

Biomarkers of Tobacco Exposure: Summary of an FDA-Sponsored Public Workshop

Cindy M. Chang¹, Selvin H. Edwards¹, Aarthi Arab¹, Arseima Y. Del Valle-Pinero¹, Ling Yang¹, and Dorothy K. Hatsukami²

Abstract

Since 2009, the FDA Center for Tobacco Products (CTP) has had the authority to regulate the manufacturing, distribution, and marketing of tobacco products in order to reduce the death and disease caused by tobacco use. Biomarkers of exposure pertain to actual human exposure to chemicals arising from tobacco use and could play an important role across a number of FDA regulatory activities, including assessing new and modified-risk tobacco products and identifying and evaluating potential product standards. On August 3–4, 2015, FDA/CTP hosted a public workshop focused on biomarkers of exposure with participants from government, industry, academia, and other organizations. The

workshop was divided into four sessions focused on: (i) approaches to evaluating and selecting biomarkers; (ii) biomarkers of exposure and relationship to disease risk; (iii) currently used biomarkers of exposure and biomarkers in development; and (iv) biomarkers of exposure and the assessment of smokeless tobacco and electronic nicotine delivery systems. This article synthesizes the main findings from the workshop and highlights research areas that could further strengthen the science around biomarkers of exposure and help determine their application in tobacco product regulation. *Cancer Epidemiol Biomarkers Prev*; 26(3); 291–302. ©2016 AACR.

Introduction

The FDA Center for Tobacco Products (CTP), established in 2009 by the Family Smoking Prevention and Tobacco Control Act (Tobacco Control Act), has the broad authority to regulate the manufacturing, distribution, and marketing of tobacco products with the ultimate goal of reducing harm caused by tobacco use (1). Under the 2009 Tobacco Control Act, the FDA regulates cigarettes, roll-your-own tobacco, and smokeless tobacco. In 2016, FDA finalized a rule extending the FDA's authority to regulate electronic nicotine delivery systems (ENDS), cigars, hookah tobacco, pipe tobacco, and nicotine gels (2).

Biomarkers could play an important role across FDA's regulatory authorities, including the review of premarket tobacco product applications and modified-risk tobacco product applications, which includes an assessment of the potential health risks of the tobacco product and the impact of product use on the population as a whole. Biomarkers could also be useful in identifying and evaluating potential product standards that involve the reduction or elimination of an additive, constituent, or other components in tobacco products. Biomarkers of exposure are defined as the "chemical, or its metabolite, or the product of an interaction between a chemical and some target molecule or cell, that is

measured in a compartment in an organism" (3). They capture actual human exposure to tobacco products or internal doses in contrast to external measures of exposure. For example, for decades, the risks of low-yield cigarettes were underestimated because those risks were assumed to be well correlated with machine-determined cigarette smoke yields. However, machine-determined smoke yields did not account for changes in people's smoking behaviors, which included taking deeper and longer puffs to get more nicotine, as well as blocking ventilation holes intended to dilute smoke concentration (4). Studies measuring exposure biomarkers would have provided more accurate assessments of risk, as these biomarkers are a result of smoking behavior and not the characteristics of the cigarette itself.

As exposure biomarkers continue to be studied extensively in tobacco research, their practical utility in the tobacco regulatory context should be carefully considered. For example, although a large number of tobacco exposure biomarkers can be measured, it may not always be practical to measure every available biomarker in every evaluation. Thus, a first step is to identify how to select the most suitable biomarkers for regulatory use by better understanding the strengths and limitations of both well-established and promising biomarkers of tobacco exposure.

Materials and Methods

On August 3–4, 2015, the FDA/CTP hosted a public workshop on biomarkers of exposure to gather information on: (i) approaches to assessing and selecting biomarkers of exposure; (ii) identifying biomarkers of exposure that may be useful in tobacco product regulation; and (iii) identifying areas of research that may help to advance the field (5). The workshop consisted of participants from government, industry, academia, and other organizations (Table 1; Supplementary Table 1). The workshop was divided into four sessions focused on: (i) approaches to evaluating and selecting biomarkers; (ii) biomarkers of exposure

¹Office of Science, Center for Tobacco Products, Food and Drug Administration, Silver Spring, Maryland. ²Department of Psychiatry, Tobacco Research Programs, University of Minnesota, Minneapolis, Minnesota.

Note: Supplementary data for this article are available at Cancer Epidemiology, Biomarkers & Prevention Online (<http://cebp.aacrjournals.org/>).

Corresponding Author: Cindy M. Chang, Food and Drug Administration, Building 71, Room G335, 10903 New Hampshire Avenue, Silver Spring, MD 20993-0002. Phone: 301-796-9267; Fax: 301-595-1138; E-mail: Cindy.Chang@fda.hhs.gov

doi: 10.1158/1055-9965.EPI-16-0675

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Table 1. FDA/CTP "Biomarkers of Tobacco Exposure" Workshop Sessions, August 3–4, 2015

Session/title	Presenter	Organization
Session 1: Strategies for Identifying Potential Biomarkers for CTP Regulatory Use		
Biomarker Basics: Validation, Qualification, Translation	William B. Mattes, PhD, DABT	FDA National Center for Toxicological Research
Biomarker Utility and Acceptance in Drug Development and Clinical Trials: An FDA Regulatory Perspective	Christopher Leptak, MD, PhD	FDA Center for Drug Evaluation and Research
Biomarkers in Risk Assessment: Use, Challenges, and Lessons Learned	Cecilia Tan, MS, MBA, PhD	EPA National Exposure Research Laboratory
Biomonitoring at the National Institute for Occupational Safety and Health	CAPT D. Gayle DeBord, PhD	CDC National Institute for Occupational Safety and Health
Characterization of Biomarkers to Assess the Utility of Potential Reduced Exposure Tobacco Products: Status Update	Dorothy Hatsukami, PhD	University of Minnesota
Integrating Biomarkers into Tobacco Regulatory Science Systems	Raymond S. Niaura, PhD	Schroeder Institute for Tobacco Research and Policy Studies
Panel Discussion on Strategies for Identifying Potential Biomarkers for CTP Regulatory Use	William B. Mattes, PhD, DABT	FDA National Center for Toxicological Research
	Christopher Leptak, MD, PhD	FDA Center for Drug Evaluation and Research
	Cecilia Tan, MS, MBA, PhD	EPA National Exposure Research Laboratory
	CAPT D. Gayle DeBord, PhD	CDC National Institute for Occupational Safety and Health
	Dorothy Hatsukami, PhD	University of Minnesota
	Raymond S. Niaura, PhD	Schroeder Institute for Tobacco Research and Policy Studies
	Stephen S. Hecht, PhD	University of Minnesota
Session 2: Biomarkers of Exposure and Relationship with Disease Risk		
A Case Study on the Use of Biomonitoring at EPA: A Cumulative Assessment on Phthalates	Matthew Lorber, MS	EPA Office of Research and Development
Biomarkers of Exposure to Tobacco Smoke and Risk of Cancer—The Shanghai Cohort Study	Jian-Min Yuan, MD, PhD	University of Pittsburgh
Coherence of Human Findings with Animal Tumor Studies	Irina Stepanov, PhD	University of Minnesota
Two Different Approaches for the Use of Biomarkers of Exposure in the Risk Assessment of Tobacco Products	Kristin M. Marano, MS, MPH, CPH	RAI Services Company
Metals as Non-specific Tobacco Biomarkers of Cardiovascular Disease	Miranda Jones, MHS, PhD	Johns Hopkins University Bloomberg School of Public Health
Relationship Between Biomarkers of Tobacco Exposure and Disease Risk	Mohamadi Sarkar, M.Pharm, PhD, FCCP	Altria Client Services
Leveraging Population Studies for Tobacco Biomarker Research	Neal Freedman, PhD, MPH	National Institutes of Health/National Cancer Institute
Panel Discussion on Biomarkers of Exposure and Relationship with Disease Risk	Matthew Lorber, MS	EPA Office of Research and Development
	Jian-Min Yuan, MD, PhD	University of Pittsburgh
	Irina Stepanov, PhD	University of Minnesota
	Kristin M. Marano, MS, MPH, CPH	RAI Services Company
	Miranda Jones, MHS, PhD	Johns Hopkins University Bloomberg School of Public Health
	Mohamadi Sarkar, M.Pharm, PhD, FCCP	Altria Client Services
	Neal Freedman, PhD, MPH	National Institutes of Health/National Cancer Institute
	Cecilia Tan, MS, MBA, PhD	EPA National Exposure Research Laboratory
Session 3: Identifying Biomarkers of Tobacco Exposure for CTP Regulatory Use		
Biomarkers of Exposure to Tobacco Carcinogens and Toxicants	Stephen S. Hecht, PhD	University of Minnesota
Biomarkers of Tobacco Exposure for CTP Regulatory Use: Utility and Challenges	Kimberly Frost-Pineda, PhD, MPH	Altria Client Services
Lessons from Clinical Studies Using Biomarkers of Exposure to Assess Toxicant Exposure	Christopher Proctor, BSc (Hon), PhD	British American Tobacco Research and Development
Nicotine and Tobacco Alkaloids: Biomarkers of Tobacco Use and Exposure	Gideon St. Helen, PhD	University of California, San Francisco
Importance of Analytical Methods for Biomarker Measurement	Benjamin Blount, PhD	CDC National Center for Environmental Health
Biomarkers of Tobacco Exposure in Exhaled Breath	Marielle C. Brinkman	Battelle Public Health Center for Tobacco Research
Panel Discussion on Identifying Biomarkers of Tobacco Exposure for CTP Regulatory Use	Stephen S. Hecht, PhD	University of Minnesota
	Kimberly Frost-Pineda, PhD, MPH	Altria Client Services
	Christopher Proctor, BSc (Hon), PhD	British American Tobacco Research and Development
	Gideon St. Helen, PhD	University of California, San Francisco
	Benjamin Blount, PhD	CDC National Center for Environmental Health
	Marielle C. Brinkman	Battelle Public Health Center for Tobacco Research

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Table 1. FDA/CTP "Biomarkers of Tobacco Exposure" Workshop Sessions, August 3–4, 2015 (Cont'd)

Session/title	Presenter	Organization
Session 4: Biomarkers of Exposure in Smokeless Tobacco and ENDS		
Biomarkers of Exposure for Smokeless Tobacco Products	Dorothy Hatsukami, PhD	University of Minnesota
A Multi-Center, Cross-Sectional Study of U.S. Tobacco Users, Including Both Exclusive and Dual Use Of Cigarettes, Moist Snuff and a U.S. Snus Brand: Biomarkers Of Exposure	Michael F. Borgerding, PhD	RAI Services Company
New Biomarkers of Tobacco Exposure	Peyton Jacob III, PhD	University of California, San Francisco
Biomarkers of Exposure to Nicotine and Toxicants Among Exclusive Users of Electronic Cigarettes and Dual Users of Electronic and Tobacco Cigarettes	Maciej Goniewicz, PharmD, PhD	Roswell Park Cancer Institute
Toxicants in Biofluids Originated from Stable Isotopes of Propylene Glycol in eCigarettes	Raymond Farmen, PhD	Celerion
Panel Discussion on Biomarkers of Exposure in Smokeless Tobacco and ENDS	Dorothy Hatsukami, PhD	University of Minnesota
	Michael F. Borgerding, PhD	RAI Services Company
	Peyton Jacob III, PhD	University of California, San Francisco
	Maciej Goniewicz, PharmD, PhD	Roswell Park Cancer Institute
	Raymond Farmen, PhD	Celerion
	Stephen S. Hecht, PhD	University of Minnesota

and relationship to disease risk; (iii) currently used biomarkers of exposure and biomarkers in development; and (iv) biomarkers of exposure and the assessment of smokeless tobacco and ENDS. A panel discussion followed each session's presentations. FDA did not solicit advice or consensus at the workshop, but rather facilitated an exchange of scientific information.

Results

This report is an integration of the different viewpoints expressed at the workshop. Topics are organized as follows: (i) biomarker validation and qualification at other agencies; (ii) the state of the literature on existing tobacco-related exposure biomarkers; (iii) evaluation of noncigarette tobacco products; (iv) biomarkers in development; and (v) biomonitoring approaches.

Biomarker validation and qualification at other agencies

Biomarkers serve different purposes at other FDA centers and other agencies. At the FDA's Center for Drug Evaluation and Research (CDER), biomarkers have been used to assess biological responses to a therapeutic intervention. The Environmental Protection Agency (EPA) and the National Institute for Occupational Safety and Health (NIOSH) use biomonitoring ("method for assessing human exposure to chemicals by measuring the chemicals or their metabolites in human tissues or specimens, such as blood and urine") to assess the magnitude of health risks of environmental and occupational exposures and to monitor impacts of regulatory action (e.g., removal of lead from gasoline) or occupational interventions (e.g., personal protective equipment for asphalt workers; ref. 6).

In any context, having a validated method for measuring a particular biomarker is essential. Validation is the "fit-for-purpose process of assessing the assay and its measurement performance characteristics, determining the range of conditions under which the assay will give reproducible and accurate data" (7). Fit-for-purpose elements of biomarker method validation include precision, accuracy, sensitivity, and specificity (8). The level of validation required depends on the category of the biomarker assay: definitive quantitative (e.g., mass spectrometry), relative quantitative (e.g., enzyme-linked immunosorbent assays [ELISA]), quasi-quantitative (e.g.,

immunogenicity immunoassays), and qualitative (e.g., immunohistochemistry; ref. 9).

Qualification has regulatory implications. A key concept is defining a context of use or the boundary within which the biomarker is used and interpreted to enable an action or decision (10, 11). At the FDA/CDER, the Biomarker Qualification Program (BQP) is a formalized process for qualifying biomarkers. A biomarker is qualified when a community (scientific, medical, and regulatory) comes to a consensus that its measurement is not only analytically valid, but also the test result has a specific physiologic, toxicologic, pharmacologic, or clinical meaning (10). A biomarker qualified through BQP for a particular context of use would not need further qualification data to be resubmitted for future submissions, such as those for new drugs; this increases efficiency of parties within and outside of the FDA. BQP also serves to foster biomarker development by groups such as consortia of industry stakeholders. Finally, BQP provides a pathway by which academic investigators can interact with the FDA in a nonregulatory manner. Thus, CDER's collaborative qualification process has advanced the field faster and more efficiently. One example of a successful BQP application (submitted by the Predictive Safety Testing Consortium) is a set of seven urinary kidney biomarkers (KIM-1, albumin, total protein, β 2-microglobulin, cystatin C, clusterin, and trefoil factor-3) found to be acceptable for detecting acute drug-induced nephrotoxicity in rats. Analysis included the use of receiver operating characteristic (ROC) curves to evaluate performance of the proposed biomarkers compared with existing biomarkers against histopathology, the gold standard for structural changes in the kidney. ROC curves showed that some biomarkers had improved specificity and sensitivity for detecting tubular and/or glomerular renal injury compared with existing biomarkers. All seven were determined to be acceptable biomarkers within a very specific context of use (12).

State of the literature on existing tobacco-related exposure biomarkers

A broad body of evidence exists for tobacco-related exposure biomarkers, ranging from analytical validation to disease risk (13–16). Analytical method validation is the first step in determining the suitability of tobacco-related exposure biomarkers, many of which are quantified by liquid chromatography tandem

Table 2. 20 HPHCs and examples of corresponding tobacco exposure biomarkers

Group	HPHC	Biomarker(s)
Variable gases	Carbon monoxide ^c	Carbon monoxide (exhaled breath); carboxyhemoglobin (blood)
Tobacco alkaloids	Nicotine ^{c,s}	Nicotine (plasma); TNEs (urine), cotinine (urine)
TSNA	NNK ^{c,s}	NNK (urine), NNAL (urine), NNAL-N-Gluc (urine), NNAL-O-Gluc (urine)
TSNA	NNN ^{c,s}	NNN (urine), NNN-Gluc (urine)
PAHs	Benzo[a]pyrene and other proxies for PAH ^{c,s}	BAP-tetrol (urine), 3-hydroxy-BAP (urine), 1-Hydroxypyrene (urine), PheT (urine)
VOCs	1,3-Butadiene ^c	Monohydroxybutenyl mercapturic acid (MHBMA; urine)
VOCs	Acetaldehyde ^{c,s}	N2-Ethylidene-dGuo (tissue, blood)
VOCs	Acrolein ^c	N-Acetyl-S- (3-Hydroxypropyl)-L-Cysteine (HPMA; urine)
VOCs	Acrylonitrile ^c	2-Cyanoethylmercapturic acid (CEMA; urine)
VOCs	Benzene ^c	N-Acetyl-S-(phenyl)-L-cysteine (SPMA; urine)
VOCs	Crotonaldehyde ^{c,s}	3-Hydroxy-1-methylpropylmercapturic acid (HMPMA; urine)
VOCs	Formaldehyde ^{c,s}	N ⁶ -Hydroxymethyl deoxyadenosine adducts (tissue, blood)
VOCs	Isoprene ^c	
VOCs	Toluene ^c	N-Acetyl-S-benzyl-cysteine (SBMA; urine), N-Acetyl-S-(benzyl)-L-cysteine (BMA; urine)
Aromatic amines	1-Aminonaphthalene ^c	1-Aminonaphthalene (urine)
Aromatic amines	2-Aminonaphthalene ^c	2-Aminonaphthalene (urine)
Aromatic amines	4-Aminobiphenyl ^c	4-aminobiphenyl (urine); 4-aminobiphenyl hemoglobin adduct (blood)
Heavy metals	Arsenic ^{c,s}	Total arsenic (urine)
Heavy metals	Cadmium ^{c,s}	Cadmium (urine, blood)
Acyclic amines	Ammonia ^c	

Abbreviations: Gluc, glucuronides; BAP, benzo[a]pyrene.

^cHPHCs in cigarette smoke or filler.

^sHPHCs in smokeless tobacco.

mass spectrometry, gas chromatography tandem mass spectrometry, and related techniques (3). The methods must exhibit suitable sensitivity to consistently detect target levels of the biomarker, specificity to ensure that the compound measured is the target compound, accuracy to ensure the measurement is close to the true value, and precision to ensure minimal variance with repeated measurements (17).

In addition to analytical method validation, tobacco-related exposure biomarkers have been further characterized with respect to tobacco product use. Specifically, the characterizations include whether or not the biomarker distinguishes between tobacco users versus nonusers; has a dose–response relationship with the amount of product use or constituent yields; and responds to cessation, reduction in use, or change in constituent yields (15, 18). Tobacco-related exposure biomarkers have also been assessed with respect to disease risk in epidemiologic studies (16, 19–23). For example, case–control studies nested in the Shanghai cohort, a prospective cohort of over 18,000 men with 25-year follow-up, examined the association between tobacco-related nitrosamines and cancer risk (24). Furthermore, the extent to which biomarkers distinguish tobacco products in the expected way (combusted vs. noncombusted products) and the convergence of findings across studies on specific tobacco biomarkers can be considered.

The FDA has identified a set of 93 harmful and potentially harmful constituents (HPHC) and an abbreviated list of 20 HPHCs in tobacco products and tobacco smoke. The criteria for HPHC selection are based on whether the constituent is identified as one or more of the following: a carcinogen, a respiratory toxicant, a cardiovascular toxicant, a reproductive or developmental toxicant, or an addictive chemical and chemical compound (25). Many widely investigated parent chemical substances or their metabolites represent HPHC exposure. HPHCs represent several chemical classes in tobacco or mainstream cigarette smoke, including nicotine and tobacco alkaloids, carbon monoxide, tobacco-specific nitrosamines (TSNAs), polycyclic aromatic hydrocarbons (PAHs), volatile organic compounds (VOCs), carcinogenic aromatic amines, and metals (Table 2).

The following sections summarize commonly used biomarkers belonging to the different chemical classes and biomarkers in development.

Nicotine and tobacco alkaloids. Nicotine is the most abundant alkaloid found in the tobacco leaf (26). It is addictive and is the primary driver of continued tobacco use. The plasma nicotine level is effective for assessing nicotine intake and its pharmacologic effects within a narrow window of exposure; however, it is not ideal for monitoring tobacco exposure over extended periods due to its short half-life (2 hours). In contrast, blood, salivary, or urinary cotinine, a metabolite of nicotine, is more advantageous as a tobacco biomarker due to its longer elimination time and half-life (16–18 hours; ref. 27). For these reasons, cotinine is widely measured. In several large epidemiologic studies of smokers and nonsmokers, blood cotinine concentrations ranged from 100 to 350 ng/mL for daily smokers (27–29). The cotinine level also correlates with cigarette-per-day (CPD) consumption and, as a marker of overall tobacco exposure, cotinine is dose-dependently associated with lung cancer risk as demonstrated in the Shanghai cohort study (20). However, blood and urinary cotinine levels are affected by several factors, including pregnancy, gender, genetic variability, and certain diseases (29–31). Demographic-specific cotinine cut points permit differentiation between smokers and from nonsmokers (32). Nicotine and cotinine measurements do not completely explain total nicotine intake due to their metabolic transformation to water-soluble N-oxides, glucuronides, and *trans*-3'-hydroxycotinine passed in urine (27).

In contrast, total nicotine equivalents (TNE) is the sum of urinary nicotine, cotinine, and several metabolites in the nicotine metabolic profile; TNE is considered the gold standard for daily nicotine intake. Unlike cotinine, TNE accommodates factors that influence nicotine metabolism and exhibits strong correlation with several tobacco exposure biomarkers, including hydroxy-PAHs and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL; refs. 33, 34).

Finally, anabasine and anatabine are minor tobacco alkaloids that are established biomarkers of tobacco constituent intake.

Neither anabasine nor anatabine are present in nicotine replacement therapy (NRT) and thus provide a reliable estimate of nicotine intake from tobacco use, even in the presence of concomitant NRT use. Scientific evidence indicates that anabasine and anatabine urinary levels exceeding 2 ng/mL are highly specific for tobacco use while using NRT (35).

Carbon monoxide (CO). CO is a product of all incomplete combustion of organic materials and is present in tobacco smoke as well as nontobacco sources (36). CO exposure can be measured in the blood as carboxyhemoglobin (as percent saturation of hemoglobin) and in exhaled breath (as concentration of CO in parts per million). Both are short-term markers with half-lives around 4 to 6 hours (37, 38). The close correlation between exhaled CO and carboxyhemoglobin is well documented and thus they serve as proxies for one another (39). CO biomarkers are well established with respect to distinguishing tobacco users from nonusers, responding to cessation and reduced use, and having a dose response with tobacco use (40). For example, in the Total Exposure Study, a cross-sectional study of U.S. cigarette smokers ($n = 3,585$) and nonsmokers ($n = 1,077$), carboxyhemoglobin distinguished smokers from nonsmokers with a 3.6-fold difference (41). Additionally, CO is considered one of the most useful biomarkers for verifying smoking cessation in clinical trials (37, 42).

TSNAs. TSNAs are N-nitroso-derivatives of pyridine-alkaloids (e.g., nicotine and nornicotine) and are present in tobacco and cigarette smoke. The most well-studied TSNAs are nicotine-derived nitrosamine ketone (NNK) and N-nitrososornicotine (NNN), which are classified as group 1 carcinogens (43).

NNAL, the primary metabolite of NNK, is the most widely investigated TSNA biomarker. Several others include NNK, NNN, NNN-glucuronide, NNAL-N-glucuronide, NNAL-O-glucuronide, N'-nitrosoanabasine (NAB), NAB-glucuronide, N'-nitrosoanatabine (NAT), and NAT-glucuronide (44–46). NNAL is particularly advantageous as a biomarker of exposure because it is completely tobacco-specific and has a long half-life in biological fluids, around 10 to 45 days (47, 48). Several large epidemiologic studies, such as the National Health and Nutrition Examination Survey (NHANES), the Total Exposure Study, and the Multiethnic Cohort Study, report typical ranges of 1 to 2 pmol NNAL/mL of urine for smokers (41, 49, 50, 51). In nonsmokers exposed to secondhand smoke, NNAL is 1% to 5% of the amount in smokers. NNAL is never found in nontobacco users who are not exposed. Studies examining NNAL as a biomarker indicate that 34% of NNAL remains up to 1 week following smoking cessation (47, 48). In addition, NNAL correlates with other tobacco-specific markers, including cotinine and TNE (21, 52). NNAL has been effective in the investigation of NNK exposure in nonsmokers, including newborns, children, and women living with smoking partners (53–55). NNAL levels and its glucuronides are elevated in nonsmokers exposed to secondhand smoke (56, 57). Two cohorts in Asia found that NNAL was associated with lung cancer risk independent of factors such as years smoked and cotinine level and with significant dose–response relationships (i.e., the greater the NNAL level, the greater is the lung cancer risk; refs. 16, 20, 23).

NNN and its glucuronides (NNN-Gluc) can be quantified in urine and toenails and generally exist at lower levels than total NNAL due to differences in NNK and NNN metabolism (44, 45).

Urinary NNN distinguishes smokers from nonsmokers and correlates with increasing cigarettes per day and urinary total cotinine levels (22, 58). However, NNN has been observed in NRT users which can be formed endogenously due to the presence of nornicotine in these products (59, 60). In one Shanghai Cohort study of smokers, total NNN was independently associated with esophageal cancer with a dose–response relationship (22). Notably, the Shanghai Cohort studies did not find associations between NNAL and esophageal cancer or NNN and lung cancer. These findings are consistent with rat studies in which NNN causes esophageal and oral cavity tumors and NNK causes lung cancer (19, 61).

PAHs. PAHs are chemicals formed by the combination of incomplete combustion and pyrolysis of organic matter, including tobacco, fossil fuels, and wood (26). Established biomarkers of PAHs are monohydroxylated metabolites of several noncarcinogenic PAHs, including pyrene, fluorene, phenanthrene, and naphthalene (62). Both noncarcinogenic and carcinogenic PAHs are formed similarly during combustion; thus, they exhibit high correlation among themselves in smoke. Evidence from NHANES and other large studies indicates that levels of PAH biomarkers are higher in smokers compared with nonsmokers (63). Although 1-hydroxypyrene is commonly used as a PAH biomarker, it is not tobacco specific due to other major sources of exposure (15, 64). Alternative biomarkers, such as tetrols of benzo[a]pyrene and phenanthrene, are effective biomarkers of PAH uptake and metabolic activation (54, 65, 66). Similar to levels for monohydroxy-PAHs, the amounts of benzo[a]pyrene-tetrols and phenanthrene-tetrols are 2 to 3 times higher in smokers than in nonsmokers (16, 21, 64, 65, 67). The urinary level of phenanthrene tetraol is independently associated with lung cancer among current smokers and lifelong never smokers (20, 68).

VOCs. VOCs are found in mainstream cigarette smoke and are formed as a result of incomplete combustion of tobacco (26). Among VOCs measured in the blood, 2,5-dimethylfuran is comparable with serum cotinine in sensitivity and specificity for current smoking, while benzene, toluene, ethylbenzene, xylene, and styrene are correlated with daily smoking and have dose–response relationships with CPD (69–71). Exposure to tobacco smoke VOCs is most commonly measured as urinary mercapturic acids of ethylene oxide, acrolein, crotonaldehyde, butadiene, benzene, acrylonitrile, and acrylamide, which are elevated in smokers compared with nonsmokers (21). For example, 3-hydroxypropylmercapturic acid (3-HPMA), a mercapturic acid metabolite of acrolein, is four times higher in smokers compared with nonsmokers (72). Less than 10% of mercapturic acid metabolites of VOCs in urine remain 1 day following smoking cessation (66, 73). Urinary mercapturic acid metabolites of VOCs (acrolein, benzene, 1,3-butadiene, crotonaldehyde, and ethylene oxide) were examined in the Shanghai Cohort Study, but were not found to be independently associated with lung cancer among smokers and life-long nonsmokers (16, 21). Finally, hemoglobin adducts of VOCs, such as acrylonitrile, acrylamide, and ethylene oxide, exhibit longer lifetimes than urinary metabolites and are significantly elevated in smokers (66, 73).

Aromatic amines and heterocyclic amines. Both aromatic amines and heterocyclic amines are combustion products in the particulate phase of tobacco smoke (26). Several aromatic and

heterocyclic amines show great potential as tobacco exposure biomarkers (74, 75). Urinary levels of ortho-toluidine, 1-naphthylamine, 2-naphthylamine, 3-aminobiphenyl, and 4-aminobiphenyl are typically low; studies reporting these biomarkers do not consistently differentiate smokers from nonsmokers. Similarly, urinary levels of all heterocyclic amines with exception of 2-amino-1,7-dimethylimidazo[4,5-b]pyridine (DMIP) and 2-amino-9H-pyrido[2,3-b]indole (A α C) do not differentiate smokers and nonsmokers (74, 76). In contrast, hemoglobin adducts of 3-aminobiphenyl, 4-aminobiphenyl, and dimethylanilines are significantly elevated in smokers compared with nonsmokers (74, 77–79).

Metals. Metals such as lead and cadmium are widespread in the environment, but tobacco is a major source of exposure, primarily due to absorption of metals from the soil into the tobacco plant (26). Tobacco is no longer a major source of arsenic due to the termination of arsenic pesticide use during tobacco cultivation (80). In cross-sectional studies, biomarkers of cadmium and lead are elevated in smokers compared with nonsmokers (81–83). Blood cadmium distinguishes clearly between current and noncurrent smokers while the dose–response relationship between urine cadmium and CPD is more subtle. This is most likely due to the longer half-life of urine cadmium (11–30 years) compared with blood cadmium (7–16 years; refs. 83–85). Thus, urine cadmium is a biomarker of cumulative, long-term tobacco exposure. At the population level, reduction in urine cadmium concentrations in the United States can be explained, in part, by reductions in smoking prevalence, increases in smoking cessation, and reduction in secondhand smoke exposure (86).

The biomarkers cadmium and lead have been associated with several health outcomes. The associations of blood and urine cadmium with cardiovascular disease mortality and incidence have been observed in cross-sectional and prospective NHANES analyses and the Strong Heart Study, a longitudinal cohort of American Indians (86–88). Blood lead is associated with adverse neurodevelopmental effects in children; no safe level has been identified (6). Lead as measured in blood, urine, and other biospecimens is also associated with cardiovascular effects. The prospective VA Normative Aging Study observed significant associations between blood and patella levels of lead and fatal and nonfatal ischemic heart disease (89). A systematic review of studies of lead exposure as assessed by biomarker, environmental measures (airborne lead levels), or indirect measure, found a causal effect of lead on hypertension and suggestive, but not sufficient, evidence of a causal effect on clinical cardiovascular outcomes (90).

Evaluation of noncigarette tobacco products

Biomarkers can also provide information about use of noncigarette tobacco products, specifically smokeless tobacco and ENDS.

Smokeless tobacco. Smokeless tobacco products are diverse and differ in constituent yields due to design factors (e.g., fine or long cut), flavors, packaging (pouched or loose), and types (moist snuff or chewing tobacco; ref. 91). Additionally, factors such as tobacco blends, agricultural practices, and tobacco processing contribute to products' chemical composition and subsequently affect exposure levels of HPHCs (92).

To date, only a few studies have been conducted comparing levels and types of biomarkers of exposure in exclusive users of smokeless tobacco with nonusers or other tobacco user groups. Studies have consistently observed higher levels of NNAL or total TSNAs in exclusive smokeless tobacco users compared with nonconsumers of tobacco products (57, 93–96). Similarly, cotinine, nicotine, some PAHs, lead, and halogenated aromatic hydrocarbons are higher in smokeless tobacco users than in nonusers. However, biomarker levels were not significantly different for many other analytes, particularly for VOCs (93–95, 97).

Several cross-sectional studies comparing cigarette smokers with smokeless tobacco users found that cigarette smokers experience greater exposure to most tobacco combustion-related compounds (including CO, PAHs, and VOCs) compared with smokeless tobacco users (94–96). However, in one study, the levels of some PAH urinary biomarkers in moist snuff (phenanthrene) and Camel Snus (naphthalene and pyrene) users were not significantly different from levels in cigarette smokers (96). In cross-sectional studies based on NHANES, blood lead levels among smokeless tobacco and cigarette smokers were comparable (94, 95). Several studies have found that smokeless tobacco users had comparable or higher levels of nicotine and NNAL or total TSNAs compared with cigarette smokers (93–95). Borgerding and colleagues observed higher urinary NNN levels in exclusive moist snuff users compared with exclusive cigarette smokers (96). However, urinary NNN levels and nicotine levels were not significantly different in Camel Snus users compared with exclusive cigarette smokers. Studies indicate that dual users of smokeless tobacco smoke fewer cigarettes and use less smokeless tobacco compared with exclusive cigarette smokers and exclusive smokeless tobacco users (95, 96). However, levels of many biomarkers of exposure are similar for dual users and exclusive smokers, and levels of nicotine and TSNA biomarkers are higher in dual users of some smokeless tobacco products (e.g., moist snuff) and cigarettes than exclusive smokers.

Some studies have switched smokers and smokeless tobacco users to smokeless tobacco products with lower TSNAs and compared biomarker levels before and after switching. For example, Sarkar and colleagues randomized smokers to a snus product (Marlboro snus) exclusively ($n = 15$), dual use with cigarettes (instructed to smoke 50% or less; $n = 60$), continued smoking ($n = 30$), or no tobacco condition ($n = 15$; ref. 98). Dual users who substantially reduced cigarette smoking experienced a 36% to 59% reduction of total NNAL, NNN, and TNE, while 78% to 89% reductions were seen in switchers to exclusive snus use. While dual users experienced a 50% reduction in most aromatic amines, VOCs, and PAHs (except o-toluidine and carboxyhemoglobin), exclusive snus users experienced a 60% to 99% reduction in the same biomarkers. Another study (99) of smokers also found that switching to exclusive use of Camel Snus, Sticks, Strips, or Orbs achieved levels of select exposure biomarkers similar to tobacco abstinence, while smokers randomized to controlled dual use (60% reduction in CPD) experienced more modest reductions.

Other biomarker studies with smokeless tobacco examined the relationship between constituent levels and biomarkers of exposure. NNN and NNK levels in the smokeless tobacco product positively correlate with total NNN and NNAL biomarker levels, respectively, even when accounting for product use patterns. In

contrast, nicotine yield does not correlate with TNE, but rather with frequency and duration of use (100).

Currently, there are nine HPHCs in smokeless tobacco products on FDA's abbreviated list for which manufacturers and importers are currently required to report (25). For most of these HPHCs, widely used biomarkers are available: nicotine (TNE or cotinine), TSNAs (NNN and NNAL), PAH (hydroxyl PAH), metals (cadmium and arsenic), and VOCs (crotonaldehyde). Given the elevated blood lead levels in smokeless tobacco users as compared with nontobacco users (95), biomarkers for lead may also be useful for evaluating smokeless tobacco exposure. Biomarkers may be selected based on expected variability of constituents in smokeless tobacco products. For example, levels of NNN, NNK, and PAH exhibit substantially greater variability among and within different brands of U.S. smokeless tobacco products (92). Biomarkers should be selected based on "fit for purpose." For example, if the intent is to compare potential health risks of combusted vs. smokeless tobacco products, then other VOCs may be considered to evaluate comparative health risks.

ENDS. ENDS products come in different forms, including electronic cigarettes (e-cigarettes), vape pens, and vaporizers, but all use a liquid [usually propylene glycol (PG) or vegetable glycerin (VG)] containing nicotine and various flavorings; the liquid is heated into an aerosol that is inhaled or "vaped" by the user. ENDS may contain carcinogenic carbonyls, acrolein, metal particles, and trace amounts of TSNAs, depending on liquid contents, product design, and the manner of consumer use. In addition to solvent effects, major sources of constituent yield variability are the heating element and battery-output voltage, which affect the temperature at which the fluid is aerosolized. Higher battery-output voltage can increase the likelihood of generating higher levels of carbonyl compounds (101). Increasing the voltage setting from 3.2 to 4.8 volts can produce 5 to 25 times more formaldehyde, acetaldehyde, and acetone depending on the e-liquid solvent composition. Users can modify the temperature of aerosol generation by changing the heating source and battery parameters (resistance, voltage, power, etc.) and the way the product is used (strength of draw, ventilation, etc.).

In two recent cross-sectional studies, urinary biomarkers of tobacco toxicants were compared in ENDS users and other tobacco users (100, 102). In a study by Hecht and colleagues, study participants who met the inclusion criteria, including having not smoked cigarettes for at least two months and having used e-cigarettes for at least 1 month and at least 4 days per week, completed a questionnaire and provided a spot urine sample (100). Biomarker levels in the e-cigarette users in this study were compared with previously published levels in cigarette smokers who were tested using the same assay methods. E-cigarette users ($n = 28$) had urinary levels of nicotine and cotinine that were significantly lower compared with cigarette smokers in one study but not in another study. However, e-cigarette users had significantly lower levels of PAH, VOC, and TSNA biomarkers than cigarette smokers in all of the comparisons. A multisite, cross-sectional study by Shahab and colleagues included five categories of users: exclusive cigarette smoking (daily for at least 6 months), dual use with NRT or e-cigarettes (for at least 6 months), and single users of NRT or e-cigarettes (one product daily for at least 6 months; ref. 102). In preliminary results based on one site, urinary levels of nicotine exposure, as reflected in TNEs, were not signif-

icantly different across all five groups, and the results did not change when the full study sample was included. However, e-cigarette users experienced significantly lower levels of exposure to other aerosol constituents, such as TSNAs and VOCs, compared with conventional cigarette smokers and exhibited levels similar to tobacco nonusers (102). In contrast, differences between dual users of e-cigarettes or NRT and cigarettes compared with exclusive cigarette smokers were not significant, despite dual users smoking a mean of 6 cigarettes less than exclusive cigarette smokers (102).

In one switching study, 20 conventional cigarette smokers naïve to e-cigarettes were provided a first-generation e-cigarette device resembling a cigarette ("cig-a-like") that contained 16 mg/mL of nicotine e-liquid and generated significant yields of nicotine (103). Participants were asked to substitute their conventional cigarettes with e-cigarettes for 2 weeks and use them *ad libitum*, but were also allowed to smoke cigarettes. The researchers found that users reported completely substituting their conventional cigarettes for e-cigarettes, despite being allowed to smoke. At two weeks, compared with baseline measures, exhaled carbon monoxide was reduced significantly to negligible levels, comparable with levels observed among nonsmokers. TNEs levels were unchanged from baseline, and levels of potential toxicants (NNAL, metabolites of VOCs, including acrylamide, 1,3-butadiene, ethylene oxide, and propylene oxide) were significantly reduced, similar to levels observed during smoking cessation. Another recent switching study recruited a larger number of smokers ($n = 40$), potentially capturing a more diverse pattern of use, including dual use (104). Smokers were assigned to "cig-a-like" e-cigarettes labeled 2.4% nicotine for 4 weeks (104). Both dual users and exclusive e-cigarette users experienced a significant reduction in acrolein exposure as measured by 3-HPMA; the magnitude of the reduction was greater among exclusive users (79% vs. 60%). Preliminary findings suggest that exposure to selective tobacco smoke toxicants is significantly reduced among smokers who switch to e-cigarettes; however, the number of studies and the types of exposure biomarkers specific to the assessment of potentially harmful constituents in e-cigarettes is limited, including assessment of exposure to flavor chemicals and formaldehyde.

Biomarkers in development

Exhaled breath biomarkers. Urine is more commonly used than blood or saliva for measuring biomarkers because levels of exposure biomarkers are higher and thus easier to detect in urine. However, exhaled breath biomarkers of semi-volatile and volatile HPHCs have several advantages, including noninvasive sampling, no need for sample clean-up or storage, sensitive detection, and potential for complementing more established methods (105). These studies are usually performed in a laboratory setting and involve acute administration of the product (105). During each smoking session, the participant's exhaled breath is continuously monitored and puff topography is recorded and used to simulate inhaled breath. Uptake or body burden of HPHCs is then estimated by subtracting exhaled from inhaled concentrations. In particular, nano- or ultrafine particles (having a diameter of less than 100 nm), which deposit more deeply in the lungs and thus diffuse more rapidly to the bloodstream, can be detected in exhaled breath. A recent study observed higher levels of ultrafine particle exposures and fine particle (>100 nm to 2.5 μ m in diameter) NNK in menthol versus nonmenthol cigarette smokers,

but no differences in ultrafine particle nicotine exposure and uptake (106). Another exhaled breath study compared the smoking of different types of cigarettes that differed by nicotine and tar yields, and filter ventilation level: very low nicotine content (nicotine, 0.05 mg per cigarette [mg/cig]; tar, 10 mg/cig; ventilation percent, 49%), low-nicotine content (0.6 mg/cig; 10 mg/cig; 1.1%), "ultra-light" (0.5 mg/cig; 6 mg/cig; 52%) and "full-flavor" (1 mg/cig; 15 mg/cig; 0%). Based on the topography data, the researchers observed that very low nicotine content cigarette smokers took larger, longer puffs when compared with the low-nicotine content, "ultra-light," and "full-flavor" cigarette smokers. Based on exhaled breath biomarker data, very low nicotine content smokers had greater-than-expected uptake of nicotine attached to fine and ultrafine particles (105, 107). Altogether, the results suggest that smokers engaged in compensation when smoking the very low nicotine content cigarettes. In summary, biomarkers in exhaled breath can be useful for comparing uptake of HPHCs in smokers of different types of cigarettes and complement more established methods.

Nicotelline/anatelline. New biomarkers are being identified and studied that can help confirm whether consumers are exclusive users of noncombusted products (e.g., smokeless tobacco, and e-cigarettes) or are using these products with cigarettes. Studies have used anabasine and anatabine as biomarkers to detect tobacco use during NRT (35). However, these biomarkers cannot be used to verify exclusive e-cigarette use because they are present in e-liquids and smokeless tobacco, with levels comparable with those in cigarette smoke (35, 108).

One constituent that shows promise is nicotelline, a non-volatile alkaloid. Researchers have developed a quantitative analytical method to measure nicotelline concentrations in tobacco, tobacco smoke, environmental samples, and biologic fluids (109). Nicotelline is found almost entirely in the particulate matter of cigarette smoke and the mass of nicotelline is highly correlated with the mass of the particulate matter. Nicotelline is metabolized to N-oxides that can be measured in smokers' urine. Furthermore, the half-life of nicotelline based on urinary excretion is about 2 to 3 hours, which would detect recent smoking.

Further research demonstrates that anatalline, a tobacco alkaloid, is structurally similar to nicotelline and is converted to nicotelline by oxidation of the central ring during tobacco curing or burning (109). Anatalline is biosynthesized in the tobacco plant; thus, much more anatalline than nicotelline is present in unburned tobacco. Based on product analysis, the ratio of exposure of anatalline to nicotelline is about 1.7 for cigarettes and 120 for smokeless tobacco use—about a 70-fold difference in the ratios (108). In a comparison of human subjects using various tobacco products (cigarettes, small cigars, hookah, e-cigarettes, and smokeless tobacco), nicotelline is highest in urine of cigarette and cigar smokers (about 1000 pg/mL) and about 10-fold lower in urine of smokeless tobacco users. In e-cigarette and hookah users, the average concentrations were present but near the limit of quantitation, probably due to tobacco use in some subjects. Current research is under way to develop an anatalline-to-nicotelline ratio as a biomarker to distinguish smokeless from combusted tobacco use and to estimate the extent of dual use. Anatalline should be selective for smokeless tobacco and other noncombusted product use and nicotelline for combusted product use.

Stable isotopes of propylene glycol in e-cigarettes. Researchers noted a lack of biomarkers specific to ENDS and e-liquids. An example of one such biomarker is for e-liquid solvents such as propylene glycol (PG) and vegetable glycerin (VG) that will distinguish endogenous levels of constituents from those originating from e-cigarettes. One approach is to replace the e-liquid solvent with solvent labeled with ^{13}C , a stable, nonradioactive isotope of carbon that can be distinguished from ^{12}C (generally used in PG and VG) using a mass spectrometer (110). In order to test the feasibility of using this biomarker to test quantitative measures of PG, a confinement study measured the presence of nicotine, PG, and $^{13}\text{C}_3$ -labeled PG in subjects' plasma. Both actively vaping ($n = 3$) and nonvaping ($n = 3$) subjects were exposed to the aerosol. Subjects were asked to vape using a 98.2% $^{13}\text{C}_3$ -PG solvent. Unlabeled PG levels at baseline (before vaping) varied between 200 and 275 ng/mL but increased after a PG-heavy meal. However, $^{13}\text{C}_3$ -PG levels peaked at around 3,500 to 4,000 ng/mL during the active vaping period and were not largely affected by a meal, suggesting that $^{13}\text{C}_3$ -PG levels were not influenced by alternate PG sources. Nonvaping subjects showed no quantifiable levels (above 5 ng/mL) of $^{13}\text{C}_3$. Consequently, $^{13}\text{C}_3$ -labeled PG and VG could be strong candidates for accurately measuring acetaldehyde, formaldehyde, and other carbonyls originating from e-liquid reactions in e-cigarettes. Future studies could enhance this evidence by directly measuring levels of $^{13}\text{C}_3$ -acetaldehyde and $^{13}\text{C}_3$ -formaldehyde levels, testing varied e-liquid solvent compositions, and increasing the subject pool size.

Biomonitoring approaches

In addition to epidemiologic studies, biomarkers may be linked to health risk by examining biomonitoring equivalents (BE), a measure that was established by the EPA. BE is "the concentration or range of concentrations of a chemical in a biological medium (blood, urine, or other medium) that is consistent with an existing health-based exposure guideline" (111). For example, BE for cadmium in urine and blood samples is based on a dose that indicates kidney effects preceding kidney damage. In an NHANES study in which blood and urinary cadmium levels were obtained from smokers, smokeless tobacco users, and nonconsumers of tobacco, a higher percentage of smokers than smokeless tobacco users exceeded the BE of 1.5 $\mu\text{g/L}$ urine and 1.7 μL blood (81). Higher percentages of smokers and smokeless tobacco users exceeded the BE for urinary cadmium than nonconsumers of tobacco products; however, the percentages of smokeless tobacco users and nonusers who exceeded the BE for blood cadmium were similar (81).

In another approach using exposure biomarker data, external exposure can be reconstructed with pharmacokinetics, which characterizes what the body does to a chemical. For example, EPA developed a cumulative approach to risk assessment for five phthalates, chemicals found in plasticizers and solvents that have adverse effects on the developing male reproductive system. Using reverse dosimetry, or the "creatinine correction" approach, daily intakes for five phthalates in the U.S. general population were determined using urine metabolite levels from NHANES. These phthalate levels were compared with a health "reference value" to determine the intake level that represents a health concern (112). This approach allowed estimation of the risk due to individual and multiple chemical contributions.

Discussion

Future research directions

Based on the current body of knowledge, a number of analytically validated exposure biomarkers represent many of the HPHCs in cigarettes, especially when taking into account the correlation among biomarkers belonging to the same class of chemicals (17, 58). However, some areas of research could further strengthen the utility of exposure biomarkers for potential regulatory use. Many exposure biomarkers have been shown to distinguish smokers from nonsmokers, but fewer exposure biomarkers have comprehensive dose-response information with frequency of product use and change after cessation or reductions in use. Although the knowledge base around exposure biomarkers is largely based on studies of cigarette smokers, there is overlap with other combusted products, such as cigars, pipe, and hookah, as observed in recent studies (113–115). However, more information would clarify the dose–response relationships between exposure biomarkers and patterns of other combusted product use. Additional biomarker studies of other tobacco product use, including smokeless tobacco and ENDS, can also further strengthen this body of knowledge.

In particular, biomarkers specific to new and emerging tobacco products, such as ENDS, have yet to be identified. Few studies examine product design and exposure levels, and a major challenge is that products are rapidly evolving and diverse. Existing biomarker studies are limited because they do not examine exposure to carbonyl compounds (e.g., formaldehyde and acetaldehyde), which are generated when the fluid is vaporized at high temperatures. Other major challenges in examining exposure to these constituents are endogenous formation and exposures from other environmental sources. Generally Recognized As Safe flavorings in e-liquids are safe for ingestion, but can undergo chemical reactions when heated and, if inhaled, may create a wide range of unforeseen issues. Thus, future research should address biomarkers for ENDS flavorings including cinnamon and butter flavors, which have been implicated as pulmonary health hazards.

Dual and poly use of cigarettes and other tobacco products is common in the United States, but exposure and health risks are not well characterized. A better characterization of their use patterns—such as whether products are used concurrently or separately and whether dual and poly use is sustained over the short-term or long-term—will provide a better understanding of exposure and, consequently, health effects. Biomarkers that can distinguish different types of tobacco products from one another, such as anattaline-to-nicotelline ratio, can serve as verification of self-reported use as well as help determine the contribution of tobacco products to each of the exposures.

To date, only a few studies of cigarette smokers have assessed the independent association between exposure biomarkers and disease risk. Additional studies based on existing cohorts with biospecimens at baseline and long-term outcomes could further elucidate this relationship. No study has examined the relationship between exposure biomarkers and risk for diseases that are specifically associated with noncigarette tobacco use, though inferences can be drawn from studies of cigarette smokers.

However, challenges include the limited number of existing cohorts, gaining access to the type and amount of samples needed, and having adequate statistical power to detect associations. A consortium approach may be valuable for overcoming such challenges and advancing the science. Another approach to linking exposure biomarkers to health risk is using BE or converting internal dose to dose intakes for comparison with a health "reference value."

Conclusions

In this inaugural CTP biomarker workshop, different biomarker approaches in regulatory and nonregulatory settings and the current state of the literature for tobacco-related exposure biomarkers were discussed. To date, many tobacco-related exposure biomarkers have been validated with respect to measurement performance characteristics. However, not all are equally informative with respect to tobacco product use and subsequent disease risk, and thus prioritization of biomarkers may be beneficial in the regulatory setting. In addition, identifying the most appropriate biomarkers depends on context of use or research question to be addressed. Finally, developing biomarkers for measurement of additional exposures relevant to emerging tobacco products and disentangling exposures in poly tobacco users could enhance tobacco product evaluation. Prioritization of biomarkers can be driven by the current state of the literature and facilitated through consensus on terminologies, definitions, and contexts of use. At the same time, the ongoing assessment of issues arising from the study of existing and emerging tobacco products can enable researchers to continue strengthening the science around biomarkers. Additional research and collaborative approaches will be helpful in determining how biomarkers of exposure can be best applied in regulating tobacco products.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Disclaimer

The findings and conclusions of this article are those of the authors and do not necessarily represent FDA positions or policies.

Acknowledgments

The authors thank CTP Office of Science (OS) and the members of the OS Biomarker Workgroup, Jiping Chen, Yu-Ching Cheng, T. Matthew Cho, Carolyn Dresler, Elizabeth L. Durmowicz, Dhanya John, Won Lee, Dana van Bommel, and Maocheng Yang, for their contributions to the planning, logistics, and execution of the Biomarkers of Tobacco Exposure Workshop. We also thank Benjamin Apelberg for reviewing and providing feedback on the manuscript.

Grant Support

For this work, all authors were supported by funds from the FDA.

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Received August 22, 2016; revised October 25, 2016; accepted October 31, 2016; published OnlineFirst November 9, 2016.

References

1. U.S. Congress. Family smoking prevention and tobacco control and federal retirement reform. Public Law 2009:111–31.
2. Deeming Tobacco Products to Be Subject to the Federal Food, Drug, and Cosmetic Act, as Amended by the Family Smoking Prevention and

- Tobacco Control Act; Restrictions on the Sale and Distribution of Tobacco Products and Required Warning Statements for Tobacco Products. Available from: <https://www.federalregister.gov/articles/2016/05/10/2016-10685/deeming-tobacco-products-to-be-subject-to-the-federal-food-drug-and-cosmetic-act-as-amended-by-the>
3. IOM. Scientific standards for studies on modified risk tobacco products. Washington, DC: The National Academies Press; 2012:2012.
 4. Hatsukami DK, Hecht SS, Hennrikus DJ, Joseph AM, Pentel PR. Biomarkers of tobacco exposure or harm: application to clinical and epidemiological studies. 2001 October 25–26, Minneapolis, Minnesota. *Nicotine Tob Res* 2003;5:387–96.
 5. Center for Tobacco Products/U.S. Food and Drug Administration. Biomarkers of Tobacco Exposure: A Public Workshop. 2015; Available from: <http://www.fda.gov/TobaccoProducts/NewsEvents/ucm447723.htm>
 6. National Research Council. Human Biomonitoring for Environmental Chemicals. Washington, DC: The National Academies Press; 2006.
 7. Wagner JA. Strategic approach to fit-for-purpose biomarkers in drug development. *Annu Rev Pharmacol Toxicol* 2008;48:631–51.
 8. Soreide K. Receiver-operating characteristic curve analysis in diagnostic, prognostic and predictive biomarker research. *J Clin Pathol* 2009;62:1–5.
 9. Chau CH, Rixe O, McLeod H, Figg WD. Validation of analytic methods for biomarkers used in drug development. *Clin Cancer Res* 2008;14:5967–76.
 10. Leptak_Presentation. Biomarker Utility and Acceptance in Drug Development and Clinical Trials: an FDA Regulatory Perspective. Center for tobacco products' biomarkers of tobacco exposure workshop; 2015 August 3–4, 2015; U.S. Food and Drug Administration, Silver Spring, MD.
 11. Mattes_Presentation. Biomarker Basics: Validation, Qualification, Translation. Center for tobacco products' biomarkers of tobacco exposure workshop; 2015 August 3–4; U.S. Food and Drug Administration, Silver Spring, MD.
 12. Center for Drug Evaluation and Research/U.S. Food and Drug Administration. Review of qualification data for biomarkers of nephrotoxicity submitted by the predictive safety testing consortium. 2009; Available from: <http://www.fda.gov/downloads/Drugs/DevelopmentApprovalProcess/DrugDevelopmentToolsQualificationProgram/UCM382536.pdf>
 13. Benowitz NL. The use of biologic fluid samples in assessing tobacco smoke consumption. *NIDA Res Monogr* 1983;48:6–26.
 14. Bernert JT, Jacob P III, Holiday DB, Benowitz NL, Sosnoff CS, Doig MV, et al. Interlaboratory comparability of serum cotinine measurements at smoker and nonsmoker concentration levels: a round-robin study. *Nicotine Tob Res* 2009;11:1458–66.
 15. Hatsukami DK, Benowitz NL, Rennard SI, Oncken C, Hecht SS. Biomarkers to assess the utility of potential reduced exposure tobacco products. *Nicotine Tob Res* 2006;8:600–22.
 16. Yuan JM, Butler LM, Stepanov I, Hecht SS. Urinary tobacco smoke-constituent biomarkers for assessing risk of lung cancer. *Cancer Res* 2014;74:401–11.
 17. Blount_Presentation. Importance of Analytical Methods for Biomarker Measurement. Center for Tobacco Products' Biomarkers of Tobacco Exposure Workshop; 2015 August 3–4; U.S. Food and Drug Administration, Silver Spring, MD.
 18. Hatsukami_Presentation. Characterization of Biomarkers to Assess the Utility of Potential Reduced Exposure Tobacco Products: Status Update. Center for Tobacco Products' Biomarkers of Tobacco Exposure Workshop; 2015 August 3–4; U.S. Food and Drug Administration, Silver Spring, MD.
 19. Stepanov I, Sebero E, Wang R, Gao YT, Hecht SS, Yuan JM. Tobacco-specific N-nitrosamine exposures and cancer risk in the Shanghai Cohort Study: remarkable coherence with rat tumor sites. *Int J Cancer* 2014;134:2278–83.
 20. Yuan JM, Gao YT, Murphy SE, Carmella SG, Wang R, Zhong Y, et al. Urinary levels of cigarette smoke constituent metabolites are prospectively associated with lung cancer development in smokers. *Cancer Res* 2011;71:6749–57.
 21. Yuan JM, Gao YT, Wang R, Chen M, Carmella SG, Hecht SS. Urinary levels of volatile organic carcinogen and toxicant biomarkers in relation to lung cancer development in smokers. *Carcinogenesis* 2012;33:804–9.
 22. Yuan JM, Knezevich AD, Wang R, Gao YT, Hecht SS, Stepanov I. Urinary levels of the tobacco-specific carcinogen N'-nitrosanornicotine and its glucuronide are strongly associated with esophageal cancer risk in smokers. *Carcinogenesis* 2011;32:1366–71.
 23. Yuan JM, Koh WP, Murphy SE, Fan Y, Wang R, Carmella SG, et al. Urinary levels of tobacco-specific nitrosamine metabolites in relation to lung cancer development in two prospective cohorts of cigarette smokers. *Cancer Res* 2009;69:2990–5.
 24. Yuan_Presentation. Biomarkers of Exposure to Tobacco Smoke and Risk of Cancer – The Shanghai Cohort Study. Center for Tobacco Products' Biomarkers of Tobacco Exposure Workshop; 2015 August 3–4; U.S. Food and Drug Administration, Silver Spring, MD.
 25. Center for Tobacco Products/U.S. Food and Drug Administration. Harmful and potentially harmful constituents in tobacco products and tobacco smoke; Established List 2012; Available from: <http://www.fda.gov/downloads/TobaccoProducts/GuidanceComplianceRegulatoryInformation/UCM297981.pdf>
 26. U.S. Department of Health and Human Services. How tobacco smoke causes disease: the biology and behavioral basis for smoking-attributable disease: A Report of the Surgeon General. Atlanta, GA; 2010.
 27. Hukkanen J, Jacob P III, Benowitz NL. Metabolism and disposition kinetics of nicotine. *Pharmacol Rev* 2005;57:79–115.
 28. Pirkle JL, Bernert JT, Caudill SP, Sosnoff CS, Pechacek TF. Trends in the exposure of nonsmokers in the U.S. population to secondhand smoke: 1988–2002. *Environ Health Perspect* 2006;114:853–8.
 29. Rostron B. NNAL exposure by race and menthol cigarette use among U.S. smokers. *Nicotine Tob Res* 2013;15:950–6.
 30. Benowitz NL, Lessov-Schlaggar CN, Swan GE, Jacob P III. Female sex and oral contraceptive use accelerate nicotine metabolism. *Clin Pharmacol Ther* 2006;79:480–8.
 31. Zhu AZ, Renner CC, Hatsukami DK, Swan GE, Lerman C, Benowitz NL, et al. The ability of plasma cotinine to predict nicotine and carcinogen exposure is altered by differences in CYP2A6: the influence of genetics, race, and sex. *Cancer Epidemiol Biomarkers Prev* 2013;22:708–18.
 32. Benowitz NL, Bernert JT, Caraballo RS, Holiday DB, Wang J. Optimal serum cotinine levels for distinguishing cigarette smokers and nonsmokers within different racial/ethnic groups in the United States between 1999 and 2004. *Am J Epidemiol* 2009;169:236–48.
 33. Benowitz NL, Dains KM, Dempsey D, Wilson M, Jacob P. Racial differences in the relationship between number of cigarettes smoked and nicotine and carcinogen exposure. *Nicotine Tob Res* 2011;13:772–83.
 34. St HG, Goniewicz ML, Dempsey D, Wilson M, Jacob P III, Benowitz NL. Exposure and kinetics of polycyclic aromatic hydrocarbons (PAHs) in cigarette smokers. *Chem Res Toxicol* 2012;25:952–64.
 35. Jacob P III, Hatsukami D, Severson H, Hall S, Yu L, Benowitz NL. Anabasine and anatabine as biomarkers for tobacco use during nicotine replacement therapy. *Cancer Epidemiol Biomarkers Prev* 2002;11:1668–73.
 36. Scherer G. Carboxyhemoglobin and thiocyanate as biomarkers of exposure to carbon monoxide and hydrogen cyanide in tobacco smoke. *Exp Toxicol Pathol* 2006;58:101–24.
 37. SRNT Subcommittee on Biochemical Verification. Biochemical verification of tobacco use and cessation. *Nicotine Tob Res* 2002;4:149–59.
 38. Sandberg A, Skold CM, Grunewald J, Eklund A, Wheelock AM. Assessing recent smoking status by measuring exhaled carbon monoxide levels. *PLoS One* 2011;6:e28864.
 39. Jarvis MJ, Russell MA, Saloojee Y. Expired air carbon monoxide: a simple breath test of tobacco smoke intake. *Br Med J* 1980;281:484–5.
 40. Hatsukami DK, Le CT, Zhang Y, Joseph AM, Mooney ME, Carmella SG, et al. Toxicant exposure in cigarette reducers versus light smokers. *Cancer Epidemiol Biomarkers Prev* 2006;15:2355–8.
 41. Roethig HJ, Munjal S, Feng S, Liang Q, Sarkar M, Walk RA, et al. Population estimates for biomarkers of exposure to cigarette smoke in adult U.S. cigarette smokers. *Nicotine Tob Res* 2009;11:1216–25.
 42. Hatsukami DK, Hanson K, Briggs A, Parascandola M, Genkinger JM, O'Connor R, et al. Clinical trials methods for evaluation of potential reduced exposure products. *Cancer Epidemiol Biomarkers Prev* 2009;18:3143–95.
 43. IARC. Personal habits and indoor combustions. Volume 100 E. A review of human carcinogens. *IARC Monogr Eval Carcinog Risks Hum* 2012;100:1–538.
 44. Kavvadias D, Scherer G, Cheung F, Errington G, Shepperd J, McEwan M. Determination of tobacco-specific N-nitrosamines in urine of smokers and non-smokers. *Biomarkers* 2009;14:547–53.

45. Stepanov I, Hecht SS. Tobacco-specific nitrosamines and their pyridine-N-glucuronides in the urine of smokers and smokeless tobacco users. *Cancer Epidemiol Biomarkers Prev* 2005;14:885–91.
46. Stepanov I, Hecht SS. Detection and quantitation of N'-nitrosonornicotine in human toenails by liquid chromatography-electrospray ionization-tandem mass spectrometry. *Cancer Epidemiol Biomarkers Prev* 2008;17:945–8.
47. Hecht SS, Carmella SG, Chen M, Dor Koch JF, Miller AT, Murphy SE, et al. Quantitation of urinary metabolites of a tobacco-specific lung carcinogen after smoking cessation. *Cancer Res* 1999;59:590–6.
48. Goniewicz ML, Havel CM, Peng MW, Jacob P III, Dempsey D, Yu L, et al. Elimination kinetics of the tobacco-specific biomarker and lung carcinogen 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol. *Cancer Epidemiol Biomarkers Prev* 2009;18:3421–5.
49. Carmella SG, Ming X, Olvera N, Brookmeyer C, Yoder A, Hecht SS. High throughput liquid and gas chromatography-tandem mass spectrometry assays for tobacco-specific nitrosamine and polycyclic aromatic hydrocarbon metabolites associated with lung cancer in smokers. *Chem Res Toxicol* 2013;26:1209–17.
50. Vogel RI, Carmella SG, Stepanov I, Hatsukami DK, Hecht SS. The ratio of a urinary tobacco-specific lung carcinogen metabolite to cotinine is significantly higher in passive than in active smokers. *Biomarkers* 2011;16:491–7.
51. Xia Y, Bernert JT, Jain RB, Ashley DL, Pirkle JL. Tobacco-specific nitrosamine 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL) in smokers in the United States: NHANES 2007–2008. *Biomarkers* 2011;16:112–9.
52. Park SL, Carmella SG, Ming X, Vielguth E, Stram DO, Le Marchand L, et al. Variation in levels of the lung carcinogen NNAL and its glucuronides in the urine of cigarette smokers from five ethnic groups with differing risks for lung cancer. *Cancer Epidemiol Biomarkers Prev* 2015;24:561–9.
53. Hecht SS. Human urinary carcinogen metabolites: biomarkers for investigating tobacco and cancer. *Carcinogenesis* 2002;23:907–22.
54. Hecht SS, Carmella SG, Le KA, Murphy SE, Boettcher AJ, Le C, et al. 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol and its glucuronides in the urine of infants exposed to environmental tobacco smoke. *Cancer Epidemiol Biomarkers Prev* 2006;15:988–92.
55. Jensen JA, Schillo BA, Moilanen MM, Lindgren BR, Murphy S, Carmella S, et al. Tobacco smoke exposure in nonsmoking hospitality workers before and after a state smoking ban. *Cancer Epidemiol Biomarkers Prev* 2010;19:1016–21.
56. Bernert JT, Pirkle JL, Xia Y, Jain RB, Ashley DL, Sampson EJ. Urine concentrations of a tobacco-specific nitrosamine carcinogen in the U.S. population from secondhand smoke exposure. *Cancer Epidemiol Biomarkers Prev* 2010;19:2969–77.
57. Wei B, Blount BC, Xia B, Wang L. Assessing exposure to tobacco-specific carcinogen NNK using its urinary metabolite NNAL measured in US population: 2011–2012. *J Expo Sci Environ Epidemiol* 2015.
58. Hecht Presentation. Biomarkers of Exposure to Tobacco Carcinogens and Toxicants. Center for Tobacco Products' Biomarkers of Tobacco Exposure Workshop; 2015 August 3–4, 2015; U.S. Food and Drug Administration, Silver Spring, MD.
59. Stepanov I, Carmella SG, Briggs A, Hertsgaard L, Lindgren B, Hatsukami D, et al. Presence of the carcinogen N'-nitrosonornicotine in the urine of some users of oral nicotine replacement therapy products. *Cancer Res* 2009;69:8236–40.
60. Stepanov I, Carmella SG, Han S, Pinto A, Strasser AA, Lerman C, et al. Evidence for endogenous formation of N'-nitrosonornicotine in some long-term nicotine patch users. *Nicotine Tob Res* 2009;11:99–105.
61. Balbo S, James-Yi S, Johnson CS, O'Sullivan MG, Stepanov I, Wang M, et al. (S)-N'-Nitrosonornicotine, a constituent of smokeless tobacco, is a powerful oral cavity carcinogen in rats. *Carcinogenesis* 2013;34:2178–83.
62. Li Z, Sandau CD, Romanoff LC, Caudill SP, Sjodin A, Needham LL, et al. Concentration and profile of 22 urinary polycyclic aromatic hydrocarbon metabolites in the US population. *Environ Res* 2008;107:320–31.
63. Suwan-ampai P, Navas-Acien A, Strickland PT, Agnew J. Involuntary tobacco smoke exposure and urinary levels of polycyclic aromatic hydrocarbons in the United States, 1999 to 2002. *Cancer Epidemiol Biomarkers Prev* 2009;18:884–93.
64. Carmella SG, Chen M, Han S, Briggs A, Jensen J, Hatsukami DK, et al. Effects of smoking cessation on eight urinary tobacco carcinogen and toxicant biomarkers. *Chem Res Toxicol* 2009;22:734–41.
65. Hecht SS, Chen M, Yagi H, Jerina DM, Carmella SG. r-1,t-2,3,c-4-Tetrahydroxy-1,2,3,4-tetrahydrophenanthrene in human urine: a potential biomarker for assessing polycyclic aromatic hydrocarbon metabolic activation. *Cancer Epidemiol Biomarkers Prev* 2003;12:1501–8.
66. Hecht SS, Yuan JM, Hatsukami D. Applying tobacco carcinogen and toxicant biomarkers in product regulation and cancer prevention. *Chem Res Toxicol* 2010;23:1001–8.
67. Zhong Y, Carmella SG, Upadhyaya P, Hochalter JB, Rauch D, Oliver A, et al. Immediate consequences of cigarette smoking: rapid formation of polycyclic aromatic hydrocarbon diol epoxides. *Chem Res Toxicol* 2011;24:246–52.
68. Yuan JM, Butler LM, Gao YT, Murphy SE, Carmella SG, Wang R, et al. Urinary metabolites of a polycyclic aromatic hydrocarbon and volatile organic compounds in relation to lung cancer development in lifelong never smokers in the Shanghai Cohort Study. *Carcinogenesis* 2014;35:339–45.
69. Jia C, Ward KD, Mzayek F, Relyea G. Blood 2,5-dimethylfuran as a sensitive and specific biomarker for cigarette smoking. *Biomarkers* 2014;19:457–62.
70. Chambers DM, Ocariz JM, McGuirk MF, Blount BC. Impact of cigarette smoking on volatile organic compound (VOC) blood levels in the U.S. population: NHANES 2003–2004. *Environ Int* 2011;37:1321–8.
71. Ashley DL, Bonin MA, Cardinali FL, McCraw JM, Wooten JV. Measurement of volatile organic compounds in human blood. *Environ Health Perspect* 1996;104Suppl 5:871–7.
72. Alwis KU, Blount BC, Britt AS, Patel D, Ashley DL. Simultaneous analysis of 28 urinary VOC metabolites using ultra high performance liquid chromatography coupled with electrospray ionization tandem mass spectrometry (UPLC-ESI/MSMS). *Anal Chim Acta* 2012;750:152–60.
73. Wang M, Cheng G, Balbo S, Carmella SG, Villalta PW, Hecht SS. Clear differences in levels of a formaldehyde-DNA adduct in leukocytes of smokers and nonsmokers. *Cancer Res* 2009;69:7170–4.
74. Turesky RJ, Le ML. Metabolism and biomarkers of heterocyclic aromatic amines in molecular epidemiology studies: lessons learned from aromatic amines. *Chem Res Toxicol* 2011;24:1169–214.
75. Riedel K, Scherer G, Engl J, Hagedorn HW, Tricker AR. Determination of three carcinogenic aromatic amines in urine of smokers and nonsmokers. *J Anal Toxicol* 2006;30:187–95.
76. Turesky RJ, Yuan JM, Wang R, Peterson S, Yu MC. Tobacco smoking and urinary levels of 2-amino-9H-pyrido[2,3-b]indole in men of Shanghai, China. *Cancer Epidemiol Biomarkers Prev* 2007;16:1554–60.
77. Castelao JE, Yuan JM, Skipper PL, Tannenbaum SR, Gago-Dominguez M, Crowder JS, et al. Gender- and smoking-related bladder cancer risk. *J Natl Cancer Inst* 2001;93:538–45.
78. Bernert JT, Jain RB, Pirkle JL, Wang L, Miller BB, Sampson EJ. Urinary tobacco-specific nitrosamines and 4-aminobiphenyl hemoglobin adducts measured in smokers of either regular or light cigarettes. *Nicotine Tob Res* 2005;7:729–38.
79. Gan J, Skipper PL, Gago-Dominguez M, Arakawa K, Ross RK, Yu MC, et al. Alkylamine-hemoglobin adducts and risk of non-smoking-related bladder cancer. *J Natl Cancer Inst* 2004;96:1425–31.
80. Jones Presentation. Metals as Non-Specific Tobacco Biomarkers of Cardiovascular Disease. Center for Tobacco Products' Biomarkers of Tobacco Exposure Workshop; 2015 August 3–4, 2015; U.S. Food and Drug Administration, Silver Spring, MD.
81. Marano KM, Naufal ZS, Kathman SJ, Bodnar JA, Borgerding MF, Garner CD, et al. Cadmium exposure and tobacco consumption: biomarkers and risk assessment. *Regul Toxicol Pharmacol* 2012;64:243–52.
82. Jones MR, Apelberg BJ, Tellez-Plaza M, Samet JM, Navas-Acien A. Menthol cigarettes, race/ethnicity, and biomarkers of tobacco use in U.S. adults: the 1999–2010 National Health and Nutrition Examination Survey (NHANES). *Cancer Epidemiol Biomarkers Prev* 2013;22:224–32.
83. Tellez-Plaza M, Navas-Acien A, Crainiceanu CM, Guallar E. Cadmium exposure and hypertension in the 1999–2004 National Health and Nutrition Examination Survey (NHANES). *Environ Health Perspect* 2008;116:51–6.

84. Jarup L, Rogenfelt A, Elinder CG, Nogawa K, Kjellstrom T. Biological half-time of cadmium in the blood of workers after cessation of exposure. *Scand J Work Environ Health* 1983;9:327–31.
85. Amzal B, Julin B, Vahter M, Wolk A, Johanson G, Akesson A. Population toxicokinetic modeling of cadmium for health risk assessment. *Environ Health Perspect* 2009;117:1293–301.
86. Tellez-Plaza M, Navas-Acien A, Menke A, Crainiceanu CM, Pastor-Barriuso R, Guallar E. Cadmium exposure and all-cause and cardiovascular mortality in the U.S. general population. *Environ Health Perspect* 2012;120:1017–22.
87. Tellez-Plaza M, Navas-Acien A, Caldwell KL, Menke A, Muntner P, Guallar E. Reduction in cadmium heart exposure in the United States population, 1988–2008: the contribution of declining smoking rates. *Environ Health Perspect* 2012;120:204–9.
88. Tellez-Plaza M, Navas-Acien A, Crainiceanu CM, Sharrett AR, Guallar E. Cadmium and peripheral arterial disease: gender differences in the 1999–2004 US National Health and Nutrition Examination Survey. *Am J Epidemiol* 2010;172:671–81.
89. Jain NB, Potula V, Schwartz J, Vokonas PS, Sparrow D, Wright RO, et al. Lead levels and ischemic heart disease in a prospective study of middle-aged and elderly men: the VA Normative Aging Study. *Environ Health Perspect* 2007;115:871–5.
90. Navas-Acien A, Guallar E, Silbergeld EK, Rothenberg SJ. Lead exposure and cardiovascular disease—a systematic review. *Environ Health Perspect* 2007;115:472–82.
91. National Cancer Institute and Centers for Disease Control and Prevention. *Smokeless tobacco and public health: a global perspective*. Bethesda, MD: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention and National Institutes of Health, National Cancer Institute; 2014.
92. Stepanov I, Hatsukami D. Call to establish constituent standards for smokeless tobacco products. *Tobacco Regulatory Science* 2016;2(1):9–30.
93. Hecht SS, Carmella SG, Murphy SE, Riley WT, Le C, Luo X, et al. Similar exposure to a tobacco-specific carcinogen in smokeless tobacco users and cigarette smokers. *Cancer Epidemiol Biomarkers Prev* 2007;16:1567–72.
94. Naufal ZS, Marano KM, Kathman SJ, Wilson CL. Differential exposure biomarker levels among cigarette smokers and smokeless tobacco consumers in the National Health and Nutrition Examination Survey 1999–2008. *Biomarkers* 2011;16:222–35.
95. Rostron BL, Chang CM, van Bommel DM, Xia Y, Blount BC. Nicotine and toxicant exposure among U.S. smokeless tobacco users: results from 1999 to 2012 National Health and Nutrition Examination Survey Data. *Cancer Epidemiol Biomarkers Prev* 2015;24:1829–37.
96. Borgerding_Presentation. a multi-center, cross-sectional study of U.S. tobacco users, including both exclusive and dual use of cigarettes, moist snuff and a U.S. snus brand: biomarkers of exposure. Center for Tobacco Products' Biomarkers of Tobacco Exposure Workshop; 2015 August 3–4, 2015; U.S. Food and Drug Administration, Silver Spring, MD.
97. Borgerding MF, Bodnar JA, Curtin GM, Swauger JE. The chemical composition of smokeless tobacco: a survey of products sold in the United States in 2006 and 2007. *Regul Toxicol Pharmacol* 2012;64:367–87.
98. Sarkar M, Liu J, Koval T, Wang J, Feng S, Serafin R, et al. Evaluation of biomarkers of exposure in adult cigarette smokers using Marlboro snus. *Nicotine Tob Res* 2010;12:105–16.
99. Krautter GR, Chen PX, Borgerding MF. Consumption patterns and biomarkers of exposure in cigarette smokers switched to Snus, various dissolvable tobacco products, Dual use, or tobacco abstinence. *Regul Toxicol Pharmacol* 2015;71:186–97.
100. Hatsukami DK, Stepanov I, Severson H, Jensen JA, Lindgren BR, Horn K, et al. Evidence supporting product standards for carcinogens in smokeless tobacco products. *Cancer Prev Res (Phila)* 2015;8:20–6.
101. Kosmider L, Sobczak A, Fik M, Knysak J, Zacierka M, Kurek J, et al. Carbonyl compounds in electronic cigarette vapors: effects of nicotine solvent and battery output voltage. *Nicotine Tob Res* 2014;16:1319–26.
102. Shahab L, Goniewicz M, Alwis U, Xia Y, Feng J, Blount B. Exposure to selected toxicants and carcinogens as a function of smoking status and long-term use of nicotine replacement therapy or electronic cigarettes. Society for Research on Nicotine and Tobacco 21st Annual Meeting, Philadelphia, PA. 2015.
103. Goniewicz_Presentation. Biomarkers of Exposure to Nicotine and Toxicants Among Exclusive Users of Electronic Cigarettes and Dual Users of Electronic and Tobacco Cigarettes. Center for Tobacco Products' Biomarkers of Tobacco Exposure Workshop; 2015 August 3–4, 2015; U.S. Food and Drug Administration, Silver Spring, MD.
104. McRobbie H, Phillips A, Goniewicz ML, Smith KM, Knight-West O, Przulj D, et al. Effects of switching to electronic cigarettes with and without concurrent smoking on exposure to nicotine, carbon monoxide, and acrolein. *Cancer Prev Res (Phila)* 2015;8:873–8.
105. Brinkman_Presentation. Biomarkers of Tobacco Exposure in Exhaled Breath. Center for Tobacco Products' Biomarkers of Tobacco Exposure Workshop; 2015 August 3–4, 2015; U.S. Food and Drug Administration, Silver Spring, MD.
106. Brinkman MC, Chuang JC, Gordon SM, Kim H, Kroeger RR, Polzin GM, et al. Exposure to and deposition of fine and ultrafine particles in smokers of menthol and nonmenthol cigarettes. *Inhal Toxicol* 2012;24:255–69.
107. Brinkman MC, Kim H, Gordon SM, Chuang JC, Kroeger RR, Clark PI. Smokers and PREPs: measurement of inhaled and exhaled tobacco smoke particulate. Joint Conference of the Society for Research on Nicotine and Tobacco and SRNT-Europe; 2009; Dublin, Ireland.
108. Jacob_Presentation. New Biomarkers of Tobacco Exposure. Center for Tobacco Products' Biomarkers of Tobacco Exposure Workshop; 2015 August 3–4, 2015; U.S. Food and Drug Administration, Silver Spring, MD.
109. Jacob P III, Goniewicz ML, Havel CM, Schick SF, Benowitz NL. Nicotelline: a proposed biomarker and environmental tracer for particulate matter derived from tobacco smoke. *Chem Res Toxicol* 2013;26:1615–31.
110. Farnen_Presentation. Toxicants in biofluids originated from stable isotopes of propylene glycol in eCigarettes. Center for Tobacco Products' Biomarkers of Tobacco Exposure Workshop; 2015 August 3–4, 2015; U.S. Food and Drug Administration, Silver Spring, MD.
111. Mattes W, Yang X, Orr MS, Richter P, Mendrick DL. Biomarkers of tobacco smoke exposure. *Adv Clin Chem* 2014;67:1–45.
112. Christensen KL, Makris SL, Lorber M. Generation of hazard indices for cumulative exposure to phthalates for use in cumulative risk assessment. *Regul Toxicol Pharmacol* 2014;69:380–9.
113. Chen J, Kettermann A, Rostron BL, Day HR. Biomarkers of exposure among U.S. cigar smokers: an analysis of 1999–2012 National Health and Nutrition Examination Survey (NHANES) data. *Cancer Epidemiol Biomarkers Prev* 2014;23:2906–15.
114. Jacob P III, Abu Raddaha AH, Dempsey D, Havel C, Peng M, Yu L, et al. Comparison of nicotine and carcinogen exposure with water pipe and cigarette smoking. *Cancer Epidemiol Biomarkers Prev* 2013;22:765–72.
115. St Helen G, Benowitz NL, Dains KM, Havel C, Peng M, Jacob P III. Nicotine and carcinogen exposure after water pipe smoking in hookah bars. *Cancer Epidemiol Biomarkers Prev* 2014;23:1055–66.