Appendix 12 - Biomarkers of Exposure - Selection and Justification

APPENDIX 12: BIOMARKERS OF EXPOSURE SELECTION AND JUSTIFICATION

In clinical settings, biomarkers of exposure (BoExp), HPHCs or their metabolites in biological fluids, are compared in adult smokers continuing to smoke their own brand, adult smokers who switch to THS 2.2, adult smokers who switch to nicotine replacement therapy (NRT) and/or to adult smokers who stop smoking entirely. The selected BoExp measured in PMI's clinical studies represent HPHCs supported by public health researchers as priority HPHCs for being reduced in CC smoke.

Biomarkers of exposure provide direct, quantitative evidence of the presence of HPHCs, or their metabolites, in the body. Ideally, BoExp should be (a) specific to the source of exposure with other sources being minor or non-existent, (b) easily detectable using reliable, reproducible, precise analytical methods, and (c) reflect a specific toxic exposure or be a reliable surrogate of exposure to HPHCs.

The selection criteria for the BoExp measured in PMI clinical studies include: (d) representation of a set of HPHCs as listed by the FDA (e) ensure assessment of both gas and particulate phase of the EHTP aerosol, (f) include a broad variety of chemical classes and organ toxicity classes (carcinogen, cardiovascular toxicant, respiratory toxicant, reproductive and development toxicant, addiction potential) (Table 1 and Table 2).

PMI clinical studies measure BoExp of the following HPHCs: 1,3-butadiene, acrolein, acrylonitrile, benzene, carbon monoxide, crotonaldehyde, ethylene oxide, o-toluidine and toluene which are present in the gas phase of mainstream cigarette smoke, and 1-aminonaphtalene, 2-aminonaphthalene, 4-aminobiphenyl, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), benzo[a]pyrene, nicotine, N-nitrosonornicotine (NNN), and pyrene which are present in the particulate phase of the mainstream cigarette smoke. These HPHCs include 14 (78%) (except acetaldehyde, ammonia, formaldehyde and isoprene) of the 18 HPHCs requested to be reported to the FDA. Seven of the nine toxicants (1,3-butadiene, acrolein, benzene, benzo[a]pyrene, carbon monoxide, NNK, and NNN) are recommended for mandated lowering in mainstream cigarette smoke according to World Health Organization .

The assessment matrix for the BoExp measured in PMI clinical studies has been evaluated with regards to validity of the available methods, feasibility and exposure time needed to assure reliable results.

The scientifically accepted and standard approach to assess exposure in tobacco users is to measure BoExp of HPHCs in urine. Urinary BoExp determine the presence of HPHCs which have a relatively short blood half-life and are extensively metabolized to water soluble metabolites that are excreted in urine. Determination of urinary BoExp to HPHCs can reliably detect differences in exposure markers of the EHTP vs. CC.

Table 1: Summary of measured HPHC biomarkers of exposure.

Appendix 1 HPHC [smoke phase]	**Appendix 2 Smoking list**	**Appendix 3 Biomarker [Matrix]**	**Appendix 4 Biomarker $t_{1/2b}$**	**Appendix 5 LOQ(1)**	**Appendix 6 Organ Class toxicity**
1,3-Butadiene [gas]	FDA-18 FDA-93 HC PMI-58 WHO-18	Monohydroxybutenyl-mercapturic acid (MHBMA) [Urine(a)]	4 to 16 h	0.1ng/mL	CA, RT, RDT
1-Aminonaphthalene [particulate]	FDA-18 FDA-93 HC PMI-58	1-Aminonaphthalene (1-NA) [Urine(a)]	Not described	2.5pg/mL	CA
2-Aminonaphthalene [particulate]	FDA-18 FDA-93 HC PMI-58 WHO-18	2-Aminonaphthalene (2-NA) [Urine(a)]	9 h	2.5pg/mL	CA
4-Aminobiphenyl [particulate]	FDA-18 FDA-93 HC PMI-58 WHO-18	4-Aminobiphenyl (4-ABP) [Urine(a)]	26 h	1.25pg/mL	CA
Acrolein [gas]	FDA-18 FDA-93 HC PMI-58 WHO-18	3-Hydroxypropyl-mercapturic acid (3-HPMA) [Urine(a)]	10 h	20ng/mL	RT, CT
Acrylonitrile [gas]	FDA-18 FDA-93 HC PMI-58 WHO-18	2-Cyanoethylmercapturic acid (CEMA) [Urine(a)]	17 h	0.275ng/mL	CA, RT
Benzene [gas]	FDA-18 FDA-93 HC PMI-58 WHO-18	S-Phenyl-mercapturic acid (S-PMA) [Urine(a)]	9 to 15 h	25pg/mL	CA, CT, RDT
Benzo[a]pyrene [particulate]	FDA-18 FDA-93 HC PMI-58 WHO-18	3-Hydroxybenzopyrene [Urine(a)]	3 to 4 h	50fg/mL	CA
Carbon monoxide [gas]	FDA-18 FDA-93 HC PMI-58 WHO-18	Carboxyhemoglobin (COHb) [Blood(b)]	2 to 4 h	0.5%	RDT, CT
Crotonaldehyde [gas]	FDA-18 FDA-93 HC PMI-58 WHO-18	3-Hydroxy-1-methylpropyl-mercapturic acid (3-HMPMA)	2 days	20ng/mL	CA

Appendix 1 HPHC [smoke phase]	**Appendix 2 Smoking list**	**Appendix 3 Biomarker [Matrix]**	**Appendix 4 Biomarker $t_{1/2b}$**	**Appendix 5 LOQ(1)**	**Appendix 6 Organ Class toxicity**
		[Urine(a)]			
Ethylene Oxide [gas]	FDA-93 PMI-58	2-Hydroxyethyl-mercapturic acid (HEMA) [Urine(a)]	5 h	2.5ng/mL	CA, RT, RDT
Nicotine [particulate]	FDA-18 FDA-93 HC PMI-58	Nicotine (NIC-P) [Plasma(a)]	1 to 2 h	1ng/mL	RDT, AD
		Cotinine (COT-P) 3-OH Cotinine (3OHCOTP) [Plasma(a)]	16 to 18 h -	1ng/mL	
		Nicotine equivalents (NEq) [Urine(a)]	16 h (estimated)	10 or 50ng/mL(2)	
NNK [particulate]	FDA-18 FDA-93 HC PMI-58 WHO-18	Total 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL) [Urine(a)]	10 to 18 days	5pg/mL	CA
NNN [particulate]	FDA-18 FDA-93 HC PMI-58 WHO-18	Total N-nitrosonornicotine (NNN) [Urine(a)]	15 h	0.2pg/mL	CA
Pyrene [particulate]	PMI-58	Total 1-hydroxypyrene (1-OHP) [Urine(a)]	6 to 20 h	10pg/mL	Nontoxic (surrogate for exposure to polycyclic aromatic hydrocarbons)
o-Toluidine [gas]	FDA-93 PMI-58	*o*-Toluidine (*o*-TOL) [Urine(a)]	10 to 16 h	20pg/mL	CA
Toluene [gas]	FDA-18 FDA-93 HC PMI-58 WHO-18	S-benzyl-mercapturic acid (S-BMA) [urine[a]]	9 h	100pg/mL	RT, RDT

Note (1): Lower limit of Quantification of the assay used
Note (2): LLOQ for Nicotine, Cotinine, trans-Hydroxycotinine, Nicotine-glucuronide = 10ng.ml. LLOQ for Cotinine-glucuronide, trans-Hydroxycotinine-glucuronide = 50ng/ml

Analytical methods: [a] liquid chromatography-tandem mass spectrometry (LC-MS/MS) [b] Spectrophotometry
Smoking list. FDA-18: FDA list of 18 HPHCs ; FDA-93:Established list of 93 HPHCs, Federal Register 2012 ; HC: Health Canada list of smoke constituents , PMI-58: PMI list of 58 HPHCs; WHO-18: priority smoke toxicants listed WHO technical report series No. 951
Organ Class toxicity : AD: addictive; CA: carcinogen; CT: cardiovascular toxicant; RDT: reproductive and developmental toxicant; RT: respiratory toxicant

Table 2: Chemical classes and Risk Classification of assessed Biomarkers of Exposure to HPHCs.

	Chemical classes of concerned HPHCs	BoExp coverage of HPHC toxicity classes					
		None	CA(1)	CT(2)	RT(3)	RDT(4)	AD(5)
Gas phase	Carbon oxides			1		1	
	Aliphatic dienes				1	1	
	Carbonyls		1	1	1		
	Acid derivates		1		1		
	Epoxides		1		1	1	
	Inorganic compounds						
	Monocyclic aromatics		1	1	1	2	
	Aromatic amines		4				
	N-nitrosamines		2				
Particulate phase	Phenols						
	Polycyclic aromatic hydrocarbons		2				
	Metals and Elements						
	Alkaloids						1
	Total	3	1,2	3	5	5	1

Note (1): Carcinogenic
Note (2): Cardiovascular toxicant
Note (3): Respiratory toxicant
Note (4) Reproductive & Development toxicant
Note (5): Addictive
Risk classification according to: WHO-18: priority smoke toxicants listed WHO technical report series No. 951 Organ Class toxicity : AD: addictive; CA: carcinogen; CT: cardiovascular toxicant; RDT: reproductive and developmental toxicant; RT: respiratory toxicant

LIST OF REFERENCES

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