WHO Prequalification of Vector Control Products

Regeneration study for ITN fabric

Factors which may affect the validity of a regeneration study:

- study not conducted in compliance with GLP;
- negative control mortality exceeds limits identified for the applicable method(s);
- identification of issues related to the health of test organisms;
- environmental conditions, e.g. temperature and humidity at which the test is conducted, or the mosquitoes are held for delayed mortality monitoring;
- storage conditions of ITNs and net samples during sample preparation, storage, and testing.

1. Purpose of the study

For the purpose of the prequalification assessment, **regeneration studies** are conducted to investigate how an ITN fabric performs after washing to determine:

- how many days after washing the surface and reservoir concentrations of AI(s) reach stability and
- how many days after washing an ITN will regain its intended entomological effect(s) using appropriate test system(s).

Three washes are conducted to deplete the insecticide present on the surface of the fabric, after which there is a re-establishment of surface concentration(s).

Historically, the entomological results of these studies were used to select the wash interval to be used in the artificial aging of ITNs in subsequent studies, i.e., wash resistance and semi-field studies. In the prequalification assessment of ITNs, both the chemical and entomological regeneration study results are used to inform the subsequent selection of the wash interval to be used in studies that require the artificial ageing of ITNs.

The chemistry component of the regeneration study is intended to characterize the amount of AI lost during a single wash from test samples which have been allowed to regenerate for different durations. This is achieved by using a before and after wash method (BAM) prior to determining total AI content *(1)*. In addition to guiding the selection of an appropriate wash interval, this establishes a baseline for estimating the total loss of AI from the reservoir over the intended lifespan of the product.

The entomology component of the regeneration study is intended to characterize the time for which the active ingredient(s) in the reservoir (bound in coating or within the yarn) becomes biologically available on the surface to the point of inducing an expected effect on the test system(s) used in the bioassay.

A narrative justification of the selected wash interval that is subsequently used for studies with artificially aged ITNs should be provided as part of the product dossier.

2. Requirement for submission of regeneration studies

It is required that a regeneration study, or studies, is conducted for each fabric used within the construction of an ITN. The specific formulation and manufacturing process for the fabric can significantly influence the behaviours of the treated fabric, especially the rate at which active ingredient(s) move from the reservoir to the surface and, as a consequence, the time taken for biological activity to be restored and for chemical content to reach stability.

Wash regeneration studies must be GLP compliant.

3. Considerations for chemistry method selection

The total AI of sampled fabric pieces 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).

3.1 Before and after wash method for chemical analysis

The before and after wash method is used to determine the amount of AI lost during a single wash *(1)*. When used in the context of a regeneration study, it allows for an estimation of the regeneration of AI over time.

Following bioassays, fabric samples are cut such that each original sample is in two pieces. One piece is wrapped in aluminium foil and stored at 4°C until chemical analysis for total AI(s) content. The second piece is washed and dried once before being wrapped in aluminium foil and stored at 4°C until chemical analysis for total AI(s) content. The difference between the two total AI content results is the amount of AI that was removed during the wash. Refer to Section 9.2.2 for sample preparation information.

4. Considerations for entomology method selection

Typically, regeneration studies utilize the cone test or tunnel test as the bioassay method(s) for experimentation. The IACT method can also be used. In designing a regeneration study, the formulation of the fabric, mode of action of the AI(s) and intent of the product should be carefully considered to determine which method or methods to use.

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.

A single bioassay method should be selected for use in the regeneration study, except when there is a necessity to use multiple bioassays to demonstrate the intended effect of multiple AIs, e.g., cone tests to demonstrate the rapid toxicity of a pyrethroid insecticide coupled with tunnel tests to demonstrate the effect of chlorfenapyr in a pyrethroid-chlorfenapyr dual-AI ITN.

As the prequalification assessment has evolved from a framework for decisions relying solely on bioassays results meeting preselected thresholds, prequalification will no longer accept results from a second bioassay method to verify sub-optimal bioassay results.

5. Selection of entomological endpoints

The potential **endpoint(s)** which may be selected for use in the regeneration study must be representative of the intended effect of the product. The selection of appropriate endpoint(s) may dictate the selection of method and/or encourage the use of multiple entomological methods.

6. Considerations for test system species/strain selection

For the purposes of the regeneration study, the selected test system species/strain should be relevant to the intended use of the product, i.e., vectors of the disease(s) intended to be impacted. The selected strains should be characterized in terms of the susceptibility to the AI(s) and the specific mechanisms of resistance, if applicable. The use of multiple species/strains in a regeneration study can provide valuable information about:

- the differences in time until effects are observed in relation to species/strain characteristics
- identification of the potential range of response (baseline) for selected endpoints measured in the bioassay in relation to species/strains.

Where multiple test system species/strains are used, the test system species/strain that will be used to determine the selected wash interval for artificial aging must be clearly identified.

Further guidance on the selection of strains for use in bioassays is provided in implementation guidance *Considerations for the selection of mosquito strains for use in bioassays and site selection for semi-field studies.*

7. Study materials

7.1 Treated fabric

The study should include samples from a minimum of three batches of each fabric used in the construction of the ITN. Depending on the design of the individual fabrics, bespoke ITN sampling plans may be necessary to adequately address different formulants and/or target effects of the various fabrics (refer to Section 7.2). For the development of a new product dossier, it is critical that the batches used

in the regeneration study are the same as those used for other data generation, e.g., the characterization of chemical and physical characteristics and semi-field trials.

Documentation of the source, receipt, handling and storage of whole ITNs and cut netting samples prior to testing is critical.

7.2 ITN sampling for regeneration studies

As the purpose of the regeneration study is to measure changes in AI(s) presentation over time, the sampling plan for the cutting of netting samples should attempt to minimize intra-batch variability as far as is possible.

Depending on the context and use of the study, samples may be cut from:

- pre-constructed treated fabrics in a described manner, or
- constructed ITNs.

Samples are cut from defined positions (Fig. 1). Measurements should be taken from the left-hand seam and bottom of the net when locating the positions from which to cut samples. First, horizontal swatches are taken from ITN panels as indicated in Fig. 1. Swatches taken from side panels should measure 30 cm x 150 cm and the bottom of the swatch should be 60 cm from the bottom of the net. Swatches taken from the roof panel should measure 60 cm x 150 cm and the swatch should be taken 30 cm from the long side. The use of horizontal swatches minimizes the potential variability of the fabric such that the changes in the AI presentation over time are easier to monitor.

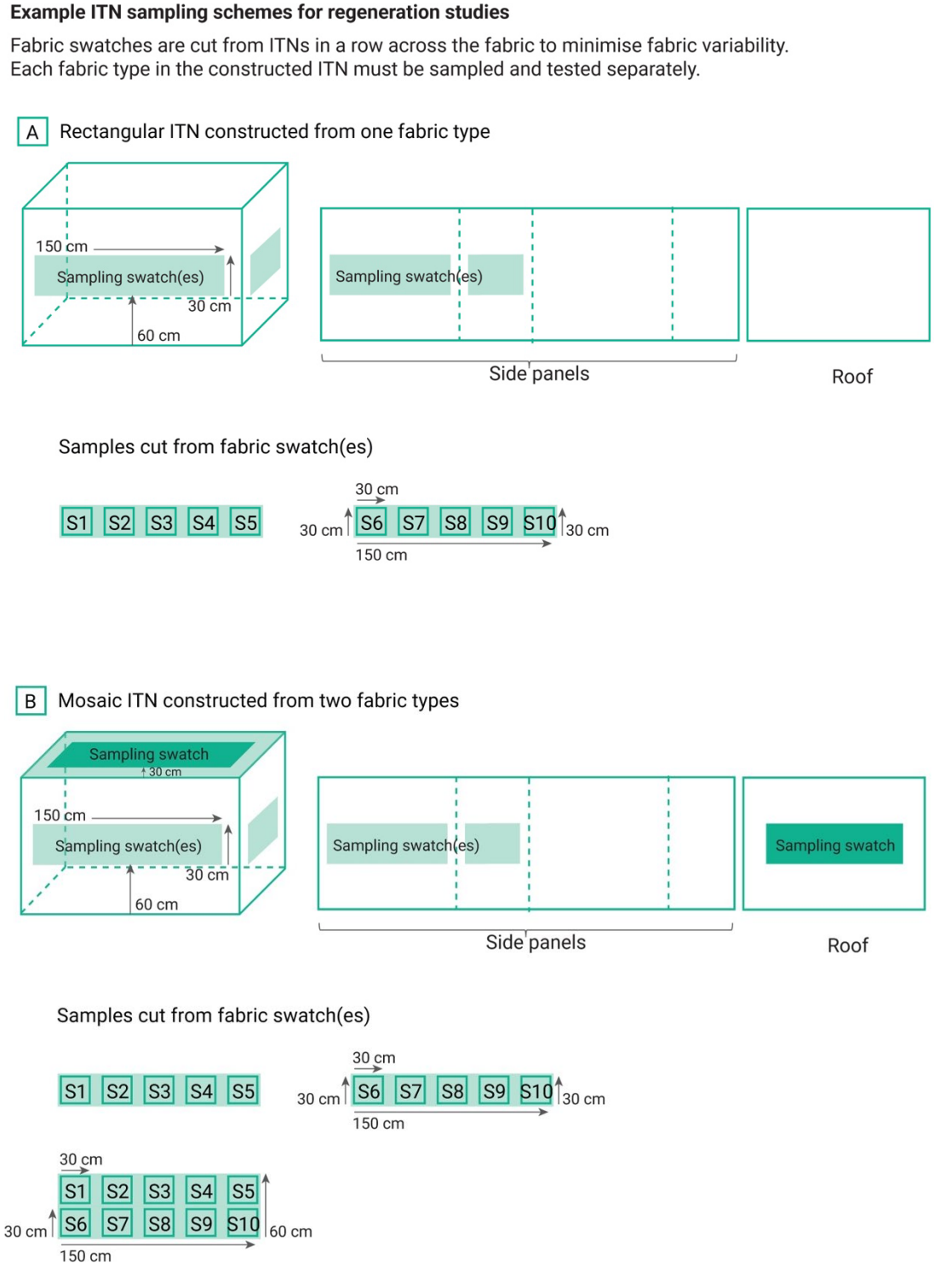
After removing the swatches, five 30 cm x 30 cm samples are removed from each swatch if the swatch was taken from a side panel, or ten 30 cm x 30 cm samples if the swatch was taken from a roof panel.

A total of sixty samples are cut per ITN fabric in the study (10 samples per net; two nets selected per production batch of three production batches).

If additional samples are required, further swatches from the same positions can be removed from the remaining ITN panels.

Swatches should not be taken across seams.

Fig. 1. Sampling schemes for wash regeneration studies



7.3 Negative control

Negative control samples should be untreated fabric made of polyethylene or polyester.

7.4 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/strains, in order to support the assessment of the validity of the study.

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

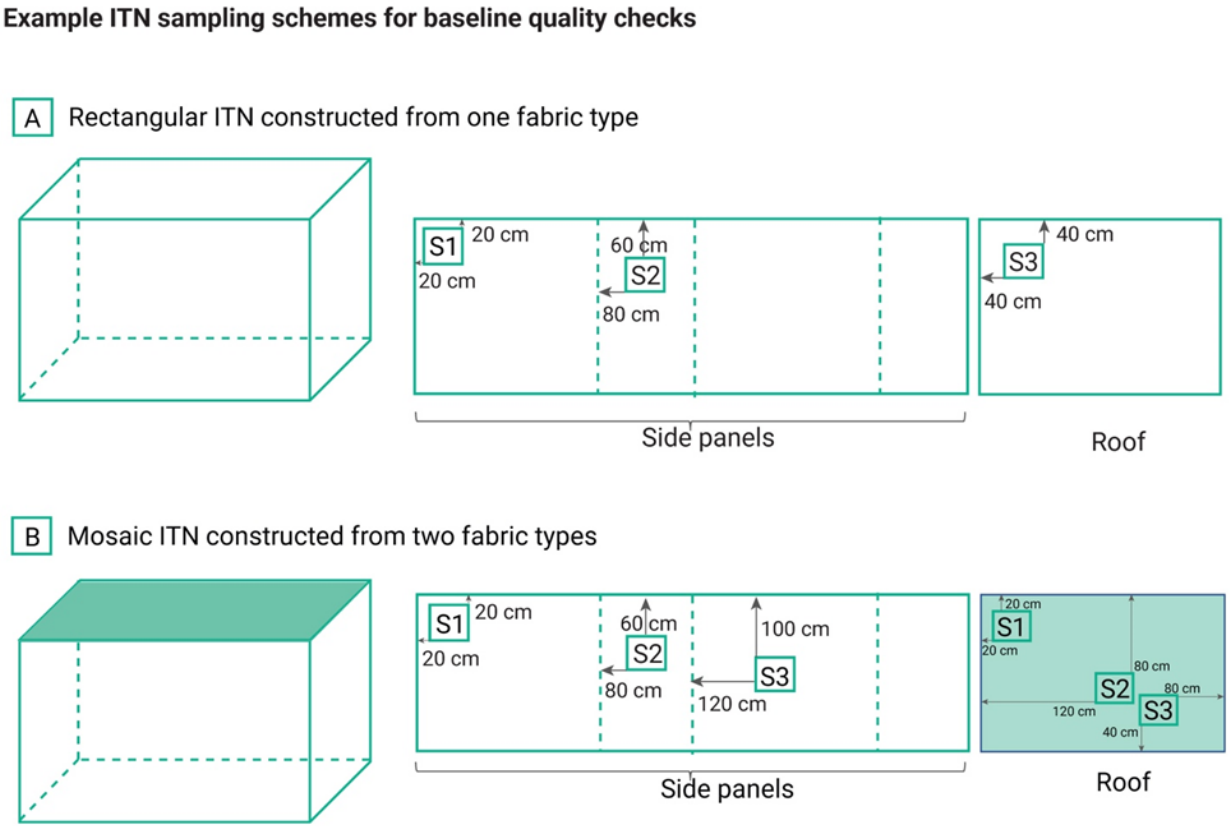
8. Baseline quality check

Prior to the commencement of the study, a baseline quality check using the selected chemical and bioassay test methods should be conducted to identify if any significant changes in the product have occurred during the transport, receipt and handling of fabric samples. The baseline quality check should be conducted on 45 samples (3 pieces per net; five nets selected per batch of three production batches) using the sampling plan as defined below (Fig. 2).

When taking samples, the location should be measured from the left-hand seam of each panel. For a rectangular, uniform ITN comprised of one fabric (roof and sides), Sample 1 is taken 20 cm from the top of the net and 20 cm from the left-hand seam, Sample 2 is taken 60 cm from the top seam and 80 cm from the left-hand seam, and Sample 3 is taken from the roof, 40 cm from the long side and 40 cm from the left-hand seam.

For a rectangular, mosaic net comprised of two fabrics (roof and sides), for Fabric 1 (sides) Sample 1 is taken 20 cm from the top of the net and 20 cm from the left-hand seam, Sample 2 is taken 60 cm from the top seam and 80 cm from the left-hand seam, and Sample 3 is taken 100 cm from the top seam and 120 cm from the left-hand seam; for Fabric 2 (roof) Sample 1 is taken 20 cm from the left hand ‘top’ corner, Sample 2 is taken 80 cm from the long seam and 120 cm from the left-hand seam and Sample 3 is taken 80 cm from the right-hand seam and 40 cm from the long seam. Each fabric is evaluated separately.

Fig 2. Sampling for baseline quality checks



As the purpose of the baseline quality check is to establish a baseline for the consistency within and between the batches of ITNs that have been received at a testing facility, fewer samples are taken from a higher number of ITNs than in other Module 3 studies.

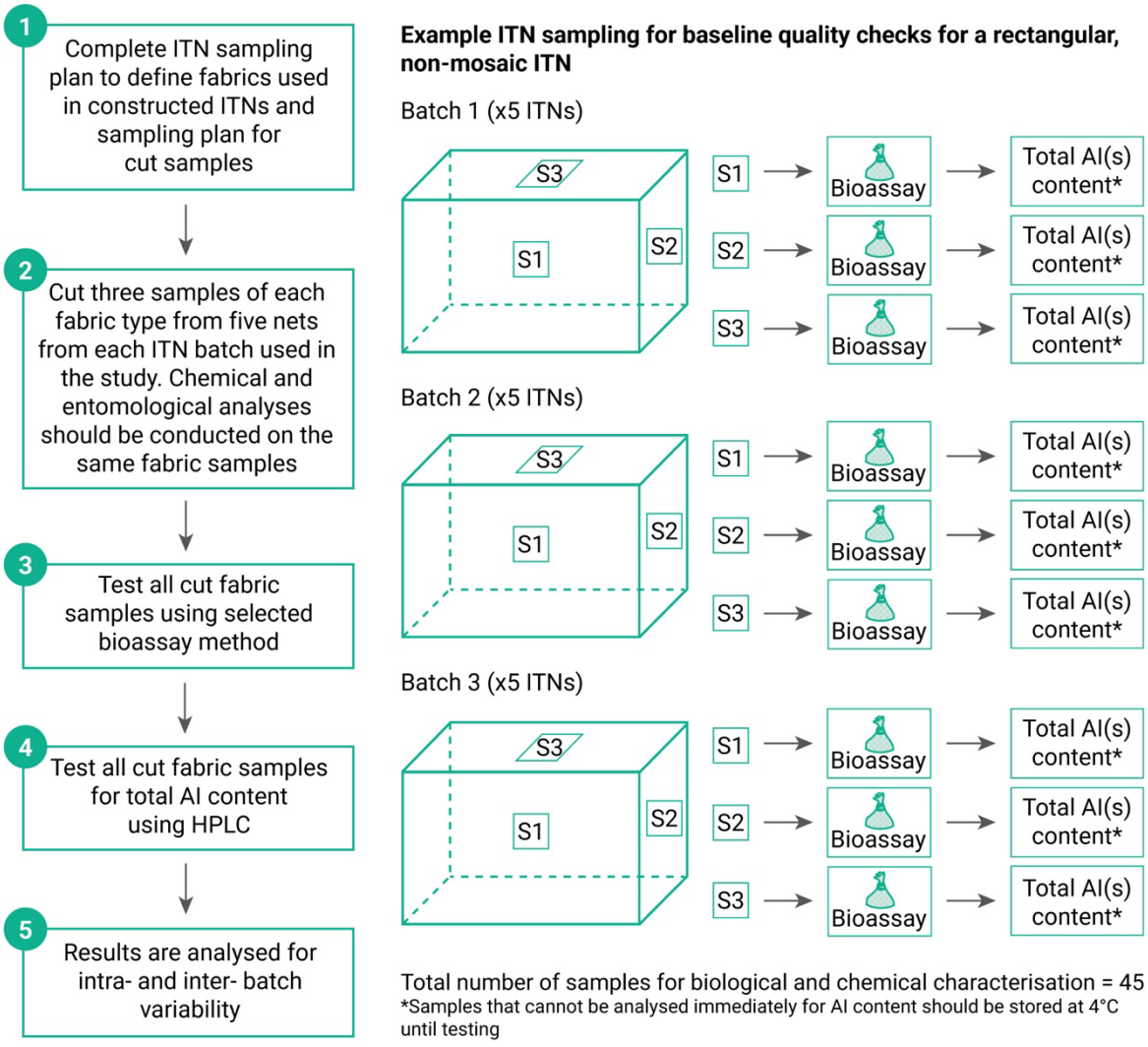
The baseline quality check should only be conducted once per testing facility, i.e., a testing facility conducting a regeneration study, a wash resistance study and a semi-field study need only conduct a single baseline quality check.

Baseline quality check results should be analysed for intra- and inter-batch variability in addition to presenting the results from each analysis with an appropriate measure of variation (Refer to Section 12).

8.1 Schematic of a baseline quality check

Fig. 3 illustrates the baseline quality check procedure for a rectangular, uniform ITN.

Fig 3. Baseline quality check



Note:Test all cut fabric samples for total AI content using HPLC or the appropriate method of analysis accoridng to the AI.

9. Sample preparation

9.1 Cutting

Fabric samples should be cut in accordance with the procedures for the selected test(s) to be performed. When cutting the test material ensure that the material is not being stretched nor compressed.

9.2 Washing of fabric samples

9.2.1 Initial washes (Day 0)

Fabric samples should be washed three times using the following established washing procedure:

- Cut ITN samples (30 cm x 30 cm) are introduced individually into 1 l beakers or glass bottles containing 0.5 l deionized water, with 2 g/l of soap (pH 10–11) added and fully dissolved just before washing. Place the beakers/bottles upright in a water bath heated to 30 ±2°C and shake horizontally for 10 minutes at 155 movements per minute.
- Remove the sample from the beaker/bottle using tweezers and remove excess fluid by gently shaking it several times in the air.
- Rinse the sample twice. For each rinse the sample is placed in a 1l beaker or glass bottle in 500ml deionized water at 30 ±2°C, shaken horizontally for 10 minutes at 155 movements per minute, and fresh water used for each rinse.
- After the second rinse, remove the sample from the beaker/bottle using tweezers and remove excess fluid by gently shaking it several times in the air. Dry the sample on a washing line for 30 minutes at 27 ±2°C away from direct sunlight. Ensure that the sample is completely dry before further washing, testing, or storage.

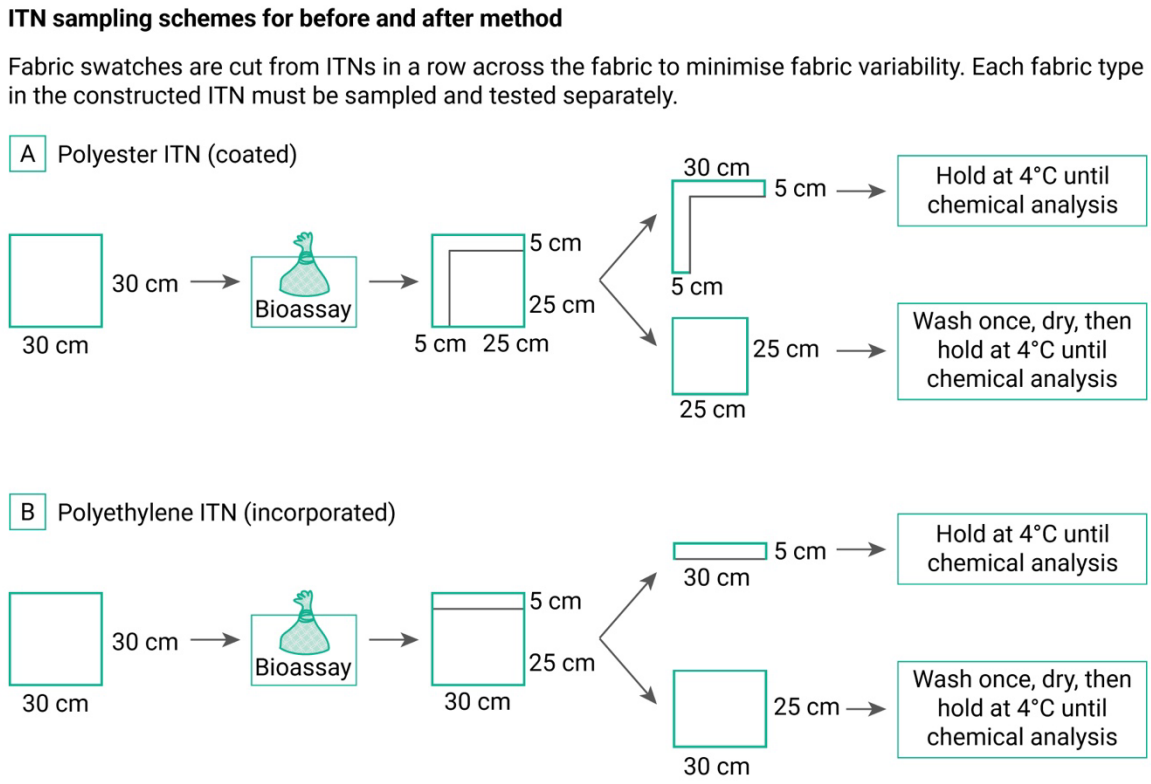
Between washes (all carried out on Day 0), samples should be wrapped in aluminium foil and stored in the dark at 30 ±2°C.

9.2.2 Before and after wash method for chemical analysis (Testing days 1-40)

On each testing day, after the bioassay testing has been completed, each 30cm x 30cm fabric sample should be cut according to the schematic in Fig. 4, depending on whether the ITN is constructed from polyester or polyethylene fabric. For polyester ITNs, an L-shaped panel 30cm long and 5cm deep is removed from each 30cm x 30cm sample (Fig. 4A). This L-shaped panel is held at 4°C until testing for total AI content. The remaining 25cm x 25cm sample is washed and stored as per the instructions below.

For polyethylene ITNs, a 30cm x 5cm panel is removed from the top of each 30cm x 30cm sample. This panel is held at 4°C until testing for total AI content (Fig. 4B). The remaining 30cm x 25cm sample is washed and stored as per the instructions below.

Fig 4. Sampling for before and after wash method



Note: It should be always ensured that the sample taken includes sufficient weight of fabric to allow for the chemical analyses. Enlargement of sample sizes may also be necessary if multiple AIs are required to be analysed. The sampling scheme and size of initial and sub-samples should be developed to support the necessary analyses. The pattern for sub-sampling of polyester vs polyethylene fabrics should be retained in the augmentation of sample size.

The cut sample piece to be washed should be washed once using established washing procedures:

- Cut ITN samples (25 cm x 25 cm or 30 cm x 25 cm, depending on the material of the ITN, as described above) are introduced individually into 1 l beakers or glass bottles containing 0.5 l deionized water, with 2 g/l of soap (pH 10–11) added and fully dissolved just before washing. Place the beakers/bottles upright in a water bath heated to 30°C ±2°C and shake horizontally for 10 minutes at 155 movements per minute.
- Remove the sample from the beaker/bottle using tweezers and remove excess fluid by gently shaking it several times in the air.
- Rinse the sample twice. For each rinse the sample is placed in a 1l beaker or glass bottle in 500 ml deionized water at 30 ±2°C, shaken horizontally for 10 minutes at 155 movements per minute, and fresh water used for each rinse.
- After the second rinse, remove the sample from the beaker/bottle using tweezers and remove excess fluid by gently shaking it several times in the air. Dry the sample on a washing line for 30

minutes at 27 ±2°C away from direct sunlight. Ensure that the sample is completely dry before further testing, or storage.

- After washing and drying, samples should be wrapped in aluminium foil and stored at 4 ±2°C until testing.

9.3 Storage of fabric samples prior to testing and between washes

Fabric samples should be stored at 30 ±2°C.

9.4 Fabric samples for chemical analysis

Fabric samples which are not immediately analysed for chemical content should individually wrapped in aluminium foil and held at 4°C.

10. Environmental conditions in testing room

The testing laboratory where bioassays are conducted should be maintained at a temperature of 27±2°C and 75% ± 10% relative humidity.

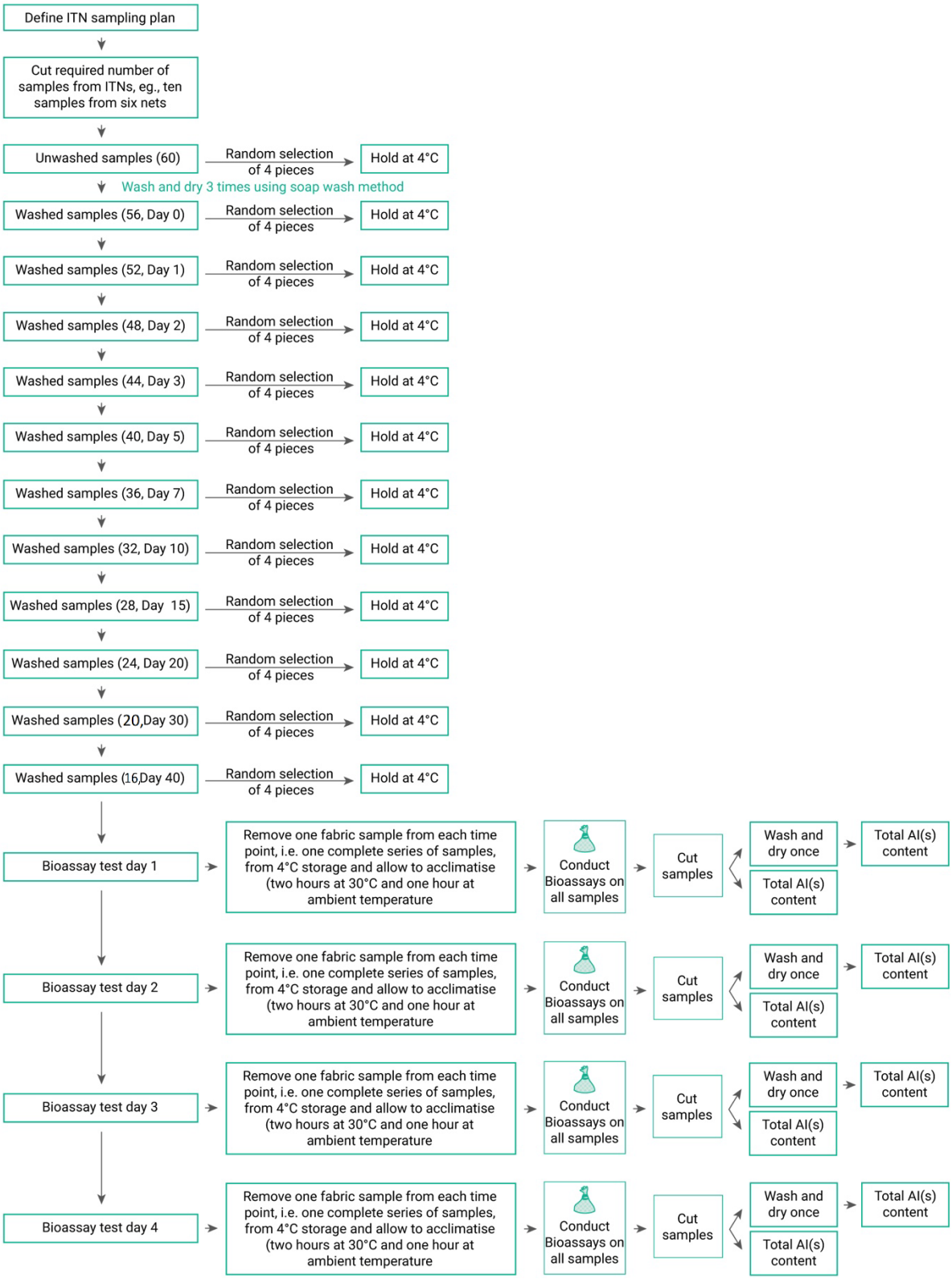
11. Experimental procedures

The duration of the regeneration study is 40 days, plus up to 4 days for bioassay testing (bioassays can all be conducted on the same day, if facilities exist). Every fabric used in the ITN construction must be tested separately. The first day of washing is Day 0 of the study.

11.1 Schematic of a regeneration study

Fig. 3 illustrates the baseline quality check procedure for a rectangular, uniform ITN.

Fig. 5. Regeneration study



11.2 Random selection of samples

The random selection of samples for testing at each stage of the study should be managed such that every at least one sample from each ITN batch that is used in the study is represented at each timepoint.

11.3 Complete series method for sample preparation

Fabric samples for a regeneration study should be prepared using the complete series method, which is a method of sample preparation that allows for all bioassays in a series or study (Days 0, 1, 2, 3, 5, 7, 10, 15, 20, 30, and 40) to be conducted on the same day. This reduces the source of variability in bioassay results that is due to bioassay test day and reduces variability due to the use of different batches of mosquitoes for bioassays in the same wash series.

To prepare samples for a wash regeneration study using the complete series method, all fabric samples for use in the study (excepting the samples selected for unwashed tests) are washed and dried three times consecutively in a single day to deplete the insecticide on the fabric surface, using the washing method described in section 9.2.1. On days 0, 1, 2, 3, 5, 7, 10, 15, 20, 30, and 40 after washing, four fabric samples are randomly selected, wrapped in aluminium foil, at stored at 4°C.

On bioassay test days, one fabric sample from each regeneration day, i.e., 0, 1, 2, 3, 5, 7, 10, 15, 20, 30 and 40, is removed from cold storage and allowed to acclimatise for two hours at 30°C followed by one hour at ambient temperature. Bioassays are conducted on all fabric samples.

After bioassays are complete, each fabric sample is cut as per the instructions in Fig 4. One piece is wrapped in aluminium foil and stored at 4°C until chemical analysis. The second piece of the sample is washed and dried once according to the method described in section 9.2.2, then wrapped in aluminium foil and stored at 4°C. Care must be taken to ensure that each piece of the sample is appropriately labelled to avoid mismatches in chemical analysis results. The chemical analysis consists of measurements of total AI.

Bioassays are conducted on all samples to Day 40 regardless of results from samples that have been allowed to regenerate for shorter periods of time in order to characterize any entomological impacts that may result from alterations in the presentation of AI(s) on the surface of the fabric(s), e.g., changes in physical form of surface AI (crystalline vs amorphous), during longer regeneration periods.

Scanning electron microscopy imaging, or other approach, may be conducted on samples and included within the study report to provide further information on the physical form of AI on the surface.

11.4 Sample sizes for bioassays

The number of replicates to be tested within the selected method must be considered as part of the protocol development and is dependent on the intent and context of the study. Sample size is estimated based on the selected endpoint for the bioassay method in use (usually mosquito mortality) and should be powered to detect a precise point estimate of the selected endpoint i.e., $\pm$ 5%. While 4 ITN samples

are recommended as a minimum, it is necessary to conduct wash resistance studies using a sufficient number of replicates per samples to obtain a sufficiently precise point estimate.

12. Results and data analysis

Results for test samples and controls (positive and negative) should be presented in both tabular and graphical format. Results of samples from each net, batch and overall should be included in the report.

If multiple fabrics are investigated in the study, the results for each fabric must be presented separately, e.g., results for the roof and sides for a mosaic net where the roof and sides have been constructed from different fabrics should be presented as [Product A roof] and [Product A sides].

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

12.1 Baseline quality results

12.1.1 Baseline chemical quality check analysis

The results to be reported for baseline chemical quality checks are:

- arithmetic mean results with respective standard deviation and range;
- percentage Relative standard deviation (RSD).

The inter- and intra-batch variability are analysed using RSD to measure the precision. RSD should be expressed as percentage. It is obtained by multiplying the standard deviation (SD) by 100 and dividing by product average (%RSD = SD *100/Mean).

A table showing the summary results (number of net pieces, mean concentration of AI, SD, range, %RSD) per net, production batch and overall should be included in the report.

12.1.2 Baseline bioassay quality checks

The results to be reported for baseline quality bioassays are:

- arithmetic mean results with 95% CIs for each selected endpoint.

A table showing the summary results (number of mosquitoes exposed, number of replicates, percentage arithmetic mean and 95% confidence intervals) per net, production batch and overall should be included in the report.

12.2 Regeneration study results

12.2.1 Chemical analysis

12.2.1.1 Chemical analysis results

The results to be reported for chemical analyses are:

- total AI for each piece in g/kg
- difference in total AI between the halves of each sample (estimated surface concentration) in g/kg.

The model-estimated mean surface concentration (12.2.1.2) of each fabric should be plotted on a graph together with results derived from the observed data (y axis – estimated surface concentration in g/kg; x axis – days) in order to visualize the observed response over time. The model and observed data should include the 95% CIs and estimates of the unwashed samples in the plot.

A table of summary results of the observed data (number of samples tested, arithmetic mean difference and Standard Deviation) for each experimental day should be included in the report.

12.2.1.2 Statistical data analysis

The mean difference between the before and after one wash (surface concentration) of the unwashed and three-times washed data should be analysed using a linear regression model, with day, production batch (factor variables) and AI of the before sample (continuous variable) as fixed effects. An additional fixed effect should be included if more than one day is used to analyse the samples.

The fitted model should be used to generate a predicted means and respective 95% CIs for the surface concentration (g/kg) for each day that experiments were carried out. The predicted estimates are averaged across the batches, AI before sample and day of analysis (if included). The means with respective 95% CIs should be plotted on a graph with the (y axis - difference in total AI in g/kg; x axis – days) together with the observed surface concentration (12.2.1.1) in order to visualize the surface concentration over time.

12.2.1.3 Interpretation of results

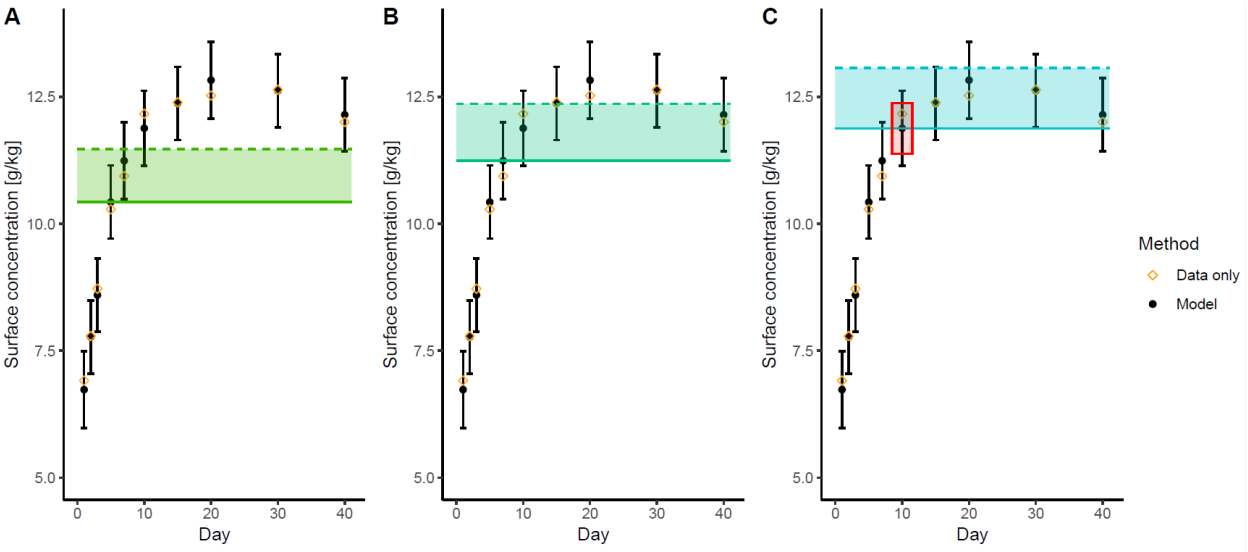
The time (days) required to reach stability in response(s) is considered as the time to re-establishment of chemical activity. Stability is defined as the earliest day when the model-derived mean surface concentrations for the later days are less than 10% higher than the concentration of that particular day.

12.2.1.3.1 Example of plots of chemical analysis results and resulting interpretation

In the example below, the chemical analysis results from the regeneration study have been plotted with the estimated surface concentration in g/kg on the y axis and time (days) on the x axis. The process to identify the timepoint at which the estimated surface concentration has stabilised is illustrated in sections A, B, and C, using a coloured zone that visually indicates concentrations that are 10% higher than the timepoint under consideration:

- In A: Day 5 is considered. However, five out of the six subsequent concentration measurements are greater than 10% higher than the Day 5 result, i.e., fall outside the coloured zone, and therefore the estimated surface concentration has not stabilised.
- In B: Day 7 is considered. However, there are two later estimates with a concentration greater than 10% higher than the Day 7 measurement, and therefore the estimated surface concentration has not stabilised.
- In C: Day 10 is considered as a candidate for the regeneration time. All of the subsequent concentration estimates fall within the coloured zone, and therefore the point at which the estimated surface concentration stabilises has been identified.

Example of chemical analysis interpretation



12.2.2 Bioassay

12.2.2.1 Bioassay results

The results to be reported for bioassays are:

- Mean results with an appropriate measure of dispersion for each selected endpoint at each time point.

If multiple bioassay methods are used, the results must be presented separately. Data generated from various bioassays should not be combined.

If multiple test systems (species/strains) are used, results must be presented in relation to the bioassay method used. Summary graphs including means and 95% CIs for the observed and model-derived (12.2.2.2) per test system should be presented.

A table showing the summary results of the observed data (number of net samples, number of mosquitoes exposed, number of replicates, percentage arithmetic mean and 95% confidence intervals)

per product and day should be included in the report for all test systems exposed to a specific fabric should be presented.

12.2.2.2 Statistical data analysis

The mortality data are analysed using a logistic regression model, with day and batch as fixed effects (factor variables). An additional fixed effect is included, if more than one technician carried out the experiments.

The fitted model is used to generate a predicted mortality and the respective 95% CI for each day that experiments were carried out. These model-derived means averaged over the batch effects (and operator effects, if included). The model-derived means and observed mortality (including the 95% CI) should be plotted on a graph (y axis – percent arithmetic mean mortality; x axis – days) in order to visualize the response over time.

12.2.2.3 Interpretation of results

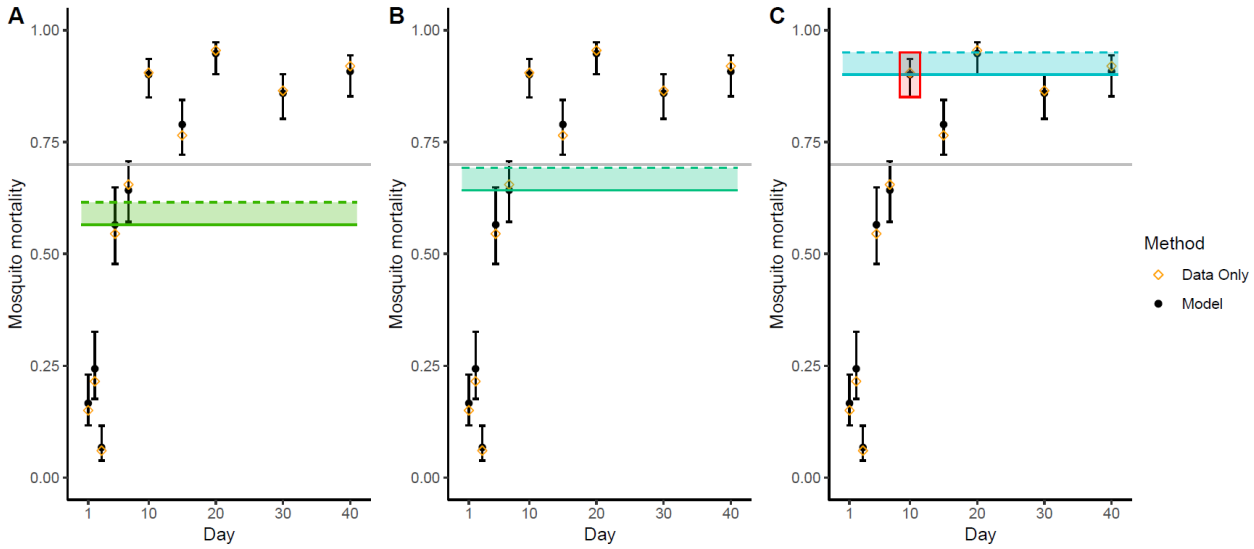
The time (days) required to reach stability in response(s) is considered as the time to re-establishment of biological activity. Stability is defined as the earliest day with the later model-derived mortality less than 5% higher than the mortality of that particular day.

12.2.2.3.1 Example of plots of bioassay results and resulting interpretation

In the example below, the bioassay results from the regeneration study have been plotted with mosquito mortality on the y axis and time (days) on the x axis. The process to identify the timepoint at which the mosquito mortality has stabilised is illustrated in sections A, B, and C, using a coloured zone that visually indicates mortality results that are 5% higher than the timepoint under consideration:

- In A, Day 7 is considered. However, all subsequent model-derived mortality estimates are greater than 5% higher than the Day 7 result, i.e., fall outside the coloured zone, and therefore the mortality has not stabilised.
- In B, Day 10 is considered. However, all subsequent model-derived mortality estimates are greater than 5% higher than the Day 10 result, i.e., fall outside the coloured zone, and therefore the mortality has not stabilised.
- In C, Day 15 is considered. All subsequent model-derived mortality estimates fall within the coloured zone, and therefore the point at which the mosquito mortality (biological response) stabilises has been identified.

Example of bioassay mortality interpretation



12.2.3 Criteria for study acceptance

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

The total AI(s) content results from the cut samples treated as 'before' samples should be higher than the total AI(s) content results for the 'after' sample.

Results for the positive and negative controls in bioassays must comply with acceptable results for the selected method. Refer to the implementation guidance documents for the selected method for details of control acceptance criteria.

13. Selection of wash interval

Selection of the wash interval to be used in subsequent artificial ageing studies is based on the results from the chemical analysis, i.e., the earliest in time from a model-estimated surface concentrations where mean (excluding CI) of later days are not more than 10% higher than the point estimate (excluding CI).

The bioassay results are used to provide information regarding the biological effect of the presentation of AI(s) on the surface of the fabric, including any changes in the AI(s) presentation over time. Bioassay results are not directly used to select the wash interval. However, both the bioassay and the chemical analysis results should be consulted when selecting the product wash interval to ensure internal consistency between study arms and as an additional study validity check.

14. Study report

14.1 Regeneration 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 regeneration 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
  - Summarise the chemical and entomological results and define and justify the selected wash interval
- List of abbreviations
- Background information
- Study rationale
- Study objectives
- Study endpoints
  - If multiple strains of tests systems have been tested, identify the strain which has been used to inform the wash interval selection, and provide a rationale
- Criteria for study acceptance
- Deviations from published guidance or study protocols and expected impact on the quality of the study results.
- Methods
  - Test systems
    - Colony maintenance and brief summarized rearing procedures
    - Description of test system. Indicate the most recent date of insecticide resistance characterization (NB. The results of the characterization are presented in the Matrix of selected mosquito strains)
    - Description of any additional test systems. Indicate the most recent date of insecticide resistance characterization (NB. The results of the characterization are presented in the Matrix of selected mosquito strains)
    - Age and physiological status of each test system used in bioassays. Separate descriptions by method if multiple bioassay methods have been used.
  - Test and reference items
    - Description of each test and reference item including:
      - Name of the items, type of net, active ingredient(s)/synergist name and their concentration
      - 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
        - Sampling plan
        - Net cutting procedures, including number and size of samples
        - Sample storage conditions
        - Washing method. Ensure that the water temperature, number of oscillations per minute of the shaker, soap and water type are reported
    - Sample shipment details for chemical analysis (if required)
    - Chemical analysis methods (if the chemical analysis was conducted on site at regeneration study testing facility)
    - Bioassays
        - Full methodological details for selected bioassay method(s). If multiple methods are used, each should be described separately
        - Sample sizes for bioassays, including the number of replicates conducted per sample and the number of mosquitoes exposed in each replicate
    - Data analysis
        - Statistical analysis methods for descriptive and inferential statistics, including all model parameters. Provide the R or Stata code used for analyses as an annex to the report
- Results
    - Baseline quality check
        - Summarised tabular results for chemical analyses and bioassays
            - If multiple bioassay methods were used, ensure that the results for each bioassay method are presented separately and that results for different fabric types and controls are clearly delineated
            - Refer to section 17 for suggested table formats for the reporting of chemical and bioassay results
        - Narrative description of results
    - Regeneration study
        - Summarised tabular results for chemical analyses and bioassays
            - If multiple bioassay methods were used, ensure that the results for each bioassay method are presented separately and that results for different fabric types and controls are clearly delineated
        - Graphical presentation of results
        - Narrative description of results
    - Data analysis and statistical results
        - Baseline quality check
            - Summary statistics
            - Statistical analysis results
        - Regeneration study
            - Summary statistics
            - Inferential statistical analysis results
    - Interpretation and selection of wash interval

- 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.

15. Related documents

- WHO PQT/VCP Implementation guidance – Considerations for the selection of controls for use in ITN studies
- WHO PQT/VCP Implementation guidance – Bioassay methods for ITNs: Cone test
- WHO PQT/VCP Implementation guidance – Bioassay methods for ITNs: Tunnel test
- WHO PQT/VCP Implementation guidance – Bioassay and semi-field methods for ITNs: IACT
- WHO PQT/VCP Implementation guidance – Considerations for the selection of mosquito strains for use in bioassays and site selection for semi-field and community studies
- WHO PQT/VCP Implementation guidance – Matrix of selected mosquito strains
- WHO PQT/VCP Matrix of selected mosquito strains - Template

16. References

1. Skovmand, O., Dang, D.M., Tran, T.Q., Bosselmann, R., Moore, SJ. Timing is everything: A simple chemical method to determine the bioavailable surface concentration of insecticide for insecticide treated net (ITN) evaluation. (https://doi.org/10.21203/rs.3.rs-2751835/v1)

17. **Annex.** Suggested table formats for summary results

17.1 Suggested table format for baseline quality check chemical analysis results

Table x. Baseline quality check chemical analysis results of ITNs received at [testing facility name] for [product name(s) and batch numbers [batch#1, batch#2, batch#3]]					
[Product name 1]					
Sample ID (net and batch identification)	Number of net samples	Mean [AI name] content (g/kg)	RSD (%)	Mean [synergist name, or second AI] content (g/kg)	RSD (%)
[sample IDs Batch 1 Net1]		[mean] ([SD][range (lower limit-upper limit)])	[this value shows the intra-net variability]	[mean] ([SD][range (lower limit-upper limit)])	[this value shows the intra-net variability]
[sample IDs Batch 1 Net2]					
[sample IDs Batch 1 Net3]					
[sample IDs Batch 1 Net4]					
[sample IDs Batch 1 Net5]					
Combined batch [1] results		[mean] ([SD][range (lower limit-upper limit)])	[this value shows the intra-batch variability]	[mean] ([SD][range (lower limit-upper limit)])	[this value shows the intra-batch variability]
[sample IDs Batch 2 Net1]					
[sample IDs Batch 2 Net2]					
[sample IDs Batch 2 Net3]					
[sample IDs Batch 2 Net4]					
[sample IDs Batch 2 Net5]					

Combined batch [2] results		[mean] ([SD][range (lower limit-upper limit)])	[this value shows the intra-batch variability]	[mean] ([SD][range (lower limit-upper limit)])	[this value shows the intra-batch variability]
[sample IDs Batch 3 Net1]					
[sample IDs Batch 3 Net2]					
[sample IDs Batch 3 Net3]					
[sample IDs Batch 3 Net4]					
[sample IDs Batch 3 Net5]					
Combined batch [3] results		[mean] ([SD][range (lower limit-upper limit)])	[this value shows the intra-batch variability]	[mean] ([SD][range (lower limit-upper limit)])	[this value shows the intra-batch variability]
Combined results for all batches		[mean] ([SD][range (lower limit-upper limit)])	[this value shows the inter-batch variability]	[mean] ([SD][range (lower limit-upper limit)])	[this value shows the inter-batch variability]
Add additional rows for additional products, if required					

17.2 Suggested table format for baseline quality check bioassay results

Table x. Baseline quality check bioassay results for [product name(s) and batch numbers [batch#1, batch#2, batch#3]] using [bioassay method] against [species/strain(s)] mosquitoes

Sample ID (net and batch identification)	Product/Fabric [A]				Product/Fabric [B]				Product/Fabric [C]			
	N [mosquitoes]	N [replicates]	Mean M24 (%) (95% CI)	Mean endpoint 2 (%) (95%CI)	N [mosquitoes]	N [replicates]	Mean M24 (%) (95% CI)	Mean endpoint 2 (%) (95%CI)	N [mosquitoes]	N [replicates]	Mean M24 (%) (95% CI)	Mean endpoint 2 (%) (95%CI)
[sample IDs Batch 1 Net1]												
[sample IDs Batch 1 Net2]												
[sample IDs Batch 1 Net3]												
[sample IDs Batch 1 Net4]												
[sample IDs Batch 1 Net5]												
Batch [1] combined results												
[sample IDs Batch 2 Net1]												
[sample IDs Batch 2 Net2]												
[sample IDs Batch 2 Net3]												
[sample IDs Batch 2 Net4]												
[sample IDs Batch 2 Net5]												
Batch [2] combined results												
[sample IDs Batch 3 Net1]												
[sample IDs Batch 3 Net2]												
[sample IDs Batch 3 Net3]												
[sample IDs Batch 3 Net4]												
[sample IDs Batch 3 Net5]												

Batch [3] combined results												
Combined results for all batches												
Add additional rows for additional products/fabrics if required												

NB. Present results for the negative control, positive control(s) and ITN under investigation. Additional rows/columns may be added for additional products/endpoints/species/strains.

17.3 Table format for regeneration study chemical analysis results

Table x. Regeneration study chemical analysis results for [product name(s) and batch numbers [batch#1, batch#2, batch#3]]							
[Product name 1]							
Wash	Net sample	Total [AI name] content for each piece sample (g/kg)	Total [AI name] content for each piece sample (g/kg) after wash and dry once	Difference in total [AI name] between the pieces of each sample in g/kg	Total [synergist name, or second AI] content for each piece sample (g/kg)	Total [synergist name, or second AI] content for each piece sample (g/kg) after wash and dry once	Difference in total [synergist name, or second AI] between the pieces of each sample in g/kg
Unwashed	1	[value]	[value]	[value]	[value]	[value]	[value]
Unwashed	2	[value]	[value]	[value]	[value]	[value]	[value]
Unwashed	3	[value]	[value]	[value]	[value]	[value]	[value]
Unwashed	4	[value]	[value]	[value]	[value]	[value]	[value]
Unwashed	1-4	[Mean value]	[Mean value]	[Mean value]	[Mean value]	[Mean value]	[Mean value]
Day 0	1	[value]	[value]	[value]	[value]	[value]	[value]
Day 0	2	[value]	[value]	[value]	[value]	[value]	[value]
Day 0	3	[value]	[value]	[value]	[value]	[value]	[value]
Day 0	4	[value]	[value]	[value]	[value]	[value]	[value]
Day 0	1-4	[Mean value]	[Mean value]	[Mean value]	[Mean value]	[Mean value]	[Mean value]
Day 1	1	[value]	[value]	[value]	[value]	[value]	[value]
Day 1	2	[value]	[value]	[value]	[value]	[value]	[value]
Day 1	3	[value]	[value]	[value]	[value]	[value]	[value]
Day 1	4	[value]	[value]	[value]	[value]	[value]	[value]
Day 1	1-4	[Mean value]	[Mean value]	[Mean value]	[Mean value]	[Mean value]	[Mean value]

Table x. Regeneration study chemical analysis results for [product name(s) and batch numbers [batch#1, batch#2, batch#3]]							
[Product name 1]							
Wash	Net sample	Total [AI name] content for each piece sample (g/kg)	Total [AI name] content for each piece sample (g/kg) after wash and dry once	Difference in total [AI name] between the pieces of each sample in g/kg	Total [synergist name, or second AI] content for each piece sample (g/kg)	Total [synergist name, or second AI] content for each piece sample (g/kg) after wash and dry once	Difference in total [synergist name, or second AI] between the pieces of each sample in g/kg
Day 2	1	[value]	[value]	[value]	[value]	[value]	[value]
Day 2	2	[value]	[value]	[value]	[value]	[value]	[value]
Day 2	3	[value]	[value]	[value]	[value]	[value]	[value]
Day 2	4	[value]	[value]	[value]	[value]	[value]	[value]
Day 2	1-4	[Mean value]	[Mean value]	[Mean value]	[Mean value]	[Mean value]	[Mean value]
Day 3	1	[value]	[value]	[value]	[value]	[value]	[value]
Day 3	2	[value]	[value]	[value]	[value]	[value]	[value]
Day 3	3	[value]	[value]	[value]	[value]	[value]	[value]
Day 3	4	[value]	[value]	[value]	[value]	[value]	[value]
Day 3	1-4	[Mean value]	[Mean value]	[Mean value]	[Mean value]	[Mean value]	[Mean value]
Day 5	1	[value]	[value]	[value]	[value]	[value]	[value]
Day 5	2	[value]	[value]	[value]	[value]	[value]	[value]
Day 5	3	[value]	[value]	[value]	[value]	[value]	[value]
Day 5	4	[value]	[value]	[value]	[value]	[value]	[value]
Day 5	1-4	[Mean value]	[Mean value]	[Mean value]	[Mean value]	[Mean value]	[Mean value]
Day 7	1	[value]	[value]	[value]	[value]	[value]	[value]
Day 7	2	[value]	[value]	[value]	[value]	[value]	[value]

Table x. Regeneration study chemical analysis results for [product name(s) and batch numbers [batch#1, batch#2, batch#3]]							
[Product name 1]							
Wash	Net sample	Total [AI name] content for each piece sample (g/kg)	Total [AI name] content for each piece sample (g/kg) after wash and dry once	Difference in total [AI name] between the pieces of each sample in g/kg	Total [synergist name, or second AI] content for each piece sample (g/kg)	Total [synergist name, or second AI] content for each piece sample (g/kg) after wash and dry once	Difference in total [synergist name, or second AI] between the pieces of each sample in g/kg
Day 7	3	[value]	[value]	[value]	[value]	[value]	[value]
Day 7	4	[value]	[value]	[value]	[value]	[value]	[value]
Day 7	1-4	[Mean value]	[Mean value]	[Mean value]	[Mean value]	[Mean value]	[Mean value]
Day 10	1	[value]	[value]	[value]	[value]	[value]	[value]
Day 10	2	[value]	[value]	[value]	[value]	[value]	[value]
Day 10	3	[value]	[value]	[value]	[value]	[value]	[value]
Day 10	4	[value]	[value]	[value]	[value]	[value]	[value]
Day 10	1-4	[Mean value]	[Mean value]	[Mean value]	[Mean value]	[Mean value]	[Mean value]
Day 15	1	[value]	[value]	[value]	[value]	[value]	[value]
Day 15	2	[value]	[value]	[value]	[value]	[value]	[value]
Day 15	3	[value]	[value]	[value]	[value]	[value]	[value]
Day 15	4	[value]	[value]	[value]	[value]	[value]	[value]
Day 15	1-4	[Mean value]	[Mean value]	[Mean value]	[Mean value]	[Mean value]	[Mean value]
Day 20	1	[value]	[value]	[value]	[value]	[value]	[value]
Day 20	2	[value]	[value]	[value]	[value]	[value]	[value]
Day 20	3	[value]	[value]	[value]	[value]	[value]	[value]
Day 20	4	[value]	[value]	[value]	[value]	[value]	[value]

Table x. Regeneration study chemical analysis results for [product name(s) and batch numbers [batch#1, batch#2, batch#3]]							
[Product name 1]							
Wash	Net sample	Total [AI name] content for each piece sample (g/kg)	Total [AI name] content for each piece sample (g/kg) after wash and dry once	Difference in total [AI name] between the pieces of each sample in g/kg	Total [synergist name, or second AI] content for each piece sample (g/kg)	Total [synergist name, or second AI] content for each piece sample (g/kg) after wash and dry once	Difference in total [synergist name, or second AI] between the pieces of each sample in g/kg
Day 20	1-4	[Mean value]	[Mean value]	[Mean value]	[Mean value]	[Mean value]	[Mean value]
Day 30	1	[value]	[value]	[value]	[value]	[value]	[value]
Day 30	2	[value]	[value]	[value]	[value]	[value]	[value]
Day 30	3	[value]	[value]	[value]	[value]	[value]	[value]
Day 30	4	[value]	[value]	[value]	[value]	[value]	[value]
Day 30	1-4	[Mean value]	[Mean value]	[Mean value]	[Mean value]	[Mean value]	[Mean value]
Day 40	1	[value]	[value]	[value]	[value]	[value]	[value]
Day 40	2	[value]	[value]	[value]	[value]	[value]	[value]
Day 40	3	[value]	[value]	[value]	[value]	[value]	[value]
Day 40	4	[value]	[value]	[value]	[value]	[value]	[value]
Day 40	1-4	[Mean value]	[Mean value]	[Mean value]	[Mean value]	[Mean value]	[Mean value]

17.4 Suggested table format for regeneration study bioassay results (observed)

Table x. Regeneration study bioassay results for [product name(s) and batch numbers [batch#1, batch#2, batch#3]] using [bioassay method] against [species/strain] mosquitoes															
Days post-wash	Product/Fabric [A]					Product/Fabric [B]					Product/Fabric [C]				
	N [net samples]	N [mosquitoes]	N [replicates]	Mean M24 (%) (95% CI)	Mean [endpoint 2] (95% CI)	N [net samples]	N [mosquitoes]	N [replicates]	Mean M24 (%) (95% CI)	Mean [endpoint 2] (95% CI)	N [net samples]	N [mosquitoes]	N [replicates]	Mean M24 (%) (95% CI)	Mean [endpoint 2] (95% CI)
UW															
0															
1															
2															
3															
5															
7															
10															
15															
20															
30															
40															

NB. Present results for the negative control, positive control(s) and ITN under investigation. Additional rows/columns may be added for additional products/endpoints/species/ strains

17.5 Suggested table format for regeneration study bioassay inferential statistics results

Table x. Wash resistance inferential statistics results for [product name(s) and batch numbers [batch#1, batch#2, batch#3]] using [bioassay method] against [species/strain] mosquitoes																		
N [washes]	Product/Fabric [A]						Product/Fabric [B]						Product/Fabric [C]					
	Mean M24	OR (95%CI)	p	Mean [endpoint 2]	OR (95%CI)	p	Mean M24	OR (95%CI)	p	Mean [endpoint 2]	OR (95%CI)	p	Mean M24	OR (95%CI)	p	Mean [endpoint 2]	OR (95%CI)	p
UW																		
1																		
2																		
2																		
3																		
5																		
7																		
10																		
15																		
20																		
30																		
40																		