Attachment I
Table of Biomarkers

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

Biomarker	Detection	Collection	Method of Analysis	Use	Half-Life
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

Biomarker	Detection	Collection	Method of Analysis	Use	Half-Life
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 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]

Biomarker	Detection	Collection	Method of Analysis	Use	Half-Life
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-carboxyethyl)-L-cysteine	Marker for acrolein exposure	Urine	LC/MS/MS	Acrolein is a toxic chemical in tobacco smoke formed by combustion of tobacco [44,45].	Acrolein is highly reactive and no 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]

Biomarker	Detection	Collection	Method of Analysis	Use	Half-Life
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].	
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].

Biomarker	Detection	Collection	Method of Analysis	Use	Half-Life
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

Biomarker	Detection	Collection	Method of Analysis	Use	Half-Life
Solanesol	Marker for total tobacco exposure	Cigarette Filters	HPLC MS	From Watson et al., 2004: "The measurement of solanesol content deposited in the filter of a cigarette butt meets the 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]	Shelf-Life of approximately 2 years [58]

HPLC: High-performance liquid chormatography

MS: Mass spectrometry

Attachment J

(1) NCI. Risks Associated with Smoking Cigarettes with Low Machine-Measured Yields of Tar and Nicotine. Smoking and Tobacco Monograph No. 13, NIH Pub. No. 02-5074. 2001. Bethesda, MD, U.S. Department of Health and Human Services, National Institutes of Health, National Cancer Institute.
Ref Type: Report

(2) Djordjevic MV, Stellman SD, Zang E. Doses of nicotine and lung carcinogens delivered to cigarette smokers. *J Natl Cancer Inst.* 2000;92:106-111.

(3) Bridges RB, Combs JG, Humble JW, Turbek JA, Rehm SR, Haley NJ. Puffing topography as a determinant of smoke exposure. *Pharmacol Biochem Behav.* 1990;37:29-39.

(4) Kolonen S, Tuomisto J, Puustinen P, Airaksinen MM. Effects of smoking abstinence and chain-smoking on puffing topography and diurnal nicotine exposure. *Pharmacol Biochem Behav.* 1992;42:327-332.

(5) Federal Trade Commission. Cigarette Report for 2001. 2003.
Ref Type: Report

(6) Djordjevic MV, Fan J, Ferguson S, Hoffmann D. Self-regulation of smoking intensity. Smoke yields of the low-nicotine, low-'tar' cigarettes. *Carcinogenesis.* 1995;16:2015-2021.

(7) Fischer S, Spiegelhalder B, Preussmann R. Influence of smoking parameters on the delivery of tobacco-specific nitrosamines in cigarette smoke--a contribution to relative risk evaluation. *Carcinogenesis.* 1989;10:1059-1066.

(8) Bernert, J. T. Biomarkers of environmental tobacco smoke exposure. 2004.
Ref Type: Slide

(9) Russell MA, Jarvis M, Iyer R, Feyerabend C. Relation of nicotine yield of cigarettes to blood nicotine concentrations in smokers. *Br Med J.* 1980;280:972-976.

(10) National Center for Chronic Disease Prevention and Health Promotion. Cigarette Smoking-Related Mortality. Tobacco Information and Prevention Source (TIPS) . 2006.
Ref Type: Electronic Citation

(11) Mendelson JH, Sholar MB, Goletiani N, Siegel AJ, Mello NK. Effects of low- and high-nicotine cigarette smoking on mood states and the HPA axis in men. *Neuropsychopharmacology.* 2005;30:1751-1763.

(12) Pickworth WB, Moolchan ET, Berlin I, Murty R. Sensory and physiologic effects of menthol and non-menthol cigarettes with differing nicotine delivery. *Pharmacol Biochem Behav.* 2002;71:55-61.

(13) Lee EM, Malson JL, Moolchan ET, Pickworth WB. Quantitative comparisons between a nicotine delivery device (Eclipse) and conventional cigarette smoking. *Nicotine Tob Res.* 2004;6:95-102.

(14) Pickworth W.B., Houlgate, P., Schorp, M., Dixon, M., Borgerding, M. F., and Zaatari, G. A review of human smoking behavior data and recommendations for a new ISO standard for the machine smoking of cigarettes. Report of the *AD HOC* WG9 Smoking Behaviour Review Team to ISO/TC 126 WG9. 8-10-2005.
Ref Type: Report

(15) Surgeon General. Tobacco use as drug dependence. Centers for Disease Control and Prevention. Chapter 4, 145-239. 1988.
Ref Type: Report

(16) Caraballo RS, Giovino GA, Pechacek TF et al. Racial and ethnic differences in serum cotinine levels of cigarette smokers: Third National Health and Nutrition Examination Survey, 1988-1991. *JAMA.* 1998;280:135-139.

(17) US Department of Commerce, Census Bureau. National Cancer Institute Sponsored Tobacco Use Supplement to the Current Population Survey (2001-2002). Data files. 2004.
Ref Type: Report

(18) Cohen J. *Statistical power analyses for the behavioral sciences*. 2nd ed. New York: Erlbaum; 1988.

(19) Ahijevych K, Parsley LA. Smoke constituent exposure and stage of change in black and white women cigarette smokers. *Addict Behav.* 1999;24:115-120.

(20) Clark PI, Gautam S, Gerson LW. Effect of menthol cigarettes on biochemical markers of smoke exposure among black and white smokers. *Chest.* 1996;110:1194-1198.

(21) Eissenberg T, Adams C, Riggins EC, III, Likness M. Smokers' sex and the effects of tobacco cigarettes: subject-rated and physiological measures. *Nicotine Tob Res.* 1999;1:317-324.

(22) Perez-Stable EJ, Herrera B, Jacob P, III, Benowitz NL. Nicotine metabolism and intake in black and white smokers. *JAMA.* 1998;280:152-156.

(23) Tabachnick BG, Fidell LS. *Using multivariate statistics*. 4th ed. Boston: Allyn and Bacon; 2001.

(24) Hecht SS, Carmella SG, Chen M et al. Quantitation of urinary metabolites of a tobacco-specific lung carcinogen after smoking cessation. *Cancer Res.* 1999;59:590-596.

(25) Benowitz NL, Jacob IP. Individual differences in Nicotine kinetics and metabolism in humans. *Pharmacokinetics, metabolism, and pharmaceutics of drugs of abuse*. NIDA Research Monograph 173, NIH Publication No. 97-4141 ed. Bethesda, MD: U.S.

Department of Health and Human Services, National Institutes of Health, National Institute on Drug Abuse; 1997:48-64.

(26) Vivometrics. Vivometrics LifeShirt® system receives U.S. Food and Drug Administration 510(K) market clearance. http://www.vivometrics.com/site/press_pr20020422.html Press Release. 4-22-2002. Ref Type: Generic

(27) Paakko P, Kokkonen P, Anttila S, Kalliomaki PL. Cadmium and chromium as markers of smoking in human lung tissue. *Environ Res.* 1989;49:197-207.

(28) Navas-Acien A, Selvin E, Sharrett AR, Calderon-Aranda E, Silbergeld E, Guallar E. Lead, cadmium, smoking, and increased risk of peripheral arterial disease. *Circulation.* 2004;109:3196-3201.

(29) Friberg L, Piscator M, Nordberg GF, Kjellstrom T. *Cadmium in environment*. 2nd ed. Boca Raton, FL.: CRC Press; 1974.

(30) Institute of Medicine. *Clearing the smoke: Assessing the science base for tobacco harm reduction*. Washington DC: National Academy Press; 2001.

(31) Murphy SE, Link CA, Jensen J et al. A comparison of urinary biomarkers of tobacco and carcinogen exposure in smokers. *Cancer Epidemiol Biomarkers Prev.* 2004;13:1617-1623.

(32) Benowitz NL, Jacob P, III. Metabolism of nicotine to cotinine studied by a dual stable isotope method. *Clin Pharmacol Ther.* 1994;56:483-493.

(33) Bernert JT, Jr., McGuffey JE, Morrison MA, Pirkle JL. Comparison of serum and salivary cotinine measurements by a sensitive high-performance liquid chromatography-tandem mass spectrometry method as an indicator of exposure to tobacco smoke among smokers and nonsmokers. *J Anal Toxicol.* 2000;24:333-339.

(34) Benowitz NL, Jacob P, III. Trans-3'-hydroxycotinine: disposition kinetics, effects and plasma levels during cigarette smoking. *Br J Clin Pharmacol.* 2001;51:53-59.

(35) Benowitz NL, Jacob P, III. Trans-3'-hydroxycotinine: disposition kinetics, effects and plasma levels during cigarette smoking. *Br J Clin Pharmacol.* 2001;51:53-59.

(36) Hecht SS. Biochemistry, biology, and carcinogenicity of tobacco-specific N-nitrosamines. *Chem Res Toxicol.* 1998;11:559-603.

(37) Hecht SS, Hoffman D. N-nitroso compounds and man: sources of exposure, endogenous formation and occurrence in body fluids. *Eur J Cancer Prev.* 1998;7:244-246.

(38) Hecht SS. Human urinary carcinogen metabolites: biomarkers for investigating tobacco and cancer. *Carcinogenesis.* 2002;23:907-922.

(39) Hecht SS. Tobacco smoke carcinogens and lung cancer. *J Natl Cancer Inst.* 1999;91:1194-1210.

(40) Hoffmann D, Hoffmann I, El Bayoumy K. The less harmful cigarette: a controversial issue. a tribute to Ernst L. Wynder. *Chem Res Toxicol.* 2001;14:767-790.

(41) Carmella SG, Akerkar S, Hecht SS. Metabolites of the tobacco-specific nitrosamine 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone in smokers' urine. *Cancer Res.* 1993;53:721-724.

(42) Carmella SG, Akerkar SA, Richie JP, Jr., Hecht SS. Intraindividual and interindividual differences in metabolites of the tobacco-specific lung carcinogen 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) in smokers' urine. *Cancer Epidemiol Biomarkers Prev.* 1995;4:635-642.

(43) Karreth S, Lenk W. The metabolism of 4-aminobiphenyl in rat. I. Reaction of N-hydroxy-4-aminobiphenyl with rat blood in vivo. *Xenobiotica.* 1991;21:417-428.

(44) Linhart I, Frantik E, Vodickova L, Vosmanska M, Smejkal J, Mitera J. Biotransformation of acrolein in rat: excretion of mercapturic acids after inhalation and intraperitoneal injection. *Toxicol Appl Pharmacol.* 1996;136:155-160.

(45) U.S.Environmental Protection Agency. Toxicological review of acrolein (CAS No. 107-02-8): In Support of Summary Information on the Integrated Risk Information System (IRIS). EPA/635/R-03/003. 2003. Washington, D.C.
Ref Type: Report

(46) Agency for Toxic Substances and Disease Registry (ATSDR). Toxicological profile for 1,3 butadiene. Public Health Service. 1992. Atlanta, GA, US Department of Health and Human Services.
Ref Type: Generic

(47) U.S.Environmental Protection Agency. Health Assessment of 1,3-Butadiene. EPA/600/P-98/001F. 2002. Washington, DC, National Center for Environmental Assessment.
Ref Type: Report

(48) Melikian AA, Qu Q, Shore R et al. Personal exposure to different levels of benzene and its relationships to the urinary metabolites S-phenylmercapturic acid and trans,trans-muconic acid. *J Chromatogr B Analyt Technol Biomed Life Sci.* 2002;778:211-221.

(49) Qu Q, Cohen BS, Shore R et al. Benzene exposure measurement in shoe and glue manufacturing: a study to validate biomarkers. *Appl Occup Environ Hyg.* 2003;18:988-998.

(50) Qu Q, Shore R, Li G et al. Validation and evaluation of biomarkers in workers exposed to benzene in China. *Res Rep Health Eff Inst.* 2003;1-72.

(51) Qu Q, Melikian AA, Li G et al. Validation of biomarkers in humans exposed to benzene: urine metabolites. *Am J Ind Med.* 2000;37:522-531.

(52) Bayliss, D., Chen, C., Sonawane, B., and Valcovic, L. Carcinogenic effects of benzene: An update. EPA/600/P-97/001A. 1997. Washington, DC, U.S. Environmental Protection Agency, National Center for Environmental Assessment.
Ref Type: Report

(53) Brzeznicki S, Jakubowski M, Czerski B. Elimination of 1-hydroxypyrene after human volunteer exposure to polycyclic aromatic hydrocarbons. *Int Arch Occup Environ Health.* 1997;70:257-260.

(54) Jongeneelen FJ, Bos RP. Excretion of pyrene and hydroxypyrene in urine. *Cancer Lett.* 1990;51:175-179.

(55) Buckley TJ, Lioy PJ. An examination of the time course from human dietary exposure to polycyclic aromatic hydrocarbons to urinary elimination of 1-hydroxypyrene. *Br J Ind Med.* 1992;49:113-124.

(56) van Schooten FJ, Jongeneelen FJ, Hillebrand MJ et al. Polycyclic aromatic hydrocarbon-DNA adducts in white blood cell DNA and 1-hydroxypyrene in the urine from aluminum workers: relation with job category and synergistic effect of smoking. *Cancer Epidemiol Biomarkers Prev.* 1995;4:69-77.

(57) Watson C, McCraw J, Polzin G, Ashley D. Development of a method to assess cigarette smoke intake. *Environ Sci Technol.* 2004;38:248-253.

(58) Jenkins RA, Guerin MT, Tomkins BA. *The chemistry of environmental tobacco smoke: Composition and measurement*. Boca Raton, FL: CRC Press LLC; 2000.