



## **CORESTA Guide N° 11**

**July 2011**

**CORESTA Smokeless Tobacco Sub-Group**

### **TECHNICAL GUIDELINE FOR SAMPLE HANDLING OF SMOKELESS TOBACCO AND SMOKELESS TOBACCO PRODUCTS**

#### **0. INTRODUCTION**

This Technical Guideline for the handling of Smokeless Tobacco and Smokeless Tobacco Products (STPs) has been developed by the CORESTA Smokeless Tobacco Sub-Group to provide guidance to the tobacco industry and independent testing laboratories.

The appropriate storage and preparation of tobacco samples is one of the most important factors with regard to the achievement of representative and reproducible test results. A laboratory can only produce high quality results if the integrity of samples is maintained.

The procedures for the representative sampling of tobacco products can be found in CORESTA Recommended Method No. 71 (CRM No. 71; Smokeless Tobacco Products - Sampling). Please note the importance of the precautions to be taken when sampling. Careful documentation during sampling is required. All relevant information, such as when and where a sample was taken and under what conditions it was taken should be clearly recorded. This is necessary because variations in sampling procedures can have a marked effect on the results of analysis.

In addition to the sampling procedure, appropriate sample handling, (i.e., storage and preparation) prior to analysis are also important to ensure representative analytical results. It is important to ensure that the passage of a sample through the laboratory's analytical system is fully documented and corresponds to each laboratory's relevant Standard Operating Procedure (SOP).

Sub-sampling or splitting of a sample into two or more aliquots may be necessary for some types of products or analytes. Different types of sample pre-treatment may also be necessary to homogenize different types of product before analysis can be performed.

## 1. FIELD OF APPLICATION

This Technical Guideline is applicable for the sample handling of Smokeless Tobacco and Smokeless Tobacco Products before analysis and retention of samples. In Annex 1 & 2, matrices display the recommended sample preparation and sample storage per product type and analyte.

## 2. NORMATIVE REFERENCES

CORESTA Recommended Method No 71 (2011): Smokeless Tobacco Products - Sampling

## 3. TERMS and DEFINITIONS

### 3.1. Laboratory Sample

A sample intended for laboratory inspection or testing.

### 3.2. Test Sample

STPs for test taken at random from the laboratory sample and which are representative of each of the increments making up the laboratory sample.

### 3.3. Test portion

A group of STPs prepared for a single determination and which is a random sample from the test sample or conditioned sample as appropriate.

## 4. PURPOSE

The objective of this guideline is to describe the sample handling (i.e. sample storage and preparation) *after* the sample has reached the laboratory.

It is difficult to recommend a guideline for sample handling that will address every possible situation. The objective of sample handling is to minimize sample deterioration. For example, the elapsed time between sampling and testing will affect sampling and handling parameters. This guideline will describe the sample storage and preparation of samples that has reached the laboratory.

Variables such as particle size, moisture content and analyte volatility can impact sampling/handling parameters. Other considerations are:

- Amount needed for all analyses - including amounts for retests.
- Containers for storage to ensure sample such as integrity-metal or plastic, clear or amber, Ziploc bag or gas/air-tight container.
- Freezer or refrigerator storage.
- Sample preparation (i.e., grinding or no grinding).

## 5. SAMPLING

This guideline describes the sample handling *after* the sample has reached the laboratory and the guideline for sampling STPs can be found in CORESTA Recommended Method No. 71

It is recommended that after the samples have been collected, they shall be packed securely with adequate protection against damage, kept cold (+1 to 8 °C) and sent to the laboratory by the most expeditious means available. The amount of each sample needed is related to the requested analysis.

## 6. SAMPLE PREPARATION AND STORAGE

### 6.1. Sample Storage

After arrival at the laboratory, but before analysis, the sample should be stored in accordance to Annex 1. However if the sample was taken or shipped refrigerated or frozen, then it should be stored in a refrigerator or freezer.

As mentioned above in purpose, it is difficult to recommend a preparation/storage scheme that will address the various characteristics of all tobacco and tobacco products. Therefore samples should be tested as soon as possible after being prepared.

### 6.2. Sample Preparation

After arrival at the laboratory, the sample should be prepared in accordance to Annex 2. However if there is specific demand of particle size or preparation in the analytical method, then that preparation should be followed.

It is important to ensure that sample integrity is maintained when being ground. Cross-contamination between samples must be prevented by implementing appropriate cleaning procedures between samples and checks where appropriate. When preparing samples; i.e., grinding, contamination of samples by metals (Cr and Ni) may occur, therefore an appropriate grinder should be selected to prevent such contamination. In addition when grinding, the temperature should be controlled, especially when grinding several samples with various particle sizes. An increase in temperature in the grinding mill could affect the sample material especially if volatile compounds are present. Some compounds are so volatile that grinding the sample while frozen will be necessary.

Moist products with small particle size (< 4 mm), like SNUS and fine cut moist snuff could normally be analysed without further preparation.

## ANNEX 1

### Sample Handling Storage: Tables 1A – C

#### Key to Tables 1A – C

General Sample Storage Categories (if not specific storage stated in the method):

1. Refrigerator (> 1 °C to < 8 °C), 3 weeks  
Analytes must be analyzed within 3 weeks, freezing may affect the results
2. Refrigerator (> 1 °C to < 8 °C), 3 weeks  
Maximum storage time 3 weeks (otherwise store in Freezer)
3. Refrigerator (> 1 °C to < 8 °C), “Best before date”  
Maximum storage time: “Best before date” (otherwise store in Freezer)
4. Freezer (< -18 °C)  
If stored longer than 5 days  
Avoid freeze and thawing
5. Room temperature

Recommended containers:

Original package  
Amber Glass jar or Amber Gas Tight Plastic bags with limited headspace

Table 1A		Chewing Tobacco									
Analyte family	Species	Loose Leaf	Plug	Twist/Roll	Bits	Gutkha	Khaini	Mawa	Pan Masala	Shamma	Zarda
Alkaloids	Nicotine	2	2	2	2	5	5	5	5	5	5
Alkaloids, minor	Nornicotine, anatabine, anabasine	2	2	2	2	5	5	5	5	5	5
Carbohydrates	Total sugars	2	2	2	2	5	5	5	5	5	5
Carbonyls	Acetaldehyde	4	4	4	4	4	4	4	4	4	4
Carbonyls	Acrolein	4	4	4	4	4	4	4	4	4	4
Carbonyls	Crotonaldehyde	4	4	4	4	4	4	4	4	4	4
Carbonyls	Formaldehyde	4	4	4	4	4	4	4	4	4	4
Humectants	Glycerol	3	3	3	3	5	5	5	5	5	5
Humectants	Propylene Glycol	3	3	3	3	5	5	5	5	5	5
Humectants	Triethylene Glycol	3	3	3	3	5	5	5	5	5	5
Hydrogen ion	pH	1	1	1	1	5	5	5	5	5	5
Inorganic	Ash	3	3	3	3	5	5	5	5	5	5
Inorganic	Calcium, potassium, sodium	3	3	3	3	5	5	5	5	5	5
Metals	As, Cd, Cr, Hg, Ni, Pb, Se )	3	3	3	3	5	5	5	5	5	5
Mycotoxins	Aflatoxins B <sub>1</sub> ,B <sub>2</sub> ,G <sub>1</sub> ,G <sub>2</sub>	3	3	3	3	5	5	5	5	5	5
Mycotoxins	Ochratoxin A	3	3	3	3	5	5	5	5	5	5
Nitrogen Compounds	Ammonia	4	4	4	4	4	4	4	4	4	4
Nitrogen Compounds	Nitrate	4	4	4	4	4	4	4	4	4	4
Nitrogen Compounds	Nitrite	4	4	4	4	4	4	4	4	4	4
Nitroso-amino acids	Nitrososarcosine	2	2	2	2	5	5	5	5	5	5
Other nitrosamines	NDMA	4	4	4	4	4	4	4	4	4	4
Oven volatiles	Moisture	1	1	1	1	5	5	5	5	5	5
PAH's	BaP	3	3	3	3	5	5	5	5	5	5
Radioisotopes	Po-210	3	3	3	3	5	5	5	5	5	5
TSNA	NAB, NAT, NNK, NNN, Total	2	2	2	2	5	5	5	5	5	5

<b>Table 1B</b>		<b>Snus</b>	<b>Moist snuff</b>	<b>Paste</b>	<b>Tooth powder</b>			<b>Dissolvable tobacco</b>	<b>Dry Snuff Scotch</b>
<b>Analyte family</b>	<b>Species</b>				<b>Quiwam</b>	<b>Bajjar</b>	<b>Gul</b>		
Alkaloids	Nicotine	2	2	2	5	5	5	5	5
Alkaloids, minor	Nornicotine, anatabine, anabasine	2	2	2	5	5	5	5	5
Carbohydrates	Total sugars	2	2	2	5	5	5	5	5
Carbonyls	Acetaldehyde	4	4	4	4	4	4	4	4
Carbonyls	Acrolein	4	4	4	4	4	4	4	4
Carbonyls	Crotonaldehyde	4	4	4	4	4	4	4	4
Carbonyls	Formaldehyde	4	4	4	4	4	4	4	4
Humectants	Glycerol	3	3	3	5	5	5	5	5
Humectants	Propylene Glycol	3	3	3	5	5	5	5	5
Humectants	Triethylene Glycol	3	3	3	5	5	5	5	5
Hydrogen ion	PH	1	1	1	5	5	5	5	5
Inorganic	Ash	3	3	3	5	5	5	5	5
Inorganic	Calcium, potassium, sodium	3	3	3	5	5	5	5	5
Metals	As, Cd, Cr, Hg, Ni, Pb, Se	3	3	3	5	5	5	5	5
Mycotoxins	Aflatoxins B <sub>1</sub> , B <sub>2</sub> , G <sub>1</sub> , G <sub>2</sub>	3	3	3	5	5	5	5	5
Mycotoxins	Ochratoxin A	3	3	3	5	5	5	5	5
Nitrogen Compounds	Ammonia	2	2	2	4	4	4	4	4
Nitrogen Compounds	Nitrate	4	4	4	4	4	4	4	4
Nitrogen Compounds	Nitrite	4	4	4	4	4	4	4	4
Nitroso-amino acids	Nitrososarcosine	2	2	2	5	5	5	5	5
Other nitrosamines	NDMA	4	4	4	4	4	4	4	4
Oven volatiles	Moisture	1	1	1	5	5	5	5	5
PAH's	BaP	3	3	3	5	5	5	5	5
Radioisotopes	Po-210	3	3	3	5	5	5	5	5
TSNA	NAB, NAT, NNK, NNN, Total	2	2	2	5	5	5	5	5

<b>Table 1C</b>		<b>Western Europe Nasal snuff</b>			<b>Asian</b>
<b>Analyte family</b>	<b>Species</b>	<b>European snuff</b>	<b>"Old style" snuff</b>	<b>Schmalzer</b>	<b>Naswar</b>
Alkaloids	Nicotine	5	5	5	5
Alkaloids, minor	Nornicotine, anatabine, anabasine	5	5	5	5
Carbohydrates	Total sugars	5	5	5	5
Carbonyls	Acetaldehyde	4	4	4	4
Carbonyls	Acrolein	4	4	4	4
Carbonyls	Crotonaldehyde	4	4	4	4
Carbonyls	Formaldehyde	4	4	4	4
Humectants	Glycerol	5	5	5	5
Humectants	Propylene Glycol	5	5	5	5
Humectants	Triethylene Glycol	5	5	5	5
Hydrogen ion	pH	5	5	5	5
Inorganic	Ash	5	5	5	5
Inorganic	Calcium, potassium, sodium	5	5	5	5
Metals	As, Cd, Cr, Hg, Ni, Pb, Se	5	5	5	5
Mycotoxins	Aflatoxins B <sub>1</sub> ,B <sub>2</sub> ,G <sub>1</sub> ,G <sub>2</sub>	5	5	5	5
Mycotoxins	Ochratoxin A	5	5	5	5
Nitrogen Compounds	Ammonia	4	4	4	4
Nitrogen Compounds	Nitrate	4	4	4	4
Nitrogen Compounds	Nitrite	4	4	4	4
Nitroso-amino acids	Nitrososarcosine	5	5	5	5
Other nitrosamines	NDMA	4	4	4	4
Oven volatiles	Moisture	5	5	5	5
PAH's	BaP	5	5	5	5
Radioisotopes	Po-210	5	5	5	5
TSNA	NAB, NAT, NNK, NNN, Total	5	5	5	5

## ANNEX 2

### Sample Handling Preparation: Tables 2A – C

#### Key to Tables 2A – C

General Sample Storage Categories (if not specific storage stated in the method):

1. No preparation of samples (particle size < 4 mm)
2. Cut sample pouches and analyzed with the pouches
3. Grind samples (particle size > 4 mm)
4. Freeze / Grind samples (particle size > 4 mm)
5. Other preparation
  - Dissolvable products:
    - If soluble in the Analytical method extraction solvent: 1
    - If not soluble in the Analytical method extraction solvent: 4

Note: For pouched products follow recommendations within brackets



<b>Table 2A</b>		<b>Chewing Tobacco</b>								
<b>Analyte family</b>	<b>Species</b>	<b>Loose Leaf</b>	<b>Plug</b>	<b>Twist/Roll</b>	<b>Bits</b>	<b>Gutkha</b>	<b>Khaini (pouched)</b>	<b>Mawa</b>	<b>Pan Masala</b>	<b>Zarda</b>
Alkaloids	Nicotine	3	3	3	3	3	1 (2)	3	3	3
Alkaloids, minor	Nornicotine, anatabine, anabasine	3	3	3	3	3	1 (2)	3	3	3
Carbohydrates	Total sugars	3	3	3	3	3	1 (2)	3	3	3
Carbonyls	Acetaldehyde	4	4	4	4	4	1 (2)	4	4	4
Carbonyls	Acrolein	4	4	4	4	4	1 (2)	4	4	4
Carbonyls	Crotonaldehyde	4	4	4	4	4	1 (2)	4	4	4
Carbonyls	Formaldehyde	4	4	4	4	4	1 (2)	4	4	4
Humectants	Glycerol	3	3	3	3	3	1 (2)	3	3	3
Humectants	Propylene Glycol	3	3	3	3	3	1 (2)	3	3	3
Humectants	Triethylene Glycol	3	3	3	3	3	1 (2)	3	3	3
Hydrogen ion	pH	3	3	3	3	3	1 (2)	3	3	3
Inorganic	Ash	3	3	3	3	3	1 (2)	3	3	3
Inorganic	Calcium, potassium, sodium	3	3	3	3	3	1 (2)	3	3	3
Metals	As, Cd, Cr, Hg, Ni, Pb, Se	3	3	3	3	3	1 (2)	3	3	3
Mycotoxins	Aflatoxins B <sub>1</sub> , B <sub>2</sub> , G <sub>1</sub> , G <sub>2</sub>	3	3	3	3	3	1 (2)	3	3	3
Mycotoxins	Ochratoxin A	3	3	3	3	3	1 (2)	3	3	3
Nitrogen Compounds	Ammonia	4	4	4	4	4	1 (2)	4	4	4
Nitrogen Compounds	Nitrate	3	3	3	3	3	1 (2)	3	3	3
Nitrogen Compounds	Nitrite	3	3	3	3	3	1 (2)	3	3	3
Nitroso-amino acids	Nitrososarcosine	3	3	3	3	3	1 (2)	3	3	3
Other nitrosamines	NDMA	4	4	4	4	4	1 (2)	4	4	4
Oven volatiles	Moisture	1	1	1	1	1	1 (2)	1	1	1
PAH's	BaP	3	3	3	3	3	1 (2)	3	3	3
Radioisotopes	Po-210	1	1	1	1	1	1 (2)	1	1	1
TSNA	NAB, NAT, NNK, NNN, Total	3	3	3	3	3	1 (2)	3	3	3

<b>Table 2B</b>		<b>Snus</b>	<b>Moist snuff</b>	<b>Paste</b>	<b>Tooth powder</b>			<b>Dissolvable tobacco</b>	<b>Dry Snuff</b>
<b>Analyte family</b>	<b>Species</b>	<b>(pouched)</b>	<b>(pouched)</b>	<b>Quiwam</b>	<b>Bajjar</b>	<b>Gul</b>	<b>Mishiri</b>		<b>Scotch</b>
Alkaloids	Nicotine	1 (2)	1 (2)	5	1	1	1	5	1
Alkaloids, minor	Nornicotine, anatabine, anabasine	1 (2)	1 (2)	5	1	1	1	5	1
Carbohydrates	Total sugars	1 (2)	1 (2)	5	1	1	1	5	1
Carbonyls	Acetaldehyde	1 (2)	1 (2)	5	1	1	1	5	1
Carbonyls	Acrolein	1 (2)	1 (2)	5	1	1	1	5	1
Carbonyls	Crotonaldehyde	1 (2)	1 (2)	5	1	1	1	5	1
Carbonyls	Formaldehyde	1 (2)	1 (2)	5	1	1	1	5	1
Humectants	Glycerol	1 (2)	1 (2)	5	1	1	1	5	1
Humectants	Propylene Glycol	1 (2)	1 (2)	5	1	1	1	5	1
Humectants	Triethylene Glycol	1 (2)	1 (2)	5	1	1	1	5	1
Hydrogen ion	pH	1 (2)	1 (2)	5	1	1	1	5	1
Inorganic	Ash	1 (2)	1 (2)	5	1	1	1	5	1
Inorganic	Calcium, potassium, sodium	1 (2)	1 (2)	5	1	1	1	5	1
Metals	As, Cd, Cr, Hg, Ni, Pb, Se	1 (2)	1 (2)	5	1	1	1	5	1
Mycotoxins	Aflatoxins B <sub>1</sub> ,B <sub>2</sub> ,G <sub>1</sub> ,G <sub>2</sub>	1 (2)	1 (2)	5	1	1	1	5	1
Mycotoxins	Ochratoxin A	1 (2)	1 (2)	5	1	1	1	5	1
Nitrogen Compounds	Ammonia	1 (2)	1 (2)	5	1	1	1	5	1
Nitrogen Compounds	Nitrate	1 (2)	1 (2)	5	1	1	1	5	1
Nitrogen Compounds	Nitrite	1 (2)	1 (2)	5	1	1	1	5	1
Nitroso-amino acids	Nitrososarcosine	1 (2)	1 (2)	5	1	1	1	5	1
Other nitrosamines	NDMA	1 (2)	1 (2)	5	1	1	1	5	1
Oven volatiles	Moisture	1 (2)	1 (2)	5	1	1	1	5	1
PAH's	BaP	1 (2)	1 (2)	5	1	1	1	5	1
Radioisotopes	Po-210	1 (2)	1 (2)	5	1	1	1	5	1
TSNA	NAB, NAT, NNK, NNN, Total	1 (2)	1 (2)	5	1	1	1	5	1

<b>Table 2C</b>		<b>Western Europe Nasal snuff</b>			<b>Asian</b>
<b>Analyte family</b>	<b>Species</b>	<b>European snuff</b>	<b>"Old style" snuff</b>	<b>Schmalzer</b>	<b>Naswar</b>
Alkaloids	Nicotine	1	1	1	1
Alkaloids, minor	Nornicotine, anatabine, anabasine	1	1	1	1
Carbohydrates	Total sugars	1	1	1	1
Carbonyls	Acetaldehyde	1	1	1	1
Carbonyls	Acrolein	1	1	1	1
Carbonyls	Crotonaldehyde	1	1	1	1
Carbonyls	Formaldehyde	1	1	1	1
Humectants	Glycerol	1	1	1	1
Humectants	Propylene Glycol	1	1	1	1
Humectants	Triethylene Glycol	1	1	1	1
Hydrogen ion	pH	1	1	1	1
Inorganic	Ash	1	1	1	1
Inorganic	Calcium, potassium, sodium	1	1	1	1
Metals	As, Cd, Cr, Hg, Ni, Pb, Se	1	1	1	1
Mycotoxins	Aflatoxins B <sub>1</sub> , B <sub>2</sub> , G <sub>1</sub> , G <sub>2</sub>	1	1	1	1
Mycotoxins	Ochratoxin A	1	1	1	1
Nitrogen Compounds	Ammonia	1	1	1	1
Nitrogen Compounds	Nitrate	1	1	1	1
Nitrogen Compounds	Nitrite	1	1	1	1
Nitroso-amino acids	Nitrososarcosine	1	1	1	1
Other nitrosamines	NDMA	1	1	1	1
Oven volatiles	Moisture	1	1	1	1
PAH's	BaP	1	1	1	1
Radioisotopes	Po-210	1	1	1	1
TSNA	NAB, NAT, NNK, NNN, Total	1	1	1	1