CONFIDENTIAL

# UK SMOKE CONSTITUENTS STUDY

## Part 11: Determination of Metals Yields in Cigarette Smoke

### Annex C - CVAAS Method

Commissioned by:

Tobacco Manufacturers Association

55 Tufton Street

London SW1P 3QL

March 2003

Arista LABORATORIES EUROPE
www.aristalabs.co.uk

## DEFINITIONS

The acronyms used in this procedure are listed and defined below.

| | |
|---|---|
| (v/v) | Volume/Volume |
| 1R4F | Industry reference cigarette produced by the University of Kentucky |
| Cal | Calibration |
| CCS | Calibration check standard |
| Cigt. | Cigarette |
| CINT | Canadian intense |
| Clean Lab | Metal free sample preparation lab |
| Conc | Concentration |
| CSC | Cigarette smoke condensate |
| CVAAS | Cold Vapor Atomic Absorption Spectrometry |
| FIMS | Flow injection mercury system |
| HCl | Hydrochloric Acid |
| $H_2SO_4$ | Sulfuric Acid |
| $HNO_3$ | Nitric Acid |
| ISO | International Standards Organisation |
| $KMnO_4$ | Potassium Permanganate |
| LFM | Laboratory fortified sample matrix |
| LOD | Limits of detection |
| LOQ | Limits of quantitation |
| MS | Mainstream Smoke |
| MSDS | Material Safety Data Sheet |
| N | Number of replicates |
| P.E. | Perkin Elmer |
| PP | Polypropylene |
| QC | Quality Control |
| RE | Relative Error |
| RSD | Relative Standard Deviation |
| SD | Standard Deviation |
| SnCl | Stannous Chloride |
| STD | Standard |

# DETERMINATION OF MERCURY IN MAINSTREAM SMOKE

## I. PURPOSE/SCOPE

Mercury is a known component of mainstream cigarette smoke. This method describes the procedure for the determination of mercury in mainstream cigarette smoke.

## II. PRINCIPLE OF METHOD

This method describes determination of trace mercury by cold vapor atomic absorption spectrometry (CVAAS). This method is used to determine the amount of mercury (Hg) in mainstream cigarette smoke.

Both particulate phase and gas phase mercury in mainstream smoke is trapped using two impingers connected in-series, each containing an acidic potassium permanganate solution. The impinger solutions containing mainstream smoke are subjected to a microwave-digestion in which the mercury compounds (organic and inorganic) are oxidized to $Hg^{+2}$. Mercury ions ($Hg^{+2}$) in the digestate are reduced by stannous chloride to elemental mercury and analyzed by cold vapor atomic absorption spectrometry.

Mercury is quantitated from mainstream smoke collected from twenty cigarettes smoked under International Standards Organisation (ISO) conditions. Smoking is performed on a Borgwaldt RM 20 CSR analytical smoking machine. The instrument used in the Cold Vapor Atomic Absorbance Spectrometry method is a Perkin Elmer FIMS-100

Mercury is reported in units of mass-to-volume (ng/mL). The measured concentration, the number of cigarettes smoked, and the sample solution volume(s) are also used to calculate the total analyte mass on a per cigarette basis. The measured concentration of the Mercury and the number of cigarettes smoked are used to calculate the concentration in units of nanograms per cigarette (ng/cigt).

All data in this SOP was collected under ISO conditions. ISO smoking refers to a 35cc puff, one puff every 60 seconds, 2-second puff duration with none of the ventilation holes blocked.

**List of Additional SOPs and Documents Referenced by this Method**

No. MS-14, *Determination of Mercury in Mainstream Tobacco Smoke*, Proposed Analytical Methods, Health Canada, 1998.
EPA method 245.1, *Determination of mercury in water by cold vapor atomic absorption Spectrometry*, revision 3.0, 1994.
EPA method 245.5, *Determination of mercury in Sediment by cold vapor atomic absorption Spectrometry*, June 1991.

## III. APPARATUS, CHEMICALS, AND LABORATORY SUPPLIES

### A. Required Chemicals

**Table 1: Required Chemicals**

| Chemical | Supplier | Grade or Purity |
|---|---|---|
| Sulfuric Acid ($H_2SO_4$), concentrated | Fisher Scientific Cat# A300S-500 (or similar) | Trace Metal |
| 10 mg/L Stock mercury Solution | SPEX Certiprep Inc. Cat# CLHG2-1AY (or similar) | Claritas PPT |
| 100 mg/L Stock mercury Solution | Similar to, but different source from above standard | Trace ICP/ICP-MS grade |
| Potassium Permanganate ($KMnO_4$) solution (5% w/v) | Fisher Scientific LC19940-2 (or similar) | APHA (for Mercury Metals) |
| Hydrochloric Acid (HCl), concentrated | Fisher Scientific Cat# No. A144-500 (or similar) | Trace Metal |
| Stannous Chloride ($SnCl_2 \bullet 2H_2O$) | Fisher Scientific Cat# T142-500 (or similar) | ACS |
| Hydroxylamine Hydrochloride ($NH_2OH \bullet HCl$) | Fisher Scientific Cat# H330-500 (or similar) | ACS (suitable for Mercury Determination) |
| Type 1 Water | In-House | 18 megohm-cm water. |

### B. Laboratory Apparatus and Supplies

1. Perkin Elmer FIMS-100
2. Microwave oven for sample preparation, CEM model MDS 2000, Matthews, NC (or similar)
3. Closed vessel microwave digestion vessels, CEM model Advanced Composite Vessel (ACV), Matthews, NC (or similar)
4. Borgwaldt RM 20/CSR smoking machine
5. 92mm Cambridge filter pads and holder
6. 10-100μL micropipette
7. 100 – 1000μL micropipette
8. 1-10mL macropipette
9. HPLC grade water or water system capable of delivering 18 MΩ-cm water
10. Keck Clips No. 29, Fisher Scientific, Cat. No. 05-880F
11. Parafilm
12. powder free Nitrile gloves
13. 100mL polypropylene volumetric flasks
14. 1000mL polypropylene volumetric flasks
15. 50mL polypropylene centrifuge tubes (Fisher Cat# 14-959-49A)
16. 15mL polystyrene centrifuge tubes (Fisher Cat# 05-527-45)
17. 100 mL polypropylene Snap Seal Containers (Fisher Cat# 1730 4H)

**NOTE: All labware and impingers should be pre-cleaned before use by soaking a minimum of 24 hours in 5% nitric acid (aqueous). After soaking, rinse thoroughly with Type I water. Dry in the clean bench and store in plastic bags until needed.**

## IV. Preparation of Analytical Solutions

### A. Working Standards

1. Calibration Blank: Using a graduated cylinder, transfer 50 mL of sulfuric acid to a pre-cleaned 500-mL polypropylene volumetric flask containing 250ml of reagent water. Then using a Micropipette add 50 μL 5%(w/v) $KMnO_4$, and dilute to volume with reagent water. Prepare daily

2. Prepare calibration standards by diluting the 10mg/L standard with appropriate aliquots of the Calibration Blank into a 50mL polypropylene centrifuge tube as follows and then mix well. Prepare daily.

**Table 2: Preparation of Calibration Standards**

| Calibration Standards | Aliquot of 10 µg/L standard | Aliquot of 10 mg/L Stock Std | Final Volume of calibration blank |
|---|---|---|---|
| 10.0 µg/L | --- | 50 µL | 50 mL |
| 5.0 µg/L | --- | 25 µL | 50 mL |
| 2.0 µg/L | --- | 10 µL | 50 mL |
| 1.0 µg/L | 5 mL | --- | 50 mL |
| 0.5 µg/L | 2.5 mL | --- | 50 mL |
| 0.2 µg/L | 1 mL | --- | 50 mL |

**B. Reagents Preparation**

1. 3% v/v Hydrochloric Acid (HCl) solution: Using a graduated Cylinder add 30 mL of Hydrochloric Acid to 500 mL of reagent water in a 1-L polypropylene volumetric flask. Dilute to volume with reagent water. Prepare as needed.

2. 10% w/v Hydroxylamine Hydrochloride: Dissolve 25g ± 0.1g of Hydroxylamine Hydrochloride with reagent water in a 250 mL polypropylene volumetric flask. Shelf life is two weeks. Store at room temperature. Prepare as needed.

3. Stannous Chloride (1.1% w/v) in 3% HCl (v/v): Using a graduated Cylinder add 15 mL Hydrochloric Acid to 250 mL of reagent water in a 500-mL polypropylene volumetric flask. Swirl to mix. Add 5.5g ± 0.1g of Stannous Chloride. Swirl until dissolved. Dilute to volume with reagent water. Prepare daily.

**C. Quality Control Dilute Stock**

1. Dilute Stock CCS (3mg/L Hg): The Dilute CCS is prepared from a source different from that used to prepare the calibration standards. Using a mechanical pipettes, transfer 3000 µL of the 100 mg/L CCS stock mercury solution and 5 mL of nitric acid to 50 mL of reagent water in a pre-cleaned 100-mL polypropylene volumetric flask. Dilute to volume with reagent water. Dilute Stock CCS is prepared and stored in the clean bench under ambient conditions and is stable for 6 months.

2. Working CCS (3 µg/L Hg): Using a micropipette transfer 50 µL of dilute stock CCS (3mg/L Hg)(A.a) To a 50 ml centrifuge tube containing approximately 25 mL of Calibration Blank (VI.A.a) then dilute to 50 ml with calibration blank (VI.A.a) and mix well. **Prepare daily.**

## V. SAMPLE COLLECTION AND WORKUP

*Authors Comment – conditions for smoke generation and collection are described elsewhere – a summary is reproduced below.*

- Cigarettes are conditioned[1] at a temperature of 22 ± 1°C and 60 ± 3 % relative humidity for a minimum of 48 hours but not exceeding 10 days.
- Butt marking will be ISO butt length specifications[2]. Filtered cigarettes will be smoked to a measured butt length equal to either the tipping paper + 3 mm or filter length + 8 mm whichever is longer. The minimum butt length will be 23 mm and this will also be used for non filter brands. All smoking shall be conducted in an environment of temperature 22 ± 2°C and 60 ± 5 % relative humidity[1].

[1] ISO 3402: 2000 - Tobacco and tobacco products – atmosphere for conditioning and testing
[2] ISO :4387: 2000 - Methods for chemical analysis of tobacco and tobacco products – Determination of total and nicotine- free dry particulate matter using a routine analytical smoking machine

- ISO conditions[3] for smoking cigarettes will apply. The smoking machine puffing parameters will be 35 ± 0.2 $cm^3$ puff volume with 2.0 ± 0.05 second puff duration once every 60.0 ± 0.5 seconds.
- A minimum of five determinations will be performed for each brand. The smoking of the cigarette brands is randomised so that samples from the same brand are smoked on different days.
- With each batch of samples a 2R4F cigarette is smoked.

Before smoke collection, the smoking laboratory must conform to the environmental conditions specified in SOP T-002, Smoke Laboratory Environmental Control.

A flow diagram of the sample collection and workup procedure is given in Appendix A.

### A. Sample Collection

1. Smoking is performed on a rotary, 20-port Borgwaldt smoking machine. For detailed operating procedures, the user should consult the manual for the smoking machine. Wear powder-free disposable gloves to assemble both impingers in series (Figure 1) then connect the assembly to the impinger adapter (figure 2) within the smoking sealing segment. Using Tygon tubing connect a 92mm pad holder to the outlet of the second impinger, then attach a back-up 44mm Cambridge filter cassette located on the pneumatic panel also using Tygon tubing.

**Figure 1: Impingers in series**

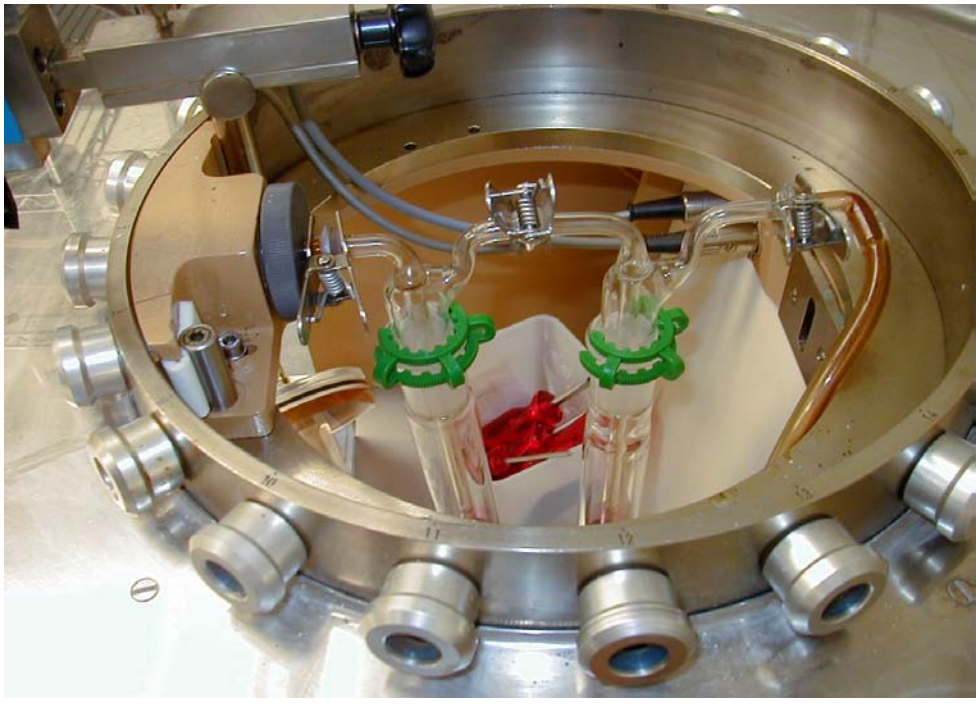

**Figure 2: Impinger adaptor**

[3] ISO 3308:2000 – Routine analytical cigarette smoking machine – 1: Definitions and standard conditions

2. Preparation of the smoke trapping system and of the smoking machine is described below. Using a dispenser Add 20 mL of 5% (w/v) Potassium Permanganate solution into each glass impingers. Slowly add 5 mL of conc. sulfuric acid to each impinger and mix well.

3. Perform leak and volume checks.

4. Correctly insert the test cigarettes in the 20-port smoking machine set for the proper mode. Position the butt length mark in coincidence with the infrared sensor. Light each cigarette and begin the smoking process.

5. The infrared sensor will stop the smoking when the predefined butt length is reached. Open the door of the smoking cabinet and remove the cigarettes and extinguish by dipping into water. Disconnect and remove the impingers from the smoking machine.

6. Method blanks are collected using the same sample collection procedure except one cigarette is placed in the cigarette holder and 200 puffs are taken. The method blank is monitored to ascertain no contamination during the smoking and analytical procedure sample preparation

**NOTE: All sample prep work must be performed in the clean lab.**

7. Quantitatively transfer the contents of the two impingers into one microwave digestion vessel and rinse the impingers with approximately 5 mL of reagent water into the microwave digestion vessel.

8. Assemble the microwave digestion vessel per the manufacturer's instruction

9. Place sample vessels in the carousel evenly. Follow the digestion program given below.

**Table 3: Operating Program of the CEM Digestion Oven**

| Stage | 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| Power* (%) | 95 | 95 | 95 | 95 |
| Pressure (psi) | 40 | 70 | 100 | 150 |
| Run Time (min.) | 15 | 15 | 15 | 15 |
| Time @ P (min.) | 5 | 5 | 5 | 5 |
| Fan Speed (%) | 100 | 100 | 100 | 100 |

*The stated power output applies to 12 vessels. As a general rule, 35% power is applied for one vessel, and a 5% increase in power for each additional vessel.

12. After the digestion is complete, wait until the digestion vessels cool to room temperature before disassembling.

13. Slowly add 7 mL of hydroxylamine hydrochloride (10% w/v) to reduce the excess permanganate.

14. Transfer the digestate to a graduated, 100 mL sample container and dilute to volume with reagent water

## VI. INSTRUMENT ANALYSIS

**NOTE: Before analysis can begin, the instrument must be calibrated by utilizing the calibration capabilities of the FIMS-100 software and standard solutions**.

### A. Sample Run Order

1. The analysis order will be designed so that an equal number of batched samples, in groups of 10 or less, be analyzed and bracketed by calibration check standards. The analyst will be contacted if there are any questions regarding the sample analysis order.

Sampling analysis order is as follows:

Calibration blank and standards
QC = Quality Control Standard
Samples 1 – 10
QC = Quality Control Standard

### B. FIMS-100 Apparatus and Operation Parameters

**Table 4: Operating Parameters of the FIMS-100**

| Step | Time (sec) | Pump Speed (rpm) | Valve Position | Read |
|---|---|---|---|---|
| Prefill | 15 | 100 | Fill | |
| 1 | 10 | 100 | Fill | |
| 2 | 20 | 0 | Inject | * |

Gas Flow: set between 50 and 100
Carrier Solution: 3% (v/v) HCl
Reducing Agent: 1.1 % $SnCl_2 \bullet 2H_2O$ in 3% (v/v) HCl
Sample Solution: $Hg^{+2}$ in acidified solution

## VII. Data Reduction and Example Calculations

This section describes the method used for determining the amount of mercury present on a per cigarette basis.

### A. Instrument Calibration Calculations

1. The analysis is carried out using the calibration capabilities of the FIMS software and assumes the operator is already familiar with the procedure for setting up a calibration on the software.

### B. Data Reduction of Raw Data from FIMS-100

1. The analyte concentration is determined by the method of external calibrations using the regression equation derived from the calibration curve. Calculation of the analyte is obtained by using the calculation capabilities of the FIMS software and assumes the operator is already familiar with the procedure for setting up the FIMS software**.**

### C. Example Calculation of a mainstream 1R4F sample:

1. Example calculation:

   Mainstream Cigarette Sample Concentration

   20 cigarettes were smoked and the final volume after digestion is 50mL. The concentration of the mercury is determined to be 0.125ng/mL.

   Mercury concentration: (ng/cigt.) = (0.125ng/ml*50mL)/20 cigt = 0.3125ng/cigt.

### D. Data Acceptance

1. In the event of poor data, (failing QC), the lead chemist will be contacted. If the poor data can be attributed to a single event, the standard or sample exhibiting the bad data will be disregarded from any calculations, and a complete explanation will be included with the data.

### E. Calibration and Quality Control Standard Acceptance Criteria

1. Each calibration curve must have a correlation coefficient ($r^2$) of 0.996 or better. Calibrations that do not meet these requirements should be brought to the attention of the lead chemist immediately. All quality control standards must be within ±10% RE of their calculated values. All quality control standards that do not meet these requirements should be brought to the attention of the lead chemist immediately. If a quality control standard is not ± 10% RE of their calculated values the quality control standard may be replaced at the operators discretion with a different vial of the same standard and re-analyzed. If the new quality control standard is within ±10% RE of its calculated value then sample analysis may continue. If the new quality control standard is not ± 10% RE of the calculated value then the sample analysis must stop, the problem corrected and the instrument recalibrated. Any samples that are bracketed with a failed quality control standard must be re-analyzed.

## VIII. APPENDIX A

### Mainstream Mercury Sample Workup