WHO Prequalification of Vector Control Products

Annex II. IRS studies

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Table 2.1 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. If multiple strains of test systems have been tested in the study, identify the test system which was used to determine the	• Primary endpoints • Secondary endpoints

Table 2.1 General		
Report section	**Description**	**Critical parameters to report**
	General	
	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 2.2 Methods

Report section	Description	Critical parameters to report
Methods		
2.2.1 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 IRS 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
2.2.2 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
2.2.3 Characterisation of vector populations		
Characterisation of local vector population (for semi-field studies)	Description and characterisation of the local vector population at semi-field 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 IRS 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
2.2.4 Test items, product information		

Table 2.2 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, e.g. WP • AI description » Name » Mode of action » Concentration in formulated product
Product preparation	Description of spray/tank mix preparation	• Volume of spray mix prepared • Water volume • Product weight/volume • Final concentration
Block preparation	Description of block preparation	• Number of blocks prepared • Block dimensions • Drying conditions • Spraying method • Storage conditions • Dose verification
2.2.5 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
2.2.6 Bioassay methods		

Table 2.2 Methods

Report section	Description	Critical parameters to report
Methods		
Bioassay methods	Description of bioassay method used: Cone tests (if used)	• Environmental conditions (temperature, relative humidity and light cycle) of testing room and/or semi-field huts • Starvation protocol (if any) • Transport protocol (if any) • Acclimatisation time and temperature(s) for test systems • Time of day when tests were conducted • Number of mosquitoes per replicate • Total number of replicates, and number of replicates per test arm • Exposure duration • Post-exposure holding duration and environmental conditions in holding room • Endpoint recording • Holding receptacle • Timing and placement of sugar sources
Bioassay methods	Description of bioassay method used: IACT (if used)	• Environmental conditions (temperature, relative humidity) • Decontamination and/or refurbishment procedure • Baseline collections and scavenging rate estimation method • Method for recording environmental conditions (temperature and relative humidity) • Application procedures » Equipment » Calibration method » Description of spray application • Dosage verification procedures, e.g. chemical analysis of filter papers, volumetric/gravimetric determination • Sample storage conditions, e.g. filter paper storage • Starvation protocol • Transport protocol • Acclimatisation time and temperature(s) for test systems and materials • Time of day when tests were conducted, including start and end times • Number of mosquitoes per replicate • Total number of replicates and number of replicates per test arm

Table 2.2 Methods		
Report section	**Description**	**Critical parameters to report**
Methods		
		• Exposure duration • Post-exposure holding duration and environmental conditions in holding room • Endpoint recording • Holding receptacle • Timing and placement of sugar sources
Bioassay methods	Description of bioassay method used: Experimental hut (if used)	• Hut design and measurements • Study arms, including application dosages • Decontamination and/or refurbishment procedure • Baseline collections and scavenging rate estimation method • Method for recording environmental conditions (temperature and relative humidity) • Application procedures » Equipment » Calibration method » Description of spray application • Dosage verification procedures, e.g. chemical analysis of filter papers, volumetric/gravimetric determination • Sample storage conditions, e.g. filter paper storage • Time of day when tests were conducted, including start and end times • Exposure duration • Transport protocol for collected mosquitoes • Mosquito identification procedure • Post-exposure holding duration and environmental conditions in holding room • Endpoint recording • Holding receptacle • Density of mosquitoes in holding receptacle • Timing and placement of sugar sources
2.2.7 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

Table 2.2 Methods		
Report section	**Description**	**Critical parameters to report**
Methods		
		• Delivery method and location on mosquito • Topical application volume • 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 • 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	Irritant or excito-repellent properties	• Concentration selection • Age and physiological status of mosquitoes • Preparation of filter papers, including solvent and carrier where relevant • Substrates used (if relevant) • Exposure method, e.g. WHO cone • Total number of replicates, number of mosquitoes in each replicate, total number of mosquitoes/replicates tested per study arm

Table 2.2 Methods		
Report section	**Description**	**Critical parameters to report**
Methods		
		• Justification for the selection of positive control • 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 insecticides	• 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	Efficacy and residual activity on relevant substrates	• Dosage selection and range of dosages used in the study • Substrate selection and justification • Selected bioassay method, e.g. WHO cone test • Total number of replicates, number of mosquitoes in each replicate, total number of mosquitoes/replicates tested per study arm, total number of mosquitoes/replicates tested per block • Study duration and testing frequency • 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	Semi-field studies	• Ethical review board permission • Environmental conditions (temperature, relative humidity)

Table 2.2 Methods		
Report section	**Description**	**Critical parameters to report**
Methods		
		• Number, gender and age range of volunteers • Latin square design » Treatment allocation » Volunteer allocation » Volunteer rotation » Collection period » Cleaning protocol • Total number of collection nights • Exposure duration • Mosquito collection method • Mosquito scoring method • Endpoint recording • Adverse effects monitoring
Study design	Supplemental bioassays to semi-field studies	• Selected bioassay method(s) • Selected chemical analysis method(s) • Total number of test days • Frequency of testing • Total number of replicates and number of replicates per hut/chamber, study arm and test day
2.2.8 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 semi-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

Table 2.2 Methods		
Report section	**Description**	**Critical parameters to report**
Methods		
		• 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
2.2.9 Data analysis		
Data analysis for intrinsic insecticidal activity and diagnostic concentration determination	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 correction of mortality using control results, if appropriate
Data analysis for determination of excito-repellent properties	Description of the statistical method used to analyse the relationship between dose and irritability	• Procedure used to estimate the log-dose probit 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	• Number of mosquitoes per study arm • Mean • Standard deviation • Range • Precision measurements, e.g. intra- and inter-net/batch variability (where appropriate)
Data analysis for inferential statistical analyses	Description of the fitted inferential model used for each endpoint (including secondary endpoints)	• For each endpoint: » 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

Table 2.3 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 • Summary results for all primary and secondary endpoints, presented by study arm and batch with appropriate measures of dispersion (tabular) • Inferential statistical results (tabular) • Graphical presentation of results • Narrative description of results • The code used for statistical analyses in the format that it was produced (separate file)
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 • Summary results for all primary and secondary endpoints, presented by study arm and batch with appropriate measures of dispersion (tabular) • Inferential statistical results (tabular) • Graphical presentation of results • Narrative description of results • The code used for statistical analyses in the format that it was produced (separate file)
Irritant or excito-repellent properties	Narrative, tabular and graphical presentation of results of investigations of irritant and/or excito-repellency properties studies	• Evaluation of the results in terms of compliance with the required sample size • Summary results for all primary and secondary endpoints, presented by study arm and batch with appropriate measures of dispersion (tabular) • Inferential statistical results (tabular) • Graphical presentation of results • Narrative description of results

Table 2.3 Results		
Report section	**Description**	**Critical parameters to report**
	Results	
		• The code used for statistical analyses in the format that it was produced (separate file)
Cross-resistance to other insecticides	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 • Summary results for all primary and secondary endpoints, presented by study arm and batch with appropriate measures of dispersion (tabular) • Inferential statistical results (tabular) • Graphical presentation of results • Narrative description of results • The code used for statistical analyses in the format that it was produced (separate file)
Efficacy and residual activity on relevant substrates	Narrative, tabular and graphical presentation of results of residual activity studies	• Evaluation of the results in terms of compliance with the required sample size • Summary results for all primary and secondary endpoints, presented by study arm and batch with appropriate measures of dispersion (tabular) • Inferential statistical results (tabular) • Dose verification results • Graphical presentation of results • Narrative description of results • The code used for statistical analyses in the format that it was produced (separate file)
Semi-field studies	Narrative, tabular and graphical presentation of results of semi-field studies	• Evaluation of the results in terms of compliance with the required sample size • Summary results for all primary and secondary endpoints, presented by study arm with appropriate measures of dispersion (tabular) • Inferential statistical results (tabular) • Non-inferiority analysis (where appropriate) (tabular, graphical)

Table 2.3 Results		
Report section	**Description**	**Critical parameters to report**
Results		
		• Dose verification results • Graphical presentation of results • Narrative description of results • The code used for statistical analyses in the format that it was produced (separate file)
Supplemental bioassays	Narrative, tabular and graphical presentation of results of bioassays supplemental to semi-field studies	• Evaluation of the results in terms of compliance with the required sample size • Summary results for all primary and secondary endpoints, presented by study arm and batch with appropriate measures of dispersion (tabular) • Inferential statistical results (tabular) • Graphical presentation of results • Narrative description of results • The code used for statistical analyses in the format that it was produced (separate file)

Table 2.4 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.