WHO PQT/VCP Implementation Guidance –

Regeneration Time Study for ITN Fabric

Factors which may affect validity of regeneration time study

- Study not conducted in compliance with GLP
- Negative control mortality exceeds limits identified for the applicable methods
- Identification of issues related to the health of test organisms

Determination of Regeneration Time of ITNs

1. Purpose of the Study

For the purpose of the prequalification assessment, regeneration studies are conducted to determine the time required for an ITN fabric to regain its intended entomological effect(s) using appropriate test species/strains after the fabric has been washed.

Historically, the results of these studies were used to inform the wash interval to be used in the artificial aging of ITNs in other studies. In the prequalification assessment of ITNs, the results from the regeneration study may inform the determination of the wash interval; however, these results may underestimate the loss of AI per wash in operational settings. Therefore, other lines of evidence should be included to inform the determination of wash interval.

2. Requirement for submission of regeneration time studies

It is required that a regeneration time study, or studies, are conducted for each fabric used within the construction of an ITN. The specific formulation and manufacturing process for the fabric can significantly influence the behaviors of the treated fabric, especially the rate at which active ingredient(s) move from the reservoir to the surface.

Studies from at least 2 independent testing facilities should be generated.

Studies must be GLP compliant.

3. What does the study tell us about the fabric(s) used in the construction of ITNs

The regeneration time 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 organism(s) used for the bioassay.

The regeneration time study does not provide information on the rate of AI translocation nor quantification of the concentration of AIs on the surface. Additionally, because the regeneration time study relies on bioassays which optimize contact of test organisms with the fabric, it should not be used to predict or estimate efficacy in operational settings.

4. Considerations for method selection

Typically, regeneration studies utilize the cone test or tunnel test as the method(s) for experimentation. In designing a regeneration time 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.

5. Selection of Endpoints

The potential endpoint(s) which may be selected for use in the regeneration time 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 methods.

6. Considerations for test organism species/strain selection

For the purposes of the regeneration time study, the selected species 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

7. Study Materials
    a. Treated fabric

The study should include samples from a minimum of 3 batches of each fabric used in the construction of the ITN. Depending on the design of the individual fabrics, differentiated protocols may be necessary to address different formulants and/or target effects of the various fabrics. For the development of a new product dossier, it is critical that the batches used in the regeneration time study are the same as those used for other data generation.

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

- Pre-constructed treated fabrics in a described manner to ensure good representation across the batch production, or
- Constructed ITNs in a manner as described in the relevant Declaration of ITN Construction and Sampling Procedure

Documentation of the source, receipt and handling of samples prior to testing is critical.

The number of samples to be tested and replicates within the selected method must be considered as part of the protocol development and is dependent on the intent and context of the study. The protocol should consider the importance of characterization of potential inter- and intra-batch variability.

b. Negative Control

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

c. 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.

8. Sample preparation
    a. Cutting

Fabric samples should be cut in accordance with the procedures for the selected bioassay(s) to be performed.

b. Washing of fabric samples

Fabric samples should be washed three times using established washing procedures:

Samples (25 cm x 25 cm) should be introduced individually into 1-l beakers containing 0.5 l deionized water, with 2 g/l of soap (pH 10–11) added and fully dissolved just before washing. The beakers should be placed into a water-bath at 30 °C and shaken for 10 min at 155 movements per minute. The samples should then be removed, rinsed twice for 10 min in clean, deionized water under the same shaking conditions as above, dried at room temperature and stored at 30 °C in the dark between washes.

c. Storage of fabric samples prior to testing and between testing days

Fabric samples should be stored at 30 °C.

d. Fabric samples for chemical analysis

Additional samples should be prepared from the source fabric for chemical analysis. Chemical analysis should include determination of initial total concentrations as well as loss of AI(s) following the three washes. In addition, chemical analyses should be considered to characterize the translocation of AI(s) over the duration of the test. Fabric samples which are not immediately analyzed for chemical content should be individually wrapped in aluminum foil and held at 4°C.

9. 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.

10. Experimental Procedures

Bioassays should first be conducted on unwashed fabric samples. Then, the fabric samples should be washed and dried three times consecutively in a single day to deplete the insecticide on the fabric surface. The day of washing is day 0 of the study. After washing, the fabric samples should be held at

30 °C before the bioassay on day 1. Bioassays should be conducted on days 1, 2, 3, 5, and 7. If needed and appropriate for the fabric under investigation, the number of days can be extended. Bioassays should be conducted every other day from the 7th day.

The results of the bioassays should be plotted on a graph (y axis - % mean response including 95% confidence intervals; x axis – days) in order to visualize the observed response over time.

11. Termination of the experimental procedures

For each combination of fabric and species/strain to be investigated in the study, the supporting protocol should define the criteria for termination of the experimental procedures.

The time (days) required to reach a plateau in response(s) is considered as the regeneration time. Data should be analyzed using logistic regression to identify the plateau.

12. Study Report

a. Regeneration Time Study Report

A template has been developed to guide the preparation of study reports. This template should be relied upon as guidance and does not need to be strictly followed.

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.

b. Results

Results for test samples and controls should be presented in both tabular and graphical forms.

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

If multiple fabrics are investigated in the study, results for each fabric must be presented separately.

If multiple test organism (species/strains) are used, results must be presented in a manner which allows for assessment of the results for each test organism in relation to the fabric to which it was exposed. Summary graphs which present the results for all test organisms exposed to a specific fabric should be considered.

c. Statistical Analysis of Results

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

The effects of inter- and intra-batch variability can be analyzed using relative standard deviation (RSD) to measure the precision of the average of the results.

Logistic regression is used to identify the response plateau.

d. 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.