WHO Prequalification of Vector Control Products

Wash resistance index test for ITN fabrics

Factors which may affect validity of a wash resistance index test:

- study not conducted in compliance with GLP.

1. Purpose of the test

The Wash Resistance Index (WRI) test is an analytical procedure used to characterize the release of the AI from the polymer/coating under the specific study conditions and methods described below. The primary purpose of the WRI test is to enable comparison of commercial batches with the batches used in pre-market data generation (Modules 3 and 5) for assessment by WHO under controlled and reproducible conditions. It is not intended to characterize release of AI during operational use of an ITN.

The information in this implementation guidance has been adapted from MT195 CIPAC Handbook O (pg 205) *(1)*. The main and only difference between this implementation guidance and CIPAC MT195 is that three washes are initially conducted to deplete the insecticide present on the surface of the fabric, and the WRI must be reported to the nearest 0.1%.

2. Requirements for performing WRI tests

The WRI must be determined for each fabric used within the construction of an ITN as part of the physical and chemical characterization studies submitted to support applications for prequalification. The WRI study must include investigation of each AI in each fabric.

WRI testing must be performed at a GLP compliant testing facility.

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

The WRI test is intended to measure the initial rate of release of AI from the reservoir after washing under controlled and reproducible conditions by measuring the total chemical content before and after a series of washes. It is not intended to measure the full extent of release nor stabilized surface concentrations.

4. Considerations for method selection

The chemical analysis methods used for determining total AI content should also be used in the WRI test. These will typically be CIPAC methods.

Alternative methods for quantification of AI may be proposed, but the alternative method must be fully validated and a detailed scientific justification for not using the same method as for the total content test must be provided.

5. Study materials

5.1.Treated fabric

The test may include samples taken from either treated fabrics or constructed ITNs. Where samples are taken from constructed ITNs, the declared sampling plan for the product should be followed.

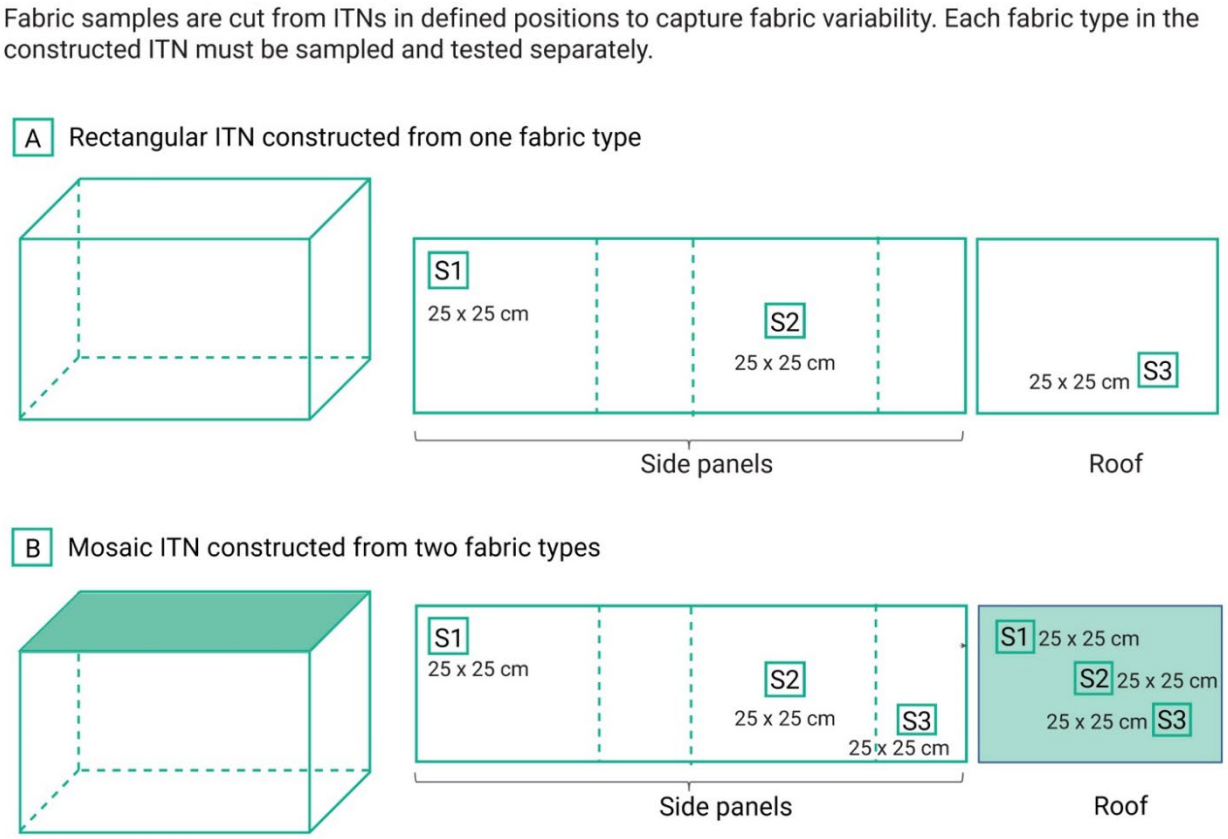
6. Sampling and test samples

6.1.Sampling

Sampling procedure for ITNs is dependent on the design and construction of the ITN, including the presence of multiple fabrics in the ITN design. The total number of samples required is dependent upon the study and the product.

For the data generation for PQ Module 3 dossier on wash resistance index for ITNs, four ITNs per batch and three samples per fabric type per ITN are required for testing. Fig. 1 shows an example of ITN sampling schemes. The position of samples should be measured from the left hand and top seams of each panel.

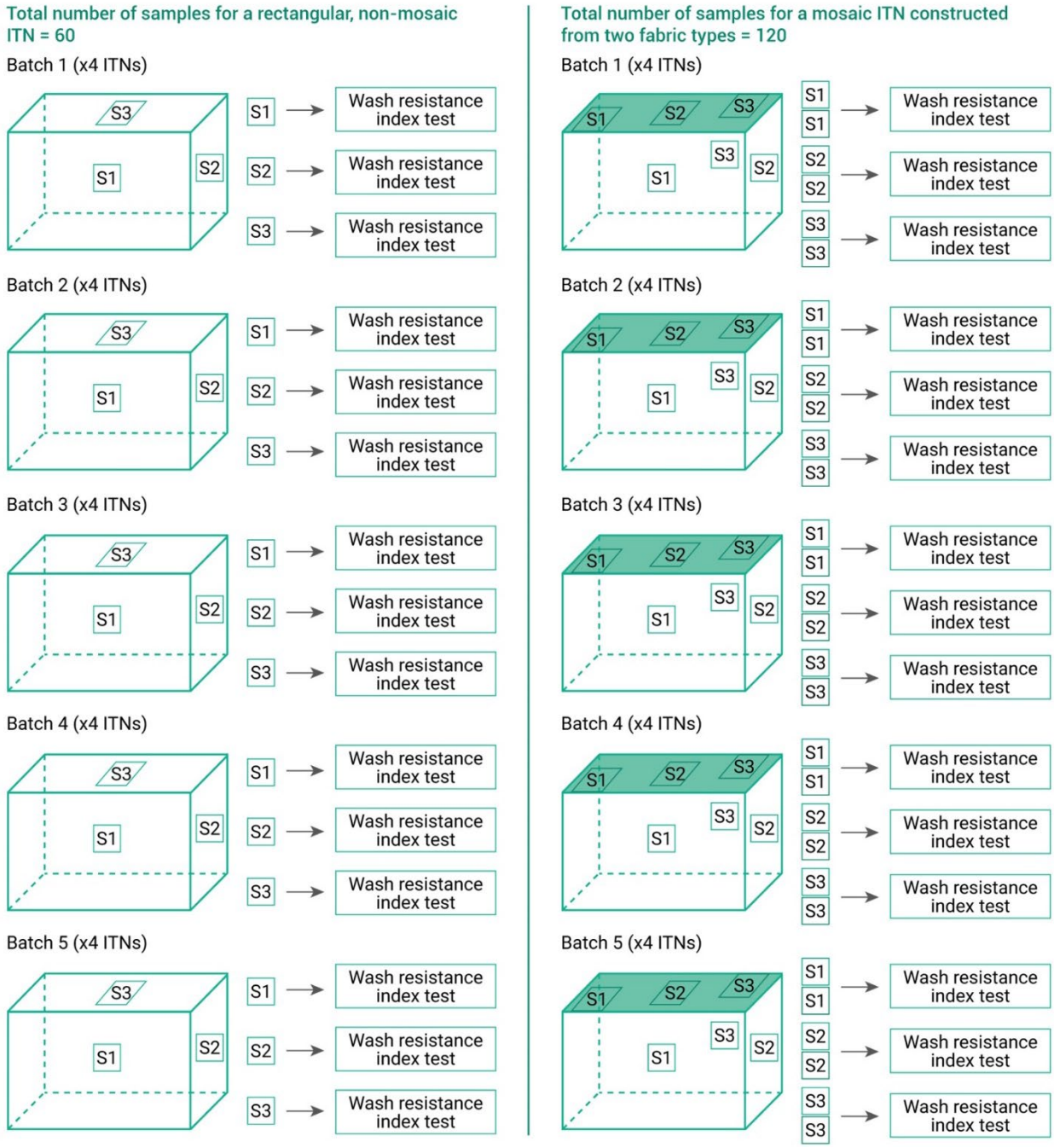
Fig. 1. Example ITN sampling schemes for wash resistance index for insecticide-treated nets.



Therefore, a total of 60 (3 samples per ITN x 4 ITNs per batch x 5 batches) samples are required for the measurements of homogeneous nets. For mosaic ITNs consisting of two fabric types, a total of 120 samples (3 samples per ITN x 4 ITNs per batch x 3 batches x 2 fabric types) are required.

Figure 2 shows an example of total number of samples required for wash resistance index for insecticide-treated nets.

Fig. 2. Example of total number of samples required for wash resistance index for insecticide-treated nets.



6.2.Sample preparation

6.2.1. Cutting

Cut 25 × 25 cm pieces from the fabric or net panel in accordance with the sampling plan.

6.2.2. Initial washes

Fabric samples should be washed three times using MT195 CIPAC washing-rinsing-heating process:

Washing agent: Heat the bottle containing polyoxyethyleneglycol monostearate to about 50°C to melt its content and reduce the viscosity thereof. Turn the bottle through 180 degrees a few times to ensure homogeneity. Weigh into a suitable glass flask containing 80 ml of de-ionised water sodium oleate (12.0 g) and polyoxyethyleneglycol monostearate (8.0 g). Heat this mixture at about 50°C turning it frequently through 180 degrees or stirring it by a magnetic stirrer until the mixture becomes clear and homogeneous. Check the pH of the washing solution; this must be typically between 8 and 10. This solution can be used for up to 4 weeks if kept sealed in the dark in a refrigerator.

Washing: Add washing agent (2.5 ml) to 500 ml of de-ionised water at 30°± 2°C in a glass bottle (1 litre). Insert an individual cut fabric sample and cap the bottle. Invert the bottle 10 times[1]. Place the bottle in the water bath in an upright position free from vibration[2] avoiding direct sunlight. After 10min, remove the sample using tweezers, and remove any remaining adherent drops of wash fluid by gentle shaking.

Rinsing: Insert the washed sample into a glass bottle (1 litre) containing de-ionised water (500 ml) at 30°C ± 2°C, cap and invert 10 times[1]. Place the bottle in the water bath in an upright position free from vibration[2] avoiding direct sunlight. After 10 min, remove the sample using tweezers, replace the deionised water, insert the sample into the 1 litre bottle and repeat this rinsing step once more.

Heating: Using tweezers pull out the sample from the rinsing fluid and carefully remove any remaining adherent water drops by gentle shaking. Allow the sample to dry at room temperature on a line for 30 min and keep protected from direct sunlight. Fold the sample carefully once or twice in each direction,

[1] The expression "invert the bottle" implies that the bottle is turned by hand through 180 degrees, and is then brought back to its original position, the whole operation being completed in approximately 2 s. An appropriate machine may be used to invert the bottle.
[2] Use a water bath as free as possible from vibration, as this may affect the result of the test. If a water bath without stirrers is used, check the temperature frequently to ensure that the water bath stays at the proper temperature. Alternatively, a thermostatic oven at 30 ± 2°C can be used.

place it loosely rolled in a glass bottle (100 to 125 ml), cap and store it in an oven at 40 ± 2°C [3] for 22 ± 2 h before starting the next washing cycle [4].

Ensure that the sample is completely dry before further washing, testing, or storage.

7. Experimental procedures

After completion of the initial washes, for each fabric, six randomly chosen samples from each batch should be wrapped individually in aluminium foil and stored at 4±2 °C. These will be analysed as the 'initial washed' samples.

Each of the remaining samples should be folded carefully once or twice in each direction, rolled loosely, placed in a glass bottle, capped, and stored in the dark at 40±2 °C. After 24, 48, 72, and 96 hours, each sample should be washed-rinsed-heated once using the procedure described above for the initial washes (section 6.2.2). Between washes, the samples should be stored in capped glass bottles in the dark at 40±2 °C. These will be analysed as the '4 x washed' samples.

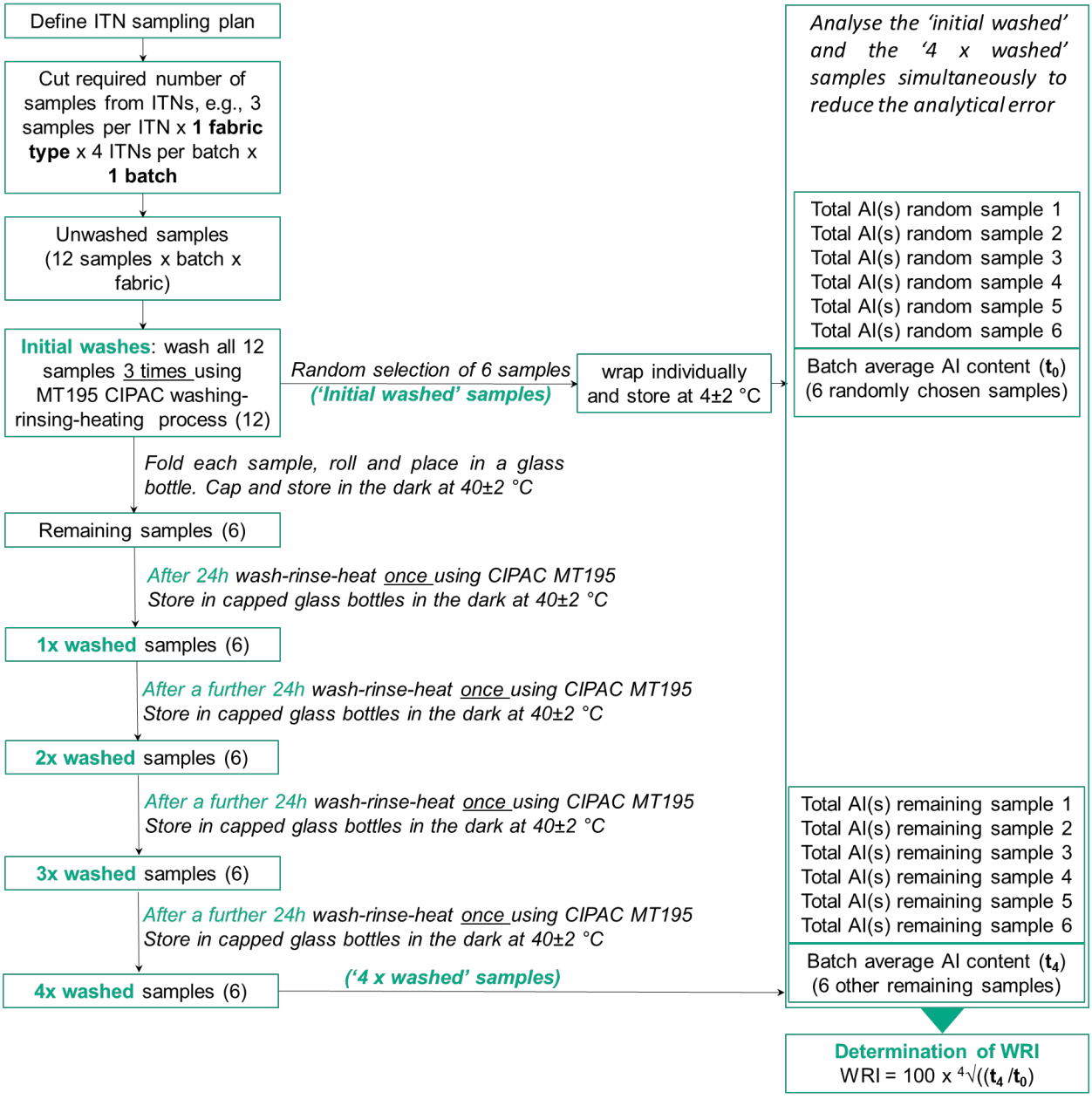
7.1.Schematic of a wash resistance index test for ITN fabrics

Fig. 3 illustrates the wash resistance index test procedure for **one batch** of a rectangular, uniform ITN.

[3] Keep the oven at 40 ± 2°C unless a lower or higher temperature is prescribed for the particular net. The storage at 40 ± 2°C is intended to allow migration of the active ingredient from the inner parts of the fibre of the net to the surface. This process is essential for both incorporated and coated LN's to attain a physico-chemical equilibrium of active ingredient between the surface of the net and the inner part of the coating or the fibre before the next washing. The average storage time until the next washing step is approximately 22 h. Lower or higher heating temperatures may be necessary and justifiable for certain net/active ingredient combinations.

[4] When longer storage times are necessary, store the sample folded and loosely rolled in a glass bottle at low temperature (0°C to 5°C) protecting it from direct sunlight. Net samples of which the scheduled washing cycles have been finished can also be stored in a glass bottle in a refrigerator at 4°C (± 3°C) awaiting analysis.

Fig 3. Wash resistance index test for ITN fabrics



8. Chemical analysis

Each of the 'initial washed' and '4 x washed' samples should be analysed separately for total content of each AI in the fabric using the appropriate analytical method(s). Analyse the 'initial washed' and the '4 x washed' samples simultaneously to reduce the analytical error. Express results in g/kg.

9. Determination of WRI

The wash resistance index (WRI) is calculated using the following equation:

$$w = 100 \times \sqrt[4]{(t_4/t_0)}$$

where: w = WRI, expressed as a percentage; t_4 = mean total active ingredient content (in g/kg) for '4 x washed' samples; and t_0 = mean total active ingredient content (in g/kg) for 'initial washed' samples.

10. Reporting

For each fabric, the results of the 'initial washed'and '4 x washes' should be reported as separate tables within the results for each batch.

For the 'initial washed', the table should include the sample ID and the measured AI content. The average AI content should be calculated to establish t_0.

For the '4 x washed', the table should include the sample ID, the measured AI content and the sample specific WRI using the established t_0. The average WRI for the batch should then be reported.

Report the WRI to the nearest 0.1% and specify the following parameters:

- temperature at which the heating procedure was carried out differed from the standard (40 ± 2°C)
- assay method used.

Data should not be aggregated for analysis across batches.

11. Inclusion of the WRI in manufacturing release specifications

The applicant is responsible for proposing a declared WRI for each AI in each fabric used in the construction of the finished ITN for the purpose of manufacturing release specifications. A justification for the proposed tolerances must be provided for assessment and should address the ability of the tolerances to ensure that commercial batches are comparable to the batches used in pre-market data generation (Modules 3 and 5).

12. Related documents

- WHO PQT/VCP Implementation guidance - Declaration of ITN construction and sampling Procedure
- WHO PQT/VCP Implementation guidance - Data requirements table – Module 3

13. References

1. Pigeon, O., Müller, M. , Kozuki, Y. & Rodler, M. (2017). CIPAC method MT 195, wash resistance index of LNs In: *CIPAC Handbook O*, Collaborative International Pesticides Analytical Council Ltd, ISBN 978-1-911009-14-6.

14. Bibliography

- Skovmand O, Dang DM, Tran TQ, Bossellman R, Moore SJ. From the factory to the field: considerations of product characteristics for insecticide-treated net (ITN) bioefficacy testing. Malar J. 2021;20(1):363. doi:10.1186/s12936-021-03897-7.

15. Annex

15.1. Suggested table formats for summary results

15.1.1. Table formats for wash resistance index test results

Table x. 'Initial washed' Results. AI content of sampled pieces of [product name] used in the wash resistance index test for ITN fabrics (batch numbers [batch#1, batch#2, batch#3, batch#4, batch#5])

Sample ID (net and batch identification)	Product/Fabric [A]		Product/Fabric [B]		Product/Fabric [C]	
	Mean [AI name] content (g/kg)	RSD (%)	Mean [AI name] content (g/kg)	RSD (%)	Mean [AI name] content (g/kg)	RSD (%)
Batch [1] average AI content (t_0) (6 randomly chosen samples IDs)	[mean] ([range lower limit] - [range upper limit])	[this value shows the intra-batch variability]				
Batch [2] average AI content (t_0) (6 randomly chosen samples IDs)						
Batch [3] average AI content (t_0) (6 randomly chosen samples IDs)						
Batch [4] average AI content (t_0) (6 randomly chosen samples IDs)						
Batch [5] average AI content (t_0) (6 randomly chosen samples IDs)						

Add additional columns for additional products/fabrics, if required.

Add additional tables for additional active ingredients (AIs), if required.

Table x. '4 x washed' Results. AI content and WRI of sampled pieces of [product name] used in the wash resistance index test for ITN fabrics (batch numbers [batch#1, batch#2, batch#3, batch#4, batch#5])

Sample ID (net and batch identification)	Product/Fabric [A]			Product/Fabric [B]			Product/Fabric [C]		
	Mean [AI name] content (g/kg)	RSD (%)	WRI(%) using t_0	Mean [AI name] content (g/kg)	RSD (%)	WRI(%) using t_0	Mean [AI name] content (g/kg)	RSD (%)	WRI(%) using t_0
Batch [1] average AI content (t_4) (6 other remaining samples IDs)	[mean] ([range lower limit] - [range upper limit])	[this value shows the intra-batch variability]	[value]						
Batch [2] average AI content (t_4) (6 other remaining samples IDs)									
Batch [3] average AI content (t_4) (6 other remaining samples IDs)									
Batch [4] average AI content (t_4) (6 other remaining samples IDs)									
Batch [5] average AI content (t_4) (6 other remaining samples IDs)									

Add additional columns for additional products/fabrics, if required.

Add additional tables for additional active ingredients (AIs), if required.