WHO Prequalification of Vector Control Products

Annex IV. Space spray studies

Table 4.1 General		
Report section	**Description**	**Critical parameters to report**
General		
Cover page		
Table of contents		
GLP compliance statement	An official statement of compliance with GLP requirements. The GLP certificate can be provided as part of this section or as an annex to the report	
Results summary	Briefly summarise the results and conclusions of the study. This can be in tabular or narrative text format.	
List of abbreviations	List of abbreviations used in the study report. The use of abbreviations should be kept to a minimum.	
Background	Relevant background information for the study. This can be a brief description of the product and its proposed use.	
Study rationale	A brief description of the rationale for conducting the study and the intent of its use	
Study objectives	List the objectives of the study. Study objectives should be clearly written and described. If the study has been conducted to meet the requirements of multiple bodies, the full list of study objectives can be provided in this section, with those study objectives related to the prequalification product assessment clearly indicated.	
Study endpoints	This section should list and describe all endpoints used in the study, including descriptions of primary and secondary endpoints where relevant.	• Primary endpoints • Secondary endpoints

Table 4.1 General		
Report section	**Description**	**Critical parameters to report**
General		
	If multiple strains of test systems have been tested in the study, identify the test system which was used to determine the validity of the study/provide the scientific determination of product performance, and provide a rationale for the selection of said test system as the decision-making strain. Endpoints should be used consistently throughout all data generation for a product, with the exception of early exploratory studies which might be submitted in a dossier as supplementary evidence.	
Criteria for study acceptance	List and describe the criteria for • Acceptance of the study as scientifically valid • Evaluation of the product as having met the requirements for prequalification for that particular study type	• Criteria for controls • Criteria for evaluation of the proposed product as having met the requirements for prequalification for that particular study type, e.g. laboratory assessment
Guidance and protocol deviations	Provide any deviations from either the study protocol (as per GLP requirements) and/or from WHO guidance	• Deviations from the study protocol » As per GLP facility requirements • Deviations from WHO guidance » Evidence-based justifications/rationales » Assessment of the impact on study validity, acceptability, robustness, with additional evidence to support the assessment where necessary » Any adjustments that were made to the study protocol in response to considerations received from WHO as part of a protocol review submission

Table 4.2 Methods

Report section	Description	Critical parameters to report
Methods		
4.2.1 Test systems		
Test systems	Description of the test systems used in the study	• Colony maintenance and brief summarised rearing procedures • Light cycle of insectary • Age and physiological status used in bioassays » If multiple bioassays have been used, report the age and physiological status for each method separately • Most recent date of insecticide resistance characterisation » NB. The matrix of mosquito strains template has currently been implemented only for ITNs; for space spray studies it is acceptable to either adapt the ITN template appropriately or to report the results of the insecticide resistance characterisation in the body of the study report • Justification for the selection of test system(s), including reference to the product AI and mode of action, and the characteristics of the test system(s) that make it a suitable choice
4.2.2 Study sites		
Description and selection of study sites (for semi-field studies)	Narrative description of semi-field study site(s), including a justification for the site(s) suitability	• Location • GPS coordinates • Description of seasonal variations and rainfall
4.2.3 Characterisation of vector populations		
Characterisation of local vector population (for operational studies)	Description and characterisation of the local vector population at operational study sites, including suitability for use in testing the proposed product	• Vector species and composition, including sibling species, if present • Description of insecticide resistance status and mechanisms » NB. The matrix of mosquito strains template has currently been implemented only for ITNs; for space spray studies it is acceptable to either adapt the ITN template appropriately or to report the results of the insecticide resistance characterisation in the body of the study report
4.2.4 Test items, product information		

Table 4.2 Methods		
Report section	**Description**	**Critical parameters to report**
Methods		
Test and reference items	Description of the batch(es) of test and reference items used in the study.	• The number of batches of test items used in the study • All batch numbers for test and reference items • The number of test and reference items received at the testing facility » The number of test items received per batch of test items • Source of all test and reference items • Date of manufacture • Date of receipt at the testing facility • Storage conditions post-receipt • Justification for the choice of positive control(s)
Test and reference items	Description of the product	• Formulation type • AI description » Name » Mode of action » Concentration in formulated product
Product preparation	Description of product preparation	• Delivery mechanism • Equipment calibration • Operational delivery parameters
4.2.5 Insecticide resistance status		
Insecticide resistance status of test systems and local vector populations	If insecticide resistance characterisation of test systems has been conducted as part of the study, describe the method.	• Insecticides tested • Insecticide dosages • Method used, i.e. WHO tube test or bottle bioassay • Total number of mosquitoes tested • Number of mosquitoes per replicate • Number of mosquitoes per test arm • Exposure duration • Post-exposure holding conditions and monitoring
4.2.6 Study design		
Study design	Intrinsic insecticidal activity	• Dosage selection and range of dosages used in the study • Age and physiological status of mosquitoes • Preparation of solutions • Solvent • Exposure method, e.g. topical application • Delivery method and location on mosquito • Topical application volume

Table 4.2 Methods

Report section	Description	Critical parameters to report
Methods		
		• Anaesthetisation procedure • Test conditions, inc. post-anaesthetisation holding temperature • Total number of replicates, number of mosquitoes in each replicate, total number of mosquitoes/replicates tested per study arm • Post-exposure holding duration and environmental conditions in testing/holding room • Holding receptacle • Density of mosquitoes in holding receptacle • Timing and placement of sugar sources • Endpoint recording
Study design	Intrinsic insecticidal activity of active ingredient(s) used as space sprays	• Dosage selection and range of dosages used in the study • Age and physiological status of mosquitoes • Preparation of solutions • Solvent • Exposure method, e.g. wind tunnel • Method for measuring droplet size • Anaesthetisation procedure • Test conditions, inc. post-anaesthetisation holding temperature • Total number of replicates, number of mosquitoes in each replicate, total number of mosquitoes/replicates tested per study arm • Post-exposure holding duration and environmental conditions in testing/holding room • Holding receptacle • Density of mosquitoes in holding receptacle • Timing and placement of sugar sources • Endpoint recording
Study design	Diagnostic concentration	• Dosage selection and range of dosages used in the study • Age and physiological status of mosquitoes • Preparation of solutions and filter papers • Solvent and carrier • Exposure method, e.g. WHO cylinder

Table 4.2 Methods		
Report section	**Description**	**Critical parameters to report**
	Methods	
		• Total number of replicates, number of mosquitoes in each replicate, total number of mosquitoes/replicates tested per study arm • Post-exposure holding duration and environmental conditions in testing/holding room • Holding receptacle • Density of mosquitoes in holding receptacle • Timing and placement of sugar sources • Endpoint recording
Study design	Cross-resistance to other active ingredients	• Selection of test systems • Identification of reference strain • Selected bioassay method • Total number of replicates, number of mosquitoes in each replicate, total number of mosquitoes/replicates tested per study arm • Post-exposure holding duration and environmental conditions in testing/holding room • Holding receptacle • Density of mosquitoes in holding receptacle • Timing and placement of sugar sources • Endpoint recording • Calculation of LD_{50}, LD_{95}, RR_{50}, RR_{95}
Study design	Small-scale studies – outdoor applications	• Application dosage(s) • Product delivery method and vehicle traverse plan • Equipment calibration • Droplet size » Method for verification of droplet size • Cage design • Monitoring method for environmental conditions (air and ground temperature, relative humidity, wind speed and direction) during study • Time of day • Mosquito transportation method and conditions (if relevant) • Acclimatisation time • Cage placements and height

Table 4.2 Methods		
Report section	**Description**	**Critical parameters to report**
	Methods	
		• Number of replicates, number of mosquitoes in each cage • Mosquito scoring method • Age and physiological status of mosquitoes • Exposure duration • Endpoint recording • Post-exposure duration and monitoring » Environmental conditions of holding room • Holding receptacle • Density of mosquitoes in holding receptacle • Timing and provision of sugar source
Study design	Small-scale studies – indoor applications	• Application dosage(s) • Product delivery method and location of nozzle within experimental room • Dimensions and volume of experimental room • Environmental conditions of experimental room • Equipment calibration • Droplet size » Method for verification of droplet size • Cage design • Time of day • Mosquito transportation method and conditions (if relevant) • Acclimatisation time • Cage placement and height • Number of replicates, number of mosquitoes in each cage • Ventilation of experimental room between replicates • Mosquito scoring method • Age and physiological status of mosquitoes • Exposure duration • Endpoint recording • Post-exposure duration and monitoring » Environmental conditions of holding room • Holding receptacle • Density of mosquitoes in holding receptacle • Timing and provision of sugar source

Table 4.2 Methods

Report section	Description	Critical parameters to report
	Methods	
Study design	Operational studies – outdoor studies	• Surveying, mapping and recording of configuration of buildings, dwellings, rooms, and vegetation characteristics » Habitat characterisation • Method for determining the allocation of treated and untreated areas » Allocation of treated and untreated areas, presented by habitat • Method for determining the flight range and endophilic/exophilic behaviour of the target species • Application dosage(s) • Product delivery method • Equipment calibration • Droplet size » Method for verification of droplet size • Monitoring method for environmental conditions (air and ground temperature, relative humidity, wind speed) during application and sampling periods • Time of day • Sampling plan and frequency, i.e. pre- and post-treatment monitoring of target species • Number of replicates • Mosquito collection method • Mosquito identification method • Mosquito scoring method • Number and placement of sentinel cages » Number of mosquitoes in each sentinel cage » Age and physiological status of mosquitoes • Endpoint recording • Post-exposure duration and monitoring » Environmental conditions of holding room • Holding receptacle • Density of mosquitoes in holding receptacle • Timing and provision of sugar source
Study design	Operational studies – indoor studies	• House plans of study houses • Application dosage(s) • Product delivery method • Equipment calibration

Table 4.2 Methods		
Report section	**Description**	**Critical parameters to report**
Methods		
		• Droplet size » Method for verification of droplet size (if used) • Monitoring method for environmental conditions • Time of day • Sampling plan and frequency, i.e. pre- and post-treatment monitoring of target species • Number of replicates • Mosquito collection method • Mosquito scoring method • Number and placement of cages » Number of mosquitoes in each cage • Age and physiological status of mosquitoes • Exposure duration • Endpoint recording • Post-exposure duration and monitoring » Environmental conditions of holding room • Holding receptacle • Density of mosquitoes in holding receptacle • Timing and provision of sugar source
4.2.7 Sample size calculations		
Sample size calculation for laboratory studies	Provide a full description of the calculations employed to arrive at the required sample size(s)	• Data source used to parameterize sample size calculations, e.g. previous studies, simulated data • Endpoint used to power study • Point estimate used • Procedure used to estimate the sample size, e.g., simulations, existing software/packages • Details of the procedure that was followed • Assumptions considered, e.g. effect size, power, variability, significance level, and justification(s) for the values of each assumption
Sample size calculations for small-scale and operational studies	Provide a full description of the calculations employed to arrive at the required sample size(s)	• Data source used to parameterize sample size calculations, e.g. previous studies, simulated data • Endpoint used to power study • Point estimate used • Simulation procedure used to estimate the sample size/number of required nights of collection • Details of the procedure that was followed

Table 4.2 Methods		
Report section	**Description**	**Critical parameters to report**
Methods		
		• Assumptions considered, e.g. effect size, power, variability, significance level, and justification(s) for the values of each assumption
4.2.8 Data analysis		
Data analysis for intrinsic insecticidal activity	Description of the statistical method used to analyse the relationship between dose and mortality	• Procedure used to estimate the log-dose probit regression, e.g. software package, parallelism test • Method for determining LC_{50} and LC_{90} • Method for correction of mortality using control results, if appropriate
Determination of diagnostic concentration	Description of the method applied to determine the diagnostic concentration using dose-response regression lines or testing of technical material against susceptible vector species	• Appropriate descriptive statistics • Procedure used to estimate the log-dose probit regression, e.g. software package • Method for correction of mortality using control results, if appropriate
Determination of cross-resistance	Description of the statistical method(s) used to determine whether cross-resistance to other active ingredients is present	• Appropriate descriptive statistics • Method for correction of mortality using control results, if appropriate
Data analysis of small-scale outdoor studies	Description of the statistical method(s) used to analyse small-scale outdoor studies	• Appropriate descriptive statistics • Method for correction of mortality using control results, if appropriate • Procedure for estimating the difference between dosages, including: » Type of model » Type of endpoint/data » Distribution » Fixed effects (including the type of variable, e.g., continuous or categorial/factor,

Table 4.2 Methods

Report section	Description	Critical parameters to report
Methods		
		» Random effects (if any) • Justifications for any deviations from published guidance
Data analysis of small-scale indoor studies	Description of the statistical method(s) used to analyse small-scale indoor studies	• Appropriate descriptive statistics • Method for correction of mortality using control results, if appropriate • Procedure for estimating the difference between dosages, including: » Type of model » Type of endpoint/data » Distribution » Fixed effects (including the type of variable, e.g., continuous or categorial/factor, » Random effects (if any) • Justifications for any deviations from published guidance
Data analysis of operational outdoor studies	Description of the statistical method(s) used to analyse operational outdoor studies	• Appropriate descriptive statistics • Method for correction of mortality using control results, if appropriate • Procedure for estimating the difference between treatments, including: » Type of model » Type of endpoint/data » Distribution » Fixed effects (including the type of variable, e.g., continuous or categorial/factor, » Random effects (if any) • Justifications for any deviations from published guidance
Data analysis of operational indoor studies	Description of the statistical method(s) used to analyse operational indoor studies	• Appropriate descriptive statistics • Method for correction of mortality using control results, if appropriate • Procedure for estimating the difference between treatments, including: » Type of model » Type of endpoint/data » Distribution » Fixed effects (including the type of variable, e.g., continuous or categorial/factor, » Random effects (if any)

Table 4.2 Methods		
Report section	**Description**	**Critical parameters to report**
Methods		
		» Justifications for any deviations from published guidance
4.2.9 Determination of diagnostic concentration		
Determination of diagnostic concentration	Description of the method applied to determine the diagnostic concentration using dose-response regression lines or testing of technical material against susceptible vector species	• Method for determining $LC_{99.9}$ and 2 x $LC_{99.9}$
4.2.10 Selection of optimum field application dosage		
Selection of optimum field application dosage	Description of the method applied to select the optimum field application dosage	

Table 4.3 Results		
Report section	**Description**	**Critical parameters to report**
Results		
Characterisation of local vector population(s)		• Composition of local vector population, including sibling species and seasonal variation (where appropriate)
Intrinsic insecticidal activity	Narrative, tabular and graphical presentation of results of investigations of intrinsic insecticidal activity studies	• Evaluation of the results in terms of compliance with the required sample size • Appropriate descriptive statistics • Narrative description of results • LC_{50} • LC_{90} • Probit analysis results (tabular)
Diagnostic concentration	Narrative, tabular and graphical presentation of results of determinations of diagnostic concentration studies	• Evaluation of the results in terms of compliance with the required sample size • Appropriate descriptive statistics • Narrative description of results • $LC_{99.9}$ • 2 x $LC_{99.9}$ • Probit analysis results (tabular)
Cross-resistance	Narrative, tabular and graphical presentation of results of determinations of cross-resistance studies	• Evaluation of the results in terms of compliance with the required sample size • Appropriate descriptive statistics • Narrative description of results • LD_{50}, LD_{95}, RR_{50}, RR_{95}
Small-scale outdoor studies	Narrative, tabular and graphical presentation of results of small-scale studies	• Evaluation of the results in terms of compliance with the required sample size • Environmental conditions • Droplet size verification • Appropriate descriptive statistics, including measures of dispersion for all primary and secondary endpoints (tabular) • Comparison of average mortality and standard deviation using appropriate statistical models, by study arm • Narrative description of results • The code used for statistical analyses in the format that it was produced (separate file)

Table 4.3 Results		
Report section	**Description**	**Critical parameters to report**
Results		
Small-scale indoor studies	Narrative, tabular and graphical presentation of results of small-scale studies	• Evaluation of the results in terms of compliance with the required sample size • Environmental conditions • Droplet size verification • Appropriate descriptive statistics, including measures of dispersion, for all primary and secondary endpoints (tabular) • Comparison of average mortality and standard deviation using appropriate statistical models, by study arm • Narrative description of results • The code used for statistical analyses in the format that it was produced (separate file)
Operational outdoor studies	Narrative, tabular and graphical presentation of results of operational studies	• Evaluation of the results in terms of compliance with the required sample size • Study site survey • Flight range and endophilic/exophilic behaviour of the target species • Environmental conditions during study • Droplet size verification • Appropriate descriptive statistics, including measures of dispersion, for all primary and secondary endpoints (tabular) • The code used for statistical analyses in the format that it was produced (separate file) • Comparison of average mortality and standard deviation using appropriate statistical models, by study arm • Narrative description of results • The code used for statistical analyses in the format that it was produced (separate file)
Operational indoor studies	Narrative, tabular and graphical presentation of results of operational studies	• Evaluation of the results in terms of compliance with the required sample size • Environmental conditions • Droplet size verification • Appropriate descriptive statistics, including measures of dispersion, for all primary and secondary endpoints (tabular)

Table 4.3 Results		
Report section	**Description**	**Critical parameters to report**
Results		
		• Comparison of average mortality and standard deviation using appropriate statistical models, by study arm • Narrative description of results • The code used for statistical analyses in the format that it was produced (separate file)
Selection of optimum field application dosage	Results of the optimum field application dosage	• Minimum dosage at which the maximum effect (immediate and residual) is achieved

Table 4.4 Discussion		
Report section	**Description**	**Critical parameters to report**
Discussion and conclusions		
Discussion	For each study or sub-study, e.g. small-scale studies in natural breeding sites, an interpretative discussion of the results must be provided.	• Interpretation of the study/sub-study results with reference to the criteria for study acceptability identified in Criteria for study acceptance, e.g. evaluation of the scientific validity of the study based on the parameters of the study and the results of controls » Specific discussions on any methodological deviations, anomalies in results, or other factors which may have impacted the results should be included. • Interpretation of the study/sub-study results with reference to the criteria for study acceptability identified in the Criteria for study acceptance with regards to the evaluation of the proposed product as having met the requirements for prequalification for that particular study type, e.g. laboratory assessment. » Specific discussions on any methodological deviations, anomalies in results, or other factors which may have impacted the results should be included.