Attachment I
Table of Biomarkers

Cigarette yield and body burden of smoke toxins

Biomarker	Detection	Collection	Method of Analysis	Use	Half-Life
Arsenic	Arsenic exposure	Urine	ICP-MS	The association between long-term arsenic exposure and peripheral vascular disease has been well documented in epidemiologic studies.	The half-life of arsenic in the body is about 4 days, and it is primarily excreted in urine. [Arsenic in Drinking Water. Commission on Life Sciences. The National Academies Press. Washington DC. 1999]
Cadmium	Cadmium exposure	Urine	ICP-MS	Cadmium is elevated in tissue from smokers and depends on smoking variables, increasing as smoking increases [27]. Recent research suggests that cadmium promotes atherosclerotic plaque deposits in mammalian arteries as well as increasing blood pressure [28].	Human kidney and whole-body half-life estimated at 10-30 years [29].
Carbon Monoxide	Smoke exposure	Exhalate	Vitalograph - Commerical Instrument	Easy to measure in exhaled air. CO (ppm): Non-Smoker: 0 ppm; Light-Smoker: 10ppm and 20ppm; Medium Smoker: 30ppm and 40ppm; Heavy Smoker: 50ppm and 60ppm [30].	4-6 hrs

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Nicotine	Nicotine Exposure	Urine	LC-MS/MS	Specific to nicotine exposure. May reflect passive exposure to tobacco smoke, use of smokeless tobacco products or nicotine replacement products [31].	1-3 hrs
Cotinine and its metabolites	Nicotine Exposure	Urine / Saliva*	LC/MS/MS	Approximately 80% of nicotine is transformed to cotinine [25,32]. Nicotine is converted to cotinine by hepatic P-450 enzyme CYP2A6 (Tyndale and Sellers, 2001). *Salivary cotinine is more directly related to serum cotinine levels, for which more nicotine exposure data has been collected in large, epidemiologic studies such as the National Health and Nutrition Examination Survey [33].	16 hrs
trans-3-Hydroxycotinine	Nicotine Exposure	Urine	LC/MS/MS	Major metabolite of nicotine	The average elimination half-life for 3-HC in urine was 6.4

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					hrs [34].
Nicotine glucuronide	Nicotine Exposure	Urine	LC/MS/MS	Metabolite of nicotine exposure	
trans-3-Hydroxycotinine glucuronide	Nicotine Exposure	Urine	LC/MS/MS	Major metabolite of nicotine	The average half-life for 3-HC-Gluc excretion was 7.2 hrs, ranging from 4.6±9.4 hrs[35].
4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL)	Carcinogen (NNK) exposure	Urine	LC/MS/MS	Marker and metabolite of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK)[36]. NNK is a well-established systemic lung carcinogen [37,38]. NNK is the most prevalent systemic lung carcinogen in tobacco products [39,40]. This assay has been widely used in urine analysis from smokers [38,39,41,42].	The elimination half-life of NNAL is approximately 40 days.[24]
NNAL-glucuronide	Carcinogen (NNK) exposure	Urine	LC/MS/MS	Marker and metabolite of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) [36].	The distribution half-life for NNAL and NNAL-glucuronide is 3-4 days while the elimination half-life is 40-45 days[24]
4-Aminobiphenyl	4-aminobiphenyl presence	Urine	LC/MS/MS	Uptake and metabolic activation of known human bladder carcinogen, 4-aminobiphenyl.	31 hours in rats[43]
N-Acetyl-S-(2-	Marker for	Urine	LC/MS/MS	Acrolein is a toxic chemical in tobacco smoke formed by	Acrolein is highly reactive and no

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carboxyethyl)-L-cysteine	acrolein exposure			combustion of tobacco [44,45].	studies were located regarding excretion in humans after inhalation exposure.
N-Acetyl-S-(3-hydroxypropyl)-L-cysteine	Marker for acrolein exposure; Marker for 1,3-butadiene exposure	Urine	LC/MS/MS		No studies were located regarding excretion in humans after inhalation exposure to 1,3-butadiene. An elimination half-life of between 2 and 10 hours has been observed in rodents.[46]
N-Acetyl-S-(3,4-Dihydroxybutyl)-L-cysteine	Marker for 1,3-butadiene exposure	Urine	LC/MS/MS	Tobacco smoke can be a significant source of 1,3-butadiene in indoor air. The Environmental Protection Agency's assessment concludes that 1,3-butadiene is carcinogenic to humans by inhalation, based on the total weight of evidence [47].	

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Biomarker	Detection	Collection	Method of Analysis	Use	Half-Life
N-Acetyl-S-(phenyl)-L-cysteine (S-Phenyl mercapturic acid)	Marker for benzene exposure	Urine	LC/MS/MS	Metabolite of benzene [48-50] and has been validated as a suitable biomarker for monitoring benzene exposure in occupational and environmental exposure as well as in tobacco smoke exposure studies [36,48-51]. Benzene is a known carcinogenic agent [52].	Elimination half-life of 12.8 hrs [49-51].
24 Hydroxy-polycyclic aromatic hydrocarbons (PAH) including 1-hydroxypryene (1-HOP)	Carcinogen exposure	Urine	LC/MS/MS	A metabolite of pyrene and an established biomarker of tobacco carcinogen (PAH) uptake [38].	Elimination half-life (T1/2) of 9.8 h[53] following inhalation of pyrene, although a longer half-life (18-20 h) has also been reported [54-56].
Thiocyanate	Cyanide exposure	Urine	Ion Chromatography /MS	Long term marker; May distinguish smokers from nonsmokers or smokers and users of smokeless tobacco.	1-2 weeks [30]
Cardiovascular Reactivity				Blood pressure, heart rate, arterial oxygen saturation	Acute reactivity to smoking
Solanesol	Marker for total tobacco	Cigarette Filters	HPLC MS	From Watson et al., 2004: "The measurement of solanesol content deposited in the filter of a cigarette butt meets the	Shelf-Life of approximately 2 years [58]

Biomarker	Detection	Collection	Method of Analysis	Use	Half-Life
	exposure			criteria for a good smoke marker. The amount of deposited solanesol is a function of the mainstream smoke delivery of nicotine and tar. Its measurement provides a noninvasive means to access mainstream cigarette smoke exposure, allows for both individual data on a per cigarette basis and cumulative exposure estimates, and does not require real-time monitoring. The data also suggest that solanesol analysis is applicable for a relatively long time after smoking has occurred. The solanesol in cigarette butt filter method appears to provide adequate sensitivity and reproducibility to provide dosage estimates. This method utilizes automated sample injection and a 7-min LC/MS run time to achieve rapid and accurate solanesol quantitation with high sample throughput."[57]	

HPLC: High-performance liquid chormatography   **MS**: Mass spectrometry