



## Data Collection Worksheet

**Please Note:** The Data Collection Worksheet (DCW) is a tool to aid integration of a PhenX protocol into a study. The PhenX DCW is not designed to be a data collection instrument. Investigators will need to decide the best way to collect data for the PhenX protocol in their study. Variables captured in the DCW, along with variable names and unique PhenX variable identifiers, are included in the PhenX Data Dictionary (DD) files.

### Sample Preparation

There are ten different categories of commercial smokeless tobacco products:

- 1) Dry snuff;
- 2) Moist (wet) snuff;
- 3) Moist (wet) snuff portion packs;
- 4) Plug;
- 5) Twist;
- 6) Loose leaf;
- 7) Dry snuff portion packs;
- 8) Snus;
- 9) Snus portion packs; and
- 10) Pellet or Compressed.

Because of their physical characteristics, some of the ten product categories must be ground (whole or in part) before nicotine, total moisture, and pH analyses can be conducted. The objective of grinding the samples is to obtain a homogeneous sample with particles measuring approximately 4 mm. Grinding to achieve this particle size should take no more than 3 minutes. To ensure proper grinding and an adequate amount of the ground sample for analysis, the minimum sample size of all commercial products to be ground should not be less than 100 grams.

To ensure precision of analyses for nicotine, total moisture, and pH, the samples that require grinding should be ground using a Robot Coupe Model RSI 2V Scientific Batch Processor or its equivalent. This is a variable speed (0 to 3,000 RPM)

processor. The variable speed motor is required to ensure proper grinding of the tobacco tissues (and in the case of pH determination, the portion pack). Elevated temperatures can result in moisture loss and an underestimated value for moisture content. Hence, care must be taken during grinding to avoid elevated temperatures. The bowl should be cleaned after each grinding to obtain accurate results. Freeze- or cryo-grinding is also an acceptable grinding method.

1) *Dry snuff*: Dry snuff samples do not need to be ground since the product is a powder. The sample must be thoroughly mixed before weighing for nicotine, total moisture, and pH analysis.

2) *Moist (wet) snuff*: Moist (wet) snuff samples do not need to be ground. The sample must be thoroughly mixed before weighing for nicotine, total moisture, and pH analysis.

3) *Moist (wet) snuff portion packs*: The tobacco contents of the moist (wet) snuff portion packs do not need to be ground for nicotine, total moisture, or pH analysis.

4) *Plug tobacco*: Break or cut apart plugs and add in portions to grinder at 2,000 RPM. Reduce RPM or stop grinding if sample bowl becomes warm. Pulse the Robot Coupe, when needed, to complete grinding. Grind samples until approximately 4 mm in size. The total grinding time should be no more than 3 minutes.

5) *Twist tobacco*: Separate twists, add to grinder, and grind at 2,000 RPM. Reduce RPM or stop grinding if sample bowl becomes warm. Continue grinding until sample particles are approximately 4 mm in size. The total time for grinding should be no more than 3 minutes.

6) *Loose leaf*: Grind in the same manner as described in 4 and 5 to obtain product with particle size of approximately 4 mm.

7) *Dry snuff portion packs*: The tobacco contents of the dry snuff portion packs do not need to be ground for nicotine, total moisture, or pH analysis.

8) *Snus*: Snus samples do not need to be ground since the product is a powder. The sample must be thoroughly mixed before weighing for nicotine, total moisture, and pH analysis.

9) *Snus portion packs*: The tobacco contents of the snus portion packs do not need to be ground for nicotine, total moisture, or pH analysis.

10) *Pellet or compressed*: Break apart compressed tobacco pellets and add in portions to grinder at 2,000 RPM. Reduce RPM or stop grinding if sample bowl becomes warm. Pulse the Robot Coupe, when needed, to complete grinding. Grind samples until approximately 4 mm in size. The total grinding time should be no

more than 3 minutes.

### Total Moisture Determination Procedure

- 1) Accurately weigh 5.00 grams of the sample (ground to pass  $\leq$  4 mm screen) <sup>20</sup> into a weighed moisture dish and place uncovered dish in oven.<sup>21</sup> The number of products sampled should reflect an acceptable level of precision.<sup>16</sup> The test material is to be representative of the product that is sold to the public and therefore should consist of sealed, packaged samples of finished product that is ready for commercial distribution. Samples are to be analyzed in duplicate.
- 2) Do not exceed 1 sample/10 sq in (650 sq cm) shelf space, and use only 1 shelf. Dry 3 hr at  $99 \pm 1.0^\circ\text{C}$ . Remove from oven, cover, and cool in desiccator to room temperature (about 30 min). Reweigh and calculate percent moisture.
- 3) Report the final moisture determination as a percentage (%), to an accuracy level of one decimal place for each brand name (e.g., Skoal Bandits Wintergreen, Skoal Long Cut Cherry, Skoal Long Cut Wintergreen, etc.). All data should include the mean value with a 95% confidence interval, the range of values, the number of samples tested, the number of lots per brand name, and the estimated precision of the mean. Information will be reported for each manufacturer and variety (including brand families and brand variations) and brand name (e.g., Skoal Bandits Wintergreen, Skoal Long Cut Cherry, Skoal Long Cut Wintergreen, etc.).

### Footnotes:

<sup>20</sup>The method is a modification of AOAC Method 966.02 (1990) in that the ground tobacco passes through a 4 mm screen rather than a 1 mm screen.

<sup>21</sup>When drying samples, do not dry different products (e.g., moist [wet] snuff, dry snuff, loose leaf) in the oven at the same time since this will produce errors in the moisture determinations.

<sup>16</sup>The testing facility is referred to ISO Procedure 8243 for a discussion of sample size and the effect of variability on the precision of the mean of the sample (ISO 8243, 1991).

### Assay Criteria for Quality Assurance:

All quality control parameters must be determined within the laboratory in which they are to be used. At least 10 within-laboratory runs must be performed to establish temporary confidence intervals for the quality control parameters. Permanent limits should be established after 20 runs and should be reestablished after each additional 20 runs.

### Good Laboratory Practice Guidelines:

The comments and notes listed below can be described as Good Laboratory Practice guidelines; they are described in detail in this protocol to ensure minimal interlaboratory variability in the determination of nicotine, total moisture, and pH in smokeless tobacco.

<sup>1</sup>This protocol assumes that the testing facility will implement and maintain a stringent Quality Assurance/Quality Control program to include, but not be limited to, regular interlaboratory comparisons, determination of the quality and purity of purchased products, and proper storage and handling of all reagents and samples.

<sup>2</sup>When a specific product or instrument is listed, it is the product or instrument that was used in the development of this method. Equivalent products or instruments may also be used. Use of trade names is for identification only and does not constitute endorsement by the Public Health Service or the U.S. Department of Health and Human Services.

### Sampling Guidelines

For total Moisture determination in multiple samples, sample each smokeless tobacco brand name according to the provided testing frequency schedule.<sup>19</sup>

<sup>19</sup> The testing frequency for each smokeless tobacco brand name (*e.g.*, Skoal Bandits Wintergreen, Skoal Long Cut Cherry, Skoal Long Cut Wintergreen, etc.) is based on the manufacturing duration (refer to table below). Each smokeless tobacco brand name will be sampled and tested for nicotine, total moisture, and pH no fewer than twice and no more than four times during a calendar year.

Manufacturing duration in weeks	Test frequency*
up to and including 4 .....	2
up to and including 28 .....	3
up to and including 52 .....	4

\* Use a statistical program to determine random sampling dates based on the total manufacturing duration during a calendar year. Sampling dates should fall on actual manufacturing days for the product when test material that is

representative of the product that is sold to the public (consisting of sealed, packaged samples) is available. If a statistically determined sampling date falls on a day that does not meet this criterion, sample the product on the next date that does meet the criteria.

<sup>11</sup>The testing facility must ensure that samples are obtained through the use of a survey design protocol for sampling “at one point in time” at the factory or warehouse. The survey design protocol must address short-, medium-, and long-term smokeless tobacco product variability (e.g., variability over time and from container to container of the tobacco product) in a manner equivalent to that described for cigarette sampling in Annex C of ISO Protocol 8243. Information accompanying results for each sample should include, but not be limited to:

For each product-manufacturer and variety (including brand families and brand variations) and brand name (e.g., Skoal Bandits, Skoal Long Cut Cherry, Skoal Long Cut Wintergreen, etc.):

- Product “category,” e.g., loose leaf, plug, twist, dry snuff, moist (wet) snuff, etc.
- Lot number.
- Lot size.
- Number of randomly sampled, sealed, packaged (so as to be representative of the product that is sold to the public) smokeless tobacco products selected (sampling fraction) for nicotine, moisture, and pH determination.
- Documentation of method used for random sample selection.
- "Age" of product when received by testing facility and storage conditions prior to analysis.

For smokeless tobacco brand names with episodic production during a calendar year, the total number of sampling dates is determined by the sum of the individual test frequencies, not to exceed four. For the purpose of the Protocol, episodic production is defined as manufacturing intervals separated by periods of 30 or more days when the smokeless tobacco brand name is not manufactured.

*Example 1:* Within a single calendar year a smokeless tobacco brand name is manufactured from January 1 to March 31 and from September 1 to December 15. The testing frequency for the first manufacturing interval is 3 and for the second manufacturing interval is 3. The Protocol allows that each smokeless tobacco brand name be tested for nicotine, total moisture, and pH no more than four times during a calendar year. Therefore, 4 random sampling dates, as described in the footnote to the above table, are determined for the smokeless tobacco brand

name. The values for nicotine, moisture, and pH determinations, and unionized (free) nicotine calculations and the mean of the 4 data points for that smokeless tobacco brand name are reported.

*Example 2:* Within a single calendar year a smokeless tobacco brand name is manufactured from April 5 to May 3 and from September 1 to December 15. The testing frequency for the first manufacturing interval is 2 and for the second manufacturing interval is 3. The values for nicotine, moisture, and pH determinations, and unionized (free) nicotine calculations and the mean of the 4 data points for that smokeless tobacco brand name are reported.

*Example 3:* Within a single calendar year a smokeless tobacco brand name is manufactured from January 1 to January 15 and from September 1 to September 22. The testing frequency for the first manufacturing interval is 2 and for the second manufacturing interval is 2. Four random sampling dates are selected to fall within the 6 weeks of manufacturing for the smokeless tobacco brand name. The values for nicotine, moisture, and pH determinations, and unionized (free) nicotine calculations and the mean of the 4 data points for that smokeless tobacco brand name are reported.

Protocol source: <https://www.phenxtoolkit.org/protocols/view/730502>