Nicotine & Tobacco Research, Volume 14, Number 1 (January 2012) *18–28*

Commentary

Research Opportunities Related to Establishing Standards for Tobacco Products Under the Family Smoking Prevention and Tobacco Control Act

Stephen S. Hecht, Ph.D.

Masonic Cancer Center, University of Minnesota, Minneapolis, MN

Corresponding Author: Stephen S. Hecht, Ph.D., *Masonic Cancer Center, University of Minnesota, Mayo Mail Code 806, 420 Delaware St SE, Minneapolis, MN 55455, USA. Telephone: 612-624-7604; Fax: 612-626-5135; E-mail: hecht002@umn.edu*

Received September 7, 2010; accepted November 25, 2010

Abstract

Introduction: This paper was written in response to a request from the U.S. National Cancer Institute. The goal is to discuss some research directions related to establishing tobacco product standards under the Family Smoking Prevention and Tobacco Control Act, which empowers the U.S. Food and Drug Administration to regulate tobacco products. Potential research related to tobacco product ingredients, nicotine, and harmful or potentially harmful constituents of tobacco products is discussed.

Discussion: Ingredients, which are additives, require less attention than nicotine and harmful or potentially harmful constituents. With respect to nicotine, the threshold level in tobacco products below which dependent users will be able to freely stop using the product if they choose to do so is a very important question. Harmful and potentially harmful constituents include various toxicants and carcinogens. An updated list of 72 carcinogens in cigarette smoke is presented. A crucial question is the appropriate levels of toxicants and carcinogens in tobacco products. The use of carcinogen and toxicant biomarkers to determine these levels is discussed.

Conclusions: The need to establish regulatory standards for added ingredients, nicotine, and other tobacco and tobacco smoke constituents leads to many interesting and potentially highly significant research questions, which urgently need to be addressed.

Introduction

This paper was written at the request of the U.S. National Cancer Institute. The goal is to summarize some potential research directions, which may be pursued to more effectively establish tobacco product standards under the Family Smoking Prevention and Tobacco Control Act, which empowers the U.S. Food and Drug Administration (FDA) to regulate tobacco products. The focus of this paper is the effects of tobacco products on cancer. Research needs related to ingredients used in manufacturing tobacco products, nicotine in tobacco products, and reduction of known or suspected harmful constituents of tobacco products are discussed.

Each section briefly summarizes the history of regulation, what is known about regulation, what the law provides, and pertinent research opportunities.

Added Ingredients

History of Regulation

Currently, cigarette manufacturers are required to report additives to the Centers for Disease Control and Prevention annually under the Federal Cigarette Labeling and Advertising Act (Public Law 89–92) and the Comprehensive Smokeless Tobacco Health Education Act (Public Law 99–252). In addition, the U.S. Department of Agriculture regulates and monitors certain pesticides, which are prohibited in the United States but which may be present on imported tobacco (U.S. General Accounting Office, 2003). There are no current regulations that establish performance standards for ingredients.

What Is Known

Since, with the exception of certain pesticides, added ingredients are not regulated, the effects of regulation are unknown. However, an extensive evidence base exists on the toxicological properties of ingredients as summarized below.

What the Law Provides

Each tobacco product manufacturer or importer shall submit to the Secretary a list of ingredients that are added by the manufacturer to the tobacco, paper, filter, or other part of each tobacco product by brand and by quantity in each brand and subbrand.

Research Opportunities

Background

Tobacco constituents are those substances that are naturally present in tobacco, while tobacco ingredients are substances

doi: 10.1093/ntr/ntq216
Advance Access published on January 10, 2011

that are added to tobacco during the manufacturing process (Baker, Pereira da Silva, & Smith, 2004a). Tobacco ingredients are classified as flavors and additives. Flavors impart a specific taste, flavor, or aroma to the product, while additives are substances used for specific technological purposes. Additives include humectants, which increase the moisture-holding capacity of the tobacco; preservatives, which protect the product from deterioration; solvents, which are used to dissolve or dilute ingredients; binders and strengtheners, which make it possible to maintain the physical state of the product; and fillers, which contribute to the volume of the product without affecting odor, taste, or flavor (Baker et al., 2004a). The effects of tobacco ingredients on smoke chemistry and toxicology have been examined in many studies as summarized in Baker et al. (2004a).

In one study, the effects of more than 450 tobacco ingredients added to tobacco on levels of toxicants and on various bioassay systems were examined by researchers at British American Tobacco (Baker, Massey, & Smith, 2004; Baker et al., 2004a, 2004b). The ingredients comprised 431 flavors, 1 flavor/solvent, 1 solvent, 7 preservatives, 5 binders, 2 humectants, 2 process aids, and 1 filler. With few exceptions, there was little significant effect of any of the additives on smoke chemistry or biological endpoints, consistent with several earlier studies, as discussed by Baker et al. (2004a). These results indicate that added ingredients have little effect on smoke toxicology. It would be prudent however to independently validate some aspects of this research, which has been supported almost exclusively by the tobacco industry.

One interesting exception to the results described above involves the generation of formaldehyde during the combustion of sugars and relative additives (Baker, 2006). This leads to increased formaldehyde levels in the mainstream smoke of cigarettes. As formaldehyde is genotoxic and carcinogenic and formaldehyde–DNA adducts are present in leukocytes of smokers, this requires further investigation (Wang et al., 2009).

With the exception of ammonium compounds, there is little published information available on the effects of flavors and additives on product characteristics, such as attractiveness, sensory perception, palatability, and addictiveness.

Opportunities

- What are the effects of added ingredients on qualities, such as attractiveness, sensory perception, palatability, inhalability, and addictiveness of tobacco products? How do they contribute to uptake by nondependent users, to maintenance of tobacco use, and to topography?
- How do consumers perceive ingredients?
- What are the effects of added ingredients, individually and in combination, on tobacco smoke toxicology and carcinogenicity?
- Broadly, what is being put into tobacco products and why?

Nicotine

History of Regulation

There is currently no regulation of nicotine in tobacco products sold in the United States.

What Is Known

Since nicotine in tobacco products is not regulated in the United States, the effects of regulation of these products are virtually unknown, although nicotine levels in nicotine replacement products such as patch, gum, inhaler, nasal spray, and lozenge are regulated by FDA. There is a vast literature on the addictiveness, biochemistry, biology, pharmacology, and other properties of nicotine in tobacco and on the effects of the availability of cigarettes with differing machine measured nicotine levels (Henningfield & Zeller, 2009; Hukkanen, Jacob, & Benowitz, 2005).

What the Law Provides

Each tobacco product manufacturer or importer shall submit to the Secretary a description of the content, delivery, and form of nicotine in each tobacco product measured in milligrams of nicotine.

Research Opportunities

Background

Based on years of use, nicotine replacement therapy products, such as gum, patch, and lozenge, which must meet regulatory standards for purity and safety, appear to be relatively safe for short-term use. However, recent studies indicate that there is endogenous formation of the carcinogen *N*′-nitrosonornicotine (NNN) in some users of these products, particularly those who use gum or lozenge, which may present some hazards, especially if the products are used for extended periods of time (Stepanov, Carmella, Briggs, et al., 2009; Stepanov, Carmella, Han, et al., 2009). Nicotine, the major known addictive substance in tobacco products, is not a carcinogen, but a number of studies suggest that it may have cocarcinogenic or tumor-enhancing properties (Schuller, 2009), although there is presently no definitive evidence in this regard. The major problem is that addiction to nicotine in tobacco products leads to chronic exposure to the harmful constituents of tobacco and tobacco smoke, which accompany nicotine in all these products.

Nicotine exposure in people who use tobacco products can readily be quantified by measuring nicotine and five of its major metabolites—nicotine glucuronide, cotinine, cotinine–glucuronide, *trans*-3′-hydroxycotinine, and *trans*-3′-hydroxycotinine glucuronide—in urine (Hukkanen et al., 2005). These metabolites account for 73%–96% of the nicotine dose. Thus, a reliable and validated biomarker of nicotine exposure exists, and this biomarker, which has been used in studies on thousands of smokers (Hecht, Yuan, & Hatsukami, 2010), can bypass many questions about machine measurement of nicotine in cigarette smoke. This is pertinent to the recommendations below.

A plan for a comprehensive long-term nicotine policy has been presented (Gray et al., 2005). This plan proposes a three-phase policy. The initial phase would involve regulation of nicotine in tobacco products. The second phase involves introduction of clean nicotine products as the main source of nicotine. The third phase suggests progressive reduction of nicotine content of cigarettes, with clean nicotine taking their place.

The gradual reduction of nicotine in cigarettes has been proposed (Benowitz & Henningfield, 1994; Henningfield et al., 1998), and recent studies support this approach (Benowitz et al., 2007; Hatsukami et al., 2010; Yuan et al., 2009). In one

study, use of a cigarette containing 0.05 mg nicotine per cigarette was not associated with compensatory smoking behavior but did lead to reduced carcinogen exposure, nicotine dependence, and product withdrawal scores. This cigarette also led to a significantly higher rate of cessation than a cigarette containing 0.3 mg nicotine per cigarette and a similar rate of cessation as the nicotine lozenge (Hatsukami et al., 2010).

Basic compounds added to tobacco could affect smoke deliveries of nicotine. There is considerable literature data on this subject (Chen & Pankow, 2009). Published studies indicate that minor tobacco alkaloids such as nornicotine may have addictive properties and that acetaldehyde may contribute to the addictive properties of nicotine (Belluzzi, Wang, & Leslie, 2005; Clemens, Caille, Stinus, & Cador, 2009).

Opportunities

- What is the level of machine-measured nicotine, which leads to a given level of nicotine metabolite biomarkers in urine and what is the quantitative relationship between these parameters? This information is critical in establishing a realistic and practical metric for nicotine uptake in people who use tobacco products and can be determined in appropriately designed clinical studies.
- What is the threshold level of nicotine in tobacco products below which dependent users will be able to freely stop using the product if they choose to do so and what would be the optimal schedule for nicotine reduction? Clinical studies that use cigarettes with progressively lower levels of machine measured and/or biomarker-confirmed nicotine levels are necessary to extend and confirm results of published studies indicating the benefits of very low nicotine delivery cigarettes with respect to dependence and cessation. The results of these studies could establish the appropriate target level for regulation of nicotine in cigarette smoke.
- What is the threshold level of nicotine in tobacco products sufficient to cause dependence in a person who was nondependent?
- What is a reliable and valid method for comparing products and ranking them in terms of nicotine abuse liability?
- What factors of product design contribute to nicotine delivery of a product? For example, what are the effects of addition of ammonia and other basic compounds in tobacco on deliveries of nicotine and on levels of nicotine biomarkers?
- Are there other potentially addictive constituents of cigarette smoke, such as minor tobacco alkaloids or nicotine analogs, and what are the best ways to assess their uptake?
- What product standards would make products less addictive than they are currently?
- Are there threshold levels of nicotine delivery associated with loss of interest in use of smokeless tobacco products?
- What are the effects of tobacco pH and additives that affect pH on nicotine absorption from smokeless tobacco?

Harmful Constituents

History of Regulation

There is currently no regulation of harmful constituents—for example, constituents that can cause disease or other untoward effects—in the United States, except for the regulation of pesticides mentioned in "Added Ingredients." However, various countries have set maximum levels for certain harmful constituents—tar, nicotine, and CO—in cigarette smoke. So far, there is no evidence that these regulations, which depend on machine measurements of constituents using the International Organization for Standardization (ISO) method, have had an effect on disease risk. A proposal for mandated lowering of toxicants in cigarette smoke under the Framework Convention on Tobacco Control (FCTC) has been presented (Burns et al., 2008). This proposal focuses on regulation of several constituents of cigarette smoke, identified by a consideration of their animal and human toxicity, concentrations in cigarette smoke, variability across brands, potential for being lowered, and other factors. The constituents proposed for regulation are acetaldehyde, formaldehyde, acrolein, benzene, benzo[*a*]pyrene, 1,3-butadiene, carbon monoxide, and the tobacco-specific nitrosamines *N*′-nitrosonornicotine (NNN) and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK). Several other constituents were identified for disclosure and monitoring. The constituents would be regulated according to their concentrations per milligram of nicotine as determined using the intense smoking regimen employed by Health Canada. The initial levels selected for regulation were the median values of NNK and NNN for brands on the market and 125% of the median for the other toxicants. These initial levels were determined as a first step of a phased in regulatory process.

What Is Known

While a great deal is known about toxicants in tobacco products, little is known about the effectiveness of measures designed to regulate them. Levels of some harmful constituents that occur in occupational environments or in the general environment are regulated, for example, by the Occupational Safety and Health Administration or the Environmental Protection Agency, and some of these same constituents such as benzene and 1,3-butadiene are found in cigarette smoke. The current mandated minimum exposure levels for such constituents might serve as a guide for tobacco product regulation.

What the Law Provides

Each tobacco manufacturer or importer shall submit to the Secretary a listing of all constituents identified by the Secretary as harmful or potentially harmful to health in each tobacco product by brand and by quantity in each brand and subbrand.

Research Opportunities

Which Constituents Should Be Listed?

Background. Table 1 summarizes carcinogens in cigarette smoke (International Agency for Research on Cancer, 2004). This table has been updated based on recently available analytic data, as cited in the table, and other comprehensive lists of tobacco smoke carcinogens (Rodgman & Perfetti, 2009; Smith, Perfetti, Rumple, Rodgman, & Doolittle, 2000; 2001). The 72 compounds listed are only those that have been evaluated for carcinogenicity by the International Agency for Research on Cancer (IARC) and placed in Groups 1 (carcinogenic to humans), 2A (probably carcinogenic to humans), or 2B (possibly carcinogenic to humans). All the compounds are carcinogenic in laboratory animals, and 16 are rated as carcinogenic to humans. There are other carcinogens in cigarette smoke that have not been evaluated by IARC. These include, for example, multiple polycyclic aromatic hydrocarbons (PAH) and aromatic amines with incompletely characterized occurrence levels and carcinogenic activities (International Agency for Research on Cancer, 1986, 2004).

Table 1. Constituents of Cigarette Smoke Classified by the IARC as Carcinogenic (updated and revised in 2010)[a]

Carcinogen	Range of representative amounts in mainstream cigarette smoke, per cigarette		IARC Monographs evaluation of carcinogenicity			IARC Monograph volume, year; and (additional references)
	Weight	mol[b]	In animals	In humans	IARC group	
Polycyclic aromatic hydrocarbons (PAH)						
Benz[*j*]aceanthrylene[c]	Present	—	Limited		2B	92, 2010; (Rodgman & Perfetti, 2009)
Benz[*a*]anthracene	2.6–26.8 ng	117 pmol	Sufficient		2B	92, 2010; S7, 1987; (Chen & Moldoveanu, 2003; Ding et al., 2005; Roemer et al., 2004)
Benzo[*b*]fluoranthene	1.3–17.0 ng	67 pmol	Sufficient		2B	92, 2010; S7, 1987; (Ding et al., 2005; Roemer et al., 2004)
Benzo[*j*]fluoranthene	1.8–24 ng	95 pmol	Sufficient		2B	92, 2010; S7, 1987; (Ding et al., 2007)
Benzo[*k*]fluoranthene	0.5–3.3 ng	13 pmol	Sufficient		2B	92, 2010; S7, 1987; (Ding et al., 2005; Roemer et al., 2004)
Benzo[*c*]phenanthrene[c]	Present	—	Limited		2B	92, 2010
Benzo[*a*]pyrene (BaP)	1.0–15.2 ng	60 pmol	Sufficient	Limited	1	92, 2010; S7, 1987; (Counts et al., 2004; Ding et al., 2005; Hammond & O'Connor, 2008; Intorp et al., 2009; Roemer et al., 2004)
Chrysene	2.6–24.7 ng	108 pmol	Sufficient		2B	92, 2010; (Chen & Moldoveanu, 2003; Ding et al., 2005)
Cyclopenta[*c,d*]pyrene[c]	Present	—	Sufficiient		2A	92, 2010; (Rodgman & Perfetti, 2009)
Dibenz[*a,h*]anthracene	ND–6 ng	22 pmol	Sufficient		2A	92, 2010; S7, 1987; (Ding et al., 2007; Roemer et al., 2004)
Dibenzo[*a,e*]pyrene	1.5–2.6 ng	8.6 pmol	Sufficient		2B	92, 2010; S7, 1987; (Ding et al., 2007; Roemer et al., 2004)
Dibenzo[*a,i*]pyrene	0.7–1.2 ng	4.0 pmol	Sufficient		2B	92, 2010; S7, 1987; (Ding et al., 2007; Roemer et al., 2004)
Dibenzo[*a,h*]pyrene[c]	5–9.5 ng	31 pmol	Sufficient		2B	92, 2010; (Smith et al., 2001)
Dibenzo[*a,l*]pyrene[c]	0.1 ng	0.3 pmol	Sufficient		2A	92, 2010; (Seidel et al., 2004)
Indeno[1,2,3-*c,d*]pyrene	0.65–11.2 ng	41 pmol	Sufficient		2B	92, 2010; S7, 1987; (Ding et al., 2007; Roemer et al., 2004)
5-Methylchrysene[c]	ND–2 ng	8.3 pmol	Sufficient		2B	92, 2010; S7, 1987; (Smith et al., 2001)
Other hydrocarbons						
1,3-Butadiene	6.4–68.7 μg	1.3 μmol	Sufficient	Sufficient	1	97, 2008; 71, 1999; (Chen & Moldoveanu, 2003; Counts et al., 2004; Hammond & O'Connor, 2008; Gregg et al., 2004; Intorp et al., 2009; Roemer et al., 2004)
Isoprene	70–586 μg	8.6 μmol	Sufficient		2B	60, 1994; 71, 1999; (Chen & Moldoveanu, 2003; Counts et al., 2004; Gregg et al., 2004; Hammond & O'Connor, 2008; Intorp et al., 2009; Roemer et al., 2004)
Benzene	6.1–58.9 μg	0.8 μmol	Sufficient	Sufficient	1	29, 1982; S7, 1987; (Chen & Moldoveanu, 2003; Counts et al., 2004; Gregg et al., 2004; Hammond & O'Connor, 2008; Intorp et al., 2009; Roemer et al., 2004)
Ethylbenzene[c]	Present	—	Sufficient	Inadequate	2B	77, 2000
Naphthalene	65–868 ng	0.068 μmol	Sufficient	Inadequate	2B	82, 2002; (Chen & Moldoveanu, 2003; Ding et al., 2006)
Styrene	ND–48 μg	0.46 μmol	Limited	Limited	2B	82, 2002; (Chen & Moldoveanu, 2003; Ding et al., 2005; Gregg et al., 2004; Intorp et al., 2009)
N-Nitrosamines						
N-Nitrosodimethylamine	ND–7.9 ng	0.11 nmol	Sufficient		2A	17, 1978; S7, 1987; (Roemer et al., 2004)
N-Nitrosoethylmethylamine[c]	ND–0.2 ng	0.0023 nmol	Sufficient		2B	17, 1978; S7, 1987; (Smith et al., 2001)
N-Nitrosodiethylamine[c]	ND–7.6 ng	0.075 nmol	Sufficient		2A	17, 1978; S7, 1987; (Smith et al., 2000)
N-Nitrosopyrrolidine	ND–19.7 ng	0.197 nmol	Sufficient		2B	17, 1978; S7, 1987; (Roemer et al., 2004)
N-Nitrosopiperidine[c]	ND–231 ng	2 nmol	Sufficient		2B	17, 1978; S7, 1987; (Smith et al., 2001)
N-Nitrosodiethanolamine[c]	ND–290 ng	2.2 nmol	Sufficient		2B	17, 1978; 77, 2000; (Smith et al., 2001)

(continued)

Table 1. Constituents of Cigarette Smoke Classified by the IARC as Carcinogenic (updated and revised in 2010)[a] (continued)

Carcinogen	Range of representative amounts in mainstream cigarette smoke, per cigarette		IARC Monographs evaluation of carcinogenicity			IARC Monograph volume, year; and (additional references)
	Weight	mol[b]	In animals	In humans	IARC group	
N'-Nitrosonornicotine (NNN)	5.0–270 ng	1.5 nmol	Sufficient	Limited	1	89, 2007; S7, 1987; (Chen & Moldoveanu, 2003; Counts et al., 2004; Gregg et al., 2004; Intorp et al., 2009; Hammond & O'Connor, 2008; Roemer et al., 2004)
4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK)	13–223 ng	1.1 nmol	Sufficient	Limited	1	89, 2007; S7, 1987; (Chen & Moldoveanu, 2003; Counts et al., 2004; Gregg et al., 2004; Hammond & O'Connor, 2008; Intorp et al., 2009; Roemer et al., 2004)
Aromatic amines						
2-Toluidine	8.6–144 ng	1.3 nmol	Sufficient	Limited	2A	S7, 1987; 77, 2000; (Roemer et al., 2004)
2,6-Dimethylaniline[c]	3.6–18 ng	0.15 nmol	Sufficient		2B	57, 1993; (Smith et al., 2001)
2-Naphthylamine	1.47–17.2 ng	0.12 nmol	Sufficient	Sufficient	1	4, 1974; S7, 1987; (Chen & Moldoveanu, 2003; Counts et al., 2004; Gregg et al., 2004; Hammond & O'Connor, 2008; Intorp et al., 2009; Roemer et al., 2004)
4-Aminobiphenyl	0.3–3.3 ng	0.012 nmol	Sufficient	Sufficient	1	1, 1972; S7, 1987; (Chen & Moldoveanu, 2003; Counts et al., 2004; Gregg et al., 2004; Hammond & O'Connor, 2008; Intorp et al., 2009; Roemer et al., 2004)
o-Anisidine[c]	Present	—	Inadequate	Sufficient	2B	73, 1999
Heterocyclic aromatic amines[c]						
A-α-C	ND–260 ng	1.4 nmol	Sufficient		2B	S7, 1987; 40, 1986; (Smith et al., 2001)
MeA-α-C	2–37 ng	0.19 nmol	Sufficient		2B	S7, 1987; 40, 1986; (Smith et al., 2001)
IQ	0.3 ng	0.0015 nmol	Sufficient		2A	56, 1993; S7, 1987; (Smith et al., 2000)
Trp-P-1	0.2–0.3 ng	0.0015 nmol	Sufficient		2B	S7, 1987; 31, 1983; (Smith et al., 2001)
Trp-P-2	ND–0.2 ng	0.0011 nmol	Sufficient		2B	S7, 1987; 31, 1983; (Smith et al., 2001)
Glu-P-1	ND–0.89 ng	0.0045 nmol	Sufficient		2B	S7, 1987; 40, 1986; (Smith et al., 2001)
Glu-P-2	0.25–0.88 ng	0.0048 nmol	Sufficient		2B	S7, 1987; 40, 1986; (Smith et al., 2001)
PhIP	11–23 ng	0.10 nmol	Sufficient		2B	56, 1993; (Smith et al., 2001)
Other heterocyclic compounds[c]						
Furan	18–65 μg	0.96 μmol	Sufficient		2B	63, 1995; (Smith et al., 2001)
Dibenz[*a,h*]acridine	ND–0.1 ng	0.36 pmol	Sufficient		2B	S7, 1987; 32, 1983; (Smith et al., 2001)
Dibenz[*a,j*]acridine	ND–10 ng	3.6 pmol	Sufficient		2B	S7, 1987; 32, 1983; (Smith et al., 2001)
Dibenzo[*c,g*]carbazole	ND–0.7 ng	2.6 pmol	Sufficient		2B	S7, 1987; 32, 1983; (Smith et al., 2001)
Benzo[*b*]furan	Present	—	Sufficient		2B	63, 1995; (Smith et al., 2001)
Aldehydes						
Formaldehyde	1.6–75.5 μg	2.5 μmol	Sufficient	Sufficient	1	88, 2006; 62, 1995; (Chen & Moldoveanu, 2003; Counts et al., 2004; Gregg et al., 2004; Hammond & O'Connor, 2008; Intorp et al., 2009; Roemer et al., 2004)
Acetaldehyde	32–828 μg	19 μmol	Sufficient		2B	71, 1999;S7, 1987; (Chen & Moldoveanu, 2003; Counts et al., 2004; Gregg et al., 2004; Hammond & O'Connor, 2008; Intorp et al., 2009; Roemer et al., 2004)
Phenolic compounds						
Catechol	5.1–89.9 μg	0.82 μmol	Sufficient		2B	71, 1999; S7, 1987; (Chen & Moldoveanu, 2003; Counts et al., 2004; Gregg et al., 2004; Hammond & O'Connor, 2008; Intorp et al., 2009; Roemer et al., 2004)
Caffeic acid[c]	Present	—	Sufficient		2B	56, 1993; (Smith et al., 2001)

(continued)

Table 1. Constituents of Cigarette Smoke Classified by the IARC as Carcinogenic (updated and revised in 2010)[a] (continued)

Carcinogen	Range of representative amounts in mainstream cigarette smoke, per cigarette		IARC Monographs evaluation of carcinogenicity			IARC Monograph volume, year; and (additional references)
	Weight	mol[b]	In animals	In humans	IARC group	
Nitrohydrocarbons						
Nitromethane[c]	0.5–0.6 μg	9.8 nmol	Sufficient		2B	77, 2000
2-Nitropropane	ND–18.7 ng	0.21 nmol	Sufficient		2B	71, 1999; S7, 1987; (Roemer et al., 2004)
Nitrobenzene[c]	25 ng	0.20 nmol	Sufficient		2B	65, 1996; (Smith et al., 2001)
Miscellaneous organic compounds						
Acetamide[c]	2.2–111 μg	1.9 μmol	Sufficient		2B	S7, 1987; 71, 1999; (Smith et al., 2001)
Acrylamide[c]	2.3 μg	0.032 μmol	Sufficient		2A	S7, 1987; 60, 1994; (Smith et al., 2001)
Acrylonitrile	0.9–19.6 μg	0.36 μmol	Sufficient		2B	S7, 1987; 71, 1999; (Counts et al., 2004; Gregg et al., 2004; Intorp et al., 2009; Roemer et al., 2004)
Vinyl chloride	ND–36.6 ng	0.45 nmol	Sufficient	Sufficient	1	97, 2008 ; S7, 1987; (Roemer et al., 2004)
Ethylene oxide[c]	Present	—	Sufficient	Limited	1	97, 2008; 60, 1994
Propylene oxide[c]	Present	—	Sufficient		2B	60, 1994; S7, 1987
Urethane[c]	10–35 ng	0.35 nmol	Sufficient		2B	S7, 1987; 7, 1974; (Smith et al., 2001)
Vinyl acetate[c]	1.6–4 μg	0.047 μmol	Limited	Inadequate	2B	63, 1995
Metals and inorganic compounds						
Arsenic	ND–5.5 ng	0.07 nmol	Sufficient	Sufficient	1	84, 2004; (Counts et al., 2004)
Beryllium	ND–0.5 ng	0.06 nmol	Sufficient	Sufficient	1	58, 1993; S7, 1987; (Smith et al., 1997)
Nickel	ND–500 ng	8.5 nmol	Sufficient	Sufficient	1	49, 1990; S7, 1987; (Smith et al., 1997)
Chromium (hexavalent)	ND–2.6 ng	0.04 nmol	Sufficient	Sufficient	1	49, 1990; S7, 1987; (Counts et al., 2004; Smith et al., 1997)
Cadmium	1.6–101 ng	0.9 nmol	Sufficient	Sufficient	1	58, 1993; S7, 1987; (Counts et al., 2004; Hammond & O'Connor, 2008)
Cobalt[c]	0.13–100 ng	1.7 nmol	Sufficient		2B	52, 1991; (Smith et al., 2001)
Lead (inorganic)	3.9–39.2 ng	0.19 nmol	Sufficient	Limited	2A	87, 2004; S7, 1987; (Counts et al., 2004; Gregg et al., 2004)
Hydrazine[c]	24–57 ng	1.8 nmol	Sufficient		2B	71, 1999; S7, 1987; (Smith et al., 2001)
Radioisotope Polonium-210[c]	0.03–1.0 pCi	—	Sufficient		1	78, 2001

Note. A-α-C = 2-amino-9*H*-pyrido[2,3-*b*]indole; GIu-P-1 = 2-amino-6-methyl[1,2-*a*:3′,2′-*d*]imidazole; GIu-P-2 = 2-aminodipyrido[1,2-*a*:3′,2′-*d*]imidazole; IARC = International Agency for Research on Cancer; IQ = 2-amino-3-methylimidazo[4,5-*f*]quinoline; MeA-α-C = 2-amino-3-methyl-9*H*-pyrido[2,3-*b*]indole; ND = not detected; S7 = Supplement 7 of the IARC Monographs; Trp-P-1 = 3-amino-l,4-dimethyl-5*H*-pyrido[4,3-*b*]indole; pCi = picoCurie; PhIP = 2-amino-l-methyl-6-phenylimidazo[4,5-*b*]pyridine; Trp-P-2 = 3-amino-l-methyl-5*H*-pyrido[4,3-*b*]indole.

[a]This table [modified from Hoffmann et al. (2001) and IARC volume 83 (International Agency for Research on Cancer, 2004) and updated in July, 2010] shows compounds or elements in mainstream cigarette smoke, with representative amounts (determined using the ISO/Federal Trade Commission smoking conditions) given per cigarette. Presence and amounts in cigarette smoke were assessed based on recent literature as cited and data given in references (Chen & Moldoveanu, 2003; Counts et al., 2004; Gregg et al., 2004; Intorp et al., 2009; Smith et al., 2000, 2001; Rodgman & Perfetti, 2009). Only constituents evaluated by IARC and included in Groups 1 (16 constituents), 2A (9 constituents), or 2B (47 constituents) are listed. Virtually, all these substances are known carcinogens in experimental animals. In combination with data on cancer in humans and—in some cases—other relevant data, IARC Monographs classifications for these agents have been established as Group 2B (possibly carcinogenic to humans), Group 2A (probably carcinogenic to humans), or Group 1 (carcinogenic to humans). When IARC evaluations were made more than twice, only the two most recent Monographs are listed, with volume number and year of publication. No entry in the column "humans" indicates inadequate evidence or no data.

[b]Based on upper limit value only; μmol = 10^{-6} mol, nmol = 10^{-9} mol, pmol = 10^{-12} mol. 1 μmol = 6.02×10^{17} molecules.

[c]Not commonly reported: Values may be estimates or unreliable for the smoke of current cigarettes.

Table 1 expresses carcinogen levels in weight amounts and in molar amounts. Molar amounts are more appropriate than weight amounts when making biological comparisons. The relationship between molar amounts and number of molecules should also be kept in mind. Thus, 1 nmol is 6×10^{14} molecules, which would be the approximate number, for example, of NNK molecules delivered in the smoke of one cigarette.

There are other important constituents of cigarette smoke that are likely involved in the genesis of tobacco-related diseases. These include phenols such as hydroquinone, resorcinol, and cresols; aldehydes such as acrolein, crotonaldehyde, propionaldehyde, and butyraldehyde; other carbonyls such as acetone and 2-butanone; and other compounds such as nitrogen oxides, ammonia, hydrogen cyanide, carbon monoxide, toluene, and pyridine. Extensive data on levels of these constituents in cigarette smoke are available (Chen & Moldoveanu, 2003; Gregg et al., 2004; Intorp, Purkis, Whittaker, & Wright, 2009).

There are also poorly characterized constituents such as free radicals (6×10^{14} spins per cigarette or about 1 nmol) and oxidants in cigarette smoke. Little is known about the specific inflammatory agents in cigarette smoke that may be involved in chronic obstructive pulmonary disease and cancer. Also, some studies demonstrate that nicotine may influence carcinogenic pathways (Schuller, 2009). For smokeless tobacco, attention to constituents in addition to tobacco-specific nitrosamines and nicotine is required. For example, some products have unusually high levels of NaCl that could play a role in irritation and inflammation and act in concert with genotoxic carcinogens (Stepanov, Jensen, Hatsukami, & Hecht, 2008).

Opportunities.

- Which known constituents of tobacco products should be reduced in concentration in order to decrease cancer risk?
- What are the potential tumor promoters, cocarcinogens, inflammatory agents, and related materials in tobacco smoke that may influence the development of cancer and other diseases?
- What are the smokeless tobacco constituents other than nicotine, PAH, aldehydes, and tobacco-specific nitrosamines that may influence the development of cancer and other diseases? How might smokeless tobacco constituents impact cancer risk in smokers under conditions of dual use of cigarettes and smokeless tobacco products?

What Are the Appropriate Levels of These Constituents?

Background. This is the crucial question. The 20th century method for determining constituent levels in cigarette smoke was machine measurement. The 21st century method introduces tobacco carcinogen and toxicant biomarkers into constituent assessment. Machine measurement is useful for comparisons of different products under standard conditions but fails completely for determining actual deliveries to a smoker. Validated tobacco carcinogen and toxicant biomarkers represent a possible solution to this problem, and their use in constituent assessment and regulation is proposed here.

The general approach suggested here involves four steps:

1. Develop methods for accurate quantitation of tobacco toxicant and carcinogen biomarkers in users of tobacco products. Many of these methods are already available and have been extensively validated with respect to tobacco use (Hatsukami, Benowitz, Rennard, Oncken, & Hecht, 2006a; Hecht et al., 2010).
2. Validate biomarkers with respect to disease by carrying out molecular epidemiology studies. Determine target levels of biomarkers associated with reduced disease risk.
3. Determine product constituent levels that correspond to target biomarker levels by performing clinical studies to determine whether people who used a product with reduced constituent levels, as determined by machine measurement, showed a corresponding reduction in biomarker levels.
4. Use these machine-measured constituent levels in regulation.

Opportunities.

- What are the levels of particular constituents below which there would be no impact on disease risk?

Which Biomarkers Should Be Used in Regulation?

Background. Biomarkers for assessing potential health effects of tobacco products have been reviewed (Hatsukami et al., 2006b; Hecht et al., 2010), and a panel of biomarkers related to tobacco carcinogenesis has been suggested (Hecht et al., 2010). Validated biomarkers of exposure related to carcinogenic constituents of tobacco smoke include parent compounds or metabolites in blood, breath, nails, hair, or urine; carcinogen–DNA adducts; and carcinogen–protein adducts. Further research is required on the relationship of these biomarkers to disease. Some studies show that biomarkers such as cotinine and total 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL), a metabolite of NNK, are related to lung cancer risk (Boffetta et al., 2006; Church et al., 2009; Yuan et al., 2009). Less is known about biomarkers of inflammation, tumor promotion and cocarcinogenesis, chronic obstructive pulmonary disease, heart disease, and other tobacco-related diseases. Exhaled carbon monoxide and total nicotine metabolites are good general short-term biomarkers of cigarette smoke exposure and may ultimately serve as excellent monitors of exposure and disease risk. For smokeless tobacco, tobacco-specific nitrosamine metabolites, total nicotine metabolites, and measures of local damage in the oral epithelium, perhaps obtained from exfoliated oral cells, would be pertinent.

Opportunities.

- What are valid biomarkers of cancer, inflammation, tumor promotion, cocarcinogenesis, chronic obstructive pulmonary disease, heart disease, and other tobacco-related diseases?

How Does One Measure Constituents?

Background. Machine measurement of cigarette smoke constituents is not intended to fully characterize smoke composition but rather to produce a standardized method of comparison of different brands. Different methods have been discussed and characterized (Burns et al., 2008). In the approach discussed here, machine methods will be related to biomarker levels. A given tobacco product such as a cigarette would be tested using standard machine smoking methods to determine the level of each constituent that would correspond to each mean corresponding biomarker target level in a panel of biomarkers such as that which we have recently suggested

(Hecht et al., 2010). Such testing would approximate the new product's potential for reduced exposure. Then, clinical studies that include a representative sample of smokers would be performed. The object would be to determine whether those who used this product actually met the mean target biomarker levels. Postmarketing epidemiological studies would also be conducted to provide a broader assessment of the mean levels of biomarkers achieved by the product. The design of such studies has been reviewed (Hatsukami et al., 2009; O'Connor et al., 2009), and some studies of this type have been published (Hecht et al., 2010). The machine measurement method that best approximated validated biomarker levels would be used in regulation.

For smokeless tobacco products, constituent determinations are simpler, although different extraction methods can produce different constituent yields (Prokopczyk, Hoffmann, Cox, Djordjevic, & Brunnemann, 1992).

Opportunities.

- Which machine smoking method or extraction method for smokeless tobacco, if any, best predicts the relationships of constituents to biomarkers and disease?

What Are the Unknown Biological Properties of Tobacco Products That Need To Be Studied?

Background. While we understand a great deal about the toxic and carcinogenic constituents of tobacco products, there are also major gaps. Cigarette smoke contains more than 5,000 individual constituents (Rodgman & Green, 2003; Rodgman & Perfetti, 2009), but only a few have been thoroughly studied with respect to their potential toxic effects. Interactions among constituents have been only sporadically investigated. For example, we know that some PAH are cocarcinogens but that PAH also inhibit each other's metabolic activation to carcinogenic products (Shimada et al., 2007). The biological properties of the whole mixture are more difficult to investigate than those of individual constituents. This work is impeded, for example, by the lack of perfect animal models of cigarette smoke inhalation, yet it is critical for our understanding of pertinent regulatory approaches. Some major constituents such as CO_2, seemingly innocuous, may have been overlooked (Schwartz et al., 2010).

Opportunities.

- What are the significant deleterious properties of the whole tobacco or tobacco smoke mixture and their subfractions?

Testing and Reporting of Tobacco Product Constituents and Ingredients

History of Regulation

There is currently no reporting in a federal regulatory framework in the United States.

What Is Known

There are no comprehensive data available on constituent reporting under a federal regulatory framework in the United States. Tar and nicotine levels have been reported to the Federal Trade Commission since 1966, but the use of the Cambridge Filter Method for determination of tar and nicotine was discontinued in 2008.

What the Law Provides

The secretary shall require testing and reporting of tobacco product constituents, ingredients, and additives, including smoke constituents, by brand and subbrand that the Secretary determines should be tested to protect the public health . . . and may require that tobacco product manufacturers, packagers, or importers make disclosures relating to the results of the testing of tar and nicotine through labels or advertising or other appropriate means and make disclosures regarding the results of the testing of other constituents, including smoke constituents, ingredients, or additives.

Research Opportunities

Background

The three machine methods used for determining cigarette smoke constituents—ISO, intense modified ISO as used by Health Canada, and Massachusetts Department of Health regimen—have been discussed (Burns et al., 2008), and the FCTC panel decided to use the Health Canada method. It is unlikely that any one method can ever capture the variation in smoking characteristics among smokers. As machine method testing is simpler and far less expensive than biomarker testing, the use of a given machine method is deemed practical, but research is needed to relate constituent levels as determined by this method to biomarker threshold levels and disease risk as discussed in "Harmful Constituents." The appropriate methods for smokeless tobacco analysis need to be determined as well.

Opportunities

- What are the appropriate methods for testing and reporting tobacco product constituents?
- How often should these constituents be analyzed in a given product?

Summary of Research Recommendations

This section summarizes the main recommendations. Further details are provided in the text.

- What are the effects of added ingredients on qualities such as attractiveness, sensory perception, palatability, inhalability, and addictiveness of tobacco products? How do they contribute to uptake by nondependent users, to maintenance of tobacco use, and to topography?
- How do consumers perceive ingredients?
- What are the effects of added ingredients, individually and in combination, on tobacco smoke toxicology and carcinogenicity?
- Broadly, what is being put into tobacco products and why?
- What is the level of machine-measured nicotine that leads to a given level of nicotine metabolite biomarkers in urine and what is the quantitative relationship between these parame-

ters? This information is critical in establishing a realistic and practical metric for nicotine uptake in people who use tobacco products and can be determined in appropriately designed clinical studies.

- What is the threshold level of nicotine in tobacco products below which dependent users will be able to freely stop using the product if they choose to do so and what would be the optimal schedule for nicotine reduction? Clinical studies that use cigarettes with progressively lower levels of machine-measured/biomarker-confirmed nicotine levels are necessary to extend and confirm results of published studies indicating the benefits of very low nicotine delivery cigarettes with respect to dependence and cessation. The results of these studies could establish the appropriate target level for regulation of nicotine in cigarette smoke.
- What is the threshold level of nicotine in tobacco products sufficient to cause dependence in a person who was nondependent?
- What is a reliable and valid method for comparing products and ranking them in terms of nicotine abuse liability?
- What factors of product design contribute to nicotine delivery of a product? For example, what are the effects of addition of ammonia and other basic compounds in tobacco on deliveries of nicotine and on levels of nicotine biomarkers?
- Are there other potentially addictive constituents of cigarette smoke such as minor tobacco alkaloids or nicotine analogs and what are the best ways to assess their uptake?
- What product standards would make products less addictive than they are currently?
- Are there threshold levels of nicotine delivery associated with loss of interest in use of smokeless tobacco products?
- What are the effects of tobacco pH and additives that affect pH on nicotine absorption from smokeless tobacco?
- Which known constituents of tobacco products should be reduced in concentration in order to decrease disease risk?
- What are the potential tumor promoters, cocarcinogens, inflammatory agents, and related materials in tobacco smoke that may influence the development of cancer and other diseases?
- What are the smokeless tobacco constituents other than nicotine, PAH, aldehydes, and tobacco-specific nitrosamines that may influence the development of cancer and other diseases? How might smokeless tobacco constituents impact cancer risk in smokers under conditions of dual use of cigarettes and smokeless tobacco products?
- What are the levels of particular constituents below which there would be no impact on disease risk?
- What are valid biomarkers of inflammation, tumor promotion, cocarcinogenesis, chronic obstructive pulmonary disease, heart disease, and other tobacco-related diseases?
- Which machine smoking method or extraction method for smokeless tobacco, if any, best predicts the relationships of constituents to biomarkers and disease?
- What are the significant deleterious properties of the whole tobacco or tobacco smoke mixture and their subfractions?
- What are the appropriate methods for testing and reporting tobacco product constituents?
- How often should these constituents be analyzed in a given product?

Funding

The author's research on tobacco carcinogenesis is supported by grants no. CA-81301, CA-92025, CA-138338, and ES-11297 from the National Institutes of Health.

Declaration of Interests

None declared.

Acknowledgments

I thank Bob Carlson for editorial assistance.

References

Baker, R. R. (2006). The generation of formaldehyde in cigarettes—Overview and recent experiments. *Food and Chemical Toxicology*, *44*, 1799–1822. doi:10.1016/j.fct.2006.05.017

Baker, R. R., Massey, E. D., & Smith, G. (2004). An overview of the effects of tobacco ingredients on smoke chemistry and toxicity. *Food and Chemical Toxicology*, *42*(Suppl.), S53–S83. doi:10.1016/j.fct.2004.01.001

Baker, R. R., Pereira da Silva, J. R., Jr., & Smith, G. (2004a). The effect of tobacco ingredients on smoke chemistry. Part I: Flavourings and additives. *Food and Chemical Toxicology*, *42*(Suppl.), S3–S37. doi:10.1016/S0278-6915(03)00189-3

Baker, R. R., Pereira da Silva, J. R., & Smith, G. (2004b). The effect of tobacco ingredients on smoke chemistry. Part II: Casing ingredients. *Food and Chemical Toxicology*, *42*(Suppl.), S39–S52. doi:10.1016/j.fct.2003.08.009

Belluzzi, J. D., Wang, R., & Leslie, F. M. (2005). Acetaldehyde enhances acquisition of nicotine self-administration in adolescent rats. *Neuropsychopharmacology*, *30*, 705–712. doi:10.1038/sj.npp.1300586

Benowitz, N. L., Hall, S. M., Stewart, S., Wilson, M., Dempsey, D., & Jacob, P., III. (2007). Nicotine and carcinogen exposure with smoking of progressively reduced nicotine content cigarette. *Cancer Epidemiology Biomarkers & Prevention*, *16*, 2479–2485. doi:10.1158/1055-9965.EPI-07-0393

Benowitz, N. L., & Henningfield, J. E. (1994). Establishing a nicotine threshold for addiction. *New England Journal of Medicine*, *331*, 123–125. doi:10.1056/NEJM199407143310212

Boffetta, P., Clark, S., Shen, M., Gislefoss, R., Peto, R., & Andersen, A. (2006). Serum cotinine level as predictor of lung cancer risk. *Cancer Epidemiology Biomarkers & Prevention*, *15*, 1184–1188. doi:10.1158/1055-9965.EPI-06-0032

Burns, D. M., Dybing, E., Gray, N., Hecht, S., Anderson, C., Sanner, T., et al. (2008). Mandated lowering of toxicants in cigarette smoke: A description of the World Health Organization TobReg proposal. *Tobacco Control*, *17*, 132–141. doi:10.1136/tc.2007.024158

Chen, C., & Pankow, J. F. (2009). Gas/particle partitioning of two acid-base active compounds in mainstream tobacco smoke: Nicotine and ammonia. *Journal of Agricultural and Food Chemistry*, *57*, 2678–2690. doi:10.1021/jf803018x

Chen, P. X., & Moldoveanu, S. C. (2003). Mainstream smoke chemical analyses for 2R4F Kentucky reference cigarette. *Beiträge zur Tabakforschung International*, *20*, 448–458. Retrieved from http://www.beitraege-bti.de/

Church, T. R., Anderson, K. E., Caporaso, N. E., Geisser, M. S., Le, C., Zhang, Y., et al. (2009). A prospectively measured serum biomarker for a tobacco-specific carcinogen and lung cancer in smokers. *Cancer Epidemiology Biomarkers & Prevention*, *18*, 260–266. doi:10.1158/1055-9965.EPI-08-0718

Clemens, K. J., Caille, S., Stinus, L., & Cador, M. (2009). The addition of five minor tobacco alkaloids increases nicotine-induced hyperactivity, sensitization and intravenous self-administration in rats. *International Journal of Neuropsychopharmacology*, *12*, 1355–1366. doi:10.1017/S1461145709000273

Counts, M. E., Hsu, F. S., Laffoon, S. W., Dwyer, R. W., & Cox, R. H. (2004). Mainstream smoke constituent yields and predicting relationships from a worldwide market sample of cigarette brands: ISO smoking conditions. *Regulatory Toxicology and Pharmacology*, *39*, 111–134. doi:10.1016/j.yrtph.2003.12.005

Ding, Y. S., Ashley, D. L., & Watson, C. H. (2007). Determination of 10 carcinogenic polycyclic aromatic hydrocarbons in mainstream cigarette smoke. *Journal of Agricultural and Food Chemistry*, *55*, 5966–5973. doi:10.1021/jf070649o

Ding, Y. S., Trommel, J. S., Yan, X. J., Ashley, D., & Watson, C. H. (2005). Determination of 14 polycyclic aromatic hydrocarbons in mainstream smoke from domestic cigarettes. *Environmental Science and Technology*, *39*, 471–478. doi:10.1021/es048690k

Ding, Y. S., Yan, X. J., Jain, R. B., Lopp, E., Tavakoli, A., Polzin, G. M., et al. (2006). Determination of 14 polycyclic aromatic hydrocarbons in mainstream smoke from U.S. brand and non-U.S. brand cigarettes. *Environmental Science and Technology*, *40*, 1133–1138. doi:10.1021/es0517320

Gray, N., Henningfield, J. E., Benowitz, N. L., Connolly, G. N., Dresler, C., Fagerstrom, K., et al. (2005). Toward a comprehensive long term nicotine policy. *Tobacco Control*, *14*, 161–165. doi:10.1136/tc.2004.010272

Gregg, E., Hill, C., Hollywood, M., Kearney, M., McAdam, K., McLaughlin, D., et al. (2004). The UK smoke constituents testing study. Summary of results and comparison with other studies. *Beiträge zur Tabakforschung International*, *21*, 117–138. Retrieved from http://www.beitraege-bti.de/

Hammond, D., & O'Connor, R. J. (2008). Constituents in tobacco and smoke emissions from Canadian cigarettes. *Tobacco Control*, *17*(Suppl. 1), i24–i31. doi:10.1136/tc.2008.024778

Hatsukami, D. K., Benowitz, N. L., Rennard, S. I., Oncken, C., & Hecht, S. S. (2006a). Biomarkers to assess the utility of potential reduced exposure tobacco products. *Nicotine & Tobacco Research*, *8*, 169–191. doi:10.1080/14622200600576628

Hatsukami, D. K., Benowitz, N. L., Rennard, S. I., Oncken, C., & Hecht, S. S. (2006b). Biomarkers to assess the utility of potential reduced exposure tobacco products. *Nicotine & Tobacco Research*, *8*, 600–622. doi:10.1080/14622200600858166

Hatsukami, D. K., Hanson, K., Briggs, A., Parascandola, M., Genkinger, J. M., O'Connor, R., et al. (2009). Clinical trials methods for evaluation of potential reduced exposure products. *Cancer Epidemiology Biomarkers & Prevention*, *18*, 3143–3195. doi:10.1158/1055-9965.EPI-09-0654

Hatsukami, D. K., Kotlyar, M., Hertsgaard, L. A., Zhang, Y., Carmella, S. G., Jensen, J. A., et al. (2010). Reduced nicotine content cigarettes: Effects on toxicant exposure, dependence and cessation. *Addiction*, *105*, 343–355. doi:10.1111/j.1360-0443.2009.02780.x

Hecht, S. S., Yuan, J.-M, & Hatsukami, D. K. (2010). Applying tobacco carcinogen and toxicant biomarkers in product regulation and cancer prevention. *Chemical Research in Toxicology*, *23*, 1001–1008. doi:10.1021/tx100056m

Henningfield, J. E., Benowitz, N. L., Slade, J., Houston, T. P., Davis, R. M., & Deitchman, S. D. (1998). Reducing the addictiveness of cigarettes. Council on Scientific Affairs, American Medical Association. *Tobacco Control*, *7*, 281–293. doi:10.1136/tc.7.3.281

Henningfield, J. E., & Zeller, M. (2009). Nicotine psychopharmacology: Policy and regulatory. *Handbook of Experimental Pharmacology*, *192*, 511–534. doi:10.1007/978-3-540-69248-5_18

Hoffmann, D., Hoffmann, I., & El Bayoumy, K. (2001). The less harmful cigarette: A controversial issue. A tribute to Ernst L. Wynder. *Chemical Research in Toxicology*, *14*, 767–790. doi:10.1021/tx000260u

Hukkanen, J., Jacob, P., III, & Benowitz, N. L. (2005). Metabolism and disposition kinetics of nicotine. *Pharmacological Reviews*, *57*, 79–115. doi:10.1124/pr.57.1.3

International Agency for Research on Cancer. (1986). Tobacco smoking. In *IARC monographs on the evaluation of the carcinogenic risk of chemicals to humans, vol. 38* (pp. 37–385). Lyon, France: . Retrieved from http://www.iarc.fr/en/publications/list/

International Agency for Research on Cancer. (2004). Tobacco smoke and involuntary smoking. In *IARC monographs on the evaluation of carcinogenic risks to humans, vol. 83* (pp. 53–119). Lyon, France: Author. Retrieved from http://www.iarc.fr/en/publications/list/

Intorp, M., Purkis, S., Whittaker, M., & Wright, W. (2009). Determination of "Hoffmann analytes" in cigarette mainstream smoke. The Coresta 2006 Joint Experiment. *Beiträge zur Tabakforschung International*, *23*, 161–202. Retrieved from http://www.beitraege-bti.de/

O'Connor, R. J., Cummings, K. M., Rees, V. W., Connolly, G. N., Norton, K. J., Sweanor, D., et al. (2009). Surveillance methods for identifying, characterizing, and monitoring tobacco products: Potential reduced exposure products as an example. *Cancer Epidemiology Biomarkers & Prevention*, *18*, 3334–3348. doi:10.1158/1055-9965.EPI-09-0429

Prokopczyk, B., Hoffmann, D., Cox, J. E., Djordjevic, M. V., & Brunnemann, K. D. (1992). Supercritical fluid extraction in the determination of tobacco-specific N-nitrosamines in smokeless tobacco. *Chemical Research in Toxicology*, *5*, 336–340. doi:10.1021/tx00027a003

Rodgman, A., & Green, C. R. (2003). Toxic chemicals in cigarette mainstream smoke—Hazard and hoopla. *Beiträge zur Tabakforschung International*, *20*, 481–545. Retrieved from http://www.beitraege-bti.de/

Rodgman, A., & Perfetti, T. (2009). *The Chemical components of tobacco and tobacco smoke* (pp. 1483–1784), Boca Raton, FL: CRC Press. doi:10.1201/9781420078848

Roemer, E., Stabbert, R., Rustemeier, K., Veltel, D. J., Meisgen, T. J., Reininghaus, W., et al. (2004). Chemical composition, cytotoxicity and mutagenicity of smoke from US commercial and reference cigarettes smoked under two sets of machine smoking conditions. *Toxicology*, *195*, 31–52. doi:10.1016/j.tox.2003.08.006

Schuller, H. M. (2009). Is cancer triggered by altered signalling of nicotinic acetylcholine receptors? *Nature Reviews Cancer*, *9*, 195–205. doi:10.1038/nrc2590

Schwartz, L., Guais, A., Chaumet-Riffaud, P., Grevillot, G., Sasco, A. J., Molina, T. J., et al. (2010). Carbon dioxide is largely responsible for the acute inflammatory effects of tobacco smoke. *Inhalation Toxicology*, *22*, 543–551. doi:10.3109/08958370903555909

Seidel, A., Frank, H., Behnke, A., Schneider, D., & Jacob, J. (2004). Determination of dibenzo[a, l]pyrene and other fjord-region PAH isomers with MW 302 in environmental samples. *Polycyclic Aromatic Compounds*, *24*, 759–771. doi:10.1080/10406630490472527

Shimada, T., Murayama, N., Okada, K., Funae, Y., Yamazaki, H., & Guengerich, F. P. (2007). Different mechanisms for inhibition of human cytochromes P450 1A1, 1A2, and 1B1 by polycyclic aromatic inhibitors. *Chemical Research in Toxicology*, *20*, 489–496. doi:10.1021/tx600299p

Smith, C. J., Livingston, S. D., & Doolittle, D. J. (1997). An international literature survey of "IARC Group I Carcinogens" reported in mainstream cigarette smoke. *Food and Chemical Toxicology*, *35*, 1107–1130. doi:10.1016/S0278-6915(97)00063-X

Smith, C. J., Perfetti, T. R., Rumple, M. A., Rodgman, A., & Doolittle, D. J. (2000). "IARC Group 2A carcinogens" reported in cigarette mainstream smoke. *Food and Chemical Toxicology*, *38*, 371–383. doi:10.1016/S0278-6915(99)00156-8

Smith, C. J., Perfetti, T. A., Rumple, M. A., Rodgman, A., & Doolittle, D. J. (2001). 'IARC Group 2B carcinogens' reported in cigarette mainstream smoke (Vol. 38, pp. 825, 2000). *Food and Chemical Toxicology*, *39*, 181–205. doi:10.1016/S0278-6915(01)00008-4

Stepanov, I., Carmella, S. G., Briggs, A., Hertsgaard, L., Lindgren, B., Hatsukami, D. K., et al. (2009). Presence of the carcinogen *N′*-nitrosonornicotine in the urine of some users of oral nicotine replacement therapy products. *Cancer Research*, *69*, 8236–8240. doi:10.1158/0008-5472.CAN-09-1084

Stepanov, I., Carmella, S. G., Han, S., Pinto, A., Strasser, A. A., Lerman, C., et al. (2009). Evidence for endogenous formation of *N′*-nitrosonornicotine in some long term nicotine patch users. *Nicotine & Tobacco Research*, *11*, 99–105. doi:10.1093/ntr/ntn004

Stepanov, I., Jensen, J., Hatsukami, D., & Hecht, S. S. (2008). New and traditional smokeless tobacco: Comparison of toxicant and carcinogen levels. *Nicotine & Tobacco Research*, *10*, 1773–1782. doi:10.1080/14622200802443544

U.S. General Accounting Office. (2003). *Pesticides on tobacco: Federal activities to assess risk and monitor residues, GAO-03–485*. Retrieved from http://www.gao.gov/products/GAO-03-485

Wang, M., Cheng, G., Balbo, S., Carmella, S. G., Villalta, P. W., & Hecht, S. S. (2009). Clear differences in levels of a formaldehyde-DNA adduct in leukocytes of smokers and non-smokers. *Cancer Research*, *69*, 7170–7174. doi:10.1158/0008-5472.CAN-09-1571

Yuan, J. M., Koh, W. P., Murphy, S. E., Fan, Y., Wang, R., Carmella, S. G., et al. (2009). Urinary levels of tobacco-specific nitrosamine metabolites in relation to lung cancer development in two prospective cohorts of cigarette smokers. *Cancer Research*, *69*, 2990–2995. doi:10.1158/0008-5472.CAN-08-4330