor host-seeking density	indoor resting density	outdoor resting density	malaria incidence	anaemia prevalence	
		LLIN	indoor host-seeking density	indoor resting density	outdoor resting density	malaria incidence	anaemia prevalence	
		IRS	indoor host-seeking density	indoor resting density	outdoor resting density	malaria incidence	anaemia prevalence	
	Syafruddin 2020	spatial repellent	host-seeking density	parity rate	nulliparous rate	first malaria incidence	malaria incidence	
tick-borne diseases	Hinckley 2016	IT ground barrier	ticks found crawling	ticks found crawling		self-reported illness in humans	self-reported illness in humans	

Note. IRS: indoor residual spraying; IT: insecticide-treated; ITC: insecticide-treated curtains; ITN: insecticide-treated nets; LLIN: long-lasting insecticidal nets; *aeg*: *Aedes aegypti*; *An.*: *Anopheles;* EIR: entomological inoculation rate*; Pv*: *Plasmodium vivax*; *Pf*: *Plasmodium falciparum*; *Lu.: Lutzomyia; P.: Phlebotomus.*

In the included studies on **dengue**, house index was most often included as an entomological endpoint. The most frequently used epidemiological endpoint was dengue incidence. Only two studies reported effect sizes expressed as ratios for house index and dengue incidence (Andersson et al., 2015; Toledo et al., 2017), resulting in three interventions (Figure 8). Two studies provided data frow which we calculated the RR (Kroeger et al., 2006; Lenhart et al., 2020). In the two studies reporting effect as ratios, there was no significant effect as measured by entomological or epidemiological endpoints. However, the point estimates largely concur between endpoint types (Figure 11A). The endpoints concurred in Kroeger et al. (2006), but did not in Lenhart et al. (2020).

All included **leishmaniasis** studies reported sandfly abundance as an entomological endpoint and leishmaniasis incidence as an epidemiological endpoint. Two studies did not report effect sizes as ratios (Kroeger et al., 2002; Gunay et al., 2014) (Figure 9). Among the studies that reported effect sizes as ratios, all but one study (Picado et al., 2010a; Picado et al., 2010b) showed both entomological and entomological effect in the same direction (Figure 11B). The calculated RR from Gunay et al. (2014) showed that the entomological endpoint slightly favoured control (i.e. exhibiting an increased risk in the intervention group). Due to an incidence of zero, de the datapoint of Kroeger et al. (2002) is not represented in the scatterplot.

Because most included studies were on **malaria,** we were able to generate forest plots for two entomological endpoints, namely vector density (Figure 10A) and entomological inoculation rate (EIR) (Figure 10B). In both cases, we compared the respective entomological endpoint with malaria incidence or prevalence. We included additional studies which did not report malaria incidence but instead reported malaria prevalence.

In Figure 10B and 11C, we can see that effect on vector density generally concurs with effect on malaria incidence, but vector density shows a slightly larger effect size. In Loha et al. (2019) and Pinder et al. (2015) we can see that the epidemiological endpoint shows little to no effect, where the entomological endpoint shows a positive effect. Figure 11D shows less indication of a positive corelation between EIR and malaria incidence or prevalence then we observed between vector density and malaria incidence or prevalence. EIR (Figure 10B) showed larger confidence intervals than vector density. These large confidence intervals could be an indication of underpowered study designs in regard to this entomological endpoint.

Because we only retrieved one study on **tick-borne diseases** we were not able to generate a forest plot for these.

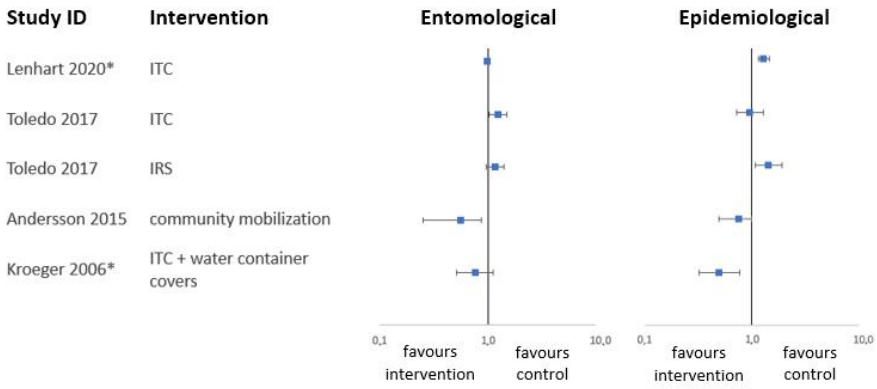

Figure 8: Forest plot comparing the effect of given intervention on house index (entomological endpoint) with the effect on dengue incidence (epidemiological endpoint). Study ID and intervention is given. A value below one indicates the endpoint favours intervention, a value higher than one favours control. Blue squares represent effect size point estimates and horizontal error bars represent the 95% confidence interval. Type of effect size is given to the right. Abbreviations: IRS = indoor residual spraying; ITC = insecticide treated curtains; RR = rate ratio. * RR calculated by reviewers

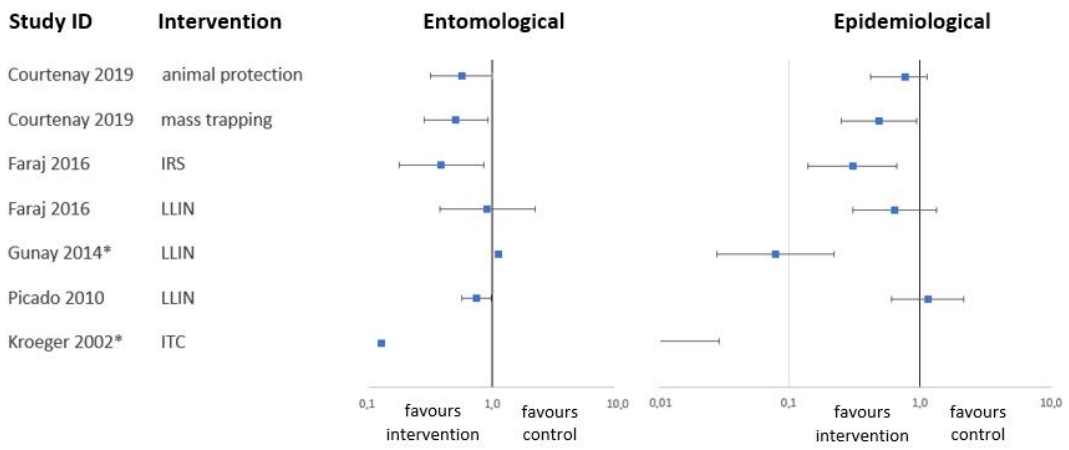

Figure 9: Forest plot comparing the effect of an intervention on vector density (entomological endpoint) with the effect on leishmaniasis incidence (epidemiological endpoint). Study ID and intervention is given. A value below one indicates the endpoint favours intervention, a value higher than one favours control. Blue squares represent the effect size point estimate and (horizontal) error bars represent the 95% confidence interval. Type of effect size is given to the right. Abbreviations: IRS = indoor residual spraying; LLIN = long lasting insecticidal nets; RR = rate ratio. * RR calculated by reviewers

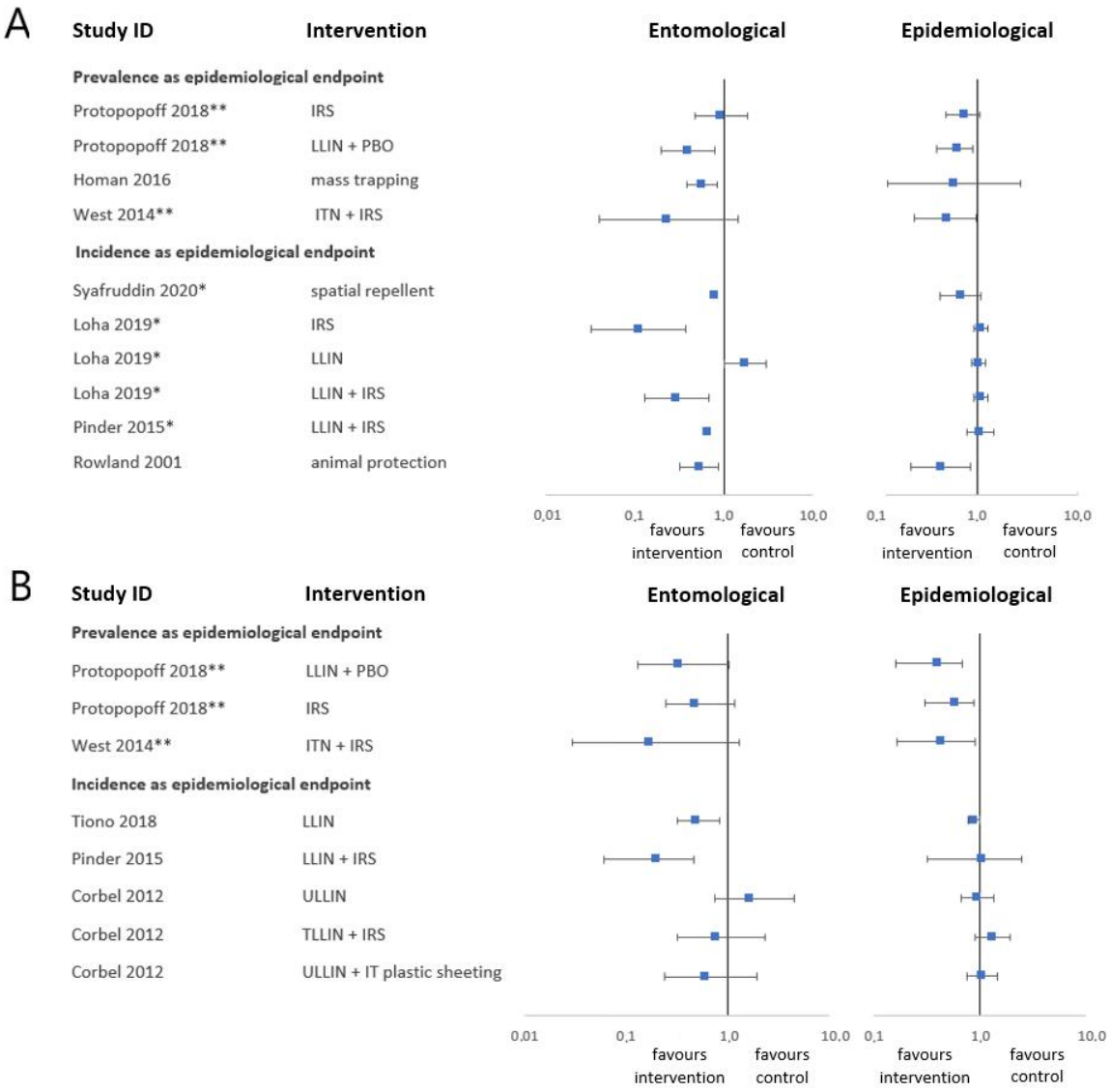

Figure 10: Forest plot comparing (A) the effect of an intervention on vector density with the effect on malaria incidence or prevalence; and (B) the effect of an intervention on entomological inoculation rate (entomological endpoint) with the effect on malaria incidence or prevalence (epidemiological endpoint). Study ID and intervention is given. Blue squares represent the effect size point estimate and (horizontal) error bars represent the 95% confidence interval. A value below one indicates the endpoint favours intervention, a value higher than one favours control. Type of effect size is given to the right. Abbreviations: IRS = indoor residual spraying; IT = insecticide treated; ITN = insecticide treated nets; LLIN = long lasting insecticidal nets; TLLIN = LLIN targeted to pregnant women; ULLIN = universal coverage of LLIN; PBO = piperonylbutoxide synergist; RR = rate ratio. * RR calculated by reviewer; ** RR converted from odds ratio

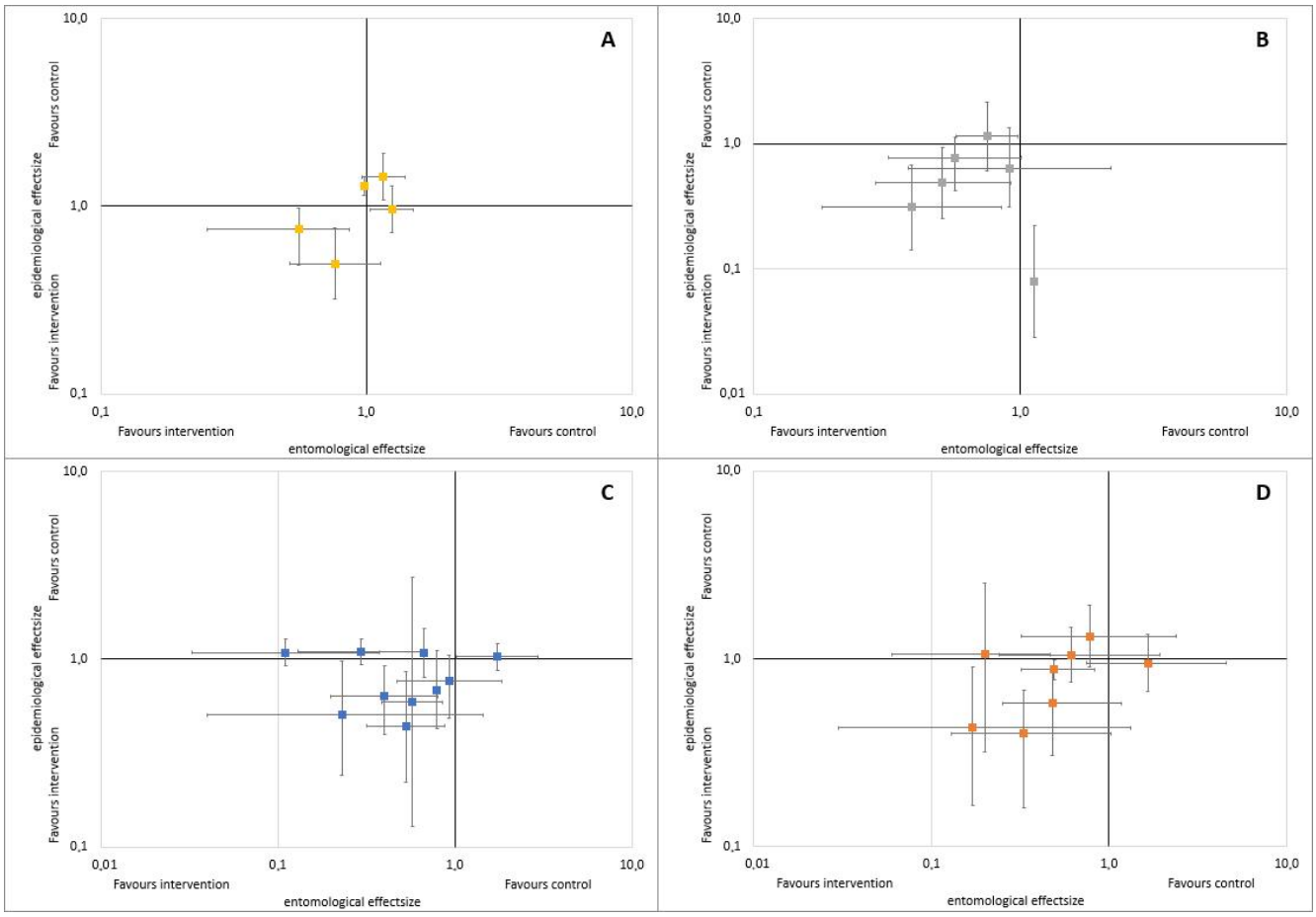

Figure 11: Scatterplot showing the effect (as RR) of the entomological endpoints (X-axis) and the epidemiological endpoints (Y-axis) and their respective confidence intervals of selected studies displaying the same data as in Figures 8 – 10. (A) Dengue with house index (entomological endpoint) versus dengue incidence (epidemiological endpoint); (B) leishmaniasis with vector density (entomological endpoint) versus leishmaniasis incidence (epidemiological endpoint); (C) malaria with vector density versus malaria incidence or prevalence; and (D) malaria with entomological inoculation rate (entomological endpoint) versus incidence or prevalence (epidemiological endpoint).

To further evaluate the importance of the entomological endpoints, we focused on the malaria intervention studies, which provided various endpoints and studies. Within each intervention, the agreement within each of the possible pairs of an individual entomological and epidemiological endpoint was assessed (e.g. if two epidemiological endpoints and three entomological endpoints are measured, effect size can be compared within six pairs) based on three categories (the same used as in Table 3) i.e. (1) "Favouring intervention" meaning a significant impact of the intervention with a decrease of the endpoint; (2) "No effect" no statistically significant effect on the endpoint; and (3) "favouring control" meaning a statistically significant increase of the endpoint indicator i.e. higher values were measured in the intervention arm. A summary was made over all studies (Figure 12).

For the endpoint "adult density", 25 of the 49 comparisons with epidemiological endpoints were in agreement, refering to an effect of the intervention (both endpoints score "Favouring intervention", n=14) or no effect of the intervention (both endpoints score "No effect", n=11). In 15 of the 49 comparisons, the adult density overestimated the effect of the intervention in the sense that the

entomological endpoint pointed towards an impact of the intervention, whereas the epidemiological endpoint did not show statistically significant results.

In 11 of the 21 comparisons made with the EIR, this entomological endpoint showed an insignificant impact of the intervention while the epidemiological endpoint showed a statistically significant one. This probably reflects the difficulty in measuring the EIR correctly and making statistical inferences (Smith et al., 2010; Tusting et al., 2014). The endpoint "parity rate" was in good agreement with the epidemiological endpoints, but only six comparisons could be made, limiting the interpretation of this endpoint.

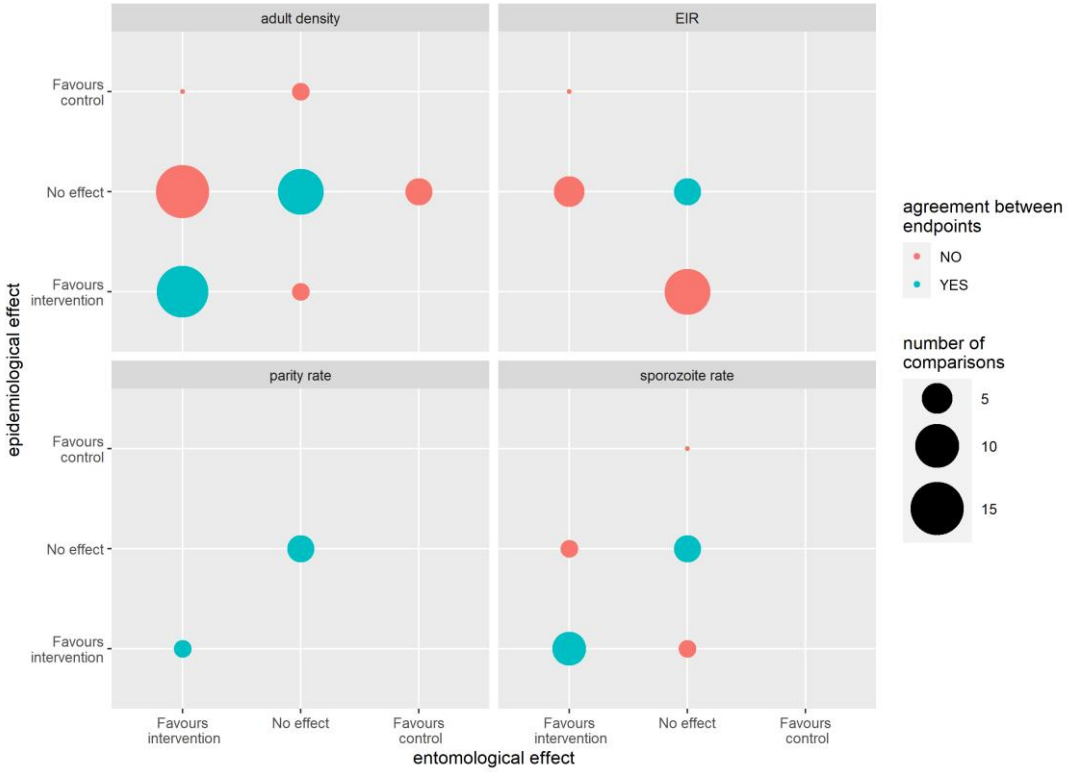

Figure 12: Comparison between four entomological endpoints and corresponding epidemiological endpoints, summarised over all malaria trials. "Favours intervention" meaning a significant impact of the intervention with a decrease of the endpoint; "No effect" indicating no significant effect on the endpoint; and "Favours control" meaning a significant increase of the endpoint indicator i.e. higher values were measure in the intervention zone. The number of comparisons per endpoint: adult density, n=49; EIR, n=21; parity rate, n=6; sporozoite rate, n=15.

4. Discussion and conclusions

In this systematic literature review, 31 studies were included for which both epidemiological and entomological endpoints were available. The studies included four disease categories, namely malaria (n=16), dengue (n=9), leishmaniasis (n=5) and tick-borne diseases (n=1). Various types of entomological endpoints were used, including vector density-related endpoints such as adult female mosquito density, host-seeking density, indoor resting density and larval density, sporozoite and parity rate and entomological inoculation rate. In malaria trials, more endpoints were used compared to leishmania or dengue trials, including measures of vector infection (sporozoite rate), vector longevity

(parity rate), vector density (*Anopheles* density) and vector host contact (biting rate). Leishmania and dengue trials focused more often on adult and larval density estimates, respectively.

Nine studies on **dengue** were retrieved. In only a few, entomological and epidemiological endpoints demonstrated a significant effect of interventions. The low proportion of studies with significant effect sizes is possibly due to the epidemic nature of dengue and fluctuations in vector abundance. This means that sample sizes need to be larger to provide sufficient power for significant results. Because only four studies, comprising five interventions (three studies with one intervention and one with two interventions) could be included in the forest plot, this also limits the interpretation. Despite this, we see that in four of the five interventions, point estimates of entomological and epidemiological endpoint concur. The one discrepancy we observed between entomological and epidemiological endpoints might be due to the use of larval indices as entomological outcomes in the assessment of interventions focusing on adult vectors.

Of the diseases included in this review, studies on vector control interventions against **leishmaniasis** had the highest proportion of significant results, with four of the five included studies having at least for one intervention both significant entomological and epidemiological effect size estimates. It is interesting to note that change in vector density was the only entomological effect measure used. The large number of significant effect estimates and the fact that almost all corresponded, gives an indication that effect on vector density might be a reliable measure of vector control efficacy on this disease. Besides this, one study (Picado et al., 2010a; Picado et al., 2010b) showed that the choice of vector species can be of importance. It measured a significant decrease in *P. argentipes* and no significant effect on *P. papatasi* density. This discrepancy between the two species can be due to a number of reasons, including different levels of anthropophagy, time of biting and vector movement. Irrespective of the reason for this difference, this study illustrates the variable effects vector control interventions can have on different vector species. Such variable effects were also observed in the (malaria) study of Homan et al. (Homan et al., 2016), who found a different effect of the intervention on *Anopheles gambiae* compared to *An. funestus.*

In the studies on **malaria**, vector density was used frequently and effects on this endpoint were slightly more often significant compared to effects on other endpoints. A possible explanation for this can be the relative ease with which these endpoints can be reliably measured compared to for example EIR or sporozoite rate, the latter being more labour intensive requiring dissection of the collected mosquitoes. Consequently, this can result in smaller sample sizes and larger confidence intervals making these endpoints less likely to provide significant results. Despite this, point estimates of effect size often pointed in the same direction as epidemiological endpoints (i.e. both favouring intervention or both favouring control). The use of vector density as an entomological endpoint proxy for epidemiological effect, however, needs to be approached with caution. In some instances, we saw that this endpoint estimated a stronger effect of vector control interventions than epidemiological endpoints.

Overall, for effect estimates on entomological endpoints, we often observed large confidence intervals. The forest plots and scatterplot indicate that entomological effect estimates often pointed in the same direction as epidemiological effect estimates. This observation shows that, in order for entomological endpoints to provide more valuable input in the assessment of vector control efficacy, there is a need for more extensive entomological sampling in studies on the efficacy of vector control interventions.

Based on the statistical inference (whether an outcome is significant or not and whether it favours control or intervention) of the results, we observed some disagreement between endpoints. For instance, vector density often favoured intervention in studies where epidemiological endpoints did not. Yet, only in rare instances the entomological and epidemiological endpoint indicated different directions of effect (e.g. one favouring intervention and one favouring control).

Despite the fact that point estimates of the endpoints where often pointing in the same direction, this review illustrated the complex relation between entomological and epidemiological endpoints. Based on this review, evaluating interventions on only entomological endpoints seems to be insufficient to

understand their potential epidemiological impact. Vector-borne disease transmission is often very focal and variable in time, making a robust design of vector control intervention studies challenging. Yet, the trial design and the choices made were not always as well described for entomological endpoints as for the epidemiological endpoints.

A limitation of this review is that the included studies were not specifically designed to evaluate the question whether entomological endpoints can be used on their own as evidence of efficacy of vector control interventions against vector-borne diseases. Further, the included studies covered different diseases, in a variety of settings, evaluating various interventions and using different endpoints resulting in a heterogenous data set. Hence, this review only gives a first appraisal of possible correlation between entomological and epidemiological endpoints.

5. Recommendations

Entomological endpoints are important in understanding the impact of an intervention on a vector-borne disease. A better description and detailed reporting of these entomological endpoints would improve the overall interpretation and understanding of intervention trials. Yet, for entomological surveys, the design and the choices made are not always well described. Factors such as the blinding of outcome assessors and the expertise of field personnel are rarely mentioned, although these can have substantial effects on outcome measures. Based on this review the following recommendations can be made:

- As trials are designed to power primary endpoints, which are most often epidemiological, the entomological data might not have enough statistical power to assess the efficacy of vector control intervention on entomological endpoints. It would be of value to consider entomological endpoints as well in the power calculations to at least improve the estimates of these endpoints.
- The reporting of entomological endpoints could be improved by better describing the actual study design in terms of the entomological aspects. The use of automated trapping methods should be encouraged to minimise human or operational bias and make entomological results more comparable between studies.
- To evaluate the question whether entomological endpoints can be used on their own as evidence of efficacy of vector control interventions against vector-borne diseases, specific studies should be designed to address this question. This could be trials such as the ones reviewed in this study but powered for both entomological and epidemiological endpoints.

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Abbreviations

aeg	*Aedes aegypti*
An.	*Anopheles*
cRCT	Cluster randomised controlled trial
EIR	Entomological inoculation rate
ENTO	Entomological
EPI	Epidemiological
IRS	Indoor residual spraying
IT	Insecticide treated
ITC	Insecticide treated curtains
ITN	Insecticide treated nets
LLIN	Long-lasting insecticidal nets
Lu.	*Lutzomyia*
OR	Odds ratio
P.	*Phlebotomus*
PBO	Piperonylbutoxide
Pf	*Plasmodium falciparum*
Pv	*Plasmodium vivax*
RR	Risk ratio
RRR	relative risk reduction
TLLIN	targeted long lasting insecticidal nets
ULLIN	Universal long lasting insecticidal nets

Annex A – Search terms

PUBMED

No.	Query	Results
1	"Pest Control"[Mesh] OR "Pest Control/methods"[MAJR] OR "Pesticides"[Mesh] OR vector management[tw] OR vector control*[tw] OR control strateg*[tw] OR control measure*[tw] OR control program*[tw] OR control tool*[tw] OR control initiative*[tw] OR Habitat control*[tw] OR environmental control*[tw] OR Reducing contact*[tw] OR Limiting exposure*[tw] OR Chemical control*[tw] OR biological control*[tw] OR biocontrol*[tw] OR Insecticides[tw] OR larvicides[tw] OR rodenticide*[tw] OR Lethal ovitrap*[tw] OR repellent*[tw] OR Insecticide treated nets[tw] OR Attractive targeted bait[tw] OR Vector traps[tw] OR Sterile insect technique[tw] OR SIT[tw] OR Microbial control[tw] OR endectocides[tw] OR "Community mobilization"[tw] OR IRS [tw] or "indoor residual spray*"[tw] OR Acaricide*[tw] OR "impregnated dog collar*"[tw]	225,457
2	"Malaria"[Mesh] OR "Plasmodium"[Mesh] OR malaria[tw] OR plasmodium[tw] remittent fever*[tw] OR paludism*[tw] OR marsh fever*[tw] OR P. falciparum[tw] OR P. vivax[tw] OR P. ovale[tw] OR P. malariae[tw] OR P. knowlesi[tw] OR "Dengue"[Mesh] OR "Dengue Virus"[Mesh] OR dengue[tw] OR "break bone fever"[tw] OR breakbone fever*[tw] OR "Chikungunya Fever"[Mesh] OR "Chikungunya virus"[Mesh] OR chikungunya[tw] OR chikv[tw] OR "Zika Virus Infection"[Mesh] OR "Zika Virus"[Mesh] OR zika[tw] OR zikv[tw] OR zikav[tw] OR zikavirus[tw] OR "congenital zika"[tw] OR "Yellow Fever"[Mesh] OR "Yellow fever virus"[Mesh] OR Yellow fever*[tw] OR YFV[tw] OR "West Nile Fever"[Mesh] OR "West Nile virus"[Mesh]	206.489

	OR WNV Infection*[tw] OR West Nile Virus[tw] OR West Nile flavivirus[tw] OR West Nile Fever[tw] OR Egypt 101[tw] OR Kunjin virus[tw] OR "Tick-Borne Diseases"[Mesh] OR "Encephalitis, Tick-Borne"[Mesh] OR "Encephalitis Viruses, Tick-Borne"[Mesh] OR "Borrelia Infections"[Mesh] OR "Lyme Disease"[Mesh] OR "Borrelia burgdorferi"[Mesh] OR "Babesiosis"[Mesh] OR "Babesia"[Mesh] OR "Hemorrhagic Fever, Crimean"[Mesh] OR "Hemorrhagic Fever Virus, Crimean-Congo"[Mesh] OR "Anaplasmosis"[Mesh] OR "Anaplasma phagocytophilum"[Mesh] OR "Rickettsia Infections"[Mesh] OR "Rickettsia"[Mesh] OR "Relapsing Fever"[Mesh] OR "Borrelia"[Mesh] OR "African Swine Fever"[Mesh] OR Tick-Borne[tw] OR Tick Borne[tw] OR encephalitis*[tw] OR TBE[tw] OR Lyme Arthritis[tw] OR Lyme disease*[tw] OR Lyme Borreliosis[tw] OR Borrelia burgdorferi[tw] OR Alkhurma virus[tw] OR "Al Khurma virus"[tw] OR Babesi*[tw] OR Piroplasmos*[tw] OR Congo Virus[tw] OR Anaplasm*[tw] OR Rickettsios*[tw] OR Rickettsial Disease*[tw] OR Rickettsia Infection*[tw] OR Relapsing Fever*[tw] OR Borrelia Infection*[tw] OR Asfivirus*[tw] OR "Wart-Hog Disease"[tw] OR "wart hog disease"[tw] OR African Swine Fever*[tw] OR ((Alkhurma[tw] OR "Al Khurma"[tw] OR Crimean-Congo[tw] OR Crimean[tw]) AND (Hemorrhagic Fever*[tw] OR Haemorrhagic Fever*[tw] OR Hemorrhagic disease*[tw] OR Haemorrhagic disease*[tw])) OR "Leishmaniasis"[Mesh] OR "Leishmania"[Mesh] OR Leishmania Infection*[tw] OR Leishmanias*[tw] OR "Bluetongue"[Mesh] OR "Bluetongue virus"[Mesh] OR bluetongue[tw] OR blue tongue[tw] OR "Ovine Catarrhal Fever"[tw] OR "Schmallenberg virus"[tw] OR "Schmallenberg disease"[tw] OR "Orthobunyavirus"[Mesh] OR "Bunyaviridae Infections"[Mesh] OR "Rift Valley Fever"[Mesh] OR "Rift Valley fever virus"[Mesh] OR "rift valley"[tw] OR "Lumpy Skin Disease"[Mesh] OR "Lumpy skin disease virus"[Mesh] OR Lumpy Skin Disease*[tw] OR Neethling Virus*[tw] OR "African Horse Sickness"[Mesh] OR "African Horse Sickness Virus"[Mesh] OR African Horsesickness*[tw] OR "African Horse Sickness"[tw] OR Equine Plague*[tw] OR "Hemorrhagic Disease Virus, Epizootic"[Mesh] OR Epizootic hemorrhagic disease*[tw] OR "Encephalomyelitis, Venezuelan Equine"[Mesh] OR "Encephalitis Virus, Venezuelan Equine"[Mesh] OR Equine Encephalomyeliti*[tw]	
3	"Clinical Trial"[Publication Type] OR "Clinical Trial, Veterinary" [Publication Type] OR "Cross-Over Studies"[Mesh] OR "Randomized Controlled Trials as Topic"[Mesh:NoExp] OR stepped-wedge[tw] OR "stepped wedge"[tw] OR step-wedge[tw] OR "cluster randomized controlled"[tw] OR "cluster randomised controlled"[tw] OR SWT[tw] OR CRCT[tw] OR "case-control"*[tw] OR "case control"*[tw] OR casecontrol*[tw] OR cohort*[tw] OR cross-over stud*[tw] OR crossover stud*[tw] OR Cross-over Trial*[tw] OR Crossover Trial*[tw] OR Cross-Over Design*[tw] OR "cross-sectional study"*[tw] OR Crossover Design*[tw] OR "controlled trial" [tw]	1.016.343
4	(#1 AND #2 AND #3)	272
5	(#1 AND #2)	10.902

WoS / CAB Abstracts

No.	Query	Results
1a	TS = ((("Pest*" OR "vector*") NEAR "control") OR ("Pest Control*" OR "Pest Control*" OR "pest control method*" OR Pesticid* OR "vector management" OR "vector control*" OR "control strategy*" OR "control measure*" OR "control program*" OR "control tool*" OR "control initiative" OR "Habitat control" OR "environmental control" OR "Reducing contact" OR "Limiting exposure" OR "Chemical control" OR "Insecticides" OR "larvicide*" OR rodenticide* OR "Lethal ovitrap*" OR repellent* OR "biological control*" OR biocontrol* OR "Insecticide treated net*" OR "Attractive targeted bait*" OR "Vector trap*" OR "Sterile insect* technique" OR SIT OR "Microbial control" OR endectocide* OR "odour baited mosquito trapping system*" OR "Community mobilization*" OR IRS OR "indoor residual spray*" OR Acaricide* OR "impregnated dog collars"))	342,680

2	TS = (Plasmodium OR "Marsh Fever" OR Paludism OR "Plasmodium Infections" OR "Remittent Fever" OR malaria OR plasmodium OR "remittent feve"r OR "P. falciparum" OR "P. vivax" OR "P. ovale" OR "P. malariae" OR "P. Knowlesi" OR Dengue OR "Dengue Virus" OR dengue OR "break bone fever" OR "breakbone fever" OR "Chikungunya Fever" OR "Chikungunya virus" OR chikungunya OR "chikungunya virus" OR chikv OR "Zika Virus Infection" OR "Zika Virus" OR zika OR zikv OR zikav OR zikavirus OR "congenital zika" OR "Yellow Fever" OR "Yellow fever virus" OR "Yellow fever" OR YFV OR "West Nile Fever" OR "West Nile virus" OR "Kunjin virus" OR "WNV" OR "West Nile Virus" OR "West Nile flavivirus" OR "West Nile Fever" OR "Egypt 101" OR "Kunjin virus" OR "Tick-Borne Diseases" OR "Central European Encephalitis" OR "European Tick-Borne Encephalitis" OR "Far Eastern Russian Encephalitis" OR "Louping Ill Encephalitis" OR "Russian Spring-Summer Encephalitis" OR "Far Eastern Russian Encephalitis" OR "Powassan Encephalitis" OR "Powassan Virus Disease" OR "Tick-Borne Encephalitis" OR "Borrelia Infections" OR "Lyme Disease" OR "Borrelia burgdorferi" OR "Borrelia burgdorferi group" OR Babesiosis OR Babesia OR proplasma OR "Crimean Haemorrhagic Fever" OR "Crimean Hemorrhagic Fever" OR "Congo Virus Infection" OR "Congo-Crimean Hemorrhagic Fever" OR "Crimean-Congo Haemorrhagic Fever" OR "Crimean Congo Hemorrhagic Fever" OR "Crimean Congo Hemorrhagic Fever Virus" OR CCHF OR Anaplasmosis OR "Anaplasma phagocytophilum" OR "Anaplasma infection" OR Anaplasmosis OR "Relapsing Fever" OR Borrelia OR "African Swine Fever" OR Asfivirus OR "Wart-Hog Disease" OR "wart hog disease" OR "Tick-Borne encephalitis" OR "Tick Borne encephalitis" OR TBE OR "Lyme Arthritis" OR "Lyme disease" OR "Lyme Borreliosis" OR "Borrelia burgdorferi" OR "Alkhurma virus" OR "Al Khurma virus" OR Babesiosis OR Piroplasmosis OR "Congo Virus" OR Rickettsiosis OR "Rickettsial Disease" OR "Rickettsia Infection" OR "Borrelia Infection" OR Asfivirus OR Leishmaniasis OR Leishmania OR "Sheep Diseases" OR Bluetongue OR "blue tongue" OR "Ovine Catarrhal Fever" OR "Schmallenberg virus" OR "Schmallenberg disease" OR Orthobunyavirus OR "Apeu virus" OR "Catu virus" OR "Guama virus" OR Bunyavirus OR "Rift Valley Fever" OR "Rift Valley fever virus" OR "rift valley" OR "Lumpy Skin Disease" OR "Lumpy skin disease" OR "Lumpy Skin Disease" OR "Neethling Virus" OR "African Horse Sickness" OR "African Horse Sickness Virus" OR "African Horse sickness" OR "African Horse Sickness" OR "Equine Plague" OR "Hemorrhagic Disease Virus" OR "Epizootic hemorrhagic disease" OR "Venezuelan Equine Encephalomyelitis" OR "Venezuelan Equine Encephalitis" OR "Equine Encephalomyelitis")	242,214
3	TS = ("Clinical Trial" OR "Cross-Over Study" OR "Randomized Controlled Trial" OR "stepped-wedge" OR "stepped wedge" OR "step-wedge" OR "cluster randomized controlled" OR "cluster randomised controlled" OR SWT OR CRCT OR "case control" OR casecontrol OR cohort OR "cross-over study" OR "crossover study" OR "Cross-over Trial" OR "Crossover Trial" OR "Cross-Over Design" OR "Crossover Design")	378,901
4	(#1 AND #2 AND #3)	224
5	(#1 AND #2)	

Cochrane library

No.	Search	Hits
#1	(("vector control" NEAR/3 (pest OR interven* OR strateg* OR measure* OR progra* OR tool* OR initiat* OR vector OR environmental OR chemical or habitat OR biological)) OR (pesticides OR "reducing contact*" OR "limiting exposure*" OR insecticide* OR "Insecticide treated net*" OR "Attractive targeted bait*" OR "Vector traps" OR "Sterile insect technique" OR SIT OR "Microbial vector control" OR endectocide* OR larvicide* OR rodenticide* OR biocontrol OR "lethal ovitrap*" OR repellent* OR "Community mobilization" OR IRS or "indoor residual spray*" OR Acaricide* OR "impregnated dog collar*")):ti,ab,kw	5919
#2	(Malaria OR Plasmodium OR "plasmodium remittent fever*" OR paludism* OR "marsh fever*" OR "P. falciparum" OR "P. vivax" OR "P. ovale" OR "P. malariae" OR "P. knowlesi" OR Dengue OR (("break bone" OR breakbone OR yellow OR "West Nile" OR Alkhurma OR	9511

	"Al-khurma" OR "African Swine" OR "Rift Valley") NEAR/3 (fever* OR virus*)) OR (tick-borne NEAR/3 (diseas* OR encephalitis)) OR ((Alkhurma OR "Al Khurma" OR Crimean-Congo OR Crimean) NEAR/3 ("Hemorrhagic Fever*" OR "Haemorrhagic Fever*" OR "Hemorrhagic disease*" OR "Haemorrhagic disease*")) OR chikungunya OR chikv OR zika OR zikv OR zikav OR zikavirus OR "congenital zika" OR "WNV Infection*" OR "West Nile flavivirus*" OR "Egypt 101" OR "Kunjin virus" OR "Borrelia Infection*" OR "Lyme Disease*" OR "Borrelia burgdorferi" OR "Lyme Arthritis" OR "Lyme Borreliosis" OR Babesi* OR Piroplasmos* OR "Congo Virus*" OR Anaplasm* OR Rickettsios* OR "Rickettsial Disease*" OR "Rickettsia Infection*" OR "Relapsing Fever*" OR Asfivirus* OR "Wart-Hog Disease*" OR "wart hog disease*" OR Leishmania OR "Leishmania Infection*" OR Leishmanias* OR bluetongue OR "blue tongue" OR "Schmallenberg virus*" OR "Schmallenberg disease*" OR Orthobunyavirus OR "Bunyaviridae Infection*" OR "Lumpy Skin Disease*" OR "Neethling Virus*" OR "African Horse Sickness*" OR "African Horsesickness*" OR "Equine Plague*" OR "Epizootic hemorrhagic disease*" OR ((Encephalomyelitis OR "Encephalitis Virus*") NEAR/3 "Venezuelan Equine") OR "Equine Encephalomyeliti*"):ab,ti,kw	
#3	((stepped-wedge OR "stepped wedge" OR step-wedge OR cross-over OR "cross over" OR "cluster Randomized Controlled" OR "cluster Randomised Controlled" OR "case control" OR casecontrol OR cohort OR controlled trial) NEAR/3 (trial* OR design* OR stud*) OR SWT OR CRCT):ti,ab,kw	63230
#4	#1 AND #2 AND #3	189
#5	#1 AND #2	896
	Cochrane reviews: 10 Trials: 179	

Annex B – Data table.

This table can be consulted in a separate Excel file.

Guidance Framework for Testing the Sterile Insect Technique as a Vector Control Tool against *Aedes*-Borne Diseases

IAEA International Atomic Energy Agency

Joint FAO/IAEA Programme Nuclear Techniques in Food and Agriculture

World Health Organization

TDR For research on diseases of poverty
UNICEF • UNDP • World Bank • WHO

Guidance Framework for Testing the Sterile Insect Technique as a Vector Control Tool against *Aedes*-Borne Diseases

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Abstract

This document is intended to be a comprehensive guide for programme managers tasked with recommending a "go/no-go" decision on testing, full deployment and scale-up of the sterile insect technique (SIT[1]) in regions of the world affected by diseases transmitted by *Aedes* mosquitoes. However, the authors hope that the material presented herein will be used more widely—by scientists, decision makers, review groups and others.

The SIT has a long history of successfully combatting many pest species without negatively impacting the environment or health. This guidance document will inform stakeholders and all persons involved with SIT testing on vectors of human diseases about how to plan, develop, test and evaluate the impacts of the technology against *Aedes* mosquitoes, the main vectors of dengue, yellow fever, chikungunya and Zika. The nine chapters of this document cover the processes for decision support—including risk assessment and regulatory aspects, technical aspects (e.g., insect mass rearing), entomological and epidemiological indicators, as well as community involvement, cost-effectiveness and programme monitoring and evaluation.

The scope of this document covers programme initiation through pilot evaluation, while touching on aspects of scale-up and full implementation. The technical and operational details of SIT implementation are beyond the scope of this guidance, but readers are referred to other sources for this information. An overview of SIT test planning is provided in **Chapter 1**. **Chapter 2** describes the requirements for assessment of environmental and health risks related to the technology. **Chapter 3** informs about regulatory frameworks, which are determined by individual countries and, in some cases, regional or local authorities within countries. Project plans, performance expectations and protection goals should be discussed with key stakeholders in individual countries, including with regulatory authorities, to determine the scope of any risk assessment and risk management activities. Protection-goal-related risk assessment and risk management for mosquito SIT are likely to include technical risks, such as radiation; entomological and epidemiological risks, such as niche replacement by other vector species, new or different disease transmission by alternative vectors, loss of immunity in the human population; and social risks, such as a complacent attitude towards vector control by communities. SIT facilities and operations also pose conventional environmental and health risks related to buildings, processing activities, waste, transport and worker safety.

The SIT requires mass production of sterile insects of high quality (**Chapter 4**). The technological package for mass rearing, sterilization, release and quality control of sterile *Aedes* mosquitoes has been developed. Standard operating procedures or guidelines are available for colonization, colony

[1] *A list of abbreviations and their definitions is provided at the end of this document.*

management, mass rearing and irradiation for sterilization. Guidelines for transportation and release, as well as for quality control, are under development. Evaluation of entomological efficacy and epidemiological impacts is key to understanding the impact of SIT (**Chapters 5** and **6**). A phased conditional approach is proposed to guide the SIT testing programme through a series of evaluation steps of increasing complexity, with "go/no-go" decisions based on robust, established evaluation methods made at each phase. Illustrative "go/no-go" criteria are presented for the key performance indicators.

Chapter 7 highlights issues of ethics and community and/or stakeholder participation in the process of testing SIT interventions to control *Aedes*-borne diseases. The two issues are mutually interlinked, but with different purpose and objectives. When doing any research that involves human subjects, researchers are obligated to follow the highest possible ethical principles and standards stipulated in international research ethics guidelines, of which informing communities and stakeholders and involving them at the early stages of any research or intervention that will affect their health, life and wellbeing is an essential component. Meanwhile, the understanding, support and collaboration of communities and stakeholders for the research and the intervention are absolutely crucial for the success of any research activity, including SIT testing activities, and for the sustained effect of those interventions. Therefore, the SIT testing team must take both issues into account at the very beginning of any SIT testing project and plan and act accordingly. SIT testing teams also need to be aware that the diverse communities where SIT testing will be conducted are embedded within different socioeconomic, political, cultural and environmental contexts and ecosystems; hence, they need to fulfil their ethical responsibilities and plan and adapt their community participation strategies and actions based on locally prevailing conditions.

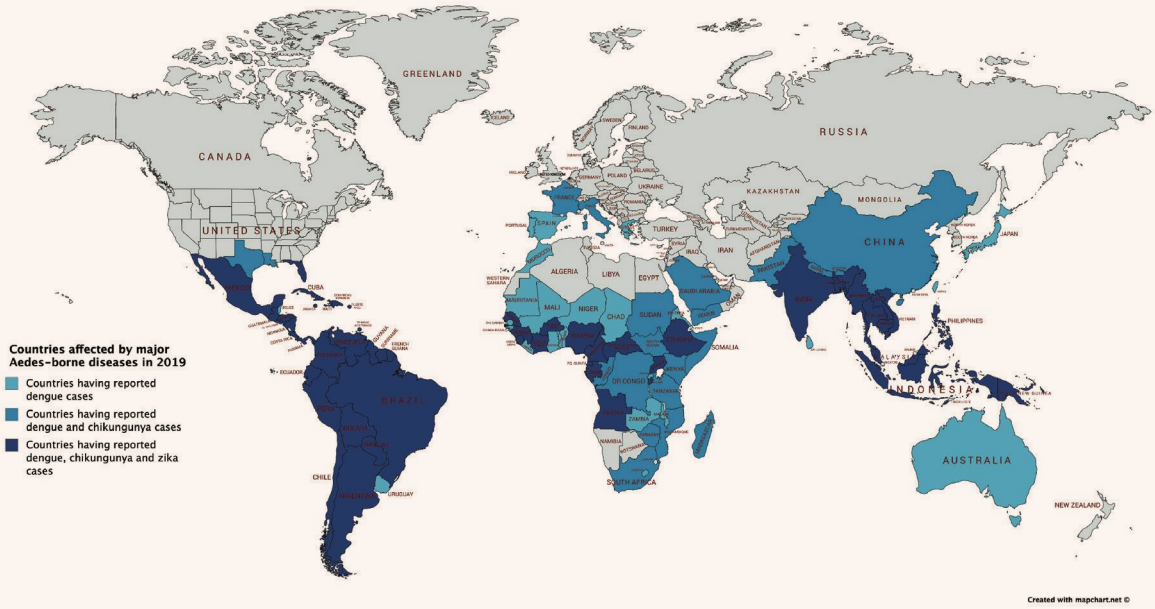
The decision to implement SIT is also linked to the cost-effectiveness of the technology, which is explicated in **Chapter 8**. And in **Chapter 9**, the general concept of monitoring and evaluation (M&E) is discussed in light of the general framework for testing SIT. The relationship between monitoring and evaluation and the requirements for a functioning M&E system are highlighted. Results should be assessed using an input-process-output-outcome-impact framework. Well-designed outputs achieve short-term effects (outcomes), which in turn will lead to the long-term effects (impact). Examples of M&E indicators useful for assessing long-term impact are given for inclusion during the planning and implementation stages. Entomological and epidemiological evaluation components also are provided.

The objectives of the guidance document on testing the sterile insect technique are to provide the necessary and sufficient information for decision-making in testing and for evaluating the outcomes and epidemiological and entomological impacts of this new vector control approach against *Aedes* mosquitoes, vectors of major arboviruses and *Aedes*-borne diseases.

Foreword

Causing more than one million deaths per year, with few new drugs or strategies to combat these emerging infectious pathogens, vector-borne diseases (VBDs) such as malaria, dengue, Zika, chikungunya, yellow fever and others account for 17% of the total morbidity from infectious diseases. The incidence of arboviral diseases transmitted by *Aedes* mosquitoes has grown dramatically in recent decades, with about one third of the world population now at risk from *Aedes*-borne epidemics (**Map 1**), 99% of which are caused by just two species, *Aedes aegyti* and *Aedes albopictus*. This increase is due to global changes that include unplanned urbanization, increased travel and climate change, coupled with a lack of efficient vector control methods. This accelerating increase prompted WHO to state the urgent need for alternative vector control methods in its Global vector control response (GVCR) 2017–2030, which was approved at the World Health Assembly in 2017 by more than 190 Member States (WHO 2017).

The general objective of vector control is the reduction of vector populations, which in turn will bring about a reduction in mortality or morbidity associated with vector-borne diseases. By acting on four key vector capacity parameters (vector density, vector longevity, number of bites and rate of infective vectors), vector control aims to prevent or reduce the intensity of transmission of pathogens at a community or regional level and to protect against infective arthropod bites at an individual level. Control strategies can apply different methods or techniques depending on the vectors and the epidemiological and socio-economic contexts.



Map 1. Countries with reported cases of dengue, chikungunya and Zika viruses. (Extracted from 2019 WHO, CDC and ECDC report data, the list of countries and data are not exhaustive.) (Map credit: Florence Fouque).

One of these alternative technologies is the sterile insect technique, "a method of pest control using area-wide inundative releases of sterile insects to reduce reproduction in a field population of the same species" (FAO 2007). Released sterile males mate with wild females, which will then not produce offspring. The SIT thus has the potential to strongly decrease the density of a target insect's natural population, sometimes to the point of eradication. This technique has been successfully implemented in agriculture against numerous insects (Dyck et al. 2006) and is presently under development against mosquitoes (Lees et al. 2015). In this guidance document, we focus on the release of irradiated sterile male insects that are exempted worldwide from product regulations due to their long safety record of conventional use in a number of applications (EFSA 2013) and are not classified as genetically modified organisms (GMOs) and/or living modified organisms (LMOs) according to the Cartagena Protocol on Biosafety to the Convention on Biological Biodiversity (Secretariat of the Convention on Biological Diversity 2000[2]). According to Protocol definitions, irradiation is not considered as one of the modern biotechnologies used for the purpose of genetically modifying the DNA of the organisms (in this case, mosquitoes).

An alternative method for sterilizing target populations is *Wolbachia*-induced cytoplasmic incompatibility, a natural phenomenon whereby mating between males carrying the *Wolbachia* bacterium and wild-type females results in embryonic lethality (McMeniman et al. 2009; Sinkins 2004). The release of *Wolbachia*-carrying males to suppress insect populations is termed the incompatible insect technique (IIT) and can be used in combination with irradiation-induced sterility (Yen and Barr 1971). However, the use of *Wolbachia*-infected mosquitoes having not yet received the full approval of legislative bodies in many countries, this technology is not included in this document.

Recent systematic reviews of the effectiveness of vector control methods against *Ae. aegypti* and *Ae. albopictus* in the context of dengue control have concluded that there is a paucity of reliable evidence (WHO 2019): Few rigorous studies are available on the impact of vector control on the vector population and on dengue incidence, hence there is a need for standardized and comparative studies (Erlanger et al. 2008; Bowman et al. 2016). The methods for which the most evidence of their effectiveness is available are source reduction—but only as one element of an integrated strategy (WHO 2012)—and house screening. In addition, there is experience in the European Region with the use of insecticides (larviciding and targeted residual spraying) to suppress or eliminate local *Aedes* populations (Schaffner et al. 2014). Emergency space spraying of insecticides is still considered to be a useful tool in emergency situations (outbreaks or epidemics) for *Aedes*-borne viruses, even though evidence on its effectiveness is lacking. Several other options such as mosquito traps (Degener et al. 2014; Perich et al. 2003; Kittayapong et al. 2008; Rapley et al. 2009), autodissemination of juvenile hormones like pyriproxyfen (Devine et al. 2009), insecticide-treated materials (Wilson et al. 2014) and topical repellents are also available for

[2] *https://bch.cbd.int/protocol/text/*

controlling the vectors of diseases. In specific settings, these methods could be considered as complementary to the main interventions.

The SIT against mosquitoes is still under development. It is not a stand-alone technique, but rather meant to complement (not replace) existing vector control measures within area-wide integrated control strategies for mosquito control. Vector control agencies should continue to carry out and promote source reduction activities additional to SIT and remain vigilant about mosquito breeding sites. A unique aspect of SIT is its inverse density-dependent efficacy, whereby the ratio of sterile to wild males increases exponentially as the target population is reduced. This sets SIT apart from most conventional control techniques and makes it a useful tool in modern integrated strategies (Feldmann et al. 2001).

For public health vector control, the sterile insect technique applied to *Aedes* mosquitoes is designed to control both the *Aedes* mosquitoes and *Aedes*-borne diseases, including dengue, chikungunya and Zika. Several Member States expressed the need for guidance on how to plan, implement and assess SIT-*Aedes* field testing. To that end, a joint collaboration was established between the Department of Nuclear Sciences and Applications (NA), the Department of Technical Cooperation (TC) of the International Atomic Energy Agency (IAEA), and the UNICEF/UNDP/World Bank/ WHO Special Programme for Research and Training in Tropical Diseases (TDR) of the World Health Organization (WHO), in partnership with the WHO Department of Control of Neglected Tropical Diseases (NTD), with the goal of providing guidance to Member States on the use of the sterile insect technique as a component of integrated vector control programmes for disease prevention.

An expert working group (WG) appointed by the collaborating agencies has developed and finalized this guidance framework to support and planning for and assessment of field testing and operational use of the sterile insect technology for *Aedes* control. This effort was facilitated by two in-person meetings of the WG in February 2019 in Tapachula, Mexico, and July 2019 in Vienna, Austria. This document presents the results of the work between this WG and the Secretariats of the participating UN Agencies (IAEA and WHO).

Chapter 01

Framework for the implementation of the sterile insect technique (SIT) for *Aedes* control

1.1 Introduction

Transformative changes such as an increased global trade, international travel, urbanization and climate change facilitate the proliferation and spread of *Aedes* mosquitoes, vectors of human pathogens that are consequently also on the rise. For example, dengue virus now causes nearly 400 million infections annually (Bhatt et al. 2013), yellow fever is experiencing a resurgence in Africa and the Americas (Massad et al. 2018; Chan et al. 2016) and chikungunya and Zika have emerged in recent years to cause outbreaks on multiple continents.
The resurgence of such *Aedes*-borne disease outbreaks underscores the limitations of conventional vector control programmes, which are heavily focused on insecticide application and the elimination of larval breeding sites. Challenges include the development of insecticide resistance, the presence of cryptic breeding sites, insufficient infrastructure or government support and high cost. Thus, there is a pressing need for innovative, sustainable and cost-effective control strategies targeting *Aedes* mosquitoes, particularly *Ae. aegypti* and *Ae. albopictus*, the two major vectors of arboviruses that together are responsible for more than 99% of arbovirus transmission within human populations.

A promising method for *Aedes* control is the sterile insect technique, which involves the mass rearing and inundative release of sterile male insects into target populations. Because mating between sterile males and wild-type (field) females does not produce viable offspring, sustained releases of sterile males, if properly conducted, will suppress vector populations, and hence reduce the risk of *Aedes*-borne disease transmission (**Figures 1.1** and **1.2**).

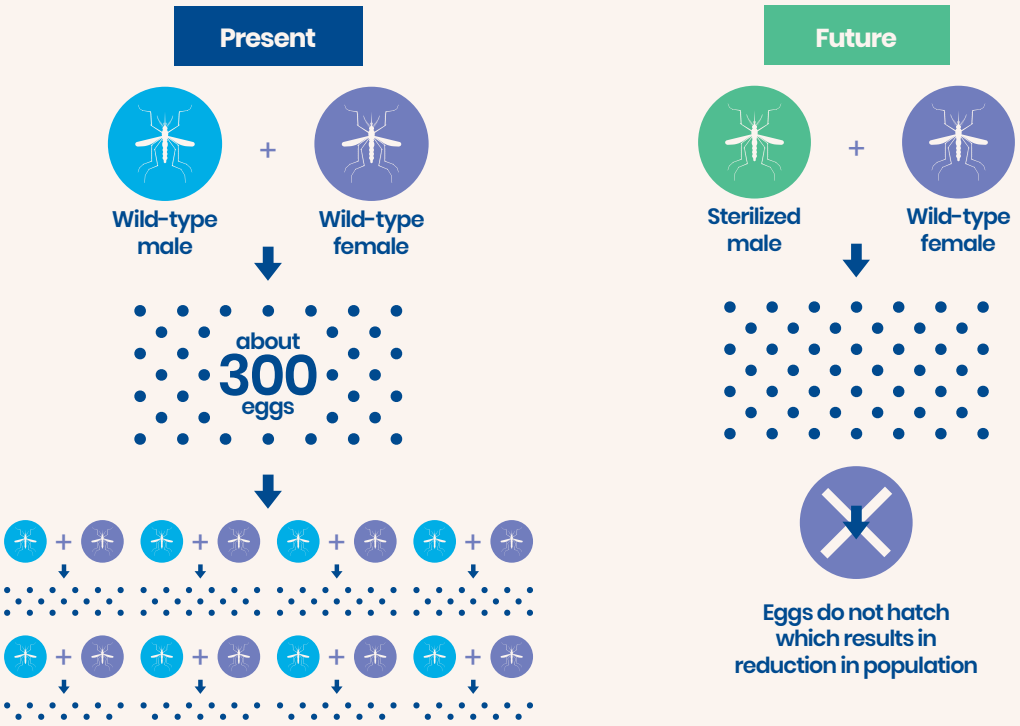


Figure 1.1. Overview of the sterile insect technique.

Figure 1.2. *Aedes* population reduction using the sterile insect technique.

SIT methods using ionizing radiation to sterilize male insects have been deployed to control and even eradicate agricultural pests, such as the New World screwworm in the Americas (Wyss 2000), medflies in Mexico (Enkerlin et al. 2015) and tsetse flies on the island of Unguja, Zanzibar (Vreysen et al. 2000).

Despite its effectiveness against agricultural pests, SIT has not yet been widely used operationally to target *Aedes* species, vectors of several diseases. A major challenge is that SIT is not currently a turnkey solution, thus strategies and protocols may need to be customized for different epidemiological settings, social contexts, legislative systems, geographical distributions and ecologies. Testing and deployment also should dovetail with existing public health priorities and vector control methods. For example, because SIT targets future generations of vectors, the technology aims mostly at decreasing or eliminating endemic disease transmission and preventing future outbreaks, although it can be used as a supplementary tool during long-standing epidemics. Therefore, when infected females are circulating, SIT must be integrated with control tools designed for the immediate removal of adult mosquitoes.

Any successful implementation of SIT for *Aedes* control will therefore require systematic, well-thought-out plans and processes to address these wide-ranging and important issues. In this chapter, we discuss some of the key principles and considerations required for the success of SIT programmes and present a comprehensive guidance framework for the testing and use of SIT for *Aedes* control (**Figure 1.3**). This framework is intended to serve as a reference for countries exploring alternative methods for *Aedes* control and to assist health authorities with making informed decisions about the feasibility of SIT for their unique contexts. The chapters that follow will discuss various components of the framework in greater detail.

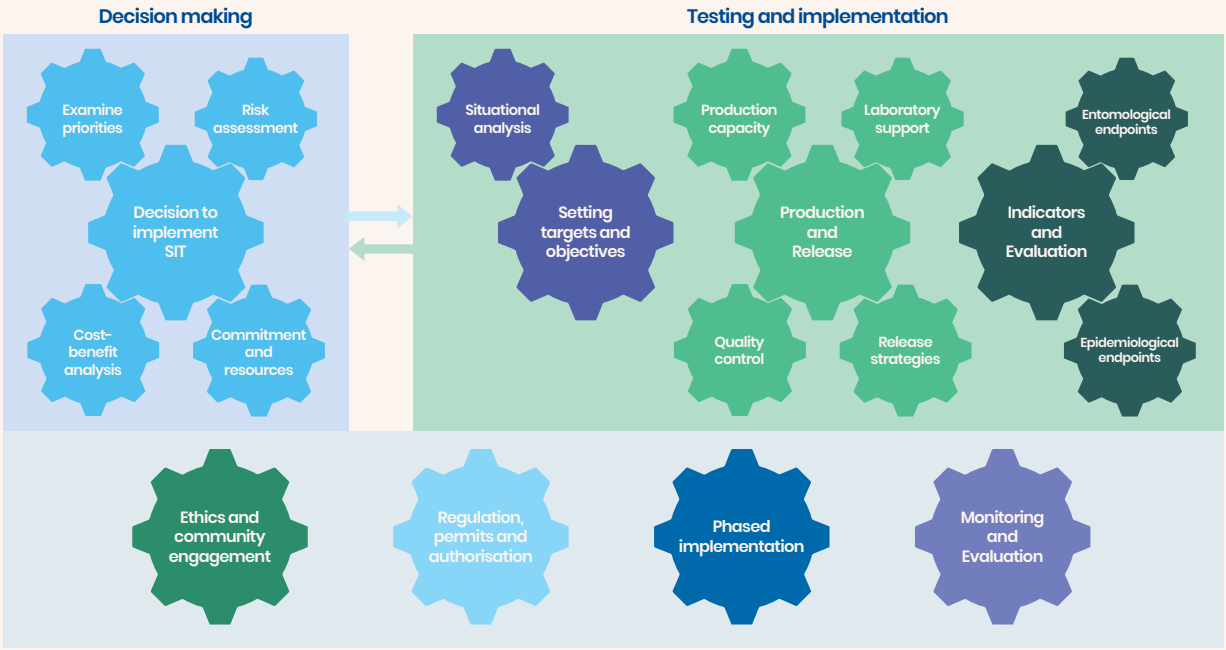


Figure 1.3. Guidance framework for the testing and implementation of SIT for *Aedes* control, visualized as an ecosystem of interlocking components, like gears working in concert.

1.2 Key framework components

The planning and implementation of an SIT programme requires implementing the full suite of framework components, as these interlink with, depend on and underpin one another (**Figure 1.4**). A poor implementation of any of the components can cause derailments or delays. To avoid this, the entire life cycle of the SIT programme should be carefully laid out and examined during the planning stages, so that required activities can be identified and timelines can be aligned.

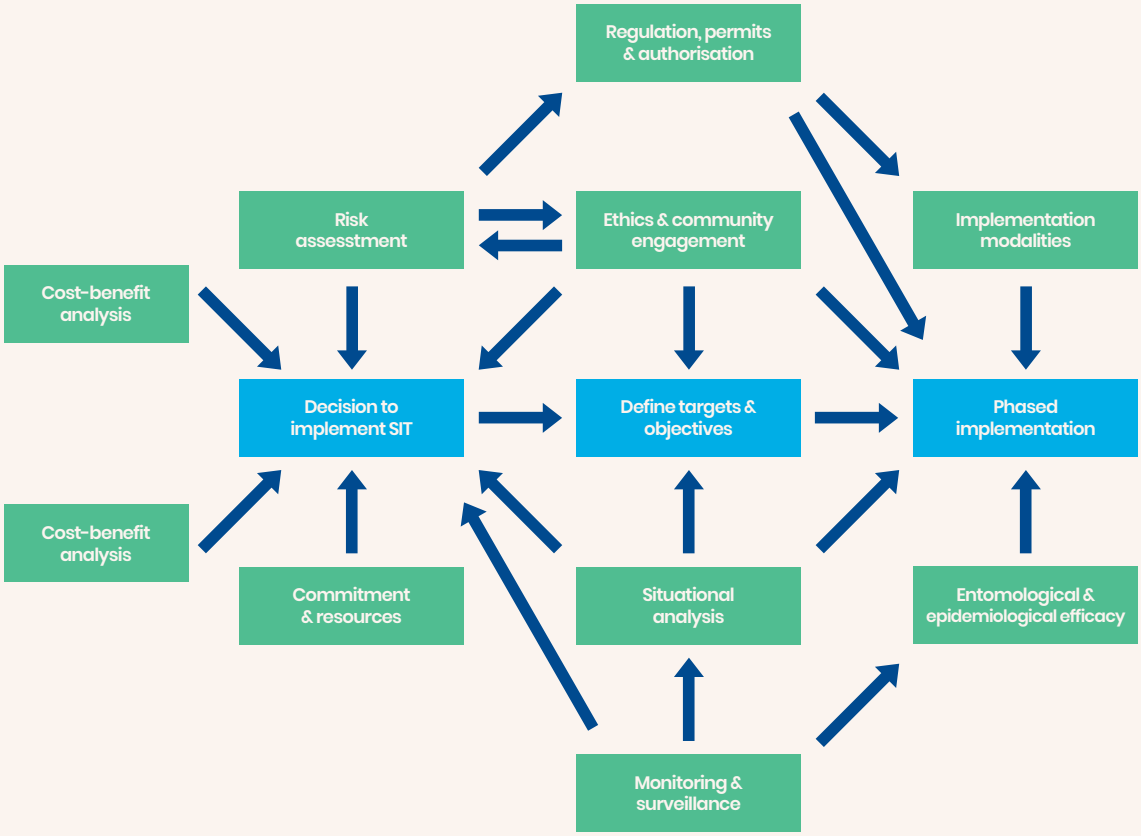


Figure 1.4. Interconnectivity and interdependency of SIT framework components.

To achieve their goals, SIT programmes should be well structured, with clearly defined roles, responsibilities and timelines for the various groups, agencies or organizations involved. At the same time, the success of the programme will depend upon communication and cooperation across the various groups, so that progress can be made in an integrated manner. To assist managers in developing an SIT programme, we provide a checklist of the framework's components (**Annex 1**), which can be used to list required actions, assign lead agencies and estimate timelines.

1.2.1 Making the decision to embark on an SIT programme

a. Assessing priorities, costs, and benefits

Given limited resources and multiple public health issues to address, health authorities must prioritize which issues to take on, using which interventions. For example, planners may need to divide resources between malaria and dengue control programmes or choose between implementing an SIT programme and stepping up existing vector control measures.

Therefore, when considering SIT, countries should undertake a systematic decision-making process early on. This process takes into consideration factors such as disease burden and risk, public perception of the disease, suitability of the SIT compared with other interventions, whether or not SIT aligns with existing national health strategies and whether or not long-term financial, political and other commitments can be made to sustain the programme.

SIT programmes often require a substantial initial investment and may only yield results in the medium or long term. Therefore, planners should undertake a thorough and transparent evaluation of the costs and benefits associated with an SIT programme and compare them with the costs and benefits associated with alternative control interventions. **Chapter 8** will review the process of performing a cost-effectiveness analysis and elaborate on how such analyses can serve as important decision-support tools.

b. Risk analysis

Like any other approaches, SIT approaches can yield secondary ecological, social or economic repercussions; hence, comprehensive risk assessments must be conducted against the backdrop of local disease epidemiology, social issues, legislation, and processes to identify any such potential impacts. Risk management activities, such as monitoring and mitigation measures, can then be planned and incorporated into the design of the programme.

For example, a secondary social impact might be the development of complacency in the wake of a successful SIT programme (NEA 2019). As with any control method, complacency could result in the public relaxing their personal source reduction efforts or policy makers allocating resources elsewhere, despite the need to sustain control efforts. To manage this risk, health authorities could design advocacy strategies and public messaging to emphasize the importance of continued vigilance and action.

To gather a broader range of perspectives on risk and to identify risks that need to be managed, focus group discussions can be held to engage diverse stakeholders, including members of the public, academic experts, medical professionals, government agencies

and non-governmental organizations. Risk assessment and stakeholder engagement are closely intertwined, thus findings from risk assessment should be promptly and transparently communicated to stakeholders.

While an initial assessment typically is needed to satisfy national regulatory requirements and obtain approval for SIT, risk assessment should be an ongoing, iterative process with regular reviews to incorporate new information and knowledge. **Chapter 2** will highlight the importance of risk assessment and management and provide guidance on principles and methodologies.

c. Obtaining long-term commitment and resources

Like most public health interventions, SIT programmes are long-term propositions, requiring sustained releases of sterilized insects and continuous monitoring. To protect the programme and ensure continuity, it is important for planners to obtain a long-term governmental commitment to trial and implement SIT as part of a national or regional integrated vector management strategy. This commitment should also include the financial resources and workforce required for implementation.

As SIT programmes comprise many dynamic components, securing the commitment and support of key stakeholders—including political leaders, grassroots leaders, implementing agencies and private sector partners—will be crucial for the SIT programme to run smoothly. Stakeholders' and implementers' roles and responsibilities for activities from mass rearing, releases, monitoring and surveillance to community engagement—should be defined from the start.

1.2.2 Testing and implementing SIT

a. Setting objectives and targets

The decision to trial and implement an SIT programme should be accompanied by the identification of the target vector, geographical region and local human population (cf. PICO in Chapter 6), as well as a clear definition of the programme's short- and long-term objectives. Short-term objectives typically include immediate activities that need to be carried out before field trials can begin, such as the selection of an appropriate sterile male strain and evaluation of its mating competitiveness. Long-term objectives may include entomological and epidemiological endpoints developed in partnership with public health programs, such as sustained suppression of the vector population and reduction of the number of cases of disease in the target population, respectively.

Objectives and implementation targets can be determined through a situational analysis of the epidemiological, entomological and environmental factors affecting disease transmission. This analysis requires comprehensive surveillance programs (discussed in more detail in **Chapters 5** and **6**) to characterize the distribution of the disease and its vectors, as well as to identify high-risk areas and outbreak epicentres.

For example, because *Ae. aegypti* and *Ae. albopictus* co-exist in many regions, a detailed understanding of their spatial and temporal distributions, as well as their relative roles in disease transmission, will help planners prioritize targets. Vector and case surveillance also can yield data on the entomological thresholds that should be reached to prevent outbreaks (Ong et al. 2019); this information can be used as a guide for setting SIT programme objectives in terms of reductions in mosquito populations.

b. Regulation, permits and authorization pathways

SIT programmes typically are subject to regulation throughout their development and implementation and to regulatory processes at the institutional, state, national and international levels. Due to their multifaceted nature, SIT programmes are likely to require a range of approvals and permits, such as authorizations to ship, import and release mosquitoes and certifications of biosafety compliance and occupational safety. **Chapter 3** will elaborate on the regulatory considerations faced by SIT approaches.

To avoid delays at later stages, it is critical for planners to conduct a comprehensive examination of the SIT programme life cycle in the initial phases of the project to identify, understand and budget for regulatory requirements. This is especially important as legislation, regulations and standards may differ from country to country and because SIT approaches may be subject to different requirements. SIT programme managers should communicate openly and regularly with regulators to enhance the latter's familiarity with SIT methodology and its associated mass rearing and field procedures.

c. Modalities of implementation

SIT implementation requires the production of large numbers of high-quality sterile male *Aedes* mosquitoes, followed by releases in the field. Optimal procedures for mass rearing and sterilization, effective systems for field delivery and robust mechanisms for quality control are therefore critical to the success of an SIT programme. **Chapter 4** will provide further guidance on these modalities of implementation.

During the planning phases, sufficient staff, budget and laboratory space should be allocated to mass rearing, with provisions for scaling up to support expanded SIT trials or deployment in the future. Scalability is an important factor that should be considered when initially deciding what techniques, technologies and procedures to incorporate into the workflow. To ensure scalability, planners may wish to consider evaluating or developing automated technologies to perform menial functions such as tray tilting, counting of larvae and pupae, and gender-sorting of pupae.

Once produced, sterile males must be released in the field in adequate but not excessive numbers and at the appropriate times, frequencies and locations. Release strategies should be developed and fine-tuned based on what is known about the behaviour of the target vector, as well as on a situational

analysis of disease risk and mosquito populations in the target area.

For example, in areas with seasonal variations in disease transmission or mosquito numbers, releases could commence before the high season. In areas with less seasonality, tiered releases could be implemented, beginning with a high-density release to crash the population, followed by lower density releases thereafter to sustain suppression. Automated release systems such as drones can also be evaluated and implemented to boost release volume and improve horizontal and vertical dispersal. Integration of SIT with other control measures to initially reduce the target population should also be considered to optimize the cost-effectiveness of the programme.

The production of high-quality sterile males and the implementation of high-quality releases are critical to the success of SIT programmes. Regular quality control checks should be implemented at various stages to ensure that all workflow components are performing optimally. Examples of parameters to be monitored include rates of female contamination after gender-sorting, longevity and mating competitiveness of sterile males, and mortality upon transport and release in the field.

d. Ethics and community engagement

Ethical acceptability of SIT programs to their host communities is based on providing adequate and sufficient information on the goals, benefits and risks of SIT and responding to public concerns about the technology. Community engagement should be a key priority throughout the SIT testing and implementation process, with the aim of sharing information and consulting with stakeholders and gaining their approval and cooperation. **Chapter 7** will discuss ethical issues surrounding SIT trials and provide guidelines for effective community engagement.

Strong community support is crucial for the success of novel public health interventions such as SIT. Engagement with various stakeholders—including residents of study sites, the public, medical and scientific communities, policy makers, government agencies and non-governmental organizations—should be initiated as early as possible, before beginning laboratory feasibility studies. Allocating time for stakeholders to familiarize themselves with the technology and raise concerns avoids misunderstandings that could derail the SIT programme at later stages. It also allows social expertise feedback to be incorporated into the design and implementation of laboratory and field studies at an early stage, thereby increasing their likelihood of success.

We further recommend a consultative approach that respects and takes into account the concerns and opinions of stakeholders, especially those of residents who will be directly in contact with the SIT trials. Activities such as dialogue sessions with residents and community leaders can help garner a spectrum of views and concerns, which can be integrated into implementation strategies. Public feedback mechanisms, such as hotlines or online reporting systems, should also be established to enable the community to pose queries and voice concerns.

Good situational awareness is a pillar of effective community engagement, as keeping tabs on the vector situation in an area and being attuned to the local community can help identify concerns from the public as they arise, so that they can be swiftly addressed. For example, a coincidental rise in the population of non-target mosquito species could lead to doubts in the community about the efficacy of the SIT programme. In this case, data on the population dynamics of non-target mosquito species, together with detection of their breeding sites, could provide the evidence needed to dissociate these experiences from the SIT programme.

e. Evaluation of epidemiological and entomological efficacy

The efficacy of the SIT approach against VBDs must be evaluated using both entomological and epidemiological endpoints (**Chapters 5** and **6**).

Referring to a reduction in the risk of disease transmission due to changes in vector population characteristics, entomological endpoints are an important outcome measure at all phases of testing and implementation. Because direct measures of transmission intensity (such as the entomological inoculation rate, sometimes defined as the number of infectious bites per person per unit of time) are difficult to measure, surrogate measures that monitor vector population parameters are often used instead, especially in the early phases of an SIT programme when sample sizes are small. These proxy measures may include egg hatch rates and ovitrap or gravitrap indices, as well as biting rates.

Referring to a reduction in the incidence of human infection or clinical disease, epidemiological endpoints typically only become informative once the SIT programme advances to larger, more advanced field trials. Study design will depend on the goal of the SIT programme and the disease being targeted, with possibilities including cluster-randomized trials, case-control analysis, longitudinal studies or seroprevalence surveys to gauge long-term impact.

f. Surveillance and monitoring

Comprehensive and robust surveillance systems (**Chapter 9**) for monitoring the progress of the programme, as well as entomological, epidemiological and environmental indicators, are required throughout the planning, testing, deployment and evaluation phases of SIT programmes. Up-to-date surveillance data enable prompt situational analyses, which in turn allow planners to track seasonal variations in cases or mosquito populations, detect high-risk areas to target and to improve release strategies, trial design and community engagement activities.

Entomological surveillance allows vector population dynamics and spatial distribution to be monitored and is often carried out via a network of ovitraps, which collect eggs, and gravitraps or other adult traps, which target adult female mosquitoes. Care must be taken to standardize handling procedures and protocols across the network, so that data obtained are

robust and comparable across different time points and geographical areas.

Epidemiological surveillance entails a notification system for the mandatory and timely reporting of arboviral disease cases, together with laboratory support to confirm cases using standardized, quality-controlled testing procedures. A passive surveillance system can be supplemented with active surveillance or other forms of monitoring, such as serological surveys to estimate the dynamics of infection rates and disease burdens and/or to detect changes in the patterns of circulating virus serotypes—not only to assess the results of SIT testing, but also to eventually provide early warning of outbreaks. For example, in Singapore, switches in the predominant dengue virus serotype have been associated with epidemics caused by the newly circulating serotype (Lee et al. 2010).

Incorporating environmental monitoring into the surveillance system will further strengthen situational analysis. Climatic variables such as temperature, rainfall and humidity affect mosquito populations and the incubation periods of *Aedes*-borne pathogens (also called extrinsic incubation), thus have the potential to impact disease transmission (Colón-González et al. 2013; Bouzid et al. 2014). Weekly climate monitoring, which is sometimes carried out in collaboration with the local meteorological service, therefore can complement entomological and epidemiological data to follow disease dynamics and eventually predict outbreaks.

g. Phased conditional approach implementation

We recommend that SIT programs be tested and deployed using a phased conditional approach (Figure 1.5) (Wilson et al. 2015) with demonstration of safety and efficacy as requirements for transitioning to the next phase. Phased implementation is analogous to the development roadmap for vaccines and drugs and has been proposed for the testing and deployment of genetically modified mosquitoes (WHO 2014) and new vector control technologies (Wilson et al. 2015).

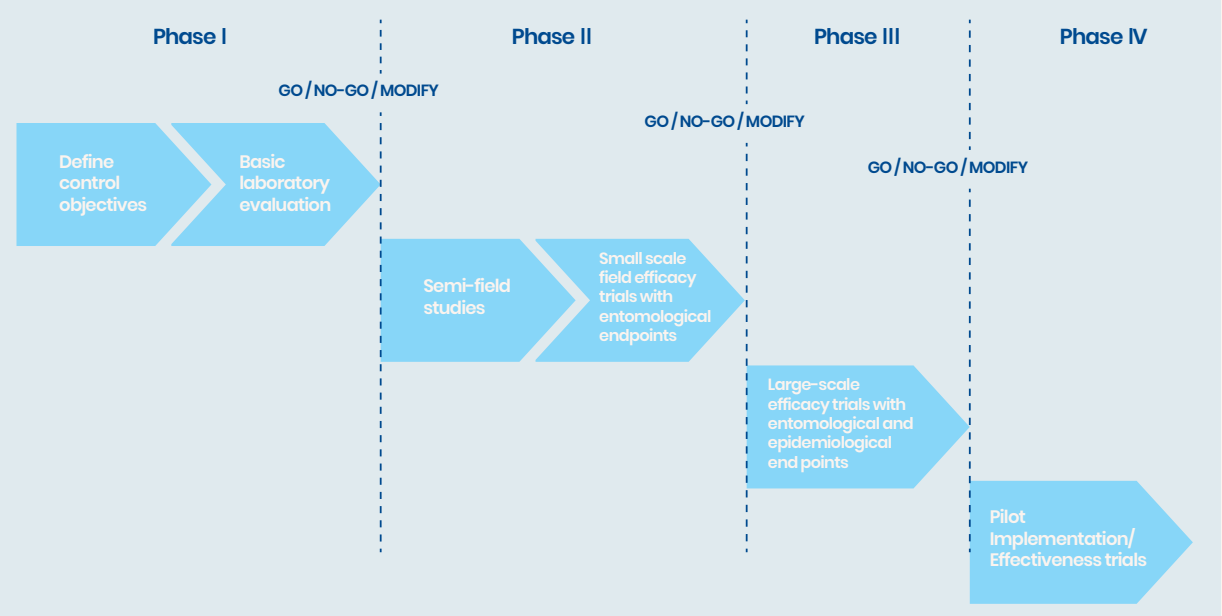


Figure 1.5. Definition of the phased conditional approach for testing SIT (adapted from Wilson et al. 2015), with a GO/NO-GO/MODIFY decision step to move from one phase to the next taken at the end of each phase until phase IV:
GO: Move to the next phase according to the original planning
NO-GO: Do not move to the next phase
MODIFY: Move to the next phase with changes in the original planning

Phase I should focus on laboratory studies to demonstrate the feasibility of the technology, with a view towards SIT's future efficacy and safety in the field. This may involve assessments of the chosen strain's biological characteristics, such as hatch rates, mating competitiveness, longevity, dose response curves, flight ability, insecticide resistance and response to lab colonization, as well as characterization of the target population.

Once feasibility has been demonstrated at the laboratory level, the programme may proceed to a **phase II** semi-field and small-scale field trial in an ecologically confined area to assess whether the SIT strain retains the desired biological characteristics in the field. This phase could include the study and calibration of release parameters, such as horizontal and vertical dispersal and release numbers and frequencies. Phase II trials also assess the competitiveness of the released sterile males in the field and the impact of releases on local mosquito populations.

Following a successful phase II, **phase III** involves scaled-up trial releases covering progressively larger areas, with the aim of demonstrating entomological and epidemiological efficacy. Trial design and release strategies will depend on the chosen objective, which may include targeting high-

risk areas, responding to seasonal variation in mosquito populations or achieving sustained suppression in the target area. Early results of phase III trials typically will include the impact of the release on mosquito populations. Data on the impact of the release on cases of human disease can start being collected in this phase, but data collection will need to continue over a longer term. In the case of testing programmes designed for disease risk reduction in areas with little or no disease, only entomological endpoints may be collected for phase III. While still developmental, phase III effectively constitutes a pre-operational deployment of the SIT approach. Already by phase III, SIT programmes should have ensured adequate capacity for producing the required numbers of sterile males and designed a robust and comprehensive surveillance and monitoring system.

Depending on the outcome of phase III, planners may decide to operationalize the SIT approach in **phase IV**, marking a transition from development to deployment as a public health intervention. Deployment should be accompanied by long-term, continuous assessment of the SIT approach's impact on entomological and epidemiological indicators, as well as monitoring to evaluate how the programme is running and to detect any potential secondary impacts on human health and the environment. The efficiency and cost-effectiveness of both the SIT approach and the integrated vector management programme of which it is a part should be routinely assessed to determine if adjustments need to be made. Assessment of the SIT approach will require feedback mechanisms and close communication between the management, field and mass-rearing teams, following an adaptive management scheme. An independent panel to review testing programme progress and validate the interpretation of the results is desirable at all stages and necessary in phase IV.

Progress from one phase to the next will require fulfilment of pre-defined criteria covering safety, efficacy, regulatory approvals, ethical approvals and social acceptance. A "go" vs "no go" decision, based on the results achieved and relevant criteria, will be taken before moving from one phase to the next until phase IV. If a "no-go" decision is made by any of the responsible parties, the technology or procedure in question will need to be improved or refined until the criteria are satisfied and a "go" decision is reached. Lead government ministries take this decision; to do so, they assess the results jointly with SIT programme managers, regulatory authorities and independent external reviewers, all of whom together factor in risk assessment and cost-benefit analyses of the technology. The final decision to progress to each subsequent phase, and especially to deployment in phase IV, requires several levels of government oversight and evaluation.

Chapter 02

Framework for health and environmental risks assessment

2.1 Introduction

Due to SIT's long history as a tool for controlling insect pests, many aspects of the technology are relatively well-known. It is an area-wide, species-specific pest control tool, whose potential risks to non-target species and ecosystems typically are significantly less than those associated with less targeted technologies, such as aerial applications of conventional, broad spectrum adulticides. However, performing risk assessment as part of SIT planning is important to acknowledge the risks and mitigate them, obtain the relevant authorizations, garner public support and ensure effective and efficient programme management.

Risk analysis is one of at least four interrelated risk processes: risk assessment, risk management, risk communication and risk policy. However, a general framework for risk assessment will include all processes. Plausible risk concerns must be identified by risk assessors and SIT programme managers, together with relevant stakeholders, and consequently inform risk planning and the formulation of risk management options. Risk analysis is also an integral part of vector control programme design and planning. Considering risk at an early phase allows programme design to reduce the likelihood or impact of any harms before the programme is deployed.

Risk analysis focuses on changes that lead to harm. Harm is an expression of a negative value associated with a change and not simply of change itself. Very small negative values may or may not be important to individuals or society, while a high likelihood of an outcome occurring beyond a defined threshold value of harm may be unacceptable. An important question for risk assessment/management is what constitutes an *acceptable* outcome? Risk assessment can provide qualitative or quantitative estimates of the probability of an outcome, including its magnitude and distribution in human communities and ecosystems, to help programme managers, stakeholders and regulators in making informed decisions that reflect social values.

Risk-assessment planning is a crucial step before the full risk assessment process (US EPA 1998; NRC 2009) (**Figure 2.1**). The goals and objectives of the risk assessment are established in the planning step; this includes not only identifying risks but also risk management options, and defining the level of uncertainty that is acceptable for risk management decisions. After establishing the objectives and scope of the assessment, the **risk assessment** starts with the **problem formulation phase**, which documents the characteristics of the technology, its operational use, the human population and ecosystem potentially at risk and the acceptable endpoints. This process takes into account the regulatory and societal objectives and values identified during planning. A conceptual model can be designed to describe the technology and the risks associated with its application, the communities and ecosystems in which it will be used, and how the technology may

directly or indirectly interact with humans and the risk-assessment-endpoint-relevant ecological entities. The **analysis plan**, which is created subsequent to problem formulation, describes the approaches that will be used to estimate risks and evaluate uncertainty. During the analysis phase, exposure to the operational events and exposure-related effects are characterized. The final step is the **risk characterization phase**, which yields a **risk description** along with an analysis (qualitative or quantitative) of the uncertainties associated with the likelihood and impact of the identified risks. The results of the **risk assessment are then communicated**. If any harm and uncertainty in the risk estimate do not exceed the level defined as acceptable during the planning stage, **the risk management decision** would be to approve the SIT programme within the regulatory framework. If they exceed the defined level, the risks must be mitigated or otherwise addressed before approval is given.

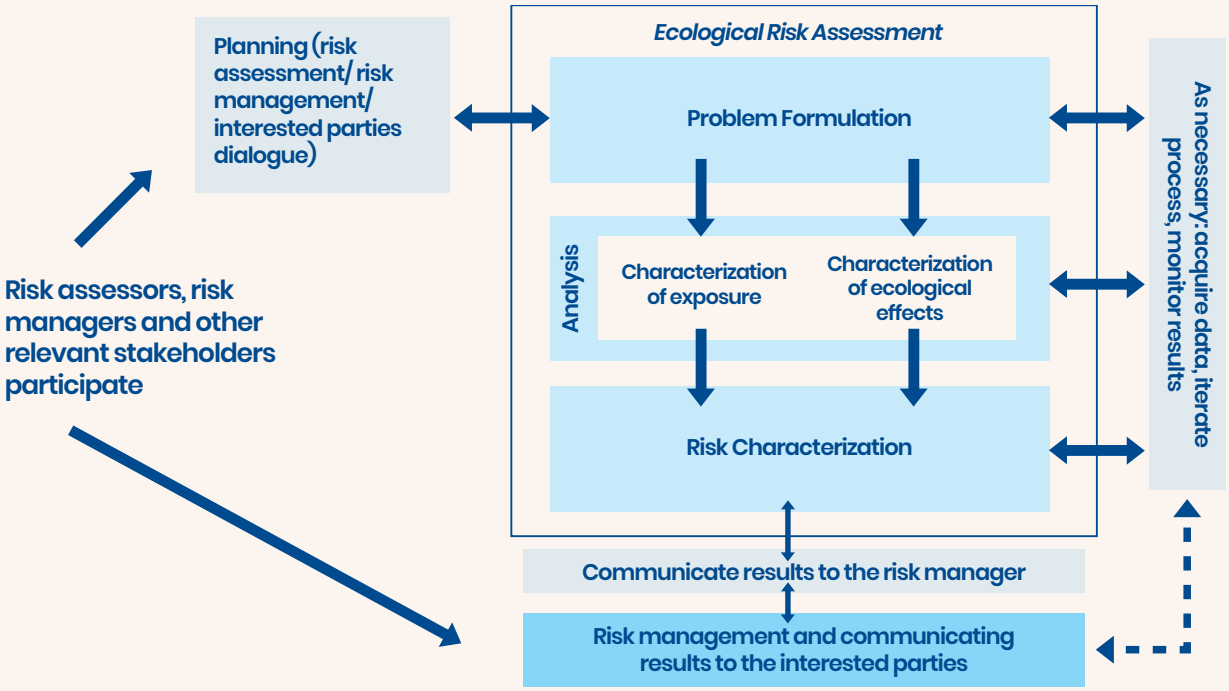


Figure 2.1. Risk assessment process (adapted from US EPA 1998).

2.2 Theoretical planning and risk assessment process

Risk assessment is an iterative process that is likely to include the steps shown in **Figure 2.1** (Mumford 2018b). **Box 2.1** shows the steps in conventional risk assessment in vector control programmes. The SIT programme managers are required to provide information relevant for this process to the national and/or regional regulatory authorities for review in accordance with their specifications for an SIT programme. The relevant regulator and/or the SIT programme manager also may ask independent groups to provide information. Consultation with stakeholders usually is required as early as possible in the risk assessment process.

Box 2.1. Conventional risk assessment in vector control programmes

Vector control has been carried out in many countries for many years, with broad public backing and participation, and strong regulatory and political support. SIT is a novel area-wide control approach for vectors in many countries and both the public and the relevant regulators will consider its adoption in the context of current conventional vector control programmes. There is a relatively simple approach to risk analysis in conventional area-wide mosquito control aimed at vector population suppression, which has had broad acceptance in many countries and includes the following steps:

Planning

- Catalogue problem mosquito species in the geographical area of responsibility;
- Describe the nature of the problem (vector, nuisance);
- Describe management criteria for each listed action;

Risk Assessment

- Map areas and time frames of mosquito concern and proposed application of insecticide;
- Delineate any areas of special protection;
- Summarize toxicity of insecticide to non-target organisms, including humans, and approaches to predict community or ecosystem harm;
- Summarize data sources and models to estimate exposure to the control actions (generally insecticides and habitat management);
- Establish a conceptual model of hazards to harm and an analysis plan for potential remedial actions;

Risk Management

- List approved control methods for each mosquito stage;
- Describe management plans for identifiable accidents or incidents (spills, application errors, etc).

2.2.1 Planning

The **proposed conditions of use**, which are the events that are expected to occur prior to, during and after an SIT release action, are described during the planning phase. During this planning process, SIT programme managers consult with a diverse range of experts, stakeholders and regulators to **identify risk concerns**, plausible pathways to potential harm, levels of acceptable risk and uncertainty in risk estimates. The **limits of concern** and the level of harm that are likely to be negligible or acceptable for regulators are defined during this phase. The planning process can also be handled by an external body of consultants, to avoid any conflict of interest.

2.2.2 Problem formulation

The SIT managers, consultants and/or regulatory authorities carry out a review of literature, technical experience and social engagement to identify **pathways to harm** (hazard-pathway-outcome chains). **Risk concerns** are considered at the planning step and a determination is made about how risk assessment endpoints, including those that cannot be empirically quantified (such as the public's perceptions or fears), can be measured. A **conceptual model** that establishes hazard source to outcome pathways is developed to assess the processes affecting the likelihood and consequences of any harm.

Relevant **comparators** can be used to demonstrate how harm may or may not arise.

The problem formulation phase results in an **analysis plan**, which estimates the time and amount of effort needed for the programme managers and independent groups (inasmuch as they have been involved) to complete the risk assessment. To determine the placement of potential outcomes in risk matrices, workshops and consultations with experts, regulators and other stakeholders can be held throughout the risk analysis process to gather input or feedback on issues such as risk concerns, pathways, endpoints, uncertainty, acceptability and hazard-pathway-outcome chains.

2.2.3 Analysis of hazard and exposure

Analysis is dependent on an accurate plan for the activities, so that the scale and exposure of any identified hazards can be estimated. Conceptual and quantitative models can be used to support analyses of the effects of exposure or events. Analysis may rely on estimates derived from expert opinion, but care should be taken to document the assumptions and justification for those opinions.

2.2.4 Risk characterization

Qualitative, semi-quantitative or quantitative descriptions of the likelihood and consequences of risk (and associated levels of uncertainty) illustrate the distribution of outcomes that would be expected from a causal event, given the assumptions and evidence used in the analysis.

Van den Brink et al. (2016) provide some recommendations and specifications for performing ecological risk assessments in landscape-scale scenarios with multiple pathways to harm and multiple endpoints. Specifications for ecological risk assessments include:

- Creating a digital map of the site that includes land use, topography and locations that are potentially sensitive to any pathways to harm;
- Mapping regions in the landscape that have similar land uses and management goals;
- Establishing a priori the cultural values and protection goals that will determine the success of the assessment and decision-making process;
- Determining interactions among species and ecological processes and functions that would be affected by any plausible pathways to harm;
- Constructing a conceptual model that reflects the plausible pathways to harm, the habitats, the expected effects and impacts on the system under investigation;
- Using the conceptual model to organize information that will inform cause-effect modelling of the plausible pathways to harm;
- Transforming the cause-effect model, as data permit, into a quantitative model that considers both deterministic and probabilistic aspects of the ecosystem.

The decision to approve vector control actions rests with the relevant national or regional regulator, who would decide whether a programme poses any unacceptable risks based on evidence provided by the programme manager and any appropriate independent sources. If the level of risk for a proposed technology when used as intended does not exceed a level of concern, then it may be approved (subject to other factors also being successfully addressed). If the risk does exceed a level of concern, management conditions may be required to reduce the level of risk to an acceptable level. It should be noted that the risk conclusion is about the acceptability of risk without consideration of any of the potential benefits of the technology, which are part of an implementation decision (cf. **Chapter 8**).

2.3 Risk planning for SIT against *Aedes* mosquitoes

During the planning stage, the relevant authorities should organize the SIT risk assessment and request the necessary information from the programme managers. The scope of what must be included in human health and ecological risk assessments is defined at the planning stage. According to Gormley et al. (2011), agreement on the scope of the risk assessment can be influenced by these factors:

- Purpose of a health and environmental risk assessment;

- Legislative and regulatory requirements;
- Boundaries of responsibility for the SIT programme and related roles;
- Environmental impact of the SIT programme;
- International, national, regional and local environmental aspects.

Risk concerns may arise upon consultation with internal and external stakeholders and/or from general categories or specific concerns indicated in national or regional regulations. Planning typically will include regulators and stakeholders relevant to the risk management decisions.

Specific protection goals will be defined, which are desired characteristics of human health or ecological values that the public wants to protect and that are relevant to the management being undertaken. Possible management goals include maintenance or improvement of individual and community health status (e.g., reduced disease transmission) and ecological integrity (cf., for example, US EPA 1998). Protection goals are defined in the planning stage and form the basis of risk assessment endpoints, which may be much more specifically stated. These protection goals will also be embedded in risk hypotheses and conceptual models defined during the problem formulation.

In the case of the SIT against vectors affecting not only human health and well-being but also animal health, there is a biodiversity concern, since the vectors may also occupy a niche with some value as part of natural or introduced ecological systems. Consequently, broad protection goals are needed for the health and environment to reflect all roles of the vector and yield endpoints related to these wider goals. For example, within the sphere of human health, objectives might include ensuring that the mosquito biting rate does not increase or that another vector species does not replace the SIT target species.

The identification of a protection goal or specific risk concern does not in itself indicate that there is an unacceptable risk; that is an issue to be addressed in later stages of the risk analysis.

At an IAEA-supported workshop on *Aedes* SIT, held in Singapore (Mumford 2018a), risk concerns related to protection goals were identified in three broad areas linked to health and the environment: human health, nuisance to humans and biodiversity.

The human health goal could be further broken down into more specific risk concerns, including:

- SIT production facility workers affected by health problems such as allergic reactions or irradiation in the production environment;
- Disease transmission not reduced after the release;
- Mutations in under-irradiated males that modify the vectorial capacity and behaviour of the mosquito vectors;
- Niche replacement by a more competent vector species;
- Complacency leading to reduced complementary vector control efforts.

The nuisance goal was related to more specific risk concerns about the biting nuisance from any female mosquitoes released

via SIT and the perceived nuisance from non-biting, sterile males.

The biodiversity goal was related to more specific risk concerns around niche replacement affecting ecological balance and the loss of all or part of an endemic or naturalized vector species with an ecological role.

Health and environmental concerns related to the request to authorize a small-scale SIT pilot against *Ae. albopictus* on Reunion Island (HCSP 2018) were considered in a national risk assessment and yielded the following risks:

- Epidemiological risks;
- Risks linked to the technology and processes;
- Risks for the workers;
- Risks for the local population;
- Ecological risks;
- Risks associated to the change of scale of the SIT operations.

Nienstedt et al. (2012) proposed an ecosystem service approach to protection goals that integrates cultural and social values and is defined by these six dimensions:

- Ecological entity: individual to ecosystem;
- Attribute: behaviour, survival, growth, abundance, biomass, process, biodiversity;
- Magnitude: negligible to large;
- Temporal scale: days to years;
- Spatial scale: local to landscape;
- Degree of certainty: low to high.

Thus, a protection goal may be more specifically defined in terms of a particular health situation, ecological level, the specific attributes of organisms in that level and the scales of protection. The levels of protection can be specified, along with the degree of certainty that could be achieved.

2.4 Risk assessment for *Aedes* SIT

2.4.1 Problem formulation

Following risk planning, the first step in the risk assessment process is problem formulation (**Figure 2.1** and Gormley et al. 2011), as it defines the planned use of the technology, the plausible hazards, the pathways to harm, the assessment endpoints and the limits to concerns. Potential effects on health and important environmental indicators are elucidated and actions are defined. Plausible pathways to harm are identified and described in this phase, with explicit risk hypotheses linking a causal event, such as the reduction in a vector population, to a potential harm of concern, such as the

replacement in the ecological niche by another vector species. Methods for measuring key indicators, such as target and non-target vector densities, are selected and decisions taken on priorities and risk outcomes, especially which outcomes would be acceptable.

Problem formulation, adapted from EFSA (2013) for SIT, should cover the following aspects:

- Description of the SIT planned rearing and release programme, including its scale in space and time and its intended outcome (prevention, reduction or suppression);
- Characteristics of irradiated released mosquitoes and production and application systems that can cause direct or indirect negative effects on human and animal health and the environment;
- Characteristics from the environment of the released area that need to be protected from harm according to environmental protection goals and identification of risk assessment endpoints;
- Identification of environmental exposure pathways and plausible links to assessment endpoints;
- Description of the methods to estimate how exposure and effects data will be collected and analysed, and how risk and uncertainty in risk estimates will be qualitatively or quantitatively characterized;
- Definition of assessment endpoints proportionate to protection goals;
- Definition of measurement endpoints for both hazard and exposure;
- Methods for evaluating assessment and measurement endpoints in relation to protection goals;
- Reference comparators for potentially harmful ecological effects;
- Limits of concern for relevant ecological effects that would not constitute harm;
- Uncertainties and their source (natural variability, knowledge, measurement).

To provide a clear route for regulatory decision-making, national and local regulatory requirements must be included and considered in any problem formulation. In addition, stakeholder input provided in the planning stage should be incorporated in problem formulation to ensure that the risk assessment is responsive to social values.

Some aspects of the abovementioned problem formulation are detailed herein. For a risk assessment to establish an objective estimate of the likelihood and impact of a potential harm, there must be a **plausible pathway** by which the planned events lead to that harm. This requires a conceptual model of a hazard-pathway-harm chain in which the probability and extent of harm can be measured or estimated. The cause-effect model includes five interconnected nodes: hazard source, environment, hazard effect on the environment, effects of exposure to the hazard, and impacts or outcomes. The source is the cause of the potentially negative response. In a SIT programme, the hazard source might include, for example, the unintended escape of mosquitoes from a production facility.

An **assessment endpoint** is an indicator of a protection goal. It describes how a harm may arise from exposure to an effect related to SIT operations. For example, an assessment endpoint related to a biodiversity protection goal could address non-target

predator organism abundance that may be affected by a decline in *Aedes* abundance as prey. A measure of exposure in that case could be the density of *Aedes* present over a season, measured by oviposition traps, larval site sampling or adult catches, while the measurement endpoint for the effects would be the density of the non-target predator species before, during and after the releases.

As noted above, SIT exposure information, including the location and type of introduction (i.e., the timing and frequency of the release and also the mechanism, such as drones or ground), together with estimates of the number of mosquitoes released and their persistence and distribution in the environment, is needed to inform SIT exposure-response relationships. Changes in population size and in the dynamics and effective breeding size of the vector over time as a result of the release are then related to endpoints, including, for example, reduction in human and animal disease, changes in biodiversity, and non-target species effects. The SIT exposure-response relationships can be assessed by combining data collected in limited field trials with deterministic or probabilistic models.

The **limit of concern** is a subjective value expressing what would constitute an acceptable level for a particular risk. Limits or levels of concern may be derived from discussions with relevant stakeholders or expressed in national regulations or policies. For example, the ratio of pesticide concentration causing 50% mortality to a non-target species population divided by the estimated upper end of the range of the pesticide's exposure level in the environment must exceed a certain (specified) value to conclude that the likelihood of an adverse effect is acceptably low. Limits of concern may also be derived from thresholds in epidemiological models (e.g., an R0 value for sustainable disease transmission) or reflect a commonly agreed level of harm, such as a biting rate below which nuisance is considered negligible.

Comparators are used to compare the potential health and environmental risks arising from a planned SIT programme with a baseline estimate of risk. This could be at the level of the characteristics of an individual irradiated mosquito compared with a wild mosquito, irradiated mosquito populations compared with wild populations or an SIT vector control operation compared to conventional control measures. The nature of the risk concern informs the selection of an appropriate comparator. Concerns related to individual behaviour, such as assortative mating or biting, would have an individual comparator. Concerns about population resurgence would have a population comparator. Finally, concerns about human immunity levels following several seasons of SIT control would be compared with such levels after a similar period of conventional control.

2.4.2 Risk analysis for *Aedes* SIT

Where a pathway to harm has been identified, the exposure to its causal events (such as sterileinsect release) should be estimated

in space and time. For example, what is the number, per area and time, of female mosquitoes that might be released in an SIT programme and how would that affect the biting rate and disease transmission? The analysis of the different categories of risks identified, as well as their pathways to harm, endpoints and limits of concern, will be then used to characterize these risks and decide upon the acceptability of the risks and mitigation measures.

2.4.3 Risk characterization for *Aedes* SIT in a likelihood–consequence matrix

Risk matrices illustrate the likelihood and consequences of risks (**Table 2.1**). They can be used to illustrate outcomes related to protection goals and inform regulators' decision making on the acceptability of risks. Each potential harm is assigned a value (and optionally an uncertainty range) for likelihood and consequence. The case shown in the table below uses subjective definitions for the likelihood and consequences scores, but scoring can also be more rigorously defined (cf., for example, OGTR 2013). Further operational risks can be characterized in activities such as insect rearing, irradiation, transport, release and monitoring; some examples are shown in **Box 2.2**.

Likelihood levels	Consecuence levels				
	1 Very low	2 low	3 Moderate	4 High	5 Very high
1 Very unlikely					
2 Unlikely		• Eradication of target *Aedes* population leading to ecosystem imbalance [2/2] • Unintentional release of sterile females leading to nuisance [2/2]		• Exposure to radiation for insectary technicians [2/4] • Unintentional release of sterile females leading to disease transmission [2/4] • Under-irradiated mutant males increase vectorial competence [1-2/4]	
3 Moderate likely				• Perception of success leading to complacent behaviour affecting disease challenge [3/4] • Niche replacement leading to invasive vectors [3/4] • Niche replacement affecting environmental balance [2-3/4]	
4 Likely		• Exposure to large numbers of sterile male mosquitoes leading to nuisance [4/2]			
5 Very likely					

Table 2.1. A matrix of health/environmental risks assessed in an IAEA-supported workshop in Singapore in June 2018 for Ae. aegypti SIT. The range in values [likelihood/consequence value ranges] are shown after each harm listed (Mumford 2018a), so the range [2/2-4] would indicate a consensus for likelihood at level 2 and the range of consequences from levels 2-4.

Box 2.2. Operational risk characterization in SIT programmes

Insect rearing

- Construction of a rearing facility will require permission from the local environmental planners and will involve the usual environmental concerns related to materials, scale, drainage, traffic etc.
- Operation of the facility will require planning for effluent, nuisance, traffic, radiation safety, insect allergens and feedstock security.

Transport

- Transport of sterile insects to the release points will require consideration of vehicle environmental effects and road (or other) safety measures for personnel.

Release

- Release of sterile insects will involve consideration of road (or other) safety measures and environmental impact.
- Release of sterile mosquitoes is likely to involve personnel moving through populated areas, thus consideration must be given to ensure the safety of bystanders and the security of the release staff.

Monitoring

- Monitoring in release areas is likely to involve monitoring personnel entering properties to conduct mosquito sampling.
- Staff security must be considered.
- Sampling methods may have an environmental or health impact, for example, if they involve collections to assess insecticide knockdown.

2.5 Risk communication for *Aedes* SIT

Risk communication depends on an accurate description of the planned SIT activities and how these relate to any risk concerns that may have been raised by the various stakeholders. Measures to mitigate those risks should be described, along with an explanation of how and why they will be effective. The protection goals likely to be associated with *Aedes* SIT should also be addressed in communication about risks and risk management.

According to the HCSP (2018), the risk related to the communication activities was considered one of the most critical risks for the acceptability of the SIT programme to the human population, and several recommendations were proposed to mitigate this risk. The development of a communication plan was strongly advocated with the following objectives and principles:

- The communication plan must be in place before any trials, to inform the entire population concerned, not only the population of the chosen field sites for testing;
- The communication plan must be developed jointly by the SIT managers and the stakeholders, taking into account all the concerns of the civil society;
- The objectives of the plan are to inform the population on the technology—in particular, to promote the benefits and inform about risks and how they are to be mitigated—in order to gain the acceptance of the populace.

The development of the communication plan must include monitoring and evaluation of the communication activities.

2.6 Risk management for *Aedes* SIT

SIT programme managers should specify standard operational activities and consider options for mitigating any unacceptable risks that have been identified. In consultation with regulators and other stakeholders, SIT programme managers must identify management actions and standards of performance that would bring risks within acceptable levels. For the above example, sorting and screening measures to limit the number of female mosquitoes in a release would be included in the operational plan.

Risk management includes both the general and specific measures taken to reduce known risks. **Box 2.3** below illustrates some general risk management measures.

Box 2.3. General risk mitigation measures adapted from the IAEA Singapore workshop for SIT and SIT/IIT (Mumford 2018a).

Measures for mitigating the risks for health and environment

- Limit release numbers, (in space and time)
- Optimize rearing quality (known performance, composition)
- Predictive modelling to design operations that minimize risk to an acceptable level
- Close monitoring of laboratory and field operations
- Monitoring of external conditions (disease, mosquito abundance) that might affect release
- Standard health and safety operating procedures for laboratory workers

Measures for mitigating operational risks

- Standardize operating procedures, ensure quality control and consistency
- Ensure staff are well trained in their roles
- Plan ahead for material needs and ensure backup supplies
- Appropriate design for facility purpose and maintenance
- Record-keeping
- Plan systematically for the whole operation to ensure efficient rearing, transport, release, monitoring
- Keep facilities clean and secure
- Learn from experience and other projects

Although risk assessment and risk management for all vector control operations, including SIT, are recommended globally; in the first *Aedes* SIT trials prior to 2018, no additional risk management measures were required from the relevant authorities, mainly because the technology was deemed to be agricultural and had been included in available biocontrol methods for decades without any adverse effects having been reported in the scientific literature (Mumford 2018b). Small-scale local releases of irradiated sterile males of *Ae. albopictus* were permitted in Italy, Germany (permission granted at local government level; Norbert Becker, pers. comm.), Montenegro (released irradiated male mosquitoes were specified as reared from locally collected eggs; EPA Montenegro and the Ministry of Agriculture stated that these releases were not subject to permit requirements for releases into the environment; Igor Pajović, pers. comm.), Albania (Enkelejda Dikolli, pers. comm) and Greece (Antonios Michaelakis, pers. comm.), as well as in Spain (Ignacio Pla Mora, pers. comm.) and Mauritius (Ambicadutt Bheecarry, pers. comm.), with no further risk assessment (based on the absence of adverse effects reported from this technology). Local releases of irradiated sterile males of *Ae. aegypti* were also permitted in Mexico in 2018—again with no further risk assessment (based on the absence of adverse effects reported from this technology) (Pablo Liedo, pers. comm.). These decisions in Mexico were based on the SIT programme managers' long experience with other sterilized insects; in each case, the relevant local, regional or national health or environmental authorities were notified of the nature and scale of intended rearing and releases.

By contrast, in France and for La Réunion Island, *Ae. albopictus* SIT releases were referred for comment and approval to authorities (AFB 2018; HCSP 2018), who noted the absence of a clearly defined regulatory framework for SIT against disease vectors in France specifically and in Europe more broadly. Previously, EPPO (2015) had also noted the lack of a clearly defined regulatory framework for biocontrol.

Generally, despite some concerns being identified, pilot SIT releases were approved, with several risk mitigation measures required to bring risks to an acceptable level. As an example, some of the risk management steps required to address the concerns raised by the proposed SIT mosquito release trials on the island of La Reunion (HCSP 2018) are reported below:

- Baseline entomological and epidemiological monitoring and monitoring of potential ecological effects after release;
- Development of standard operating procedures for technical processes, with review and revision as needed;
- Worker protection via application of good laboratory practices and standard operating procedures;
- Well-defined trial releases, on a relatively small scale in relatively isolated areas;
- Precautions to prevent escapes during transport;
- Development of a communication plan for acceptability of the trials to the local population.

2.7 Risk assessment responsibilities and governance

National authorities are responsible for the specification of SIT risk assessment requirements within their jurisdictions, in line with the regulations for their country (cf. **Chapter 3**). However, experience has shown that there may be alternative or conflicting roles for different national agencies in some countries, and in some cases, no clear regulatory pathway for this technology. Clarifying a pathway for regulatory oversight is an essential early step for any *Aedes* SIT project in a country.

The International Risk Governance Council[3] provides some general guidance on risk governance (IRGC 2017) and highlights the following areas of challenges:

- Consistent and appropriate methodologies to assess similar risks across different cases;
- Distribution of risks, benefits and trade-offs;
- Consequences and interconnections of risks and opportunities;
- Efficiency of regulation/management;
- Inclusion of stakeholders and their perceptions;
- Public trust.

Given the range of locations where control actions may be considered and the different regulatory systems involved, global harmonization of the regulations is challenging. While national regulatory requirements preponderate; wherever possible, projects should make use of appropriate precedents in other countries and for related species to help ensure consistent approaches to risk. In many cases, national regulations prescribing environmental risk assessments preclude consideration of benefits alongside harm, with benefit and efficacy at times only considered at a later pre-deployment decision stage. This may also apply to the scope of assessments and the extent to which secondary interactions are considered. Inclusion of stakeholders is necessary at the planning stage and getting value from their views requires proper resources and facilitation. Public trust is derived from the successful interaction of those developing SIT and those responsible for formal approval and community acceptance.

[3] *http://www.irgc.org/*

2.8 Risk analysis checklist

1. Determine the appropriate body (or bodies) to oversee and/or undertake the risk analysis. This body (or these bodies) may be the regulatory authority or external consultant with regulatory oversight.

2. Ensure that risk management procedures are in place and adaptable to the increasing scales of insect rearing, transportation, release events and monitoring required for executing phases I through IV of *Aedes* SIT testing.

3. Ensure that stakeholder and community input are included in the planning stage. This is likely to be a series of meetings as the SIT programme progresses from experimental/preliminary releases to area-wide suppression, with concomitant risk assessments.

4. Share the public health goals of SIT, including feasibility of alternatives; describe how SIT works and where/when the approach is best suited; describe how sterile male mosquitoes are produced and the proposed location of the production; describe how the sterile mosquitoes will be released and the likely locations; identify protection goals for human health and the environment.

5. Perform the risk assessment, i.e., problem formulation, characterization of SIT release and potential human health and ecological effects, and risk characterization. Given the nature and history of SIT in agricultural and livestock pest management programmes without adverse human health or ecological effects, the risk characterization will likely be qualitative and deterministic.

6. Develop a risk communication plan: How will the conclusions of the risk assessment be shared with SIT developers/implementers and the public? Are there any restrictions on how, when and where SIT releases can be made? Will release periods and locations be announced publicly? How will the results of the SIT release be shared with the public?

7. Monitor and mitigate the risks by developing a monitoring and evaluation plan.

Chapter 03

Permits and authorization pathways

3.1 Introduction

The role of governments in creating or cultivating a policy and regulatory environment to oversee sterile mosquito techniques management is essential for ensuring a predictable permitting process and maintaining the public trust. Mosquito production, release, monitoring of outcomes, stakeholder engagement and underlying human health and environmental risk analyses can all be subject to regulatory oversight and support.

The primary focus of this chapter will be the authorizations for SIT technology that must be given by the local or national decision maker(s). Since an authorization may be necessary for each step of the technology, a description of the process is essential. Below, we describe the stages of development from production to irradiated mosquito release and highlight areas for regulation at the local or national levels.

When seeking an authority's approval of permits for field releases, **transparency** is essential. Objectives should be clearly explained with a precise economical model.

3.2 Different levels of authorization pathway

3.2.1 Four phases of development before license

The concept of phased testing (Wilson et al. 2015) previously described in **Figure 1.5** is important to underline how local authorizations can be managed. Irradiated sterile insects are not considered to be transgenic insects and are exempted from specific regulation because of their long safety records (Lutrat et al. 2019).

a. Phases I and II (defined in Chapter 1)

These phases may not require any specific authorization except that of the funder or the research organization supporting the development, as long as the phase II semi-field releases are done into a closed and controlled environment. However, some countries do deliver approvals—including for the production and experimentation with mosquitoes—for phase I and II. In case of semi-field releases into open spaces, the same authorization as for phase III may be needed, according to the country. Depending on the institutions involved, an ethics committee may be called upon, especially for the use of an irradiated source and the release in phase II. Field trials with containment may require a permit from a local authority.

b. Phases III and IV (defined in Chapter 1)

Phases III and IV should demonstrate the efficacy and effectiveness of the new prevention tool, and thus, its epidemiological impact on a population exposed to vector transmission of a pathogen. Particularly important in terms of the regulatory framework, phases III and IV must be supported by regional and national authorities, respectively, depending on their scale and the number of insects handled.

To document the efficacy of SIT for mosquitoes, it is essential to perform a robust evaluation and demonstrate evidence of a decline in pathogen transmission. This is usually the goal of phase III trials. These tests measure the efficacy of a vector control tool based on epidemiological indicators. Efficacy and environmental safety are the key parameters for issuance of a license after completion of phases III and IV.

Concerning the license for release of mosquitoes, each country may already have possibilities for adapting an existing regulation or for delegating relevant tasks to the appropriate authority (e.g., US experience in **Box 3.1**). A local or national authority can mobilize a dedicated agency for supervision of trial evaluation.

Box 3.1. Regulation by US federal agencies

Some countries, such as the USA, have 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 (APHIS). An argument can be made that irradiated, sterile insects could be deemed biological control agents, which, as pesticides, are exempt from regulation by the United States Environmental Protection Agency (US EPA), as long as the EPA determines that regulation by another federal agency (e.g., APHIS) is adequate.

However, previous experience shows that since the application of this technology is very new in vector control, there are no overall rules: In some cases, specific regulations were used; in others, no specific regulations were applied. As an example of the latter case, previous field trials in Asia, the EU (including overseas territories) and the USA for phases III and IV neither were subject to nor gave rise to specific regulations. A review of the processes employed to authorize these trials demonstrates that these processes can be modified when applying for national or local permits.

Overall, it appears that although the authorization for release must be requested, it may not be necessary to build a specific regulatory structure to implement phases III and IV for the release of sterile irradiated mosquitoes as was done with "traditional/ agricultural" SIT for several arthropods for long-term use in the field.

Nevertheless, authorization should be based on these key elements:

- Clear definition of the objective(s) to be achieved from releasing sterile irradiated mosquitoes;
- Clear definition of the required authorization (per location);
- Clear description of insects' production (quality control, standard operating procedures, permits);
- Risk assessment by phase for the workers, the human populations of the field releases and the environment;
- Transparency throughout all the development steps;
- Mitigation measures in case of adverse effects;
- Citizen engagement and input;
- Post-release surveillance (note: an epidemiological survey is not compulsory for authorization).

3.2.2 Development of the technology: Scale-up and additional authorizations

The SIT requires a factory for irradiation (and the associated irradiation process), transportation of mosquitoes and a release system (**Figure 3.1**). As SIT implementation progresses and its scale increases, each of these steps is subject to increased oversight based on country's existing or adapted regulations.

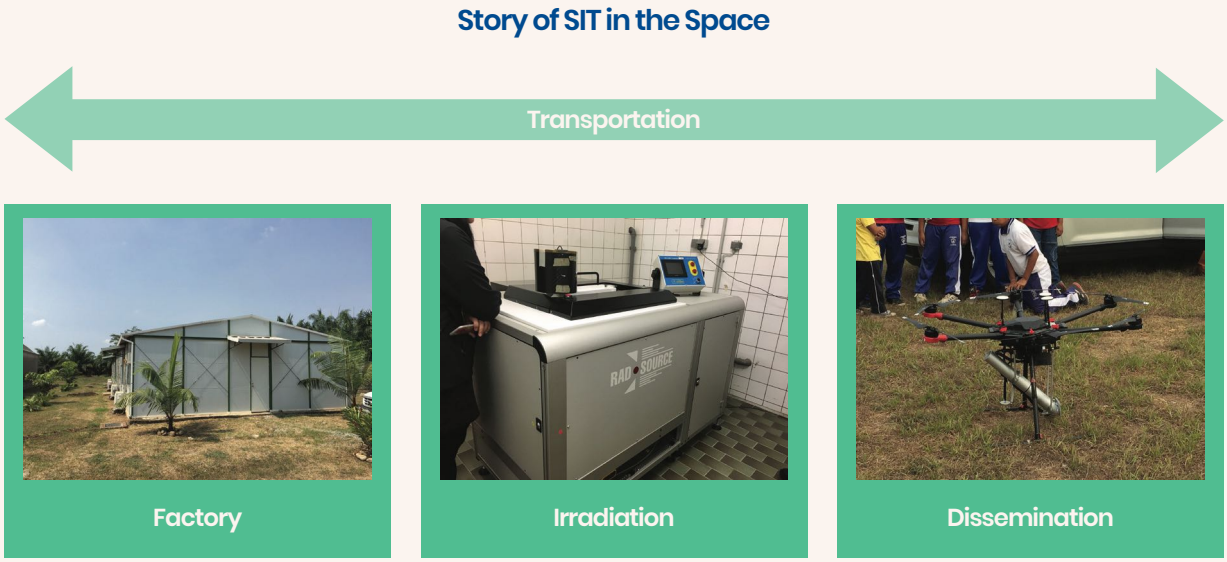


Figure 3.1. Scale-up of SIT applied to mosquitoes. Mass production for deliberate release requires a factory capable of producing millions of mosquitoes weekly. If irradiation is not performed in situ, transportation and storage of mosquitoes must be organized until releases via automated systems. Active post-release surveillance also must be set up with the acceptance of local citizens. (Photo credit: P. Boireau and F. Fouque)

a. The factory step

A production permit is required to evaluate risks to workers and the environment and to ensure that the appropriate information is conveyed to citizens. Typically, regulations already exist for mass non-domestic animal rearing. Some countries may have an existing classification for arthropod production for species that are non-domestic animals and not deemed to be pests. In most parts of the world, many species of arthropod are considered to be domestic due to their use in production (e.g., the silkworm, *Bombyx mori*; domestic varieties of the bee, *Apis spp.*; and domestic varieties of fruit flies, *Drosophila spp.*), local culinary customs or other specific uses (e.g., *Locusta migratoria migratorioides, Acheta domesticus, Tenebrio molitor*).

Mosquitoes, however, are considered to be pests. Thus, in several countries, they are regulated by the authorities in charge of the environment, public health worker safety and/or agriculture, who manage permits for mass production in a factory, which typically includes restricted access control. The definitions of possible nuisance and emergency plans must be elaborated within this framework. For example, the accidental escape of female mosquitoes must be covered in an emergency plan to mitigate risks to human health and the environment.

In Europe, these factories are classified and authorized according to the quantity of insects produced per day (1.5 kg/day, 150 kg/day or more). Such a classification makes it possible to define the overall risk of nuisances (intensity of transport for raw material, odours, noise level etc.). With regard to mosquito production, it is expected that most factories will not produce more than 1.5 kg per day of the arthropods.

Depending on local regulations, permits may be given before and after building (e.g., in China, for authorization of a factory posing risks to the environment) (Pascal Boireau, pers. comm.). In most countries, the authorization to implement such a factory is issued after a formal inspection of the premises and the procedures used to ensure environmental protection.

b. The irradiation procedure

Existing regulations in countries should support this activity. Local irradiation is always preferred, whenever possible, since it avoids transportation constraints like the transport of fertile material. The country's radiation authority ensures the proper use of radiation, prevents harmful effects of radiation on human health and promotes protection of the environment. It is emphasized that the benefits of employing radiation must outweigh the associated detriments. The use of radiation should be optimized, such that the ionizing radiation exposure is as low as practically achievable, taking into account technological knowledge and social and economic factors.

c. The transportation of irradiated mosquitoes

Containment of mosquitoes should be maintained during transport, with traceability. For example, in the USA, the movement of insects, mites and ticks that affect humans or cause human diseases requires permits from the Center for Disease Control and prevention (CDC). It is to be expected that the transport of mosquitoes will be regulated locally, before and after irradiation.

d. The release of irradiated male mosquitoes

Various systems can be used for mosquito release; as an example, drones can be used for efficient release, but all systems are subject to local regulations (**Box 3.2**).

Box 3.2. Regulation of civil drones in Europe

The European Commission's proposal for an **Implementing Act** defined the regulations around operating Unmanned Aircraft Systems (UAS) in Europe and the registration of drone operators and certified drones. The Implementing Act is accompanied by a **Delegated Act**, which defines the technical requirements for drones. It was adopted by the European Commission on 12 March 2019 and specifies three categories with a gradient of constraints. Spreading mosquitoes with drones should fulfill the specific requirements of the requested category. Operating drones over populated urban areas requires higher levels of safety precautions and specific authorizations.

e. Post-release surveillance

Post-release surveillance typically is necessary for continuing authorization of an SIT programme. As SIT is a self-limiting technology—given the short lifespan of the sterile males released (most probably less than 10 days)—surveillance is only applied during the active phase of release and as long as SIT mosquitoes are found in the release site. The participation of citizens is particularly important and should be promoted by the relevant authority to sustain success in the field. Sentinels sites can be selected and followed to measure programme impact on disease incidence and prevalence (cf. **Chapter 6**).

3.3 SIT applied to mosquitoes and existing authorizations/ regulations (past 10 years)

To provide a representative view across countries of the current experience regarding regulatory pathways, it was found that the relevant national authority typically belongs to one of three ministries: agriculture, environment and/or health. Sometimes agencies are included for their expertise and ability to give recommendations to the certifying authority. Local authorities represent the government in a defined geographic area. In the various examples described below, mostly university or research institute teams supported the request for release authorizations. Private companies or academic consortia were also involved in the development of mosquitoes with a "sterile" phenotype.

3.3.1 Montenegro: Importation of irradiated sterile mosquitoes

Authorization to import *Ae. albopictus* was requested from the Directorate for Environment (under the Ministry of Sustainable Development and Tourism) with expert input from the Agency for Nature and Environment Protection (EPA Montenegro) and the Ministry of Agriculture and Rural Development. EPA Montenegro indicated there was no prior authorization for *Ae. albopictus* importation according to the law on nature protection. *Ae. albopictus* does not appear on a list of not-protected wild animals, plants and fungi that can be used for commercial purposes (Official Gazette of Montenegro, No. 62/10), nor is this species protected by a decision about the protection of designated plant and animal species (Official Gazette of Montenegro, No. 54/16). The Ministry of Agriculture (Directorate for Food Safety, Veterinary and Phytosanitary Affairs) replied that the release of sterile mosquitoes from the species *Ae. albopictus* in the specific area described in the request was not subjected to a permit, since the released mosquitoes were reared from eggs collected in Montenegro and thus there was no possibility of introducing new genomes from other *Ae. albopictus* lines.

3.3.2 Germany: Importation of irradiated sterile mosquitoes

The authorization to release sterile mosquitoes of the species *Ae. albopictus* describes the location of release, density of mosquitoes per hectare and designated scientific leader and was delivered by the local authorities. The permit was compulsory for release and listed additional requirements: no impact on the existing ecosystem; the release species is alien; invasive species should decrease after the release (effectiveness); no risks were identified, since male mosquitoes do not bite. (Concerns about transport were not developed in this authorization.) After the experiment ends, a report must be completed and submitted to the regulatory body.

3.3.3 France: Experiments on the island of La Réunion

Local production of sterile mosquitoes was initiated in 2009 on the island of La Réunion, but no permit has been given for field release, because it was considered to be in phase I development until recently. In 2017, following a round table between the directorates of different ministries and the High Council for Biotechnology (HCB), the request for sterile insect release was formulated again. The two ministries responsible for health and environment sought expertise from two agencies—the High Council for Public Health (HCSP) and the French Biodiversity Agency (AFB)—on the following items:

- Assessment of the risks associated with this technique for the workers and the local population;
- The participatory and information procedures to be put in place to facilitate acceptance of sterile mosquito releases by the local human population;
- The precautionary measures to be taken to supervise the releases of sterile mosquitoes.

The two agencies replied in June 2018 and the Prefecture of La Réunion issued a formal decision to authorize the release of irradiated sterile males, according to phase II of the testing process and under relevant regulations, through Arrêté No. 2019-2213.[4]

One of the main conclusions of the HCSP response to the item on the participatory and information procedures to be put in place to facilitate acceptance of sterile mosquito releases by the local human population was that "Any public health programme must be presented in a way that is accessible to all communities for its acceptance. This information is essential, even if the methods used do not require active direct individual participation or acceptance of invasive treatments. This is even more true for programmes that require significant community participation to succeed, and for programmes

[4] *http://www.reunion.gouv.fr/IMG/pdf/arrete_no_2019-2213-sg-drecv_du_13.06.2019.pdf*

that use new technologies that may be viewed with suspicion, or at least not easily understood and accepted by affected communities".
The HCSP insisted on the need to know, in advance, the perceptions and reactions of the population of La Réunion to SIT. Targeting the content of SIT communications, including information and training, to different audiences (general population, youth population, health professionals and vector control staff, as well as local political actors) is a crucial and indispensable aspect to ensure acceptance by the populace and is essential for a successful phase II SIT application for mosquitoes (HCSP 2018).

3.3.4 Italy: Trial with sterile mosquitoes

The best-documented recent demonstrations of SIT against mosquitoes were made via pilot tests on Ae. albopictus in Italy between 2004 and 2009 and yielded encouraging results (Bellini et al. 2013). No specific authorization was required for this field trial. Data on the application of the SIT strategy were collected primarily through entomological surveillance.

3.3.5 Other trials

Table 3.1 summarizes examples of mosquito trials conducted in other countries. No specific rule emerges from these various field trials. Several ministries were involved, but the relevant Ministry of Health was rarely mobilized for most of the examples shown in Table 3.1.

Table 3.2 provides examples of SIT projects on non-mosquito species over the last 15 years.

Table 3.1. Examples of field trials (HCB report, CNEV Scientific Committee Working Group, pp. 196212 with updated data) (cf. also Table 4.1).

Years	Species	Location	Phase	Institution / Programme	Reference/ Source	Authorization
1959-62	*Anopheles quadri-maculatus*	Florida, USA	I		Dame and Ford 1964	N/A
1970	*Anopheles albimanus*	El Salvador	I		Lofgren et al. 1974, 99% efficacy	Pilot field trial
1973-75	*Culex pipiens quinque-fasciatus*		I	Indian Council of Medical Research and WHO/USPHS	Pal and Lachance 1974	N/A
1973-75	*Ae. aegypti / Anopheles stephensi*	India (Liston)	I	Indian Council of Medical Research and WHO/USPHS	Pal and Lachance 1974	N/A
1970	*Ae. aegypti*	Kenya	I		Lorimer et al. 1976	N/A
1977-1983	*Culex tarsalis*	California, USA	I		Zalom et al. 1981	N/A
2018	*Ae. albopictus*	Montenegro	II	University of Montenegro, Faculty of Biotechnology	Importation of sterile *Ae. Albopictus* males	Not necessary
2015-2017	*Ae. albopictus*	China, Guangzhou (Guangdong)	II	Zhongshan School of Medicine, Sun Yat-sen University of Medical Sciences	Zheng et al. 2019 Release on two small islands	Yes (for the IIT component)
2005-2009	*Ae. albopictus*	Italy	III	Centro Agricoltura Ambiente "G. Nicoli"	Bellini et al. 2013b	Yes (local authority)
2012	*Anopheles arabiensis*	Sudan	III	Tropical Medicine Research Institute	IAEA Technical Cooperation project SUD5034, EU grant COFUND (Ageep et al. 2014)	N/A

2015	*Ae. albopictus*	Mauritius	III	Ministry of Health and Quality of Life / Vector Biology and Control Division	IAEA Technical Cooperation project MAR5019 (no published data)	Yes
In progress	*Ae. albopictus*	Germany	III	German Mosquito Control Association (KABS)	Research project with AIEA RER5022 (FAO/IAEA 2017)[5]	Yes
Project (2018)	*Ae. aegypti*	Cuba	-	Pedro Kourí Tropical Medicine Institute	Research project with AIEA RLA5074 (FAO/IAEA 2017)	N/A
Project (2017-2018)	*An. arabiensis*	South Africa	-	National Institute for Communicable Diseases	Research project with AIEA SAF5014 (FAO/IAEA 2017)	N/A
Project (2018)	*Ae. aegypti*	Brazil	-	Biofábrica Moscamed Brasil	Research project with AIEA RLA5074 (FAO/IAEA 2017)	N/A
Project (2018)	*Ae. aegypti*	Mexico	-	CRISP - Instituto Nacional de Salud Pública	Research project with AIEA MEX5031 (FAO/IAEA 2017)	N/A
Project (2018)	*Ae. albopictus*	Spain (Valencia)	-	Grupo Tragsa	Research project with AIEA RER5022 (FAO/IAEA 2017)	Yes (Local)

[5] *http://www.deutschlandfunk.de/sterile-maennchen-und-klebrige-fallen-wie-deutschland-die.724.de.html?dram:article_id=364179*
http://www.srf.ch/sendungen/wissenschaftsmagazin/sterile-maennchen-gegen-die-tigermuecke

Table 3.2. SIT programmes on species other than mosquitoes: examples of SIT projects on non-mosquito species over the last 15 years (supported by IAEA and FAO) (HCB/CNEV report and additional data).

Years	Country	Species	Outcome
2005	Chile	Mediterranean fruit fly, Ceratitis capitata	Eradication from northern Chile
2006	Thailand	Oriental fruit fly, Bactrocera dorsalis and B. correcta	Successful pilot suppression projects
2007	Israel	Mediterranean fruit fly, C. capitata	Area-wide suppression in Arava valley
2008	Argentina	Mediterranean fruit fly, C. capitata	Eradication from southern provinces
2009	Peru	Mediterranean fruit fly, C. capitata	Eradication from southern provinces
2010	Mexico	Cactus moth, Cactoblastis cactorum	Eradication of an outbreak in Yucatan
2011	South Africa	False codling moth, Thaumatotibia leucotreta	Area-wide suppression in Western Cape province, expanding to Eastern Cape
2012	Spain	Mediterranean fruit fly, C. capitata	Area-wide suppression in Valencia province
2013	Guatemala	Mediterranean fruit fly, C. capitata	Eradication from Western Guatemala
2014	Morocco	Mediterranean fruit fly C. capitata	Successful pilot suppression project, triggering an area-wide programme
2015	Croatia	Mediterranean fruit fly, C. capitata	Area-wide suppression in Neretva Valley
Ongoing	Senegal	Tsetse fly, G. palpalis gambiensis	Eradication from the Niayes well advanced
Ongoing	Dominican Republic	Mediterranean fruit fly, C. capitata	Eradication of a large outbreak in eastern region
Ongoing	Ethiopia	Tsetse flies Glossina pallidipes and Glossina fuscipes	Suppression in the Deme Valley in Southern Rift Valley

3.4 The next steps in permits and authorization pathways for *Aedes* SIT

Garnering permits and authorization to release SIT mosquitoes currently is on a country-by-country basis and subject to different regulatory authorities. Consequently, not only are the different regulatory authorities looking at different issues, but the authorizations also are based on very different perspectives. Criteria for the approval of releases of high scientific quality are shown in **Box 3.3**.

Box 3.3. Criteria for High Scientific Quality Release Approval (extract from Reeves et al. 2012, modified for irradiated mosquitoes)

- Complete scientific details of the proposed field trial can be made available during pre-approval public consultations and notifications (phasing approval).
- A complete list of all potential hazards considered by regulators is published along with their determined risk classification.
- A substantial body of relevant interdisciplinary research is cited from multiple independent groups with no serious gaps in areas of importance for assessing potential impact on human health and environment.
- Documents concentrate on the issues that are truly significant and specific to the case under consideration, rather than on amassing needless details.
- Data cited in regulatory documents are published, ideally in peer-reviewed journals and are in reports validated according standard operating procedures.
- No scientific points of fundamental importance for human health and environmental protection are left apart at any stage of the process.
- Any prior data obtained from field trials in other countries, cited in support of permit approval, are widely recognized as having been collected in an ethical manner and with input from citizens. Any existing international protocols should have been followed.
- Percentage of females released must be clarified, because it is of importance as it is a key

component with consequences for all citizens where the trial will be done.
• Information in documents provided by the regulator is clear, understandable and accurate with respect to all points of fundamental importance for human health and environmental protection.
• Efficacy in reducing the density of target mosquitoes up to elimination: A threshold should be defined and maintained according to standard operating procedures.
• Effectiveness in reducing vector borne pathogens through animal sentinel survey and blood donor survey.
• Go/no-go and risk benefit analysis.

The governments which are currently creating policies and regulatory environment to oversee sterile mosquito techniques management may participate in an international committee to discuss the different pathways for permits, and eventually propose standard procedures and steps for all the elements required to reach a policy decision.

Transparency in the decision-making process will ensure widely accepted technology, the overall mitigation of risks and public acceptance. Mosquito production, irradiation and release already have good bases, however the risk assessment of impact on health and the environment, and the monitoring of outcomes, are subject to very different regulatory oversight and support. The need for a global approach on this subject will be one of the challenges on the way to full deployment of this technology, if proven efficient.

Chapter 04

Mass production and release of sterile male *Aedes*

The SIT requires mass production of sterile insects of high quality. The technological package for the mass rearing, sterilization, release and quality control of sterile *Aedes* mosquitoes has been developed. Standard operating procedures or guidelines are available for colonization, colony management, mass rearing and irradiation for sterilization. Guidelines for transportation and release, as well as for quality control, are under development.

4.1 Background

The success of the SIT strategy depends on the mass production in dedicated facilities of large numbers of high-quality sterile males of the target species. For SIT, a high-quality male means that the male is capable of flying, surviving and dispersing in the environment; mixing with the wild population; competing with its wild counterparts in courting, mating with and inseminating wild females, thus reducing the probability of those females mating with fertile wild males.

The production phase, including the separation of sexes to ensure males-only releases and the sterilization of males before field release, determines the quality of the sterile males. The mass rearing, handling and release processes consist of a complex series of highly standardized steps (**Figure 4.1**) organized to yield the most efficient and optimal production and successful release of the target species.

Figure 4.1. Main steps in the mass rearing of *Aedes* mosquitoes.

The appropriate organization of the mass production, handling, irradiation and release phases is crucial for maintaining the most cost-effective processes. The methods and tools that have been developed to maintain and check male quality specify the standard parameters used to ensure high-quality products for vector control (e.g., survival, mating capacity, mating competitiveness and flight ability in controlled conditions).

This chapter provides a general overview of the key parameters to consider in the colonization, mass rearing and release of the target species. For more detailed SOPs, readers are advised to consult the IAEA guidelines referenced below.

4.2 Strain selection and colonization

It is advisable to start colonies with genetically diverse "local material". The minimum recommended to start a colony is 500 pairs, but the greater the number and genetic diversity, the better. To produce high-quality males, it is best to avoid starting the colony with material obtained from an old established laboratory colony. If foreign strains are being considered, it is important to pay attention to national regulatory requirements (cf. **Chapter 3**).

Aedes mosquitoes can be collected easily from the field with simple entomological tools such as ovitraps or ovicups to collect eggs; dippers or water nets to collect larvae and pupae; and aspirators, sweeping nets or adult traps to collect adults.

For all stages, it is essential to verify the species before introducing wild-caught mosquitoes into the insectary. For more specifications, we refer to the **Guidelines for Colonization of Aedes Mosquito Species (Version 1.0)**[6] produced by the Joint Food and Agriculture Organization/ International Atomic Energy Agency (FAO/IAEA) Insect Pest Control Subprogramme (IPCS).

Note: If a genetic sexing strain (GSS) is available, backcrossing with the local strain is recommended (at least 6 generations). A GSS is a strain where a phenotypic trait is associated with one sex only (e.g., temperature sensitive lethal or coloured pupae) and can be used to achieve sex separation, if possible at an early stage (e.g., egg or larvae), such that only male insects are mass reared. Obtainable by classical genetics, as well as transgenic means, GSS mosquitoes are not released into the field. If a GSS is to be used, mass rearing should be supported by a mother colony or filter colony system (Fisher and Caceres 2000; Gilles et al. 2014), in order to eliminate recombinants

[6] *http://www-naweb.iaea.org/nafa/ipc/public/Guidelines-for-colonisation-of-Aedes-mosquito-species-v1.0.final.pdf*

where the phenotypic trait becomes associated with the wrong sex.

Successful mass production depends on optimal management of the mother colony, which, as stated above, ideally should be genetically diverse and also be free of pathogens. Even if the risk of pathogens affecting mosquito colonies is deemed to be low; due to the critical importance of the initial colony being free of infection, a specialized laboratory should be employed to check its health and infection status.

At the same time, the insects produced should maintain the biological and behavioural attributes favourable for their survival, dispersal and sexual competitiveness in the field. Optimal colony management will ensure standardized and cost-effective production of high-quality sterile males with enhanced field performance. For standard operations for mosquito colony management, please refer to the **Guidelines for Routine Colony Maintenance of Aedes Mosquito Species**[7] produced by the FAO/IAEA IPCS.

4.3 Mass production

When planning the mass rearing facility, the appropriate arthropod containment level requirements (American Committee of Medical Entomology American Society of Tropical Medicine and Hygiene 2019) must be determined in collaboration with the local/regional/national authorities (cf. **Chapter 3** on regulations).

The routine introduction of wild mosquitoes to renew the colony should be avoided to prevent the introduction of undesired effects or pathogens. However, a routine refreshment calendar of the colony is sometimes necessary to maintain its competitiveness and wild traits. For example, when the wild populations are insecticide-resistant, the use of local insecticide-resistant strains in mass rearing is preferred (in agreement with the public health authorities) to better match with the wild strains and have the same resilience to other control methods. This characteristic then requires refreshment to be maintained. For outcrossing, wild males should be used (i.e., wild females excluded) and reared apart for at least six generations. This prevents the introduction of diseases or unfavourable traits.

Efficient production, including synchronized pupation, homogeneous size and high quality, is only possible when all the parameters are fully optimized and standardized and kept under strict continuous control, including:

- Air and water temperatures are controlled in the larval rearing section and in the larval trays;
- Larval density is managed to be in the predetermined range (**Figure 4.2**);
- Larval diet provides well-balanced nutrients to satisfy the species-specific requirements and an appropriate dose is administered regularly;

[7] *http://www-naweb.iaea.org/nafa/ipc/public/guidelines-for-routine-colony-maintenance-of-Aedes-mosquito-species-v1.0.pdf*

- Adult cage size and setting follow the species-specific requirements, particularly regarding environmental conditions (temperature and relative hygrometry) (**Figure 4.2**).

Figure 4.2. Larval trays piled in racks (l.); adult cage (r.)

Animal blood used to feed the females must be checked for safety to prevent the introduction of any contaminants and/or pathogens. Irradiation of the blood can reduce bacterial load and improve the health of the colony (**Improved and Harmonized Quality Control for Expanded Tsetse Production, Sterilization and Field Application, IAEA TECDOC No. 1683**[8]).

The male sorting system should guarantee that residual female contamination is held to a minimum, i.e., below the predetermined threshold agreed with the public health authorities (Focks 1980; Zacarés et al. 2018).

Note: Guidelines for Mass Rearing of *Aedes* Mosquitoes (Version 1.0) are slated for publication on the Insect Pest Control website under **Manuals & Protocols**.[9]

[8] *https://www.iaea.org/publications/8725/improved-and-harmonized-quality-control-for-expanded-tsetse-production-sterilization-and-field-application*
[9] *http://www-naweb.iaea.org/nafa/ipc/public/manuals-ipc.html*

4.4. Sterilization

Each species and each developmental stage (i.e., pupae or adults) have specific susceptibility to irradiation doses (Yamada et al. 2019). The parameter of desired male sterility level (not necessarily full sterility) must be set in consideration of competitiveness, induced sterility, risk of population transformation and cost.

The reliability of the irradiator in the context of the local operational conditions should be validated by conducting dosimetry studies on pupae or adults, always using untreated males as controls (Balestrino et al. 2010; Bond et al. 2019; Lebon et al. 2018; Machi et al. 2019; Parker and Metha 2007; Yamada et al. 2014).

For more precise technical information on dosimetry, please refer to International Atomic Energy Agency, **Gafchromic® Dosimetry System for SIT—Standard Operating Procedure (2004)**.[10]

[10] *http://www-naweb.iaea.org/nafa/ipc/public/Dosimetry_SOP_v11.pdf*

4.5. Transport and release

Whenever possible, the mass rearing facility should be close to the field sites. If the release area is far from the mass rearing facility, the sterile adult males can be packaged and transported by air or ground.

In case of a transnational sterile male shipment, all the necessary administrative steps should be clarified with the authorities and the transport company. To ensure that the sterile males suffer the least amount of stress, transportation should be organized to guarantee the best possible conditions for the packaged sterile males and the shortest time en route (Chung et al. 2018; Culbert et al. 2018).

The release strategy must specify the numbers (e.g., number of sterile males per hectare) and periodicity (e.g., once a week or more often) of sterile males to be released based on the wild population density and the target objectives. The wild population density must be estimated in different seasonal periods by mark [11]-release-recapture trials (Bellini et al. 2010; IAEA Guidelines).

Homogeneous distribution of sterile males is particularly important in urban settings, where numerous obstacles may hamper dispersal, and must be achieved regardless of the release method (i.e., aerial or ground). **Figure 4.3** summarizes the steps in the SIT process from breeding to release.

Figure 4.3. The steps of the SIT process

[11] *ISPM 3 Guidelines for the export, shipment, import and release of biological control agents and other beneficial organisms. p.10, Section 3.1.8 "In the case of sterile insect technique (SIT), the sterile insect may be marked to differentiate it from the wild insect."*

The distribution of the sterile males can be monitored by marking the sterile males as suggested by the ISPM 3 (cf. Footnote 11), however this might negatively affect the sterile males by making them more susceptible to predation or by affecting their quality due to the technique used to mark them. One option is intermittent marking to monitor survival and the ratio compared to wild males.

4.6. Quality control

Quality control (QC) measures are essential for optimizing mass rearing and the production of sterile males with good performance. It is essential for mass rearing facilities to keep records and databases of their production and the quality of their sterile mosquitoes. This will facilitate diagnosis of problems and identification of measures to solve them (Caceres et al. 2007; FAO/IAEA/USDA 2014; Mumford et al. 2018) (cf. **Chapters 2** and **5**).

The QC checks that might be adopted in the mass rearing facility include:

– Female fecundity of the strain (e.g., once every ten generations) (Bond et al. 2019);
- Egg fertility, expressed as egg hatch (e.g., once every ten generations);
- Female and male longevity of the strain (e.g., once every 20 generations) (Bond et al. 2019);
- Male wing length (e.g., once every ten generations);
- Male mating competitiveness (e.g., once every ten generations);
- Male flight ability (e.g., every generation via reference flight test) (Culbert et al. 2018);
- Percentage of residual females (e.g., every batch with declaration).

The QC checks that might be applied at the release site include:

- Male mating competitiveness (e.g., at least once every three months);
- Male flight ability (e.g., every release) (Culbert et al. 2018);
- Male longevity (e.g., at least once every five releases) (Bond et al. 2019);
- Percentage of residual females (e.g., every release batch).

4.7 Guidelines and SOPs

Guidelines or standard operating procedures have been developed for the different stages of the SIT process.

Processes

1. Colonization	Guidelines for Colonization of *Aedes* Mosquito Species (Version 1.0)[12]
2. Colony management	Guidelines for Routine Colony Maintenance of *Aedes* Mosquito Species (Version 1.0)[13]
3. Mass rearing	Guidelines for Mass Rearing of *Aedes* Mosquitoes (Version 1.0)[14]
4. Sterilization	Guidelines for Small Scale Irradiation of Mosquito Pupae in SIT Programmes (Version 1.0)[14]
5. Mark-Release-Recapture	Guidelines for Mark-Release-Recapture procedures of *Aedes* mosquitoes (Version 1.0)[14]

Guidelines or SOPs for transportation and quality control are in preparation at the Insect Pest Control Laboratory of the FAO-IAEA Joint Division. They will be available online at http://www-naweb.iaea.org/nafa/ipc/public/manuals-ipc.html

[12] *http://www-naweb.iaea.org/nafa/ipc/public/Guidelines-for-colonisation-of-Aedes-mosquito-species-v1.0.final.pdf*
[13] *hhttp://www-naweb.iaea.org/nafa/ipc/public/guidelines-for-routine-colony-maintenance-of-Aedes-mosquito-species-v1.0.pdf*
[14] *Slated for publication at http://www-naweb.iaea.org/nafa/ipc/public/manuals-ipc.html*

4.8 Ongoing SIT projects

Table 4.1 shows a list of ongoing SIT pilot projects against *Aedes* species.

Country	City	Size of release area	Inhabitants in the release area	Avg. release density	Avg. production	Current status	Species	Species
Brazil	Recife (PE) Carnaiba (BA)	56 ha	18,300 residents	5-10k males/ha/week (anticipated)		BLDC, mass rearing and irradiation capacity, suppression prior release	autodissemination traps	*Ae. aegypti*
Cuba	La Habana	15 ha				BLDC, insectary and irradiation capacity		*Ae. aegypti*
Malaysia	Melaka state	4 ha	16,000 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	*Ae. aegypti*
Mexico	Tapachula	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	*Ae. aegypti*
US	Captiva island (Lee County, FL)	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 surveillance, arbovirus sentinel stations	*Ae. aegypti*
France	Reunion Island	32 ha		3k males/ha/week	100k males/week	BLDC, operational research, communication, authorizations, insectary with irradiation capacity, MRR	Deltamethrin ULV spraying+ sanitation of larval breeding sites	*Ae. albopictus*
Germany	Heidelberg Freiburg	4 ha (2016) Freiburg		3k males/ha/week	30k males/week reared in Italy	BLDC, sustained releases in 2016-2019	Bti treatment of larval breeding sites	*Ae. albopictus*
Greece	Vavrona (Athens)	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	*Ae. albopictus*
Italy	Caselline, Boschi, Budrio, Santamonica Bologna	6-45 ha 25 ha		0.9-1.6k males/ha/week 0.6-2.1k males/ha/week	50-100k males/week	Field pilot completed in 2013(Upscaling field trials ongoing in Bologna	door to door	*Ae. albopictus*
Mauritius	Panchvati	3 ha		20k males/ha/week	60k males/week	BLDC, small insectary, irradiation capacity, 9 months of releases in 2018	weekly larviciding Bti and biweekly fogging before releases	*Ae. albopictus*
Spain	Valencia	44 ha (Polinya) +35 ha (Vilavella)	2,500 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	*Ae. albopictus*

Table 4.1. List of ongoing SIT pilot projects against Aedes species at the time of this writing. (Source: derived from IAEA thematic plan 2020-2025.)

Chapter 05

Evaluation of entomological efficacy

Evaluation of entomological efficacy is key to understand the impact of SIT. This chapter provides broad guidelines for generating entomological evidence to enable decision makers to advance an Aedes SIT programme from the initial stages to operational use. A phased conditional approach is proposed in Chapter 1 to guide the SIT testing programme through a series of evaluation steps of increasing complexity, with “go/no-go” decisions made at each phase. Established methods are available to provide robust entomological evaluation at each testing phase. Illustrative “go/no-go” criteria are presented for the key entomological performance indicators.

5.1 Introduction

Unlike short-term vector control interventions, e.g., spraying with ultra-low-volume (ULV) adulticides, SIT acts over the medium to long term to drive down vector density across multiple generations. The SIT offers no personal protection and requires large-scale implementation to deliver community-wide benefits. Thus, the quantification of entomological impact is a central component in the development and evaluation of an SIT programme. While the ultimate impact is measured in terms of epidemiological outcomes (with studies necessarily conducted on large scales), the initial development and evaluation steps focus on entomological outcomes in laboratory, semi-field and field settings.

The suggested strategy follows a phased conditional approach, where the SIT programme progresses through a series of evaluation steps of increasing complexity (and cost) with go/no-go decisions made at each step (**Figure 1.5**). This strategy emphasizes evidence-based decision making designed to reduce risk and increase decision makers' confidence and willingness to support continued investment in SIT technology through a multi-stage process towards the ultimate goal of deployment.

The phased development progresses through the following stages:

- Phase I: Laboratory assays to confirm mode of action;
- Phase II: Semi-field and small-scale field trials;
- Phase III: Studies (large-scale field trials) to assess the efficacy of the intervention;
- Phase IV: Pilot implementation studies.

Phase IV studies monitor the effectiveness of the vector control tool when it is used under real-world conditions and collect information on entomological and epidemiological impacts and operational feasibility, including data on acceptability, cost-effectiveness, long-term production, safety and other relevant data.

Table 5.1 summarizes the key endpoints and evaluation criteria for each of these phases. Sections 5.2 through 5.5 expand on the information presented in the table. The specific methods and protocols on how to conduct the entomological assessments are beyond the scope of this document; however, for more detailed information, readers are referred to various resource texts (with examples given below). The aim of this chapter is to provide a roadmap of which entomological work needs to be done and which factors ought to be considered to answer the question "Does SIT work in my particular context?" The focus of this chapter is on entomological efficacy and effectiveness of SIT. Nevertheless, SIT efficiency is also based on epidemiological effectiveness (cf. **Chapter 6**) and cost-effectiveness, which is an operational decision (cf. **Chapter 8**).

Table 5.1. Range of studies and key endpoints for evaluating the entomological efficacy of SIT through the phased conditional testing process. Since there are no hard and fast rules for whether SIT is performing sufficiently well, especially in later phases of evaluation, the go/no-go criteria will be established for a specific context, i.e., the criteria included in the table are only illustrative examples.

Phase of Testing Stage	Outcome or Endpoint	Indicator	Go/No-Go Criteria
Phase I: Laboratory studies	Performance of irradiated males	Pupal mortality during radiation process	<10% instantaneous mortality at target radiation doses
		Survival/longevity	Sterile irradiated male adults suffer <10% reduction in median survival times compared with equivalent non-irradiated males
		Flight ability	<10% reduction in flight activity compared to non-irradiated males
		Sterility level	Asymptotic dosimetry curve calculated to deliver >99% sterility with minimal impact on other performance traits
	Population suppression potential	Mating competitiveness in cage (1:1:1 ratio)	Fried C Index[15]>0.7
Phase II: Contained and small-scale field trials	Performance of irradiated males	Rate of induced sterility in females	>90% reduction in viable egg production in lab-cage populations for an over-flooding ratio of 10:1
		Rate of induced sterility in females	Fried C Index >0.5
		Longevity in large cages	Sterile irradiated males suffer <10% reduction in average survival times compared with non-irradiated males
	Entomological efficacy in the field	Survival rate	Strongly system dependent, but should be measured to guide subsequent release frequency required to achieve a homogeneous ratio of sterile:wildtype males
		Dispersal rate	Strongly system dependent, but should be measured to guide subsequent spatial distribution of release to achieve a homogeneous ratio of sterile:wildtype males
		Mating competitiveness in the field	Fried C Index >0.2
		Induced sterility rate in the local population	Statistically significant induced sterility (absolute value is difficult to define a *priori*)
		Vector density in treated and control areas	Statistically significant suppression of local vector population (measured as eggs or adults)

[15] *Fried's Competitiveness Index (cf. Glossary).*

Phase III: Large-scale field trials to determine entomological (and epidemiological) efficacy	Entomological efficacy in the field	Vector density in treated and control areas	Statistically significant reduction in vector density between treated and control areas (a threshold may be needed or not, and, if needed, it is determined by local/site-specific transmission ecology).
		Transmission potential	Evaluation criteria could include declines in the number of blood-fed females collected in the treated area, proportion of infected vectors and bites per person per day.
	Epidemiological efficacy	cf. **Chapter 6**	cf. **Chapter 6**
Phase IV: Large-scale trials to evaluate effectiveness under operational conditions	Entomological effectiveness in the field	Vector density in treated and control areas	Significant reduction in vector density between treated and control areas
	Epidemiological effectiveness in the field	cf. **Chapter 6**	cf. **Chapter 6**

5.2 Outline of phase I studies

Phase I laboratory tests examine the intrinsic biological activity of the vector control tool. In the case of SIT, the aim is to characterize the negative consequences of mass rearing in artificial conditions and irradiation on the performance of male mosquitoes and the positive consequences of irradiation on reducing the reproductive output of a natural population of female mosquitoes (i.e., the control potential). If, in controlled laboratory settings, irradiated male mosquitoes have limited capacity to fly and mate or fail to induce marked reductions in female fecundity, there is little point in scaling up to subsequent evaluation phases, and this will result in a no-go decision in terms of advancing to the next stage.

Note: These evaluation phases assume that mosquito production and irradiation processes have been developed to produce a suitable SIT product for testing (cf. **Chapter 4**).

5.2.1 Survival and longevity

Survival and longevity of the released sterile male mosquitoes are important parameters that may affect the success of an SIT programme. The aim is to use a radiation dose sufficient to generate high levels of male sterility (assessed based on a dose response curve), but with minimal impact on other traits (Yamada et al. 2014). Survival in controlled laboratory settings in small cages likely will be high, making it difficult to observe subtle differences compared with non-irradiated males and females. Nonetheless, the longer the sterile males can survive, the higher the probability of mating with a wild female (assuming they remain sexually active as they age). A typical method of comparison would involve simultaneously recording daily mortality of irradiated and non-irradiated adult male mosquitoes in laboratory cages, with all mosquitoes having constant access to sugar water (Bellini et al. 2013a; Bond et al. 2019).

Indicative go/no-go criteria:

(1) <10% instantaneous male mortality at target radiation doses;
(2) <10% reduction in average survival times of sterile irradiated males compared with equivalent non-irradiated males;
(3) >99% sterility of irradiated males.

5.2.2 Mating competition and impact on female fecundity

Another go/no-go decision point is whether the irradiated sterile males are sufficiently competitive with the non-irradiated males, such that they reduce the reproductive output of females when released at appropriate ratios. The fertility of the males can be assessed by measuring the hatching rate of eggs produced in controlled conditions by a known number of virgin females after mating with an equivalent number of males over one gonotrophic cycle (Bellini et al. 2013a; Bond et al. 2019).

Mating capacity of irradiated males can be measured by determining the number of females a single male can successfully inseminate over a pre-determined number of days (Bellini et al. 2013a). Overall competitiveness can be assessed through the calculation of the Fried Competitiveness Index (Fried 1971; Pagendam et al. 2018). Typically, the male mating competitiveness index (Fried's C Index) would be estimated by comparing the percentage of hatched eggs from cage experiments combining non-irradiated females and males, a mixture of non-irradiated females and irradiated males, and a mixture of non-irradiated females and both irradiated and non-irradiated males. However, recent research indicates that Fried's C Index can be estimated effectively using experiments from mixed

mating cages alone (Pagendam et al. 2018). By using different ratios of non-irradiated and irradiated males to mate with fertile females, the experiments can help to determine the release ratio of sterile males during field trials in phases III and IV.

Indicative go/no-go criteria:

(1) >0.7 for the competitiveness of irradiated males relative to non-irradiated males.

5.2.3 Flight ability

High radiation doses can reduce flight ability and mating performance of males. Estimating flight ability in the laboratory is challenging, but a flight cylinder assay previously used for other insects recently has been adapted to mosquitoes and proven to be not only a robust tool for evaluating flight ability, but also a rapid assay method that correlates well with multiple measures of male mosquito quality (Balestrino et al. 2017; Culbert et al. 2018; Bond et al. 2019).

Indicative go/no-go criteria:

(1) <10% reduction in flight ability of irradiated males relative to non-irradiated males.

5.3 Outline of phase II studies

5.3.1 Semi-field evaluations

Phase II semi-field studies add more ecological realism than can be achieved within simple laboratory environments, while still retaining a relative high level of experimental control. This research can generate valuable insights in situations where there is no prior experience with SIT. Studies could include experiments in large field cages to measure female fecundity, percent egg hatching, and capacity to induce sterility, calculated by placing different combinations of non-irradiated (fertile) and irradiated (sterile) males into semi-field enclosures with fertile female mosquitoes and comparing them with an analogous combination of non-irradiated males and females in control enclosures (for illustrative examples of semi-field studies, cf. Chambers et al. 2011, Olivia et al. 2012 and Bellini et al. 2013a). Survival can be measured either

by recovering mosquitoes at the end of the observation period or by counting daily survival of males housed in individual cages within the semi-field setup.

Indicative go/no-go criteria:

(1) >90% reduction in viable egg production in mosquito populations in semi-field studies for an over-flooding ratio of 10:1;
(2) Fried C Index >0.5;
(3) <10% reduction in average survival times of sterile irradiated males compared with non irradiated males.

5.3.2 Small-scale pilot field trials

Release strategies for SIT aim for a homogeneous ratio of sterile to wild males over time and space. The dispersal distance of the released males determines the optimal density of release sites, with the optimal frequency of release determined by survival rate. The greater the dispersal distance and the greater the survival rate, the less intensive the required release rates. Dispersal rate and survival rate can be estimated using mark-release-recapture of sterile males (Bellini et al. 2010). In addition, as a tool for estimating egg density or calculating the egg hatch rate or percentage of sterile eggs collected, ovitraps placed in treated versus control areas can be used to determine the capacity of irradiated males to induce sterility in the local population (Bellini et al. 2013b). Adult mosquitoes also can be sampled to provide direct measures of density and the egg hatch rate of gravid females (O'Connor et al. 2012).

Indicative go/no-go criteria:

(1) Survival rate (will be system dependent, but should be measured);
(2) Dispersal rate (will be system dependent, but should be measured);
(3) Male competitiveness (Fried C) index >0.2;
(4) Statistically significant induced sterility (measured as reduced egg hatch rate);
(5) Significant suppression of local vector population (measured as reduced egg or adult density), although this will likely depend on the scale of releases, as small-scale studies will be more affected by immigration of mosquitoes from adjacent populations.

5.4 Outline of phase III studies

Phase III evaluations involve large-scale field trials that aim to achieve sustainable suppression of the target vector population over a large area. Such studies must include epidemiological evaluations (cf. **Chapter 6**), when SIT is tested in areas with disease transmission, and be combined with measurement of entomological indicators such as mark-release-recapture measures of adult population density, sterility rate (egg hatch rate), ratio of sterile to wild males, egg density and competitiveness index. In cases where SIT is being considered as a preventive tool to reduce the risk of disease establishment or outbreak (i.e., the disease is not necessarily present in the area at the time of implementation), the focus will be on entomological indicators. Phase III should provide solid evidence for decision makers as to whether the SIT programme should be integrated into a national vector control operation.

Indicative go/no-go criteria:

(1) The primary evaluation criterion for efficacy in large-scale entomological trials is a significant reduction in mosquito population density in the treatment areas relative to the control areas;
(2) Secondary entomological criteria could include declines in measures such as oviposition rate, number of blood-fed females collected in the treated area and proportion of infected vectors.

5.5 Outline of phase IV studies

Phase IV studies evaluate effectiveness as the experimental scale expands from trial to operational implementation. Effectiveness studies aim to evaluate an intervention under realistic operational conditions and, in so doing, provide additional insights for policy and practice. Typically, the entomological measures would be similar to those of phase III but might become less intensive as phase IV moves closer to routine monitoring and evaluation. National vector and disease surveillance programmes could provide routine monitoring and evaluation once the SIT programme is considered for integration into the national vector control operation (cf. **Chapter 9**).

Indicative go/no-go criteria:

(1) Demonstrated ability to sustain large-scale mass production and release protocols;
(2) Demonstrated ability to perform entomological and epidemiological surveillance at scale;
(3) Most critically, significant suppression of vector population density;
(4) Where relevant, epidemiological evidence indicating reduction in disease transmission.

5.6 General considerations and guiding principles

5.6.1 Defining objectives

A key starting point in the evaluation process is to clearly define the goals of the SIT programme overall (i.e., what control objective SIT is meant to deliver), as well as the objectives for each phase of testing. The primary objective is to produce irradiated mosquitoes that are sterile, competitive with wild males and able to suppress wild populations. The SIT works by reducing vector density, which, in turn, can reduce disease transmission. However, because the relationship between vector density and human disease can be complex, the epidemiological consequences need not be straightforward, even if SIT delivers measurable reductions in vector density. Moreover, different disease outcomes and targets potentially cover a range of objectives, such as reduction in disease incidence, reduction in disease prevalence, reduction in frequency and/or size of epidemics, local eradication and prevention or reducing risk of disease where the diseases are not yet present. Having clearly defined targets from the outset is important for framing and gauging the success of the control programme and for comparing the results of each phase against appropriate go/no-go criteria to progress through the phased conditional approach.

5.6.2 Selection of study site

Appropriate study sites must be selected for each of the phases of evaluation. As every study site will have unique features, general guidelines are difficult to convey. Nonetheless,

achieving and demonstrating impact will tend to be easier if:

- There is some level of geographic or ecological isolation for studies in phases II and III;
- The target mosquito species is the main vector in the selected site;
- Sites are of manageable size for surveillance and monitoring;
- There is good cooperation of the local government and local communities.

Consideration should be given to the location of treatment and control areas in order to minimize contamination or spillover effects due to the movements of mosquitoes or humans.

5.6.3 Baseline interventions as comparators

The SIT is not generally considered to be a stand-alone intervention. It is more cost-effective and easier to implement effectively if the density of the local vector population is low. Due to its mechanism of action, SIT acts too slowly to be an effective response tool for epidemics or outbreaks. Accordingly, SIT should be viewed as part of an integrated vector management strategy and its impact should be considered over the medium to long term and measured against a baseline of existing control tools.

5.6.4 Outcome (endpoints and effect size)

The SIT works by reducing vector density, but reductions in density alone do not necessarily lead to significant epidemiological impacts. This disconnect can make it difficult to interpret effect sizes, as there is no clear threshold that relates to transmission, which can be very heterogeneous in time and space for diseases such as dengue, as well as strongly influenced by the susceptibility of the human population.

Moreover, many indices are available to estimate vector abundance, including:

- Percent premises/houses positive for adults;
- Percent premises/houses positive for females;
- Percent houses positive for blood-fed females;
- Percent houses positive for males;
- Mean number of females per house;
- Mean number of blood-fed females per positive house;
- Mean number of males per positive house;
- Percent houses positive for immatures (pupae);
- Number of immatures (pupae) per house;
- Number of immatures (pupae) per number of household inhabitants;
- Container index (CI) = [number of containers with immatures/wet containers inspected] x 100;
- House index (HI) = [number of houses with immatures/houses inspected] x 100;
- Breteau index (BI) = number of positive containers per 100 houses inspected;
- Pupae per person index (PPPI) = ratio of pupae to persons living in each experimental cluster computed at cluster level.

Some of these measures are not necessarily good indicators of the mosquito population characteristics that are the most important for transmission, namely female mosquito density and longevity. Ovitrap data, for example, do not always correlate with adult density in most situations (Focks 2004). Similarly, indices such as the container index, house index and the Breteau index fail to take into account variations in container productivity and provide little information on transmission risk (Focks 2004). At least during efficacy testing, the emphasis should be on measures such as pupae per person, density of parous females, adult vector density or number of bites per person (pursuant to the ethical regulations for human landing catches).

5.6.5 Trial design

The quality of evidence for evaluating efficacy depends to a large extent on trial design. Details of methods for planning and conducting entomological trials are beyond the scope of this document, but readers are directed to the WHO manual on study design of field trials for vector control interventions (WHO 2017) and other resources (Wilson et al. 2015; WHO 2018).

In **Figure 5.1**, we present a hierarchy of study designs utilized by WHO for evaluating the efficacy and methodological quality of vector control interventions (Wilson et al. 2015; WHO 2017). Studies with limited replication, poor randomization and inappropriate control treatments will tend to provide poor-quality evidence, and thus risk failing to show an impact where there should be one, or, alternatively, suggest an impact when in fact there isn't one. One of the challenges is that well-replicated studies with good controls and appropriate statistical power tend to be large in scale (certainly for phase III) and take a lot of resources to implement. The quality of evidence required ultimately is a programmatic decision, but it is important to appreciate the possible trade-off between cost and quality. Further discussions of trial design in the context of epidemiological outcomes are included in **Chapter 6.**

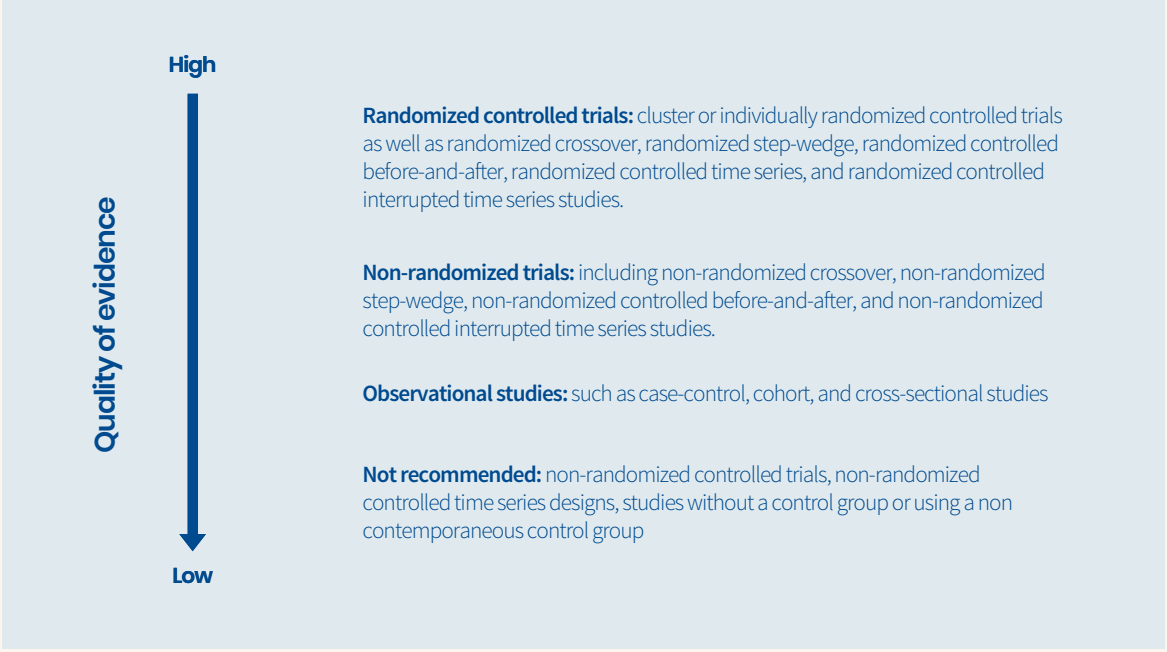


Figure 5.1. Hierarchy of study designs for evaluating the entomological and epidemiological impact of a vector control intervention (in this case, SIT) implemented at large scale (phase III). The study designs vary in terms of the methodological quality of evidence they provide (modified from Wilson et al. 2015).

5.6.6 Understanding of confounders

The SIT is species-specific. If there is more than one species responsible for transmission of the target vector-borne disease (e.g., *Ae. albopictus* is a competent vector of many of the same arboviruses as *Ae. aegypti*), the epidemiological impact of SIT could be diluted, even if the entomological impact is strong. This issue underscores the need to characterize study sites prior to intervention. Baseline data on mosquito species, seasonal density, dispersal of females and egg hatch rates, measures of disease transmission (incidence, prevalence, dispersal of females and possibly seroconversion rate), habitat features including density and distribution of households and potential mosquito breeding sites, existing control operations etc. should be collected for both treatment and control sites. These data can help guide study design (e.g., restricted randomization of treatment and control clusters) and inform the interpretation of results.

5.6.7 Quality control

For the efficacy studies for **phases I** to **III**, quality control checks should be in place to ensure that the SIT intervention, along with any control arm interventions, is being implemented in an optimal manner. The aim of these initial phases is to evaluate the intervention under optimal conditions. Without good quality control, it is difficult to determine whether, for example, poor results stem from SIT having limited impact in a particular location or because it wasn't implemented rigorously. **Phase IV** studies provide information on how robust the effects are under realistic operational conditions (i.e., field setting).

A key element in quality control is to ensure the quality of the SIT mosquitoes themselves. Regular checks should be conducted to assess and quantify flight ability and longevity and to demonstrate the capacity of the irradiated sterile males to induce sterility in the females (Balestrino et al. 2017; Culbert et al. 2018) (cf. **Chapter 4**).

Chapter 06

Epidemiological efficacy and effectiveness trials to evaluate SIT against *Aedes*-borne diseases

For any new vector control tools in new product classes, two control trials are the minimum number needed to assess their generalizability in a region (WHO 2017). Selection of appropriate intervention and control sites is a crucial step that impacts the success of any SIT programme. Besides addressing epidemiological and entomological aspects, trials must reflect practical considerations around, but not limited to, funding, infrastructure and ethical and socio-economic factors. Epidemiological and entomological indicators and endpoints also need to be determined/assessed before trial initiation, at baseline and again post-intervention. Establishing an independent expert group to validate the interpretation of the results is recommended.

This chapter highlights various steps to be undertaken while planning and conducting epidemiological trials—in due consideration of the available funding, existing infrastructure, feasibility, acceptability, as well as ethical, social, legal and other considerations—to determine the efficacy and effectiveness of the intervention.

6.1 Introduction

The SIT is aimed at suppressing the mosquito vector population to an extent that will significantly reduce infection and/or disease from *Aedes*-borne viruses, i.e., dengue, chikungunya, yellow fever, Zika and any other arboviruses transmitted by this mosquito genus (Almeida et al. 2019; Jansen & Beebe 2010; Lees et al. 2015). The SIT's efficacy and effectiveness will be a critical determinant for decision making about deployment (Almeida et al. 2019; Alphey et al. 2010; Benelli et al. 2016; Bonizzoni et al. 2013). In addition, if it is to be used as a public health intervention tool, SIT must be shown not to be detrimental to human health or the environment.

Epidemiological efficacy trials test the efficacy of SIT against the diseases in selected geographical sites by comparing intervention sites with control sites under stringent study conditions. In phase IV, effectiveness trials test the operational effectiveness of SIT over larger geographical areas under local programmatic, i.e., real-world, conditions and assess its capacity to reduce infection and/or disease burden (which are the epidemiological endpoints).

In addition to assessing operational feasibility, effectiveness trials collect information on the release mechanisms, acceptability and economics (including a cost effectiveness analysis). The entomological outcomes and a subset of the epidemiological outcomes assessed during the efficacy trial should be continuously monitored to ascertain whether the positive effects on human populations are being sustained. Plans should include scale-up of disease surveillance and monitoring systems to assess SIT impact at a population level.

The process of carrying out an efficacy study on epidemiological outcomes for SIT targeted at *Aedes* mosquitoes includes several steps (WHO 2017), which are described below.

6.2 Step 1: Develop the PICO question

PICO (Population Intervention Comparator Outcome) is the standard epidemiological question (Huang et al. 2006), which, applied in the SIT context, will answer the following clinical question: Is SIT efficient and effective in reducing the incidence of *Aedes*-borne infection and/or diseases in human populations, including, but not limited to, dengue, chikungunya, yellow fever and Zika?

Efficacy will be a critical determinant for decision-making about deployment.

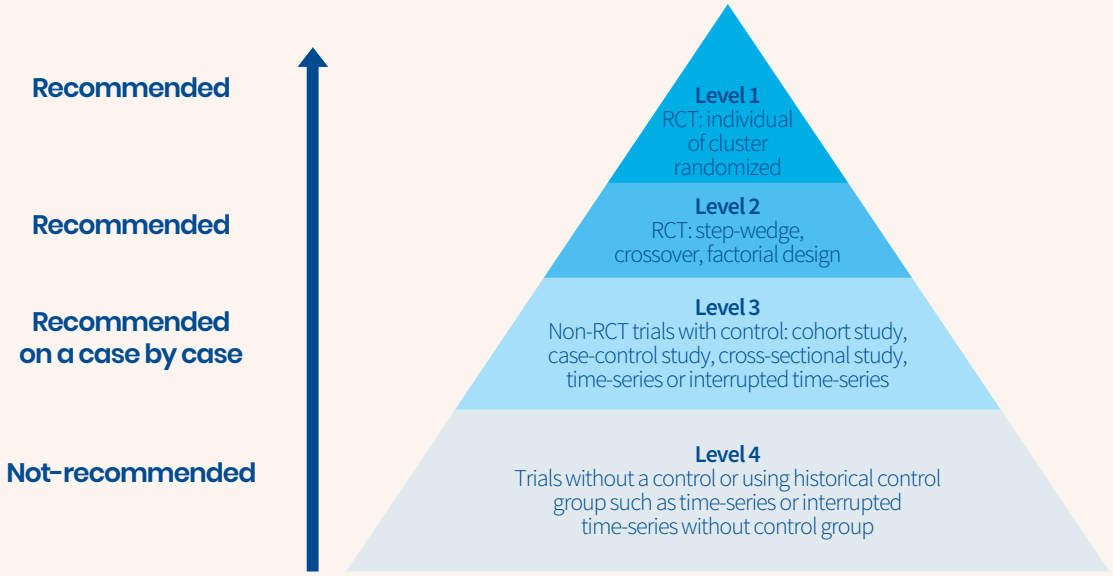
Without a well-focused PICO question to define the population, the intervention, the comparator and the outcome (**Box 6.1**), it can be very difficult and time-consuming to identify appropriate resources and search for relevant evidence. Practitioners of evidence-based practice (EBP) use the specialized PICO framework to formulate the question, facilitate the literature search and provide the relevant answer.

Box 6.1. Defining PICO: Population, Intervention, Comparator and Outcome for SIT against *Aedes*-borne diseases

- **Population**: Any community affected by *Aedes*-borne infection(s) with documented reporting of cases for at least a few years, and not in an epidemic situation or moving out of an epidemic. Stable populations with minimum mobility are preferable. Due to the importance of accurate disease surveillance, the health agencies of the country must have a surveillance system in place to capture all information related to cases occurring in that population.
- **Intervention**: Releases of sterile *Aedes* mosquitoes (via SIT). This intervention can be in the context of prevalent routine vector control measures in a particular area.
- **Comparator**: Comparison with stand-alone prevalent routine vector control measures.
- **Outcome**: Reduction in infection and/or disease caused by *Aedes* mosquitoes, i.e., dengue, chikungunya and/or others.

6.3 Step 2: Design of the study

Depending on disease pattern, variability, temporal trends, country infrastructure, available funding, personnel and logistics, intervention trials can have varied study designs like randomized control trial (RCT), cluster-randomized trial (CRT), stepped wedge randomized trial or the rolling-carpet principle or wave principle methods (Grayling et al. 2017; Hemming et al. 2015; Heintze et al. 2007; Kroeger et al. 2006; Vanlerberghe et al. 2009; WHO 2017). Randomized controlled trials (individual or cluster) and follow-up over at least two transmission seasons are WHO-recommended trial designs to demonstrate the public health value of new tools that do not fall within an already existing class; these are followed by stepped wedge and non-randomized control trials on a case-by-case basis (**Figure 6.1**) (WHO 2017).



Hierarchy of study designs recommended and not-recommended to evaluate the public health value of new vector control tools (RCT = Randomized Control Trials)

Figure 6.1. Hierarchy of trial designs recommended by WHO and which type of design WHO considers sufficiently robust to generate evidence on the efficacy and public health value of vector control products (extracted from WHO 2017).

6.3.1 Randomized controlled trials and cluster-randomized controlled trials

The term randomized controlled trials (RCT) refers to interventions implemented in villages or any other units of population in which the intervention (programme of releases of sterile male mosquitoes) and the control units are randomly allocated, whereas the term cluster-randomized controlled trials (CRT) refers to interventions implemented in village, ward or urban administrative units called clusters (WHO 2017), which are randomly allocated to intervention arms or control arms. CRT is a commonly used study design for measuring the efficacy of vector control.

6.3.2 Stepped wedge cluster-randomized trial

In stepped wedge cluster-randomized trial (SW-CRT), the intervention is rolled out to clusters in a stepwise fashion, whereby the order in which clusters receive the intervention is determined by randomization (Grayling et al. 2017; Hemming et al. 2015; WHO 2017). An SW-CRT may be used when logistical, practical or financial constraints make the staged roll-out of an intervention desirable. SW-CRT should be performed only if a standard CRT cannot be carried out and good evidence already exists indicating that the intervention is effective and should be rolled out to the entire population.

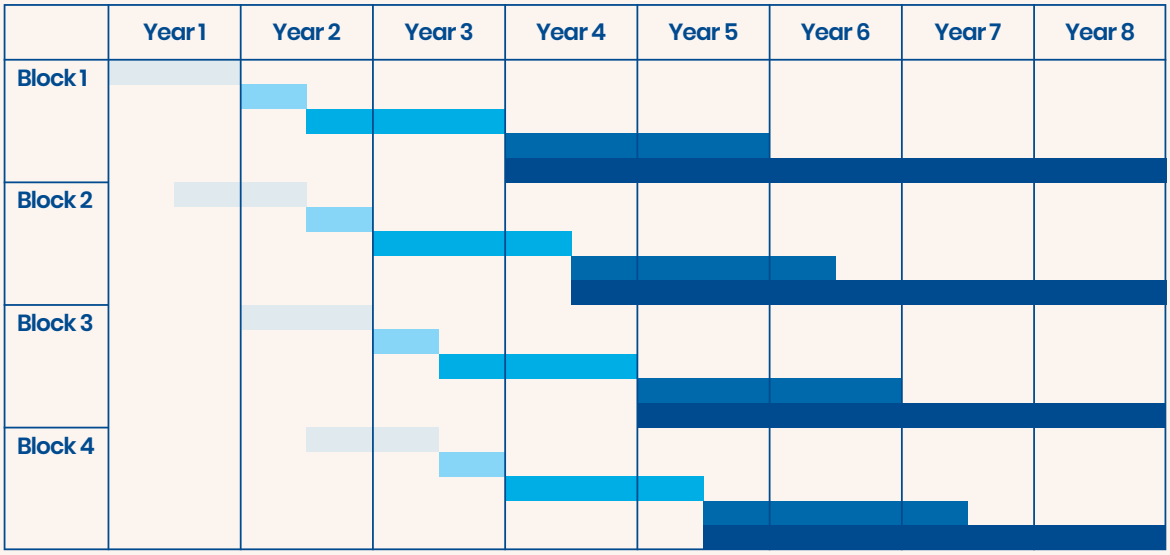
6.3.3 Rolling-carpet and wave principle methods

If baseline entomological studies have found that the target Aedes populations are distributed continuously, the rolling-carpet principle (Box 6.2) or the wave principle (Box 6.3) methods can be used to test SIT against Aedes-borne disease (Hendrichs et al. 2005). The rolling carpet principle entails a unidirectional front in interventions, whereas the wave principle employs a bidirectional or multidirectional front (Multerer et al. 2019).

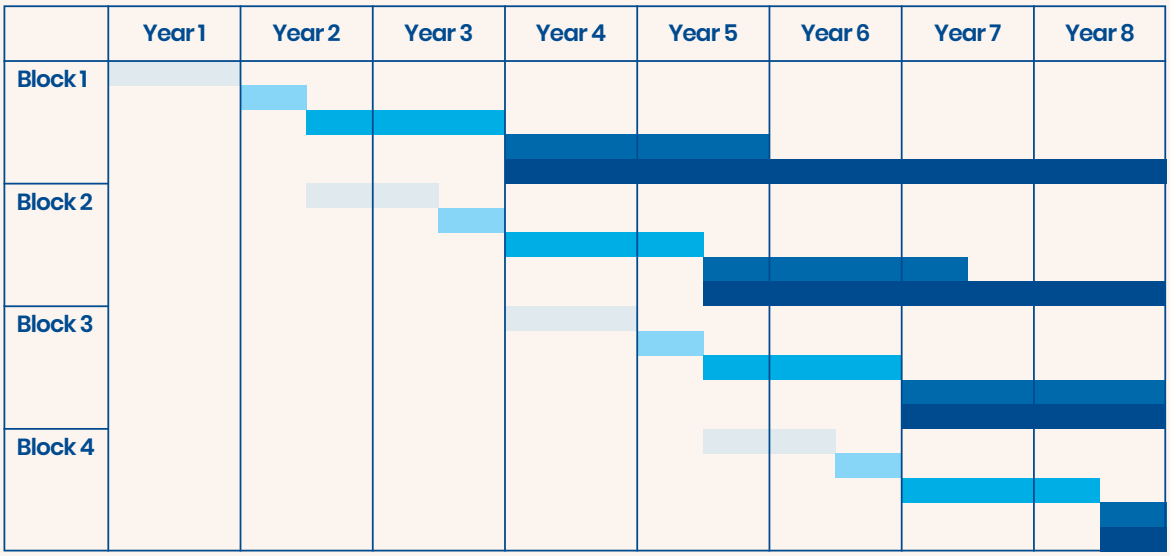
Box 6.2. Rolling carpet method

This method is more dynamic than CRT. In the rolling carpet method, the intervention area is divided into blocks (**Figure 6.2**). The estimation of baseline entomological and epidemiological indicators, vector control measures to bring down *Aedes* density, release of SIT against *Aedes* and post-release determination of entomological and epidemiological indicators are carried out simultaneously in a sequential manner in different blocks. This approach can be more cost-efficient than a static CRT approach, in which each of the four different phases would be implemented in a given block, before proceeding to the next block (Hendrichs et al. 2005).

Overlap of releases in adjacents blocks



NO overlap of releases in adjacents blocks



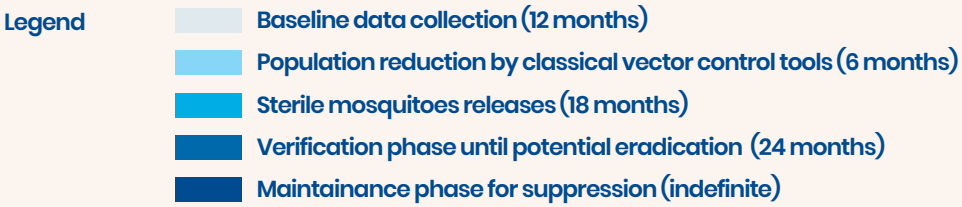


Figure 6.2. Temporal (upper, with or without overlap of releases in adjacent blocks) and spatial (lower) diagrams of the rolling carpet principle applied in four intervention blocks using eradication and suppression against a pest population distributed continuously (adapted from Hendrichs et al. 2005).

Box 6.3. Wave principle

One type of area-wide integrated pest management (AW-IPM) is tested/deployed according to the wave principle, whereby the intervention moves in expanding operational block sizes at each stage. The intervention develops along a multidirectional front beginning from Stage 1 (collection of baseline data) and continuing to Stage 2 (reductions in vector population), Stage 3 (releases of SIT *Aedes*) and Stage 4 (areas with population suppression) (**Figure 6.3**). Since each successive phase requires increasing amounts of sterile males and abundant resources are needed to sustain the expansion, the wave principle method is more resource-intensive than rolling-carpet (Hendrichs et al. 2005). Mobile insectaries may be needed to overcome the logistical hindrances.

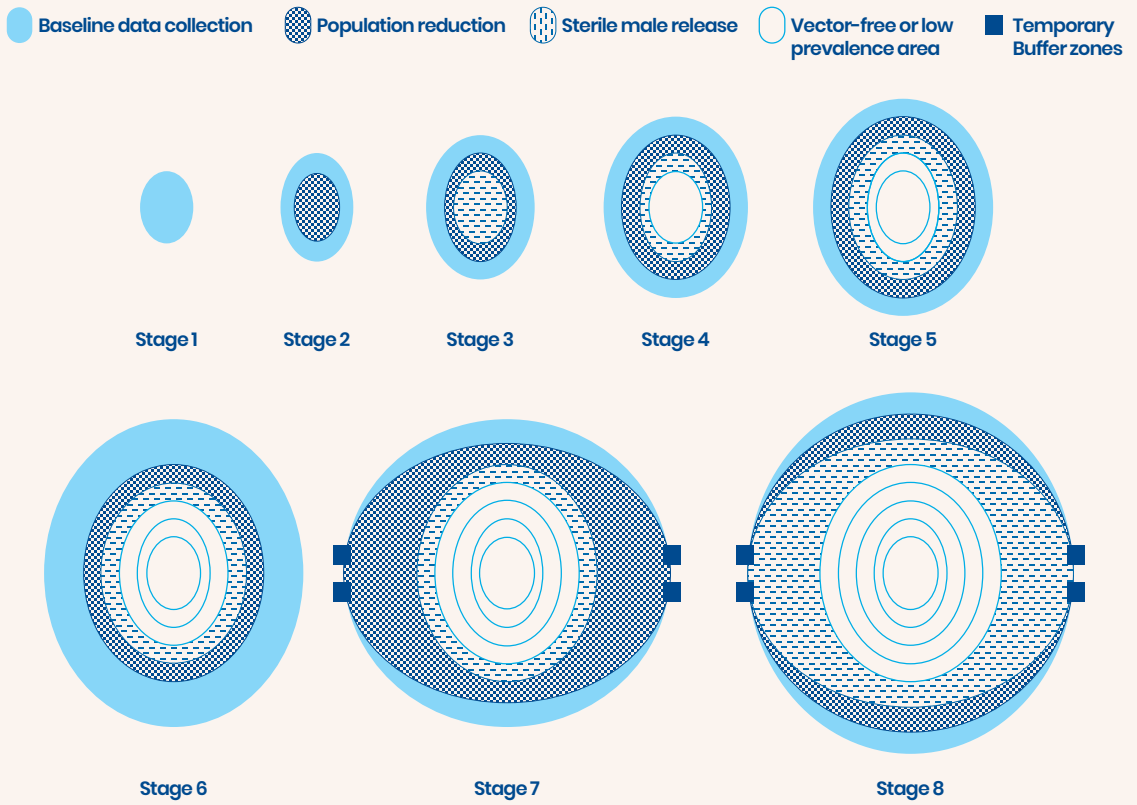


Figure 6.3. Diagram with the different stages of an AW-IPM programme using SIT according to the wave principle against a pest population with a continuous distribution. In this theoretical example, the intervention develops along a multidirectional front in the first stages until full production capacity of sterile males is reached. Beginning in Stage 1, the intervention continues along a circle front and requires the establishment of temporary buffer zones (Hendrichs et al. 2005).

Because the results are available more quickly and accrue over time, the planners and implementers of an SIT programme may find it advantageous to deploy the rolling-carpet or wave methods. The sequential nature of these approaches means the requirements for personnel, logistics and sterile mosquitoes are staggered, thus implementation becomes less challenging in comparison to a CRT approach of one phase across all blocks simultaneously (Heintze et al. 2007; Vanlerberghe et al. 2009). Moreover, the indicators estimated at different time points in different blocks through these approaches may give a better picture of variation in entomological and epidemiological parameters.

Important note: Before selecting any of the aforementioned study designs, a country's resources (e.g., public health surveillance system, trained workforce, sterile mosquito production capacities, funding and infrastructure) must be evaluated. The selected epidemiological trial must be statistically robust and designed to measure reductions in an endpoint such as seroconversion/seroprevalence.

6.4 Step 3: Determine the sample size

The RCT or CRT are recommended as the best methods to assess the comparative clinical efficacy and effectiveness of SIT; they can also provide data for estimating cost effectiveness. The optimal design of an RCT is dependent on the sample size calculation, which must ensure that the study not only has a high probability of producing significant results, but also is based on the ethical recruitment of study participants, to avoid imposing clinical trials on more patients than necessary. One recently adopted approach specified a target difference between treatments that was considered realistic or important by one or more key stakeholder groups. Called DELTA (Cook et al. 2018), this approach allows a sample size calculation which ensures that the trial will have the required statistical power to identify the existence of a difference of a particular magnitude.

Another element to take into account in a CRT sample size calculation is the variation between clusters. Outcomes measured in individuals or sampling sites within the same cluster are likely to be more similar than if they are measured between clusters; therefore, the sample size calculation needs to account for the additional variation in outcomes between clusters (WHO 2017). The degree of variation in the outcomes between clusters is measured by the coefficient of variation, which is defined as the ratio of between-cluster standard deviation to the mean:

$$k=cv=(\text{standard deviation}/\text{mean}) \times 100$$

The sample size is thus estimated as a function of the coefficient of variation within an interval of confidence (Kristunas et al. 2017). The number of clusters should be sufficient to allow differences between the test and control arms to be detected statistically.

6.5 Step 4: Site selection for intervention and control

Selecting appropriate intervention and control sites is a crucial step that will influence the success of the SIT release programme. Hence, the choice of the site should be based on the criteria described below and in consideration of the local situation (Benedict at al. 2008; Hendrichs et al. 2005; Iyaloo et al. 2014).

The three primary epidemiological criteria upon which to base the choice of sites for SIT testing are: (1) an isolated transmission situation, (2) a single vector species and (3) significant disease transmission at more or less periodic intervals (Malcolm et al. 2009).
It may not be possible to meet all of the above criteria in communities where arboviral diseases are circulating, i.e., mostly urban and peri-urban areas. Multiple vectors with different ecologies (inter alia Ae. aegypti, Ae. albopictus, Aedes polynesiensis) may be involved in transmission (Eder et al. 2018; Telle et al. 2016) across local, focal and heterogeneous sites. Although they vary in space and time, transmission foci are connected at short distances by a combination of human and mosquito movement patterns (WHO 2017). The human populations of both the intervention and control sites need to be fully characterized with regard to various parameters, such as:

- Socio-demographic (population characteristics, age and socio-economic structures, health-seeking behaviour);
- Epidemiological (disease pattern, number of reported cases, dengue and severe dengue cases, age distribution);
- Environmental (meteorological conditions, housing type, housing conditions, water storing habits, presence of potential breeding sites, general layout of the houses).

Note: It is recommended that the intervention and control sites have similar availability, accessibility and level of healthcare.

A small-scale pilot trial allows assessment of the practical and technical challenges associated with i) initiating and sustaining a more extensive suppression programme, ii)

measuring the effect of the intervention and iii) controlling for any natural fluctuation in target populations unrelated to the trial (Wilson et al. 2015).

Allocation: Since SIT against *Aedes* is also a community-based intervention, allocation of selected sites to intervention or control arms needs to be at the cluster/village level (Iyaloo et al. 2014).

Practical considerations around epidemiological indicators:

- Functioning infrastructure for disease surveillance and reporting: Costs can be saved if facilities which can be used for the project are already in place;
- Study sites of manageable size and favourable topography, with regard to disease transmission: The strength and validity of the study must be balanced with the available resources and workforce;
- Ethical, social, legal and other considerations (cf. Chapter 7): Criteria for selecting a field location may include a previous record of authorization to work in a particular area. This approval may be granted by local communities, local authorities and appropriate regulatory and government agencies (cf. **Chapters 1** and **7**).

6.6 Step 5: Baseline parameters

Epidemiological data must be generated for both the intervention and control sites before initiating the intervention. Depending on the type and number of vector species present, the difference in the ecology of transmission across the sites needs to be taken into account.

6.6.1 Epidemiological endpoints

The major epidemiological indicators are detected through active or passive surveillance and include incidence, prevalence and the number of cases, seroconversion rates, serotypes, size and speed of outbreaks. The indicators best adapted to the situation must be chosen to assess the impact of SIT as an intervention strategy. Once endpoints have been decided upon, baseline parameters must be assessed for both intervention and control sites with equal uniformity, robustness and rigour.

6.6.2 Contamination effect

The movement of vectors and humans between clusters can cause contamination effects between study arms. Major interference would be a direct contamination of the control group, while partial interference is spillover effects within the same treatment group. However, when spillover occurs, participants (or units) in the control group may experience a direct or indirect treatment effect from the programme that can make it difficult to accurately interpret study findings (Wilson et al. 2015).

Movement of mosquitoes, specifically the immigration of wild infected female mosquitoes into the release clusters and the emigration of sterile male mosquitoes and sterile females inseminated by sterile males, can confound the interpretation of releases with regard to disease transmission and prevent a positive trial outcome (Kittayapong et al. 2019). Measurements of dispersal (mark-release-recapture) can guide the selection of conditions that provide sufficient isolation or the implementation of appropriate buffers to prevent such immigration (cf. **Chapter 5**).

Since the SIT intervention discussed in this guidance document targets *Aedes* mosquitoes, confounding factors have to take *Aedes* mosquito behaviour into account. Since *Aedes* mosquito females bite during the day when people are active, the movements of people

(daily or occasional) can influence the impact and assessment of the intervention. For this reason, it is important to capture data about not only the number of people who live in the experimental cluster, but also their activities and displacement in and out of the cluster.

The above problem can be mitigated by selecting a large enough study area to include large numbers of individuals, which dilutes the risk of infection outside their respective area. Further trial planning includes social surveys to identify individuals who may become infected outside of the trial area. Collecting participants' travel histories allows statistical analysis of the number of people exposed to the risk of infection and the number of people less exposed because they have travelled for significant periods of time and spent a relatively brief period of time being exposed.

Monitoring epidemiological outcomes in a less mobile sentinel cohort (women, children) within a larger cluster area is one of the strategies for improving the reliability of the data.

6.7 Step 6: Blinding

Studies are single blind when the participants do not know which treatment group they have been assigned to, double blind when study participants and investigators are unaware of which group is control vs. intervention, or triple blind when study participants, investigators, laboratory staff and those analysing the data are all blinded (Wilson et al. 2015). Blinding the participants, healthcare providers (or outcome assessors) and researchers to the intervention received by participants can reduce two important forms of bias: performance bias and detection bias.

Performance bias occurs when there are systematic differences in the diagnosis/care received by participants in the intervention and control arms. This can be due to differences between the study arms in terms of participants' willingness to seek diagnosis/treatment or use personal protective measures or the level of diagnosis/care provided by healthcare staff.

Detection bias occurs when there are systematic differences in how outcomes are assessed between participants in the intervention and control arms.

In SIT trials, blinding of study participants is not possible, since community engagement programmes must be in place and communities informed about the intervention proposed. At the same time, due to a sense of security/protection from the intervention, the population living in intervention cluster may reduce its use of routine protective measures against vectors, such as taking personal protective measures against mosquito bites during the day. This type of behavioural difference may have an impact on the

transmission force of the diseases and must be acknowledged via studies to detect any difference between the personal protection behaviour in both intervention and control arms.

However, even if performance bias cannot be avoided, to prevent/mitigate detection bias, it is still important to maintain the blinding of those assessing the outcome as much as possible.

6.8 Step 7: Implementation of the intervention and partnerships to be established for field testing

Since the trial is to be undertaken at multiple sites, related partner organizations should be identified to be involved in the trial at the various geographical points. Engaging a trial design specialist is helpful in planning and conducting the trial. Various approvals and clearances must be in place before embarking on implementation of the intervention in a community.

Institutional ethical review committee clearances are needed from all participating centres. Trial planning should take into consideration the ethical issues covered in **Chapter 7**. Intervention trials must be registered in the country's clinical trial registry and results presented to the local authorities at regular intervals (Weijer et al. 2012; Taljaard et al. 2013).

The following partnerships are requested before the implementation is started and at all phases of testing:

- Regulatory approval. The identity of this authority may differ from country to country. In view of the novelty of the technology, national legislation may entrust this responsibility to a board/commission representing several ministries like health, environment and vector control (cf. **Chapter 3**);
- In-depth interactions with all relevant stakeholders, including media. (cf. **Chapter 7**);
- Community engagement exercises to cultivate understanding and acceptance by communities;
- Meticulous planning for releases and subsequent fieldwork as well as collection of data on epidemiological parameters.

6.9 Step 8: Measuring the outcome and effectiveness of the technology

6.9.1 The epidemiological outcome indicators

The endpoint is a reduction of disease. Reduction of disease can be measured by various means, including infection incidence, clinical disease incidence or prevalence of infection in at-risk populations and seroprevalence in a surveyed population (Cromwell et al. 2017).

At least two years of data (exclusive of baseline data) are required to effectively demonstrate abatement in areas where disease transmission is highly variable from year to year, with epidemic waves alternating with low prevalence periods. However, even two years may not be enough, with supplementary data from additional years of study needed.

The epidemiological parameters used as outcome indicators are decided upon at the beginning of the trial (**Box 6.4**) and assessed at periodic intervals, with the frequency of assessment depending on the parameters and other factors. The epidemiological endpoints could be the number of cases detected through active or passive surveillance, seroconversion rates, circulating serotypes and outbreaks. It is essential for data to be collected with equal rigor in both the intervention and control sites.

Box 6.4. Examples of outcome indicators

At locations with historically high levels of dengue infection, where the population is exposed to dengue virus (DENV), the population develops homotypic protection against the infecting serotype and temporary cross-protection (heterotypic protection) to other serotypes lasting up to two years (WHO 2018). In this previously exposed population, the transient rise in IgM and a 4-fold rise of IgG, which are the indicators required for confirming a recent dengue infection, would be difficult to capture and equally difficult to interpret. Moreover, in locations where more than one flavivirus is transmitted (inter alia, yellow fever, Japanese encephalitis, West Nile virus, Zika) or where people have been vaccinated against other flaviviruses (e.g., yellow fever, Japanese encephalitis), interpretation of DENV seroconversions needs to account for cross-immunity to closely related viruses.

Therefore, in endemic countries where a large portion of the population is affected by high levels of infection acquired during regular outbreaks, it would be more efficient to assess the impact of the intervention (in terms of a reduction in dengue infection) in a dengue-naïve population which has not been exposed to dengue infection, typically children of less than a determined age. This dengue-naïve population (age cut-off depending on endemicity level) can be monitored by any of the different tests available for dengue diagnosis over different time points to assess for seroconversion, reflecting the exposure to dengue viral infection.

The baseline data must be collected in both the intervention and control arms before the start of the intervention. The same indicators must be followed during and after the intervention, at an adequately chosen time interval, such as one-year interval (primarily to cover the dengue transmission season). It is important that the same individual children, in both the intervention and control sites, are tested at the defined regular intervals always with the same methodology as was done at baseline. The results will provide the proportion of dengue-naïve population (children) who have seroconverted within a pre-defined time period, such as at one-year intervals, for example. Repeating this procedure at regular time intervals enables following the seroconversion dynamic in both the intervention and control sites, comparing the impact of the intervention (SIT) on disease infection into a naïve population.

To complement the more classical epidemiological indicators, other indicators can be collected, with the caveat that such indicators require additional resources in terms of funding, materials and personnel. Performing a cost-effectiveness analysis will assist in choosing the best indicator to follow. Other indicators include:

– Routine febrile surveillance consisting of one to three visits per week per household of people living near cohort participants to enable longitudinal comparisons of people with documented arboviral illness (Reiner at al. 2016);
– Geographical cluster studies that screen people living within a designated radius (ca. 100 m) of a person with a laboratory-diagnosed dengue virus infection (the index case) to measure variation in fine-scale spatial patterns of DENV transmission (Reiner et al. 2016);
– Serological plaque reduction and neutralization assays for virus detection, along with active surveillance, performed on a subgroup of people with clinically apparent infection may yield more accurate information on dengue risk. However, in areas which are endemic for other flaviviruses, cross-reactivity with dengue virus is a common occurrence, making it difficult to evaluate impact on the four different dengue virus serotypes (Yung et al. 2016; Jewell et al. 2018);
– The epidemiological impact of SIT also is assessed by test-negative design where dengue cases and arbovirus-negative controls are sampled concurrently from within the population of patients presenting with undifferentiated febrile illness, with case or control status classified retroactively based on the results of laboratory diagnostic testing.

Efficacy can be estimated by comparing the exposure distribution (the probability of living in an SIT-treated area among virologically confirmed dengue cases versus the exposure distribution in test-negative controls) (Anders et al. 2018; WHO TDR 2014).

An independent expert group should be established to validate the interpretation of the results and conduct technical reviews and assessments of epidemiological outcomes, as per standard procedures for all research activities.

When the intervention is moving from experimental to programmatic, its ongoing effectiveness in a public health programme must be determined. In this phase, the effectiveness of the vector control tool in operational use under real-world conditions is measured, as well as information collected on the feasibility, release mechanisms, acceptability, economics and safety of the tool (WHO 2017).

If the coverage area is wide, it may be necessary to conduct longitudinal bolstered case surveillance, managed by the public programme with/without support of research organizations.

The entomological and epidemiological indicators that were assessed during the efficacy trials must be monitored continually to determine whether the positive effects on human populations are being sustained (WHO TDR 2014). Epidemiological indicators can be followed in a subset/representative group of the exposed population (cf. **Chapter 9**).

Chapter 07

Ethics and community/ stakeholder engagement

This chapter highlights the topics of ethics and community and/or stakeholder participation in the process of testing the SIT intervention to control *Aedes*-borne diseases. While interlinked, the two topics have different purposes and objectives. When doing any research that involves human subjects, researchers must follow the highest possible ethical principles and standards stipulated in international research ethics guidelines. An essential component of these ethical principles is to inform communities and stakeholders and involve them in any research or intervention that will affect their health, life and wellbeing. Meanwhile, communities' and stakeholders' understanding of, support for and collaboration with the research and intervention is crucial for the successful implementation of any research activity, including SIT testing, and for sustaining the effect of the interventions. Therefore, the SIT testing team must take the subjects of ethics and community/stakeholder engagement into account from the very beginning of an SIT testing project.

7.1 Introduction

Testing SIT to control *Aedes*-borne diseases is, by nature, research on an intervention that may involve multiple stakeholders and communities. Based on international ethics guidelines for health-related research involving humans (CIOMS 2016); researchers, sponsors and health authorities have the moral obligation to ensure that all research is carried out in ways that uphold human rights, and that study participants and the communities in which the research is conducted are treated respectfully, protectively and fairly. Thus, the SIT testing team needs to follow research ethics guidelines and to consider, plan, prepare and execute various community and stakeholder engagement activities.

It is essential to comply with research ethics guidelines along every step of the SIT testing process, because the production and deployment of SIT may bring potential health and other risks to communities, depending on where the SIT mosquito factory is located or where the sterilized male mosquitoes will be released to control diseases. To be successful, the SIT intervention—as a new mosquito control tool—needs the understanding, support and collaboration of involved stakeholders and communities (WHO 2017; Bartumeuse et al. 2018). The SIT testing team needs to be aware that the diverse communities living where SIT testing is to be conducted are embedded within different socioeconomic, political, cultural, environmental and ecosystem contexts. This understanding will allow the team to fulfil its ethical responsibilities and plan and adapt its community participation strategies and actions based on locally prevailing conditions.

Using a life cycle approach, this chapter first highlights ethics challenges that may arise for the teams testing SIT interventions, then focus on community/stakeholder engagement and participation, which is indispensable for the implementation and long-term success of any SIT intervention.

7.2 Ethical issues

In any research project involving human subjects, researchers have an ethical responsibility and a moral obligation to protect the rights, dignity and welfare of the research participants. Any research involving human subjects or animals needs to adhere to international, national and institutional standards, principles and regulatory requirements for research ethics. The WHO Manual (Section XV.2) defines research with human subjects as:

"Any social science, biomedical, behavioural, or epidemiological activity that entails systematic collection or analysis of data with the intent to generate new knowledge, in which human beings:

i) are exposed to manipulation, intervention, observation, or other interaction with investigators either directly or through alteration of their environment; or
ii) become individually identifiable through investigator's collection, preparation, or use of biological material or medical or other records."

Based on the above definition, the testing of SIT to control *Aedes*-borne diseases with epidemiological outcomes falls into the category of research with human subjects, therefore SIT testing teams need to follow research ethics principles and conduct the SIT intervention and research in an ethical manner. Institutions and organizations with strict research ethics requirements include, but are not limited to, international organizations such as the World Health Organization (WHO); national organizations such as the National Institutes of Health (NIH), the National Science Foundation (NSF), the Food and Drug Administration (FDA), the Environmental Protection Agency (EPA), and the US Department of Agriculture (USDA) in United States, and similar bodies in other countries. Examples of research ethics guideline documents and policies, inter alia, include the International Ethical Guidelines for Health-related Research Involving Humans[16] (CIOMS 2016), the Singapore Statement on Research Integrity, the Code of Ethics of the American Society for Clinical Laboratory Science, the American Psychological Association's Ethical Principles of Psychologists and Code of Conduct, the American Anthropological Association's Statement on Ethics and Principles of Professional Responsibility, the Nuremberg Code and the World Medical Association's Declaration of Helsinki.

Among others, the International Ethical Guidelines for Health–related Research Involving Humans sets out 25 ethics guidelines for research and covers a broad range of ethical issues, including scientific and social value and respect for rights; research conducted in low-resource settings; equitable distribution of benefits and burdens in the selection of individuals and groups of participants in research; potential individual benefits and risks of research; caring for participants' health needs; community engagement; collaborative partnership and capacity-building for research and research review; informed consent; requirements for establishing research ethics committees and for conducting ethics reviews of protocols; public accountability for health-related research; and conflicts of interest.

[16] The International Ethical Guidelines for Health-related Research Involving Humans was written by the Council for International Organizations of Medical Sciences (CIOMS) in collaboration with WHO

Although these ethical codes, guidelines, policies and principles are important and useful, like any set of rules, they do not cover every situation and sometimes conflict and require considerable interpretation. Therefore, it is important for SIT testing teams to learn how to interpret, assess and apply diverse ethics rules for research and to make decisions and act ethically in various situations.

All research involving human beings should be reviewed by one or more independent ethics review committee(s) to ensure that the appropriate ethical standards are being upheld. Informed consent must be obtained from research participants, either as individuals or groups, as applicable (Creswell and John 2014). Since SIT testing contains many research components involving human subjects, SIT testing teams need to consider applying for an ethics review or clearance from one or several relevant ethics committee(s) for research and obtain ethics approval before beginning any research operation. The information submitted to the ethics committee for research includes, but is not limited to, the research protocol and disclosure of any conflicting interests (CIOMS 2016). However, different countries have different approaches to ethics reviews for research. In some countries, reviews occur only at an institutional level, in others at both a national and institutional level, in still others at a regional level (WHO 2011), therefore the SIT testing team needs to figure out the applicable requirements and act accordingly.

Currently, the process of testing SIT to control *Aedes*-borne diseases is divided into four phases (cf. **Figure 1.5**), with the SIT testing team potentially facing different ethical issues in each phase that need to be considered, planned for and acted upon.

Phase I (cf. **Chapter 4**) includes laboratory studies and laboratory mosquito populations in cages. No human subjects from the community population are involved during this phase. The laboratory staff members are subject to the obligations and rules of their working contracts, which in most countries include all required ethical considerations. Nonetheless, SIT testing teams must carefully assess and minimize risks to researchers and technical staff by specifying and explaining the risks; they also must be prepared to provide adequate compensation in case of an injury as a result of the research (CIOMS 2016). Ethical requirements, such as informed consent, are usually not needed in phase I. By contrast, communication strategies targeting different stakeholders and communities must be developed during this phase or even earlier.

Phase II is implemented in confined field trials and/or ecologically confined field trials and involves the communities situated where the trials are to be conducted. Consequently, it is necessary to obtain informed consent from community populations as a group or as individuals, unless a waiver can be obtained from an ethics committee. The SIT testing may require both individual and community consent. Individual informed consent refers to the voluntary and informed consent of individual research participants. Community/group consent is delivered to the SIT testing team by the leaders of the communities to authorize the trials with irradiated sterile male *Aedes* mosquito releases. On the other hand, the establishment of a mass-rearing facility typically requires an industrial permit from the relevant regulatory institution either at the local or national level (**Box 7.1**).

Community/group consent can serve several purposes. It can be used as a form of consultation with the community before individuals are approached, as a method of obtaining permission from leaders, or as an additional means of providing information. Community consent may be crucial in certain cases, but this must be in addition to, not instead of, properly informed individual consent. Most research ethics guidelines agree that informed consent must be obtained from research participants and that community consent does not replace informed individual consent (Nuffield Council on Bioethics 2004). In phase II, communication activities need to be undertaken to inform the communities about the nature, objectives, significance and implications of the trial, the possible risks and benefits that this trial will bring to them, and their rights to refuse or withdraw from the trial. The SIT testing team needs to answer questions and respond to concerns raised by the community.

Box 7.1. Informed consent process and rules

Informed consent—at both the community/group and individual levels—does not simply mean obtaining the signed informed consent form from the community or individuals, but signifies a process for providing adequate information about SIT testing to the community and individuals in an appropriate manner by the appropriate persons to allow the community and individual participants to understand the nature, potential benefits and risks of the SIT intervention, and, based on this understanding, to freely decide on participation or refusal.

The key success element for obtaining informed consent is effective communication between the research team and the community/participants. Researchers need to do their best to communicate balanced, understandable and objective information about the research activities and operations accurately, intelligibly and appropriately, taking into account local knowledge and beliefs.

Most research ethics guidelines recommend written informed consent, but thumbprint or verbal consent with a witness is acceptable in situations where participants are illiterate. Further, for research involving human subjects not capable of deciding because of their youth or disabilities, informed consent must be given by the responsible person. Even after informed consent is obtained, information about SIT testing must continue to be provided throughout the entire process.

In **phase III**, larger open field releases of irradiated sterile male mosquitoes in natural conditions will involve broader communities and more stakeholders. The SIT testing team will face increasing ethical obligations, potentially requiring informed consent from more stakeholders and intensified communication activities. To give just one example, if sterilized male mosquitoes need to be transported via a vehicle from the SIT factory to the sites where they will be released into the environment, the relevant authorities, such as administrators and traffic police, will need to be informed about this intervention.

In **phase IV,** a plethora of entomological, epidemiological and other disciplinary

research activities may involve many human subjects during implementation and post-implementation. People in the community may be surveyed to get their perception of the effects of the SIT intervention or asked to provide biological samples to determine its epidemiological impact, thus the SIT testing team must ensure that it is fulfilling its ethical obligations. In this phase, as in all others, the SIT testing team is responsible for keeping the research participants and their communities informed of the research progress via appropriate means, at suitable timeframes, in a language that people can understand (WHO 2011).

Throughout the entire SIT testing process, the SIT team needs to not only fulfil its ethical obligations, but also proactively inform communities about SIT monitoring, engage them into complementary control activities and encourage their acceptance of the technology. Fulfilling SIT programme staffing requirements by hiring from local communities (in conjunction with adequate training) is another effective way of fostering participation.

Ethics principles must include honesty, objectivity, integrity, carefulness, openness, respect for intellectual property, confidentiality, responsible publication, responsible mentoring, respect for colleagues, social responsibility, non-discrimination, competence, legality, animal care and the protection of human subjects; the International Ethical Guidelines for Health-related Research Involving Humans requires researchers to be sensitive to and respect communities' culture, traditions and religious practices (WHO 2011). For any research conducted on human subjects, researchers must take care to minimize harms and risks and maximize benefits; to respect human dignity, privacy and autonomy; to take special precautions with vulnerable populations; and to strive to distribute the benefits and burdens of the research fairly (Shamoo and Resnik 2015).

In order to conduct SIT testing in an ethical manner, the SIT testing team needs to take the actions outlined in **Box 7.2**.

Box 7.2. Minimum package of requirements to conduct ethical SIT testing

- Submit all required materials, including the research protocol, to the appropriate ethical review committee(s) (ERC) for review and approval before the starting the SIT testing.
- Plan communication strategies and prepare communication materials to inform communities and relevant stakeholders where the SIT testing will be implemented, on the nature of the testing, the potential benefits and risks associated with the testing.
- Obtain informed consent from the research participants and communities/groups (leaders), whenever necessary.
- Report to the ERC whenever there is any significant change in the research protocol and seek renewal of the ERC approval as needed.
- Regularly undertake structured ethics reflection within the SIT testing team; conduct research and the intervention ethically and responsibly.

7.3 Communities and stakeholder engagement or participation

The process of testing SIT to control *Aedes*-borne diseases will involve different communities and many different stakeholders. Communities are living near the sites where mosquito factories will be located, and in the villages, towns or parts of cities where the sterile male mosquitoes will be released. Stakeholders are defined as the individuals, groups, institutions, organizations, government bodies or other entities who have some decision-making power and can influence or are affected by the execution or results of SIT testing. Involving communities and stakeholders in SIT testing is not only an ethical requirement, but also imperative for the smooth implementation of SIT testing. Thus, stakeholder and community engagement are critically important for the success of an SIT programme (**Box 7.3**).

Box 7.3. Example of vector control intervention failure caused by a lack of stakeholder and community engagement

In Kenya, an initial distribution of insecticide-treated bed nets to protect people from malaria-infected mosquitoes faced challenges, not because the technology and distribution plan were not sound, but because the engagement of the community members was overlooked in the initial development of the technology (Chuma 2010). Further investigations performed after the initial distribution discovered that people had rejected the white-coloured bed nets because they mimicked the burial shrouds used by the local population. When new bed nets were manufactured in a different colour, adoption rates—and thus the impact of the technology on protecting people—increased dramatically (Gore-Langton et al. 2015). This exemplifies the importance of ongoing and iterative engagement with communities, particularly the value of creating partnerships with communities at an early phase of project/technology design and implementation to get their input and buy in (National Academies of Sciences, Engineering, and Medicine 2016).

The fourth edition of the International Ethical Guidelines for Health–related Research Involving Humans states that "from the inception of research planning, it is important to ensure full participation of communities in all steps of the project, including discussion of the relevance of the research for the community, its risks and potential individual benefits, and how any successful product and possible financial gain will be distributed, for example though a benefit-sharing agreement" and its Guideline 7 states: "Researchers, sponsors, health authorities and relevant institutions should engage potential participants and communities in a meaningful participatory process that involves them in an early and sustained manner in the design, development, implementation, design of the informed consent process and monitoring of research, and in the dissemination of its results".

Stakeholder and community participation[17] must continue throughout the entire process of SIT testing as show in **Figure 7.1.** However, the diversity of communities in different settings—in terms of socioeconomic development level, political system, culture and social norms, environmental conditions and ecosystem—affects and determines community members' values, beliefs, attitudes, knowledge, perceptions and behaviour towards diseases and diseases control. The SIT testing teams need to be aware of this and, before designing community engagement strategies, must conduct research using methods such as a situational analysis (UNICEF and WHO 2012) to understand the communities involved in SIT testing.

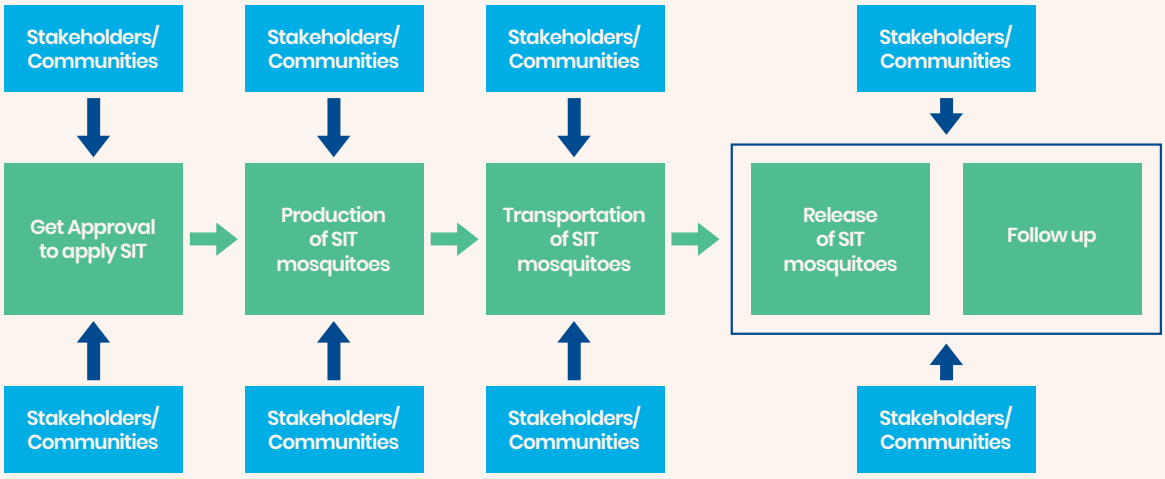


Figure 7.1. The SIT process and involved stakeholders and communities

[17] *Stakeholder and/or community participation are used interchangeably with stakeholder and/or community engagement.*

7.3.1 Definition of community and/or stakeholder participation

In its broad sense, community participation can be defined as "the process by which individuals, families or communities assume responsibility for their own welfare and develop the capacity to contribute to their own and the community's development" (Oakley 1987). In the context of development, community participation also refers to an active process whereby the beneficiaries influence the direction and execution of development projects, rather than merely receive a share of the benefits (Samuel 1987). In the context of SIT testing, community participation and stakeholder engagement can be understood as community and/or stakeholders being accepting of and/or actively involved in the development, trials and release/deployment of sterile male SIT *Aedes* mosquitoes. Community, here defined as a broad term, includes not only the rural or urban communities where the SIT mosquitoes are to be released, but also the scientific community, the health practitioner community, the mass media and any other group of people to be considered in the context. The SIT testing team must engage as early as possible with the different community/stakeholder categories (maybe at different phases of the project) and find out how best to communicate and engage with and tailor messages to each.

7.3.2 The purpose of community and/or stakeholder participation

There are at least two reasons for involving community and/or stakeholders in research. First, communities have the right to be provided with adequate information about research that may affect their health, environment and ecosystem and to freely decide whether to participate in the research or not. This ethical obligation is well stipulated by many international, national and institutional research ethics guidelines and principles, which state that for research involving humans, researchers have ethical responsibilities to inform the communities which will be affected either beneficially or negatively by the research and to obtain their informed consent for participation in the research. Second, real operational experience has demonstrated that community engagement and stakeholder acceptance and/or participation are essential for realizing project goals and objectives.

Community participation can build and maintain trust between researchers and communities, help researchers adapt research design to the local context, facilitate the communication of information to participants and discourage inappropriate inducements (Nuffield Council on Bioethics 2004). Therefore, acceptance and/or active community and/or

stakeholder involvement in SIT testing helps to ensure its ethical and scientific quality and to bring about the successful completion of the proposed research and/or intervention (**Box 7.4**). It also helps the SIT testing team understand and appreciate the community context, promotes smooth study deployment, contributes to a community's capacity to understand the research process, enables members to raise questions or concerns and builds credibility between the community and the researchers, which is a critical success factor for any SIT intervention.

Box 7.4 Objectives of community and/or stakeholder engagement

- To fulfill researchers' ethical obligations, obtaining the consent, understanding, collaboration and trust of communities and stakeholders
- To improve the project and ensure the achievement of objectives

7.3.3 How to foster community and/or stakeholder participation

Testing SIT to control *Aedes*-borne diseases is a process that will involve different communities and stakeholders at the different phases of implementation. Thus, community and/or stakeholder acceptance, engagement and/or participation need to be ongoing processes rather than a one-off action. Effective communication between the SIT testing team and community members is the foundation for community/stakeholder participation; hence, a forum/platform or other appropriate mechanism, such as a community advisory group, needs to be established to foster ongoing communication between the SIT testing team and the communities/stakeholders. Community members should be encouraged to raise any concerns that they have from the outset of the project and as the research proceeds (**Box 7.5**).

Box 7.5. Example of community engagement in a malaria project

"As co-development is one of our core values, we decided that the community itself should design its own acceptance model. Early on, a dialogue was established to agree on a set of principles—transparency, inclusiveness, openness to different perspectives—and the community of Bana elaborated its own acceptance model. They chose to establish a reference community group, representing the whole community and communicating the community's decision to the project, after their consultation. The acceptance to participate to the small-scale release of sterile mosquitoes was given by this reference group in May 2018". Extracted from a target malaria project in Bana, Burkina Faso (Diabate 2019)

There are a number of principles the SIT testing team can take into consideration to foster community acceptance/participation activities when planning and implementing the projects, among them:

- Sensitivity to local social norms and culture, including gender and other social strata;
- Striving to understand the community via situational analysis;
- Early engagement with the community;
- Clear and complete information/transparency;
- Respect for the community;
- Responsiveness to the community's concerns;
- Involvement of social scientists and communication experts, whenever possible;
- Consideration of the characteristics of communities, e.g., whether rural or urban;
- Allocating resources for community engagement activities.

The life cycle of implementing SIT provides many opportunities (cf. Figure 1.5) for pursuing active community and stakeholder participation to ensure better outcomes.

Opportunities for fostering community and stakeholder participation are listed below:

- Development of a communications strategy;
- Communication with community leaders or representatives to gain access to the community;
- Preparation of Information Education Communication (IEC) materials for stakeholders and community members, since social mobilization strategies designed in collaboration with the target audience will be more effective than those imposed without consultation or opportunities for meaningful dialogue (WHO 2012);
- Solicitation of individual informed consent for epidemiological surveys (cf. Chapter 6);
- Communication on risks and risks management (cf. Chapter 2);
- Dissemination of SIT intervention testing results.

The SIT testing team should select communication activities appropriate for the local conditions and within available resources. Activities that the SIT testing team can undertake to facilitate active community and

stakeholder participation include, but are not limited to, the following:

- Conduct stakeholder analysis to identify who will be affected, in what way and to what extent;
- Encourage stakeholder consultation and public dialogue;
- Set up a community advisory board to facilitate community participation;
- Develop communication strategies and plans that include, inter alia, target audience, purpose and frequency of the communication; what information via what channels and techniques; when and where, by whom;
- Design and select the channels of communication : Mass media (radio, television, newspaper); mobile phone; Internet-based communication tools; community outreach activities such as individual interviews, focus group discussions, community meetings, street theatre and performance, awareness raising campaigns, talk shows; and other means can be employed, as appropriate, to communicate information to stakeholders and communities;
- Invite community members to report and share their observations and perceptions of the SIT intervention with the SIT testing team (i.e., participatory monitoring and evaluation);
- Listen and respond to community concerns.

"You would never produce communication materials and products without getting to know the people affected". - WHO 2012

[18] *Communication strategies will be highly dependent on the socioeconomic and cultural conditions of the communities.*

Chapter 08

Cost-effectiveness assessment

To successfully and sustainably scale up interventions, decision makers require evidence not only of an intervention's impact on health outcomes, but also of its cost and cost-effectiveness. From a policy perspective, **cost analysis** provides information relevant for the financial planning, implementation and affordability of evidence-based interventions, whereas **cost-effectiveness analysis** indicates the relative efficiency or the relative value for money of interventions (IAEA 2008). The SIT is envisioned to be deployed as an intervention complementary to other mosquito vector control methods in the context of integrated programmes, in order to increase the effectiveness of current vector control efforts against *Aedes*-borne diseases (Flores and O'Neill 2018). With pilot releases of sterile mosquitoes occurring at multiple sites in different countries across the world, much progress has been made in scaling SIT to phases II and III field trials for evaluation (Lees 2015; Bellini 2013; Bond 2019; Boyer 2012). In the coming years, SIT will be subject to extensive testing in phase III and IV studies targeting *Ae. aegypti* and *Ae. albopictus*, the most important vectors within the genus *Aedes*, in a range of contexts and settings. In this chapter, we introduce and review the methods for conducting cost and cost-effectiveness analyses to inform future programming and deployment of SIT as a new vector control intervention for the control of arboviral diseases transmitted by *Aedes* mosquitoes.

8.1 The use of cost-effectiveness analysis for priority-setting in health

Policy makers in all healthcare systems face difficult decisions about which interventions to fund, because available resources will never be sufficient to deliver all possible means of improving health to all people who might benefit from them. Cost-effectiveness analysis is a method widely used in the public health sector to evaluate the economic efficiency of a new intervention relative to current practice or alternative interventions. The primary objective of cost-effectiveness analysis is to compare the costs and the health benefits of interventions to guide the allocation of limited healthcare resources by prioritizing those that offer the largest health benefit for the least amount of money (Jamison 2006; Musgrove 2006). To achieve this, the estimated cost-effectiveness of a new intervention is compared either with the cost-effectiveness of a set of existing interventions or with a fixed, context-specific benchmark representing the maximum willingness to pay for an additional unit of health benefit (Musgrove 2006; Woods 2016). Prioritizing interventions that are more cost-effective over those that are less allows the highest possible overall level of health to be generated for the population served (Drummond 2005). Although it is just one of the many decision-making criteria in a complex policy-making process, cost-effectiveness is an important consideration for priority-setting in health policy, whose objective is to decide how to spend public funds to improve population health (Jamison 2006; Musgrove 2006).

8.2 The use of cost-effectiveness analysis in economic evaluation of SIT

Cost-effectiveness analysis applies strictly to interventions. An intervention is broadly defined as a deliberate action to improve health by reducing the risk, duration or severity of a health problem (Jamison 2006). The SIT has a number of unique characteristics not usually found in other vector control interventions:

1) It provides area-wide vector control without requiring access to households or other private property (Flores 2018);
2) It operates in an inverse density-dependent manner, i.e., as the size of the target vector population becomes smaller and smaller, the effectiveness of a constant rate of sterile insect release increases (Feldmann 2001);
3) It requires important capital investment for infrastructure and capacity building (IAEA 2008);
Further and as for other vector control interventions, it necessitates a high degree of multisectoral organization and management at the level of large-scale vector or disease control programmes to maintain community protection from *Aedes*-borne diseases (Flores 2018).

Although SIT is often perceived as a stand-alone intervention method, past and current practice from agricultural pest management shows that SIT rarely has been used that way (Alphey 2010). Particularly, the inverse density-dependence of the method suggests that SIT would work synergistically with other vector control methods, particularly those targeting the adult females or immature stages of *Aedes* vectors (Feldmann 2001). Therefore, rather than analysing the costs and the effects of SIT individually, we can use cost-effectiveness analysis to estimate the additional cost of incorporating SIT into an integrated vector control programme as a principal component and quantify the additional health benefit that is expected to result. In this case, a package of interventions, including an SIT component, is subject to economic evaluation. Adding a new intervention to an existing package of interventions to address the same health problem can be considered a change in the integrated vector control strategy. It is possible to evaluate various combinations of interventions to determine which combination is the most cost-effective and how the cost-effectiveness of the new intervention depends on the other intervention(s) with which it is combined. An example would be combining

SIT (or any other vector control measure) with other interventions aimed at controlling the *Aedes*-borne diseases such as vaccination (Fitzpatrick 2017).

The outcome of a cost-effectiveness analysis depends on the comparator chosen. If there is no reference to a comparator (i.e., the null value being the natural course of disease without intervention), the main outcome of the analysis would be an average cost-effectiveness ratio, evaluating the total costs of an intervention relative to its total health benefits (Drummond 2005). Promoted at one point by the World Health Organization, this type of generalized cost-effectiveness analysis can be used to select a mix of interventions to maximize health within a fixed health budget (WHO 2003). However, the dearth in most countries of reliable information on intervention coverage, costs and benefits (Murray 2000) makes it difficult to obtain accurate cost-effectiveness results from such analyses.

In many cases, the main outcome of a cost-effectiveness analysis is an incremental cost-effectiveness ratio comparing the costs and the health benefits of at least two competing interventions or packages of interventions, one of which typically reflects current practice (Drummond 2005), whereby a lower ratio indicates more health benefits per incremental dollar spent (**Box 8.1**). If the incremental cost-effectiveness ratio falls into an acceptable range, the intervention or package of interventions is determined to be more economically efficient than current practice, thus warranting consideration for adoption from a policy perspective (Musgrove 2006). The development of this evidence base is important, as it has significant implications for the affordability, scalability and sustainability of effective interventions, particularly in low resource settings with several competing health priorities where *Aedes*-borne diseases are endemic (Liyanage 2019).

Box 8.1. Definition of an incremental cost-effectiveness ratio (ICER)

• The ICER is the ratio of the change in costs to incremental benefits of an intervention:

$$ICER = (Cost_{intervention} - Cost_{current\ practice}) / (Effect_{intervention} - Effect_{current\ practice})$$

• Costs are usually described in monetary units, while effects are measured in terms of cases, fatalities or disability-adjusted life years (DALYs) averted.
• If the intervention is more effective and less expensive than current practice, the ratio is positive, i.e., the intervention is preferred over current practice.

8.3 The estimation of cost-effectiveness

Using cost-effectiveness analysis for resource allocation requires the health benefits of interventions to be measured in common units in order to facilitate comparisons across interventions or diseases. Most analyses typically start with some natural health unit such as cases of disease or deaths (Drummond 2005). Evaluation of vector control interventions may also focus on intermediate outcomes such as entomological endpoints—for example, vector density. When the cost-effectiveness analysis focuses on the effects of disease, the common unit of health loss or gain resulting from an intervention should also consider the duration and the severity of disease and the preference for timing of health benefits (Fox-Rushby 2001).

To that end, disability-adjusted life years (DALYs) is the health gap metric most commonly used as a quantitative measure of health benefits (**Box 8.2**). The DALYs metric incorporates assumptions and measurements about the severity of non-fatal conditions, the age at the time of disease or death, the duration of disease sequelae with or without intervention, and the remaining life expectancy at that age (Fox-Rushby 2001). DALYs are calculated as the sum of the present value of future years of healthy life lost through illness/disability (years of life lived with a disability or YLDs, weighted by the severity of disability) and the future years of life lost through premature mortality (years of life lost, YLLs) as a result of a disease or a condition (Murray 1996). Health interventions aim to reduce DALYs.

Box 8.2. Disability-adjusted life years (DALYs)

- One DALY represents the loss of the equivalent of one year of full health.
- DALYs for a disease are the sum of the years of life lost as a result of premature mortality in the population and the years lost as a result of disability for incident cases of the health condition:

$$DALYs = YLL + YLD_{weighted}$$

- DALYs is a health gap measure that extends the concept of potential years of life lost as a result of premature death to include equivalent years of "healthy" life lost to states of less than full health, broadly termed disability.

Developed for the Global Burden of Disease (GBD) study by the World Health Organization (Murray 1996), the DALYs approach was introduced in the World Development Report in 1993 (World Bank 1993). Although the primary application of DALYs is in estimating trends in the global and regional burden of disease, the metric has also been widely used as an outcome measure for cost-effectiveness analysis over the past twenty-plus years. Nevertheless, the DALYs approach has received considerable criticism because of the value choices built into the metric through the disability weights, the age weighting and discounting (Mont 2007).

The disability weights range from zero (perfect health) to one (death) and measure the limitations from a disease or a condition in the absence of intervention (World Bank 1993). The GBD study recently has re-estimated the disability weights for 220 different health states to address the long-standing criticisms over their validity (Salomon 2010). The age weighting, another controversial value choice that gives less weight to years of healthy life lost at young and older ages, are no longer applied in cost-effectiveness analyses (Murray 2010). Discounting reflects inherent uncertainty about the future (Drummond et al. 2005). However, it has been argued that there is no intrinsic reason to assign less value to a year of health because it is anticipated in the future (Tsuchiya 1999). In 2012, WHO also dropped the use of discount rates to further simplify the calculation method for DALYs in the global burden of disease calculations (Murray 2010). Yet, discounting is still applied to future health benefits at a rate of 3-6%, and most cost-effectiveness guidelines recommend discounting future costs and benefits at the same discount rate (Attema 2018).

Another controversy in DALYs calculations is the choice of a life table, because life tables with high life expectancies yield more life years than life tables with shorter life expectancies and may overstate the health benefits of interventions (Musgrove 2006). In practice, life expectancies may be taken from life tables that are country-specific or standardized across larger regions; these are published by WHO in the **Global Health Observatory** data repository.[19]

DALYs averted during the intervention period can be computed using the standard methods and formulations (Murray 2010) or recently developed, easy-to-use tools aimed at public health professionals interested in quantifying disease burden (Devleesschauwer 2014; Center for the Evaluation of Value and Risk in Health 2018).

To demonstrate the full potential of SIT as a complementary intervention, pilot projects theoretically should be located in areas with high disease burden and be of sufficient scale and duration to allow reasonable projections of the intervention costs and effects on disease, ideally using epidemiological endpoints such as disease incidence.

[19] *https://www.who.int/gho/en/*

8.4 Determining the costs

The analysis of the comparative costs of alternative interventions is common to all forms of economic evaluation, including cost-effectiveness analysis. The primary purpose of the cost analysis is to compile information on the costs of introducing a new intervention singly or in combination with other interventions. While the identification, measurement and valuation of costs often occur simultaneously in practice, it is best to view each as a separate step in a cost analysis. The choice of the study perspective affects the costing method (Drummond 2005). For example, if a payer perspective is adopted, the most relevant costs are the expenditures in the payer's budget.

There are typically four main cost categories (Drummond 2005; Johns 2003) which would also apply to a mosquito-SIT intervention:

(1) Personnel costs are calculated for staff involved with laboratory colonization, mass rearing, production and release of sterile mosquitoes and for supervision, monitoring and evaluation of the intervention;
(2) Consumable costs include costs of all consumable items, such as rearing diet, general supplies, transport, quality control, as well as small equipment that has no resale value after one year;
(3) Overhead costs include utilities (e.g., water, electricity, communications), rent and maintenance costs of production facilities and equipment;
(4) Capital items include rearing and irradiation equipment, sex sorting machinery, vehicles for transportation and release, buildings for mass production and storage, and other relevant capital items with an expected useful life of more than one year.

The first three categories (personnel, consumables, overhead costs) are typically referred to as recurrent costs, while the fourth category (capital items) falls under capital costs (Johns 2003). Sterile insect mass rearing, irradiation and release is a continuous process, hence associated recurrent costs should be tracked and quantified over time. Once the important and relevant costs in each of the four categories have been identified, resource usage must be measured in appropriate physical units over time (Drummond 2005), bearing in mind that resources are divisible and can be shared across interventions. To address the measurement challenge posed when resources are shared by different interventions, an appropriate basis of allocation related to the joint use of shared resources should be chosen and applied to apportion the costs associated with such resources (Drummond 2005). For example, the percentage of time devoted to the activities for different vector control interventions can be used to allocate personnel and equipment costs.

The capital costs of an SIT production facility include land, building materials, construction labour and equipment costs. Some of these costs are locally determined, whereas the price of building materials may be set internationally (IAEA 2008). Some highly specialized equipment, such as oviposition cages, racks and trays for mass rearing, may or may not be produced locally. Encouragingly, the cost of adult mosquito cages has fallen significantly from over US $2,220 to US $220 per cage (Zheng et al. 2019). Annual depreciation costs should be calculated for each capital item, assuming an appropriate useful life for each item, and apportioned according to the estimated share of its use if the production facility is shared for other purposes (Drummond 2005). To estimate the total cost of SIT over the intervention period, the last step in the costing analysis is to add recurrent costs and annualized capital costs (Drummond 2005; Johns 2003).

There may be considerable variation in such costs in different locations (Johns 2003). In the long run, it may be informative to generate general guidelines based on an analysis of SIT facilities built at a range of production capacities. A financial model was previously developed by the IAEA to examine the relationship between the costs, level of production and sale price of sterile insects, using information from sterile medfly production facilities in operation (IAEA 2008). However, the most common arrangement so far has been government-sponsored production, whereby costs have been absorbed into insect management budgets (IAEA 2008), which likely will be the case for countries affected by *Aedes*-borne diseases.

Clearly, there may be significant cost savings associated with large-scale production facilities. Recurrent costs not directly related to the level of production, such as administrative and other full-time personnel, may provide some economies of scale. Further, reductions in other types of recurrent costs are expected to occur over time. For instance, the cost of larval diets has decreased by about 90%, with further reductions in operational costs expected due to aerial release of mosquitoes by drones and monitoring via ovitraps at a lower density (Zheng et al. 2019). Second, major reductions in mass rearing costs are anticipated through the application of emergent and novel technologies, such as the development of automated pupae sex sorter machines, which would facilitate separation of male from female mosquitoes on an industrial scale (Zheng et al. 2019).

The objective in valuing costs is to estimate the value of resources used by an intervention (Drummond 2005). Costs are typically assessed in the local currency and can be extracted from intervention or programme budgets. All costs can be converted to US dollars (US $) to facilitate international comparisons and must be expressed in constant dollars of some base year, usually the present year, to remove the effects of inflation from the analysis (Drummond 2005; Johns 2003).

In a cost analysis, costs incurred during the start-up period, which typically include the costs of activities conducted before the deployment of SIT (e.g., planning, recruitment and training of staff) should be identified and quantified separately (Drummond 2005). As discussed above, the capital costs of a mass

rearing facility for SIT are likely to fall under the start-up costs and include the costs of land, building materials, construction labour and equipment. To estimate the cost of intervention scale-up necessitates distinguishing between start-up costs and post-start-up costs. A distinction also should be made between research costs and routine monitoring and evaluation costs in phase IV trials. Ideally, all research costs in phase III and IV studies should be excluded from the cost analysis; however, routine monitoring and evaluation costs can be included, as it is expected that these costs would also be incurred in intervention replication and scale-up (Johns 2003). Staff time spent on research (e.g., data collection) should be recorded separately from time spent on intervention delivery.

The costs of pilot-scale SIT programmes can facilitate an estimation of costs at larger implementation scales. Given the potential economies of scale, pilot-scale costs are expected to be higher than post-pilot-scale costs; both should be estimated to inform SIT planning and implementation, as was done in Zheng et al. (2019) for a combined Incompatible Insect Technique (ITT)/SIT programme for *Ae. albopictus* control in China (**Box 8.3**).

Box 8.3. Actual and predicted future costs (in US $) of pilot and operational trials for a combined ITT/SIT *Ae. albopictus* control programme in China (Zheng et al. 2019).

Pilot trial, based on combined IIT/SIT release in Sites 1 and 2 in 2016 and 2017		
Production cost / million males,		
including quality control in laboratory and field	1,105	
Release cost / ha / week	20	
Monitoring cost / ha/ week	21	
Number of HC males released / ha / week		
(Minimum, Maximum)	11,640	158,136
Totals cost / ha / week (Minimum, Maximum)	54	216
Operational trial: initial supression for first two years		
Predicted reduction of production cost, %	90	
Predicted improvement of competitiveness	30	
Predicted reduction of release rate, %	67	
Release cost / ha / week	1	
Monitoring cost / ha/ week	1	
Totals cost / ha / week (Minimum, Maximum)	2	6
Totals cost / ha / year (Minimum, Maximum)	119	314
Operational trial: continued supression for next eight years		
Predicted reduction of release density, %	90	
Totals cost / ha / year (Minimum, Maximum)	106	125
Operational trial: overall cost for all ten years		
Totals cost / ha / year (Minimum, Maximum)	108	163

8.5 Computing incremental cost-effectiveness ratios

For a meaningful comparison, it is necessary to examine the additional costs that a new intervention imposes on another, compared with the additional benefits it delivers. The cost effectiveness analysis of SIT will rely on the cost and health outcome data from phase IV trials conducted in different contexts and settings. The analysis will centre on incremental cost-effectiveness ratios (cf. B**ox 8.1**), where the numerator represents the incremental costs of SIT relative to the existing vector control strategy, and the denominator represents its incremental benefits expressed in DALYs averted due to the reduced incidence of an *Aedes*-borne disease of interest. To that end, a cost-effectiveness analysis of SIT examines the extra amount to be paid to avert an extra DALYs by strengthening the existing vector control strategy with SIT.

Determining whether a given intervention will be cost-effective in a specific implementation setting normally rests on a local standard or a cost-effectiveness threshold that reflects the opportunity cost of resources in terms of the health benefits forgone if SIT is adopted as an additional measure of vector control (Woods 2016). As discussed previously, in cases where evidence about an intervention's epidemiological effectiveness is lacking, entomological endpoints can be used to compute incremental effects in a cost-effectiveness analysis.

However, it must be emphasized that a decision to use one technology vs. another against *Aedes*-borne diseases will be based primarily on its impact on disease transmission. Box 8.4 provides an example of such an analysis from a provider perspective, focusing on the effectiveness and incremental costs of an SIT-supported strategy relative to the conventional strategy for the control of *Aedes* mosquitoes in urban areas in Italy (Canali 2019).

Box 8.4. Comparative cost-effectiveness analysis of SIT in Italy

A recent cost-effectiveness analysis focused on the integration of SIT into the conventional vector control strategy against *Ae. albopictus* in Italy. The analysis examined the incremental cost per resident of achieving a 1% reduction in the mean annual egg density in ovitraps, which is an entomological endpoint. Three strategies were compared:

- Strategy A: Conventional control (e.g., spraying)
- Strategy B: Door-to-door (mainly source reduction, but also anti-larval treatment of large containers)
- Strategy C: SIT supplemental to Strategy A

Incremental costs: Based on expert opinion, it was estimated that an SIT facility working 25 weeks per year with a production capacity of 10 million sterile *Ae. albopictus* males per week would allow the integration of SIT into the conventional vector control strategy at €3.80 per 1,000 sterile males. The incremental cost per resident was calculated for three different sterile/wild male ratios (20:1, 10:1 and 5:1), which determine the number of sterile males per hectare that need to be released weekly in the treated urban area, given its population density.

Incremental effects: Based on the results of recent trials conducted in the intervention area, mathematical models were used to define the relationship between the sterile/wild male ratios and the expected reduction in vector density in ovitraps, considering the reduction in mosquito egg fertility, seasonal mosquito population dynamics and net mosquito immigration from bordering areas under conventional control. These models allowed the estimation of the number of sterile mosquitoes to be released weekly each year to maintain the desired sterile/wild ratio based on the daily death rate of sterile males.

Incremental cost-effectiveness ratios: ICERs compared the incremental cost per resident of achieving a 1% reduction in the mean annual egg density in ovitraps with an SIT-augmented strategy relative to the conventional strategy. ICERs were computed for the three different sterile/wild male ratios, using a discount rate of 2.85% and 3%. The results indicated that the SIT-supported vector control strategy was more effective in reducing vector density in ovitraps than the conventional strategy alone (which did not achieve a satisfactory reduction), but at a higher cost per resident. However, this increased cost was still lower than that of the alternative new technology tested in Strategy B.

Conclusion: For all the considered scenarios, even for extreme worst vs. best case evaluations, Strategy C (SIT+conventional) was more cost-effective than either Strategies A or B.

There are various types and sources of uncertainties relating to the effectiveness and cost data used in cost-effectiveness analyses. In its Model Business Plan for a Sterile Insect Production Facility, the IAEA estimated the initial capital costs and the recurrent costs for a sterile insect production facility under a range of scenarios, which could be incorporated into a cost-effectiveness analysis (IAEA 2008). An important feature of economic evaluation studies, sensitivity analysis is used to handle parameter uncertainty and assess the robustness of the cost-effectiveness results. There are a number of forms of sensitivity analysis, namely, one way and multi-way sensitivity analysis and scenario analysis (Drummond 2005). Cost effectiveness analyses increasingly incorporate probabilistic sensitivity analysis, where probability distributions are applied to the specified ranges for the key parameters, and samples are randomly drawn from these distributions to generate the empirical distributions of the costs and health benefits (Drummond 2005). The main advantage of this approach is that it allows the combined effect of all parameter uncertainties in the cost-effectiveness analysis to be characterized and the implications for a decision based on mean costs and benefits of an intervention to be reported. However, it should be noted that sufficient data for estimating probability distributions around mean parameter estimates are rarely available, particularly in low- and middle-income countries (Jamison 2006; Musgrove 2006).

As mentioned in the introductory remarks for this chapter, decision makers require evidence on the effectiveness of interventions on health outcomes, such as disease incidence. However, before scaling up and deploying an intervention, decision makers must also know if the intervention is affordable. The **cost analysis** provides information relevant for the financial planning, implementation and affordability of evidence-based interventions, whereas the **cost effectiveness analysis** indicates their relative efficiency or their relative value for money. While the burden that disease places on populations can be expressed by health gap measures such as DALYs, the true health impact is presumed to be considerably higher due to the broader societal impacts that are not directly related to health and remain uncaptured by these summary measures.

Chapter 09

Programme monitoring and evaluation

This chapter discusses the general concept of monitoring and evaluation for the SIT testing framework, highlights the relationship between monitoring and evaluation (M&E) and outlines the requirements for a functioning M&E system based on an input-process-output-outcome-impact pattern. M&E indicators for the planning and implementation stages and for long-term impact are provided as entomological and epidemiological evaluation components.

9.1 General concept

Similar to other vector control strategies, SIT requires monitoring and evaluation to guide the planning and implementation of the system, measure its effectiveness, seek improvement and evaluate the integrated resources (WHO 2012). M&E enables responding to deficiencies or failure by replacing them with more functional efforts, methods and/or techniques.

The implementation of SIT as a vector control strategy follows a stepwise phased conditional approach (**Figure 1.5**), in which each phase needs to be successfully completed and embedded into decision-making and operational processes before proceeding to the next one (**Figure 9.1**). The validity a ess.nd progress of each element will be assessed by the monitoring and evaluation process.

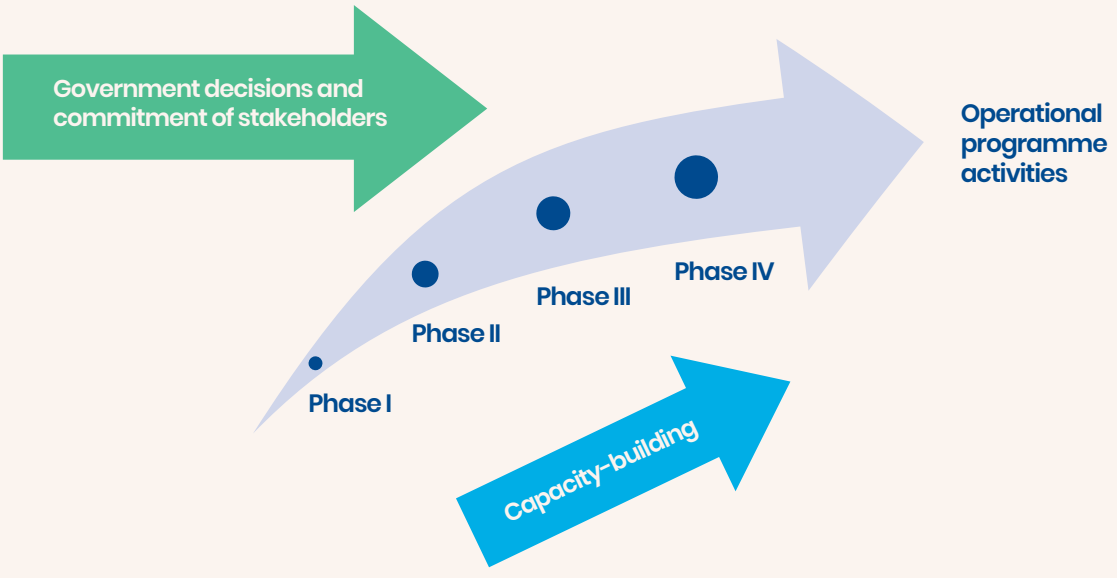


Figure 9.1. General framework for M&E of testing SIT.

9.2 Specificities of monitoring and evaluation

The components of an SIT programme interlock with and depend on one another (**Figure 1.3**). Together, monitoring and evaluation elucidate the cause-and-effect relationships between activities and impact. While interrelated, monitoring and evaluation differ in their approaches. Monitoring is used at the programmatic level to identify weaknesses in implementation, while evaluation is more at a global level and indicates whether the programme shows success or failure. The differences between monitoring and evaluation are shown in **Table 9.1**.

Table 9.1. Specificity of monitoring and evaluation for the following elements: frequency, function, purpose, focus, methods, information source and cost (adapted from the Global Fund Report, 2011).

Element	Monitoring	Evaluation
Frequency	Periodic, occurs regularly	Intermittent
Function	Tracking/oversight	Assessment
Purpose	Improve efficiency, provide information for reprogramming to improve outcomes	Improve effectiveness, impact, value for money, future programming, strategy and policymaking
Focus	Inputs, outputs, processes, work plans (operational implementation)	Effectiveness, relevance, impact, cost-effectiveness (population effects)
Methods	Routine review of reports, registers, administrative databases, field observations	Scientific, rigorous research design, complex and intensive
Information source	Routine or surveillance system, field observation reports, progress reports, rapid assessment, programme review meetings	Same sources used for monitoring + population-based surveys, special studies
Cost	Steady and regular	Occasional

9.3 Requirements for a functioning M&E system

The key to success for SIT testing is objectively and systematically assigning measurable indicators, which can be either quantitative (numbers) or descriptive (e.g., absent or present). The best scenario uses a balanced combination of both categories. However, the analysis of quantitative data is more immediate, and more easily provides results that can be statistically significant. By contrast, descriptive/qualitative data may need to be transformed before being analysed, and statistical significance is not always achieved.

9.3.1. Organization in charge of M&E

The organization(s) responsible for the SIT testing programme and M&E must be clearly defined at the different phases and globally for the full process. It is recommended that the organization responsible for M&E is not the same as the one in charge of SIT testing, to avoid conflict of interest. In some cases, the authorities provide the oversight and coordination and assign technical bodies to draft the standard operating procedures (SOPs), mechanisms of data collection and the M&E plan, which will describe stakeholders, address responsibilities and define indicators and data collection methods.

9.3.2. Operational steps for M&E of a vector control programme

Monitoring and evaluation of control programmes should start with the planning of the programme and continue through the implementation and operational stages to validate the stepwise progress of the operation using process indicators (**Figure 9.2**). Monitoring identifies hindrances to further actions and indicates modifications. The evaluation of both outcome and impact indicators points out whether programme goals are being achieved.

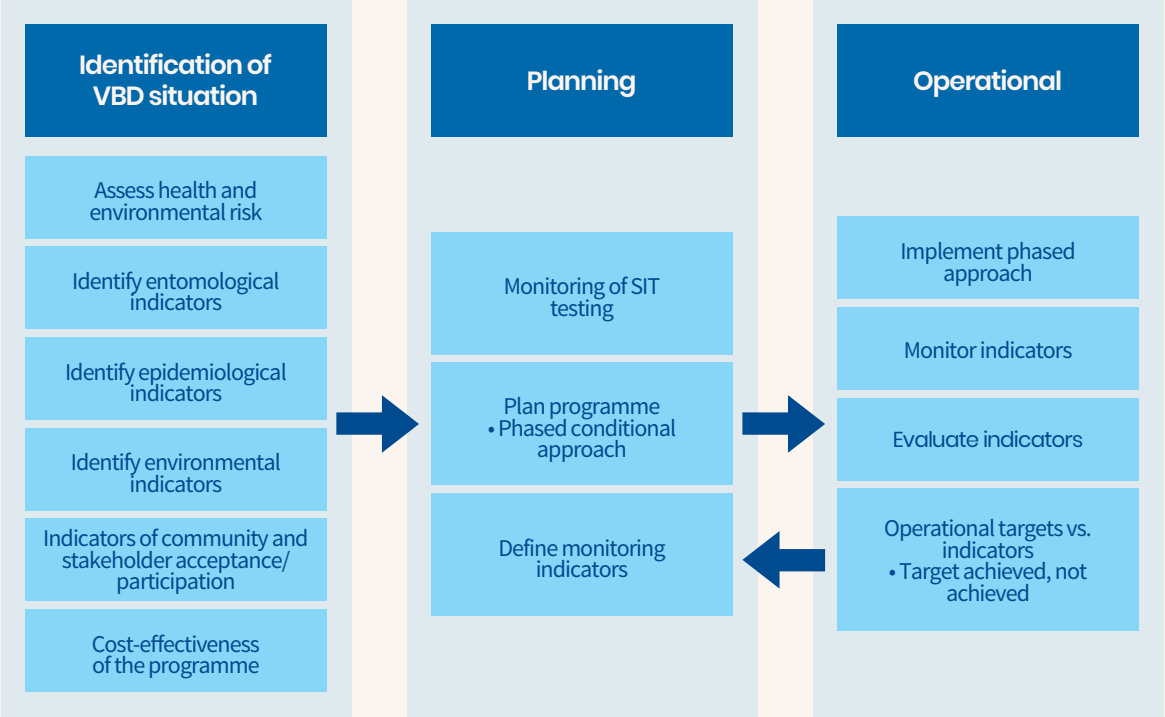


Figure 9.2. Steps for planning and implementation of M&E for SIT.

9.3.3. Collection and dissemination of data

Data collection mainly involves official maps, reports and documents from the corresponding national authorities. Via regularly collection of data from sentinel sites, epidemiological surveillance systems are essential for monitoring the impact of the control measures on vectors, disease, cost and the environment.

To determine weaknesses or areas of improvement, data must be interpreted and disseminated to help in programme planning and policy formulation. In addition, data analyses help to evaluate the status of implementation and to validate outcomes/impacts. Data dissemination not only involves the policymakers responsible for planning, but also considers feedback from the public. To achieve goals efficiently, data must be communicated in a timely manner and in the proper form (WHO 2012).

9.4 M&E of outcomes and expected impact of the success of SIT

The components of an M&E programme for SIT include input indicators, process indicators, output indicators, outcome indicators and impact indicators. The progress of all the components of the SIT testing programme should be monitored by regular data collection, reporting and analysis. The M&E plan can be developed based on the WHO framework (WHO 2016). **Figure 9.3** provides examples of indicators for each component.

By adding extra indicators to the existing ones, integrating SIT within other vector management strategies increases the complexity of monitoring and evaluation.

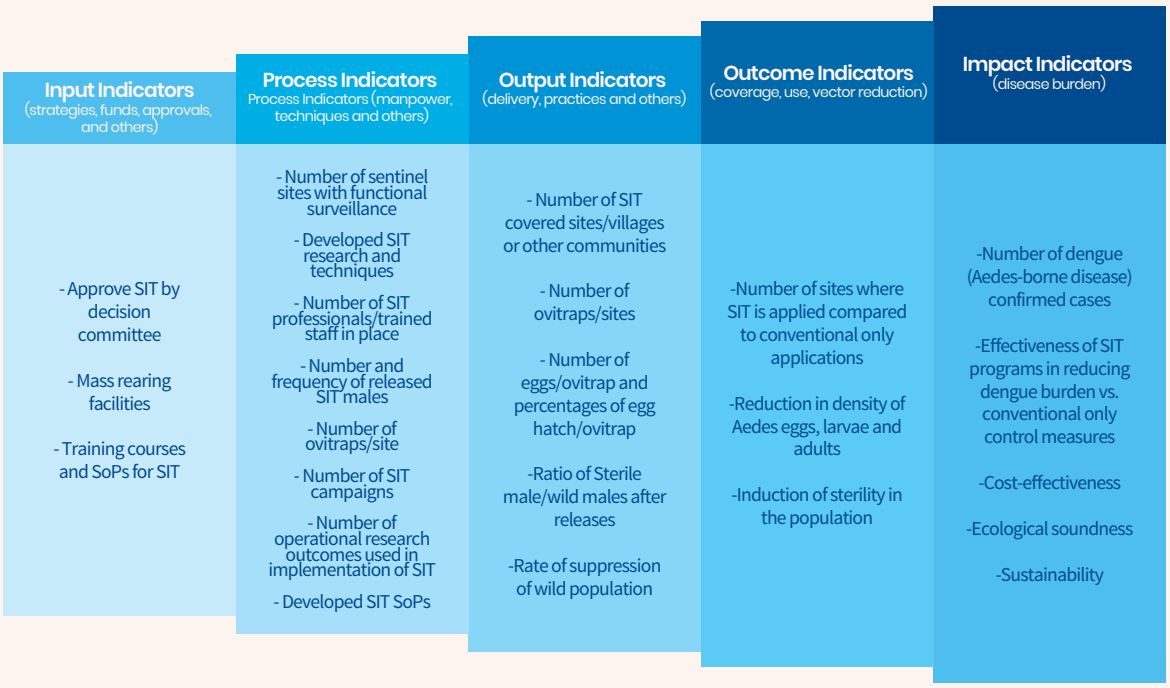


Figure 9.3. Examples of the relationship between inputs, process, outcomes and impacts of SIT to control *Aedes* (adapted from WHO 2012, 2016).

9.4.1. Definition of the different indicators and framework of a M&E programme

According to WHO (2016), the definitions of indicators and processes are listed below:

1) Input indicators reflect resources mobilized to support the process and include strategies, policies, funds, guidelines, authoritative bodies;
2) Process is the transformation of all resources to output efforts include manpower, training, techniques;
3) Output indicators are higher level resources using the inputs such as knowledge, delivery, practice;
4) Outcome indicators are the tangible direct results such as coverage, use, vector reduction;
5) Impact indicators are the final objectives of the programme: disease burden.

The performance framework of the programme should be based on an input-process-output-outcome-impact pattern (**Figure 9.3**). Inputs and processes will result in outputs; well designed outputs achieve short-term effects (outcomes), which in turn lead to long term effects (impact) (Global Fund Report 2011).

Based on data collected by national M&E systems, indicators should align with national plans for disease control (intervention- and disease-specific indicators) and include programme coverage and effect of the intervention on vector population and disease control (WHO 2012, 2016). Indicators should align with targets and programme outcomes.

9.4.2. Targets for indicators

Setting representative targets for indicators is a key factor of the planning process. Proper targets should rely on a recent inclusive analysis of the epidemiological situation, including defining target and at-risk populations. Targets should be set according to the national disease strategy framework.

Factors to be considered when setting targets include, but are not limited to, at risk population, type of epidemic, main transmission factor, number of people at risk, mapping, appropriate intervention method and coverage and gaps (Global Fund Report 2011).

9.5 Data sources to support M&E

9.5.1. Surveillance indicators in SIT testing for *Aedes*-borne diseases

Surveillance data is the baseline source of information in control programme evaluation. Vector surveillance based on routine monitoring is set to assess certain risks and provide a description of spatial and temporal risk. In addition, it is the method for capturing inconsistencies that arise in the course of evaluation (Tabbabi and Daaboub 2017). **Table 9.2** shows dengue surveillance indicators, which could be used as outcome indicators for SIT.

Table 9.2. Example of indicators and what they are representing for the evaluation of the effectiveness of dengue prevention and control programmes (cf. **Chapters 5** and **6**) (adapted from WHO 2009).

Data Source	Purpose	Surveillance Indicator
Epidemiological data	Determine effectiveness of the programme Identify areas in need of resource allocation Determine coverage of the programme	No. of suspected cases No. of laboratory-confirmed cases No. of hospitalized cases No. of at-risk health centres reporting No. of cases per health centre
Alternative indicators	Ensure accuracy of dengue classification: dengue fever (DF), dengue haemorrhagic fever (DHF), dengue shock syndrome (DSS) Determine the public's understanding of disease and areas of concern Identify problem areas	Clinical syndromic case definition as fever Surveillance of community awareness and/or participation, through Focus Group Discussion on other methods Based on epidemiological data
Entomological data	Determine changes in vector abundance and distribution; obtain vector population trends	Adult indices Pupal indices Larval indices (HI, BI, CI)*

*HI : House Index, BI : Breteau Index, CI : Container Index

9.6 M&E of an SIT operational plan

The success of SIT relies on introducing enough sterility into the wild population to bring about a strong decrease in the vector population, resulting in a decrease in the transmission of pathogens. Standardized quality control methods are used to monitor the quality and effectiveness of the technique and assess its suitability for applications (Balestrino et al. 2017). As one of the most accepted and successfully used techniques for decades, radiation (together with the usual mass rearing adaptation, handling and transportation of mosquitoes that constitute SIT programmes) may negatively affect male mating capacity and competitiveness (Bakri et al. 2005; Bull 2015; Proverbs 1969; Helinski et al. 2009). Therefore, careful quality monitoring must be done on each of these SIT components.

Surveillance methods are required to assess programme progress and determine the effectiveness of the releases of sterile mosquitoes. **Table 9.3** shows epidemiological and entomological as well as more general evaluation components for SIT, along with examples of evaluation values (Bond et al. 2019).

Table 9.3. Entomological and epidemiological indicators and examples of target values for the evaluation of the different components for SIT mosquito programmes.

Type of Components	Outcome or Endpoint	Indicator	Example of target values for indicators defined in Chapters 4, 5 and 6
Entomological components	Phase I to all phases and Laboratory quality control	Pupal survival	Depending on the species and sex, 90 to 98%
		Flight ability	Escape rate, 0.7 to 0.9
		Fecundity	90 – 100 eggs/ females over a single gonotrophic cycle.
			Egg hatch > 70%
		Adult survival	Median survival time 46 to 73 days depending on species and sex
		Pupa or adult size	Based on cephalothorax width. Base line data are necessary.
		Mating competitiveness	Fried C index > 0.7
	Phase II and Field cage	Mating competitiveness	Fried C index >0.5
	Phases III and IV and Open field	Sterile:wild ratio	> 10:1
		Survival and dispersal	Recapture of released males should be >1% and mean dispersal distance from release point 70 m.
		Sterility induction	Decrease in egg hatch should be progressive. To achieve less than 20% egg hatch (> 80% induced sterility) is desirable. When population is low, hatch rate is not meaningful, also the results may be delayed and have erratic values.
		Suppression	Should be progressive. Based on systematically collected data from ovitraps (eggs and hatched larvae) and adult traps. Data should be compared with historical data from the site and data from control sites. Should be >80%.
Epidemiological components	all phases	Disease incidence/ prevalence studies before, during and after intervention trials	Disease incidence/prevalence studies during intervention trials.
			Longitudinal passive case detection of targeted disease and other mosquito-borne diseases.
			Post-treatment active and/or passive disease incidence/prevalence
General	all phases	Cost-effectiveness and monitoring and evaluation of the programme	Cost effectiveness analysis
			Multi-year trials evaluation
			Effectiveness of SIT when combined with other control measures in IVM

For vector-borne disease prevention and control, the implementation of the different activities through a pre-determined plan that has been previously tested under real-world conditions, must include M&E at all stages. Both internal and external evaluation processes and indicators will measure how the plan is being implemented (with realistic and adapted timelines) and how effective the activities are (alone and/or combined into the plan) and allow adaptation and improvement. The M&E process also will be used to better understand whether the human and technical resources are adequate, to identify where the weaknesses, deficiencies or failures are, and (potentially) to modify the plan accordingly.

In the specific case of SIT as a vector control strategy following a stepwise phased conditional approach, M&E must be done for each of the different phases, but also more globally for the full process, to look at how the testing is moving from one phase to the next according to the best decision-making options. The complexity of layering an M&E process into a phased approach increases the necessity of building up the approach as early as possible and testing it as many times as required.

Concluding Remarks

Prior to being used on mosquitoes, the sterile insect technique has been used successfully to suppress numerous insects, including the crop pest *C. capitata*, the Mediterranean fruit fly, which was prevented from invading North America at the Guatemala-Mexico border. This agricultural success yielded huge benefits, with the cost ratio of control estimated to be US $150 of benefit for every US $1 spent on control. A veterinary pest, the New World screwworm fly, *Cochliomyia hominivorax*, was eradicated from both North and Central America and North Africa, where it was accidentally introduced. The screwworm fly is responsible for deadly injuries to cattle. And the latest and more recent SIT success was achieved against the tsetse fly, *Glossina austeni*, vector of animal and human trypanosomoses, which was eradicated from one island of Zanzibar.

The SIT is applicable against: i) insects reproducing through sexual mating, since only sterile individuals are released to mate with wild ones; ii) insects that can reproduce in confined and industrial conditions; iii) insects for which sexing of a large number of individuals is available (when only males are released); and finally, iv) insects where male mating competitiveness is similar in artificially bred vs. natural males. Moreover, methods for mass rearing, irradiation, sex separation, handling and release must be cost-effective. Until very recently, the cost of all these requirements were prohibitive for making this technology feasible for mosquitoes.

However, the lack of proven efficient vector control tools against some types of vectors, such as the mosquitoes *Ae. aegypti* and *Ae. albopictus*, has spurred the International Atomic Energy Agency, through its Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture (NAFA), to put consistent efforts towards overcoming the technological issues impeding the deployment of this technology against mosquitoes. Once the technology was found to be mature enough, a collaboration was initiated with the Special Programme for Research on Tropical Diseases (TDR) at WHO and the Department of Neglected Tropical Diseases of WHO (NTD/WHO) to test SIT against *Aedes*-borne diseases, major vectors of arboviruses worldwide.

The first step of the collaboration is the development of this guidance document to inform the Member States, research institutions and interested stakeholders about the basics of the how, why and when of testing SIT against *Aedes* mosquitoes. The challenge of this guidance is to translate the findings and methods developed for agriculture into the health field. Further, injuries caused by crop and herd pests are mostly related to density, which is not the case for the transmission of diseases. The vectorial capacity of an insect species, i.e., the capacity of the vector population to propagate a disease, is linked not only to the density of the vector, but to its longevity (survival), infection rate, attraction to the host

(human beings) and competency to amplify the pathogen. Consequently, the evaluation of the impact of SIT on the vector population must take into account all of these parameters. Further, the evaluation of SIT on disease impact will also consider the incidence of the relevant disease(s) and the level of immunity in the human population.

The epidemiological and entomological indicators used to estimate the impact of SIT on a specific disease are variable within a human population exhibiting specific behaviours and living in specific socio-economic environments, both of which are of primary importance in the testing the sterile insect technology. The social/environmental factors encompass the risk assessment and regulatory and/or authorization pathways which must be included in the test planning.

This Guidance Framework for Testing the Sterile Insect Technique as a Vector Control Tool against *Aedes*-Borne Diseases was developed with the objective of considering all of the specificities of SIT in relation to human disease transmission, so that this technology could be tested in suitable environments and conditions, under the application of the most adequate processes and options. While its target audience is decision makers and the stakeholders' technical experts, we hope that this document will be useful for a larger population of users as well, including, but not limited to, researchers, vector control agencies and technical staff.

Glossary[20]

Aerial release: Release of insects, e.g. sterile insects, from the air using aircraft or drones.

Area-wide control: A synonym for area-wide integrated pest management adapted from plant pest control. Control measures applied against a given plant pest over a geographically defined area that includes all known or potential 5 hosts with the objective of preventing pest build-up while minimizing damage to commercial host. Control actions are conducted whenever and wherever the target pest exists regardless of host seasonality (Enkerlin 2007).

Autodissemination: Sterile insects are inoculated with electrostatically charged powder formulated with entomopathogens or slow-acting insecticides, which would be spread throughout the pest population through intraspecific interactions (Robinson and Hendrichs, SIT Glossary 200521).

Colony: Individuals of one species living in close association in space and time (Gordh and Headrick 2001). For insect mass-rearing, a colony of a species consists of all stages of the insect kept in a rearing facility.

Competitiveness: Ability of an organism to compete with conspecific (i.e., belonging to the same species) organisms for a limited environmental resource (FAO/IAEA/USDA 2003).

Cost-effective: An activity that generates sufficient value to offset its cost (Friedman 2007).

Density: The number of individuals of a species per unit of habitat (Resh and Cardé 2003, Pedigo 2002).

Disability-adjusted life year index (DALY): An index that measures the burden of a disease in life years lost due to the disease (Murray 1994).

Dispersal: A non-directional movement of insects within or between habitats (Gordh and Headrick 2001).

Disease transmission: In medical and veterinary entomology, transmitting or passing on a disease, e.g. malaria, nagana, sleeping sickness. Transmission may be biological or mechanical. The passage of an infective parasite from an intermediate host (insect vector) to a definitive host (e.g. human), or vice versa (Gordh and Headrick 2001, Torre-Bueno 1978).

Efficacy: an intervention measured when it is implemented under ideal, highly controlled circumstances; efficacy is typically measured in phase III studies.

[20] Most definitions were drawn from the SIT Glossary (https://nucleus.iaea.org/sites/naipc/dirsit/Documents/sit-glossary-updated-9-6-10.pdf). Please refer to the full SIT Glossary for additional terms and definitions.

Effectiveness: the degree of benefit of an intervention measured when it is delivered and used operationally under routine, "real-world" conditions; effectiveness is typically measured in phase IV studies.

Elimination: see 'Population elimination'.

Eradication: A type of regulatory-control programme in which a target pest is eliminated from a geographical region (Gordh and Headrick 2001).

Evaluation is a periodic "rigorous assessment of the impacts that can be attributed to a programme or strategy, to demonstrate its value" (WHO, 2012).

Filter colony: From the filter rearing system (FRS) concept, which involves maintaining a small colony at a low density, or even under semi-natural conditions, and therefore assumedly a low-selection pressure. Surplus insects from this low-density mother stock or clean stream are fed into a high-density amplification chain, leading up to the final insects to be released. The important feature is that no individuals are ever fed from the amplification stages back to the mother stock.

Fried C Index: Fried's Competitiveness Index is a simple measure for quantifying the mating competitiveness of sterilized males compared to wildtype males (Fried 1971).

Genetic sexing: Also 'genetic sexing system' (GSS). Genetic method to produce unisexual progeny.

Genetically modified organism (GMO): Food or plants with a genetic composition that has been altered in purpose (that is not accidentally) through genetic engineering (Collin 2001).

Ground release: Release of sterile insects from the ground.

Impacts: Long-term effects

Implementation: An act or instance of implementing something: the process of making something active or effective (Merriam-Webster).

Infective: Capable of producing infection, a term commonly applied to pathogens or to the vector (mosquito) (adapted from WHO 2016).

Intervention: A deliberate action to improve health by reducing the risk, duration or severity of a health problem (Jamison et al. 2006).

Inundative release: The release of large numbers of mass-produced biological control agents or

beneficial organisms with the expectation of achieving a rapid effect (FAO 2006).
Life cycle: The sequence of stages in the growth and development of an organism, eventually resulting in the reappearance of the first stage (Whiteside et al. 1988; Hill 1997).

Living modified organism: Any living organism that possesses a novel combination of genetic material obtained through the use of modern biotechnology (Secretariat of the Convention on Biological Diversity 2000).

Living organism: Any biological entity capable of transferring or replicating genetic material, including sterile organisms, viruses and viroids (Secretariat of the Convention on Biological Diversity 2000).

Local strain/local material: Colonized mosquitoes which originated from specimens collected in the "general" area targeted for release of sterile males in the SIT programme. The term "general" is subject to interpretation by regulatory bodies.

Longevity: Also 'lifespan'. The length of life of an individual or a population (Hill 1997).

Mark-release-recapture: A technique of estimating insect population size by marking, releasing and recapturing of individuals and counting their proportional abundance (Daly et al. 1998).

Mass rearing: Mass rearing is a systematic enterprise accomplished with machinery in integrated facilities for the purpose of producing a relatively large number of insects for releases (Leppla et al. 1982). In mass-rearing the objective is to produce large numbers of 'acceptable' insects at the lowest possible cost (Singh 1977).

Migration: Long-range dispersal, either away from a declining resource or as part of a seasonal cycle (Gordh and Headrick 2001, Auburn 2008).

Modern biotechnology: The application of: a) in vitro nucleic acid techniques, including recombinant deoxyribonucleic acid (DNA) and direct injection of nucleic acid into cells or organelles, or b) fusion of cells beyond the taxonomic family, that overcome natural physiological reproductive or recombination barriers and that are not techniques used in traditional breeding and selection (Secretariat of the Convention on Biological Diversity 2000).

Monitoring is continuous tracking of programme performance and involves checking progress against pre-determined objectives and targets (WHO, 2016).It involves routine collection and reporting of data on programme implementation to understand how programme implementation is going.

Mosquito genetically modified (MGM): Mosquito in which specific genetic material has been altered in a way that does not occur naturally by mating and/or natural recombination (from the definition in Directive 2001/18/EC). Irradiated mosquitoes are not genetically modified organism (GMO) but randomly mutated. They are excluded from GMO referential.

Mother colony: A colony of insects that is the original colony established from field-collected insects. The progeny of a mother colony are used to create other colonies called daughter colonies. The mother colony is usually kept smaller in size, and reared differently, than daughter colonies, i.e. the mother colony is kept under conditions that are as similar as possible to field conditions (Parker 2007; Franz 2005).

Outcomes: Short-term effects

Oviposition: The act or process of laying eggs, ovipositing, depositing; the passage of an egg from the median oviduct to outside the insect's body (Resh and Cardé 2003, Gordh and Headrick 2001).

Population: A potentially interbreeding group of organisms of a single species, occupying a particular space at the same time (USDA 1993, MCC 1996, Wikipedia 2008, Pedigo 2002, Resh and Cardé 2003).

Population elimination: One objective of a vector control strategy. Local population elimination means the disappearance, in a given area, of an isolated population of vectors. This concept must be distinguished from eradication of a species. (HCB 2017).

Population modification: Vector control strategy intended to reduce the inherent ability of individual vectors in a population to transmit a given pathogen (after WHO 2014, 'population replacement'). The aim is mainly to reduce a vector population's vector competence without necessarily altering the size of the population, as opposed to population reduction strategies. (HCB 2017).

Population reduction: Vector control strategy intended to reduce the size of a vector population below the threshold required for transmission of a pathogen (after WHO 2014, 'population suppression') without affecting the vector competence of the remaining individuals, as opposed to population modification strategies. (WHO 2014; HCB 2017).

Quality control: A systematic process whereby management critically evaluates the elements of production, establishes standards and tolerances, obtains, analyses and interprets data on production and product performance, and provides feedback so as to predict and regulate product quality and quantity (FAO/IAEA/USDA 2003).

Release: Intentional liberation of an organism into the environment (FAO 2006).

Rolling carpet principle: The various operational phases of pest management are carried out simultaneously in a phased manner. Intervention entails a unidirectional front (Hendrichs et al. 2007).

Self-limiting: A vector control technique is said to be self-limiting if its effects are limited in space and time unless application of the technique is maintained. For control techniques involving release of modified insects, the modification will disappear from the population unless it is reintroduced by regular releases of modified insects. (HCB 2017).

Stakeholder: Anyone with an interest, concern or 'stake' in something, in an entity or in what the entity does (Oxford Dictionary 2008).

Sterile insect: An insect that does not produce viable offspring; an insect that, as a result of a specific treatment, is unable to reproduce (FAO 2017), irrespective of its mate.

Sterile:wild ratio: Also 'overflooding ratio'. The ratio of sterile insects to wild insects in the population in an SIT programme (FAO/IAEA/USDA 2003).

Suppression: A type of regulatory control programme in which a target-pest population is decreased within a geographical region (Gordh and Headrick 2001). Reduction of a pest population to below some predetermined economic threshold (USDA 1993).

Surveillance: An official process which collects and records data on pest occurrence or absence by survey, monitoring or other procedures (FAO 2006). The watch kept on a pest for detection of the species' presence and determination of population density, dispersion, and dynamics (Pedigo 2002, Gordh and Headrick 2001).

Target population: The population of an organism that is the intended target, object or focus of an action or programme. In the context of the SIT, the target population is the wild population that the sterile insects are being released against (FAO/IAEA/USDA 2003).

Vector (insect): An organism capable of transmitting or transporting a micro-organism or pathogen or parasite from one host to another (Pedigo 2002, Resh and Cardé 2003, Gordh and Headrick 2001).

Vector density: see 'Density'

Vector longevity: see 'Longevity'

Vectorial capacity (VC): A measure of the intensity of the transmission, calculated via the mathematical formula VC = ma2×pn/log(p), where m= mosquito density/person, a=number of bites, p=daily survival rate of the mosquito and n=the duration (in days) needed for amplifying the virus into the mosquito (also called the extrinsic incubation period) (MacDonald 1952).

Wave principle: The wave principle entails an expanding operational block size with each phase of an AW-IPM programme using the SIT (Hendrichs et al. 2007).

Abbreviations

ABDs	Aedes-borne diseases
AFB	French Biodiversity Agency
AW-IPM	Area-wide integrated pest management
CRT	Cluster randomized trial
DALY	Disability-adjusted life year
DENV	Dengue virus
DtD	Door-to-door
EBP	Evidence-based practice
EFSA	European Food Safety Authority
EPPO	European and Mediterranean Plant Protection Organization
ERC	Ethical review committee
FAO	Food and Agriculture Organization of the United Nations
GBD	Global Burden of Disease (title of a WHO study)
GMO	Genetically modified organisms
GSS	Genetic sexing strain
GVCR	Global vector control response
HCSP	Haut Conseil de la Santé Publique [French Public Health Council]
IAEA	International Atomic Energy Agency
ICER	Incremental cost-effectiveness ratio
IRGC	International Risk Governance Council
IIT	Incompatible insect technique
IPCS	Insect Pest Control Subprogramme (refers to FAO/IAEA IPCS)
LMO	Living modified organism
M&E	Monitoring and evaluation
NTD	WHO Department of Control of Neglected Tropical Diseases
pers. comm.	Personal communication
PICO	Population Intervention Comparator Outcome
QC	Quality control
RCT	Randomized control trial
SIT	Sterile insect technique
SOPs	Standard operating procedures
SW-RCT	Stepped wedge cluster-randomized trials
TDR	WHO Special Programme for Research and Training in Tropical Diseases
USDA	United States Department of Agriculture
US EPA	US Environmental Protection Agency

VBDs	Vector-borne diseases
WG	Working group
WHO	World Health Organization
YLD	Years of life lived with a disability
YLL	Years of life lost

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Annex 1. SIT Checklist

SIT checklist

Country / region:
SIT approach:
Planned phase I testing start date:

Framework component	Timeline (months before phase I testing commences)	Issue	Lead agency	Action required	Deadline	Status	Is funding required/ available?	Priority (high/medium/low)
Making the decision to implement SIT	12	**Examining public health priorities** What public health issues do health authorities want to address given limited resources?						
	12	**Cost-benefit analysis** What actions need to be taken to thoroughly understand the costs and benefits associated with the SIT programme?						
	12	**Risk assessment** What actions need to be taken to gather a broad range of perspectives on risk? What is an acceptable level of risk, and what risk mitigation measures can be taken?						
	12	**Commitment and resources** What actions need to be taken to obtain long-term commitment and adequate resources from stakeholders? What is each stakeholder's level of commitment, roles, and responsibilities?						

Programme objectives/targets	10	**Situational analysis** What information is needed for a thorough situational analysis of *Aedes*-borne disease transmission? What actions need to be taken to obtain this information?						
	10	**Establishing the target vector, geographical region, and population** What information and actions are needed to establish the target vector, geographical region and population?						
	10	**Short- and long-term objectives** What information and actions are needed to define the SIT programme's short- and long-term objectives?						
Regulation, permits, and authorisation	10	**Identifying and obtaining necessary approvals and permits** What steps need to be taken to identify and fulfil regulatory requirements at various stages of testing and implementation?						
	10	**Engaging regulators** What steps need to be taken to establish a working relationship with domestic and international regulators?						

Modalities of implementation	8	**Production capacity** What actions need to be taken to ensure adequate mass-rearing capacity for trials and implementation? What technologies need to be adopted to improve scalability?						
	8	**Laboratory support** What laboratory procedures need to be established to support SIT-related research and testing?						
	8	**Manpower and training** What needs to be done to ensure adequate, appropriately trained personnel to support the SIT programme?						
	8	**Release strategies** What information is required to design an optimal release strategy, and what steps are required to obtain this information?						
	8	**Quality control mechanisms** What quality control mechanisms need to be established to ensure that mass rearing and releases are performing optimally?						

Ethics and community engagement	12	**Ethics** What are the ethical obligations of the SIT programme to the host community, and what steps need to be taken to ensure that these obligations are met?						
	12	**Key messages** What steps need to be taken to develop key messages for community engagement?						
	12	**Targeting stakeholders** Which stakeholder groups are to be targeted, and what is the outreach plan for each group?						
	12	**Public feedback** What actions need to be taken to establish public feedback mechanisms? How will the SIT programme respond to public feedback and use it to inform implementation strategies?						
Surveillance, monitoring, and evaluation	12	**Establishing surveillance and monitoring frameworks** In the absence of an existing surveillance programme, what steps need to be taken to establish a robust and comprehensive disease surveillance framework?						
	12	**Data collection and handling** What types of data will be collected, and how will the data be handled and disseminated?						

	6	**Endpoints** What information and actions are required to establish entomological and epidemiological endpoints for evaluation of efficacy?						
Phased implementation	4	**Decision criteria** What actions need to be taken to establish pre-defined decision criteria for progress to a more advanced phase? Which stakeholders will have oversight of the evaluation and decision-making process?						
	4	**Long-term monitoring** What steps need to be taken to establish a system for long-term monitoring and assessment of the SIT approach?						
	4	**Feedback mechanisms** What actions need to be taken to establish feedback mechanisms and communications channels between management, field and laboratory teams?						
	4	**Expert review** What actions need to be taken to establish a review committee to assess and provide expert guidance on the SIT programme?						

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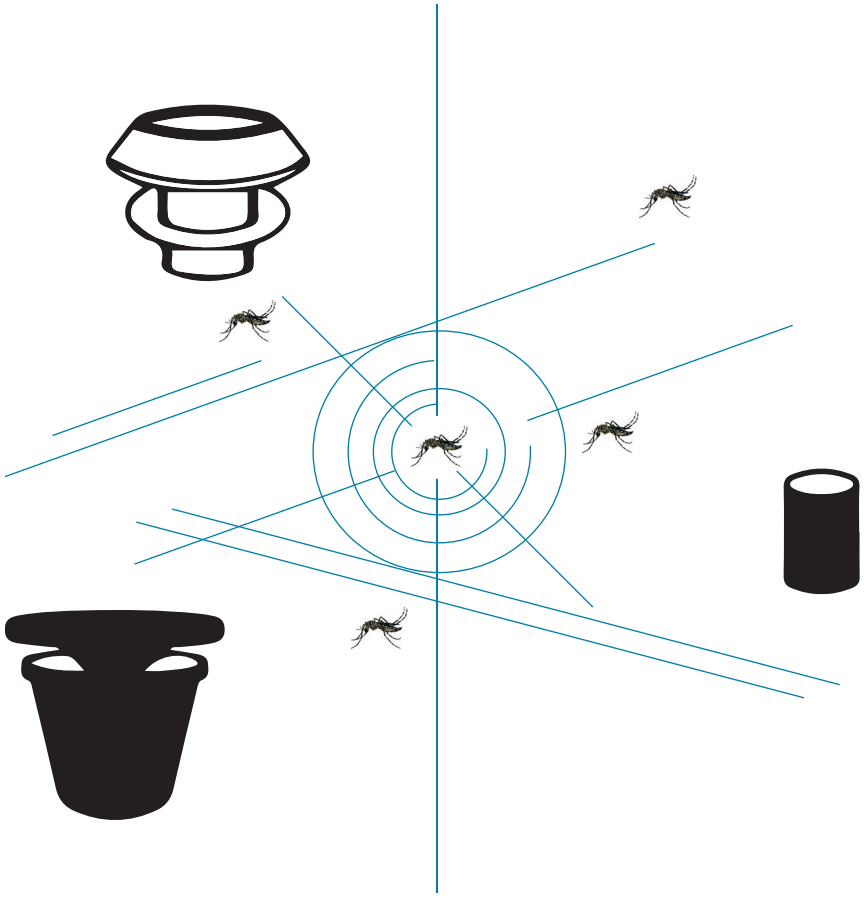
EFFICACY-TESTING OF TRAPS
FOR CONTROL OF *AEDES SPP.* MOSQUITO VECTORS



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FOR CONTROL OF *AEDES SPP.* MOSQUITO VECTORS

World Health Organization

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CONTENTS

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WHO recognizes that as the first guidelines provided for a rapidly developing area of mosquito vector control, we anticipate these to evolve with the field, and actively encourage feedback and suggestions for improvement.

ABBREVIATIONS AND ACRONYMS

ABV	*Aedes*-borne virus
AC50	concentration that attracts 50% of insects
AC90	concentration that attracts 90% of insects
AI	active ingredient
EI	emergence inhibition
EI50	concentration that prevents emergence of 50% of adults
EI90	concentration that prevents emergence of 90% of adults
FT	time to first take-off
IgG ELISA	immunoglobulin G enzyme-linked immunosorbent assay
IGR	insect growth regulator
LC50	concentration that kills 50% of insects
LC90	concentration that kills 90% of insects
NS1	nonstructural protein 1
PCR	polymerase chain reaction
VCAG	WHO Vector Control Advisory Group

GLOSSARY

Active ingredient. The part of a product that has the primary action on the insect (e.g. pesticidal, behavioural, attractant).

Attractant. A biological or chemical (e.g. odorant) or other attractive element (e.g. visual, acoustic) that attracts mosquitos to a trap (also referred to as "bait").

Attractive oviposition trap. Trap designed to attract and kill gravid or ovipositing mosquitos.

Autodissemination. Picking up by adult mosquitos of an active ingredient from treated surfaces of a device or trap and transferring it to aquatic habitats in sufficient quantities to kill larvae or prevent pupae from emerging to adults. Also known as "horizontal transfer (of chemicals)" by mosquitoes (HTM), or "mechanical dissemination by mosquitoes (DSM)".

Autodissemination devices. Devices designed to lure and contaminate mosquitos with a disseminating agent (e.g. an insect growth regulator) for its transfer to additional oviposition sites.

Bait. See "attractant".

Autodisseminant. See "disseminating agent".

Discriminating concentration. Concentration of an insecticide that, during a standard length of exposure, discriminates the proportions of susceptible and resistant phenotypes in a mosquito population.

Disseminating agent (or "autodisseminant"). An active ingredient that is topically picked up by mosquitos from treated surfaces, retained and transferred to aquatic mosquito habitats.

Durability. In relation to vector traps, the physical integrity of a trap and its components over time.

Efficacy. With regards to traps, efficacy is the impact in lowering the mosquito population and/or disease incidence/prevalence in humans.

Efficacy trial. Study to estimate the effect of an intervention under the ideal conditions that can usually be achieved only in a trial, for example, by ensuring maximal coverage of the target population and adherence to the intervention.

Fast-acting insecticide. An insecticide that causes ≥ 80% mortality in susceptible target populations within 24 h of a 30-min exposure to the compound or its active ingredient.

First-in-class. Refers to the first trap for vector control with a novel entomological effect that is validated for public health value by the WHO Vector Control Advisory Group (VCAG) based on demonstration of entomological and epidemiological efficacy.

Incidence. The number of new cases of infection or disease arising in a population per unit time.

Insecticide (see also "Pesticide"). Chemical product (natural or synthetic) that kills insects on contact or by fumigation. Ovicides kill eggs; larvicides kill larvae; pupacides kill pupae; and adulticides kill adult mosquitos. "Residual" insecticides remain active after application. Insecticides can be categorized as fast- or slow-acting.

Insect growth regulator. Compounds such as juvenile hormone analogues (juvenoids) and chitin synthesis inhibitors that prevent the emergence of viable adult insects from larval or pupal stages by disrupting adult development or transformation.

Large-cage studies. Trials conducted in large screened cages or rooms under controlled conditions of temperature and humidity.

Next-in-class (see also "first in class"). Any new subsequent vector trap product having the same mode of action as the first-in-class trap product for which a VCAG recommendation has been made.

Pesticide. Any substance or mixture of chemical or biological agents intended for repelling, destroying or controlling any pest. The term includes microorganisms, insect and plant growth regulators, pesticide synergists and "safeners" that are integral to the satisfactory performance of the pesticide. The term "formulated pesticide" refers to any formulation containing a pesticide (*1*).

Semi-field trials. Trials conducted in screened enclosures in the natural ecosystem of a target disease vector.

Seroincidence. Rate of occurrence of new infections (e.g. number of seroconversions) in the population over a period of time.

Seroprevalence. Proportion of population with serological evidence of a previous infection.

Slow-acting insecticide. An insecticide that has its primary effect on mosquito mortality > 24 h after exposure.

Trap. Structure or device unto which vectors enter and/or make contact with, which ultimately results in their their capture, death and/or sterilization. Traps may work by capturing and retaining mosquitos inside a physical structure ("capture–kill") or by attracting and releasing mosquitos exposed to an insecticide or autodisseminant that will kill, sterilize or otherwise reduce vector populations after individuals leave the trap ("capture–release").

Vector trap for disease control. A trap, as defined above, implemented with the aim of reducing vector density and vectorial capacity and ultimately decreased infection or disease in humans.

Vector trap for surveillance. A trap, as defined above, used to monitor the distribution, abundance and infection rates of vector populations.

1. INTRODUCTION

The geographical distribution of important human disease vectors is expanding, and new vectors and arthropod-borne diseases have emerged. *Aedes aegypti* is the primary vector for many arboviral diseases, including dengue fever, Zika, chikungunya and yellow fever, and is a growing global public health threat. New and improved tools and strategies are needed to suppress vector populations and reduce the transmission of *Aedes*-borne diseases.

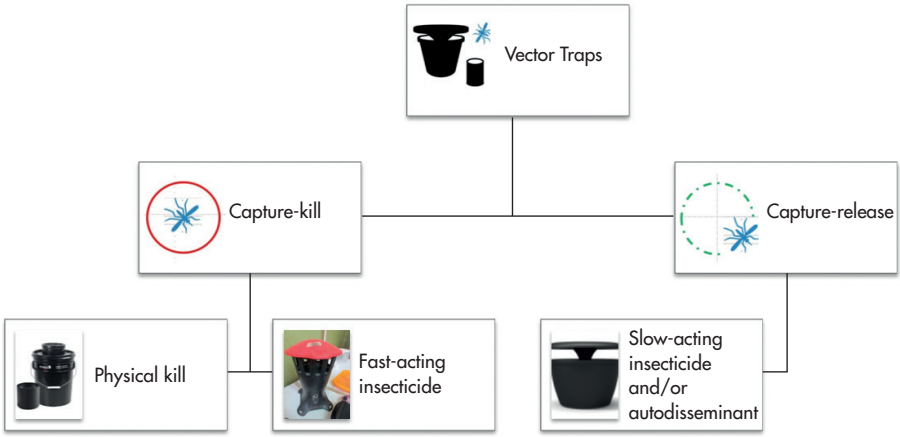
Traps are commonly used in vector surveillance to monitor the distribution, abundance and infection rates of vector populations. Several traps have been developed recently with the aim of vector control rather than surveillance; however, there are few trap-based control programmes, and evidence of a demonstrable effect in the field is required. Traps could help to reduce disease transmission by lowering vector densities below a transmission threshold or selectively targeting the older female mosquitos responsible for transmission, shifting the age structure and reducing the abundance of infectious vectors.

The purpose of this document is to provide procedures and criteria for testing the efficacy of and evaluating vector traps for disease control. It includes the design of laboratory and small-scale field trials to assess the attraction and killing effects of vector traps and of large-scale community trials to determine the efficacy of traps in reducing mosquito populations in the field and disease transmission. This document focuses on traps for container-inhabiting *Aedes* spp. mosquitos (*Ae. aegypti* and *Ae. albopictus*). Other species of mosquitos, with different larval aquatic habitats (e.g. *Anopheles*, *Culex*, floodwater mosquitos), are not yet included; however, the general testing framework described could be extended to other traps after some modification, including those for other vector species.

Vector traps are devices into which vectors enter or otherwise make contact, which ultimately result in their death or sterilization. Traps target different stages of mosquito life (eggs, larvae, pupae or adults) or physiological stages (e.g. host-seeking or gravid females). The ability of traps to attract vectors may be a function of their physical design or chemical attractant; similarly, killing may be achieved through physical design with or without insecticides. In this document, the strategy of killing vectors in traps is referred to as "capture–kill", whereby mosquitos that enter the trap are physically confined and exposed to a "fast-acting" chemical or biological insecticide (illustrated in **Fig. 1**). The trapping strategy whereby mosquitos enter the trap, come in contact with an insecticidal or sterilizing agent and then leave the trap are referred to as "capture–release" (*2*). In an autodissemination strategy, adult capture and exposure are amplified by transfer of the disseminating agent to wider aquatic habitats, where it kills larvae or prevent adults from emerging (*3*).

The WHO Vector Control Advisory Group (VCAG) has reviewed initial evidence on two broad classes of traps for control of *Aedes* vector populations: adulticidal oviposition traps, which target gravid female mosquitos (*4, 5*), and autodissemination devices, in which gravid adult females attracted to traps are contaminated with a "slow-kill" insecticide and a larvicide (IGR) for dissemination (*6*). Other traps, with new designs, attractants and insecticides, are being developed by manufacturers. The efficacy claims of new traps (on

Fig. 1. Vector traps covered in this document



the product label or elsewhere) must be validated, and the traps shown to adequately reduce *Aedes* populations and *Aedes*-borne disease before WHO can issue a policy recommendation for the broad public health use of traps for vector control. Once a policy recommendation has been developed, it is envisioned that vector trap products that are "next-in-class", thus having the same mode of action as a first-in-class product, will be assessed on entomological data only, and in most cases will not need to present epidemiological data for assessment.

This document, prepared in response to recommendations of VCAG, is intended to provide support to product developers, programmes and testing institutions in generating robust entomological evidence of the efficacy of vector traps for control and, for a first-in-class vector trap, evidence of the public health impact in reducing arboviral disease. The guidelines will be the basis for WHO evaluation of new traps and assist countries in testing the effectiveness of traps for vector control locally. The guidelines may be modified once proof of principle is established (i.e. the public health value of vector traps for controlling vector-borne disease) and as new designs, attractants, insecticides and test methods become available.

With the rapid spread of *Aedes*-borne arboviral diseases, new tools for selective targeting and suppression of *Aedes* populations are required to protect people living in areas of risk. Traps and target-based strategies have been used successfully to reduce tsetse-borne trypanosomiasis (*7*). If vector traps are proven to be effective, they could supplement current methods and improve control of *Aedes*-borne arboviral diseases. Vector traps will be most effective when used as one component in a package of interventions, and when implemented by control programmes to ensure proper use, monitoring, servicing and deployment coverage to have the desired effects on mosquitos and disease.

2. GENERAL CONSIDERATIONS FOR TESTING

As biological tests are subject to variation, they should be conducted under the close supervision of personnel who are familiar with methods for testing vector control products and compounds, using sound scientific and experimental procedures. Use of standard operating procedures for testing and for data processing, management and validation is advisable, and training of laboratory and field personnel should be documented. WHO recommends testing according to good laboratory practice as defined by the Organisation for Economic Co-operation and Development (*2*). When possible, testing institutions certified as adhering to good laboratory practice should be used for testing vector traps for WHO evaluation and prequalification listing.[1]

These guidelines are designed for evaluation of whole traps and associated attractants and/or insecticides that have already been assessed for risk and hazard. It is important that, before testing, investigators review material safety data sheets, draft product labels and certificates of compliance with manufacturing specifications and any supporting data. Independent physical and chemical assessment for compliance with the manufacturer's product specifications may be required.

Data should be collected and reported in such a way as to allow comparisons among numerous evaluation sites. For field trials, the number of replicates should be based on sample size estimates to ensure that a statistical evaluation has enough power to demonstrate efficacy. At a minimum, the data to be reported are a measure of centrality (e.g. mean, median or proportion), sample size and a measure of variability (e.g. standard error, 95% confidence interval or interquartile range).

Evaluations of vector traps should be conducted in accordance with applicable national ethical regulations, including experimental use permits for field trials. Any adverse effects on humans or potential non-target effects during relevant phases of testing should be recorded and reported.

The criteria and methods described in these guidelines will be updated by WHO as new traps, assessment methods and efficacy data become available. The test requirements for vector traps are summarized in **Table 1**.

1. The WHO prequalification team for vector control products, also known as PQT-VC, should be consulted for advice on risk assessments, specifications and prequalification requirements (http://www.who.int/pq-vector-control/en/, accessed September 2018).

Table 1. Types of studies for testing the efficacy of vector traps

Testing stage	Outcome or end-point	Applicable to	Indicator
Laboratory studies	Intrinsic activities of new AIs	New AIs only	LC_{50} and LC_{90} (both adulticides and larvicides), IE_{50} and IE_{90} (IGR), AC_{50} and AC_{90} (attractants)
	Excito-repellency	New AIs only	FT_{50} and FT_{90}
	Transfer of autodisseminant	New AI for auto-dissemination only	LC_{50} or LC_{90} or EI of susceptible larvae exposed via transfer of the autodisseminant
	Discriminating concentration	New AIs only	Discriminating concentration of AI
	Cross-resistance	New AIs only	Cross-resistance to other insecticides in unrelated insecticide classes
	Bioefficacy of formulation	All traps, AI formulations	Percentage efficacy and duration efficacy is maintained to product claims
Contained and small-scale field trials	Trap efficacy	All traps: CK, CR	Immediate and delayed mortality (adults and/or larvae) or EI
		All traps: CK, CR	Trap oviposition rates (# eggs per trap)
		CR traps only	Adult EI (%) from secondary containers[a]
	Effective trap duration	All traps: CK, CR	Number of days or weeks during which efficacy end-points meet product claims
	Effective trap density	All traps: CK, CR	Optimal number of traps per unit area
Field trials for entomological end-points	Entomological efficacy in the field	All traps: CK, CR	Significant difference in mosquito population density between treated and control areas
		All traps: CK, CR	Significant decrease in proportion of older female (parous) mosquitos
	Durability and attrition	All traps: CK, CR	Day on which efficacy indicators are not different from no trap
	Non-target effects	All traps: CK, CR	Observed negative effects on non-target organisms
Community trials for epidemiological end-points	Public health efficacy	First-in-class only	Target disease incidence or transmission Entomological outcomes (above) Community perceptions and acceptance of the intervention Adverse events per person exposed to traps and/or control

AC, attractant concentration; AI, active ingredient; CK, capture–kill; CR, capture–release; EI, emergence inhibition; FT, time to first take-off; LC, lethal concentration;

[a] This step is required only when traps include an autodissemination component.

3. LABORATORY STUDIES

Laboratory studies include tests on new active ingredients (AIs) and formulated products only. The efficacy of whole traps is studied in contained and small-scale field trials (section 4) and in large-scale field testing (section 5).

For vector traps, laboratory studies determine the intrinsic biological activity of new active ingredient(s) used in the traps, discriminating concentration and any cross-resistance with known insecticide resistance mechanisms. Laboratory studies also include determination of the efficacy and residual activity of formulated trap component products

The following objectives are relevant only for new molecules (AIs) for which evidence in the target vector has not been previously generated:

- to establish dose–response relations and determine the lethal concentration (LC) of fast- and slow-acting insecticides for 50% (LC_{50}) and 90% (LC_{90}) mortality or emergence inhibition (EI) of susceptible larval and adult mosquitos;
- to establish dose–response relations and determine the attractant concentration (AC) of a bait active for 50% (AC_{50}) and 90% (AC_{90}) attraction of mosquitos towards a chemical stimulus;
- to determine the "time to first take-off" (FT) for 50% (FT_{50}) and 90% (FT_{90}) of mosquitos after exposure to the insecticide-treated substrate;
- to establish the dose–response relation of an AI for autodissemination on adult mosquitos to achieve LC_{50} and LC_{90} of susceptible mosquito larvae that are exposed by transfer of the autodisseminant from the adult to the larval habitat;
- to assess cross-resistance of the insecticide against unrelated classes of insecticide; and
- to establish discriminating concentrations for monitoring susceptibility.[1]

Additionally, for formulated trap component products, the objective is to determine the efficacy and residual activity of a formulated AI or other agent (e.g. adulticide-treated netting, larvicide product).

3.1 GENERAL CONSIDERATIONS FOR TESTING

In order to standardize test outcomes at the laboratory stage as far as possible, laboratory tests should be conducted on well-characterized susceptible laboratory strains of *Ae. aegypti* or *Ae. albopictus*. The mosquito species and colony strain used in the test must be reported. If tests are done with other species of vectors (e.g. *Anopheles* or *Culex*), well-characterized laboratory strains should also be used and the species and colony strain reported.

1. Discriminating concentrations are already known for many insecticides. They should be determined only when they are not yet known for the target vector species.

Standardized mosquito rearing and testing conditions are essential to ensure the reliability and reproducibility of data. Existing institutional standard operating procedures (8–11) should be followed or adapted as necessary. Mosquitos are usually reared at 27 °C ± 2 °C, at 80% ± 10% relative humidity and a 12:12 h light:dark photoperiod. Test mosquitos are maintained on sugar meals (e.g. 10% sucrose) and can be non-blood-fed or blood-fed, depending on the mosquito physiological stage that is targeted by the trap. Most ovitrap AIs and components should be evaluated in 6–8-day-old gravid female mosquitos that took their first blood meal 2–4 days before the experiments. Host-seeking mosquitos are usually 3–5 day-old non-blood-fed females that have been sugar-starved for 24 h. Institutional protocols should be followed for rearing mosquitos to the desired physiological stage.

When possible, each test should include a negative control, with no insecticide or attractant, and a positive control, such as a reference attractant or insecticide for which there are data.

Equipment must be thoroughly cleaned between tests to ensure that residual material does not bias the test results.

3.2 ASSESSMENT OF INTRINSIC ACTIVITY

Intrinsic activities are assessed for novel AIs only when the biological activity against mosquitoes has not already been shown. The tests are not relevant for non-chemical components that are not produced to a manufacturing standard (e.g. hay infusion as attractant) or for formulated products (e.g. treated netting, water-soluble larvicide granules) used in traps. The relevant testing methods are summarized below.[1]

3.2.1 ADULTICIDES

To evaluate the intrinsic biological activity of a mosquito adulticide, laboratory-reared adult female mosquitos are exposed to a range of concentrations of the AI applied topically, and mortality is recorded. Topical application is used to differentiate the toxicity from confounding effects on insect behaviour. Details of testing procedures for intrinsic activity can be found in the WHO guidelines for testing mosquito adulticides for indoor residual spraying and treatment of mosquito nets (*12*); refer to section 2.1 of referenced guidelines and relevant SOPs. The bioassay procedures are the same for slow-acting as for fast-acting adulticides, except that, for the latter, mortality is monitored every 24 h until the full effect has been achieved.

3.2.2 LARVICIDES

The objective is to measure the inherent biopotency of a mosquito larvicide against the target species. Laboratory-reared mosquito larvae are exposed to a range of

1. For each referenced WHO guidelines, the most recent version should be followed and, where available, the relevant WHO standard operating procedures.

concentrations, and mortality or EI is recorded. Details of testing procedures and larval bioassays can be found in the WHO guidelines for testing mosquito larvicides (*13*); refer to section 2.1 of referenced guidelines and relevant SOPs.

3.2.3 ATTRACTANTS

The intrinsic activity of attractant AIs (including dose-response) may be a critical component of trap efficacy. Evidence should be provided demonstrating the basic ability of a new synthetic active ingredient to attract mosquitos. Laboratory-reared adult mosquitos are exposed to at least five concentrations within the activity range defined by the manufacturer or published literature. Attraction is measured in a Y-tube olfactometer in the absence and presence of the candidate compound. Tests are done on host-seeking or gravid mosquitos, depending on the physiological state targeted. Details on testing procedures can be found in WHO guidelines for testing spatial repellents (*25*); refer to section 2.1 of referenced guidelines and any relevant SOPs. These methods may need further development and validation as new attractant molecules are brought forward for use in vector control.

3.2.4 ASSESSMENT OF EXCITO-REPELLENT ACTIVITY

The repellent and irritant effects of an insecticide can modify the tarsal contact time with a treated substrate, which may reduce the lethal effect of an adulticide or reduce the probability that adult mosquitos will be contaminated and subsequently transfer the autodissemination agent. WHO cone assays may be used to assess the time between first landing and take-off for individual mosquitos exposed to technical-grade insecticide on filter paper and relevant formulations. Further details and test procedures are described in reference (12); refer to section 2.3 of referenced guidelines and relevant SOPs.

3.2.5 AUTODISSEMINATION

For AIs and formulations for autodissemination, modified bottle bioassays can provide information on the transfer of an AI to the adult mosquito (see supplemental materials). In brief, adult females are exposed to concentrations of the autodisseminant in glass bottles and then placed in screened cages with bioassay containers holding susceptible immature mosquitos. Larval mortality or EI is measured to establish the dose–response relation for 50% and 90% mortality in susceptible larvae.

Additional development and validation of bioassays may be required for different autodissemination agents and to evaluate other effects on mosquito physiology, such as chemosterilization. Suggested efficacy indicators for chemosterilization include total and mean number of eggs laid, hatchability and oviposition inhibition.[1]

1. The 2018 WHO guidelines for laboratory and field-testing of long-lasting insecticidal nets currently under development and related SOPs should be consulted for details on assessment of reproductive output.

3.3 DISCRIMINATING CONCENTRATION AND CROSS-RESISTANCE

Discriminating concentrations of all new insecticides for vector control are required for monitoring insecticide resistance in the vectors and to assess whether an intervention will be effective against local mosquitos (*14*). Test procedures should accord with WHO standard procedures for establishing discriminating concentrations (*15*).

New AIs submitted for evaluation should also be tested to determine whether there is cross-resistance with known resistance mechanisms. New compounds can first be tested against multiresistant strains of mosquitos and then against insect strains carrying one or more resistance mechanisms, as per WHO guidance (*12, 15*).

3.4 BIOEFFICACY AND RESIDUAL ACTIVITY OF FORMULATED PRODUCTS

Before testing a whole trap in small-scale field studies, the efficacy of formulated products in traps (e.g. adulticide-treated netting, sticky surface inserts, attractant sachets, larvicides or other trap components) should be validated in controlled laboratory studies. Tests should measure the initial efficacy against laboratory-reared, susceptible mosquito populations of the targeted physiological stage (e.g. gravid females, host-seeking females, larvae) and verify the proposed duration of efficacy of the products. For adulticide-treated trap components, methods can be adapted from cone bioassays (*12*, section 2.4.2). Formulated larvicides and insect growth regulators should be in accordance with laboratory methods described in the WHO guidelines for testing mosquito larvicides (*13*).

Procedures for testing attractant formulations, autodisseminants and sticky surfaces may require additional development and validation of published bioassays (e.g. *16–19*).

3.5 STATISTICAL METHODS AND DATA ANALYSIS

An appropriate estimate of centrality (mean, 95% confidence interval or median, interquartile range) are calculated and reported for the outcomes. The activity of the test compound (e.g. adulticide, larvicide) against a particular vector strain can then be compared with values for other compounds.

3.5.1 ADULTICIDES, LARVICIDES, INSECTICIDE GROWTH REGULATORS, ATTRACTANTS

The relation between dose and mortality can be analysed by log–dose probit regression with relevant statistical software packages to estimate LC_{50}, LC_{90} (or AC_{50}, AC_{90}) and 95% confidence intervals.

For insect growth regulators, total or mean emergence inhibition (EI) can be calculated from the number of larvae exposed and the overall emergence of adults. EI is calculated from:

$$EI\ (\%) = 100 - \frac{(T \times 100)}{(1/C)}$$

where T = percentage survival or emergence in treated batches and C = percentage survival or emergence in the control.

3.5.2 REPELLENT AND EXCITO-REPELLENT ACTIVITY

The relation between dose and percentage repellent and take-off due to irritability (excito-repellency) is analysed by log–dose probit regression.

3.5.3 DIAGNOSTIC CONCENTRATION

The diagnostic or discriminating concentration is determined from the dose–response regression lines obtained by testing a technical material in a susceptible vector species. The diagnostic concentration is double that of the estimated $LC_{99.9}$ estimated by probit.

3.5.4 CROSS-RESISTANCE

The LC_{50} value for susceptible mosquito strain is compared with those for several resistant strains to estimate the existence and level of cross-resistance (resistance ratio of 50% or 95%) of the new candidate insecticide (*20*).

3.6 INDICATORS FOR LABORATORY STUDIES

The values listed below should be reported where appropriate from laboratory tests.

For new AI molecules for use in vector traps:

- intrinsic activity: LC_{50} and LC_{90} (both adulticides and larvicides); EI_{50} and EI_{90} (insect growth regulators); AC_{50} and AC_{90} (attractants);
- excito-repellency: FT_{50} and FT_{90};
- transfer of autodisseminant: LC_{50} or LC_{90} or EI_{50} or EI_{90} of larvae exposed via transfer of the autodisseminant;
- discriminating concentration of AI; and
- cross-resistance to insecticides in unrelated classes.

For all formulated components for vector traps:

- bioefficacy of formulation: % mortality, EI or attraction of the target mosquito in the laboratory and the number of days the effect is maintained, according to product claims.

4. SMALL-SCALE FIELD TESTING (CONTAINED AND OPEN-FIELD TRIALS)

Small-scale, controlled evaluations of whole traps are performed with target mosquitos under contained field conditions and in small open field studies. Data collected in this phase are used to validate the claims of the manufacturer regarding efficacy and use, and to plan the next phase of testing in large-scale efficacy trials. The aims of small-scale studies are to determine the efficacy and duration of the effect of the whole trap against target vectors under controlled conditions and the effective trap application density (i.e. number of traps per unit area).

4.1 GENERAL CONSIDERATIONS FOR TESTING

4.1.1 TEST SET-UP FOR SMALL-SCALE TRIALS

Traps can be tested in large-cage or semi-field systems (contained trials) to simulate indoor or outdoor use conditions or in small-scale open field trials, depending on the end-point. Contained trials have the advantage of involving laboratory-reared mosquitos (reference or F1 of field-collected mosquitos) that are pathogen-free and of known age and physiological condition (e.g. gravid). Tests of oviposition traps in large cage and semi-field system experiments have shown good correlation with data from field tests; however, some end-points, such as effective trap density, can be measured only in open-field studies (**Table 2**). To estimate the duration of trap activity, traps are exposed to conditions of natural use (e.g. temperature, sunlight) and retested in contained trials or small-scale open field trials at set times after first use to measure their efficacy over time.

- **Large-cage trials in laboratory enclosures**: trials conducted in screened enclosures or free-flight rooms in a controlled laboratory environment with set temperature, light, humidity and air movement (*21–23*).
- **Semi-field trials in natural ecosystems**: trials conducted in screened enclosures in the natural ecosystem of the target disease vector, in local conditions of ambient temperature, light, humidity and air movement. The environment should emulate the natural habitats of the target vectors (e.g. with endemic plants and vegetation, artificial containers) (*24–25*).
- **Small-scale open-field trials**: trials conducted in local settings at limited scale, e.g. a single village. They allow collection of data on end-points that may not be feasible in enclosed studies, such as effective trap density. Small-scale field trials should be conducted in settings that represent the environments in which traps are to be deployed (e.g. back yards, in and around houses) and where the target vector is endemic.

Table 2. Small-scale contained and open-field studies on vector traps

End-point	Evaluation	Trap type	Indicator
Trap efficacy	Large cage, semi-field	Capture–kill / capture–release	Immediate and delayed mortality (adults and/or larvae) or EI
		Capture–kill / capture–release	Trap oviposition rates (number of eggs per trap)
		Capture–release	Adult EI from secondary containers (dissemination)
Effective trap duration	Large cage, semi-field and open field	Capture–kill / capture–release	Number of days or weeks for which efficacy end-points meet product claims
Effective trap density	Open field	Capture–kill / capture–release	Optimal number of traps per unit area

EI, emergence inhibition

4.1.2 MOSQUITOS AND COLLECTION METHODS

For trials in large cages or semi-field systems, well-characterized laboratory-reared strains or F1 generation offspring of mosquitoes collected in the field should be used. Appropriate arthropod containment guidelines should be followed (*26*). For open-field trials, traps are assessed against local field populations of mosquitos at trial sites.

For contained trials, it is important to be consistent in the timing of mosquito release and data collection. Ideally, trials are conducted in the afternoon, with mosquitos released around 16:00 h and the traps monitored the following morning to minimize heat stress on the mosquitos. Running contained trials for longer should be justified in the trial protocol. For traps targeting gravid mosquitos, 6–8-day-old gravid females that took their first blood meal 2–4 days before the experiments and held with access to a sugar solution can be used. Tests of traps for host-seeking mosquitos can involve 3–5-day-old nulliparous females that have been starved of sugar solution for 24 h. The conditions under which mosquitos are reared and held before use in experiments should be recorded, as this may influence the efficacy of traps (supplemental materials).

Trials in large cages or semi-field systems should aim to recapture recapture all mosquitos that were released so that the investigators can calculate the percentage mortality (including delayed mortality) and remove the remaining mosquitos via aspiration before further bioassays. Large cages or semi-field systems should be designed to allow collection of released mosquitos, through use of white netting, lowered ceilings, careful sealing of release chambers or placement of refuges such as black cloth-lined resting boxes in semi-field systems. It may also be necessary to use ant channels and daily cleaning to prevent scavenging of dead mosquitos. In all trials of this nature, there should be a wash-out period or other means of clearing all mosquitos between trials if they are not recovered through aspiration.

Trained technicians skilled in the use of aspirators should perform collections, aiming to catch all mosquitos, knocked down or resting. The total number of recaptured mosquitos should be recorded to indicate if there is some unaccounted loss. Resting mosquitos can be captured with mechanical aspirators, sweep-nets or other methods and sampling repeated until, as far as possible, all the released mosquitos are recaptured.

4.1.3 STUDY DESIGN CONSIDERATIONS

For longitudinal trap evaluations, it is important to sample systematically (e.g. weekly) throughout the test period. It is advisable to monitor the fitness (response to odour cues, egg laying or retention) of the released mosquitos. Wind speed and direction, temperature, relative humidity and precipitation should be recorded for each trial. Care should be taken to mount instruments out of direct sunlight, in the same location in each compartment for consistent comparisons of measurements. Between evaluations, products should be stored according to the label instructions or under environmental conditions similar to those used for evaluating the traps.

Experimental controls should be considered carefully. A standard negative control should be used in planned efficacy trials; for example, for gravid mosquitos, a black 1-L container with 400 mL of deionized water is suggested. Alternatively, permutations of the trap with and without AIs can be used. Currently, there is no standardized active comparator for traps; however, commonly used surveillance traps for which published efficacy data are available, could be used as a reference to compare the performance of other traps.

The number of replicates per product evaluated should be based on sample size estimates, which are required to ensure that a statistical evaluation has sufficient power (*27*). It is highly desirable that the study be fully randomized and that all field operatives be "blinded" to the allocation of treatments in order to avoid bias in the evaluation. If blinding is not possible because of the characteristics of the product (e.g. odour, colour), data should be blinded before analysis (*28*).

4.2 EVALUATION OF TRAP EFFICACY AND EFFECTIVE TRAP DURATION

Trap efficacy is assessed for candidate traps in semi-field settings or large-cage or free-flight rooms. The primary efficacy indicators are adult and/or larval mortality or EI, and trap oviposition. Where relevant, dissemination efficacy is indicated by mortality or EI from secondary oviposition containers.

To measure adult and/or larval mortality (or EI), the candidate trap is tested against a control trap in a no-choice test (i.e. either the candidate test trap or the negative control is used in one of two experimental areas or chambers). Choice tests, in which mosquitos choose between a test trap and a control in the same experimental area, are used when measuring oviposition in traps and autodissemination efficacy.

Factors that could influence preference for a trap, such as location bias, should be controlled (e.g. in a Latin square design where possible (*29*)). Care should be taken to place the traps in the same way and out of direct sunlight so as not to alter their attractiveness or the efficacy of the AIs. If multiple traps are used in choice assays, trap distance – especially with attractants – should be considered, to account for interference among traps. Gravid test traps can be placed equidistantly at a minimum of 1 m apart, while host-seeking mosquito traps can be separated by longer distances (e.g. 10 m), depending on the product claims (*30*). Mosquitos can be released in the centre of the set-up so that they have an equal probability of encountering any of the traps (test or control). Traps can be labelled with unique identification numbers and assigned randomly to an experiment or sampling station with a random number generator.

The number of replicates should be determined a priori by sample size calculation. For each replicate, at least 50 mosquitos (reared as described in section 4.1.1) are released into each large cage or semi-field compartment. For autodissemination trap trials, a maximum of 50 mosquitos should be used for each replicate. Each trial is terminated after the exposure time (usually the following morning or after 24 h). Standardized start and end times for trap operation should be used and recorded on data forms (see example in supplemental materials). At the end of the contained trial, the investigators should recapture all mosquitos, both in and outside traps, and record their status (alive, dead, gravid). A minimum recapture of 50% of released females is required for an assay to be valid.

4.2.1 MORTALITY – ADULTS IN TRAPS

For capture–kill traps, such as sticky traps or traps that prevent mosquitos from exiting, adults retained in the traps should be identified and counted. For traps in which mosquitos are killed with an adulticide, recaptured mosquitos (in and outside the trap) may be held under optimum conditions, i.e. 27 ± 2 °C and 80 ± 20% relative humidity, for a standard period defined by the AI to measure mortality after the specified holding period, e.g. 24–72 h. The performance of the trap, as measured by the proportion of retained mosquitos (percentage of females trapped) or mortality (percentage of females dead per trap), is compared with that of a negative control, in which mortality should not exceed 20%. Traps designed to kill mosquitos by retention should be monitored for mosquito escape by appropriate methods, such as video recording or holding traps in small cages.

4.2.2 MORTALITY – LARVAE IN TRAPS

In traps intended to kill larval stages and prevent adult emergence, females are allowed to lay eggs, and the performance of the trap is measured by the number of eggs laid and the percentage hatching, larval mortality and/or EI. The maximum acceptable mortality in the control is 20%, and emergence in the control group should be 80% for the test to be valid.

4.2.3 ATTRACTION – OVIPOSITION

To measure attraction to ovipositing females and to rule out repellency, the performance of a trap can be measured in an oviposition choice test, in which an alternative oviposition container is provided, such as black, 1-L pots each holding a clear glass bowl with 400 mL of water (*6*). The number of eggs laid in each oviposition site (candidate trap and secondary container) is used to calculate the percentage of eggs in the candidate trap and in the water-only controls.

If standardized recording of the first choice of oviposition location for *Ae. aegypti* is required, an additive (e.g. 0.07% Aquatain silicone oil) is applied to lower the surface tension of the water, which will cause female mosquitos to drown while ovipositing; however, the compound should be carefully selected to ensure that it does not deter ovipositing females. The performance of the trap is compared with that of the negative control and, if relevant, a standard (positive control).

4.2.4 AUTODISSEMINATION

The efficacy of autodissemination traps and devices for killing mosquitos can be measured as described above. To avoid contamination, treatment and control traps should be tested in separate testing compartments (e.g. semi-field, large cage, free-flight room).

The efficacy in disseminating insecticide to secondary (or alternative) oviposition sites is measured in a choice test, in which two alternative oviposition sites (also called "secondary containers") are provided. Secondary containers, such as black, 1-L pots each holding a clear glass bowl with 400 mL of water (*6*), can be placed at fixed locations a minimum of 1 m from the dissemination device, with two secondary containers per device.

To assess the efficacy of dissemination, 25 *Ae. aegypti* larvae (late L3 or early L4) and a larval food source are added to each secondary container. The following morning (or after a specified interval such as 24 h), the containers with larvae are removed and larval mortality and EI are monitored in the laboratory (*13*). The presence of eggs in all available oviposition sites is recorded.

Care should be taken when setting up an experiment to avoid contamination of secondary containers or control traps by handling, for instance by changing gloves between handling devices and decontamination procedures for moving devices between experimental compartments.

4.2.4 DURATION OF TRAP ACTIVITY

In order to evaluate the duration of efficacy, traps should be tested (mortality, capture, oviposition, dissemination efficiency) weekly to determine whether the efficacy targets are met, either for the duration specified on the product label or, if no claim has been made, the day on which the efficacy target falls below 50% of the initial level. Between tests, traps should be stored under normal conditions of temperature and sunlight.

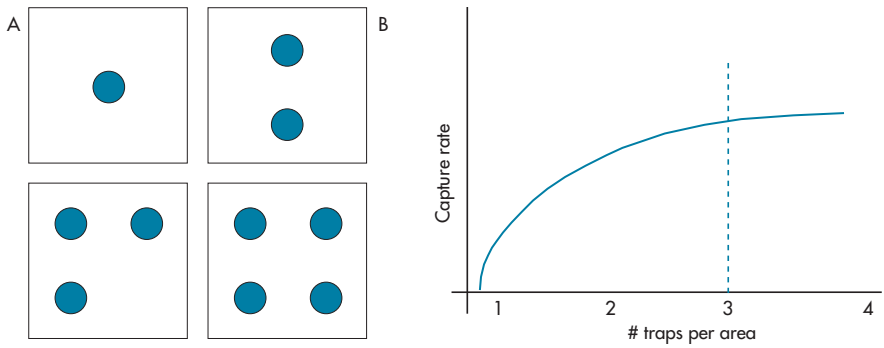
4.3 EVALUATION OF TRAP DENSITY

The likelihood that an individual mosquito will come into contact with a vector trap depends on the local abundance of mosquitos, their habitat, the presence of competing aquatic sites and also the number of traps deployed in a given area. Trap density is an important consideration for efficient deployment, as a high density of traps might be expected to maximize the likelihood of mosquito capture, but reducing trap density would lower costs. The number of traps required in an area depends on the type of larval habitat, density of houses, housing characteristics, mosquito species and amount of open space available. Small-scale open-field trials are conducted to confirm the proposed trap density (number of traps per unit area) before large-scale field testing of traps.

The number of traps per defined area (a back-yard, for example) can be studied by comparing the trap capture rate with increasing trap densities (one, two, three or four traps per area). The optimal number of traps is reached when the number of mosquitos captured per trap reaches a plateau (Fig. 2). Trap density should be evaluated in both rainy and dry seasons, especially for devices that mimic oviposition sites, as they compete with larval development sites.

For autodissemination devices, the number of devices needed in a defined area can be

Fig. 2. Measurement of trap density: (A) boxes represent defined areas with an increasing number of traps (circles); (B) in this example, the number of traps that capture mosquitos in each area plateaus at three traps (dashed vertical line), indicating the optimal number of traps to be used. This graph represents a predicted relationship assuming constant recruitment into mosquito population and density of competing attractants (e.g. host sources); the relationship between trap density and catch would also be influenced by these factors.



estimated from the larval and pupal mortality and EI in sentinel aquatic sites (secondary containers) placed at known distances from the candidate autodissemination device, compared with similarly set-up in uncontaminated control test sites. As in contained testing assays (section 4.2.4), larval bowls from sentinel sites are taken back to the laboratory for bioassay.

4.4 STATISTICAL ANALYSIS

The primary analysis should be a comparison of a candidate trap with a negative control. The statistical approach should include control for clustering and sources of variation in the experiment, such as replicate or location, in a mixed-effects or generalized linear model (i.e. distribution of families that are not necessarily normal).

Measures of centrality (e.g. mean, median, proportion) should be presented, with 95% confidence intervals or interquartile range, in addition to the results of statistical analysis, by giving the coefficient or odds ratio, value of the test statistic, associated P value and degrees of freedom.

Many of the outcome variables measured in laboratory experiments are proportions (e.g. proportion of adults or larvae dead). These data can be analysed in a binomial model, but the denominator must be specified. Other variables measured are counts (e.g. number of eggs laid, number of captured mosquitos), which can be modelled with a Poisson or negative binomial distribution, depending on the degree of overdispersion. For slow-kill AIs, daily mortality rates can be assessed by Kaplan-Meier or Cox regression to determine whether the survival of the test groups differs significantly.

Other appropriate tests include probit analysis to calculate the LC_{50} and LC_{90} if a dose–response relation is required. Survival analysis (Cox proportional hazards, Kaplan–Meier) may be appropriate to define the duration of effect.

4.5 SUMMARY OF EFFICACY INDICATORS FOR SMALL-SCALE AND SEMI-FIELD EVALUATIONS

The association between abundance and age structure of *Aedes* and disease transmission is not clearly defined and is likely to vary by ecological and epidemiological setting. Consequently, further evidence is required to set threshold values for the proportion of the *Aedes* population that a trap should remove in order to affect disease transmission

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Trap developers should seek to maximize efficacy in small-scale testing to ensure that the product has the highest possible mosquito catch rate in the field. For capture–kill traps, a consistent rate of 70% mortality or capture for the claimed duration of efficacy is desirable before proceeding with large-scale field testing (section 5). For autodissemination devices, guidance will be revised as further data are generated.

A candidate trap is assessed against its efficacy in semi-field or large cage tests for the following variables:

- adult mortality (immediate or delayed);
- larval mortality or EI;
- attraction-oviposition: trap oviposition rates (eggs in trap relative to control);
- auto-dissemination: percentage adult EI from secondary containers;
- duration of activity: number of days or weeks for which efficacy end-points meet product claims; and
- density of application: optimal number of traps per unit area.

5. LARGE-SCALE ENTOMOLOGICAL FIELD TRIALS OF VECTOR TRAPS

Candidate vector traps that are efficacious in small-scale field trials should be validated in large-scale entomological field trials against natural *Aedes* spp. populations. These trials are intended to demonstrate whether use of the traps over an area can control local populations of *Aedes* spp. and/or change the age composition of adult female mosquitos. The experimental design must be statistically robust and have the power to demonstrate a specified reduction or difference in key parameters between treatment and control clusters. The tests should also indicate the physical durability and attrition of traps, user acceptance and effects on non-target organisms.

Details of methods for planning and conducting entomological trials are beyond the scope of this document, and the WHO manual on study design of field trials for vector control interventions (*28*), other resources (e.g. *31, 32*) and a specialist in trial design and implementation for vector control should be consulted.

The objectives of such tests are to:

- confirm the efficacy and duration of the effect of traps to reduce vector populations and/or alter population structure under field conditions at the defined trap density;
- assess the physical durability and attrition of traps in field conditions;
- observe and record the ease of application, handling and perceived adverse effects during product application and use;
- for traps that include an insecticide component, determine insecticide resistance before and after the trial; and
- observe and record the effects on non-target organisms, including pests (e.g. *Culex* mosquitos) and beneficial insects (e.g. bees).

The design of large-scale entomological field trials must be robust and preferably be a cluster randomized trial that meets the criteria of replication, randomized trap allocation and adequate sample size. Vector traps in the treatment clusters are distributed at the intended density, coverage (i.e. number and placement of traps per unit area) and position inside and/or outside houses. Efficacy is assessed by comparing differences in vector population density and age structure (including sex ratio and parity) in the treatment and control (no traps) arms of the trial. Tests will also demonstrate the physical durability and attrition rates of traps, acceptability by users and effects on non-target organisms.

5.1. GENERAL CONSIDERATIONS FOR TESTING

The entomological outcomes of field trials on vector control interventions are specific for the setting in which the trial was conducted. Full assessment of the efficacy of candidate traps might require testing in several ecological settings and in different seasons, depending on the product claims. The area and location of trial sites should be representative of the target species' habitat and the expected conditions of human

exposure. Generally, at least two well-conducted large-scale field trials are required for a WHO policy recommendation.[1]

The WHO Vector Control Advisory Group recommends that first-in-class vector traps intended for public health initiatives be tested in trials that include epidemiological end-points (section 6), in addition to field trials with entomological end-points described here. Next-in-class traps do not need to show data on epidemiological efficacy and can be assessed from entomological data alone.

5.2. ETHICAL CONSIDERATIONS AND COMMUNITY SENSITIZATION

Ethical approval should be received from the appropriate ethical committees before any trial procedures are started. The design of the study, participant information sheets and consent forms should undergo ethical review. Key considerations include: increased exposure to vector-borne diseases from additional aquatic sites or diversion of vectors, potential adverse effects associated with human exposure to the traps (as described by the manufacturer; see section 6) and effects on non-target organisms such as pollinators.

Human use protocols should clearly describe the potential risks associated with use of and exposure to the traps and strategies to mitigate such risks. Examples include: instructions for trap monitoring by project personnel and appropriate disposal (e.g. on completion of the study) to ensure that traps do not become larval habitats; exclusion criteria for households that cannot provide access for trap monitoring; and provision of clear descriptions of potential health risks to study participants when obtaining consent, including the contact details of study personnel and instructions for participants if they experience any physical symptoms associated with exposure to the traps. Households must be informed about the procedures and the frequency of monitoring visits associated with their participation in the trial.

Engagement strategies should include working with community leaders and members to inform them about the trial objectives. Informed consent must be obtained from individual households and/or the communities when appropriate. If trials are conducted in areas with possible virus transmission, control and treatment sites should continue to receive vector control according to the standard of care, including emergency control (e.g. space spraying) interventions. Coordination with local health authorities to keep the lines of communication open can mitigate the impact of these activities on trial results to ensure that all activities are properly documented and that all study clusters receive any emergency control measures equally.

Risk assessments that take into account the type of device, the attractant, the insecticide used and the environment in which the trap will be set may be required before testing, according to the protocols of the testing institutions. If during the field tests evidence arises that other insects (e.g. honey bees) are being collected or their populations reduced, further studies may be required to measure the impact.

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1. Applicants are strongly encouraged to contact VCAG (vcag@who.int) to ensure that the appropriate procedures for generating evidence for public health are followed and to consult the latest guidance on its website and that of the WHO prequalification team for vector control products.

5.3. STUDY DESIGN

WHO guidance on the design of phase 3 vector control field trials (*28*) should be referred to for additional guidance.

5.3.1 SAMPLE SIZE

Before a trial begins, the necessary sample size should be estimated to ensure that the trial has enough power to quantify the effectiveness of vector traps against the entomological indicators of interest (*33, 34*). Local heterogeneity in *Aedes* numbers may influence the sample size and number of replicates.

5.3.2 DURATION OF TRIAL

Entomological field trials should be conducted over a minimum of a full transmission season, as trap performance is likely to depend on mosquito density and environmental conditions. Baseline characterization of local field sites is recommended; the data collected can be used for stratification and allocation of treatment and control clusters. For more details of study designs that require baseline data collection, see Wilson et al. (*31*). The benefits of planning longer trials should be considered, to account for the risk that atypical meteorological events (e.g. hurricanes), political or civil unrest or disease outbreaks (*Aedes*-borne or other infections) will confound or disrupt the trial. For first-in-class products, where both entomological and epidemiological field trials are planned, VCAG recommends a 2-year trial duration, excluding baseline data collection, to generate data on the consistent entomological and epidemiological outcomes across consecutive high and low transmission seasons.

5.3.3 STUDY AREAS

Study sites should be carefully selected to ensure that treated areas and controls are independent but comparable (e.g. in terms of ecology, housing type, predominant larval habitats and meteorological conditions). Clusters should be of similar sizes, with a minimum size equal to the flight range of *Aedes* spp. (150–200 m radius, e.g. a few blocks in urban sites and a single village in rural sites). Clusters should be spatially separated and not contiguous or adjacent. A separation of two to three times the flight range of *Aedes* spp. is ideal (300–600 m).

If isolated areas cannot be used for testing, traps can be deployed over a sufficiently large experimental area so that entomological assessment can be restricted to a central zone where the impact of treatment will be greatest. Mosquito density just outside the experimental area can then be measured and compared with that in the centre of the treated area.

The environmental conditions of temperature, humidity, rainfall and wind speed should be monitored and reported during evaluations for multivariate analyses and to quantify trends. Ideally, environmental conditions should be monitored at several points in the trial site. If this is not possible, data may be collected from weather stations in the study area. If the device being tested includes insecticides, the insecticide resistance profile of the target species in the testing area should be considered (*15*).

5.3.4 PRE-INTERVENTION SITE CHARACTERIZATION

As trials must have comparable treatment and control areas, preliminary characterization of the areas is essential. The length of the survey will depend on the study design. Baseline surveys are conducted to characterize parameters such as vector abundance (section 5.3.6), insecticide resistance and housing and other relevant characteristics to control for underlying sources of variation in the analysis or stratify allocation on variables with wide variation among clusters. For designs that compare study areas before and after trap deployment, the pre-intervention survey should be long enough to capture temporal variation in the study area.

5.3.5 TRAP PLACEMENT

Trap density (per area or dwelling), coverage (percentage of area or dwellings with traps) and placement (preferred locations) are decided on the basis of manufacturers' recommendations and the evidence provided by the small-scale trials. The number of traps required per study area in each trial depends on the type of trap, the size of the trial area, the estimated area covered by traps and the estimated adult mosquito population density before trapping. At a minimum, an adequate trial should achieve 80% of the planned coverage (i.e. the predetermined number of traps required) in each study area; any shortfall in coverage should be recorded and reported. If the study design specifies that trap placement be accompanied by larval source reduction (e.g. removal of tyres and other secondary containers), similar source reduction should be undertaken in the control arms of the trial.

5.3.6 ADVERSE EFFECTS (SEE ALSO SECTION 6)

Adverse effects and events due to use of the trap product, general acceptance by local inhabitants and attrition (missing or destroyed traps) in the trial area should be observed and recorded, such as for instance records of people who did not accept to participate or dropped out and those who were retained (35). A GIS database may be useful for monitoring traps and trap attrition.

5.3.7 SAMPLING AND MONITORING

Ideally, more than one monitoring method should be used for assessing effects on *Aedes* populations or mosquito survival. Sampling schemes (number of days sampled per week) should be standardized for all study areas. For interventions targeting *Ae. aegypti*, sampling should be conducted in or around households. *Ae. albopictus* is found in a wider range of habitats both near and far from human population centres (urban, rural and forested). Methods for sampling should be evaluated under local conditions before use and with consideration of the local ecology of the target vector. If traps are used for monitoring in field trials, these should be placed at a distance far enough from the intervention trap that there is no competition between the two (e.g. not in the same household or room).

The recommended sampling methods are adult aspiration for *Ae. aegypti* (e.g. CDC Backpack, Prokopack) and traps for *Aedes* surveillance (e.g. BG Sentinel traps, autocidal gravid ovitrap, gravid *Aedes* trap, infusion-baited ovitraps) (*36*) (**Fig. 3**).

Fig. 3. Common sampling devices for *Aedes* spp. mosquitos. (trap images adapted from reference *36*)

BG-Sentinel 2 | Autocidal gravid ovitrap | Gravid *Aedes* trap | Prokopack

Larval or pupal surveys provide valuable supplementary information on *Aedes* ecology in study areas, but indices for such immature stages should be considered secondary measures. The presence of eggs in ovitraps can indicate the presence or absence of *Aedes* spp. and is used in many programmes; however, because the density of both larvae and eggs in ovitraps depends on the availability of containers and is not necessarily directly related to changes in adult density, this measure is not recommended for assessing the effect of vector traps on populations.

Human landing collection of *Aedes* mosquitoes is not recommended where there is the risk of exposure of field collectors to arbovirus and the lack of prophylaxis for *Aedes*-borne diseases. Some researchers have used double nets or electrified nets to collect *Aedes* mosquitos in the field, thus preventing human baits from being bitten (*41,42*). Sweep net collections have been used for collecting adult *Ae. albopictus* (*43*).

Methods for surveillance of *Aedes* mosquitos have been described comprehensively elsewhere (*4, 36, 44*), including the use of infusion-baited ovitraps.

5.4. MEASURING EFFICACY OF TRAPS AGAINST ENTOMOLOGICAL END-POINTS

The objective of entomological evaluations is to determine whether the adult female *Aedes* population or mosquito survival is reduced significantly by the vector trap intervention. To determine the effect of traps on the target vector population, adult densities and age structure should be evaluated by collecting samples in treatment and control areas by the same standardized sampling scheme used for baseline characterization of the site. Sampling should be frequent enough to account for temporal and spatial variation in the mosquito population throughout the trial. For guidance, sampling intervals of 1–3 weeks should be used.

5.4.1 ASSESSMENT OF ADULT POPULATION DENSITY

Adult mosquito densities in and around houses in treatment and control areas can be monitored at fixed trapping points (in adult traps) or in house-to-house surveys (by aspiration). House-to-house surveys cover more houses per unit time and ensure better spatial coverage than fixed traps, but they are labour-intensive and depend strongly on the skill and diligence of the operator. Fixed trap methods better capture short-term temporal variation.

Sampling procedures should be standardized as far as possible to maximize consistency in the results. Detailed procedures for household surveys with aspirators are provided in the WHO guidelines on evaluation of space sprays (*45*). The aim of the procedures is to sample the adult vector population in the study areas reliably, as expressed by the average number of mosquitos per room, per house or per other defined unit sampling point.

5.4.2 ASSESSMENT OF MOSQUITO POPULATION STRUCTURE AND PHYSIOLOGY

The age structure of the mosquito population in the field can be estimated from the frequency of nulliparous and parous mosquitos. The proportion of parous females is an indirect measure of the probability of daily survival of mosquitos in the population. Parity is a useful indicator in mosquito populations that are stable over time, as demonstrated by surveillance in the study area, for example during site characterization before the intervention.

5.4.3 AUTODISSEMINATION EFFICACY

While the aim of large-scale entomological field trials is to detect entomological effects on the population due to the presence of vector traps, for traps that function by autodissemination, it may also be useful to monitor the efficacy of autodissemination over time. Autodissemination monitoring ovicups (*46*) or larval bioassays in water sampled from natural aquatic habitats (i.e. water bodies with *Aedes* larvae) can be used to measure autodissemination efficacy. Laboratory-reared *Aedes* larvae added to these samples and *Aedes* larvae collected from natural sites are monitored for emergence inhibition. For ovicup monitoring, a trap:ovicup ratio of no more than 1:5 is recommended to avoid an effect of ovicups on the overall mosquito population. Autodissemination efficacy may increase with time due accumulation of the autodisseminant (e.g. pyriproxifen) from multiple visits of mosquitos to the oviposition site or ovicup.

5.5. PHYSICAL INTEGRITY AND DURATION OF EFFECT

Most traps require periodic servicing or maintenance. Trap durability and efficacy should be assessed during the servicing interval (i.e. the time in days or months for which products are effective without servicing), and a longer-term assessment should be done to determine trap integrity and retention or loss and to confirm the duration of trap efficacy. The duration of each study should be appropriate for validating the manufacturers' claims. During servicing, physical integrity and trap presence should be recorded, and quality assurance assays can be conducted on certain components of the traps. Alternatively, assessments can be made of manufacturers' claim by simple random sampling of traps in the study area.

A standard sampling questionnaire should be used to collect data on the integrity, durability and attrition of traps. Mobile devices and GIS databases may be helpful for data collection and tracking and should be explored. The aspects listed below should be investigated.

- Physical integrity: A standardized form should be prepared for recording the general condition of the trap, including (where relevant) condition of insecticide components or adhesive strips (e.g. presence, whether torn or have holes), water levels, presence of larvicide or attractant.
- Trap functionality: presence of adult and immature mosquitos and other insects.
- Quality assurance of trap components (see section 3.5): bioassays with insecticide-treated materials in traps, assessment of adhesives and evaluation of larvicidal activity.
- Trap attrition: whether traps have been lost or moved, whether residents have washed or modified the traps against study instructions.
- Household retention: withdrawals and coverage rates.

5.6. OBSERVED NON-TARGET EFFECTS

Candidate traps tested under field conditions must be assessed for ecological and human toxicity before a field study is conducted. Detailed treatment and analysis of these data are beyond the scope of this document; however, during large-scale trials, when appropriate, qualitative observations should be recorded on non-target species that are protected or would affect allied species such as bees and other pollinators (*47*). For example, non-target organisms found in traps or any noticeable impact on cohabiting organisms found during larval sampling (e.g. fish, copepods, other mosquito larvae) could be noted.

5.7. EFFICACY INDICATORS FOR ENTOMOLOGICAL FIELD TRIALS

A candidate trap, with bait and/or insecticide, is tested for efficacy in large-scale entomological trials against the following primary criteria:

- local adult *Aedes* mosquito population density: significant difference in mosquito population density between treated and control areas;
- local adult *Aedes* mosquito population structure: significant decrease in the proportion of older female (parous) mosquitos.

The following secondary indicators support efficacy assessments, and, when possible, the results should be reported.

- sex ratio shift: a significant increase in the proportion of males in the treated area;
- oviposition rates: significant decrease in mean egg catch in the treated area;
- physiological status: significant decrease in the number of blood-fed females collected in the treated area; and
- infection rate: proportion of vectors infected (see section 6.3).

6. COMMUNITY TRIALS OF IMPACT ON DISEASE

The public health effect of first-in-class vector traps against natural vector populations is assessed in community trials of the epidemiological impact on the incidence of *Aedes*-borne virus (ABV) or *Aedes*-borne disease in study clusters with and without traps.

Before traps can be recommended for public health programmes, evidence is required to support the principle that a vector trap strategy can reduce infection and/or disease. To that end, the Vector Control Advisory Group recommends that at least two well-implemented, randomized, controlled trials be conducted of epidemiological outcomes in different eco-epidemiological settings for a full assessment of the public health value (i.e. reduction of infection and/or disease) of this intervention strategy (*48*). The duration of epidemiological assessment, excluding the baseline period, should cover at least 2 years, to account for inter-annual variation in transmission. Individual next-in-line traps may not require such evidence, and applicants are strongly encouraged to contact the relevant WHO programmes (i.e. VCAG and PQT-VC) to ensure that the appropriate evidence is generated.

Large-scale entomological field trials are described in the previous section. As detailed methods for planning and conducting epidemiological trials are beyond the scope of this document, the WHO manual on study design of field trials for vector control interventions (*28*), other resources (e.g. *31, 32*) and a specialist in epidemiological trial design and implementation for vector control should be consulted.

Study designs are affected by conditions that are impossible to control, including household access and coverage, heterogeneous housing, movement of people, security issues and other public health programme activities, as well as unpredictable virus transmission dynamics. Accurate evaluation of interventions requires a robust study design.

The objectives of a community trial are to:

- demonstrate the protective efficacy of traps for ABV transmission and/or *Aedes*-borne disease incidence;
- monitor severe and adverse events in the human population; and
- observe and record acceptability, coverage and maintenance during product application and use (the trap itself and the bait and/or insecticide), ease of application and handling, associated costs and any consequences associated with maintenance failure (trap loss and conversion into a larval habitat).

In this section, we describe measurement of virus transmission, disease and related proxies, ethical considerations, human safety, blinding and trap effectiveness.

6.1. MEASURING TRAP EFFICACY AGAINST EPIDEMIOLOGICAL END-POINTS

The primary epidemiological end-point is demonstration of the protective efficacy of the trap intervention. As an expected rate of protective efficacy is required for calculating sample size, a minimum of 30% is recommended.[1] Entomological end-points should be consistent with the mode of action of the traps (see section 5). Defining strategies for monitoring virus transmission or disease in human populations is particularly challenging for *Aedes*-borne diseases. In these guidelines, we focus on diseases caused by dengue, Zika and chikungunya viruses. Dengue and Zika viruses are in the family *Flaviviridae*, whereas chikungunya virus is in the family *Alphaviridae*.

During the first 5 days of acute infection, virus can be detected by cell culture or polymerase chain reaction (PCR) of key RNA sequences. Effective disease surveillance systems and confirmatory laboratory diagnostic capacity are required to identify and test potentially infected individuals. When possible, several strategies should be used to measure infection (section 6.1.2) and/or disease (section 6.1.3) in community-based trials. All residents in the study area can be monitored for disease. For infection, a subset of residents most likely to be susceptible (e.g. children) is identified during the baseline screening study. Blood samples from these individuals are tested at regular intervals to monitor seroincidence.

Monitoring multiple epidemiological parameters will increase the probability of detecting PE if an intervention is effective, but monitoring both disease and infection is not a requirement and may not be feasible or appropriate in certain locations. PE can be sufficiently demonstrated with a single epidemiological endpoint.

6.1.1 BASELINE AND SCREENING STUDIES

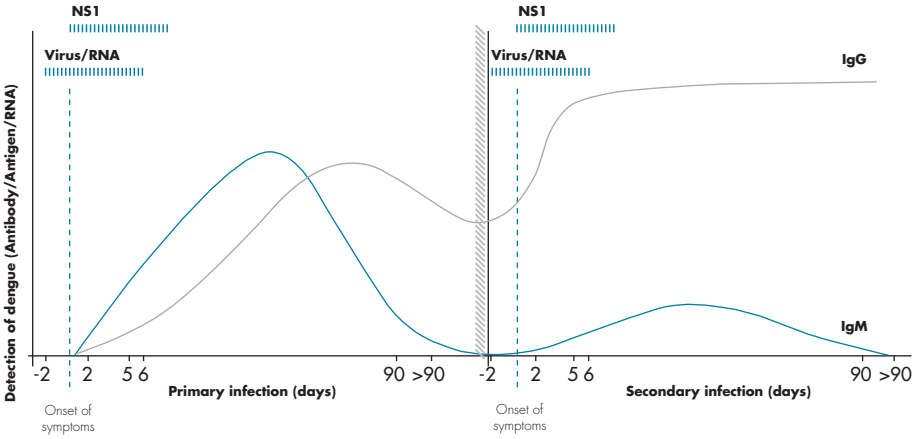
As *Aedes*-borne viruses cause "sterilizing immunity" to the infecting virus serotype, the age-specific seroprevalence of arbovirus serotypes in the study population should be known to understand heterogeneity among clusters and to stratify the allocation of traps. Residents in the study cluster(s) should be screened to determine prior ABV exposure, and only those showing negative or monotypic ABV response should be included as participants in the sero-incidence studies (section 6.1.2). Because of significant cross-reactivity in diagnostic tests between dengue serotypes and Zika virus infections, inclusion of participants with a multi-typic response is not recommended.

The serological status of study residents is used to identify a longitudinal cohort (see section 6.1.2) and to characterize the susceptibility of the human population in each study cluster to ABV infection. This information can be used to stratify clusters before trap allocation (e.g. clusters can be stratified into high, medium and low seroprevalence groups), and allocation to treatment and control be balanced within each stratum. Serology is the method used to detect ABV infection after the acute phase of infection is over. Plaque reduction neutralization or microneutralization assays should be performed,

1. The value of 30% protective efficacy was intended as a conservative, practically achievable rate of protection, as recommended during expert consultation and a review of similar efficacy trials of targeted interventions against *Aedes*.

when possible, to determine serotype specificity. Alternatively, an immunoglobulin G enzyme-linked immunosorbent assay (IgG ELISA) can be used to distinguish between naive and previously exposed individuals, although this assay is not serotype-specific. Fig. 4 summarizes the timelines for antibody responses and detection methods after dengue infection. The WHO guidelines on dengue diagnosis, treatment, prevention and control (36) should be consulted for details and resources.

Fig. 4. Approximate timelines of primary and secondary dengue virus infections and methods that can be used to detect infection



Adapted from the WHO guidelines for diagnosis, treatment, prevention and control of dengue (*36*) and www.cdc.gov/dengue/clinicalLab/laboratory.html

IgM, immunoglobulin M; NS1, nonstructural protein 1

6.1.2 SEROINCIDENCE STUDIES

A subset of the population in the study clusters should be recruited for longitudinal blood sampling. Blood samples collected at 6–12-month intervals from the same individuals are tested for virus in plaque reduction neutralization or microneutralization assays, as described above for baseline surveys. Ideally, the cohort members should have had no prior ABV infection, exception for dengue virus, when inclusion of individuals who have been exposed to a single virus serotype would be appropriate. Studies suggest that in most dengue-endemic regions > 90% of adults have had at least one dengue infection. Therefore, paediatric cohorts are recommended, as the risk of infection increases with time.

When an individual has antibody titres above the threshold level after a previously negative blood sample, he or she is assumed to have developed an infection during the time between the two samples. Individuals who show no change in antibody titres are assumed not to have seroconverted. Seroincidence rates can be calculated by cluster and time interval from all individuals who provide paired blood samples:

Seroincidence rate = number of seroconversions / sum person-time.

Alternatives to this approach include IgG ELISA or haemagglutination inhibition assays on blood samples taken at 3–6-month intervals to identify seroconversion. IgG ELISA of saliva samples has been used as a proxy to identify dengue virus transmission, but positive samples do not necessarily represent new infections (*49*).

6.1.3 DISEASE SURVEILLANCE

A second strategy for measuring public health impact is quantification of cases of *Aedes*-borne disease within study clusters. Study participants can be instructed to present to local health facilities when they have symptoms, or an active disease surveillance system can be set up. Historically, fever has been a clear trigger for either presenting to a health facility or as a key criterion for identifying individuals to be screened for ABV. As many cases of Zika virus disease do not present with fever, study participants can be told to watch for rash and/or fever accompanied by joint pain and/or red eyes (50). It is critical that the surveillance protocol and case inclusion criteria be consistent for all study clusters throughout the study.

Blood samples from both acute and convalescent cases should be obtained for laboratory diagnosis. Samples taken during the first 5 days of illness should be tested by PCR or nonstructural protein 1 (NS1) testing. When individuals present with clinical symptoms but test negative by PCR, a further blood sample should be taken 14–21 days later to test for virus-specific IgM or IgG antibodies (**Fig. 4**).

In all trials, disease surveillance protocols must be consistent and the population participating in surveillance be well characterized. The most commonly used disease surveillance strategies are listed below.

Passive surveillance in health facilities or by study personnel

Study participants can be given clear instructions to notify study personnel or to present to a designated local study clinic if they have fever or other specific symptoms. Usually, they are given a card that identifies them as study participants and provides contact information. This strategy works well if access to facilities or study personnel is readily available. As the method relies on the initiative of study participants, it can be improved by periodic phone calls or reminders. Health-seeking behaviour varies, especially by age. Passive case-finding should be considered a complementary outcome, and active surveillance is preferred to avoid treatment-seeking bias.

Surveillance in schools or workplaces

Absence from school or work has been used as a trigger for visiting study participants and obtaining samples for diagnosis if the absence is due to illness. Although this system may be effective for epidemiological studies, it is not a recommended strategy unless the experimental units are schools or workplaces. This type of surveillance may reveal cases, but it greatly restricts the size of the surveillance cohort. For community interventions, surveillance at the household level conducted by active house visits is preferred.

Active house visits

Households in the study clusters may be visited once to three times a week to ask whether individuals have fever or other symptoms. Although this system is labour-intensive, it is the most sensitive approach for identifying potential cases. Furthermore, individuals who are ill who do not agree to provide samples can be counted to identify potential participation bias in clusters.

Household census (denominator)

Calculation of seroincidence rates requires reliable, precise estimates of the number of individuals under surveillance. This requires household censuses and monitoring of residents' movements in and outside households to document their presence and absence in the study area. Census information must therefore be updated periodically.

Time in house (exposure)

As traps are deployed at cluster level, additional studies are required to determine the proportion of time individuals in the population under surveillance are exposed to the vector control intervention at both household and cluster level. This information can be collected through interviews or methods such as GPS tracking. Both seroconversion and seroincidence calculations will have to be adjusted to person–time data to account for time not exposed to the intervention.

6.1.4 CROSS-SECTIONAL SURVEYS

During periods of very high transmission, a series of standardized cross-sectional surveys across clusters could be used to identify infected people in order to determine the public health impact of an intervention. Although this strategy is not recommended as the primary or only epidemiological method, it would be appropriate after the introduction of a novel virus or serotype into a study area (for example, during periods of epidemic transmission). To increase the probability of detecting a significant public health impact, a protocol including sample size calculations could be prepared in advance for use in the case of an outbreak. If high rates of ABV infection are documented, the duration of the trial could be shortened. A random selection of individuals in each cluster under surveillance would provide blood samples each month to be tested for evidence of acute infection (PCR, IgM and NS1).

6.1.5 CHANGES IN MOSQUITO INFECTION RATE

As part of entomological monitoring in a trial, adult female mosquitos may be tested for ABV by PCR or NS1. Mosquitos that have had recent blood meals should be tested separately from those without evidence of a recent feeding and from gravid mosquitos. All species of mosquito, including abundant *Culex* mosquitos, with recent blood meals can be tested for ABV, as these will test positive if they have recently fed on an infected person even if they are not vectors of the disease. Positivity in gravid and non-gravid *Ae. aegypti* (or other known *Aedes*) females is used to estimate the number of infectious mosquitos in each cluster. The mosquito infection rate is potentially a proxy for human infection. At present this would be an appropriate secondary outcome, but it cannot substitute for seroconversion or disease incidence.

6.1.6 BLINDING

To reduce potential study bias, blinding to the intervention is usually recommended in clinical trials. When traps are used, blinding of study participants and field staff may not be practical; however, measures should be in place to ensure blinding of laboratory data, both virological and entomological, as well as data management and analysis. A standard of care alternative, such as larviciding, is recommended for comparison in all study clusters, both intervention and control. Equal, standardized treatment must be used in all study clusters for disease surveillance. Mock trap devices (with no water, no insecticide, easy escape) could be used; however, this approach is limited because participants must have information on trap components and their risks before they provide informed consent.

Teams responsible for different components of the study (disease monitoring, entomological monitoring, trap deployment and maintenance, laboratory) should work independently to avoid unintentional bias. For example, different teams should be responsible for implementation and for evaluation.

6.2. ETHICAL CONSIDERATIONS, STUDY REGISTRATION AND MONITORING

Full ethical considerations are not covered in this document, and appropriate sources and experts should be consulted during the planning of trials. Guidance on the ethical design and conduct of cluster randomized trials is provided in the Ottawa Statement (*51*).

6.2.1 STUDY REGISTRATION

It is strongly recommended that community trials (randomized controlled trials) be registered as clinical trials in an appropriate registry before they are initiated. This step has a number of important implications: (i) compliance with local regulatory institutions by passing all protocols through national institutional review boards responsible for clinical trials; (ii) a clear plan for allocation of the intervention, including a method for

generating an allocation sequence, a list of the factors used for stratification and a method for implementation; (iii) a clear statement of who will be blinded (participants, study personnel, data analysts) and how; (iv) data monitoring and audits; and (v) monitoring of safety and structures for implementation, e.g. a data safety monitoring board. In addition, it is best practice to have documented procedures (standard operating procedures) for all aspects of trial conduct and data collection, e.g. for procedures such as drawing blood, trap deployment, mosquito collection and data management.

6.2.2 MONITORING OF ADVERSE EVENTS

Although most anticipated trap designs are not expected to be associated with more than minimal risk, many contain chemical insecticides or parts that could be ingested or cause allergic or physical reactions on physical contact. Severe adverse events must be distinguished from expected minor-to-moderate adverse events described in the manufacturer's brochure.

Examples of severe adverse event include death or severe injury after choking on a trap component, asthma requiring hospitalization induced by exposure to a chemical component of a trap or serious injury due to tripping over a trap. The rules for reporting severe adverse events depend on the institutional review board or ethics committee; however, a severe adverse event that is likely or potentially to be attributable to the intervention must be reported within 24 h and be reported formally within 5 working days (these times might vary). Severe adverse events that are not likely to be associated with the intervention should also be reported to institutional review boards and to data and safety monitoring boards under their defined conditions (annually or quarterly). The events will be analysed by these independent boards for any unusual patterns or unexpected association with the intervention.

A critical component of a community trial is quantification of adverse effects of special interest. Examples include mild skin or eye irritation after contact with the trap, allergic reactions or increased symptoms of mild asthma. Unexpected adverse events, even if they are not severe, must be reported promptly. Clear reporting and recording protocols are required for complaints from participants about such events to study personnel. If possible, complaints should be followed up by study medical personnel for better characterization. Study databases should include tables for recording events linked to affected participants. As many such events are mild, participants may not report them to study personnel; therefore, at the time of consent, expected adverse events should be described and participants encouraged to report them to study personnel. Further, when participants withdraw from a study, they should be asked about the occurrence of adverse events and whether they were a factor in their decision to withdraw. Separate questionnaires or a complement to disease surveillance could also be used. In all cases, it is important not to introduce bias or potentially unblind studies.

6.2.3 INDEPENDENT MONITORING

It is recommended that independent entities be engaged through a contract research organization to monitor trials, such as a data and safety monitoring board for adverse events and independent quality assurance.

6.3. TRAP MONITORING, MAINTENANCE, COVERAGE AND SCALE-UP

The objective of the community trials is to test traps under the most closely controlled conditions possible. This often requires that trap maintenance be managed by study personnel, which may not be feasible in national vector control programmes. We recommend, if possible, use of pilot studies to examine how the community or the programme staff will be involved in trap maintenance.

6.3.1 TRAP MAINTENANCE

Trap specifications must be clearly defined, including the requirements for their use (addition of water, baits) and frequency of maintenance (cleaning and/or recharge). Compliance with these specifications should be monitored and recorded, as should movement and alteration of traps and those that are no longer effective, for example traps that have been tipped over or emptied and then returned to their position without larvicide.

6.3.2 TRAP COVERAGE

Coverage must be monitored throughout the trial, including the proportion of lots (housing and other) with traps; the proportion of lots with traps in place, functioning as planned and cleaned or recharged successfully; and traps that have disappeared and households that withdraw from the study. A monitoring system should be in place that tracks individual traps.

Although the coverage required for a public health impact is unknown, it is recommended that studies maintain 80% of the planned coverage. For example, for area-wide protection, the aim would be to include at least 80% of the planned houses or properties in the study area. Importantly, for the households participating in disease monitoring, studies should demonstrate that traps were in place and properly maintained 80% of the time and that at least 80% of the households were retained for the duration of the study. It may be difficult to achieve this proportion in field trials. The total numbers of traps, participating households and properly maintained traps must be recorded throughout the study (**Box 1**).

Box 1. EXAMPLE OF CALCULATING AND MAINTAINING TRAP COVERAGE

Initial coverage

In a study with a lethal ovitrap that requires that the larvicide component be changed every month and an optimal density of three traps per property in a study cluster of 100 houses, the cluster should have 300 traps, with three on each property. After initial deployment, spatial coverage can be calculated from:

number of houses with traps / number of houses in the cluster.

For example, if 80 of 100 household accepted traps, coverage would be 80%; alternatively, 240 of 300 traps, would be 82% coverage.

Follow-up

Each household would be visited monthly for 1 year. For the 80 participating houses, the larvicide would have to be changed 960 times. As some people might not be at home, in this example there should be a minimum of 768 successful visits (768/960 = 80%).

Some households withdraw or traps are lost. For example, if 10 household withdraw, the coverage rate would drop to 70%. Coverage should be monitored by cluster and at each appropriate monitoring visit.

Houses may have damaged traps. If 50/80 houses lose one of three traps, (30 x 3) + (50 x 2) = 190 traps would remain (63% coverage). Trap density during follow-up should therefore also be calculated.

6.3.3 SCALING-UP TRAP INTERVENTIONS

Extending the use of vector traps may require a wide array of measures that are not included in this document. One issue relevant to traps is ensuring distribution and maintenance. Distribution schemes should be tested in effectiveness trials, with coverage as the relevant end-point. Consideration should be given to trap maintenance (ideally by the community), monitoring and evaluation procedures and plans for disposing of used and unused traps.

6.3.4 COMMUNITY PERCEPTIONS AND ACCEPTANCE

A social component to assess communities' reaction to the intervention should be included. A variety of qualitative research techniques are available, such as focus group discussions and key informant interviews. Additionally, periodic quantitative surveys should be carried out of community perceptions about the acceptability and efficacy of the traps.

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SUPPLEMENTAL MATERIALS

S1. SAMPLE PROCEDURE FOR EVALUATING AUTODISSEMINATION AGENTS

Autodissemination is the ability of adult mosquitos to pick up a contaminant from treated solid surfaces and to retain and transfer it to aquatic habitats in sufficient quantities to contaminate the habitats, rendering them unproductive, either by killing larvae or preventing pupae from emerging to adults.

The aim of this assay is to establish the dose–response relation of the autodisseminant on the adult mosquitos to achieve 50% and 90% mortality of susceptible mosquito larvae that are exposed by transfer of the autodisseminant from the adult to the larval habitat. The protocols are adapted from Sihuincha et al. (*1*), Lwetoijera et al. (*2*) and WHO (*3*); however, further independent validation of this assay may be needed.

Mosquito species and test conditions

Tests should be conducted on well-characterized, strains of mosquito that are susceptible to all major insecticide classes with no detectable resistance mechanisms, reared according to standard institutional protocols (e.g. 27 °C ± 2 °C, 80% ± 10% relative humidity and photoperiod 12 h light:12 h dark). For autodissemination experiments, blood-fed and gravid mosquitos (e.g. 6–8-day-old females that took their first blood meal 2–4 days before the experiments) on sugar meals (e.g. 10% sucrose) should be used.

Methods

A modified bottle bioassay (*4*) is used for testing active ingredients (AIs) for autodissemination. In this assay, 1 mL of a solution of either the carrier or solvent alone (e.g. acetone) or of the desired concentration of insecticide in the same carrier or solvent is placed in a 250-mL glass bottle (e.g. Wheaton®). Dilutions of AIs should represent five to six test concentrations that cause 0–100% inhibition of emergence of larvae. A minimum of four replicates of each serial concentration and two control bottles (solvent only) should be prepared.

Groups of 5 female mosquitos are added to each bottle and exposed for 30 min and 1 h. Control mosquitos are maintained in bottles containing only the solvent for 1 h. The bottles are turned every 15 min to maximize the chances that the mosquitos will pick up the candidate autodisseminant.

After exposure, the mosquitos are removed from each bottle and transferred to screened cages with bioassay containers (*3*) containing 200 mL water and 25 late-stage L3 / early L4 *Ae. aegypti* larvae with a larval food source. The containers are lined with filter paper as a substrate for oviposition, and mosquitos provided with access to 10% sugar solution. After a specified time (e.g. 24 h), adult mosquitos are removed, and mortality and inhibition of larval emergence are monitored in standard larval bioassays (*3*).

If adult emergence in the controls is < 80%, the test should be discarded and repeated. If the percentage in controls is 80–95%, the data may be corrected with Abbott's formula. Cumulative totals of dead larvae and pupae from each assay are pooled for dose–response analysis by probit analysis.

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S2. SAMPLE DATA REPORTING FORM FOR SMALL-SCALE TRIALS

Date/time start:_____ Temperature/relative humidity: _____ Location: _____

Time stop: _____ Temperature/relative humidity: _____

Test item:_____ Control 1: _____ Control (If positive standard used): _____

Test system/strain: _____ Mosquito age: _____ Time blood-fed: _____

Number replicates: _____ Mosquitos released/cage: 1_____ 2_____ 3_____ 4_____

	Trap location								
Cage	A	B	C	D	E	F	No. alive	No. dead	Total recovered
1 Treatment									
No. trapped/ No. eggs									
2 Treatment									
No. trapped/ No. eggs									
3 Treatment									
No. trapped/ No. eggs									
4 Treatment									
No. trapped/ No. eggs									

Specificities of attractant (if any): _____

Collector(s): _____ Notes:

Data recorded by: _____

Control mortality: _____ Acceptable range is < 10% for adulticide

New tools to target and suppress *Aedes* populations are needed to protect people living in areas of risk for arboviral disease. The purpose of this document is to provide procedures and criteria for testing the efficacy of and evaluating vector traps for disease control. It includes the design of laboratory and small-scale field trials to assess the attraction and killing effects of vector traps and of large-scale community trials to determine the efficacy of traps in reducing mosquito populations in the field and disease transmission. This document is intended to support product developers, programmes and testing institutions generate robust entomological evidence of the efficacy of vector traps for control and, for a first-in-class vector trap, evidence of the public health impact in reducing arboviral disease.

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GUIDELINES FOR LABORATORY AND FIELD TESTING OF MOSQUITO LARVICIDES

WORLD HEALTH ORGANIZATION
COMMUNICABLE DISEASE CONTROL, PREVENTION AND ERADICATION
WHO PESTICIDE EVALUATION SCHEME

CONTENTS

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1. INTRODUCTION

The purpose of this document is to provide specific and standardized procedures and guidelines for testing larvicides, including bacterial larvicides and insect growth regulators (IGRs), against mosquitoes. Its aim is to harmonize the testing procedures carried out in different laboratories and institutions to generate data for the registration and labelling of larvicides by national authorities.

The document is an expanded and updated version of the guidelines recommended by the WHO Pesticide Evaluation Scheme (WHOPES) Informal Consultation on the evaluation and testing of insecticides, held at WHO headquarters (HQ), Geneva, 7–11 October 1996 (*1*). The guidelines were reviewed and recommended by the Eighth WHOPES Working Group Meeting, held at WHO-HQ, Geneva, 1–3 December 2004 (*2*).

The document provides guidance on laboratory studies and small-scale and large-scale field trials to determine the efficacy, field application rates and operational feasibility and acceptability of a mosquito larvicide. The table below summarizes the sequence and objectives of the studies and trials. The procedures provide some information on the safety and toxicity of the larvicides for non-target organisms, but it is presumed that preliminary eco-toxicity and human assessments have been undertaken before any field study is carried out – detailed treatment and analysis of these extra data are beyond the scope of this document.

Table 1.1
Sequence of the stages of evaluation of mosquito larvicides

Phase	Type of study	Aim
Phase I	Laboratory studies	• Biopotency and activity • Diagnostic concentration and assessment of cross-resistance
Phase II	Small-scale field trials	• Efficacy under different ecological settings • Method and rate of application • Initial and residual activity • Effect on non-target organisms
Phase III	Large-scale field trials	• Efficacy and residual activity • Operational and community acceptance • Effect on non-target organisms

2. PHASE I: LABORATORY STUDIES

The objective of laboratory testing is to determine the inherent biopotency of the technical material or, in the case of formulated larvicides, their activity. It is assumed that the compound's mode of action has already been established. Information on the speed of activity is important, as this will determine the type of testing procedures to be employed.

To evaluate the biological activity of a mosquito larvicide, laboratory-reared mosquito larvae of known age or instar (reference strains or F1 of field-collected mosquitoes) are exposed for 24 h to 48 h or longer in water treated with the larvicide at various concentrations within its activity range, and mortality is recorded. For IGRs and other materials with delayed activity, mortality should be assessed until the emergence of adults. It is important to use homogenous populations of mosquito larvae or a given instar. These are obtained using standardized rearing methods (see Annex 1).

The aims of the tests are:

- to establish dose–response line(s) against susceptible vector species;
- to determine the lethal concentration (LC) of the larvicide for 50% and 90% mortality (LC_{50} and LC_{90}) or for 50% and 90% inhibition of adult emergence (IE_{50} and IE_{90});
- to establish a diagnostic concentration for monitoring susceptibility to the mosquito larvicide in the field; and
- to assess cross-resistance with commonly used insecticides.

2.1 Determination of biological activity

2.1.1 Larvicides other than bacterial products and insect growth regulators

2.1.1.1 Materials required for testing

- One pipette delivering 100–1000 µl.
- Disposable tips (100 µl, 500 µl) for measuring aliquots of dilute solutions.
- Five 1 ml pipettes for insecticides and one for the control.
- Three droppers with rubber suction bulbs.
- The following materials to make a strainer: two wire loops, one piece of nylon netting (30 cm^2) and one tube of cement. It is suggested that two pieces of netting be cut and cemented to opposite sides of the larger end of the wire loops. More cement should then be applied around the edges of the loops to join the two pieces of netting. When dry, the netting may be trimmed with scissors.
  If a strainer is not available, a loop of plastic screen may be used to transfer test larvae into test cups or vessels.
- Data recording forms (see Annex 4).
- Disposable cups (preferred as they avoid contamination) or, if not available, glass bowls or beakers of two capacities: 120 ml (holding 100 ml) and 250 ml (holding 200 ml).
- Graduated measuring cylinder.
- Log–probit software or paper.

2.1.1.2 Preparation of stock solutions or suspensions and test concentrations

The technical materials of many organic compounds are insoluble in water. These materials have to be dissolved in appropriate organic solvents such as acetone or ethanol (the manufacturer should be consulted) in order to prepare dilute solutions for laboratory testing. The formulated materials are, however, miscible with water. Suspending or mixing these formulations in water requires no special equipment – homogeneous suspensions can be obtained by gentle shaking or stirring.

The volume of stock solution should be 20 ml of 1%, obtained by weighing 200 mg of the technical material and adding 20 ml solvent to it. It should be kept in a screw-cap vial, with aluminium foil over the mouth of the vial. Shake vigorously to dissolve or disperse the material in the solvent. The stock solution is then serially diluted (ten-fold) in ethanol or other solvents (2 ml solution to 18 ml solvent). Test concentrations are then obtained by adding 0.1–1.0 ml (100–1000 µl) of the appropriate dilution to 100 ml or 200 ml chlorine-free or distilled water (see Table A2.1). For other volumes of test water, aliquots of dilutions added should be adjusted according to Table A2.1. When making a series of concentrations, the lowest concentration should be prepared first. Small volumes of dilutions should be transferred to test cups by means of pipettes with disposable tips. The addition of small volumes of solution to 100 ml, 200 ml or greater volumes of water will not cause noticeable variability in the final concentration.

When a test is carried out using formulated materials, distilled water is used in the preparation of the 1% stock solution or suspension and in subsequent serial dilutions, according to the content of the active ingredient.

2.1.1.3 Bioassays

Initially, the mosquito larvae are exposed to a wide range of test concentrations and a control to find out the activity range of the materials under test. After determining the mortality of larvae in this wide range of concentrations, a narrower range (of 4–5 concentrations, yielding between 10% and 95% mortality in 24 h or 48 h) is used to determine LC_{50} and LC_{90} values.

Batches of 25 third or fourth instar larvae are transferred by means of strainers, screen loops or droppers to small disposable test cups or vessels, each containing 100–200 ml of water. Small, unhealthy or damaged larvae should be removed and replaced. The depth of the water in the cups or vessels should remain between 5 cm and 10 cm; deeper levels may cause undue mortality.

The appropriate volume of dilution is added (see Table A2.1) to 100 ml or 200 ml water in the cups to obtain the desired target dosage, starting with the lowest concentration. Four or more replicates are set up for each concentration and an equal number of controls are set up simultaneously with tap water, to which 1 ml alcohol (or the organic solvent used) is added. Each test should be run three times on different days. For long exposures, larval food should be added to each test cup, particularly if high mortality is noted in control. The test containers are held at 25–28 ^{o}C and preferably a photoperiod of 12 h light followed by 12 h dark (12L:12D).

After 24 h exposure, larval mortality is recorded. For slow-acting insecticides, 48 h reading may be required. Moribund larvae are counted and added to dead larvae for calculating percentage mortality. Dead larvae are those that cannot be induced to move

when they are probed with a needle in the siphon or the cervical region. Moribund larvae are those incapable of rising to the surface or not showing the characteristic diving reaction when the water is disturbed. The results are recorded on the form provided (Fig. A4.1), where the LC_{50}, LC_{90} and LC_{99} values, and slope and heterogeneity analysis are also noted. The form will accommodate three separate tests of six concentrations, each of four replicates.

Larvae that have pupated during the test period will negate the test. If more than 10% of the control larvae pupate in the course of the experiment, the test should be discarded and repeated. If the control mortality is between 5% and 20%, the mortalities of treated groups should be corrected according to Abbott's formula (*3*):

$$\textit{Mortality}\ (\%) = \frac{X - Y}{X}\ 100\ ,$$

where X = percentage survival in the untreated control and Y = percentage survival in the treated sample.

2.1.1.4 Data analysis

Data from all replicates should be pooled for analysis. LC_{50} and LC_{90} values are calculated from a log dosage–probit mortality regression line using computer software programs, or estimated using log–probit paper. Bioassays should be repeated at least three times, using new solutions or suspensions and different batches of larvae each time. Standard deviation or confidence intervals of the means of LC_{50} values are calculated and recorded on a form (Fig. A4.1). A test series is valid if the relative standard deviation (or coefficient of variation) is less than 25% or if confidence limits of

LC_{50} overlap (significant level at $P < 0.05$). The potency of the chemical against the larvae of a particular vector and strain can then be compared with the LC_{50} or LC_{90} values of other insecticides.

2.1.2 Insect growth regulators

Testing methods for the juvenile hormone (JH) analogues (juvenoids) and the chitin synthesis inhibitors differ. JH analogues interfere with the transformation of late instar larvae to pupae and then to adult, whereas chitin synthesis inhibitors inhibit cuticle formation and affect all instars and immature stages of the mosquito. The delayed action of IGRs on treated larvae means that mortality is assessed every other day or every three days until the completion of adult emergence. The effect of both types of IGR on mosquito larvae is expressed in terms of the percentage of larvae that do not develop into successfully emerging adults, or adult emergence inhibition (IE%).

2.1.2.1 Preparation of stock solutions or suspensions and test concentrations

The preparation of the test solutions or suspensions and bioassay set-ups are the same as for the fast-acting compounds (see Sections 2.1.1.1 and 2.1.1.2). Technical materials are generally soluble in organic solvents and stock solution (1%) should be made by dissolving 200 mg in 20 ml. Formulated materials should be diluted with water and serial dilutions made in the same manner.

2.1.2.2 Bioassays

Third instar larvae are used for testing JH analogues and chitin synthesis inhibitors. The accurate initial count of larvae is essential because of the cannibalistic or scavenging behaviour of larvae during the long exposure period. The long duration of the test also means that the larvae have to be provided with a small amount of food (finely ground yeast extract, rabbit pellets, or ground fish or mouse food) at a concentration of 10 mg/l at two-day intervals until mortality counts are made. The food powder should be suspended in water and one or two drops added per cup. The larvae in the control are fed in the same manner as those in the treated batches. If necessary, all the test and control cups should be covered with netting to prevent successfully emerged adults from escaping into the environment. Mortality or survival is counted every other day or every three days until the complete emergence of adults. The test containers are held at 25–28 ^{o}C and preferably for a photoperiod of 12L:12D.

At the end of the observation period, the impact is expressed as IE% based on the number of larvae that do not develop successfully into viable adults. In recording IE% for each concentration, moribund and dead larvae and pupae, as well as adult mosquitoes not completely separated from the pupal case, are considered as "affected". The number of successfully emerged adults may also be counted from the empty pupal cases. The experiment stops when all the larvae or pupae in the controls have died or emerged as adults. Data are entered on a form (Fig. A4.2). Any deformities or morphogenetic effects that occur in either the moulting immature mosquitoes or the emerging adults are also recorded.

2.1.2.3 Data analysis

The data from all replicates of each concentration should be combined. Total or mean emergence inhibition can be calculated on the basis of the number of third stage larvae exposed. The overall emergence of adults reflects activity. IE% is calculated using the following formula (*4*):

$$IE(\%) = 100 - \left(\frac{T \times 100}{C} \right) ,$$

where T = percentage survival or emergence in treated batches and C = percentage survival or emergence in the control.

If adult emergence in the control is less than 80%, the test should be discarded and repeated. Where the percentage is between 80% and 95%, the data are corrected using Abbott's formula (see Section 2.1.1.3). IE values obtained at each concentration should be subjected to probit regression analysis to determine IE_{50} and IE_{90} values (using computer software programs or estimated from log–probit paper). The data analysis procedures stated in Section 2.1.1.4 should be followed.

2.1.3 Bacterial larvicides

The laboratory bioassay procedures for bacterial products are the same as those for chemical larvicides, except in the preparation of stock suspensions.

2.1.3.1 Principles

The biopotency of the material is first examined by comparing mosquito larval mortality produced by the product under test with the mortality produced by the corresponding reference standard or other technical or formulated product. The toxicity of preparations based on *Bacillus thuringiensis* subsp. *israelensis (B. thuringiensis* subsp. *israelensis)* can be determined against a standard product that has been calibrated using *Aedes aegypti (A. aegypti)* larvae. The potency of products tested is determined by the following formula:

$$\text{Potency of product "X"} = \frac{\text{Potency standard (ITU) x } LC_{50} \text{ (mg/1) standard}}{LC_{50} \text{ (mg/l) of "X"}}$$

When the international reference standard is used, potency is expressed in International Toxic Units per milligram (ITU/mg). The biopotency of products based on *B. thuringiensis* subsp. *israelensis* is compared with a lyophilized reference powder (IPS82, strain 1884) of this bacterial species using early fourth instar larvae of *A. aegypti* (strain Bora Bora). The potency of IPS82 has been arbitrarily designated as 15 000 ITU/mg powder against this strain of mosquito larva.

The biopotency of products based on *Bacillus sphaericus* (*B. sphaericus)* is determined against a lyophilized reference powder (SPH88, strain 2362) of this bacterial species using early fourth instar larvae of *Culex pipiens pipiens (C. pipiens pipiens)* or *Culex quinquefasciatus*. The potency of SPH88 has been arbitrarily set at 1700 ITU/mg of powder against this mosquito strain.

The use of other bacterial larvicide reference powders and/or alternative strains of mosquito in this test is possible but must be approached warily, because it is inevitable that different results will

obtain. Such alternatives must be the subject of careful cross-calibration with the reference powders and strains identified above. Ideally, such cross-calibration should be conducted by a group of independent expert laboratories. The alternative powders or strains, and the cross-calibration data that support them, should be made available to anyone who wishes to use, or check, the test.

In general, it is not necessary to calibrate with or test against the standard if comparing the activity of a bacterial product with other larvicide products. Bioassay results providing LC_{50} and LC_{90} values of products are sufficient to enable comparison among different products.

2.1.3.2 Additional materials required for testing

- Top-drive homogenizer or stirrer for lyophilized products
- Ice bath (container of crushed ice) for grinding or sonication
- Micropipette
- 10 ml pipette
- 12 ml plastic tubes with stoppers or caps
- 120 ml or 250 ml plastic or wax-coated paper cups to hold 100 ml or 200 ml water

2.1.3.3 Preparation of reference standard suspensions for calibration of the bioassays

To prepare a “stock suspension”, weigh 200 mg or 1000 mg of the solid product, place in a vial (30 ml) or volumetric flask, and add 20 ml or 100 ml distilled water, yielding 1% stock suspension, or 10 mg/l. Most powders do not need blending or sonication. Vigorous shaking or stirring will facilitate suspension. If placed in

tubes, the stock suspension can be frozen for future bioassays. Frozen aliquots must be homogenized thoroughly before use, because particles agglomerate during freezing.

From the "stock suspension", any necessary subsequent dilutions (see Table A2.1) are prepared by serial dilution. Plastic or paper cups are filled with 100 ml deionized water. Twenty-five late third or early fourth instar larvae of *A. aegypti* or *C. pipiens* (depending on the bacterial species to be tested: *Aedes* larvae for *B. thuringiensis* subsp. *israelensis* and *Culex* larvae for *B. sphaericus*) are added to each cup. Using micropipettes, 400 µl, 300 µl, 200 µl, 100 µl, 80 µl and 50 µl of a given suspension (see Table A2.1) are added to the cups and the solutions mixed to produce final concentrations of 0.04 mg/l, 0.03 mg/l, 0.02 mg/l, 0.01 mg/l, 0.008 mg/l and 0.005 mg/l, respectively, of the reference standard powder. Four or more replicate cups are used for each concentration and the control, which is 100 ml deionized water.

2.1.3.4 Preparation of suspensions of the product to be tested

For bioassays of technical (solid or liquid) products of unknown potency, an initial homogenate is made simply by mixing without reducing particle size. For assays of liquid formulations, 20 ml water is added to 200 mg in a vial. Serial dilutions are made and cups and larvae are prepared as described in the previous section.

Range-finding bioassays are performed using a wide range of concentrations of the product to determine its approximate toxicity. The results are then used to determine a narrower and more refined range of concentrations for precise bioassay.

2.1.3.5 Bioassays

To prepare a valid dose–response curve, only concentrations giving values between 10% and 95% mortality should be used. A minimum of two concentrations above and two below the LC_{50} level must be used. Each bioassay series should involve at least four concentrations; and each concentration should be tested in four replicates of 25 late third or early fourth instar larvae per replicate.

No food is added to larval vessels when the exposure period is 24 h. Food may be required if the exposure period is longer. Finely ground yeast extract or ground mouse or rabbit pellets suspended in water (1.5 mg) is added to the water in test vessels at 10 mg/l. Mortality is determined at 24 h for *B. thuringiensis* subsp. *israelensis* and 48 h for *B. sphaericus* by counting the live larvae remaining. The results of the tests at different concentrations (including LC values) are entered on the form (Fig. A4.1). If more than 10% of larvae pupate, the test is invalidated because late instar larvae do not ingest 24 h before pupation and too many larvae may have survived simply because they are too old. All tests should be conducted at 25–28 °C, preferably with a 12L:12D photoperiod.

2.1.3.6 Data analysis

If the control mortality is between 5% and 20%, the mortalities of treated groups should be corrected according to Abbott's formula (see Section 2.1.1.3). Tests with control mortality greater than 20% or pupation greater than 10% should be discarded. A mortality–concentration regression is made using log–probit analysis software or log–probit paper. Bioassays should be carried out at least three times and the validity of the results assessed as for the other

larvicides. LC values (Fig. A4.1) are determined and compared to examine the activity of one product versus another.

2.2 Determination of the diagnostic concentration

The diagnostic or discriminating concentration is determined from the dose–response regression lines of testing a technical material against susceptible vector species according to the procedures outlined in Section 2.1. The diagnostic concentration is double that of the estimated $LC_{99..9}$ value.

2.3 Cross-resistance assessment

New, candidate larvicides are tested simultaneously against a small number of distinct, multi-resistant mosquito strains and a susceptible strain, according to the procedures outlined in Section 2.1. If cross-resistance is detected, its exact nature will be determined by testing the larvicide against strains that each possess a single resistance mechanism. The mechanism of resistance may be assessed following the procedures outlined in the WHO document *Techniques to detect insecticide resistance mechanisms (field and laboratory manual)* (*5*).

Susceptible strains of some mosquito species are kept in laboratories. Otherwise, any susceptible strains should be collected in the field (if truly susceptible populations still exist). If not, susceptible strains may be artificially selected using bioassays, assays for individual resistance mechanisms and selection between lines derived from individually mated females.

The resistant strains should be identified using well established assay techniques. The strains should preferably be homozygous for one or more known resistance mechanisms. If homozygosity cannot be achieved, periodic selection is usually necessary to prevent natural selection favouring the susceptible at the expense of the resistant. Established reference strains should be regularly monitored by bioassays and biochemical and/or molecular assays so that any changes in resistance or underlying mechanisms can be assessed and rectified by selection.

3. PHASE II: SMALL-SCALE FIELD TRIALS

Larvicides that show promise in laboratory studies (Phase I) may be subjected to small-scale field testing (Phase II). In Phase II, field trials of formulated products are performed on a small scale against target mosquitoes, preferably in representative natural breeding sites or, where such trials are not feasible, under simulated field conditions (see Section 3.2).

Evaluation procedures should be selected on the basis of the breeding sites and the behaviour of mosquitoes. The formulations are tested at three–five concentrations and the Phase I studies will guide the dosages chosen for use in the Phase II trials. Usually, this will be multiple concentrations of LC_{90} for the target species. Treatment concentrations are calculated on the basis of the amount of active ingredient per volume of water (if known or measurable) or surface area of the habitat.

The objectives of small-scale field trials are:

- to determine efficacy, including residual activity, against different mosquito vectors in different breeding sites and ecological settings;
- to determine the optimum field application dosage(s);
- to monitor abiotic parameters that may influence the efficacy of the product; and
- to record qualitative observations on the non-target biota cohabiting with mosquito larvae, especially predators.

3.1 Trials in natural breeding sites

The field efficacy of the larvicide under various ecological conditions is determined by selecting representative natural breeding habitats of the target species. These include stagnant drains (cement lined and unlined), soakage pits, cesspits, cesspools, domestic service tanks collecting sewage water, pools, wetlands, irrigated fields and unused wells for *Culex* spp.; cement tanks, drums, cisterns, water storage containers and air coolers for *A. aegypti*; and disused wells, garden pits, ponds, curing yards, rice plots, stream pools, wetlands, marshes, irrigated fields and seepages for *Anopheles*spp.

A minimum of three replicates of each type of habitat should be randomly selected for each dosage of the formulation, with an equal number of controls. The size of the plot should be recorded, taking account of surface area and depth. As far as possible, the plots selected should be similar and comparable. Each of the confined breeding sources or containers can be considered as a discrete plot or replicate. Habitats such as drains, irrigation canals, irrigated fields, rice fields, streams and seepages may be divided into discrete areas of 4–50 m^2 and replicated for treatment and control.

Pretreatment immature abundance (first and second instar larvae, third and fourth instar larvae, and pupae) should be recorded in both experimental and control sites (minimum of two observations at equal intervals). The sampling method should be appropriate to the type of breeding habitat, and the appropriate number of samples should be taken from each habitat based on the type and size of the habitat. Larval instars and pupae from each sample are counted and recorded. At least three different dosages of the larvicide should be applied to the breeding habitats. These can be applied using small atomizers, compression sprayers or, in most cases, plastic squeeze bottles. Granules, pellets, tablets and briquettes can be manually broadcast or thrown in the water.

Post-treatment immature abundance (all stages) should be monitored on day 2 and then weekly until the density of fourth instar larvae (or pupae in the case of IGRs) in the treated habitats reaches a level comparable to that in the control. Data are recorded on the form (Fig. A4.3).

Characterization of the habitats in terms of abiotic and biotic factors aids the interpretation of results. Rainfall and any change in water level or other parameters, such as algal bloom or predators in the habitats, should be recorded.

The efficacy and residual activity of the larvicide at different dosages are determined from the post-treatment counts of live larvae and pupae in treated and control sites compared with the pretreatment counts or the control, taking into consideration the dynamics of change occurring in the treated and the control batches (see below).

The assessment of an IGR's efficacy is based on the level of inhibition of emergence of adults and the percentage reduction in larval and pupal densities. Larvae and pupae are sampled as described above. Adult emergence can be monitored directly in the field by floating sentinel emergence traps in treated and untreated habitats (see Fig. A4.4), by pupal isolation, or by sampling and counting pupal skins. Adult emergence may also be assessed by collecting pupae (20–40 per replicate) and bringing them to the laboratory in glass containers with the water from the respective habitats, then transferring them to small cups inside the holding cages. Dead larvae and pupae found in the cups should be removed and any morphological abnormalities recorded.

When monitored directly in the field, the pretreatment and post-treatment data on adult emergence in treated and untreated habitats are analysed for IE%. The following expression (*6*) is used to calculate IE% values:

$$IE(\%) = 100 - \left(\frac{C1}{T1}\right) \times \left(\frac{T2}{C2}\right) \times 100 \quad ,$$

where $C1$ is the number of adults emerged in control habitats before treatment, $C2$ the number of adults emerged in control habitats at a given interval after treatment, $T1$ the number of adults emerged in treated habitats before treatment and $T2$ the number of adults emerged in treated habitats after treatment.

When adult emergence is monitored in the laboratory using pupae collected from treated and untreated habitats, IE% is calculated using the following formula, on the basis of determining adult emergence from the number of pupae isolated (see also Section 2.1.1.3):

$$IE(\%) = \left(\frac{C - T}{C} \right) \times 100 \ ,$$

where C = percentage emerging or living in control habitats and T = percentage emerging or living in treated habitats.

3.1.1 Data analysis

The mean number of pupae or larvae collected per dip for each replicate of each treatment and the control is calculated for each day of observation. The percentage reduction in larval and pupal densities, or the IE% on post-treatment days, will be estimated for each replicate of each treatment using Mulla's formula. The difference between treatments treatments can be compared by two-way analysis of variance (ANOVA) with treatment and number of days as independent factors. The ANOVA should be carried out after transforming the percentage reduction to arcsine values.

The post-treatment day up to which 80% or 90% reduction is observed for each treatment or dosage will then be compared to determine the residual effect and optimum application dosage (see Section 3.3).

3.2 Simulated field trials

In these trials, multiple artificial containers (jars, bucket, tubs, cylinders, etc.) of water are placed in the field or under simulated field conditions and the materials are tested against laboratory-reared or field-collected larvae. The type and size of the container will depend on the natural larval habitat of the target mosquito species. The water-filled containers are given at least 24 h for

conditioning or ageing. A batch of 25–100 laboratory-reared third instar larvae of the mosquito species to be tested is released into each container or replicate and larval food is added. After 2–3 h of larval acclimation, the containers are treated with selected dosages in a randomized manner using pipettes or appropriate hand atomizer sprays, or by broadcasting solid materials over the water surface. The containers are covered with nylon mesh screen or solid covers to prevent other mosquitoes or other insects from laying eggs and to protect the water from falling debris. The water level in the containers must be sustained. A minimum of four replicates of each dosage and four controls are to be used. For fast-acting agents all the containers are examined after 48 h and live larvae are counted to score post-treatment larval mortality. For slow-acting materials, such as IGRs, the survival of larvae, pupae and pupal skins is assessed seven days or more after treatment, by which time all larvae would have pupated and emerged as adults. The pupal skins provide the best gauge of final or overall effectiveness. To test residual activity, a new batch of laboratory-reared, late third instar larvae of the same mosquito species is introduced to each container, and mosquito larval food is added on alternate days or weekly. Larvae survival is assessed 48 h post addition, and pupal skins are counted seven days or more after addition. This process continues until no mortality is noted.

Data are recorded on the form in Fig. A4.2. For the IGRs under test, pupae are removed from the treated and control containers every other day and put into vials or cups with water from the respective containers, then placed in cages and adult emergence is recorded. Another precise method of assessing emergence is to count and remove pupal skins from containers (Fig. A4.4). Adults not freed from pupal skins are considered dead. The test is terminated when there is no statistically significant residual activity in terms of larval mortality or inhibition of adult emergence when comparing the

treated (at the highest dosage tested) batches and the untreated controls. Values of pH and water temperature are recorded throughout the evaluation.

Alternatively, tests can be conducted by exposing third instar larvae in small natural breeding sites to selected dosages of larvicides using screened floating cages (minimum of three replicates, two cages per replicate). These cages should have screened portholes to allow the movement of water and food into the cage from outside. For each dosage, at least three treated and three untreated control habitats are selected. The habitats are treated with the selected dosages of the material to be tested. Twenty-five laboratory-reared or, preferably, field-collected third instar larvae are placed in each cage. The number surviving is counted every other or every third day until all larvae have pupated and emerged. Percentage mortality or IE% is calculated. To test residual activity, 25 third instars are set weekly in treated and untreated control cages. As with the initial batches of larvae, assessments of mortality should be made every other or every third day post introduction. The weekly settings of larvae continue until no difference in mortality is recorded between untreated controls and treated batches.

3.2.1 Data analysis

The method given in Section 3.1.1 can also be used to analyse data collected under simulated trials. However, since the denominator is known for simulated trials, a probit or logistic regression analysis is more suitable than ANOVA and is described below.

The data on the number of live and dead larvae and pupae from all replicates of each dosage on one day should be combined and percentage mortality or IE% calculated. Logistic or probit

regression of the percentage mortality or IE% on dosage and number of post-treatment days can be used to determine the post-treatment day (and its 95% CI) up to which 80% or 90% (the desired level of control) is achieved for a given dosage. This analysis can be done using appropriate statistical software packages.

3.3 Selection of optimum field application dosage

From the dosages tested against a target species in the small-scale or simulated field trials, the minimum dosage at which the maximum effect (immediate as well as residual) is achieved should be selected as the optimum field application dosage for each type of habitat. The frequency of larvicidal treatment is determined based on the reappearance of fourth instar larvae or pupae, in the case of common larvicides and bacterial larvicide products, or the day reduction in inhibition of emergence falls below 90% for IGRs.

4. PHASE III: LARGE-SCALE FIELD TRIALS

The efficacy of larvicides found to be suitable in small-scale field trials (Phase II) should be validated in larger scale field trials against natural vector populations in natural breeding habitats. In this phase, the larvicide is applied to the breeding sites of the target mosquito at the optimum field dosage(s) selected in the small-scale field trials using appropriate application equipment, depending on the formulation.

The objectives of the trial are:

- to confirm the efficacy of the larvicide at the selected field application dosage(s) against the target vector when applied to large-scale plots in natural breeding sites;
- to confirm residual activity and application intervals;
- to record observations on the ease of application and dispersal of the insecticide;
- to observe community acceptance;
- to record any perceived side-effects on operators; and
- to observe the effect of the treatment on non-target organisms.

4.1 Selection of study sites

The experimental plots selected will depend on the type of larval habitat and the environment. Care should be taken that all the representative habitats of the target vector species are included in the trial. A minimum of 25–30 replicates or plots of each type of larval habitat of the target species should be selected for control and then again for treatment. Just as for the small-scale trials, each confined habitat can be considered as an individual replicate; larger habitats can be subdivided into replicates of about 10 m^2.

4.2 Assessment of pretreatment density

Pretreatment larval and pupal abundance (and adult emergence in the case of IGRs) in the treatment and control habitats should be carried out for a week on at least two occasions before treatment. The immature population and adult emergence should be estimated in different types of larval habitat by using appropriate sampling devices (as in the small-scale field trials with natural populations).

4.3 Application of larvicide

All the breeding sites within the unit should be treated at the optimum field application dosage determined in Phase II, using equipment that is appropriate to the formulation and its operational use. The optimum dosage for the major or most important larval habitat of the target species in the area can be used for all the habitats. Where small-scale trials found wide variation between optimum dosages for each type of habitat, the specific optimum dosage should be applied to each type of habitat.

4.4 Assessment of post-treatment density

The impact of larvicidal treatments on the larvae and pupae of mosquitoes (and the inhibition of adult emergence) should be evaluated by sample collection at 48 h and then at weekly intervals using a fixed number of dips or sentinel cages. Sampling procedures are similar to those followed for small-scale trials conducted in natural breeding habitats. Data should be recorded on the relevant form (Figs. A4.3 or A4.4).

4.5 Effect on non-target organisms

Specific, separate trials have to be carried out to assess the impact of larvicides on non-target organisms. However, during the large-scale trial, and where appropriate, non-target organisms cohabiting with mosquito larvae can be counted and examined for impact of treatments while sampling mosquito larvae. Larvivorous fish, snails, polychaetes, shrimps, cray fish, crabs, mayfly naiads, copepods, dragonfly naiads, coleopterans and heteropterans,

ostracods and amphipods are some of the non-target organisms that coexist with mosquito fauna.

4.6 Operational and community acceptability

During the trial, observations should be made on the ease of storage, handling and application of the insecticide formulation on the breeding sites, and of the effects of the insecticide formulation on the proper functioning of application equipment such as nozzle tips and gaskets, rotors, blowers, etc.

Observations are also recorded on the acceptability of the insecticide treatments to the residents of the area, particularly on domestic and peridomestic breeding sites.

4.7 Data analysis

The mean number of pupae or larvae or non-target organisms collected per dip on each day of observation is calculated for each replicate in treatment and control. The statistical analysis to determine residual efficacy – including the number of post-treatment days over which the desired level of control is achieved at the selected dosage – is carried out following the method described in Section 3.1.1.

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ANNEX 1
PRODUCTION OF TEST LARVAE

Use of homogenous batches of mosquito larvae is of prime importance in laboratory studies and is crucial in determining the activity and biopotency of synthetic larvicides, IGRs, bacterial larvicides and natural products. The following standard procedure is proposed for rearing *A. aegypti* and *Culex* spp. Other species may be reared according to these procedures, subject to any modifications necessary to fit the biological requisites of the test species.

For *A. aegypti*, eggs are laid in a cup lined with filter paper strips and one third filled with deionized or tap water. About one third of the paper strip should be in water. This will keep the strips moist where the eggs are laid above the water line. The paper strips are dried at room temperature and stored at room temperature for several months in a sealed plastic bag. When larvae are needed, the paper strip is immersed in de-chlorinated or distilled water. To synchronize and promote hatching, add larval food to the water 24 h before adding the eggs. The bacterial growth will de-oxygenate the water and this triggers egg hatching. This process usually induces the first instars to hatch within 12 h of hydration. The hatched larvae are then transferred to shallow pans or trays containing 2 l de-chlorinated water. The aim is to create a population of 500 to 700 larvae per container. Larval food may be flakes of protein as used for aquarium fish, rabbit pellets, chicken mash or powdered cat biscuit. The containers are held at 25 $\pm$ 2 °C. It is important that the amount of food is kept low to avoid strong bacterial growth (which kills the larvae), increasing food provision as the larvae grow. Several feeds at intervals of one or two days and daily observation of the larvae are optimal. Provision of solid pellets (chicken mash or rabbit pellets) prevents turbidity and scum. If the

water becomes turbid (in the case of powdered food), replace all water by filtering out the larvae and then transferring them to a clean container with clean water and food, a process that may result in larval mortality. A homogenous population of late third or early fourth instars (5 days old and 4–5 mm in length) should be obtained five to seven days later.

The materials and procedures necessary to rear *Culex* larvae, especially those that are severe pests or vectors of disease, are essentially the same as for *A. aegypti*, except that *Culex* eggs are deposited on water as egg rafts and will hatch 1–2 days after deposition. They require no conditioning and cannot be dried. If they do not hatch in two days they will die. It is more difficult to obtain a homogenous population of third or fourth instars of *Culex* spp. larvae. First, a large number of egg rafts must be laid and collected on the same day. These can be stored at 15–18 °C in order to accumulate more eggs for hatching over a day or two. The first instars are fragile and thus should not be handled. Development to the second instar usually takes 3–4 days at 25 $\pm$ 2 °C after the eggs are hatched. In trays containing 2–3 l de-chlorinated water at 4–6 cm depth, 400–600 larvae per tray are reared. Food (see above) is provided as needed. Early fourth instars suitable for testing are usually obtained within 7 days, although sometimes 8 or 9 days are required.

ANNEX 2
DILUTIONS AND CONCENTRATIONS

Table A2.1
Aliquots of various strength solutions added to 100 ml water to yield final concentration

Initial solution		Aliquot (ml)[a]	Final concentration (PPM) in 100 ml
%	PPM		
1.0	10 000.0	1.0	100.0
		0.5	50.0
		0.1	10.0
0.1	1 000.0	1.0	10.0
		0.5	5.0
		0.1	1.0
0.01	100.0	1.0	1.0
		0.5	0.5
		0.1	0.1
0.001	10.0	1.0	0.1
		0.5	0.05
		0.1	0.01
0.0001	1.0	1.0	0.01
		0.5	0.005
		0.1	0.001
0.00001	0.1	1.0	0.001
		0.5	0.0005
		0.1	0.0001

[a] For 200 ml double the volume of aliquots.

ANNEX 3
MEASUREMENTS AND CONVERSIONS

Volume
l l = 1000 ml
1 ml = 1000 µl
1 cubic foot = 7.5 gallons = 28 l
1 gallon = 4 quarts = 8 pints = 128 ounces = 3785 ml

Surface
1 ha = 10 000 m^2 = 2.2 acres
1 acre = 43 560 square feet
1 square foot = 0.111 square yard = 0.105 m^2

Length
1 km = 0.62 miles = 1093 yards
1 m = 39.7 inches
1 inch = 2.54 cm = 0.0254 m
1 foot = 0.333 yards = 0.3048 m
1 yard = 91.44 cm = 0.9144 m
1 mile (statute) = 1760 yards = 5280 ft = 1609.3 m

Weight
1 pound = 0.454 kg
1 kg = 2.2 pounds
1 g = 0.035 ounces

Conversion factors
Square inches to square centimetres, multiply by 6.5.
Square yards to square metres, multiply by 0.8.
Square feet to square metres, multiply by 0.09.
Acres to hectares, multiply by 0.4.
Square miles to square kilometres, multiply by 2.6.

ANNEX 4
DATA RECORDING FORMS

Fig. A4.1

Laboratory evaluation of the efficacy of larvicides against mosquito larvae

Experiment No: _____ Investigator: _____ Location: _____ Treatment date: _____

Material: _____ Formulation: _____ Temp: _____ Lighting: _____

Species: _____ Larval instar: _____ Larvae/cup or vessel: _____

Water: Tap/Distilled Volume of water: _____ ml Food: _____ Date stock solution made: _____

		No of dead larvae at various conc. (mg/L) post exposure (hr.)													
		24 hr							48 hr						
Date	Replicate	0.00							0.00						
	1														
	2														
	3														
	4														
	5														
	6														
	7														
	8														
	9														
	10														
	11														
	12														
	Total														
	Ave.														
	% mortality														

LC50 (CL 95%): _____ LC50 (CL 95%): _____

LC90 (CL 95%): _____ LC90 (CL 95%): _____

LC99: _____ LC99: _____

Slope: _____ Heterogeneity: _____ Slope: _____ Heterogeneity: _____

Fig. A4.2

Laboratory evaluation of the efficacy of insect growth regulators against mosquito larvae

Experiment No: ____________ Investigator: _________________ Location: ___________________ Treatment date: ___________

Material:_________________________ Formulation:_________________ Sampling technique: ____________________

Species: ___________________ Larval instar: ________________ No. of larvae released/exposed: __________ Setting date: ______________

Cumulative number of dead / alive mosquitoes after treatment (date or days pre or posttreatment or setting) L=larvae, P=pupae, A=adults

	Date:											Grand total	
Conc.		Alive	Dead	Alive	Dead	Alive	Dead	Alive	Dead	Alive	Dead	Alive	Dead
(mg/L)	Rep	L P A	L P A	L P A	L P A	L P A	L P A	L P A	L P A	L P A	L P A	L P A	L P A
0.0	1												
	2												
	3												
	4												
	Total												
	Mean												
T1	1												
	2												
	3												
	4												
	Total												
	Mean												
T2	1												
	2												
	3												
	4												
	Total												
	Mean												
T3	1												
	2												
	3												
	4												
	Total												
	Mean												
T4	1												
	2												
	3												
	4												
	Total												
	Mean												
T5	1												
	2												
	3												
	4												
	Total												
	Mean												

Fig. A4.3

Small-scale field testing and evaluation of larvicides against mosquito larvae

Experiment No: _____ Starting date: _____ Location: _____ Investigator: _____
Assessment date: _____ Pre or days posttreatment: _____ Types of Habitat:_____Species _____

Live larvae (L3-4) and pupae (P)/sample							
Treatments		Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Grand total
Dosage ( )	Sample	L3-4* P*	L3-4 P	L3-4 P	L3-4 P	L3-4 P	L3-4 P
Control	1						
	2						
	3						
	4						
	5						
	Total						
	Mean						
	%red						
T1	1						
	2						
	3						
	4						
	5						
	Total						
	Mean						
	%red						
T2	1						
	2						
	3						
	4						
	5						
	Total						
	Mean						
	%red						
T3	1						
	2						
	3						
	4						
	5						
	Total						
	Mean						
	%red						
T4	1						
	2						
	3						
	4						
	5						
	Total						
	Mean						
	%red						
T5	1						
	2						
	3						
	4						
	5						
	Total						
	Mean						
	%red						

Fig. A4.4

Small-scale field testing and evaluation of insect growth regulators against mosquito larvae

Experiment No: _____ Starting date: _____ Location: _____ Investigator: _____
Assessment date: _____ Pre or days posttreatment: _____ Type of Habitat: _____ Species:_____

		Live larvae (L3-4), pupae (P) and adult emergence (A) /sample or cage or trap												
Treatments		Rep 1			Rep 2			Rep 3			Rep 4			Rep 5			Grand total			Visual count	
Dosage ( )	Sample	L3-4*	P*	A*	L3-4	P	A	L3-4	P	A	L3-4	P	A	L3-4	P	A	L3-4	P	A	Pupae	Pupal skins
Control	1																				
	2																				
	3																				
	4																				
	5																				
	Total																				
	Mean																				
	%red																			IE%	
T1	1																				
	2																				
	3																				
	4																				
	5																				
	Total																				
	Mean																				
	%red																			IE%	
T2	1																				
	2																				
	3																				
	4																				
	5																				
	Total																				
	Mean																				
	%red																			IE%	
T3	1																				
	2																				
	3																				
	4																				
	5																				
	Total																				
	Mean																				
	%red																			IE%	
T4	1																				
	2																				
	3																				
	4																				
	5																				
	Total																				
	Mean																				
	%red																			IE%	
T5	1																				
	2																				
	3																				
	4																				
	5																				
	Total																				
	Mean																				
	%red																			IE%	