Applying Tobacco Carcinogen and Toxicant Biomarkers in Product Regulation and Cancer Prevention

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Tobacco carcinogen and toxicant biomarkers are metabolites or protein or DNA adducts of specific compounds in tobacco products. Highly reliable analytical methods, based mainly on mass spectrometry, have been developed and applied in large studies of many of these biomarkers. A panel of tobacco carcinogen and toxicant biomarkers is suggested here, and typical values for smokers and nonsmokers are summarized. This panel of biomarkers has potential applications in the new and challenging area of tobacco product regulation and in the development of rational approaches to cancer prevention by establishing carcinogen and toxicant uptake and excretion in people exposed to tobacco products.

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Introduction

Tobacco products present a perfect storm consisting of an addictive constituent, nicotine, and a complex mixture of toxicants and carcinogens. Attesting to their addictive power is the fact that more than one billion people in the world smoke cigarettes and other tobacco products, while hundreds of millions use smokeless tobacco (*1*). The consequences are enormous: 33% of all cancer mortality in the U.S. and 21% worldwide is due to tobacco products (*2*). The latest evaluation by the International Agency for Research on Cancer lists 19 cancers for which there is sufficient evidence that tobacco smoking is a cause, and 3 are caused by smokeless tobacco use (*3*). On average, three thousand people succumb daily to lung cancer in the world, about 90% of which is caused by cigarette smoking (*4*).

The U.S. President's Cancer Panel Report states: "Ridding the nation of tobacco is the single most important action needed to dramatically reduce cancer mortality and morbidity" (*5*). Tobacco control efforts in the U.S. have been quite successful, as the combination of smoke free legislation, taxation, and aggressive anti-tobacco advertising among other approaches has decreased smoking prevalence to the current level of 20.6%; four states have a prevalence of less than 15% (*6*). Worldwide, the results are varied, and there are still major areas of high tobacco use such as China which has more male smokers than there are people in the U.S. (*1*). There is a great deal of work left to do in tobacco control.

This perspective will discuss the potential use of tobacco carcinogen and toxicant biomarkers in tobacco product regulation with respect to cancer. Tobacco products are also a cause of cardiovascular and pulmonary disease, but those effects and their biomarkers are not considered here (*7, 8*). Major recent regulatory legislative actions have changed the landscape with respect to tobacco. The World Health Organization in its Framework Convention on Tobacco Control (FCTC) recognized the need for tobacco product regulation. In 2009, the U.S. Congress passed and President Obama signed into law, the Family Smoking Prevention and Tobacco Control Act which gives the FDA unprecedented power to regulate tobacco products.

This perspective will also discuss the application of tobacco carcinogen and toxicant biomarkers in cancer prevention. Assessment of nonsmokers' exposure to secondhand tobacco smoke is already a success story of biomarkers in cancer prevention. Biomarkers also promise to increase our understanding of the mechanisms by which tobacco products cause cancer. This can lead to innovative approaches to cancer prevention by identifying and targeting those individuals who are particularly susceptible to the cancer causing effects of tobacco products.

The term biomarker has varied meanings. In the cancer research field in particular, this term is often associated with early detection of cancer. That is not the context here. Merriam-Webster's Collegiate Dictionary defines biomarker as "a distinctive biological or biologically derived indicator (as a metabolite) of a process, event, or condition." A biomarker in this perspective is any quantifiable substance, such as a metabolite, that can be specifically related to the uptake or effects of tobacco carcinogens or toxicants.

A Panel of Biomarkers

A panel of tobacco toxicant and carcinogen biomarkers that could be used in product regulation and studies on the prevention of tobacco-induced cancer is presented in Table 1, and their structures are illustrated in Figure 1. All biomarkers have been validated analytically. Most have been used in multiple studies on hundreds or even thousands of smokers and nonsmokers. (The exceptions are HBMA, HEMA, N^6-hydroxymethyl-dAdo, and N^2-ethylidene-dGuo.) Some typical recent data are summarized in Table 1. Although some of the ranges of values overlap between smokers and nonsmokers for certain biomar-

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Table 1. A Panel of Biomarkers for Investigating Tobacco Carcinogen and Toxicant Uptake and Their Possible Relationship to Cancer

urinary biomarkers	source	range of recent mean values (nmol/24 h unless noted otherwise[a]) smokers	nonsmokers	references (smokers)	references (nonsmokers)
nicotine equivalents[b]	nicotine	70.4−154 μmol/24 h	NA[c]	*42, 52, 77−81*	
total NNAL	NNK	1.1−2.9	NA	*16, 42, 52, 79, 81−85*	
total NNN	NNN	0.049−0.24	NA	*13, 83, 84, 86*	
1-HOP	pyrene	0.50−1.45	0.18−0.50	*16, 42, 52, 79, 80, 82, 87, 88*	*42, 52, 80, 87, 88*
MHBMA	1,3-butadiene	15.5−322	0.65−7.5	*16, 42, 78, 82*	*16, 42, 82*
SPMA	benzene	3.2−32.1	0.17−3.14	*16, 52, 78−80, 82, 87, 89*	*16, 52, 78, 80, 82, 87, 89*
HPMA	acrolein	5,869−11,190	1,131−1,847	*16, 42, 52, 78−80, 82, 89*	*16, 42, 52, 78, 80, 82*
HBMA	crotonaldehyde	1,965−26,000	242−3,200	*16, 78, 90*	*16, 78, 90*
HEMA	ethylene oxide	19.1−102	6.51−38.8	*16, 89*	*16, 89*
Cd	cadmium	2.3−12.8	1.34−8.04	*91−94*	*91−94*
8-epi-$PGF_{2\alpha}$[d]	oxidative damage	1.48−2.80	0.62−1.13	*95−97*	*95, 97*
PGE-M	inflammation	54−60	31.6−45.3	*27, 98*	*27, 98*

hemoglobin adducts	source	recent data (pmol/g globin; mean ± SD) smokers	nonsmokers	references (smokers)	references (nonsmokers)
cyanoethylvaline	acrylonitrile	112 ± 81	6.5 ± 6.4	*52, 99*	*52, 99*
carbamoylethylvaline	acrylamide	84.1 ± 41.8	27.8 ± 7.1	*52, 99*	*52, 99*
hydroxyethylvaline	ethylene oxide	132 ± 92	21.1 ± 12.7	*52, 99*	*52, 99*
4-aminobiphenyl-globin	4-aminobiphenyl	0.26 ± 0.006[e]	0.067 ± 0.009[e]	*42, 99*	*42, 99*

leukocyte DNA adducts	source	recent data (fmol/μmol dN; mean ± SD) smokers	nonsmokers	references (smokers)	references (nonsmokers)
N^6-hydroxymethyl-dAdo	formaldehyde	179 ± 205	15.5 ± 33.8	*100*	*100*
N^2-ethylidene-dGuo	acetaldehyde	1,310 ± 1,720	705 ± 438	*101*	*101*

other	source	mean concentrations smokers	nonsmokers	references (smokers)	references (nonsmokers)
exhaled CO	carbon monoxide	17.4−34.4 ppm	2.6−6.5 ppm	*34, 52*	*34, 52*
carboxyhemoglobin	carbon monoxide	3.4−7.1%	0.35−1.45%	*34, 42, 52, 81*	*34, 42, 52, 81*

[a] On the basis of 1.3 g of creatinine per 24 h in smokers and 1.5 g of creatinine per 24 h in nonsmokers, or 1.5 L of urine per 24 h. Creatinine determinations were mainly by a modified Jaffe reaction using a certified automated clinical analyzer. [b] Abbreviations: nicotine equivalents, the sum of nicotine, cotinine, 3′-hydroxycotinine, and their glucuronides; total NNAL, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol and its glucuronides; total NNN, *N*′-nitrosonornicotine and its glucuronides; 1-HOP, 1-hydroxypyrene and its glucuronides/sulfates; MHBMA, the sum of 1-hydroxy-2-(*N*-acetylcysteinyl)-3-butene and 1-(*N*-acetylcysteinyl)-2-hydroxy-3-butene; SPMA, *S*-phenyl mercapturic acid; HPMA, 3-hydroxypropyl mercapturic acid; HBMA, 4-hydroxybut-2-yl mercapturic acid; HEMA, 2-hydroxyethyl mercapturic acid; 8-epi-$PFG_{2\alpha}$, 9,11,15-trihydroxyprosta-5,13-dien-1-oic acid; PGE-M, 11α-hydroxy-9,15-dioxo-2,3,4,5-tetranorprostane-1,20-dioic acid. [c] NA = not applicable as these are not detected in the urine of nonsmokers unless they use other tobacco products, nicotine replacement products (for nicotine equivalents and sometimes NNN (*13*)), or are exposed to secondhand smoke, in which case levels are usually less than 5% of smoker levels (*102, 103*). [d] Determined by mass spectrometry. [e] Weighted mean ± SD.

kers, biomarker levels are consistently higher in smokers compared to those in nonsmokers in individual studies. Biomarkers of the tobacco-specific compounds are similar in smokers and smokeless tobacco users, while those of the volatile organic compounds are considerably lower in smokeless tobacco users, on the basis of our unpublished data.

"Nicotine equivalents", the sum of nicotine, cotinine, 3′-hydroxycotinine and their glucuronides, comprise 73−96% of the nicotine dose received by a tobacco user (*9*), and is a superb biomarker of nicotine uptake directly measuring a high percentage of the nicotine dose. This is obviously crucial for any study of a tobacco product since nicotine is the major known addictive constituent. Total NNAL and total NNN, the sum of free and glucuronidated NNAL and NNN, respectively, are biomarkers of uptake of the carcinogenic tobacco-specific nitrosamines NNK and NNN (*10*). NNK and NNN always occur together in tobacco products and are found in the particulate phase of tobacco smoke (*11*). NNK is a potent lung carcinogen in rodents and also induces tumors of the pancreas, liver, and nasal mucosa in rats (*12*). NNN causes esophageal and nasal tumors in rats and respiratory tract tumors in mice and hamsters (*12*). Among the biomarkers discussed here, nicotine equivalents, total NNAL, and total NNN are unique because of their tobacco-specificity. They are only detected in people exposed to tobacco products or (for nicotine equivalents and occasionally NNN) in people who use nicotine replacement products (*13*).

1-HOP is a biomarker of exposure to polycyclic aromatic hydrocarbons (PAH), particulate phase constituents of tobacco smoke and products of incomplete combustion, many of which are potent carcinogens inducing tumors of the rat lung, hamster trachea, and mouse forestomach and skin among other sites (*14, 15*). The prototypic PAH carcinogen is benzo[*a*]pyrene (BaP). PAH always occur as mixtures, and 1-HOP, a metabolite of the noncarcinogen pyrene, a component of these mixtures, is widely accepted as a biomarker of PAH exposure. The mercapturic acids MHBMA, SPMA, HPMA, HBMA, and HEMA are biomarkers of the tobacco smoke gas phase constituents 1,3-butadiene, benzene, acrolein, crotonaldehyde, and ethylene oxide, respectively (*16*). 1,3-Butadiene is a multiorgan carcinogen in mice and rats (*17, 18*). Sites of tumor

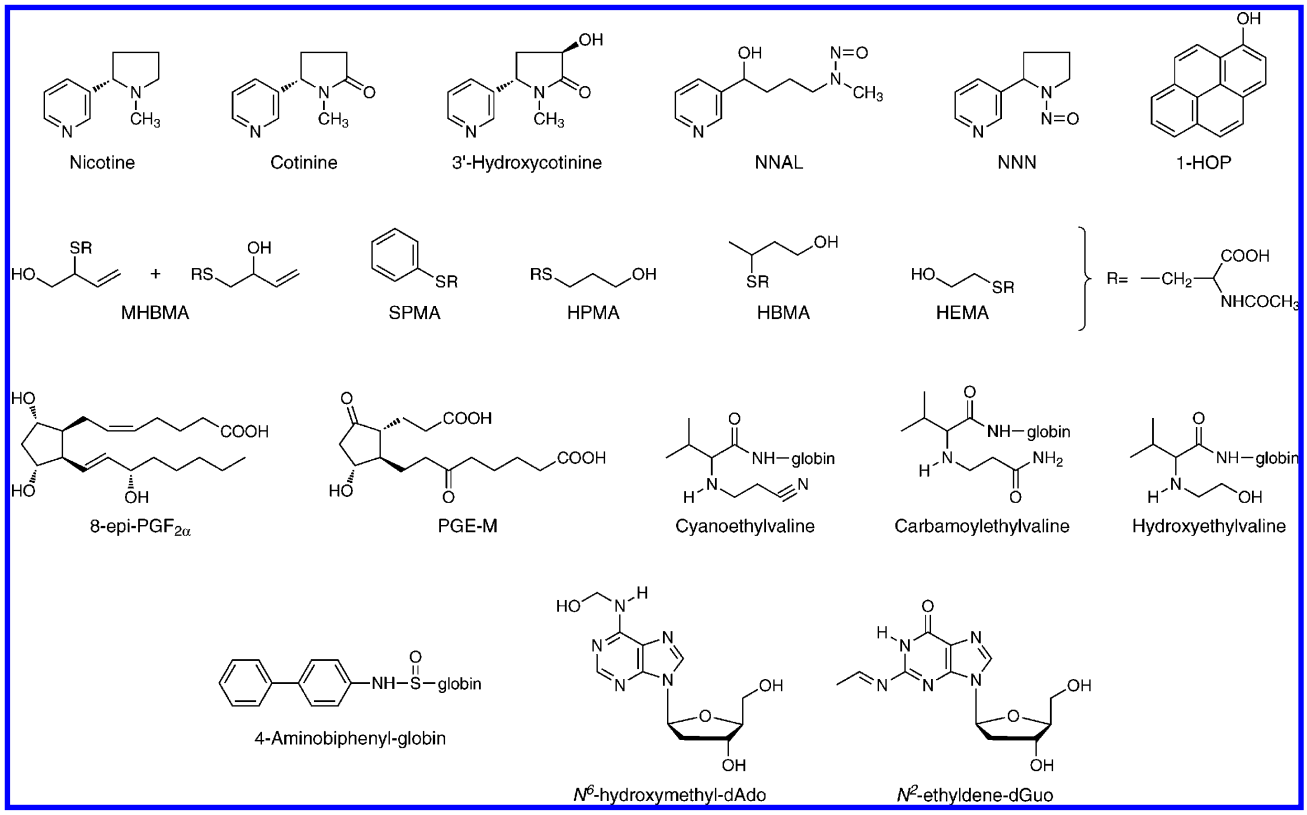

Figure 1. Structures of the biomarkers.

induction in mice include the hematopoietic system, heart, lung, forestomach, Harderian gland, preputial gland, liver, mammary gland, ovary, and kidney, while in rats, tumors are observed in the pancreas, testis, thyroid gland, mammary gland, uterus, and Zymbal gland. Benzene causes multiple types of tumors in both rats and mice exposed by various routes including oral administration, inhalation, injection, and dermal application (*19*). Ethylene oxide administered by inhalation causes alveolar/bronchiolar adenomas and carcinomas of the lung in male and female $B6C3F_1$ mice. Tumors of the Harderian gland, malignant lymphomas, uterine adenocarcinomas, and mammary gland carcinomas are also observed (*18*). Acrolein is an intense irritant and has a range of toxic effects including cilia-toxicity to the lung (*20*). Both acrolein and crotonaldehyde are associated with lipid peroxidation and perhaps inflammation (*21, 22*). Acrolein reacts with the *p53* gene at hot spots associated with lung cancer, a phenomenon which has also been observed in studies of PAH diol epoxides (*23*). Inhalation exposure to cadmium compounds causes lung tumors in rats (*24, 25*). 8-epi-$PGF_{2\alpha}$ is one of the isoprostanes, prostaglandin-like compounds which are formed by nonenzymatic free radical-induced peroxidation of arachidonic acid. It is an accepted biomarker of oxidative damage (*26*). PGE-M is a metabolite of cyclooxygenase-derived prostaglandin E_2 (PGE_2) (*27*). PGE_2 is associated with inflammation, tumor development, and a variety of other physiological responses (*27*).

Acrylonitrile is an important industrial chemical as well as a tobacco smoke constituent. It induces tumors at multiple sites in rats including forestomach, central nervous system, and mammary gland (*28, 29*). Acrylamide occurs widely in cooked starchy foods as well as in tobacco smoke. It causes a variety of tumors in rats including mesotheliomas of the testes, thyroid tumors, and mammary gland tumors (*30*). 4-Aminobiphenyl induces bladder tumors in rabbits and dogs and causes neoplasms at various sites in mice, while administration to rats produces tumors of the mammary gland and intestine (*31*).

Formaldehyde is genotoxic in multiple systems and causes squamous cell carcinomas of the nasal cavities in rats, while other studies produced mixed results (*32*). Acetaldehyde is genotoxic in a variety of in vitro systems and produces adenocarcinoma and squamous cell carcinoma of the nasal mucosa in rats, and laryngeal carcinoma in hamsters upon administration by inhalation (*33*). Carbon monoxide competes with oxygen for binding to Hb and impairs the release of oxygen from Hb. Although acute CO related symptoms are unlikely to occur in smokers (*34*), CO is believed to reduce oxygen delivery and promote complications of atherosclerosis and other cardiovascular diseases in smokers (*8*).

Overall then, the biomarkers in Table 1 represent a broad cross-section of carcinogens and toxicants in tobacco products. Among these compounds, NNK and NNN, BaP, 1,3-butadiene, benzene, ethylene oxide, cadmium, 4-aminobiphenyl, and formaldehyde are considered "carcinogenic to humans" by IARC (*11, 18, 25, 31, 32, 35*) and are likely causes of different types of cancer caused by tobacco use, a topic which is beyond the scope of this perspective but has been discussed elsewhere (*36*−*38*). Many of these compounds also have considerable toxic effects. While these constituents represent only a small percentage of the over 5000 identified compounds in cigarette smoke (*39*), they are collectively a powerful group and include all of those singled out by the WHO for regulation under the FCTC: acetaldehyde, acrolein, benzene, BaP, 1,3-butadiene, carbon monoxide, formaldehyde, NNN, and NNK (*40*). It is virtually inconceivable that a major reduction in their biomarker levels would not significantly impact cancer incidence in smokers.

Some fairly well-known biomarkers are not included in Table 1. BaP-DNA and Hb adducts, 4-hydroxy-1-(3-pyridyl)-1-butanone (HPB) releasing DNA and Hb adducts, other PAH metabolites such as phenanthrene tetraol and hydroxyfluorenes, DNA adducts as determined by ^{32}P postlabeling or immunoassay, and 3-ethyladenine in urine are examples. These have been omitted because they may require further validation, either

analytically or with respect to tobacco use in large studies, or may provide information similar to those listed in Table 1.

Some Strengths and Limitations of Biomarkers

It is axiomatic that dose is related to risk in toxicology. In the setting of this perspective, toxicant and carcinogen doses are expected to be related to cancer risk. The key advantage of the biomarkers discussed here is that they are reliable metrics of dose in a person who uses tobacco products. This is evident from the studies listed in the Table, most of which find significantly higher levels of all biomarkers in the high risk group, smokers, compared to those in the low risk group, nonsmokers.

Tobacco product use is highly complex. There are many different types of smoked and smokeless products, and each has differing amounts of the carcinogens and toxicants listed in Table 1. These have been classically measured in cigarette smoke by machine smoking protocols. These protocols are useful for comparing different products under standard conditions, but they may fail when one tries to reproduce complex and varied human smoking conditions or relate machine measured values to cancer risk. These aspects are beyond the scope of this perspective but have been discussed in detail in a recent review (*41*). Biomarkers have the potential to bypass these uncertainties and provide a realistic and direct assessment of carcinogen and toxicant dose in an individual. This is particularly true in cases such as nicotine equivalents where one is directly measuring most of the toxicant dose. But nicotine dose alone is not likely to be a good biomarker with respect to carcinogenic potential because cigarette smoke from different brands differs in the amounts of the other constituents in Table 1 when expressed per amount of nicotine (*40*). Thus, one large study found a strong relationship between nicotine equivalents and total NNAL ($R^2 = 0.5$) and HPMA ($R^2 = 0.48$), but only moderate or poor correlations with several other biomarkers including COHb, MHBMA, 1-HOP, and 4-ABP Hb adducts (*42*). Therefore, a panel of biomarkers selected from those in Table 1 likely will be necessary to obtain comprehensive and accurate information on toxicant and carcinogen dose.

The biomarkers listed in Table 1 measure different points in the continuum from exposure to internal dose to cellular effects. Nicotine equivalents, total NNAL, total NNN, 1-HOP, the mercapturic acids, and Cd in urine are all measures of exposure or dose, but to differing extents. Nicotine equivalents capture 73−96% of nicotine dose (*9*), total NNAL about 12−17% of NNK dose (*43, 44*), and total NNN an estimated 1% of NNN dose (*45*). Similar data do not seem to be available for the mercapturic acids and Cd. The DNA and Hb adducts are biomarkers of internal dose, indicating how much material reaches a cellular target (DNA) or its surrogate (Hb). 8-epi-$PGF_{2\alpha}$ and PGE-M are not directly related to cigarette smoke constituents but rather to their cellular effects, oxidative damage, and inflammation. While it is often assumed that biomarkers further along the continuum from exposure to cellular effects would be more closely related to risk, that is not necessarily the case and must be validated for each biomarker. For example, levels of the exposure biomarkers cotinine and total NNAL have been related to lung cancer risk in recent studies, discussed in more detail in the next section (*46*−*48*), but none of the other biomarkers discussed here have yet been validated with respect to cancer risk.

A limitation of the biomarker approach to estimation of dose is interindividual differences in metabolism, particularly in cases where a nearly complete metabolite profile is not being obtained. An example is total NNAL. It is a measure of the metabolism of NNK by carbonyl reduction and glucuronidation. But it does not take into account the pathways of metabolic activation of NNK, which lead to different metabolites. Increased metabolic activation of NNK would presumably increase cancer risk but would decrease total NNAL levels, thus blunting the effect of total NNAL as a risk biomarker. The solution to this problem is the use of a metabolic activation biomarker such as NNK-DNA adducts in conjunction with total NNAL. The effects of metabolism on some of the biomarkers listed in Table 1 require further investigation.

Potential Application of Biomarkers in Tobacco Product Regulation

The panel of biomarkers in Table 1 or perhaps a subset should be used as part of a strategy to regulate tobacco products. The critical question is, what are the mean biomarker values below which one would see a decrease in tobacco-induced cancer? As an ultimate goal, we should aim for the levels observed in nonsmokers, who are clearly at far lower risk for almost all tobacco-related cancers. But, recognizing that this is not likely to be feasible, one should set a realistic yet meaningful target level.

One approach to setting this target level is to carry out prospective epidemiologic studies or cohort studies of biomarkers and disease risk. In these studies, samples from healthy subjects are collected and stored, and demographic and lifestyle data are obtained using questionnaires. The subjects are then followed for years, and eventually, cancers will occur in some of them. The stored samples from these subjects are retrieved, along with samples from appropriately matched controls without cancer, to form a nested case-control study. These samples can be analyzed for the biomarkers to determine their relationship to disease. The magnitude of the relationship to disease risk for each biomarker and/or their combinations can be evaluated using standard statistical analysis methods. Mean target biomarker levels related to minimal risk of disease could be established. Although there are certain limitations of this approach which have been discussed (*49*), such epidemiologic studies with prospective study design and objective measurements of biomarkers in biospecimens would provide a direct link of the disease of interest to the biomarker and its parent compound. The relationship of tobacco carcinogen and toxicant biomarkers such as those discussed here to cancer has been examined in only limited prospective studies to date. Examples are cotinine and total NNAL with respect to lung cancer. In one prospective study, serum cotinine was related linearly to lung cancer risk, with no suggestion of a plateau at high exposure levels (*46*). Two recent molecular epidemiology studies related total NNAL to lung cancer risk. In one study, urinary levels of total NNAL were significantly associated with risk of lung cancer in a dose-dependent manner in smokers (*48*). Relative to the lowest tertile, risks associated with the second and third tertiles of total NNAL were 1.43 [95% CI, 0.86−2.37] and 2.11 (95% CI, 1.25−3.54], respectively (P for trend = 0.005) *after* adjustment for number of cigarettes per day, number of years of smoking, and total cotinine (cotinine plus its glucuronide). Smokers in the highest tertiles of urinary total NNAL and total cotinine exhibited an 8.5-fold increased risk for lung cancer relative to smokers in the lowest tertiles but otherwise comparable in smoking history. On the basis of this study, if urinary levels of both total NNAL and cotinine were reduced to the lowest third for all smokers, lung cancer incidence would have decreased by approximately three-fourths in smokers

of the target population. Similar results were obtained using prospective measurements of total NNAL in serum, although no relationship with cotinine was seen (*47*). Further studies are needed to explore the relationship to cancer risk to total NNAL, nicotine equivalents, and the other biomarkers in Table 1. This is feasible because there are many ongoing prospective cancer epidemiology studies in the world, and analytical methods for the panel listed in Table 1 are for the most part amenable to large sample sizes.

Once the mean biomarker target levels have been established using approaches such as those just discussed, the next step would be to design a tobacco product that met those target levels. This tobacco product, a cigarette, for example, would be tested using standard machine smoking methods to determine the level of each constituent that would correspond to each mean biomarker target level in the panel. Such testing would provide an approximation of the new product's potential for reduced exposure. Then, clinical studies which included a representative sample of smokers would be carried out to determine whether those who used this product actually met the mean target biomarker levels. Furthermore, subsequent postmarketing epidemiologic studies would also be conducted to provide a broader assessment of the mean levels of biomarkers achieved by the product and their relationship to cancer. The design of such studies is a science in itself and is beyond the scope of this perspective. Recent reviews discuss this subject comprehensively (*50, 51*).

While measurements of cigarette tar and nicotine using smoking machines generally do not correlate well with biomarker levels in smokers (*52*−*55*), it should be possible to establish predictable relationships of individual cigarette smoke constituents and the desired mean biomarker levels, as measured by smoking machines under defined conditions. If this can be accomplished, then these constituent levels as determined on smoking machines could be used in a practical approach to regulation.

The suggested regulatory approach under the FCTC is more conservative than that discussed here (*40*). The WHO study group on tobacco regulation decided against the use of biomarkers in regulation because "distinguishing the differences in biomarker levels due to variations between products from the differences due to smoker behavior (e.g. who uses the product and how they use it), is a formidable scientific challenge." The ultimate recommendation of the panel was to regulate on the basis of the levels of certain constituents as measured on smoking machines under the "intense smoking regimen" used by Health Canada and expressed per mg nicotine since smokers will adjust their pattern of smoking to obtain their desired dose of nicotine (*40*). The panel also took into account the number of cigarette brands that would be eliminated from the market by their proposed standards. This aspect is not considered here.

When light cigarettes began to appear on the market in the 1970s and were heralded by many, including some in the public health community as less harmful, biomarkers such as those discussed here were mainly unavailable. Cancer risk from smoking light cigarettes did not decrease, nor did most biomarkers (*52*−*57*). If these biomarkers had been available and applied at the time light cigarettes were introduced, then these cigarettes could not have been accepted as less harmful, and large numbers of cancer deaths presumably could have been prevented.

In summary, the approach described here comprises 3 major steps: (1) set a panel of mean target biomarker levels based on molecular epidemiologic studies of biomarkers and cancer risk; (2) determine the product constituent levels that correspond to the mean target biomarker levels in the panel; and (3) regulate on the basis of these determined constituent levels.

Application of Biomarkers in Cancer Prevention

Multiple epidemiologic studies, buttressed by tobacco carcinogen and biomarker studies, particularly cotinine and total NNAL, have established that secondhand smoke (SHS) causes immediate and long-term adverse health effects in nonsmokers, including lung cancer and heart disease (*58*). Exposure occurs mainly in homes and workplaces (*6*). These facts have spurred legislation which has had a major impact on tobacco control. In one recent survey of 11 states, most people reported having smoke-free home rules (*6*). Anti-tobacco legislation, such as clean air statutes that make indoor public places and worksites completely smoke-free are now law in 21 states of the U.S. and the District of Columbia (*6*). These positive developments, which are still in progress, would have been unimaginable in the 1980s when the first studies demonstrating the presence of cotinine in the serum and urine of nonsmokers exposed to SHS were published (*59*−*61*). According to the National Health and Nutrition Examination Survey (NHANES) study, the percentage of nonsmokers aged >4 years in the U.S. with detectable serum cotinine declined from 83.9% in 1988 to 46.4% in 1999−2004 (*6, 62*). The specificity of cotinine as a biomarker of nicotine exposure is the obvious strength of these studies. Cotinine and nicotine are, however, not carcinogenic. Total NNAL emerged in the 1990s as a biomarker of SHS exposure with impact because it is not only tobacco-specific but also represents the uptake of NNK, a lung carcinogen (*10, 63*). An additional advantage of total NNAL is its relatively long elimination half-life of up to 40−45 days compared to 3 days for cotinine (*64, 65*). Thus, cotinine and total NNAL have played a significant role in establishing secondhand smoke as a cause of disease, thus providing impetus for smoke free legislation, which has been important in decreasing cigarette smoking in the U.S., resulting in a steady decline in lung cancer mortality (*66*).

Although smoking causes up to 90% of lung cancer, about 11−24% of smokers will get lung cancer, and presently there is no way to reliably identify which user is susceptible (*4*). Tobacco carcinogen and toxicant biomarkers have the potential to identify those smokers at highest risk for cancer, but have yet to be applied for this purpose. Susceptibility must be related, at least in part, to carcinogen dose and processing (e.g., extent of metabolic activation, DNA adduct formation, and repair). Several recent studies have described approaches to the development of a risk prediction model for lung cancer, and these have been summarized (*67*). Variables in these models include mainly traditional epidemiologic parameters such as family history of cancer, smoking history, dust exposure, prior respiratory disease, and others (*68*−*70*). One recent model also includes host DNA repair capacity (*67*). The results to date are modest with respect to the prediction of lung cancer susceptibility. The inclusion of traditional smoking data such as pack-years of smoking detracts from the utility of such models, as it necessarily makes them retrospective in nature. Being able to predict lung cancer susceptibility in a young person who has just embarked on a regular pattern of smoking would be potentially much more powerful in cancer prevention. Biomarker measurements in these young smokers could perhaps provide a risk profile, thus leading to targeted smoking cessation intervention. What is needed are further prospective molecular epidemiology studies of the type discussed earlier in order to establish

a panel of biomarker levels demonstrably related to cancer incidence in smokers.

It seems likely that genetic polymorphism data ultimately will be included along with biomarkers in a tobacco and cancer risk algorithm. Many individual gene candidate studies have been carried out examining polymorphisms and cancer susceptibility in smokers, but to date with the possible exception of polymorphisms in cytochrome P4501A1 and glutathione transferases, the results have been generally quite modest (*71, 72*). Genome wide association studies in contrast have identified a locus at 15q24/15q25.1 which includes the nicotinic acetylcholine receptor A subunits 3 and 5 (*CHRNA3* and *CHRNA5*) genes associated with lung cancer (*73*−*75*). Our biomarker data demonstrated that carriers of these variants extract a greater amount of nicotine and have higher levels of total NNAL per cigarette than noncarriers (*76*). In this case, a combination of genotyping and biomarker studies provided mechanistic insight. The power of current genomic sequencing techniques argues for the potential inclusion of genetic data together with tobacco carcinogen and toxicant biomarker data in a predictive algorithm for cancer in tobacco users.

Conclusions

The use of tobacco carcinogen and toxicant biomarkers has expanded rapidly in recent years, and large amounts of new data are available. Sensitive, quantitative, and reliable analytical methods, mainly using mass spectrometry, are now available for assessment of these biomarkers, which are almost uniformly elevated in smokers. The methods are now sufficiently routine that their application in large studies is feasible, and this has been demonstrated for many of the biomarkers in the panel. The biomarkers can assess dose or dose plus metabolic processing, factors demonstrably related to risk, in people who use tobacco products. Therefore, they have great potential in tobacco product regulation and in the identification of individuals at high risk for cancer upon exposure to tobacco products.

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