# <u>SMOKELESS TOBACCO REFERENCE PRODUCTS PROGRAM</u> Sample Storage and Handling Protocols

#### Storage at the Tobacco Analytical Services Lab

The Smokeless Tobacco Reference Products (STRP's) are stored at -20 °C in a large walk-in freezer at the NCSU Tobacco Analytical Services Laboratory.

#### **Recommended Procedure for Storage upon Receipt**

Upon receipt, investigators should immediately place the sealed STRPs in plastic bags and store them in a laboratory freezer at approximately -20 °C until analyses can be performed. The STRP's may be refrigerated for short term storage of approximately 1-week or less.

## Handling Immediately Prior to Laboratory Use

The samples should be removed from -20 °C and placed in a refrigerator for a minimum of 24 hours. This will allow water to become thoroughly re-equilibrated throughout the product. After removal from the refrigerator, the samples should be allowed to equilibrate to ambient conditions before opening. This will typically require 2 hours. After opening, the samples can be placed in sealed container for short term storage in the refrigerator. Individual researches must determine the stability of the reference products after removal from -20 °C. Stability will be dependent upon the analyses that will be conducted.

The CORESTA Smokeless Tobacco Subgroup recommends the following procedure for subsequent handling of the CORESTA reference Products (CRPs):

- 1. Snus pouches (CRP1 & CRP 1.1) should be cut into two halves and the tobacco and pouch material be added directly to the extraction vessel for chemical analyses. Furthermore, unit pouches should be analyzed.
- 2. Moist Snuff reference products (CRP2 & CRP 2.1) and Dry Snuff reference products (CRP3 & CRP 3.1) do not require sample grinding and can be used directly for sample analysis.
- 3. The 2009 CRP4 is typical of a loose leaf chewing tobacco consisting of large pieces of the tobacco blend components. Reproducible analysis of this product requires grinding an entire pouch to ensure within-pouch sample homogeneity. It is recommended that CRP4 be frozen in liquid nitrogen before being ground in a standard laboratory mill. Cryogrinding will ensure the sample does not degrade due to the generation of heat during the grinding process. The 2016 CRP4.1 was produced with the product characteristics of a loose leaf chewing tobacco; however, it was produced in a chopped format. For this reason, CRP4.1 does not require grinding prior to analysis. This product is similar in appearance to moist snuff.

### **Other Analytical Considerations**

Please note that each of the STRPs have specific moisture content (see <u>Product Information</u> for approximate values). The 2009 CRPs have been shown to be stable when stored at  $-20^{\circ}$ C. Stability reports can be found at <u>https://www.coresta.org</u>. Depending upon how these products are transported and stored, moisture content and chemical constituents may change over time. Furthermore, each product varies in particle size and size distribution. These variables should be taken into account in the development of robust analytical chemistry protocols as they may affect analytical reproducibility.