WHO Prequalification of Vector Control Products

Annex III. Larvicide studies

Table 3.1 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 3.1 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 3.2 Methods		
Report section	**Description**	**Critical parameters to report**
Methods		
3.2.1 Test systems		
Test systems	Description of the test systems used in the study	• Colony maintenance and brief summarised rearing procedures • Light cycle of insectary • Instar used in bioassays/field studies » If multiple bioassays have been used, report the larval instar 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 larvicide 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
3.2.2 Study sites		
Description and selection of study sites (for small- and large-scale studies in natural breeding sites)	Narrative description of site(s) selected for small- and large-scale studies in natural breeding sites, including a justification for the site(s) suitability	• Location • GPS coordinates • Description of seasonal variations and rainfall • Habitat type • Ecological conditions
3.2.3 Characterisation of vector population(s)		
Characterisation of local vector population (for small- and large-scale studies in natural breeding sites)	Description and characterisation of the local vector population at breeding sites, including suitability for use in testing the proposed product	• Vector species and composition, including sibling species, if appropriate • Description of insecticide resistance status and mechanisms, if appropriate » NB. The matrix of mosquito strains template has currently been implemented only for ITNs; for larvicide 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

Table 3.2 Methods

Report section	Description	Critical parameters to report
Methods		
3.2.4 Test items, product information		
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	• Product type, e.g. dispersible granules, slow release tablet, etc. • AI description » Name » Mode of action, e.g. bacterial, Insect Growth Regulator (IGR) » Concentration in formulated product
Product preparation	Description of method of application	• Calculations for determining the volume/weight of product required • Application method
3.2.5 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
3.2.6 Study design		
Study design	Determination of biological activity for larvicides other than	• Method for preparation of stock solution(s) • Solvent • Method for preparation of test concentrations • Dosage selection and range of dosages used in the study

Table 3.2 Methods		
Report section	**Description**	**Critical parameters to report**
	Methods	
	bacterial products and IGRs	• Larvae instar • Total number of replicates, number of larvae in each replicate, total number of larvae/replicates tested per study arm • Number of test days • Water volume and dilution method • Procedure for adding larval food, if appropriate • Total exposure duration • Test room conditions, including environmental conditions and the light cycle of the testing room • Holding receptacle • Endpoint recording
Study design	Determination of biological activity for IGRs	• Method for preparation of stock solution(s) • Solvent • Method for preparation of test concentrations • Dosage selection and range of dosages used in the study • Larvae instar • Total number of replicates, number of larvae in each replicate, total number of larvae/replicates tested per study arm • Number of test days • Water volume and dilution method • Procedure for adding larval food • Concentration of larval food provided • Rearing method or other method for monitoring adult emergence • Total exposure duration • Test room conditions, including environmental conditions and the light cycle of the testing room • Post-exposure holding duration and environmental conditions in testing/holding room, if appropriate • Holding receptacle • Endpoint recording • Method for identifying and recording morphological abnormalities

Table 3.2 Methods

Report section	Description	Critical parameters to report
	Methods	
Study design	Determination of biological activity for bacterial larvicides	• Procedure for measuring the biopotency of the material • Method for preparation of reference standard suspensions, stock solution(s) and serial dilutions • Method for preparation of test suspensions • Dosage selection and range of dosages used in the study • Larvae instar • Total number of replicates, number of larvae in each replicate, total number of larvae/replicates tested per study arm • Number of test days • Water volume and dilution method • Procedure for adding larval food, if appropriate • Total exposure duration • Test room conditions, including environmental conditions and the light cycle of the testing room • Holding receptacle • Endpoint recording
Study design	Cross-resistance to other insecticides	• Selection of test systems • Selected bioassay method • Total number of replicates, number of mosquitoes in each replicate, total number of mosquitoes/replicates tested per study arm • Holding receptacle • Endpoint recording • Calculation of LD_{50}, LD_{95}, RR_{50}, RR_{95}
Study design	Small-scale studies in natural breeding sites	• Study arms • Breeding site identification, number and allocation to study arms • Method for recording pre-treatment immature abundance • Treatment application method • Treatment dosage(s) • Method for, and parameters of, habitat characterisation, e.g. abiotic factors, biotic factors, environmental conditions and changes • Method for post-treatment immature abundance measurements

Table 3.2 Methods

Report section	Description	Critical parameters to report
Methods		
		» Sampling plan, including frequency and number of samples » Sampling method • Larval instar and pupae identification method • Rearing method or other method for monitoring adult emergence (for IGR products) • Method for identifying and recording morphological abnormalities (for IGR products) • Total number of collections • Endpoint recording
Study design	Small-scale studies in simulated breeding sites	• Study arms • Number and type of containers and allocation to study arms » Breeding site identification, number and allocation to study arms for studies conducted in natural breeding sites using screened cages of laboratory test systems • Method for recording pre-treatment immature abundance • Treatment application method • Treatment dosage(s) • Water volume • Water exchange procedure • Method for, and parameters of, habitat characterisation, e.g. abiotic factors, biotic factors, environmental conditions and changes (for studies conducted in natural breeding sites using screened cages of laboratory test systems) • Method for post-treatment immature abundance measurements » Sampling plan, including frequency and number of samples » Sampling method • Larval instar and pupae identification method • Rearing method or other method for monitoring adult emergence (for IGR products) • Method for identifying and recording morphological abnormalities (for IGR products)

Table 3.2 Methods		
Report section	**Description**	**Critical parameters to report**
Methods		
		• Total number of collections • Endpoint recording
Study design	Large-scale studies in natural breeding sites	• Study arms • Breeding site identification, number and allocation to study arms • Method for recording pre-treatment immature abundance • Treatment application method • Treatment dosage (optimum field dosage should be used) • Method for, and parameters of, habitat characterisation, e.g. abiotic factors, biotic factors, environmental conditions and changes • Method for post-treatment immature abundance measurements » Sampling plan, including frequency and number of samples » Sampling method • Method for the assessment of effects on non-target organisms • Larval instar and pupae identification method • Rearing method or other method for monitoring adult emergence (for IGR products) • Method for identifying and recording morphological abnormalities (for IGR products) • Total number of collections • Endpoint recording
3.2.7 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

Table 3.2 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
Sample size calculations for small- and large-scale studies in natural breeding sites and simulated field 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 • Assumptions considered, e.g. effect size, power, variability (e.g. differences between huts/chambers, sleepers, collection nights), significance level, and justification(s) for the values of each assumption
3.2.8 Data analysis		
Data analysis for determination of biological activity for larvicides other than bacterial products and IGRs	Description of the statistical method(s) used to determine biological activity for larvicides other than bacterial products and IGRs	• Procedure used to estimate the log-dose probit regression, e.g. software package • Procedure used to determine LC_{50}, LC_{90}, and LC_{99} • Method for correcting mortality using control results, if appropriate
Data analysis for determination of biological activity for IGRs	Description of the statistical method(s) used to determine biological activity for IGRs	• Procedure used to estimate the %IE on adult emergence (for IGR products) • Procedure used to estimate the log-dose probit regression, e.g. software package
Data analysis for determination of biological activity for bacterial larvicides	Description of the statistical method(s) used to determine biological activity for bacterial larvicides	• Method used to produce dose-response curve(s) • Method for correcting mortality using control results, if appropriate • Procedure used to estimate the mortality-concentration regression using log-probit analysis, e.g. software package
Data analysis for small-scale studies in natural breeding sites and large-scale studies in	Description of the statistical method(s) used to analyse efficacy and residual activity	• Procedure used to estimate the %IE on adult emergence (for IGR products) • Procedure for estimating the difference between treatments, including: » Type of model » Type of endpoint/data

Table 3.2 Methods

Report section	Description	Critical parameters to report
Methods		
natural breeding sites		» 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 for small-scale studies in simulated breeding sites	Description of the statistical method used to analyse efficacy and residual activity	• Procedure used to estimate the %IE on adult emergence (for IGR products) » Procedure used to estimate the log-dose probit or logistic regression, e.g. software package • Procedure for estimating the difference between treatments » Procedure used to estimate the log-dose probit or logistic regression, e.g. software package,
Data analysis for descriptive statistical analyses	Description of the descriptive statistical methods used to summarise and describe data in the report, including measurements of dispersion	• Mean mortality (or other primary endpoint, if applicable) • Mean number of pupae or larvae collected per dip for each replicate of each treatment and for each day of observation for small- and large-scale field studies • Reduction in pupal and larval densities/%IE • Number of mosquitoes per study arm • Standard deviation and 95% CI • Range
3.2.9 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 $LC_{99.9}$
3.2.10 Selection of optimum field application dosage		
Selection of optimum field application dosage	Description of the method applied to select	

Table 3.2 Methods		
Report section	**Description**	**Critical parameters to report**
Methods		
	the optimum field application dosage	

Table 3.3 Results		
Report section	**Description**	**Critical parameters to report**
Results		
Determination of biological activity for larvicides other than bacterial products and IGRs	Narrative, tabular and graphical presentation of results of investigations of biological activity studies	• Appropriate descriptive statistics • LC_{50} • LC_{90} • LC_{99} • Slope • Heterogeneity analysis • Probit analysis results (tabular)
Determination of biological activity for IGRs	Narrative, tabular and graphical presentation of results of investigations of biological activity studies	• Appropriate descriptive statistics • %IE on adult emergence • Probit analysis results (tabular) • Difference between treatments
Determination of biological activity for bacterial larvicides	Narrative, tabular and graphical presentation of results of investigations of biological activity studies	• Appropriate descriptive statistics • Dose-response curve • Mortality-concentration regression results (tabular) • LC_{50} • LC_{90} • LC_{99}
Determination of diagnostic concentration	Narrative, tabular and graphical presentation of results of determinations of diagnostic concentration studies	• $LC_{99.9}$ • 2 x $LC_{99.9}$
Small-scale studies in natural breeding sites	Narrative, tabular and graphical presentation of results of small-scale studies	• Size, surface area and depth of each breeding site • Pre-treatment immature abundance (tabular, figures if desired), presented by breeding habitat and mosquito species • Habitat characterisation, e.g. abiotic factors, biotic factors, environmental conditions and changes over the course of the study • Post-treatment immature abundance (tabular, figures if desired) presented by breeding habitat, mosquito species and dosage (pupae for IGRs)

Table 3.3 Results		
Report section	**Description**	**Critical parameters to report**
Results		
		• Evaluation of the results in terms of compliance with the required sample size • The code used for statistical analyses in the format that it was produced (separate file) • Narrative description of results
Small-scale studies in simulated breeding sites	Narrative, tabular and graphical presentation of results of small-scale studies	• Pre-treatment immature abundance (tabular, figures if desired), presented by container material and mosquito species • Habitat characterisation, e.g. abiotic factors, biotic factors, environmental conditions and changes over the course of the study (for studies conducted in natural breeding sites using screened cages of laboratory test systems) • Post-treatment immature abundance (tabular, figures if desired) presented by container material, mosquito species and dosage (pupae for IGRs) • Evaluation of the results in terms of compliance with the required sample size • The code used for statistical analyses in the format that it was produced (separate file) • Narrative description of results
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 • Frequency of larvicidal treatment
Large-scale studies in natural breeding sites	Narrative, tabular and graphical presentation of results of large-scale studies	• Size, surface area and depth of each breeding site • Pre-treatment immature abundance (tabular, figures if desired), presented by breeding habitat and mosquito species • Habitat characterisation, e.g. abiotic factors, biotic factors, environmental conditions and changes over the course of the study

Table 3.3 Results		
Report section	**Description**	**Critical parameters to report**
Results		
		• Post-treatment immature abundance (tabular, figures if desired) presented by breeding habitat, mosquito species and dosage (pupae for IGRs) • Effects on non-target organisms (tabular, figures if desired) • Evaluation of the results in terms of compliance with the required sample size • The code used for statistical analyses in the format that it was produced (separate file) • Narrative description of results

Table 3.4 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.