Characterization of Mainstream Tobacco Smoke

Analytical Test Report

Labstat®
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Prepared for *Philip Morris International R&D*

Project Code: NS367-H

Original Date: February 7, 2018

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1 TABLE OF CONTENTS

2 USE OF LABSTAT'S[1] ANALYTICAL REPORTS

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3 REVISION HISTORY

The revised report was required as part of a client inquiry (CIR-512-18) questioning the detection of NPIP in P1 products (IQOS sample) where previously reported data was below the method limit of quantification. A re-analysis of the products was conducted.

4 ADMINISTRATIVE INFORMATION

4.1 QUOTATION IDENTIFICATION

Quotation Number: 556 (1)

Date of Quotation: December 21, 2017

Recipient's Name: Cyril Jeannet

4.2 CLIENT IDENTIFICATION

Philip Morris International R&D

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2000 Neuchatel

Switzerland

5 SAMPLE HANDLING

5.1 CHAIN OF CUSTODY

The samples to be tested for project NS367-H were received on January 04, 2018 via DHL.

5.2 SAMPLE CHARACTERIZATION AND CODING

5.2.1 SAMPLE CHARACTERISTICS

The shipment received on January 04, 2018 consisted of 10 cartons of each of 2 products. Labstat International ULC supplied one product for testing –"Kentucky Reference Cigarette – 3R4F". There was no physical damage to the cartons or packages. Individual cigarettes were normal in appearance.

5.2.2 SAMPLE IDENTIFICATION

The following sample codes have been used to identify the products associated with the results in each of the tables that are part of this report.

Sample ID	Sample Description	Qty/Type Received	Type of Product	Type of Analysis
1700174	3R4F Kentucky Reference Cigarette	N/A	Cigarettes	Mainstream Smoke
1700175	P1/SMP095215	10/cartons	Tobacco Sticks	Mainstream Smoke
1700176	P1/SMP095216	10/cartons	Tobacco Sticks	Mainstream Smoke

5.2.3 PROJECT-SPECIFIC INSTRUCTIONS PHYSICAL MEASUREMENTS

Physical measurements were performed on the sticks and tobacco rods.

A representative test sample of 10 sticks was selected haphazardly from the client-submitted laboratory sample. The sticks from the test sample went through a physical characterization process, based on 10 observations (1 observation per stick) in which the measurements were recorded to the nearest 0.5mm. The following represents the characteristics which were recorded for this process:

- Total Stick Length (mm)
- Filter Length (mm)
- Overwrap Length (mm)
- Stick Diameter (at 9.0mm from the mouth end)
- Weight of Tobacco (g/unit)

These 10 observations were averaged and recorded to the nearest 0.1mm. The expected measurement variability is mean ± 1mm or mean ± 0.1g, depending on the parameter measured.

For the tobacco rods, the weight of tobacco per rod was measured.

For this project, the variability of the physical parameters measured was within the acceptance limits.

6 PROJECT-SPECIFIC INSTRUCTIONS

6.1 PLATFORM I TESTING

Test sticks were used in conjunction with a "tobacco heating system" by inserting the consumable (test stick) into a cigarette holder (CH). The CH was used in conjunction with a lighter bar to smoke the test sticks. Therefore, the following requirements of the ISO standard (see section 6.2.3) were either not applicable or could not be fulfilled for technical reasons:

- Air velocity control since the consumable is inserted into the CH.
- Butt length requirement since the heating of the consumable has no effect on the consumable length which remains unchanged after smoking.
- There was no ignition of the cigarette by an external lighter.

The use of the CH, in conjunction with the lighter bar for the test samples, required the reference cigarettes to be smoked on an independent run. All reference cigarettes were smoked to the ISO standard requirements for air velocity, butt length and ignition.

6.2 PLATFORM I END POINTS

Test sticks (Labstat sample ID's 1700175 and 1700176) are puffed to a fixed puff number: 12 puffs per stick under Health Canada Intense (HCI) smoking regime[2].

7 EXPERIMENTAL DESIGN AND METHODS

The following is a summary of the instructions that have been received from the client in regard to the smoking and analysis of the tobacco products in this project.

7.1 SAMPLE GENERATION

All tobacco products were conditioned and smoked under the smoking regimes outlined in the following sub-sections.

7.1.1 CIGARETTE BUTT MARKING

Prior to testing, all test and reference cigarettes were marked with the standard butt length as specified in ISO 4387 (2000) *"Cigarettes -- Determination of total and nicotine-free dry particulate matter using a routine analytical smoking machine".*

7.1.2 CIGARETTE CONDITIONING AND SMOKING ENVIRONMENTS

Cigarettes were conditioned and smoked under the environmental conditions specified in ISO 3402 (1999) *"Tobacco and tobacco products – Atmosphere for conditioning and testing".* With respect to conditioning, this document states "*The conditioning atmosphere shall be as follows: temperature 22 ± 1 ℃; relative humidity 60 ± 3%*". Smoking requires an environment in which the temperature is 22 ± 2°C and the relative humidity 60 ± 5%.

7.1.3 MACHINE SMOKING CONDITIONS

Smoking of test and reference cigarettes were carried out on either a rotary smoking machine or a linear smoking machine. The smoking parameters and smoking machine specifications which were used are set out in the International Organization for Standardization standard ISO 3308:12, ***Routine analytical cigarette-smoking machine - Definitions and standard conditions*** with modifications as noted in the table below.

[2] Health Canada Intense: (55.0±0.5)ml puff volume, (30±0.5)s puff frequency, (2.00±0.02)s puff duration, bell-shaped puff profile, no vent blocking; Canadian Tobacco Reporting Regulations: 21 June, 2000, Part 3(6)(b)(iii) - *Canada Gazette Part II, Vol. 134, No. 15*

The following table is a summary of the smoking parameters that were employed in this project.

Variable	"Health Canada Intense"
Puff Volume (ml)	55
Interval (sec)	30
Duration (sec)	2
Vents[3]	"fully blocked"

Note: Heat-not-burn test brands (Labstat sample ID's 1700175 and 1700176) did not have any vent blocking applied. Vent blocking for "Health Canada Intense" was only applied to the cigarette test brands.

Mainstream yields (MS) were obtained under "Health Canada Intense" conditions (as defined above). Yields obtained using "Health Canada Intense" smoking parameters are referred to as "non-standard" (n). Data files ending in (n) denote results obtained under the condition as noted in the previous sentence and in the above table.

7.2 ANALYTICAL METHODS[4]

7.2.1 SMOKE ANALYSIS

Test methods for the analysis of mainstream tobacco smoke are referenced in the table(s) below and were practiced as written unless otherwise indicated (see "Method Modifications").

OTHER METHODS FOR THE COLLECTION OF EMISSION DATA ON MAINSTREAM SMOKE[5]

Item	Emission	Official/ Labstat Method	Method Description
1.	Ammonia	T-101/ TMS-00101 Appendix E	*Determination of Ammonia in Mainstream Tobacco Smoke*

[3] Health Canada 100% Vent Blocking Method
Canadian Tobacco Reporting Regulations: 21 June 2000, Part 3(6)(b)(iii) all ventilation holes must be blocked by placing over them a strip of Mylar adhesive tape, Scotch Brand product no. 600 Transparent Tape, and the tape must be cut so that it covers the circumference and is tightly secured from the end of the filter to the tipping overwrap seam, or by another method of equivalent efficiency.

[4] The most current version available at the time of testing was used for all test methods listed.

[5] Methods marked with an * are not included in the accreditation scope.

Item	Emission	Official/ Labstat Method	Method Description
2.	*(a)* Formaldehyde *(b)* Acetaldehyde *(c)* Acetone *(d)* Acrolein *(e)* Propionaldehyde *(f)* Crotonaldehyde *(g)* MEK (methyl ethyl ketone)	T-104/ TMS-00104 Appendix G	*Determination of Selected Carbonyls in Mainstream Tobacco Smoke*
3.	Hydrogen cyanide	T-107/ TMS-00107	*Determination of Hydrogen Cyanide in Mainstream Tobacco Smoke*
4.	Mercury	T-108/ TMS-00108 Appendix F	*Determination of Mercury in Mainstream Tobacco Smoke*
5.	*(a)* Lead *(b)* Cadmium *(c)* Chromium *(d)* Nickel *(e)* Arsenic *(f)* Selenium *(g)* Beryllium *(h)* Cobalt	T-109/ TMS-00109	*Determination of Ni, Pb, Cd, Cr, As and Se in Mainstream Tobacco Smoke*
6.	*(a)* Quinoline *(b)* Styrene *(c)* Benzo(b)furan *(d)* Nitrobenzene *(e)* Acetamide *(f)* Acrylamide	T-112/ TMS-00112 Appendix G	*Determination of Pyridine, Quinoline and Styrene in Mainstream Tobacco Smoke*
7.	*(a)* Tar *(b)* Nicotine *(c)* Carbon Monoxide *(d)* Water	T-115/ TMS-00115a	*Determination of "Tar", Nicotine and Carbon Monoxide in Mainstream Tobacco Smoke*

Item	Emission	Official/ Labstat Method	Method Description
8.	*(a)* Naphthalene *(b)* 1- methylnaphthalene *(c)* 2- methylnaphthalene *(d)* Acenaphthylene *(e)* Acenaphthene *(f)* Fluorene *(g)* Phenanthrene *(h)* Anthracene *(i)* Fluoranthene *(j)* Pyrene *(k)* Benzo(a)anthracene *(l)* Chrysene *(m)* Benzo(b)fluoranthene *(n)* Benzo(k)fluoranthene *(o)* Benzo(a)pyrene *(p)* Benzo(e)pyrene *(q)* Perylene *(r)* Indeno(1,2,3-cd)pyrene *(s)* Dibenz(a,h)anthracene *(t)* Benzo(g,h,i)perylene *(u)* Benzo(c)phenanthrene *(v)* Cyclopenta(c,d)pyrene *(w)* Benzo(j)aceanthrylene	TMS-00120 Appendix H	*Determination of Selected Polynuclear Aromatic Hydrocarbons in Mainstream Tobacco Smoke*
9.	*(a)* 1,3-Butadiene *(b)* Isoprene *(c)* Acrylonitrile *(d)* Benzene *(e)* Toluene *(f)* Ethylbenzene *(g)* Ethylene Oxide *(h)* Vinyl Chloride *(i)* Propylene Oxide *(j)* Furan *(k)* Vinyl Acetate *(l)* Nitromethane	TMS-00124 Appendix G	*Determination of Vinyl Chloride, 1,3-Butadiene, Isoprene, Acrylonitrile, Benzene, Toluene, Styrene, and Acetamide in Mainstream Tobacco Smoke (expanded list)*
10.	2-Nitropropane	TMS-00126	*Determination of 2-Nitropropane in Mainstream Tobacco Smoke*
11.	*(a)* o-toluidine *(b)* 1- aminonaphthalene *(c)* 2- aminonaphthalene *(d)* 4- aminobiphenyl *(e)* 2,6-dimethylaniline *(f)* o-anisidine	TMS-00128 Appendix G	*Determination of Aromatic Amines in Mainstream Tobacco Smoke (expanded list)*

Item	Emission	Official/ Labstat Method	Method Description
12.	*(a)* *N*-nitrosonornicotine *(b)* 4-(*N*-nitrosomethylamino)-1-(3-pyridyl)-1-butanone	TMS-00135 Appendix I	*Determination of Tobacco Specific Nitrosamines in Mainstream Tobacco Smoke by High-Performance Liquid Chromatography-Tandem Mass Spectrometry*
13.	*(a)* Catechol *(b)* Phenol *(c)* *m*- Cresol *(d)* *p*-Cresol *(e)* *o*-Cresol	TMS-00139 Appendix F	*Determination of Phenolic Compounds In Mainstream Tobacco Smoke by a Modified High Performance Liquid Chromatography Method*
14.	Caffeic acid	TMS-00143	*Determination of Caffeic Acid in Mainstream Tobacco Smoke*
15.	Ethyl Carbamate	TMS-00145 Appendix D	*Determination of Ethyl Carbamate (Urethane) In Mainstream Tobacco Smoke*
16.	*(a)* IQ *(b)* Glu-P-2 *(c)* Glu-P-1 *(d)* PhIP *(e)* Trp-P-2 *(f)* AαC *(g)* Trp-P-1 *(h)* MeAαC	TMS-00146	*Determination of Heterocyclic Aromatic Amines (Haas) in Mainstream Tobacco Smoke by Liquid Chromatography-Tandem Mass Spectrometry*
17.	Hydrazine	TMS-00147 Appendix D	*Determination of Hydrazine in Mainstream Tobacco Smoke*
18.	*(a)* NDMA *(b)* NEMA *(c)* NDEA *(d)* NPIP *(e)* NPYR *(f)* NMOR *(g)* NDELA	TMS-00148 Appendix D	*Determination of Volatile Nitrosamines (VNAs) And N-Nitrosodiethanolamine (NDELA) in Mainstream Particulate and Vapour Phase Emissions by Liquid Chromatography-Tandem Mass Spectrometry*
19.	Polonium-210	N/A	* External Laboratory
20.	Uranium	N/A	* External Laboratory
21.	Chlorinated Dioxins and Furans	N/A	* External Laboratory

7.2.2 INTERNAL METHOD REFERENCES AND SYNOPSES

7.2.2.1 AMMONIA (LABSTAT METHOD TMS-00101)

7.2.2.1.1 REFERENCE(S)

Risner, C.H., Conner, J.M. Collection of Ammonia in Indoor Air by Means of a Weak Cation Exchange Cartridge. Environmental Toxicology and Chemistry, Vol. 10, pp. 1417-1423, 1991.

Nanni, E.J., Lovette, M.e., Hicks, R.D., Fowler, K.W. and Borgerding, M.F. Separation and Quantitation of Monovalent Anionic and Cationic Species in mainstream Cigarette Smoke Aerosols by High-Performance Ion Chromatography. Journal of Chromatographic Science, Vol. 28, August 1990.

IonPac CS12A Analytical Column, Installation Instructions and Troubleshooting Guide, Document No. 031132, Revision 01, Dionex Corporation, 1995.

TMS-00115 Labstat Test Method: Determination of "Tar", Nicotine and Carbon Monoxide in Mainstream Tobacco Smoke.

7.2.2.1.2 METHOD SYNOPSIS

Five conditioned sticks (cigarettes) were smoked on a standard rotary smoking machine. The mainstream smoke was passed through a conditioned, pre-weighed 92mm glass fiber filter disk (pad) and then into two impingers placed in series after the pad, each containing 15mL of 0.1N sulfuric acid. The pad, after being weighed to determine the mainstream total particulate matter (TPM), was extracted with the contents of the two impingers. The mixture was then filtered through a 0.45 µm syringe filter into an auto-sampler vial and analyzed by cation exchange chromatography using an external standard calibration.

For the analysis of "heat-not-burn" products, the sticks were smoked on a standard 20 port linear smoking machine using two impingers per port as smoked on a rotary machine.

7.2.2.2 CARBONYLS (LABSTAT METHOD TMS-00104)

7.2.2.2.1 REFERENCE(S)

Houlgate, P. R., Dhingra, K. S., Nash, J. S., and Evans, W. H. (1989): Determination of Formaldehyde and Acetaldehyde in Mainstream Cigarette Smoke by high-performance Liquid Chromatography; Analyst 114, 355-360.

Manning, D.L., Maskerinec, M.P., Jenkins, R.A., and Marshall, A.H. (1983): High Performance Liquid Chromatographic Determinations of Selected Gas Phase Carbonyls in Tobacco Smoke" Journal of Assoc. of Anal. Chem ., 66, 8-12.

TMS-00115 Labstat Test Method: Determination of "Tar", Nicotine and Carbon Monoxide in Mainstream Tobacco Smoke.

Intorp, M., Steve Purkis, S., and Wagstaff, W. (2012): Determination of Carbonyl Compounds in Cigarette Mainstream Smoke. The CORESTA 2010 Collaborative Study and Recommended Method, Beiträge zur Tabakforschung International/Contributions to Tobacco Research, Volume 25, no. 2, 361-374.

Coresta Recommended Method No. 74. (2011): Determination of Carbonyl Compounds in Cigarette Mainstream Smoke by High Performance Liquid Chromatography (HPLC), CRM No. 74, 1-16.

7.2.2.2.2 METHOD SYNOPSIS

Two conditioned sticks (cigarettes) were smoked on a standard 20 port linear smoking machine that was fitted with Drechsel-type bottles or traps with fritted impingers. The unfiltered mainstream tobacco smoke was scrubbed of volatile carbonyls by passing each puff through an impinger into a trap containing 80mL of an acidified solution of 2,4-dinitrophenylhydrazine in acetonitrile. An aliquot of the reacted DNPH-smoke extract was then syringe-filtered and diluted with 1% trizma base in aqueous acetonitrile. The samples were subjected to reverse phase high performance liquid chromatography (HPLC) and quantified via ultra violet detection.

For the analysis of "heat-not-burn" products, no additional changes to the sample generation, collection or sample preparation of the base method for cigarettes were required.

7.2.2.3 HYDROGEN CYANIDE (LABSTAT METHOD TMS-00107)

7.2.2.3.1 REFERENCE(S)

Collins, P.F. et al. 1973. A Trapping System for the Combined Determination of Total HCN and Total Gas Phase Aldehydes in Cigarette Smoke. Beitrage zur Tabakforschung, Vol 7 No.2.

Rickert, W. S., and P. B. Stockwell (1979). Automated determination of hydrogen cyanide, acrolein, and total aldehydes in the gas phase of tobacco smoke. J. Autom. Chem. 1:152-154.

TMS-00115 Labstat Test Method: Determination of "Tar", Nicotine and Carbon Monoxide in Tobacco Smoke.

7.2.2.3.2 METHOD SYNOPSIS

Five conditioned sticks (cigarettes) were smoked per port on a standard 20 port linear smoking machine, onto a conditioned, pre-weighed 44mm glass fiber filter disc (pad), with a trap containing 0.1N NaOH located directly behind the pad. The pad was extracted with 40 mL of 0.1N NaOH on a wrist action shaker for 30 minutes. Both the pad extract and impinger trapping solutions were analyzed by an automated continuous flow colorimetric analyzer where each sample undergoes on-line dilution. Hydrogen cyanide in the sample was converted to cyanogen chloride by an aqueous solution of chloramine-T. The cyanogen chloride then reacted with pyridine to give glutaconic aldehyde, which, upon reaction with a pyrazolone reagent, formed a coloured complex. A single channel monitored the complex, which was quantified by comparison to an external standard calibration.

For the analysis of "heat-not-burn" products, no additional changes to the sample generation, collection or sample preparation of the base method for cigarettes were required.

7.2.2.4 MERCURY (LABSTAT METHOD TMS-00108)

7.2.2.4.1 REFERENCE(S)

Varian Instruments at Work: Rapid Determination of Mercury in Fish Tissue, a Rapid, Automated Technique for Routine Analysis, No. AA-60, May 1986.

Varian Instruments at Work: Automated Cold Vapor Determination of Mercury: EPA Stannous Chloride Methodology, No. AA-51, September 1985.

Van Delft, W. & Vos G. (1988) Comparison of Digestion Procedures for the Determination of Mercury in Soils by Cold-Vapour Atomic Absorption Spectrometry, Analytica Chimica Acta 209, 1988. pp 147-156

Determination of ultratrace-level mercury in sediment and tissue by microwave digestion and atomic fluorescence detection. CEM reference R105.

The Determination of Total Mercury (Hg) in Air Sampling Solutions, Regulation respecting Mercury - made under the Occupational Health and Safety Act, O. Reg. 23/87, 1987. pp. 47-55.

TMS-00115 Labstat Test Method: Determination of "Tar", Nicotine and Carbon Monoxide in Mainstream Tobacco Smoke.

7.2.2.4.2 METHOD SYNOPSIS

Twenty conditioned sticks (cigarettes) were smoked on a standard rotary smoking machine. The mainstream smoke was passed directly through two impingers placed in series, each containing 30mL of an acidified potassium permanganate solution. The impinger solutions were then subjected to microwave digestion. Excess potassium permanganate was reduced with hydroxylamine hydrochloride and made to a final volume of 100mL. The digestate was then analyzed via cold vapour atomic absorption spectroscopy at 253.7 nm using a continuous flow vapour generator to reduce the divalent mercury to its atomic state with stannous chloride.

For the analysis of "heat-not-burn" products, ten sticks were smoked per port on a standard 20 port linear smoking machine instead of a rotary smoking machine. The mainstream smoke was passed through two midget impingers, each containing 10mL of the acidified potassium permanganate solution. The final volume of samples was 50mL instead of 100mL.

7.2.2.5 TRACE METAL (LABSTAT METHOD TMS-00109)

7.2.2.5.1 REFERENCE(S)

Environmental Carcinogens - Selected Methods of Analysis, Volume 8 - Some Metals: As, Be, Cd, Cr, Ni, Pb, Se, Zn. IARC Scientific Publication No. 71, 1986. pp 129-138

Perinelli, M.A. & Carugno, N. (1978) Determination of Trace Metals in Cigarette Smoke by Flameless Atomic Absorption Spectrometry, Beitrage zur Tabakforschung International, Band 9, Heft 4, Juli 1978. pp 214-217

Bell, Paul & Mulchi, Charles L. (1990) Heavy Metal Concentrations in Cigarette Blends, Tobacco Science, Vol. 34, 1990. pp 32-34.

NIOSH Method 7300, Elements (ICP), NIOSH Manual of Analytical Methods, Volume 2, Third Edition, 1984

Varian Analytical Methods for Graphite Tube Atomizers, Varian Australia Pty Ltd, Publication No. 85-100848-00 (1988).

Gawalco et al. (1997). Comparison of Closed-Vessel and Focused Open-Vessel Microwave Dissolution for Determination of Cadmium, Copper, Lead and Selenium in Wheat, Wheat Products, Corn Bran, and Rice Flour by Transverse-Heated Graphite Furnace Atomic Absorption Spectrometry, Journal of AOAC International, Vol. 80, No. 2, 1997. pp. 379-387.

TMS-00115 Labstat Test Method: Determination of "Tar", Nicotine and Carbon Monoxide in Mainstream Tobacco Smoke.

Krivan V., Scheneider G., Baumann H., Reus U. (1994): Multi-element characterization of tobacco smoke condensate, Fresenius J. Anal. Chem. 348, 218-225.

Chang MJ, Naworal J, Walker K, Connell C (2003): Investigations on the direct introduction of cigarette smoke for trace elements analysis by inductively coupled plasma mass spectrometry, Spectrochimica Acta, B58, 1979-1996.

7.2.2.5.2 METHOD SYNOPSIS

Twenty conditioned sticks (cigarettes) were smoked on a standard rotary smoking machine equipped with an electrostatic precipitation generator to electrostatically precipitate the particulate matter onto a glass electrostatic precipitate (EP) tube. The total particulate matter (TPM) was extracted into 25 mL methanol. The methanol extract was then evaporated using gentle heating while under a constant stream of filtered ultra high purity (UHP) nitrogen. The sample was then subjected to microwave digestion using a mixture of hydrochloric acid, nitric acid and hydrogen peroxide. The gaseous phase metals were trapped by placing an impinger of a 10% v/v nitric acid solution between the EP tube and the puff drawing mechanism. The impinger solution was added to the same digestion vessel as the EP tube product and subjected to microwave digestion. The digestates were then analyzed by Inductively Coupled Plasma - Mass Spectrometer (ICP-MS) or Inductively Coupled Argon Plasma - Atomic Emission Spectrometer (ICP-AES).

For the analysis of "heat-not-burn" products, due to the nature of the sample, collection of the total particulate matter (TPM) required the use of two EP tubes. Each EP tube was extracted using half of the volume of methanol (12.5mL) that would have been used if only one EP tube was employed. The EP tubes and impinger samples were then collected in a single digestion vessel and subjected to the digestion process. For sample generation, sample preparation, and analysis high purity chemical reagents (Ultrapure Fisher Optima chemical grade) were used.

7.2.2.6 SEMI VOLATILES (LABSTAT METHOD TMS-00112)

7.2.2.6.1 REFERENCE(S)

White, E., Uhrig, M., Johnson, T., Gordon, B., Hicks, R., Borgerding, M., Coleman, W., and Elder, J. (1990). Quantitative Determination of Selected Compounds in a Kentucky 1R4F Reference Cigarette Smoke by Multidimensional Gas Chromatography and Selected Ion Monitoring - Mass Spectrometry. Journal of Chromatographic Science 26, 393-399.

TMS-00115 Labstat Test Method: Determination of "Tar", Nicotine and Carbon Monoxide in Mainstream Tobacco Smoke.

7.2.2.6.2 METHOD SYNOPSIS

Twenty conditioned sticks (cigarettes) were smoked on a standard rotary smoking machine. The mainstream smoke was passed through a conditioned, pre-weighed 92mm glass fiber filter disc (pad) and then into two cryogenic traps placed in series after the pad, each containing 20mL of methanol. The pad was then cut into quarters, spiked with the internal standard solution (containing d5-pyridine, d8-styrene and d7-quinoline) and extracted with the 40 mL of methanol from the two cryogenic traps. An aliquot of the extract was syringe filtered into an auto-sampler vial and analyzed using gas chromatography – mass spectrometry (GC/MS) with ion trap under full scan mode.

For the analysis of “heat-not-burn” products, ten sticks were smoked per port on a standard 20 port linear smoking machine using a 44mm pad instead of a rotary smoking machine. The pad was replaced after five sticks were smoked per port for HCI smoking conditions (two pads per replicate for HCI). d5-acetamide and d3-acrylamide were added as internal standards and analysis was done by gas chromatography – mass spectrometry (GC/MS) using a single quadrupole mass detector operating under selective ion monitoring (SIM) mode.

7.2.2.7 TAR, NICOTINE AND CARBON MONOXIDE (LABSTAT METHOD TMS-00115)

7.2.2.7.1 REFERENCE(S)

N/A

7.2.2.7.2 METHOD SYNOPSIS

Five conditioned sticks (cigarettes) were smoked using a standard 20 port linear smoking machine equipped with a CO analyzer, onto a conditioned, pre-weighed 44mm glass fiber filter disc (pad). The gas phase was collected into a Vapour Phase (VP) collection bag and then introduced into a Non-Dispersive Infra-Red (NDIR) analyzer and the % CO determined. The pad was re-weighed with the difference calculated as the Total Particulate Matter (TPM). The pad was extracted with isopropanol (IPA) containing the internal standards (trans-anethole for nicotine and methanol for water), and the extract analyzed for nicotine and water by gas chromatography (flame ionization detector (FID) for nicotine and thermal conductivity detector (TCD) for water). The nicotine-free dry particulate matter (NFDPM; 'tar') value was determined by subtracting the water and nicotine from the TPM result.

For the analysis of “heat-not-burn” products, no additional changes to the sample generation, collection or sample preparation of the base method for cigarettes were required.

7.2.2.8 POLYNUCLEAR AROMATIC HYDROCARBONS (LABSTAT METHOD TMS-00120)

7.2.2.8.1 REFERENCE(S)

G. Gmeiner, G. Stehlkik, H. Tausch, Determination of Seventeen Polycyclic Aromatic Hydrocarbons in Tobacco Smoke Condensate, *J. Chromatogr.* A 767 (1997) 163-169.

T-115 Health Canada Test Method: Determination of "Tar", Nicotine and Carbon Monoxide in Mainstream Tobacco Smoke.

7.2.2.8.2 METHOD SYNOPSIS

Ten conditioned sticks (cigarettes) were smoked using a standard rotary smoking machine, onto a conditioned, pre-weighed 92mm glass fiber filter disc (pad). The pad was spiked with internal standards (d12-benzo[a]pyrene, d14-dibenz[a,h]anthracene, d10-anthracene and d10-pyrene) and extracted with 50 mL of methanol. The methanol extracts were filtered through a filter paper. A portion of filtered extract was cleaned up by Solid Phase Extraction (SPE) using a RapidTrace SPE Workstation and analyzed by gas chromatography-mass spectrometry for quantification. The mass detector was operated under Selected Ion Monitoring (SIM) mode. The ions of interest (i.e. molecular ions and in some cases specific fragment ions) were mass-selected and used for quantification.

For the analysis of "heat-not-burn" products, five sticks were smoked per port on a standard 20 port linear smoking machine using a 44mm pad instead of a rotary smoking machine. After adding the internal standards solution to the pad, the pad was extracted with 20mL of methanol. The sample was then analyzed by GC/MS as described above.

7.2.2.9 VOLATILE ORGANICS (LABSTAT METHOD TMS-00124)

7.2.2.9.1 REFERENCE(S)

T-116 Health Canada Test Method: Determination of Selected Volatiles (1,3-Butadiene, Isoprene, Acrylonitrile, Benzene and Toluene) in Mainstream Smoke.

Byrd, G.D., K.W. Fowler, R.D. Hicks, M.E. Lovette and M.F. Borgerding (1990). Isotope dilution gas chromatography-mass spectrometry in the determination of benzene, toluene, styrene and acrylonitrile in mainstream cigarette smoke. *J. Chromat.* 503, 359-368.

Brunnemann, K.D., M.R. Kagan, J.E. Cox, and D. Hoffmann (1990). Analysis of 1,3-butadiene and other selected gas phase components in cigarette mainstream and sidestream smoke by gas chromatography-mass selective detection. *Carcinogenesis* 11, 1863-1868.

Brunnemann, K.D., M.R. Kagan, J.E. Cox, and D. Hoffmann (1989). Determination of benzene, toluene and 1,3-butadiene in cigarette smoke by GC-MSD. *Exp. Pathol.* 11, 108-113.

T-115 Health Canada Test Method: Determination of "Tar", Nicotine and Carbon Monoxide in Mainstream Tobacco Smoke.

7.2.2.9.2 METHOD SYNOPSIS

Ten conditioned sticks (cigarettes) were smoked on a standard rotary smoking machine. The mainstream smoke was passed through a conditioned, pre-weighed 92mm glass fiber filter disc (pad) and then into two cryogenic traps placed in series after the pad, each containing 10mL of methanol. An aliquot of the solution from the two

traps was spiked with the internal standards (d6-benzene, d8-styrene and d5-pyridine) and analyzed by gas chromatography – mass spectrometry (GC/MS) with ion trap under full scan mode.

A second aliquot of the solution from the two traps was derivatized with 48% hydrobromic acid, then spiked with internal standards (d6-benzene and d4-2-bromoethanol) and analyzed for ethylene oxide via gas chromatography – mass spectrometry (GC/MS) operating under selective ion monitoring (SIM) mode.

For the analysis of "heat-not-burn" products, ten sticks were smoked per port on a standard 20 port linear smoking machine using a 44mm pad instead of a rotary smoking machine. The pad was replaced after five sticks were smoked per port for HCI smoking conditions (two pads per replicate for HCI). Lower calibrations standards were prepared and analysis of the trapping solution was done by gas chromatography – mass spectrometry (GC/MS) using a single quadrupole mass detector operating under selective ion monitoring (SIM) mode.

7.2.2.10 2-NITROPROPANE (LABSTAT METHOD TMS-00126)

7.2.2.10.1 REFERENCE(S)

INBIFO standard operating procedure AC 183/4, "Determination of 2-Nitropropane in Cigarette Smoke", Jan.16, 2002, T. Ottmüller (TOM), B. Dimitrow (BDI).

T-115 Health Canada Test Method: Determination of "Tar", Nicotine and Carbon Monoxide in Mainstream Tobacco Smoke.

7.2.2.10.2 METHOD SYNOPSIS

Mainstream smoke was drawn through a cartridge filled with silica gel and 2-nitropropane was eluted from the cartridge. The concentration of 2-nitropropane in the eluate was analyzed by gas chromatography using a TEA as detector. The results were quantified on the basis of an internal standard.

7.2.2.11 AROMATIC AMINES (LABSTAT METHOD TMS-00128)

7.2.2.11.1 REFERENCE(S)

Pieraccini, G., F. Luceri, and G. Moneti (1992). New Gas-Chromatographic/Mass-Spectrometric Method for the Quantitative Analysis of Primary Amines in Main- and Sidestream Cigarette Smoke. I. *Rapid Communications in Mass Spectrometry. 6, 406-409.*

T-115 Health Canada Test Method: Determination of "Tar", Nicotine and Carbon Monoxide in Mainstream Tobacco Smoke.

7.2.2.11.2 METHOD SYNOPSIS

Ten conditioned sticks (cigarettes) were smoked on a standard rotary smoking machine. The mainstream total particulate matter (TPM) was collected on a conditioned, pre-weighed 92mm glass fiber filter disc (pad). The pad was quartered and extracted with 100mL of 5% hydrochloric acid solution. The extraction flask was shaken for 30

minutes on a wrist-action shaker and the contents filtered into a 500mL separatory funnel. The internal standards (d5-aniline, d9-o-toluidine, d7-o-anisidine, d7-4-aminobiphenyl, d8-benzidine) were spiked into the solution. The filtrate was washed with dichloromethane, made basic with sodium hydroxide solution and extracted with hexane. The hexane extracts were dried with sodium sulphate, derivatized with pentafluoropropionic acid anhydride (PFPA) and trimethylamine, concentrated by rotary evaporation, passed through a florisil column, and quantified using gas chromatography – mass spectrometry (GC/MS) with ion trap under full scan mode.

For the analysis of "heat-not-burn" products, a standard 20 port linear smoking machine and a 44mm pad was used instead of a rotary smoking machine. The 44mm pad was replaced after five sticks were smoked per port for HCI smoking conditions (two pads per replicate for HCI). Lower calibrations standards were prepared with the addition of d7-1-aminonaphthalene, d7-2-aminonaphthalene as internal standards. Quantification was achieved using negative chemical ionization (NCI) gas chromatography – mass spectrometry (GC/MS) operating in selective ion monitoring (SIM) mode.

7.2.2.12 TOBACCO SPECIFIC NITROSAMINES (LABSTAT METHOD TMS-00135)

7.2.2.12.1 REFERENCE(S)

Wu, W.; Ashley, D. L.; Watson, C. H. Anal. Chem. 2003, 75, 4827-4832.

Wagner, K. A.; Finkel, N. H.; Fossett, J. E.; Gillman, I. G. Anal. Chem. 2005, 77, 1001-1006.

Lee, J-M.; Shin, J-W.; Oh, I-H.; Lee U-C.; Rhee M-S. 2004 CORESTA Congress Kyoto. Paper SS20; full text available on CORESTA CD-ROM Vol. 22; abstract available on the Internet at http://www.coresta.org/Past_Abstracts/Kyoto2004-SmokeTech.pdf (accessed December 29, 2006).

Chwojdak, C. A.; Self, D. A.; Wheeler, H. R. A Collaborative, Harmonized LC-MS/MS Method for the Determination of Tobacco Specific Nitrosamines (TSNA) in Tobacco and Tobacco Related Materials. 61st Tobacco Science Research Conference, Charlotte, NC. USA. September 24, 2007.

NIH Guidelines for the Laboratory Use of Chemical Carcinogens; NIH Publication 81-2385, 1981.

Wu. J.; Joza, P.; Sharifi, M.; Rickert, W. S.; Lauterbach, J. H. Anal. Chem. 2008, 80, 1341-1345.

T-115 Health Canada Test Method: Determination of "Tar", Nicotine and Carbon Monoxide in Mainstream Tobacco Smoke.

7.2.2.12.2 METHOD SYNOPSIS

Five conditioned sticks (cigarettes) were smoked per port on a standard 20 port linear smoking machine. The mainstream total particulate matter (TPM) was collected on a conditioned, pre-weighed 44mm glass fiber filter disc (pad). The pad was spiked with a deuterium labeled internal standard solution (containing NNN-d4, NAT-d4, NAB-d4, and NNK-d4) and then extracted with a 100mM ammonium acetate solution. The extract was then filtered and subject to LC-MS/MS analysis with positive electrospray ionization (ESI). Two mass transition pairs for each analyte can be used to assist analyte confirmation and quantification. The most intense pairs are used for quantification while the less intense transition pairs are used as qualifiers for further compound confirmation.

For the analysis of "heat-not-burn" products, no additional changes to the sample generation, collection or sample preparation of the base method for cigarettes were required.

7.2.2.13 PHENOLIC COMPOUNDS (LABSTAT METHOD TMS-00139)

7.2.2.13.1 REFERENCE(S)

Wu, J. and Rickert, W. B. "A New High Performance Liquid Chromatography – Fluorescence Detection Method for the Determination of Phenolic Compounds in Cigarette Smoke and Smokeless Tobacco Products." *63rd Tobacco Science Research Conference (**TSRC**)*. September 27-30, **2009**, Amelia Island, Florida USA.

Risner, C.H. and Cash, S.L. "A High Performance Liquid Chromatographic Determination of Major Phenolic Compounds in Tobacco Smoke", *Journal of Chromatographic Science*, 28 (**1990**) and the references cited within this refs.

Nanni, E.J.; Lovette, M.E.; Hicks, R.D.; Fowler, K.W and Borgerding, M.F. "Separation and quantitation of phenolic compounds in mainstream cigarette smoke by capillary gas chromatography with mass spectrometry in the selected ion mode". *Journal of Chromatography*, 505 (**1990**), 365-374.

Moldoveanu, S.C. and Kiser, M. "Gas chromatography/mass spectrometry versus liquid. chromatography/fluorescence detection in the analysis of phenols in mainstream cigarette smoke". *Journal of Chromatography A*, 1141 (**2007**), 90-97.

T-115 Health Canada Test Method: Determination of "Tar", Nicotine and Carbon Monoxide in Mainstream Tobacco Smoke.

T-114 Health Canada Test Method: Determination of Phenolic Compounds in Mainstream Tobacco Smoke.

7.2.2.13.2 METHOD SYNOPSIS

Five conditioned sticks (cigarettes) were smoked per port on a standard 20 port linear smoking machine. The mainstream total particulate matter (TPM) was collected on a conditioned, pre-weighed 44mm glass fiber filter disc (pad). The pad was then extracted with 20 mL of 1% acetic acid (HOAc). An aliquot of the TPM extract was syringe filtered, diluted and subjected to reversed-phase gradient liquid chromatography. Phenols were monitored using selective fluorescence detection and quantified by comparison to an external standard calibration.

For the analysis of "heat-not-burn" products, ten sticks were smoked per port on a standard 20 port linear smoking machine. An impinger containing 20mL of 1% acetic acid (HOAc) was added in the smoke train after the pad. The pad was replaced after five sticks were smoked per port for HCI smoking conditions (two pads per replicate for HCI). The collection pad for ISO smoking conditions was extracted with the impinger solution (20mL). An additional 20mL of 1% acetic acid was added to the impinger solution to extract the two pads per replicate for HCI smoking conditions (total of 40mL per replicate).

7.2.2.14 CAFFEIC ACID (LABSTAT METHOD TMS-00143)

7.2.2.14.1 REFERENCE(S)

M. Snook and O. Chortyk, "An Improved Extraction-HPLC Method for Tobacco Polyphenols," *Tobacco Science,* xxvi, 1982, 25-29.

Z. Li, L Wang, G. Yang, H Shi, C. Jiang and W. Liu, "Study on the Determination of Polyphenols in Tobacco by HPLC Coupled with ESI-MS After Solid Phase Extraction," *Journal of Chromatographic Science*, 41, 2003, 1-5.

7.2.2.14.2 METHOD SYNOPSIS

Cigarettes were smoked on a standard 20-port linear smoking machine. The mainstream total particulate matter (TPM) was collected on a 44-mm conditioned filter pad. The pad was shaken for 30 minutes with 40mL of Type I water in an Erlenmeyer flask covered with aluminum foil and with the aid of a wrist action shaker. The TPM extract was then filtered with a 0.45µm syringe filter and aliquots were transferred to autosampler vials for HPLC analysis. Caffeic acid quantification was accomplished using UV detection in combination with an external calibration method.

7.2.2.15 ETHYL CARBAMATE (LABSTAT METHOD TMS-00145)

7.2.2.15.1 REFERENCE(S)

N/A

7.2.2.15.2 METHOD SYNOPSIS

Mainstream cigarette smoke was passed through a 44mm glass fiber filter disc and into two traps each containing 25mL of an aqueous sulfamate buffer solution. At the end of smoking, the filter pad was spiked with a solution of d5-ethyl carbamate (used as Internal Standard) and then both trap solutions were combined and used to extract the filter pad. The extract was then passed through a ChemElut® cartridge using dichloromethane as the eluent. The eluate was concentrated to 1mL and an aliquot was analyzed by GC-MS-MRM.

7.2.2.16 HETEROCYCLIC AROMATIC AMINES (LABSTAT METHOD TMS-00146)

7.2.2.16.1 REFERENCE(S)

Zhang, L.; Ashl;ey, D. M. S.; Watson, C. H.; *Nicotine Tob. Res.,* **2011**, *13(2)*, 120-126.

Saha, S.; Mistri, R.; Ray, B. C., *J. Chromatogr. A.,* **2009**, *1216*, 3059-3063.

Alaejos, M. S.; Ayala, J. H.; Gonzalez, V.; Afonso, A. M.; *J. Chromatogr. B.,* **2008**, *862*, 15-24.

Ni, W.; McNaughton, L.; LeMaster, D. M.; Sinha, R.; Turesky, R. J., *J. Agric. Food Chem.* **2008**, *56*, 68-78.

Barcelo-Barrachina et al. *J. Chromatogr. A.,* **2006**, *1125*, 195-203.

Turesky, R. J.; Taylor, J.; Schnackenberg, L.; Freeman, J. P.; Holland, R. D., *J. Agric. Food Chem.* **2005**, *53*, 3248-3258.

NIH Guidelines for the Laboratory Use of Chemical Carcinogens; NIH Publication 81-2385, **1981**.

7.2.2.16.2 METHOD SYNOPSIS

The mainstream smoke of 5 cigarettes under ISO conditions (2 cigarettes under Canadian Intense conditions) was collected onto a 44mm glass fibre filter disc (pad). The pad was spiked with a certain amount of isotope labeled internal standard solution (containing 12 analogues of 12 HAAs) and then extracted with 20mL of extraction solution (0.1M hydrochloric acid).

The extract was filtered and 5mL of the filtered solution was used for sample clean up and concentration by the mixed mode solid phase extraction (SPE) procedures.

The concentrated sample was analyzed by LC-MS/MS analysis with positive electrospray ionization (ESI). Two mass transition pairs for each analyte can be used to assist analyte confirmation and quantification. The most intensive pairs are used for quantification; the less intense transition pairs are used as qualifiers for further compound confirmation.

7.2.2.17 HYDRAZINE (LABSTAT METHOD TMS-00147)

7.2.2.17.1 REFERENCE(S)

Davis W., (2008), Analysis of Hydazine in Drinking water by isotope Dilution Gas Chromatography /Tandem Mass Spectrometry with Derivatisation and Liquid- Liquid Extraction, Analytical Chemistry, vol. 80, no.14, pp. 5449-5453.

Diekmann J., Biefel C., Rustemeier K., (2002), Analysis of Cigarette Mainstream Smoke for 1,1 Dimethylhydrazine and Vinyl Acetate by Gas Chromatography – Mass Spectrometry, Journal of Chromatographic Science, vol.40, pp 509-514.

Liu Y.Y., Schmeltz I., Hoffman D. (1974), Chemical studies on Tobacco Smoke. Quantitative Analysis of Hydrazine in Tobacco and Cigarette Smoke, Analytical Chemistry, vol. 46, no.7, pp.885-889.

Plunkett S., Parrish M., Shafer K., Shorter J., Nelson D., Zahnister M., (2002) Hydrazine Detection Limits in the Cigarette Smoke Matrix using Infrared Tunable Diode Laser Absorption Spectroscopy, Spectrochimica Acta Part A, 58, pp. 2505-2517.

7.2.2.17.2 METHOD SYNOPSIS

Mainstream cigarette smoke was passed through a 44mm glass fiber filter disc and into a trap with 40mL of an aqueous buffer:methanol (55:45, v/v) solution containing 2-nitrobenzaldehyde (10 g/L) and $^{15}N_2$-hydrazine used as internal standard (100 ng/mL). Immediately after smoking, the filter pad was extracted with impinger solutions and the extract was incubated for 30 minutes at 35°C. An aliquot of the extract was centrifuged and the resultant

hydrazone (i.e. dinitrophenyl-hydrazone) was quantified by liquid chromatography tandem mass spectrometry (LC-MS/MS). The triple-quadrupole mass analyzer, operating under multiple-reaction-monitoring (MRM) mode, allows mass-specific determination and quantification of hydrazone by monitoring specific parent/daughter fragmentation patterns.

7.2.2.18 VOLATILE NITROSAMINES (LABSTAT METHOD TMS-00148)

7.2.2.18.1 REFERENCE(S)

Applied Biosystem/MDS Sciex, Determination of N-nitrosamines in Baby Bottle Rubber Teats by Liquid Chromatography-Atmospheric Pressure Chemical Ionization Mass Spectrometry, http://www.appliedbiosystem.com/ , Application Note 040502.

7.2.2.18.2 METHOD SYNOPSIS

Mainstream cigarette smoke was passed through two traps each containing 25mL of an ammonium sulfamate/sulfuric acid buffer solution and then a 44mm filter pad. At the end of smoking, the first trapping solution was spiked with a mixture solution of 7 Internal Standards (NDMA-d6, NEMA-d3, NDEA-d10, NDPA-d14 and NPYR-d8, NDBA-d18, and NDELA-d8), and then both trap solutions were combined and used to extract the filter pad. A portion of the extract (12mL) was acidified, treated with ammonium sulphate and then subject to ChemElut® cartridge clean-up using ethyl formate:ethanol (98:2, v/v) as eluent. The eluate was spiked with 0.8mL of a 0.01% formic acid solution and the solvent was evaporated on a rotary evaporator to approximately 0.8mL. The concentrated sample was volumized to 1mL with 0.01% formic acid solution and an aliquot was analyzed by liquid chromatography-APCI$^+$-tandem mass spectrometry detection. The mass detector was operated under multiple reaction monitoring (MRM) mode.

7.2.2.19 POLONIUM-210[6] (EXTERNAL LABORATORY)

7.2.2.19.1 METHOD SYNOPSIS

Conditioned cigarettes were smoked on a standard 20-port linear smoking machine. The mainstream total particulate matter (TPM) was collected on a 44-mm conditioned filter pad. Each sample was spiked with a traceable standard containing a known amount of Po-209 as a 'tracer'. All sample matrices were normally digested (3-5 gms) with concentrated hydrofluoric acid until all organic material was dissolved, and then heated to dryness. Aqueous dilute hydrochloric acid was then added to the residue. The Po-209/210 was then deposited on silver or nickel foil in the presence of dilute hydrochloric acid and alpha spectrometry performed on the foil (209Po α-particle energy = 4.9 MeV and 210Po α-particle energy = 5.3 MeV). Results for Po-210 were calculated according to Po-209 measured.

[6] The method synopsis for polonium was provided by the external laboratory that provided the test results (b) (4)

7.2.2.20 URANIUM-238 AND URANIUM-235[7] (EXTERNAL LABORATORY)

7.2.2.20.1 SYNOPSIS - NEUTRON ACTIVATION

For analysis of uranium-238, neutron activation analysis method was used. The samples were submitted for exposure to a flux of neutrons at a nuclear reactor. This was performed at the (b) (4) which has flux of $8x10^{12}$ neutrons/cm^2/sec. These bundles were inserted into the core of a nuclear reactor for up to twenty minutes. In the (b) (4) reactor sites, the bundles were rotated during irradiation so that there was no horizontal flux variation (The vertical flux variation was monitored with the individual flux monitors.). This irradiation caused many of the elements in the sample to become radioactive and begin to emit radiation in the form of penetrating gamma rays whose energies (or wavelengths) were characteristic of particular elements.

After a decay period of six days, the irradiated samples were loaded onto the counting system. The sample was placed close to a gamma-ray spectrometer with a high resolution, coaxial germanium detector. Gamma rays radiate continuously and the interaction of these with the detector lead to discrete voltage pulses proportional in height to the incident gamma-ray energies. A multichannel analyzer sorted out the voltage pulses from the detector according to their size and digitally constructed a spectrum of gamma-ray energies versus intensities. The counting time was twenty to thirty minutes per sample. By comparing spectral peak positions and areas with library standards, the elements comprising the samples were qualitatively and quantitatively identified.

7.2.2.20.2 SYNOPSIS - DELAYED NEUTRON COUNTING ANALYSIS

For the determination of Uranium-235, the packaged samples were irradiated thermally at the (b) (4) (b) (4) which has flux of $8x10^{12}$ neutrons/cm^2/sec. The delayed neutrons that were produced were counted at the (b) (4) The data was collected and processed at (b) (4) to calculate the U-235 concentration by comparison with standards carried through the entire procedure.

Principle: The fission of U by thermal neutrons is due to fission of the U-235 isotope since the major uranium isotope, U-238, is not fissioned with thermal neutrons. Some of the fission products of U-235, such as La-147 and Br-87, are unstable and obtain stability through one or more beta decays. When these beta decay daughter products have excitation energy greater than their nuclear binding energy, a neutron is emitted. The delay in neutron emission is determined by the beta decay half-life of the parent, hence these are called delayed neutrons.

This method is very selective because there are few elements that decay in this manner, specifically U, Th, and Pu; and Pu is not found in “natural” material.

[7] The method synopsis for uranium was provided by the external laboratory that provided the test results (b) (4)

7.2.2.21 CHLORINATED DIOXINS[8] (EXTERNAL LABORATORY)

7.2.2.21.1 METHOD SYNOPSIS

Mainstream total particulate matter (TPM) was collected on Cambridge filter pads, which were spiked with 9 13C12 -labeled PCDD/PCDF internal standards and Soxhlet-extracted for 16 hours. The extract was subjected to an acid/base clean-up procedure followed by clean-up on micro columns of silica gel and alumina. The extract was then spiked with 0.1ng 13C-1,2,3,4-TCDD and 13C-1,2,3,4,7,8,9-HCDD (to determine extraction efficiencies achieved for the 13C-labeled internal standards) and then concentrated to 20µL for HRGC-HRMS analysis in a 1mL conical Reacti-vial. The set of sample extracts was subjected to HRGC-HRMS selected ion monitoring (SIM) analysis using a 60-m DB-5 MS fused silica capillary column to determine the sampler efficiency, extraction efficiency, and the concentrations or the DLs achieved for the PHDDs/PHDFs. Defined identification criteria and QA/QC criteria and requirements were used in evaluating the analytical data.

The method itself was true isotope dilution for the analytes reported (C-13 labeled), and tuning of the instrument had to be performed to one in 10,0000 mass resolution, and run with a perfluorokerosene (PFK) lock. The method required that each run be performed after a column performance run was performed in which SIM cycle time had to be lower than 1 second, peak separation for co-eluting 2,3,7,8-TCDD isomers was resolved with a valley of 25% or more and chromatographic integration windows were within the required limits and calibrations were run daily.

7.2.2.21.2 ESTIMATED DETECTION LIMIT (EDL)

The EDL is calculated for each isomer that was not identified, regardless of whether or not any isomers in that homologue were present. The EDL was also calculated for those isomers where responses for both of the quantitation ions were <2.5 times (2.5x) the background levels, and therefore do not meet the identification criteria.

The formula below was used to calculate an EDL for each absent CDD/CDF. The background level (H_X) was determined by measuring the height of the noise at the expected Retention Times (RTs) of both of the quantitation ions of the particular isomer. The expected RT was determined from the most recent analysis of the midpoint standard (CS3) performed on the same High Resolution Gas Chromatograph/High Resolution Mass Spectrometer (HRGC/HRMS) system that was used for the analysis of the samples that were associated with the EDL calculations.

$$EDL(ng/Kg) = \frac{2.5 \times Q_{IS} \times (H_{X1} + H_{X2}) \times D}{W \times (H_{IS1} + H_{IS2}) \times \overline{RR}}$$

EDL = Estimated Detection Limit

Q_{IS} = Quantity (pg) of appropriate internal standard added prior to sample extraction

[8] The method synopsis for chlorinated dioxins and furans was provided by the external laboratory that provided the test results (b) (4)

H_{X1}, H_{X2} = Peak heights of the noise for both quantitation ions of the CDD/CDF

H_{IS1}, H_{IS2} = Peak heights of the internal standard ions

D = Dilution Factor

W = Weight extracted in grams

$\overline{RR}$ = The mean Relative Response for the isomer of interest from the initial calibration.

Note that for Estimated Maximum Possible Concentrations (EMPC) where the ratio has failed originally, the weight of the ion which has the higher response was corrected to meet the "classical ratio" for the homologue group. This total area was used to calculate the EMPC for the isomer and was flagged accordingly.

7.3 METHOD MODIFICATIONS

7.3.1 MERCURY (HEALTH CANADA METHOD T-108)

Ten conditioned cigarettes were smoked on a linear smoking machine instead of a rotary smoking machine. The analyte was collected by passing the mainstream smoke through two midget impingers, each containing 10mL of an acidified potassium permanganate solution. Final volume of the samples was 50mL.

8 ACCEPTANCE OF DATA

8.1 EVALUATION OF RESULTS FROM CONTROL MATERIALS

Data obtained using control materials are deemed acceptable if the data are in keeping with Labstat's database for the control material and the specific method of analysis[9]. This is not a simple problem since there is no "yes" or "no" answer but rather one which is phrased in terms of probabilities. In the approach taken by Labstat, the measure of random variation in the procedure is taken to be the sample standard deviation (S.D. or "s").

To evaluate control data accuracy, a "*Z score*" statistic is determined as follows:

$$Z\text{-}score = \frac{\text{Sample Average} - \text{Historical Average}}{\text{Historical Standard Deviation}}$$

To evaluate control data precision, a "*Chi-square*" statistic is determined as follows:

$$Chi\text{-}square = (\text{Sample Size} - 1) \times \frac{(\text{Sample Standard Deviation})^2}{(\text{Historical Standard Deviation})^2}$$

[9] A minimum of 30 results is normally required for the purpose of this comparison.

P values are generated and the cut-off point (α) chosen in such a way as to minimize the chance of rejecting data which are legitimate members of the set (i.e. type 1 error). Thus, in most cases where the number of observed control samples is greater than or equal to 5, z-score p-values are generated from the Standard Normal distribution.

The standard deviation rather than the standard error for the mean has been chosen when determining the 'Z score'. This allows for both project-to-project variation, which is inherent in the historical data, and the 'normal' run-to-run variability, which is present in the data set. The cut-off point for P values is a matter of judgment and has been set at 0.005 assuming the probability of falsely rejecting a data point is 0.5% (i.e. α=0.01) or less for a two tailed test.

In instances where expected values are not known, a decision to accept the data is made based on observed levels of precision in comparison with that determined for similar analyses. Also, there are circumstances where the expected value may be "Below Detection Limits". In this case the decision to accept or reject the data is made upon the ability of the method to recover the analyte of interest either in the form of a laboratory fortified blank (LFB) or laboratory fortified matrix (LFM). Acceptable recoveries are close to 100%, but vary depending on the analyte.

8.2 IDENTIFICATION OF OUTLIERS[10]

8.2.1 OUTLIER DEFINITION

An outlying observation, or "outlier," is one that appears to deviate markedly from other members of the sample in which it occurs. In this case, there are two alternatives:

1. An outlying observation may be merely an extreme manifestation of the random variability inherent in the data. If this is true, the value is retained and processed in the same manner as the other observations in the sample.
2. The observation may be the result of gross deviation from prescribed experimental procedure or an error in calculating or recording the numerical value. In such cases, an investigation must be carried out. When the experimenter is clearly aware that a gross deviation from prescribed experimental procedure has taken place, the resultant observation is discarded (assignable cause) without recourse to a statistical test. A statistical test may always be used to support a judgment that a physical reason does actually exist for an outlier, or the statistical criterion may be used routinely as a basis to initiate action to find a physical cause.

8.2.2 STATISTICAL CRITERIA

There are a number of criteria for testing outliers. In all of these, the doubtful observation is included in the calculation of the numerical value of a sample criterion (or statistic) that is then compared with a critical value. The critical value is that which would be exceeded by chance with some specified (small) probability on the assumption that all the observations did indeed constitute a random sample from a single parent population, distribution or

[10] The term "outlier" has been defined in International Standard ISO 3534-1:2006 entitled "*Statistics - Vocabulary and symbols - Part 1: General statistical terms and terms used in probability*"

universe. The specified small probability is called the "significance level" and can be thought of as the risk of erroneously rejecting a good observation. A level of significance of 0.02 has been chosen in conjunction with the statistical test and tables described in ASTM E178-08[11].

Significant departures from the expected results (i.e. “outliers”) are viewed seriously, requiring an investigation for an assignable cause. This is a documented procedure that, at a minimum, consists of the following steps:

1. Review of all associated calculations to ensure that arithmetic errors have not been made
2. Review of linearity range for any standards
3. Assessment of instrument status
4. Review of reagents, columns, standards etc. to ensure that contamination or decomposition has not occurred
5. Review of sample preparation and handling procedures as they relate to the result in question

If the outlier is present in the analyte data and an assignable cause is found, the test result is removed from the data set but recorded in the quality control section of the laboratory’s record of test results for that project. The analysis must then be repeated. If the outlier is present in the ancillary[12] data and an assignable cause is found, the test result is not removed, but rather the outlying observation is replaced by the designation “AC” (Assignable Cause). If this investigation fails to determine an assignable cause, the test result is assumed to be a legitimate member of the data set and is included in all subsequent calculations.

9 RESULTS

9.1 QUALITY CONTROL

The control results for the variables of interest were acceptable as defined in section 7.1. Consequently it is reasonable to assume that the values determined for the test samples are reflective of the characteristics of the products as received and tested as described in the Analytical Methods section.

9.2 ANALYTICAL DATA

Individual results and the corresponding sample statistics (consisting of means, standard deviations, and coefficients of variation or 95% confidence limits) may be found in the data files, labeled *NS367-H_ms_dataCF_R1.xls* and *NS367-H_ms_ControlsCF.xls*, which accompany this report.

[11] ASTM Designation: E178-08. Standard Practice for Dealing with Outlying Observations

[12] Data, which are related, but not normally required as part of the reporting process (e.g. puff counts, TPM, cigarette weights etc.). Outliers in the analyte data that have an assignable cause are always repeated.

9.2.1 SAMPLE STATISTIC CALCULATIONS

In cases where a sample result is below the limit of detection (LOD), the average of the value zero (0) and the LOD is used in the sample statistic calculation. In cases where a sample result is between the LOD and the limit of quantification (LOQ), the average of the LOD and the LOQ is used in the sample statistic calculation.

10 ACCREDITATION

Labstat International ULC has been accredited by the Standards Council of Canada to International Standard ISO/IEC 17025:2005 "*General requirements for the competence of testing and calibration laboratories*" with a scope[13] that includes all of the mandated tobacco-related Health Canada methods (see Tobacco Reporting Regulations dated 26 June 2000, Canada Gazette Part II, Vol. 134, No. 15 Schedules 1, 2 and 3 pages 1780 – 1785). The testing included in this report is within the scope of this accreditation, unless otherwise noted.

Accredited LAB 368

(SCC Accreditation & Design Mark is an Official Mark of the Standards Council of Canada, used under license)

11 AUTHORIZATION

11.1 ORIGINAL

This report has been reviewed by me and is certified, to the best of my knowledge, to be a true and accurate description of the procedures, protocols and test methods used to arrive at the data and/or findings that accompany this report.

[13] Labstat's accreditation scope is available on Standards Council of Canada website at: http://palcan.scc.ca/specs/pdf/180_e.pdf

Dated: February 07, 2018

Mingliang Bao,
Scientist
Labstat International ULC

11.2 REVISION 1

This report has been reviewed by me and is certified, to the best of my knowledge, to be a true and accurate description of the procedures, protocols and test methods used to arrive at the data and/or findings that accompany this report.

Dated: March 29, 2018

Peter Joza,

Chief Technical Officer, Chemistry

Labstat International ULC

12 APPENDIX A: "RAW" DATA AND SUMMARY STATISTICS

See Accompanying Data Files or Enclosed CD