APPENDIX A3.2.1-12

MAINSTREAM AEROSOL CONSTITUENT ASSESSMENT

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Document / Subject

Chemical assessment of Modified Tobacco Products Mainstream Aerosols (MMA)[1] for both comparative (i.e., vs. conventional lit-end cigarettes market maps - SCMM) and comparability assessment (i.e., vs. established Modified Tobacco Products "benchmarks").

Review findings and recommendations

1 Criteria for Comparative and Comparability Assessment under GLP requirements

Criterion 1: Smoke constituents based on International Organization for Standardization (ISO) methods

Criterion 2: Priority toxicants in tobacco smoke as listed by regulatory bodies, or proposed by cognizant authorities

Criterion 3: Smoke constituents with established biomarkers of exposure

Criterion 4: Potentially harmful aerosol constituents which are predominantly formed below 400°C, and which are NOT included in Criterion 2

Criterion 5: Potentially harmful aerosol constituents which are predominantly formed above 400°C, and which are NOT included in Criterion 2

Criterion 6: Aerosol characterization to identify aerosol constituents which have the potential to introduce novel hazards compared to conventional lit-end cigarettes, and semi-quantitative analysis of 'potentially harmful/harmful' aerosol constituents

Criterion 7: Product-specific analytes

2 Smoking regimen

1 ISO 3308
2 Health Canada Intense
3 Human Puffing Behavior (HPB) conditions, i.e., smoking machine settings to produce nicotine yields corresponding to the 10^{th} – 90^{th} percentile of nicotine uptake distribution determined in the clinical Biomarker of Exposure Study (BEX1).
4 Worst case nicotine yield target derived from prior knowledge on nicotine uptake distribution of established Modified Tobacco Products "benchmark": 99^{th} percentile during prototype screening

3 Experimental conditions

For the DDA1, to be conducted in compliance with GLP principles;

Otherwise, to be conducted in compliance with ISO 17025

4 Constituent reporting

1 ISO 3308: per cigarette basis, equal nicotine basis
2 Health Canada Intense: per cigarette, equal nicotine basis,
3 Human Puffing Behavior (HPB) conditions: per cigarette basis

[1] Current nomenclature of Modified Tobacco Products (MTP) aerosols:
Modified Tobacco Products – Environmental Aerosol (MEA); CoF Recommendation September 27, 2010
Modified Tobacco Products – Mainstream Aerosol (MMA); this recommendation

5 List of analytes for determination of MMA composition according to Criteria 1-7

Criterion 1: Smoke constituents based on ISO methods	
Benzo[*a*]pyrene	ISO Standard 22634, 1st ed., 2008. Cigarettes -- Determination of benzo[*a*]pyrene in cigarette mainstream smoke -- Method using gas chromatography/mass spectrometry
Carbon monoxide	ISO Standard 8454, 2nd ed., 1995. Cigarette -- determination of carbon monoxide in the vapor phase of cigarette smoke -- NDIR method
Nicotine	ISO Standard 10315, 2nd ed. and Corrigendum I, 2000. Cigarettes -- determination of nicotine in smoke condensates -- Gas chromatographic method.
TPM, water and NFDPM	ISO Standard 4387, 3rd ed., 2000. Cigarettes -- determination of total and nicotine free dry particulate matter using a routine analytical smoking machine.

Criterion 2: Priority toxicants in tobacco smoke as listed by regulatory bodies, or proposed by cognizant authorities [a]		**Criterion 3: Constituent with established biomarkers of exposure (smoke/aerosol constituents or metabolites)**
Health Canada	**WHO TobReg 2008**	
1-aminonaphthalene		
2-aminonaphthalene (2-NA) [b]	•	• (2-NA)
3-aminobiphenyl		
4-aminobiphenyl (4-ABP) [b]	•	• (4-ABP)
Acetaldehyde [b]	•	
Acetone		
Acrylonitrile [b]	•	(CEMA, in development)
Acrolein [b]	•	• (3-HPMA)
Ammonia [b]		
1,3-butadiene [b], #	•	• (MHBMA)
Benzene [b], #	•	• (*S*-PMA)
Benzo[a]pyrene [b], #	•	• (Total 1-OHP)*
Butyraldehyde		
Carbon monoxide [b]	•	• (COHb)
Catechol [b],	•	
m–cresol		
p-cresol		
o-cresol		
Crotonaldehyde	•	• (HMPMA)
Formaldehyde [b]	•	
Hydrogen cyanide [b], #	•	
Hydroquinone	•	
Isoprene [b], #		
Methyl ethyl ketone		
Propionaldehyde		
Nitric oxide		
N-nitrosoanabasine (NAB)		
N-nitrosoanatabine (NAT)		

4-(N-nitrosomethylamino)-1-(3-pyridyl)-1-butanone (NNK) [b)]	•	• (Total NNAL)
N-nitosonornicotine (NNN) [b)]	•	(Total NNN, in development)
Nitrogen oxides [b)]	•	
Phenol [b)]		
Pyridine		
Quinoline		
Resorcinol		
Styrene [b), #]		
Toluene [b), #]		
Arsenic [b)]		
Cadmium [b)]	•	
Chromium [b)]		
Lead [b)]		
Mercury		
Nickel [b)]		
Selenium		
Criterion 3: Constituent with established biomarkers of exposure (constituent or metabolites)		
Pyrene [#]		• (Total 1-OHP)*
o-toluidine [b)]		• (*o*-toluidine)
Criterion 4: (Potentially) harmful aerosol constituents which are predominantly formed below 400°C, and which are NOT included in Criterion 2		
Acrylamide		
Acetamide [b)]		
Propylene oxide		
Ethylene oxide		
Nitrobenzene		
Vinyl chloride [b)]		
Criterion 5: (Potentially) harmful aerosol constituents which are predominantly formed above 400°C, and which are NOT included in Criterion 2 [c)] and 3		
Dibenz[a,h]anthracene [b), #]		
Benz[a]anthracene [b), #]		
Criterion 6: Aerosol characterization to identify aerosol constituents which have the potential to introduce novel hazards compared to conventional lit-end cigarettes, and semi-quantitative analysis of 'potentially harmful/harmful' aerosol constituents		
Non-Targeted Differential Screening (NTDS) and Targeted Fingerprinting (TF) as defined by the Draft 'Guideline for Pre-Clinical MTP Assessment'		
Criterion 7: Product-specific analytes (examples only)		
Glycerin		
Menthol		
'Particle breakthrough'		
Droplet Size Distribution		

Abbreviations: 3-HPMA, 3-hydroxypropylmercapturic acid; CEMA, 2-cyanoethylmercapturic acid; COHb, carboxyhemoglobin; HMPMA, 3-hydroxy-2-methylpropyl mercapturic acid; MHBMA, 1-hydroxy-2-(*N*-acetyl-cysteineyl)-3-butene; MS, mainstream smoke; MTP, Modified Tobacco Product; NNAL, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol; *S*-PMA, *S*-phenylmercapturic acid .

* Total 1-OHP is a surrogate for the exposure to polycyclic aromatic hydrocarbons, including benzo[a]pyrene

High temperature pyrolysis marker include 1,3-butadiene, benzo[*a*]pyrene, dibenz[*a,h*]anthracene, benz[*a*]anthracene, pyrene, hydrogen cyanide, isoprene, benzene, toluene, styrene.

a) Health Canada 2008. Tobacco Industry Reporting: Tobacco Reporting Regulations. Schedule 2

TobReg, 2008. *The scientific basis for tobacco product regulation*. Second report of a WHO study group. Geneva, World Health Organization (WHO Technical Report Series, No. 951).

b) PMI List of potentially harmful smoke constituents; GENERAL OVERVIEW OF TOXICOLOGICAL ASSESSMENT BEST PRACTICES - Cigarette Ingredients, Product Designs and Manufacturing, TPA, PMI R&D, August 2009.

c) Until further specified by the 'Draft Guideline for MTP Pre-Clinical Assessment' the appearance of pyrolysis/combustion markers, when detected above their respective Limits of Detection (LODs), is taken as an indicator of "burning" rather than "heating" of the tobacco-based sensorial media: 1,3-butadiene, benzo[*a*]pyrene, hydrogen cyanide, isoprene, benzene, toluene, styrene, dibenz[*a,h*]anthracene, benz[*a*]anthracene, pyrene.

6 Rationale for Substance Selection - Criteria 4-7

6.1 Criterion 4[2]

The following constituents were chosen on the basis of both their IARC classification[3] and abundance in cigarette mainstream smoke (or relative abundance with regard to constituents from the same chemical class): ethylene oxide (Group 1); vinyl chloride (Group 1); acrylamide (Group 2A); acetamide (Group 2B); propylene oxide (Group 2B); nitrobenzene (Group 2B).

6.2 Criterion 5

The following constituents were chosen on the basis of their IARC classification, abundance in cigarette mainstream smoke (or relative abundance with regard to constituents from the same chemical class): dibenz[*a*,*h*]anthracene (Group 2A); benz[*a*]anthracene (Group 2B).

(Note: Benzo[*a*]pyrene (Group 1) is covered by Criteria 1 & 2, and pyrene (Group 3) by Criterion 3).

6.3 Criterion 6

MMA non-targeted fingerprinting is a relevant approach for detecting novel compounds and potentially novel hazard compared to cigarette mainstream smoke. Based on a semi-quantitative analysis, the targeted fingerprinting assay extends the 'List of 58 Aerosol Constituents' to include additional 'harmful/potentially harmful' aerosol constituents, e.g., smoke constituents proposed by cognizant authorities, e.g., FDA (2011)[4].

6.4 Criterion 7

The list under Criterion 7 is not exhaustive, but provides some examples from current or past products for which specific design features may lead to the presence of aerosol constituents in quantities that require measurement according to best practice.

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[2] Criteria 4 & 5 cover all currently listed *Group 1* Carcinogens by IARC (2010), which are known to be present in cigarette smoke with the exception of beryllium and radio-isotopes:
http://monographs.iarc.fr/ENG/Classification/ClassificationsGroupOrder.pdf

[3] IARC Classification:
Group 1 — The agent (mixture) is carcinogenic to humans.
The exposure circumstance entails exposures that are carcinogenic to humans.
Group 2A — The agent (mixture) is probably carcinogenic to humans.
The exposure circumstance entails exposures that are probably carcinogenic to humans.
Group 2B — The agent (mixture) is possibly carcinogenic to humans.
The exposure circumstance entails exposures that are possibly carcinogenic to humans.
Group 3 — The agent (mixture or exposure circumstance) is not classifiable as to its carcinogenicity to humans.

[4] Food and Drug Administration, 2011. Food and Drug Administration [Docket No. FDA–2011–N–0271] Harmful and Potentially Harmful Constituents in Tobacco Products and Tobacco Smoke; Request for Comments. Available at:
http://www.gpo.gov/fdsys/pkg/FR-2011-08-12/pdf/2011-20502.pdf (accessed 27.09.2011)

7 Consolidated list of 58 constituents for MMA assessment[5]

#	Aerosol deliveries according to ISO 4387; 8454; 1035	Unit
	Glycerol[6]	mg/stk
1	Nicotine	mg/stk
2	Nicotine Free Dry Particulate Matter (NFDPM)[2]	mg/stk
3	Total Particulate Matter (TPM)	mg/stk
4	Water	mg/stk
	HPHCs	**Unit**
5	1,3-Butadiene	µg/stk
6	1-Aminonaphthalene	ng/stk
7	2-Aminonaphthalene	ng/stk
8	3-Aminobiphenyl*	ng/stk
9	4-(Methyl-nitrosamino)-1-(3-pyridyl)-1-butanone (NNK)	ng/stk
10	4-Aminobiphenyl*	ng/stk
11	Acetaldehyde	µg/stk
12	Acetamide	µg/stk
13	Acetone	µg/stk
14	Acrolein	µg/stk
15	Acrylamide	µg/stk
16	Acrylonitrile	µg/stk
17	Ammonia	µg/stk
18	Arsenic*	ng/stk
19	Benz[a]anthracene	ng/stk
20	Benzene	µg/stk
21	Benzo[a]pyrene*	ng/stk
22	Butyraldehyde	µg/stk
23	Cadmium*	ng/stk
24	Carbon monoxide (CO)[3]	mg/stk
25	Catechol	µg/stk
26	Chromium**	ng/stk
27	Crotonaldehyde	µg/stk
28	Dibenz[a,h]anthracene*	ng/stk

[5] Test/reference items comparisons, 'Prototype Screening', 'Due Diligence Assessment (DDA)', and applicable 'Pass/Fail Criteria' are documented in the Draft 'Guideline for MTP Pre-clinical Assessment'

[6] Product Sepcific

#	Aerosol deliveries according to ISO 4387; 8454; 1035	Unit
29	Ethylene oxide*	µg/stk
30	Formaldehyde	µg/stk
31	Hydrogen cyanide	µg/stk
32	Hydroquinone	µg/stk
33	Isoprene	µg/stk
34	Lead*	ng/stk
35	m-Cresol	µg/stk
36	Mercury	ng/stk
37	Methyl-ethyl-ketone (MEK)	µg/stk
38	Nickel**	ng/stk
39	Nitric oxide (NO)	µg/stk
40	Nitro benzene*	µg/stk
41	Nitrogen oxides (NOx)	µg/stk
42	N-nitrosoanabasine (NAB)*	ng/stk
43	N-nitrosoanatabine (NAT)	ng/stk
44	N-Nitrosonornicotine (NNN)	ng/stk
45	o-Cresol	µg/stk
46	o-Toluidine	ng/stk
47	p-Cresol	µg/stk
48	Phenol	µg/stk
49	Propionaldehyde	µg/stk
50	Propylene oxide	ng/stk
51	Pyrene*	ng/stk
52	Pyridine	µg/stk
53	Quinoline*	µg/stk
54	Resorcinol	µg/stk
55	Selenium*	ng/stk
56	Styrene	µg/stk
57	Toluene	µg/stk
58	Vinyl chloride*	ng/stk

APPENDIX A - Smoke Constituent Formation (based on tobacco pyrolysis experiments)

Health Canada List and additional constituents	Predominant temperature of formation (Torikai et al. 2004; Senneca et al. 2007; Adam et al. 2009)
Health Canada List constituents (n=48)*	
Ammonia	< 400 °C
1-aminonaphthalene	300 – 500 °C
2-aminonaphthalene	300 – 500 °C
3-aminobiphenyl	300 – 500 °C
4-aminobiphenyl	300 – 500 °C
1,3-butadiene	> 400 °C
Isoprene	> 400 °C
Benzo[a]pyrene	> 400 °C
Formaldehyde	300 – 500 °C
Acetaldehyde	300 – 500 °C
Acetone	300 – 500 °C
Acrolein	300 – 500 °C
Propionaldehyde	300 – 500 °C
Crotonaldehyde	300 – 500 °C
Butyraldehyde	300 – 500 °C
Methyl ethyl ketone	300 – 500 °C
Acrylonitrile	> 400 °C
Hydrogen cyanide	> 400 °C
Mercury	already in tobacco – transfer depends on physical properties of element
Lead	already in tobacco – transfer depends on physical properties of element
Cadmium	already in tobacco – transfer depends on physical properties of element
Nickel	already in tobacco – transfer depends on physical properties of element
Arsenic	already in tobacco – transfer depends on physical properties of element
Chromium	already in tobacco – transfer depends on physical properties of element
Selenium	already in tobacco – transfer depends on physical properties of element
NO	200 – 500 °C
NOx	200 – 500 °C
NNN	already in tobacco – boiling point < 400 °C

Health Canada List and additional constituents	Predominant temperature of formation (Torikai et al. 2004; Senneca et al. 2007; Adam et al. 2009)
NNK	already in tobacco – boiling point < 400 °C
N-nitrosoanatabine	already in tobacco – boiling point < 400 °C
N-nitrosoanabasine	already in tobacco – boiling point < 400 °C
Pyridine	> 400 °C
Quinoline	> 400 °C
Benzene	> 400 °C
Toluene	> 400 °C
Styrene	> 400 °C
Hydroquinone	< 400 °C
Resorcinol	No specific temperature
Catechol	< 400 °C
Phenol	No specific temperature
m-cresol	> 400 °C
p–cresol	> 400 °C
o-cresol	> 400 °C
NFDPM	-
TPM	-
Nicotine	-
Carbon monoxide	> 300 °C
Water	n/a
Additional constituents (N=10)	
o-toluidine	Probably 300 – 500°C (like the other aromatic amines)
Ethylene oxide	< 400 °C
Propylene oxide	< 400 °C
Acrylamide	< 400 °C
Acetamide	< 400 °C
Vinyl chloride	< 400 °C
Nitrobenzene	< 400 °C
Dibenz[*a*,*h*]anthracene	> 400 °C
Benz[*a*]anthracene	> 400 °C
Pyrene	> 400 °C

* Heatlh Canada method = 47 constituents, as *m*,*p*-cresol are reported together by Health Canada method, and in PMI, those two compounds are reported separately.

8 References

Reference List

Adam T, Mitschke S, Baker RR (2009). Investigation of Tobacco Pyrolysis Gases and Puff-by-puff Resolved Cigarette Smoke by Single Photon Ionisation (SPI) – Time-of-flight Mass Spectrometry (TOFMS). *Beiträge zur Tabakforschung International* **23**: 203-226.

Senneca O, Ciaravolo S, Nunziata A (2007). Composition of the gaseous products of pyrolysis of tobacco under inert and oxidative conditions. *Journal of Analytical and Applied Pyrolysis* **79**: 234-243.

Torikai K, Yoshida S, Takahashi H (2004). Effects of temperature, atmosphere and pH on the generation of smoke compounds during tobacco pyrolysis. *Food Chem Toxicol* **42**: 1409-1417.

CoF Recommendation on benzidine and the PMI-58 smoke chemistry list _November 2013

9 Revision history

Version N°	Author	Description of change (including reason for change)	Type of change
1.0	Schorp, Matthias, Tricker Anthony	Original issue	1
1.1	Schorp, Matthias, Tricker Anthony	Alignment with *Draft* 'Guideline for Pre-Clinical MTP Assessment'	2
2.1	Stabbert, Regina	1. Recommended by the Council of Fellows (CoF Recommendation, 2013) and after informing the SOT, **benzidine** was removed from the list (Dec. 2013). 2. Due to a method improvement ***m*- and *p*-cresol** yields are reported individually;	1
3.0	Cyril Jeannet	Better discrimination between HPCHs and other product specific constituents/parameters, replace Tar by NFDPM. Add signature page and reviewers	1
3.0 SV	Cyril Jeannet	Submission Version: removal of hyperlinks and internal references	2

(1. Major change/new version; 2. Minor change; 3 Review without change)

10 Approval

This document has been approved by:

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