Semi-field studies for spatial emanator products

Factors which may affect validity of **semi-field studies**:

- study not conducted in compliance with Good Laboratory Practice (GLP)
- negative control mortality exceeds limits defined for the semi-field method
- failure to conduct power calculations based on local mosquito densities
- inadequate sample sizes due to low mosquito densities
- local vector population not suitable for testing with the proposed spatial emanator product
- environmental or holding conditions outside of target range
- tests not conducted in alignment with the test system's circadian rhythm
- resistance status of local vector population not characterised prior to the commencement of the study *(1)*.

1. Purpose of the study

For the purposes of the prequalification assessment, semi-field studies are conducted to investigate the entomological efficacy and chemical consistency of a spatial emanator product by means of:

- investigation of the biological activity of a spatial emanator product under simulated user conditions by observing the relevant effects on wild, free-flying mosquitoes, and
- chemical analysis to determine the retention/release of active ingredient(s) (AIs) during and after artificial and/or operational ageing.

Semi-field studies are conducted to generate semi-field efficacy data for Module 5. For the purposes of the prequalification assessment and listing, the biological effect of the spatial emanator product on the target vector population at the study site after artificial/or operational ageing is considered the key efficacy criterion for the demonstration of entomological efficacy.

2. Requirement for submission of semi-field studies

Three semi-field studies are required for Module 5 submissions, which must be conducted in diverse geographic regions.

Semi-field studies must be GLP-compliant.

3. Considerations for study site selection

Manufacturers should consider the composition of local mosquito populations, and the characteristics of these populations, in the selection of sites for semi-field studies. The vector population(s) at the selected sites should exhibit traits in alignment with the defined primary target(s) based on the mode of action of the AI(s) and intended effects of the product. To assist with study site selection, characterization data for the vector population's target traits, for example, WHO susceptibility tests, insecticide resistance intensity assays, genomic screening, etc., generated by the relevant contract research organization (CRO) should be considered.

Additionally, manufacturers should consider potential requirements from other departments within WHO and National Regulatory Authority requirements for product registration in order to prioritize the generation of efficacy data which can be used to support registration and/or selection decisions across multiple countries/organizations.

4. Considerations for method selection

4.1. Considerations for entomology method selection

The method for a semi-field study should be selected based on the mode of action of the AI(s) and the intent of the product. Typically, the method selected for use in semi-field studies for spatial emanator products is an experimental hut study.

Other existing or novel methods can be proposed in situations where the standard methods are not appropriate. If another method is being considered or augmentations to standard methods are necessary, WHO recommends that substantiating documentation be provided with a Protocol review request submission.

4.2. Considerations for chemistry method selection

Chemical analysis is conducted to quantify the AI content of spatial emanator products before and after use in semi-field studies. The chemical analysis of samples at intermediate time points may be useful in determining the rate of AI loss under the specific environmental conditions at the study site.

In supplementary chemical analysis, the total AI of the product should be measured using the available/validated enforcement analytical method (validation may be in-house and could require bridging to CIPAC or other methods if being validated concurrently).

5. Study materials

5.1. Test and reference items

5.1.1. Test items

Semi-field studies conducted for Module 5 data generation should include spatial emanator products from a minimum of three production batches that have been both fully characterized as part of the data generation for Module 3 and used in Module 5 flight room studies. For the development of a new product dossier, it is critical that the batches used in the semi-field studies are the same as those used for other data generation, for example, the characterization of chemical and physical characteristics.

Documentation of the source, receipt and handling, and storage of spatial emanator products prior to testing is critical.

The environmental conditions of the storage room/area and the duration of storage prior to the use of products in studies should be documented and reported in the study report.

5.1.2. Reference items

The purpose of the **reference items** (positive and negative controls) is to validate the experimental procedures.

The means by which **reference items** were obtained and the storage conditions prior to testing should be documented, with certificate of conformity and batch numbers reported.

5.1.2.1. Negative control

Negative control samples should be untreated product samples/untreated product casings, depending on the design of the product. In the case of active spatial emanator products, an empty device can be used as the negative control.

5.1.2.2. Positive control

The positive control(s) should be selected based on the intent and design of the study, including the selection of method(s), endpoint(s), and species, in order to support the assessment of the validity of the study. All positive controls should be prequalified products that have an entomological mode of action consistent with the entomological mode of action and the intended effect of the product that is under investigation.

It is critical that the selected positive control(s) is used consistently in other studies for data generation.

The selected positive control should be as similar as possible with respect to AI(s), intended useful life and type of product (passive/active emanation).

6. Baseline quality check

There are no specific baseline quality check requirements for spatial emanator products used in semi-field studies. CROs receiving products for use in studies should ensure that the Certificates of Analysis (COA) for the product batches used in the studies are provided and that the values reported in the COA are within the specified limits.

To ensure that product samples used in studies remain within the manufacturing release limits prior to the commencement of the study(ies), attention should be paid to the storage of products after receipt.

7. Sample preparation

The test materials to be used in semi-field studies should be the finished product used in accordance with the intended directions for use. Ensure that all prepared samples are adequately labelled and stored appropriately, as improper storage may impact the results of the test and invalidate the study.

7.1. Sample ageing

Semi-field studies conducted for Module 5 data generation use spatial emanator products that are unused and samples that have been either artificially or operationally aged.

Products to be used as unaged samples should be kept in packaging until the first day of testing.

Product samples may be artificially aged for use in semi-field studies. In these cases, it is recommended that a PQ200 application be submitted prior to the commencement of sample preparation.

Products that are aged in real-time should be aged in environmental conditions as similar as possible to those that will be found during the semi-field study, for example, similar temperature and humidity conditions, and with standardized airflow/air exchange.

7.2. Product samples for chemical analysis

Product samples which are not immediately analysed for chemical content should be individually wrapped and stored in a manner which has been shown to limit changes in the product in advance of chemical analysis, for example, in aluminium foil and held at 4°C. In the case of shipment to an external testing facility for the determination of chemical content, samples should be shipped under conditions shown to limit changes in the product so as to ensure accurate and robust chemical analysis results.

8. Human volunteers

Semi-field studies require the presence of human volunteers as an attractant force. Volunteers should not smoke, drink alcohol or use perfumed skin care products for the duration of their involvement in the study, as these factors can affect human attractiveness to mosquitoes. If female volunteers are involved, then provision should be made to ensure that no pregnant women are involved in the study due to the complications of malaria in pregnancy.

Institutional ethical approval for the study must be sought from the local Ethical Review Board and/or local authorities. Written informed consent must be obtained from each volunteer prior to their participation in the study. The consent form is explained to each volunteer in their local language by an interpreter.

9. Experimental procedures

9.1. Study designs for semi-field studies

Typically, semi-field studies for spatial emanator products are conducted using human landing catches (HLC) and volunteer sleepers, depending on the endpoint(s) to be measured.

9.1.1. Considerations for Latin square designs (LSD)

Semi-field studies for spatial emanator products are conducted using LSDs. LSDs are block study designs for use with multiple treatments and subjects that allow for each subject to be measured with each treatment the same number of times and for the same number of hours. This study design enables sources of variability, for example, differences among hut locations and volunteer sleepers/HLC collectors, to be appropriately handled in the eventual statistical analyses.

9.1.1.1. Treatment allocation

Treatments should initially be allocated randomly to huts.

9.1.1.2. Sleeper rotation

Volunteer sleepers should initially be allocated randomly to huts. Sleepers rotate sequentially among huts each night of the study following a prepared roster.

9.1.1.3. Treatment rotation

Rotation of treatments during the study is not recommended due to the potential for cross-contamination.

Data should be collected for consecutive nights to allow each volunteer sleeper to rotate through each hut.

9.2. Study arms for semi-field studies

Study arms for semi-field studies are dependent on whether products of different ages are tested sequentially or simultaneously.

In sequential semi-field studies, the same product samples are tested periodically at intervals dependent on the duration of the intended useful life, for example, once a month for 12 months for a product with an intended useful life of 12 months.

In simultaneous semi-field studies, product samples that have been aged for different durations are tested all at the same time within the semi-field study, for example, samples aged (operational or equivalent artificial ageing) for 0, 6, and 12 months (for a product with an intended useful life of 12 months) are tested in parallel.

At minimum, the study arms to be used in semi-field studies for spatial emanator products to be tested sequentially are:

1. Negative control
2. Positive control
3. Test item

At minimum, the study arms to be used in semi-field studies for spatial emanator products to be tested simultaneously are:

1. Negative control
2. Unused positive control
3. Intermediate aged positive control
4. End-of-life aged positive control
5. Unused test item
6. Intermediate aged test item
7. End-of-life test item

Alternative LSDs incorporating additional study arms can be used depending on the intent and design of the study.

WHO recommends that a Protocol review request submission with accompanying substantiating documentation be made for all semi-field studies for spatial emanator products.

9.3. Sample size calculations for semi-field studies

Sample sizes are based on the primary endpoint. A power calculation should be conducted to determine the required sample size and the required number of nights of collection.

Sample size calculations should be conducted using simulations based on the primary endpoint, thus simplifying power calculations. Data from recent hut trials or pilot studies should be used to parameterize sample size estimations.

Note that the total number of nights required for data collection may be influenced by the power calculation conducted using local mosquito densities. In cases where mosquito densities are lower than expected, interim power analyses in order to identify the required number of additional nights of data collection are recommended.

9.4. Selection of entomological endpoints

The potential endpoint(s) which may be selected for use in a semi-field study must be representative of the intended effect of the product. Table 1 provides information pertaining to relevant endpoints which may be observed and measured when conducting semi-field studies. The endpoint to be used for decision-making purposes must be selected based on the intended entomological mode of action of the proposed product and be used consistently across all free-flight room and semi-field studies. Justification for the selection of the decision-making endpoint must be presented in the study report. The selection of appropriate endpoint(s) may dictate the selection of the method and/or encourage the use of multiple entomological methods.

Regardless of the intended entomological effect of the product, 24-hour mortality (M24) should be observed and documented for the purpose of monitoring the experimental controls and thereby experimental acceptability.

Please note that it is the responsibility of the applicant to determine the relevant endpoints to be used in the semi-field study(ies) and to propose scientific justifications, i.e., it is not a requirement to measure every endpoint that is listed in Table 1.

9.5. LC_{50} and LC_{90} measurements of local vector populations

The LC_{50} and LC_{90} of the local vector population to the AI(s) of the spatial emanator product should be conducted prior to the beginning of the semi-field study. Results are reported in the matrix of selected mosquito strains.

Table 1. Semi-field study endpoints

Endpoint	Method and time of measurement	Purpose/Definition	Considerations
Blood-feeding inhibition	Method: Volunteer sleepers Measurement time: At the end of the exposure period	The proportion of unfed females. Blood-fed includes partially or fully blood-engorged mosquitoes. Blood-feeding inhibition is the proportion of mosquitoes that are not fed. Blood-feeding inhibition induced by the proposed product may also be calculated as follows: 1. Calculate the average blood feeding in the control (C) arm. 2. Calculate the blood feeding for each observation for each intervention (T) relative to the average blood-feeding rate in the control using the formula 100x(C-T/C). 3. Calculate the mean blood-feeding inhibition (% and 95% CI) from all the observations in each arm.	

Endpoint	Method and time of measurement	Purpose/Definition	Considerations
Landing inhibition	Method: HLC Measurement time: Continuously during exposure period	The reduction in mosquito landings in the treatment arm(s) relative to the control arm(s)	
Personal protection	Method: Volunteer sleepers	The reduction in blood-fed mosquitoes in the treatment arm(s) relative to the control arm(s)	
Protective efficacy	Method: Volunteer sleepers or HLC	The protection elicited by the proposed product relative to the control arm	Protective efficacy can be measured using either the reduction in landing or the reduction in blood feeding. The calculation used must be specified in the study report.
Knockdown	Method: Volunteer sleepers Measurement time: At the end of the exposure period	The proportion of mosquitoes knocked down at the end of the exposure period.	

Endpoint	Method and time of measurement	Purpose/Definition	Considerations

Mortality at 24 hours (M24)	Method: Volunteer sleepers
	
	Measurement time: 24 hours after the semi-field exposure has ended
		The measurement of mortality is an indicator of the lethal effects of the product (if any) and is additionally used to validate experimental procedures.
		
		Mortality is observed by the following indicators:
		
		• No sign of life; immobile; cannot stand.
		• Moribund mosquitoes are also classed as dead after 24 hours of holding, as it is unlikely that they would survive in nature:
		  • any mosquito that cannot stand, for example, has 1 or 2 legs
		  • any mosquito that cannot fly in a coordinated manner
		  • a mosquito that lies on its back, moving legs and wings but unable to take off
		• a mosquito that can stand and take off briefly but falls down immediately.
			Extension of the exposure time or inclusion of multiple exposure times must be declared and scientifically justified in the context of the product being tested and the study being conducted.
			
			The standard holding time post-exposure in semi-field studies is 24 hours. Control mortality should not exceed 10% after 24 hours. Otherwise, the test is invalidated.
			
			Extension of the post-exposure holding time must be declared and scientifically justified in the context of the product being tested and study being conducted.
			
			For example, mortality at x hours after exposure - Mx
			
			Control mortality should not exceed 20% after extended holding times. Otherwise, the test is invalidated.

Other	Applicants may propose other endpoints to be measured by means of semi-field studies with adequate justification.		

10. Results and data analysis

Results for the proposed product and controls (negative, positive) should be presented in both tabular and graphical format.

Descriptive and inferential statistics with appropriate error measurements should be used to present results.

10.1. Semi-field study statistical analysis

10.1.1. Descriptive statistics

All endpoints should be presented with an appropriate measure of centrality and dispersion, for example, arithmetic mean % and 95% confidence intervals for percentages; median and interquartile range for count data. Data for the control arm provides data needed to appraise the quality of study conduct and should always be presented.

The number of replicates conducted per arm should be presented.

Presentation of test environmental conditions is useful to enable understanding of test conduct.

10.1.2. Inferential statistics

To ensure standardization of analytical approaches, a specific model must be used when performing the analysis and presenting the results for assessment. To relate the outcome variables to the intervention and covariates, generalized linear regression models (GLMs) should be used. The choice of model will depend on the endpoint(s) under investigation. For binary endpoints, such as the proportion of mosquitoes dying, a logistic model is appropriate. For outcomes that are counts, a Poisson or negative binomial model may be more appropriate.

It is recommended that the covariates for treatment, volunteer and day are fitted as fixed effects and that the covariate for hut is fitted as a random effect.

Full details of the statistical methodologies employed, and the statistical results should be reported as part of the study report. The full outputs from statistical models and the statistical code in the format in which it was produced should be submitted as part of the product dossier.

10.2. Criteria for study validity and acceptance

Acceptance of chemical analysis results is based on the criteria for the selected available/validated enforcement analytical method.

Proposed spatial emanator products are expected to fulfil the following criteria in the semi-field study in order to meet the requirements for prequalification:

1. Study power:
    - The study must demonstrate sufficient power for decision-making, based on the conducted power calculation and the sample sizes of mosquitoes collected during the study.

2. Results for free-flying mosquitoes:
    - The results for the spatial emanator product at the end-of-life ageing at the selected endpoint must be statistically significantly higher than the results for the negative control, **and**
    - The results for the spatial emanator product at the end-of-life ageing at the selected endpoint must **either**:
        - » Be statistically non-inferior to the end-of-life aged positive control, **or**
        - » Demonstrate efficacy against the target vector that is statistically equal to or better than the selected epidemiologically relevant threshold, for example, a 30% reduction in blood feeding.

    Please note that the choice of using non-inferiority methods of statistical analysis or a threshold-based statistical analysis may be dependent on the availability of positive controls of sufficiently similar design to the proposed product. The selected statistical method of analysis (non-inferiority/threshold-based) must be incorporated into the sample size calculations and the statistical analysis plan prior to the commencement of the study.

11. Study report

The study report must be a comprehensive description of the study, procedures and include justification for specific scientific approaches and/or deviations from standardized methods.

The suggested study report sections for semi-field study reports are below. These sections are provided for guidance and do not need to be strictly followed.

- Cover page
- Table of contents
- GLP compliance statement
- Results summary
- List of abbreviations
- Background information
- Study rationale
- Study objectives
- Study endpoints
    - » If multiple vector species are present at the study site, identify the strain which has been used to determine the validity of the study, and provide a rationale.
- Criteria for study acceptance
- Methods

- Study site and local vector population
  - Description of study site
  - Description of local vector population. Indicate the most recent date of insecticide resistance characterization (NB. The results of the characterization are presented in the Matrix of mosquito strains.)
- Tests and reference items
  - Description of each test and reference item, including:
    - Batch numbers
    - The number of test items received per batch
    - Source
    - Date of manufacture
    - Date of receipt
    - Storage conditions since receipt
    - Justification for choice of positive control(s)
- Sample preparation
  - Sample storage/and or ageing conditions
  - Artificial ageing methodology (if employed)
- Sample shipment details for chemical analysis (if required)
- Chemical analysis methods (if chemical analysis was conducted on site at the testing facility)
- Semi-field study method
  - Study design
  - Study arms
  - LSD design
- Data analysis
  - Statistical analysis methods

- Results
  - Semi-field study
    - Summarized tabular results from the semi-field test
    - Graphical presentation of results
    - Narrative description of results
  - Data analysis and statistical results
    - Semi-field study
      - Summary statistics
      - Inferential statistics
      - Non-inferiority analysis (if employed)
  - Results interpretation and demonstrated efficacy criteria
- Discussion and conclusions
  - The study report must include an interpretive analysis of the results. Specific discussions on any methodological deviations, anomalies in results or other factors which may have impacted the results should be included.

12. Related documents

- WHO PQT/VCP Implementation guidance – Considerations for the selection of controls for use in spatial emanator product studies
- WHO PQT/VCP Implementation guidance – Free-flight room studies
- WHO PQT/VCP Implementation guidance – Semi-field methods for spatial emanator products – Experimental huts
- WHO PQT/VCP Implementation guidance – Matrix of selected mosquito strains
- WHO PQT/VCP Implementation guidance – Template MSMS

13. References

1. Manual for monitoring insecticide resistance in mosquito vectors and selecting appropriate interventions. Geneva: World Health Organization; 2022. Licence: CC BY-NC-SA 3.0 IGO (https://iris.who.int/bitstream/handle/10665/356964/9789240051089-eng.pdf?sequence=1).
2. Guidelines for efficacy testing of spatial repellents. Geneva: World Health Organization & WHO Pesticide Evaluation Scheme; 2013 (https://www.who.int/publications/i/item/9789241505024).