Characterization of Mainstream Tobacco Smoke

Analytical Test Report

Labstat®
Since 1976

Prepared for *Philip Morris International*

Project Code: NS308-H

Original Date: April 13, 2016

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2 USE OF LABSTAT'S[1] ANALYTICAL REPORTS

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3 REVISION HISTORY

3.1 REVISION 1

This revision was required to correct the method descriptions in the written report, to reflect the inclusion of the method modifications for “heat-not-burn” products as appendices to the standard methods.

4 ADMINISTRATIVE INFORMATION

4.1 QUOTATION IDENTIFICATION

Quotation Number: T5428

Date of Quotation: January 25, 2016

Recipient's Name: Cyril Jeannet

4.2 CLIENT IDENTIFICATION

Philip Morris International

Quai Jeanrenaud 56

2000 Neuchatel

Switzerland

5 SAMPLE HANDLING

5.1 CHAIN OF CUSTODY

The samples to be tested for project NS308-H were received on March 11, 2016 via DHL.

5.2 SAMPLE CHARACTERIZATION AND CODING

5.2.1 SAMPLE CHARACTERISTICS

The shipment received on March 11, 2016 consisted of one box of one product and 7 cartons of another product. There was no physical damage to the box, packages or cartons.

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.

Labstat Sample ID	SMP Description	Description	Qty/Type Received	Type of Product	Type of Analysis
1680386	SMP060595	Marlboro	1/box	sticks	mainstream smoke
1680387	SMP060596	Monitor P1M1	7/cartons	sticks	mainstream smoke

5.2.3 PHYSICAL MEASUREMENTS

Physical measurements were performed on the sticks.

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

6 PROJECT-SPECIFIC INSTRUCTIONS

A client table was followed for testing/running the samples in different days.

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 are puffed to a fixed puff number: 6 puffs per stick under ISO standard smoking regime[2] and 12 puffs per stick under Health Canada Intense (HCI) smoking regime[3].

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 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***

[2] ISO 3308:2012: (35.0±0.3)ml puff volume, (60±0.5)s puff frequency, (2.00±0.02)s puff duration, bell-shaped puff profile, no vent blocking

[3] 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*

machine - Definitions and standard conditions with modifications as noted in the table below. A single port linear smoking machine was employed in the analysis of the NO/NO_x contents of mainstream smoke.

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

Variable	"Canadian Modified"
Puff Volume (ml)	55
Interval (sec)	30
Duration (sec)	2
Vents[4]	"fully blocked"

Note: Test samples did not have any vent blocking applied. Vent blocking for "Canadian Modified" was only applied to the reference cigarettes.

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

7.2 ANALYTICAL METHODS[5]

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").

OFFICIAL METHODS FOR THE COLLECTION OF EMISSION DATA ON MAINSTREAM SMOKE[6]

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

[4] 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.

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

[6] Canadian Tobacco Reporting Regulations: 2000-07-19 Canada Gazette Part II, Vol. 134, No. 15 Part 3: Emissions from Designated Tobacco Products. Test method numbers refer to Health Canada methodologies which have been posted by Health Canada on the internet at site http://www.hc-sc.gc.ca/hl-vs/tobac-tabac/legislation/reg/indust/index_e.html

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) *(h)* Butyraldehyde	T-104	*Determination of Selected Carbonyls in Mainstream Tobacco Smoke*
3.	Hydrogen cyanide	T-107	*Determination of Hydrogen Cyanide in Mainstream Tobacco Smoke*
4.	Mercury	T-108	*Determination of Mercury in Mainstream Tobacco Smoke*
5.	*(a)* Lead *(b)* Cadmium *(c)* Chromium *(d)* Nickel *(e)* Arsenic *(f)* Selenium	T-109	*Determination of Ni, Pb, Cd, Cr, As and Se in Mainstream Tobacco Smoke*
6.	*(a)* NO *(b)* NOx	T-110	*Determination of Oxides of Nitrogen in Mainstream Tobacco Smoke*
7.	*(a)* Pyridine *(b)* Quinoline *(c)* Styrene *(d)* Nitrobenzene	T-112/ TMS-00112	*Determination of Pyridine, Quinoline and Styrene in Mainstream Tobacco Smoke*
8.	*(a)* Tar *(b)* Nicotine *(c)* Carbon Monoxide *(d)* Glycerol	T-115/ TMS-00115a	*Determination of "Tar", Nicotine and Carbon Monoxide in Mainstream Tobacco Smoke*

OTHER METHODS FOR THE COLLECTION OF EMISSION DATA ON MAINSTREAM SMOKE

Item	Emission	Labstat Method	Method Description
1.	*(a)* Pyrene *(b)* Benzo(a)anthracene *(c)* Benzo(a)pyrene *(d)* Dibenz(a,h)anthracene	TMS-00120	*Determination of Selected Polynuclear Aromatic Hydrocarbons in Mainstream Tobacco Smoke*

Item	Emission	Labstat Method	Method Description
2.	*(a)* 1,3-Butadiene *(b)* Isoprene *(c)* Acrylonitrile *(d)* Benzene *(e)* Toluene *(f)* Ethylene Oxide *(g)* Vinyl Chloride *(h)* Propylene Oxide	TMS-00124	*Determination of Vinyl Chloride, 1,3-Butadiene, Isoprene, Acrylonitrile, Benzene, Toluene, Styrene, and Acetamide in Mainstream Tobacco Smoke (expanded list)*
3.	*(a)* 1- aminonaphthalene *(b)* 2- aminonaphthalene *(c)* 3- aminobiphenyl *(d)* 4- aminobiphenyl *(e)* o- toluidine	TMS-00128	*Determination of Aromatic Amines in Mainstream Tobacco Smoke (expanded list)*
4.	*(a)* *N*-nitrosonornicotine *(b)* 4-(*N*-nitrosomethylamino)-1-(3-pyridyl)-1-butanone *(c)* *N*-nitrosoanatabine *(d)* *N*-nitrosoanabasine	TMS-00135	*Determination of Tobacco Specific Nitrosamines in Mainstream Tobacco Smoke by High-Performance Liquid Chromatography-Tandem Mass Spectrometry*
5.	*(a)* Acetamide *(b)* Acrylamide	TMS-00137	*Determination of Acetamide and Acrylamide in Mainstream Tobacco Smoke*
6.	*(a)* Hydroquinone *(b)* Resorcinol *(c)* Catechol *(d)* Phenol *(e)* *m*- Cresol *(f)* *p*-Cresol *(g)* *o*-Cresol	TMS-00139	*Determination of Phenolic Compounds In Mainstream Tobacco Smoke by a Modified High Performance Liquid Chromatography Method*

7.2.2 INTERNAL METHOD REFERENCES AND SYNOPSES

7.2.2.1 POLYNUCLEAR AROMATIC HYDROCARBONS (LABSTAT METHOD TMS-00120)

7.2.2.1.1 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.

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

7.2.2.1.2 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.

7.2.2.2 VOLATILE ORGANICS (LABSTAT METHOD TMS-00124)

7.2.2.2.1 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.

7.2.2.2.2 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.

The instrument operating parameters are presented in the following table:

GC/MS in SIM mode: Peak Areas Used for Quantification and Qualification

Component	Target (m/z)	Qualifier ion(s) (m/z)	Dwell Time (ms)
1,3-butadiene	53	54, 51	50
Isoprene	67	68, 53	50
Acrylonitrile	52	53	50
Benzene	78	77, 51	50
Toluene	91	92	50
D6-benzene	84		50

For the ethylene oxide analysis, a second aliquot of the trapping solution was dried on anhydrous sodium sulphate, derivatized with 48% hydrobromic acid, neutralized with sodium carbonate and spiked with the internal standards (D_6-benzene and d4-2-bromoethanol). After centrifugation, the supernatant was analyzed with a capillary column by GC/MS operating under selective ion monitoring (SIM) mode.

The instrument operating parameters are presented in the following table:

GC/MS in SIM mode: Peak Areas Used for Quantification and Qualification

Component	Target (m/z)	Qualifier ion(s) (m/z)	Dwell Time (ms)
2-bromoethanol	53	107, 95	100
D6-benzene	84		100

7.2.2.3 AROMATIC AMINES (LABSTAT METHOD TMS-00128)

7.2.2.3.1 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.*

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

7.2.2.3.2 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.

The instrument operating parameters are presented in the following table:

NCI-GC/MS in SIM mode: Peak Areas Used for Quantification

Component	Retention Time (min)	Target (m/z)	Dwell Time (ms)
1-aminonaphthalene	13.035	269	100
2-aminonaphthalene	13.8	269	100
3-aminobiphenyl	15.601	295	100
4-aminobiphenyl	15.95	295	100
o-toluidine	7.532	233	100
benzidine	19.279	456	100
D7-1-aminonaphthalene	12.997	276	100
D7-2-aminonaphthalene	13.761	275	100
D7-4-aminobiphenyl	15.911	304	100
D9-o-toluidine	7.453	240	100
D8-benzidine	19.268	464	100

7.2.2.4 TOBACCO SPECIFIC NITROSAMINES (LABSTAT METHOD TMS-00135)

7.2.2.4.1 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.

7.2.2.4.2 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.

7.2.2.5 ACETAMIDE AND ACRYLAMIDE (LABSTAT METHOD TMS-00137)

7.2.2.5.1 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.

7.2.2.5.2 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.

7.2.2.6 PHENOLIC COMPOUNDS (LABSTAT METHOD TMS-00139)

7.2.2.6.1 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.

7.2.2.6.2 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).

7.3 METHOD MODIFICATIONS

7.3.1 MERCURY (HEALTH CANADA METHOD T-108)

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.

7.3.2 TRACE METALS (HEALTH CANADA METHOD T-109)

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.

7.3.3 OXIDES OF NITROGEN (HEALTH CANADA METHOD T-110)

For the analysis of "heat-not-burn" products, the mainstream smoke from a single stick was exhausted puff by puff into an evacuated 500mL smoke mixing chamber. Calibration was done with three levels of concentration including 9, 30, and 50 ppm (NO/NOx).

7.3.4 SEMI-VOLATILE ORGANICS (HEALTH CANADA METHOD T-112/LABSTAT METHOD TMS-00112 & LABSTAT METHOD TMS-00137)

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.

7.3.5 GLYCEROL (HEALTH CANADA METHOD T-115/LABSTAT METHOD TMS-00115A)

A second independent aliquot of the IPA extract was used to determine glycerol by gas chromatography (GC) with flame ionization detector (FID) using a megabore DB-Wax fused silica column that has a polyethylene glycol (PEG) stationary phase, as per Health Canada method T-304. Quantification was achieved using the internal standard calibration (internal standard: trans-anethole).

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.

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[7]. 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:

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

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

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

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.

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

8.2 IDENTIFICATION OF OUTLIERS[8]

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 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[9].

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

[8] 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*"

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

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[10] 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 8.1. 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 *NS308-H_ms_dataCF.xls* and *NS308-H_ms_controlsCF.xls*, which accompany this report.

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] 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.

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[11] 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.

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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.

Dated: April 13, 2016

Peter Joza,

Director, Science & Technology

Labstat International ULC

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

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: May 27, 2016

Peter Joza,

Director, Science & Technology

Labstat International ULC

12 APPENDIX A: "RAW" DATA AND SUMMARY STATISTICS

See Accompanying Data Files or Enclosed CD