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Perspective

The Less Harmful Cigarette: A Controversial Issue. A Tribute to Ernst L. Wynder

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The dose–response relationship between number of cigarettes smoked and risk for lung cancer was established in 1950 by epidemiological studies. Laboratory assays with tobacco tar on mouse skin and smoke inhalation experiments with hamsters provided further evidence for this relationship. In cigarette smoke, among 4800 identified compounds, 69 are carcinogens, and several are tumor promoters or cocarcinogens. The major toxic agents are nicotine, carbon monoxide, hydrogen cyanide, nitrogen oxides, some volatile aldehydes, some alkenes, and some aromatic hydrocarbons. Public health information and education have led to a reduction of cigarette smokers among U.S. adults from 40 to 25%. However, in high school students, smoking increased to 35% and in adults with less than a high school education it remains high at 33.3%. Intervention studies were augmented with attempts of risk reduction by changing the tobacco composition and makeup of cigarettes. This led to cigarettes that, according to the FTC, reduced the tar and nicotine yields from an average of 37 and 2.7 mg to 12 and 0.85 mg. The anticipated reduction of mortality rates from chronic diseases among cigarette smokers did not occur, primarily, because of a major adjustment in smoking intensity and depth of inhalation by the habitual smokers. It is, therefore, imperative that smoking control efforts are intensified and that, short of banning cigarette sales, cigarettes delivering smoke with the lowest potential for toxicity, addiction, and carcinogenicity are declared a matter of public health policy.

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I. Introduction

In developed countries, lung cancer is the major cause of mortality from cancer in men and women. Despite curative surgery and with the best therapeutic approaches, less than 14% of all lung cancer patients survive 5 years (1–3). In the United States, smoking contributes to more than 90% of all deaths from lung cancer in men and to about 80% of all deaths from lung cancer in women (2, 4). Cigarette smoking is also causally associated with cancer of the larynx, oral cavity, esophagus, pancreas, renal pelvis, and urinary bladder, and it is linked to cancer of the cervix (2–5). In the United States in 1991, more than 30% of 514 000 cancer deaths were attributed to cigarette smoking (5, 6). The Surgeon General's report for the year 1989 concluded that cigarette smoking accounted also for 81.8% of all deaths from chronic obstructive pulmonary disease (COPD),¹ for 21.5% of deaths from coronary heart disease (CHD), and for 18% of deaths from stroke (2). In 1990, smoking in 44 developed countries, as a whole, was responsible for 24% of all male deaths and 7% of all female deaths. These rates increased to over 40% in men in some East European countries and 17% in women in the U.S. (7).

The most promising avenue for the reduction of early death from cancer, COPD, CHD, and stroke among cigarette smokers is the vigorous implementation of comprehensive preventive strategies (8). These encompass increased taxation for all tobacco products, severe restriction or, better yet, elimination of advertisements, preventing minors to gain access to any tobacco product, banning of smoking at work sites, in public buildings, and public conveyances. Moreover, such measures would include a mandate for providing young people from kindergarten classes throughout high school with curricula about the harmful effects of smoking and use of other addictive substances. Whereas, scientists and physicians have only indirectly influenced the enactment of antismoking regulations, they should fully participate in interventions aimed at treating tobacco dependence for those who cannot stop smoking on their own.

Clinical smoking cessation programs should include instruction about the specific hazards of smoking and chewing of tobacco. Such instructions are to be conducted by trained psychologists and, where indicated, be accompanied by hypnosis, acupuncture, or treatment with drugs, including nicotine (8). In recent years, the development of chemopreventive agents for smoking-related diseases has advanced (9, 10). Such agents could be especially helpful toward reducing the risk for neoplastic disease among ex-smokers. This is an important goal in view of the fact that there are 45 million ex-smokers in

the U.S. alone who will face an increased risk for smoking-related cancers compared to the risk of life-long nonsmokers and who never reach the low risk of a nonsmoker (7, 8).

In 1950, there were 55 million cigarette smokers in a U.S. population of 151.3 million and, in 1990, 50.1 million cigarette smokers among 248.8 million U.S. residents. The annual consumption of cigarettes rose from 511.2 billion in 1964, the year of the Surgeon General's first report, to a high of 640 billion in 1981. Since then, annual consumption has declined to 465 billion in 1998 (11, 12). In 1972, consumption of cigarettes per adult (≥ 18 years) amounted to 3700 in the US, to 3280 in the U.K., and to 3900 in Canada. By 1990–1992, these figures declined to 2670, 2210, and 2540, respectively, and they continue to fall (1, 13). A similar decline of cigarette consumption has occurred in most West European countries (7). While this is encouraging, the figures unfortunately also reflect the limited success of smoking cessation efforts. Contrary to the trend of lower cigarette consumption among adults, there has been, since 1990, a significant increase in cigarette use among teenagers and adolescents in the U.S. In 1999, 9.2% of all middle school students and 34.8% of all high school students had smoked cigarettes in the month prior to being surveyed; overall, smoking and/or smokeless tobacco consumption was reported by 12.8 and 38.8% of the students, respectively (14).

The concept of "the less harmful cigarette" has been, and continues to be, an alternate approach toward reducing the morbidity and early deaths caused by cigarette smoking. However, "the less harmful cigarette" can, at best, be considered a compromise (15). Nevertheless, it is a necessary compromise for smokers who remain addicted to nicotine or will not give up their habit. The tobacco industry appears now to have decided to work toward a modified cigarette. Research on a "less harmful cigarette" actually began about 50 years ago by E. L. Wynder and his associates but it was always fully realized that there can be no "safe cigarette" (16).

This article discusses the controversy that has developed ever since the concept of "the less harmful cigarette" has emerged; with emphasis on the chemistry of tobacco smoke. Public health officials and some physicians and scientists were not and are not willing to accept this concept as a compromise. In fact, some scientists strongly opposed efforts toward the development of "the less harmful cigarette". This is evident from the enforced closing of the Tobacco Working Group of the National Cancer Institute (17). The major reasons for opposition to the concept of "the less harmful cigarette" lie in the assumption that any cigarette considered to be "less harmful" would inhibit a smoker's incentive to quit and that more of such cigarettes would be smoked. Moreover, the very term "less hazardous cigarette" may entice even more young people to start the addictive cigarette smoking habit. The counter-argument remains that men and women who continue to smoke should find on the market nothing but products with the least toxicity and the lowest carcinogenic activity that can be achieved with advanced science and technology. Those who view "the less harmful cigarette" as a compromise may agree, at least in part, with the sentiment of a 1989 editorial in a leading U.S. newspaper which reads, "Obviously, not smoking is better than smoking, but the best should not be the enemy of the good. There is a strong social case

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¹Abbreviations: AC, adenocarcinoma; BaA, benz(a)anthracene; BaP, benzo(a)pyrene; CHD, coronary heart disease; COHb, carboxyhemoglobin; COPD, chronic obstructive pulmonary disease; cpi, cuts per inch; CPS, cancer prevention study; CORESTA, Centre de Coopération pour les Recherches Scientifiques Relatives au Tabac; DMNA, N-nitrosodimethylamine; EHC, electrically heated cigarette; ET, expanded tobacco; DMBA, 7,12-dimethylbenz(a)anthracene; FDA, Federal Drug Administration; FTC, Federal Trade Commission; GRAS, generally regarded as safe; IARC, International Agency for Research on Cancer; MS, mainstream smoke; NAB, N-nitrosoanabasine; NAT, N-nitrosoanatabine; NNAL, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol; NNK, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone; NNN, N-nitrosornicotine; PAH, polycyclic aromatic hydrocarbons; PG, 1,2-propylene glycol; RT, reconstituted tobacco; SCC, squamous cell carcinoma; TSG, tobacco study group; TSNA, tobacco-specific N-nitrosamines; TWG, tobacco working group; VNA, volatile N-nitrosamines.

for encouraging manufacturers to develop safer cigarettes that will sell" (18).

As scientists who have studied "the less harmful cigarette" for many years, we have proposed various ideas toward this concept. Working with the late E. L. Wynder, our group was the first to publish a reproducible bioassay method for estimating the carcinogenic potential of the particulate matter of cigarette smoke. We were the first to isolate from cigarette smoke, in crystalline form, a major carcinogen, benzo(a)pyrene (BaP). Our group was also the first to report that nicotine gives rise to highly carcinogenic nitrosamines that induce cancer of the lung, upper aerodigestive tract, and pancreas (1, 2). These findings were initially challenged by the industry. Scientists outside the industry also demonstrated, for the first time, that charcoal filter tips have the capacity for selective removal of ciliotoxic smoke constituents and that cellulose acetate filter tips selectively reduce volatile phenols, which are tumor promoting agents. We reported that during tobacco processing and during smoking a portion of the sucker growth inhibitor maleic hydrazide diethanolamine (MH-30) turns into a strong carcinogen, *N*-nitrosodiethanolamine and that in the course of smoking, as also reported by others, the human carcinogen ethylene oxide is formed from the humectant ethylene glycol. These are only a few findings that have contributed significantly to a better understanding of the chemistry, toxicity, and carcinogenicity of cigarette smoke (1, 2).

In Section II, we summarize our present knowledge of the chemical composition of cigarette smoke, list the known carcinogens in cigarette smoke, and discuss those agents that are likely contributors to the disorders induced by cigarette smoking. Section III presents, in some detail, the changes that have occurred since 1950 in the makeup and the chemical composition of the U.S. cigarette and discusses concurrent changes in the smoke.

II. Chemical Composition and Toxic and Carcinogenic Agents in Cigarette Smoke

In 1950, two large-scale case control studies demonstrated that cigarette smoking is associated with cancer of the lung (19, 20). This finding was supported by the induction of skin tumors in mice treated with cigarette "tar" (21, 22).² Moreover, in 1973, the formation of benign and malignant tumors in the larynx of hamsters exposed to whole cigarette smoke confirmed the link (23). These reports led to intensive research on the chemical composition of cigarette smoke and to the identification of toxic and carcinogenic agents in the smoke. The high interest in the physicochemical nature of tobacco smoke and in its toxicology and carcinogenicity is reflected in the progressive identification of smoke constituents. In 1959, Johnstone and Plimmer reported that about 600 compounds had been found in cigarette smoke (24). In 1968, Stedman listed 1000 smoke compounds (25). In 1980, Ishiguro and Sugawara claimed 1889 compounds and in 1988, Roberts listed 3794 cigarette smoke constituents (26, 27). In 1996, Green and Rodgman reported that 4800 compounds had been identified in tobacco

Table 1. Compounds Identified in Tobacco and Smoke^{a,b}

functional groups	no. in tobacco	no. in smoke	no. in tobacco and smoke
carboxylic acids	450	69	140
amino acids	95	18	16
lactones	129	135	39
esters	529	456	314
amides and imides	205	227	32
anhydrides	10	10	4
aldehydes	111	106	48
carbohydrates	138	30	12
nitriles	4	101	4
ketones	348	461	122
alcohols	334	157	69
phenols	58	188	40
amines	65	150	37
<i>N</i> -nitrosamines	23	18	19
sulfur compounds	3	37	2
<i>N</i> -heterocyclics			
pyridines	63	324	46
pyrroles and indoles	9	88	3
pyrazines	21	55	18
non-aromatics	13	43	7
polycyclic aromatic	1	36	0
others	4	50	2
ethers	53	88	15
hydrocarbons			
saturated aliphatics	58	113	44
unsaturated aliphatics	38	178	10
monocyclic aromatics	33	138	25
polycyclic aromatics	55	317	35
pesticides	28	25	25
miscellaneous	112	110	19
inorganics and metals	105	111	69

^a D. L. Roberts (27). ^b Two additional groups have been added, *N*-nitrosamines and pesticides.

smoke (28). Roberts listed the identified smoke components according to their functional groups; a slightly modified version of this list is presented in Table 1.

Cigarette smoke is composed of a vapor phase and the particulate phase. The vapor phase is arbitrarily defined as that portion of the smoke aerosol which passes through a Cambridge glass fiber filter. The particulate phase is that portion which is trapped on the glass fiber filter. Its particle sizes range from 0.1 to <1.0 μm in diameter. This definition does not fully reflect the conditions prevailing in freshly generated cigarette smoke. Some semivolatile agents, such as phenol for example, appear to some extent in the vapor phase. Some of the substituted phenols, the semi-volatile *N*-nitrosamines, and volatile compounds, such as hydrogen cyanide and low-boiling aldehydes are partially trapped as aerosol inclusions in the particulate matter. The vapor phase accounts for 90–96% of the weight of the mainstream smoke of a nonfilter cigarette with the following compounds as major constituents: nitrogen ~60%, oxygen ~13%, carbon dioxide 13%, carbon monoxide 3.5%, water 2%, argon 1%, hydrogen 0.1–0.2%, acetone ~1%, nitrogen oxides (NO, NO₂, N₂O) < 0.1%, and volatile sulfur compounds likewise < 0.1% (Table 2). Major components of the particulate phase include nicotine (0.2–0.6% of the weight of the total smoke); the remaining *Nicotiana* alkaloids amount to ~0.02%, and compounds specific to solanaceae, namely *n*-hentriacontane (C₃₁H₆₄) and solanesol (0.1–0.2%). In addition, the particulate phase contains catechols (~1%), 3- and 4-ring noncarcinogenic aromatic hydrocarbons (~0.0003–0.007% = 3–7 ppm) and the carcinogenic PAH 0.00002–0.00007% (0.3–0.7 ppm) (Table 3). These relative proportions of smoke components are approximate figures.

²The term "tar" has been chosen as a descriptive noun only. It does not reflect the freshly generated particulate matter in cigarette smoke, but is arbitrarily defined as the portion of the smoke trapped on a Cambridge glass fiber filter CM-20 with water and nicotine deducted.

Table 2. Major Constituents of the Vapor Phase of the Mainstream Smoke of Nonfilter Cigarettes

compd	concentration/cigarette (% of total effluent)
nitrogen	280–320 mg (56–64%)
oxygen	50–70 mg (11–14%)
carbon dioxide	45–65 mg (9–13%)
carbon monoxide	14–23 mg (2.8–4.6%)
water	7–12 mg (1.4–2.4%)
argon	5 mg (1.0%)
hydrogen	0.5–1.0 mg
ammonia	10–130 μ g
nitrogen oxides (NO _x)	100–600 μ g
hydrogen cyanide	400–500 μ g
hydrogen sulfide	20–90 μ g
methane	1.0–2.0 mg
other volatile alkanes (20) ^a	1.0–1.6 mg ^b
volatile alkenes (16)	0.4–0.5 mg
isoprene	0.2–0.4 mg
butadiene	25–40 μ g
acetylene	20–35 μ g
benzene	6–70 μ g
toluene	5–90 μ g
styrene	10 μ g
other volatile aromatic hydrocarbons (29)	15–30 μ g
formic acid	200–600 μ g
acetic acid	300–1700 μ g
propionic acid	100–300 μ g
methyl formate	20–30 μ g
other volatile acids (6)	5–10 μ g ^b
formaldehyde	20–100 μ g
acetaldehyde	400–1400 μ g
acrolein	60–240 μ g
other volatile ketones (3)	80–140 μ g
methanol	100–650 μ g
other volatile ketones (3)	50–100 μ g
methanol	80–180 μ g
other volatile alcohols (7)	10–30 μ g
acetonitrile	100–150 μ g
other volatile nitriles (10)	50–80 μ g ^b
furan	20–40 μ g
other volatile furans (4)	45–125 μ g ^b
pyridine	20–200 μ g
picolines (3)	15–80 μ g
3-vinylpyridine	7–30 μ g
other volatile pyridines (25)	20–50 μ g ^b
pyrrole	0.1–10 μ g
pyrrolidine	10–18 μ g
N-methylpyrrolidine	2.0–3.0 μ g
volatile pyrazines (18)	3.0–8.0 μ g
methylamine	4–10 μ g
other aliphatic amines (23)	3–10 μ g

^a Parentheses show the number of individual compounds identified in a given group. ^b Estimate.

In 1954, Cooper, Lindsey, and Waller identified the polycyclic aromatic hydrocarbon (PAH) BaP as the first carcinogen in cigarette smoke (29). Advances in chemical analytical techniques and increased knowledge of genotoxic environmental agents brought the number of carcinogens identified in tobacco smoke to 69 by the year 2000. These include, in addition to BaP, another nine PAH, and four aromatic amines, among them two known human bladder carcinogens; they also include nitrosamines, aldehydes, and several other organic and inorganic compounds (Table 4). The carcinogenic potential of these compounds has been assessed according to the classification of carcinogens by IARC, the International Agency for Research on Cancer (30). Accordingly, there are among the identified smoke constituents 69 animal carcinogens; 48 of these are possibly also carcinogenic to humans, 8 are probably carcinogenic to humans, and 11 are proven human carcinogens. Two of these compounds

Table 3. Major Constituents of the Particulate Matter of the Mainstream Smoke of Nonfilter Cigarettes

compd	μ g/cigarette
nicotine	100–3000
normicotine	5–150
anatabine	5–15
anabasine	5–12
other tobacco alkaloids (1.7) ^a	n.a. ^d
bipyridyls (4)	10–30
n-hentriacontane (n-C ₃₁ H ₆₄)	100
total nonvolatile hydrocarbons (45) ^b	300–400 ^b
naphthalene	2–4
naphthalenes (23)	3–6 ^b
phenanthrenes (7)	0.2–0.4 ^b
anthracenes (5)	0.05–0.1 ^b
fluorenes (7)	0.3–0.5 ^b
pyrenes (6)	0.3–0.45 ^b
fluoranthrenes (5)	0.1–0.25
carcinogenic polynuclear aromatic hydrocarbons (11)^c	80–160
phenol	60–180 ^b
other phenols (45) ^b	200–400
catechol	100–200 ^b
other catechols (4)	200–400 ^b
other dihydroxybenzenes (10)	15–30
scopoletin	n.a.
other polyphenols (8) ^b	40–70 ^b
cyclotenes (10) ^b	0.5
quinones (7)	600–1000
solanesol	200–350
neophytadienes (94)	30–60
limonene	n.a.
other terpenes (200–500) ^b	100–150
palmitic acid	50–75
oleic acid	40–110
linoleic acid	150–250
linolenic acid	150–250
lactic acid	60–80
indole	10–15
skatole	12–16
other indoles (13)	n.a.
quinolines (7)	2–4
other aza-arenes (55)	n.a.
benzofurans (4)	200–300

^a Parentheses show the number of individual compounds identified in a given group. ^b Estimate; n.a., not available.

have not yet been evaluated for their carcinogenicity by the IARC (15, 30).

Of the 69 carcinogens listed in Table 4, all but 1,2-propylene oxide have been identified independently by at least two research teams. Scientists in the industry have confirmed the presence in tobacco smoke of at least 50 of the 69 carcinogens even though they published their findings in many cases several years after the first reports on the identification of such carcinogens in the literature. For example, vinyl chloride, first reported as a tobacco smoke constituent at the 28th Tobacco Chemists' Research Conference in 1975 and published in 1976, was not acknowledged by industry scientists until the 51st Tobacco Chemists' Research Conference in 1997 (31, 32).

However, the turn of events and changing policies of the industry are reflected in the fact that, in 1999, scientists from the Research Laboratories of the R. J. Reynolds Company listed all but 2 of the 69 cigarette smoke carcinogens in their publications. The exceptions were polonium-210 and 1,2-propylene oxide (PO) (33–36). Levels of ²¹⁰Po in the lungs of cigarette smokers were found to be generally three times higher than those in the lungs of nonsmokers (37). The U.S. National Council on Radiation Protection and Measurement ascribed about 1% of the risk for lung cancer after 50 years of cigarette

Table 4. Carcinogens in Cigarette Smoke^d

agent	concentration in smoke of nonfilter cigarette	IARC evaluation of carcinogenicity		group ^a
		in lab animals	in humans	
PAH				
benz(<i>a</i>)anthracene	20–70 ng	sufficient		2A
benzo(<i>b</i>)fluoranthene	4–22 ng	sufficient		2B
benzo(<i>j</i>)fluoranthene	6–21 ng	sufficient		2B
benzo(<i>k</i>)fluoranthene	6–12 ng	sufficient		2B
benzo(<i>a</i>)pyrene	20–40 ng	sufficient	probable	2A
dibenz(<i>a,h</i>)anthracene	4 ng	sufficient		2A
dibenzo(<i>a,l</i>)pyrene	1.7–3.2 ng	sufficient		2B
dibenzo(<i>a,e</i>)pyrene	present	sufficient		2B
indeno(1,2,3- <i>cd</i>)pyrene	4–20 ng	sufficient		2B
5-methylchrysene	0.6 ng	sufficient		2B
heterocyclic hydrocarbons				
furan	18–37 μg	sufficient		2B
quinoline ^b	1–2 μg			
dibenz(<i>a,h</i>)acridine	0.1 ng	sufficient		2B
dibenz(<i>a,j</i>)acridine	3–10 ng	sufficient		2B
dibenzo(<i>c,g</i>)carbazole	0.7 ng	sufficient		2B
benzo(<i>b</i>)furan	present	sufficient		2B
N-nitrosamines				
N-nitrosodimethylamine	2–1000 ng	sufficient		2A
N-nitrosoethylmethylamine	3–13 ng	sufficient		2B
N-nitrosodiethylamine	ND–2.8 ng	sufficient		2A
N-nitrosodi- <i>n</i> -propylamine	ND–1.0 ng	sufficient		2B
N-nitroso-di- <i>n</i> -butylamine	ND–30 ng	sufficient		2B
N-nitrosopyrrolidine	3–110 ng	sufficient		2B
N-nitrosopiperidine	ND–9 ng	sufficient		2B
N-nitrosodiethanolamine	ND–68 ng	sufficient		2B
N-nitrosornicotine	120–3700 ng	sufficient		2B
4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone	80–770 ng	sufficient		2B
aromatic amines				
2-toluidine	30–337 ng	sufficient		2B
2,6-dimethylaniline	4–50 ng	sufficient		2B
2-naphthylamine	1–334 ng	sufficient	sufficient	1
4-aminobiphenyl	2–5.6 ng	sufficient	sufficient	1
N-heterocyclic amines				
AaC	25–260 ng	sufficient		2B
MeAaC	2–37 ng	sufficient		2B
IQ	0.3 ng	sufficient		2A
Trp-P-1	0.3–0.5 ng	sufficient		2B
Trp-P-2	0.8–1.1 ng	sufficient		2B
Glu-P-1	0.37–0.89 ng	sufficient		2B
Glu-P-2	0.25–0.88 ng	sufficient		2B
PhIP	11–23 ng	sufficient	possible	2B
aldehydes				
formaldehyde	70–100 μg	sufficient	limited	2A
acetaldehyde	500–1400 μg	sufficient	insufficient	2B
phenolic compounds				
catechol	90–2000 μg	sufficient		2B
caffeic acid	<3 μg	sufficient		2B
methyleugenol ^b	20 ng			
volatile hydrocarbons				
1,3-butadiene	20–75 μg	sufficient	insufficient	2B
isoprene	450–1000 μg	sufficient		2B
benzene	20–70 μg	sufficient	sufficient	1
styrene	10 μg	limited		2B
nitrohydrocarbons				
nitromethane	0.5–0.6 μg	sufficient		2B
2-nitropropane	0.7–1.2 μg	sufficient		2B
nitrobenzene	25 μg	sufficient		2B
misc. organic compounds^c				
acetamide	38–56 μg	sufficient		2B
acrylamide	present	sufficient		2B
acrylonitrile	3–15 μg	sufficient	limited	2A
vinyl chloride	11–15 ng	sufficient	sufficient	1
DDT	800–1200 μg	sufficient	probable	2B
DDE	200–370 μg	sufficient		2B
1,1-dimethylhydrazine	present	sufficient		2B
ethyl carbamate	20–38 μg	sufficient		2B
ethylene oxide	7 μg	sufficient	sufficient	1
propylene oxide	0–100 ng	sufficient		2B

Table 4. (Continued)

agent	concentration in smoke of nonfilter cigarette	IARC evaluation of carcinogenicity		group ^a
		in lab animals	in humans	
inorganic compounds				
hydrazine	24–43 ng	sufficient	inadequate	2B
arsenic	40–120 µg	inadequate	sufficient	1
beryllium	0.5 ng	sufficient	sufficient	1
nickel	ND–600 ng	sufficient	sufficient	1
chromium (only hexavalent)	4–70 ng	sufficient	sufficient	1
cadmium	7–350 ng	sufficient	sufficient	1
cobalt	0.13–0.2 ng	sufficient	inadequate	2B
lead	34–85 ng	sufficient	inadequate	2B
polonium-210	0.03–1.0 pCi	sufficient	sufficient	1

^a IARC Monographs on the Evaluation of Carcinogenic Risks. Vol. 1–77 and Supplements 1–8, 1972–2000. (1) Human carcinogens; (2A) probably carcinogenic in humans; (2B) possibly carcinogenic to humans; (3) not classifiable as to their carcinogenicity to humans. ^b Unassigned line in column IARC Evaluation Carcinogenicity in lab animals; so far not reviewed. ^c In 1982, IARC assigned di(2-ethylhexyl)phthalate as sufficient to Group 2B. However, more recently, its carcinogenicity was reevaluated and it was classified as not carcinogenic (IARC, 1982; 2000). ^d Abbreviations: ND, not detected; PAH, polynuclear aromatic hydrocarbons; AaC, 2-amino-9H-pyrido[2,3-*b*]indole; MeAaC, 2-amino-3-methyl-9H-pyrido[2,3-*b*]indole; IQ, 2-amino-3-methylimidazo[4,5-*b*]quinoline; Trp-P-1, 3-amino-1,4-dimethyl-5H-pyrido[4,3-*b*]indole; Trp-2, 3-amino-1-methyl-5H-pyrido[4,3-*b*]indole; Glu-P-1, 2-amino-6-methyl[1,2-*a*:3',2''-*d*]imidazole; Glu-P-2, 2-aminodipyrro[1,2-*a*:3',2''-*d*]imidazole; PhIP, 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine.

Table 5. Probable Causative Agents for Cigarette Smoke-Related Disorders^a

disorder	contributing agents	possible enhancing or associated agents
tobacco dependence	major: nicotine minor: secondary <i>Nicotiana</i> alkaloids, flavor components	acetaldehyde
cardiovascular disease	major: carbon monoxide, nitrogen oxides, hydrogen cyanide, tar minor: cadmium, zinc, carbon monoxide, tar	nicotine, alkylating species
chronic obstructive lung disease	hydrogen cyanide, volatile aldehydes, nitrogen oxides, carbon monoxide, tar	
lung and larynx cancer	major: PAH, NNK minor: ²¹⁰ polonium, formaldehyde, acetaldehyde, butadiene, metals (Cr, Cd, Ni)	catechol, tumor promoters acetaldehydes, diet, alkylating species
oral cavity cancer	major: NNN, NNK minor: PAH	<i>herpes simplex</i> , irritation ethanol, diet
esophageal cancer	NNN	ethanol, diet
urinary bladder	4-aminobiphenyl, 2-naphthylamine, other aromatic amines	
pancreas cancer	NNK, NNAL	diet

^a Abbreviations: PAH, polynuclear aromatic hydrocarbons; NNK, 4-(methylnitrosamine)-1-(3-pyridyl)-1-butanone; NNN, *N*-nitrosornicotine; NNAL, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol.

smoking to ²¹⁰Po inhaled as a smoke constituent (38). Propylene oxide was identified in cigarette smoke in 1999. It is derived, in large part, from 1,2-propylene glycol which is present in cigarette tobacco at a level of ~1%. However, the 1,2-propylene glycol used as a tobacco humectant already contains traces of PO (36).

Table 5 presents a list of disorders induced in cigarette smokers, the major and minor contributing agents, and the possible enhancing agents. This table serves as a guide for studies on “the less harmful cigarette”; it makes no claims for completeness (39).

III. The Changing Cigarette

Epidemiological studies in the 1950s reported an association of cigarette smoking with cancer of the lung. Mounting epidemiological evidence in the following 3 decades revealed that cigarette smoking was not only causally related to lung cancer but that it was also causally associated with cancer of the larynx, oral cavity, esophagus, pancreas, kidney, and urinary bladder; moreover, it showed an association with cancer of the cervix (3). Shopland et al. estimated that out of 514 200 deaths

from cancer at all sites, a total of 157 200 (~ 31%) were attributable to cigarette smoking. There were 123 100 (~ 24%) cases of lung cancer among the 514 200 deaths (5). Thus, studies on tobacco carcinogenesis have focused primarily on lung cancer (39).

Factors regarded to be of major importance in the identification of key lung carcinogens include (1) the unambiguous identification of lung carcinogens in biologically significant amounts, (2) the uptake of significant doses of the suspected lung carcinogen as deduced from reliable biomarkers of exposure in physiologic fluids, (3) the induction of AC or SCC of the lung by the suspected carcinogen in 2 animal species, and (4) in vivo adduct formation of metabolite(s) of the suspected carcinogen with DNA in the lungs of mice and/or rats or at least in vitro DNA adduct formation in human lung cell culture.

On the basis of these criteria, the carcinogenic PAH in cigarette smoke as well as certain TSNA, typified by NNK and NDMA, can be viewed as important and highly relevant lung carcinogens for cigarette smokers.

In addition, consideration needs to be given to several elements that play a role as lung carcinogens in occupational environments. These are arsenic, beryllium,

nickel, chromium, cadmium, cobalt, and polonium-210, albeit most of these are present in tobacco and in cigarette smoke at low concentrations (Table 4). Another group of smoke carcinogens with a likely role as contributors to lung cancer risk in humans are free radicals that induce oxidative DNA damage.

In the 1950s, studies in the U.K. and the U.S.A. reported on the identification of the carcinogen BaP in cigarette smoke. These reports were challenged as merely representing BaP-like UV absorption spectra (40). Subsequent tedious analytical work finally led to the isolation and chemical identification of crystalline BaP from the smoke of several thousand cigarettes (41). Chemical-analytical studies led to the isolation and identification of the nicotine-derived nitrosamines NNN and NNK that proved to be highly carcinogenic in bioassays (42). For more than 10 years, the tobacco industry challenged the evidence for nicotine-derived carcinogens while research data emanating from groups outside the industry clearly pointed to organ-specific carcinogenicity and routes of metabolic activation of the tobacco-specific *N*-nitrosamines NNN, NNK, and five other alkaloid-derived nitrosamines. NNK induces primarily adenoma and adenocarcinoma of the lung in mice, rats, hamsters and mink independent of its route of application, i.e., regardless of whether it is injected intraperitoneally, intravenously, intravesically, or given by gavage, swabbed onto oral surfaces, or administered in the drinking water. NNK also induced tumors in the nasal cavity of rats and hamsters and in the pancreas of rats (43, 44).

While the industry now has acknowledged the existence of TSNA (45) and even endeavors to prevent or at least reduce TSNA formation in smoke, it suggests that any possible carcinogenic action of TSNA is counteracted by inhibitors, including nicotine, nornicotine, and other smoke constituents. This contention is based on significant reduction of mutagenic activity of NNK and NDMA, but not NNN, by nicotine, nornicotine, or cotinine in *in vitro* assays. Similarly, SEC induction in mammalian cells by NNK was also markedly reduced by co-application of nicotine or cotinine (46). The concurrent application of NNK with nicotine or NNK with cotinine to perfused rat liver resulted in significant inhibition of NNK clearance, and in decreased metabolic activation by α -hydroxylation; it also caused a significant increase in *N*-oxidation of NNK and in the formation of NNAL-glucuronides (47).

In contrast, neither the NNK clearance from perfused rat lung nor its pattern of metabolites were substantially affected by the co-administration of nicotine or cotinine (47). Isolated rat lungs perfused with NNK revealed only small differences in pulmonary clearance and pattern of NNK metabolites between fed and starved animals (47). Rats on a high-fat diet who were given NNK in their drinking water developed lung adenoma and adenocarcinomas sooner but the overall incidence of lung tumors was similar to that in rats given NNK and a low-fat diet. However, the role of induction of carcinoma of the pancreas by NNK was significantly higher in the group of rats on the high-fat diet than in those on a low-fat diet (48). As to underlying mechanisms in NNK lung carcinogenesis, it is thought that 11β -hydroxysteroid dehydrogenase reduces the carbonyl group of NNK to yield NNAL whose secondary alcohol group is subject to glucuronidation so that it gets excreted as a urinary constituent. Inhibition of 11β -hydroxysteroid dehydrogenase raises

the rate of α -hydroxylation of NNK, thus, increasing its carcinogenicity. On the other hand, activation of the 11β -hydroxysteroid dehydrogenase decreases α -hydroxylation of NNK, thus diminishing its carcinogenic potential (49). When rats were treated with a low dose of NNK and given drinking water that contained 0.002% nicotine, the alkaloid did not diminish DNA methylation (50). Additional well-designed bioassays are needed to clarify how nicotine and other smoke constituents affect the carcinogenicity of NNK.

The impetus for changing the makeup of cigarettes can be traced back to the landmark articles by Ernst L. Wynder and Evarts A. Graham in the United States and by Richard Doll and A. Bradford Hill in the United Kingdom in 1950 (19, 20). Both studies revealed a dose-response relationship between the number of cigarettes smoked and the risk for developing lung cancer. In 1953 and 1957, these findings were supported by bioassays showing a dose-response between the amount of tar applied to mouse skin and the induction of skin tumors (21, 22). In 1973, inhalation studies with hamsters at the Research Institute of the West German Cigarette Industry demonstrated a dose-response between the amount of cigarette smoke inhaled and the induction of tumors in the upper respiratory tract (23). On the basis of the epidemiological data and the bioassays with mice and hamsters, initial emphasis was placed on reducing the smoke yields of tar and nicotine as a measure that should lead to a less harmful cigarette. Because tar as a whole is the major carcinogen, and nicotine is the major toxic and addictive agent in tobacco smoke, measures of tar and nicotine were chosen as analytical parameters for each marketed brand.

The Federal Trade Commission (FTC) adopted, with some modifications, the standard machine smoking conditions for cigarettes developed by Bradford et al. in 1936 (51, 52). These prescribe taking one puff per minute with a 35 mL volume over 2 s. The butt lengths for nonfilter cigarettes were set at 23 mm and those for filter cigarettes at the length of the filter, plus overwrap, plus 3 mm. CORESTA, the International Organization for Research on Tobacco, developed a standard machine-smoking method in 1968 that is widely used in most of the developed countries (53). The CORESTA method differs from that of the FTC only in respect to the butt length to which filter cigarettes are smoked (CORESTA, length of filter, plus 8 mm). The standard machine-smoking conditions are otherwise identical to those of the FTC (52, 53). The data presented here are based on the FTC standard smoking conditions, except when otherwise specified.

A. Filter Tips

Figure 1 displays the sales-weighted average smoke yields for tar and nicotine of U.S. cigarettes for 1953–1996. The tar yields decreased from 38 to 12 mg and the nicotine yields from 2.7 to 0.85 mg (39). Figure 1 also approximately marks the year for the introduction of specific changes in the makeup of commercial U.S. cigarettes. Filter cigarette sales increased from 0.5% of all cigarettes on the U.S. market in 1950 to more than 97% as of 1997 (11, 12, 54, 55). Similar consumer acceptance of filter cigarettes has been recorded in other developed countries with the exception of France, where it had been delayed. In 1975, only about 70% of all French

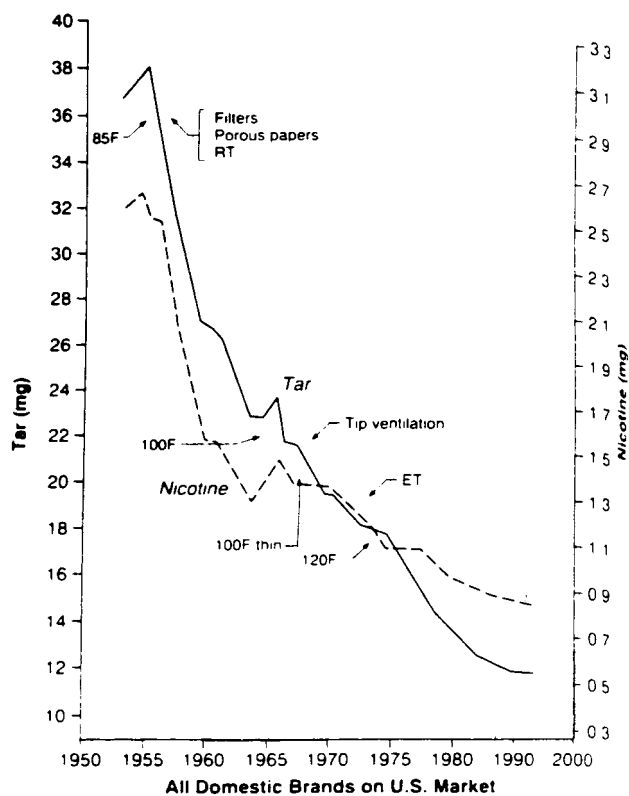


Figure 1. Sales-weighted average tar and nicotine deliveries, 1953–1993, U.S.

cigarettes had filter tips (56). There are basically three types of filter tips for cigarettes.³ These are filters made from paper, from cellulose acetate (57) and filter tips that contain charcoal. The latter consist either of two or three sections, cellulose and granulates of activated charcoal, or cellulose acetate, activated charcoal and cellulose acetate. In addition, instead of granulates of charcoal in one section of the filter tip, charcoal has been dusted onto the cellulose acetate of the inner section.

In 1959, Haag et al. reported that charcoal filter tips selectively remove certain volatile agents from the smoke (58). Some of these are strong ciliotoxic agents, such as hydrogen cyanide, formaldehyde, and acrolein (59–62). In vitro studies have demonstrated that the smoke from cigarettes with charcoal filter tips is less ciliotoxic than smoke from other types of filter cigarettes and smoke from plain cigarettes. Thus, the movement of the cilia in the bronchi and trachea is significantly less impaired by the smoke of charcoal filter cigarettes than by the smoke of the other types of cigarettes (59–62). In the United States, and in most other countries, cigarettes with charcoal filters amount to less than 1–2% of total sales. However, of all the cigarettes on the market in Japan and Venezuela, 95% have charcoal filter tips, and so have 90% in South Korea and Hungary (63, 64).

Cigarettes with cellulose filters account for less than 1% of all cigarettes sold in the developed countries, whereas cigarettes with cellulose acetate filter tips, primarily made from secondary cellulose acetate, amount to more than 90% of all cigarettes (with the exception of Japan, Venezuela, South Korea, and Hungary) (12). Cellulose acetate filters retain up to 80% of semivolatile

phenols. The retention of phenols was desirable because these compounds are active as tumor promoters in the experimental setting (65–67). Cigarette smoke contains the parent phenol and about 20 additional volatile phenols. Their total amount in the smoke of an 85-mm nonfilter cigarette amounts to about 300 μg . The individual, semi-volatile phenols range from 80 to 200 μg from phenol itself to less than 1 μg of *o*-chlorophenol (26). Boutwell and Bosch bioassayed some volatile phenols for their tumor-promoting activity on mouse skin initiated with 7,12-dimethylbenz[*a*]anthracene. The highest tumor-promoting activity was found for the following semivolatile phenols that had been identified in cigarette smoke: phenol, *o*-, *m*-, and *p*-cresol; 2,4-, 2,6-, 3,4-, and 3,5-dimethylphenol (68, 69); *o*-chlorophenol, 2-ethylphenol, and 2-*n*-propylphenol had lower tumor-promoting activities (68, 69). When bioassayed and compared for effects on a gram-to-gram basis, the tars from cellulose acetate filter-tipped cigarettes were slightly more toxic but less carcinogenic than tars collected from the smoke of plain cigarettes or from cigarettes with charcoal filter tips (69). Cellulose acetate filter tips also remove from the smoke up to 75% of the carcinogenic, volatile *N*-nitrosamines (VNA) (70). Twice daily exposure of Syrian golden hamsters for over 60 weeks to the air-diluted total MS from cellulose filter cigarettes induced a significantly lower incidence of tumors of the larynx than did exposure to the air-diluted total MS from nonfilter cigarettes. In contrast, whole smoke from cigarettes with charcoal filter tips induced carcinoma of the larynx to a similar extent as the whole smoke from nonfilter cigarettes (23).

Around 1965, perforation of the filter tips of cigarettes was introduced as a measure toward reducing toxins in the smoke. The smoke of cigarettes with filter perforation is indicated to be consumer acceptable up to about 50% air dilution. The velocity of the air flow through the burning cone of these filter cigarettes slows down as part of the negative pressure generated by the puff drawing. These changes result in more complete combustion of the tobacco (71–73). In 1999, more than 65% of all U.S. cigarettes had filter tips with various degrees of perforation. In the 70s, the analysis of the smoke of the experimental cigarette was completed for the Tobacco Working Group of the National Cancer Institute by M. R. Guerin, R. A. Jenkins et al. from the National Laboratory in Oak Ridge, TN (69, 74–76) (a) with cellulose acetate filter tips, (b) with perforated cellulose acetate filter tips, (c) with porous paper and with a perforated cellulose acetate filter tip, and (d) a nonfilter cigarette. The same tobacco blend was used as a filler for all four cigarettes. Table 6 shows that the smoke of filter cigarettes with perforation delivers the lowest yields of CO, hydrogen cyanide, nitrogen oxides (NO_x), acetaldehyde, and acrolein of all types of cigarettes. However, the yields of carcinogenic PAH in the smoke of the cigarettes with perforated filter tips were comparable with those from cigarettes without filter tips. Thus, the perforation of the filter tip results in a selective reduction of volatile, toxic agents but no major changes in the yields in particulate components (77, 78).

B. Cigarette Paper

Since about 1960, higher cigarette paper porosity and treatment of paper with citrate has significantly contributed to the reduction of the yields of several smoke

³In the People's Republic of China the leading material for the filter tip is polypropylene; there is little use in other countries for this polymer as filter material for cigarettes.

Table 6. Comparison of Experimental Cigarettes (yields/cigarette)^{a,b,c}

smoke components	nonfilter cigarette (72–75)	cellulose acetate filter cigarette (91)	cellulose acetate filter 2/perforation (89)	cellulose acetate filter w/perforation and highly porous paper (96)
carbon monoxide (mg)	16.2	19.2	8.52	6.66
hydrogen cyanide (μg)	338	296	201	109
nitrogen oxides-NO _x (μg)	439	438	364	224
formaldehyde (μg)	36.0	20.9	31.7	21.4
acetaldehyde (μg)	1170	1290	608	550
acrolein (μg)	109	104	58.6	48.6
tar (mg)	27.2	14.7	19.2	19.5
nicotine (mg)	1.8	0.94	1.31	1.50
phenol (μg)	170	61.7	122	129
benz(a)anthracene (ng)	40.6 (1.40)	35.3 (2.25)	38.5 (1.88)	40.1 (1.91)
benzo(a)pyrene (ng)	29.9 (1.09)	19.6 (1.25)	29.2 (1.13)	23.9 (1.14)

^a U.S. National Cancer Institute (69). ^b The composition of the cigarette tobacco is identical in all four experimental cigarettes. ^c Numbers in parentheses (μg of benz(a)anthracene or benzo(a)pyrene in 1 g dry tar).

components. During and between puff drawing, porous paper enhances the outward diffusion through the paper of hydrogen, NO, CO, CO₂, methane, ethane, and ethylene. On the other hand, it accelerates the diffusion of O₂ and N₂ from the air into the tobacco column; this, in turn, causes more rapid smoldering during puff intervals (15, 77). Table 6 compares smoke data from cigarettes with perforated filter tips but regular cigarette paper vs cigarettes with perforated filter tips and highly porous paper, with the tobacco blends in both cigarettes being identical (69). Whereas porous cigarette paper causes a significant decrease of volatile toxic agents, it hardly changes the smoke yields of tar, nicotine, BaA, and BaP. Importantly, the significant reduction of nitrogen oxides in the smoke of these cigarettes reduces the formation and, thus, significantly lowers the yields of VNA and TSNA (77–79).

In several countries, the use of hand-rolled cigarettes has risen significantly (80, 81). Making hand-rolled cigarettes requires sturdy cigarette paper. Such papers have low porosity. Rickert et al. compared the smoke yields of hand-rolled cigarettes with those of manufactured cigarettes, some of these two types of cigarettes contained the same tobacco; all cigarettes were machine-smoked under the same conditions. The handmade cigarettes, weighing 26% more, delivered mainstream smoke yields that were 100, 85, and 86% higher in tar, nicotine, and carbon monoxide, respectively, than those of manufactured cigarettes (80).

C. Cigarette Construction

Smoke yields also depend on physical parameters, such as length and circumference of the cigarette, and the width of the cut (number of cuts per inch; cpi) of the tobacco filler. Extending the cigarette length from 50 to 130 mm produces an increase in the level of oxygen in the mainstream smoke, while the levels of hydrogen, CO, CO₂, methane, ethane, and ethylene decrease. The major reason for this occurrence lies in the diffusion of oxygen through the paper into the smoke stream (82). This phenomenon is also reflected in increased CO delivery with ascending number of puffs, while the available surface area of the paper diminishes. With an increase in the length of the cigarette, the overall yields of tar, nicotine, PAH, and other particulate components also rise (83, 84). Circumference of cigarettes below the regular 24.8–25.5 mm (e.g., 23 mm or less) translates not only into less tobacco being burned but also into greater

volume of oxygen available during combustion (83, 84). Thus, the smoke yields of tar, nicotine, and other particulate components are lowered (15, 83, 84). Cigarettes with small circumference also have a lower ignition propensity toward inflammable materials than cigarettes that have the ~25 mm circumference (79). It has been estimated that in 1980 of the almost 5200 U.S. residents who died from fires, about 1200 of these deaths occurred in fires started by cigarettes (85).

The number of cpi applied to the filler tobacco of cigarettes has no major impact on the carcinogenicity of the tars. The first investigation on the importance of tobacco cuts per inch, with regard to smoke yields and tumorigenicity of the resulting tars, was published in 1965. It compared the smoke yields of tar and BaP when 8, 30, 50, or 60 cpi of leaf were applied. Tar yields per cigarette decreased from 29.1 to 23.0 mg and BaP from 37 to 21 ng. In a large-scale study of cigarettes filled with an identical blend, and cuts at 20 and 60 cpi, respectively, the smoke yields per cigarette of tar, nicotine, volatile aldehydes, BaA, and BaP were significantly reduced for the fine-cut (60 cpi), however, hydrogen cyanide was insignificantly increased. Gram-to-gram comparison of tumorigenicities of the two tars on mouse skin revealed statistically insignificant differences (74).

D. Tobacco Types

The botanical genus *Nicotiana* has two major subgenera: *N. rustica* and *N. tabacum*. *N. rustica* is primarily grown in Russia, the Ukraine, and other East European countries, as well as in South America, and, to a limited extent, in India. In the rest of the world, *N. tabacum* is grown as the major tobacco crop; it comes as *flue-cured type* (often called bright, blond, or Virginia tobacco), *air-cured type* (often called burley tobacco), light air-cured tobacco (grown in Kentucky), and dark air-cured tobacco (grown in parts of Tennessee and Kentucky, South America, Italy, and France), as well as *sun-cured* (often called oriental or Turkish) tobacco (primarily grown in Greece and Turkey). In addition, there are special classes of air-cured tobaccos for cigars, chewing tobacco, and snuff (86).

Until the recent 2 decades, only flue-cured tobaccos were used for cigarettes in the U.K. and in Finland, and they were the predominate type used in Canada, Japan, China, and Australia. Air-cured tobaccos were preferred for cigarettes in France, southern Italy, some parts of Switzerland and Germany, and South America; cigarettes

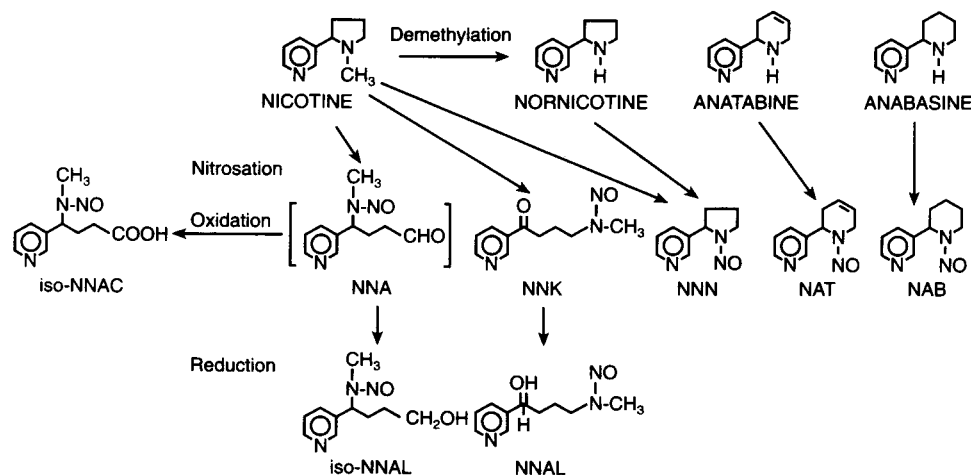


Figure 2. Formation of tobacco-specific *N*-nitrosamines (TSNA), 1994 (43).

made exclusively from sun-cured tobaccos are popular in Greece and Turkey. In the rest of Western Europe and in the U.S., cigarettes contain blends of flue-cured and air-cured tobaccos as major components. Today, in many countries, including the U.K, France, and other developed nations, the U.S. blended type of cigarette is gaining market shares. In the U.S., the composition of the cigarette blend has undergone gradual changes. In the sixties and early seventies, 45–50% of the cigarette blends were flue-cured (Virginia) tobaccos, 35% air-cured (burley) tobaccos, and a few percent were Maryland air-cured and oriental tobaccos. By 1980, blends averaged 38% flue-cured, 33% air-cured, and a few percent each of Maryland and oriental tobaccos and up to 30% reconstituted and expanded tobacco. In the early nineties, these proportions were 35%, 30%, and, again, a few percent of Maryland and oriental tobaccos. The blended cigarette is preferred in many countries, in part, because each of the three major *N. tabacum* types lends a specific aroma to the smoke.

In regard to the toxicity and carcinogenicity of tobacco and tobacco smoke, the difference in the nitrate content of the tobaccos is of primary significance. Flue-cured tobacco can contain up to 0.9% of nitrate; yet, as it is used for regular cigarettes, it contains <0.5% of NO_3 . In oriental tobaccos one finds up to 0.6% NO_3 , in air-cured tobaccos between 0.9 and 5.0%, but generally below 3% in commercial cigarettes. The highest concentration of nitrate is present in the ribs, the lowest concentration is in laminae, especially in laminae harvested from the top stalk positions of the tobacco plant (87, 88). With the utilization of a greater proportion of air-cured tobacco in the blended U.S. cigarette, the average nitrate content of the blended U.S. cigarette tobacco has risen from below 0.5% in the fifties to 1.2–1.5% in the late eighties (15, 89, 90).

The concentrations of nitrogen oxides (NO_x) and methyl nitrite in smoke depend primarily on the nitrate concentrations of the tobacco; although a portion of the nitrogen oxides is formed during smoking from amino acids and certain proteins (87, 91–94). Cigarettes made entirely with flue-cured tobaccos deliver up to 200 μg of NO_x and 20 μg of methyl nitrite in the smoke. Smoking U.S. blended cigarettes produces up to 350 μg of NO_x and 160 μg of methyl nitrite, and the smoke of air-cured tobacco cigarettes contains up to 700 μg of NO_x and 470 μg methyl nitrite. The stems of air-cured tobaccos are especially rich

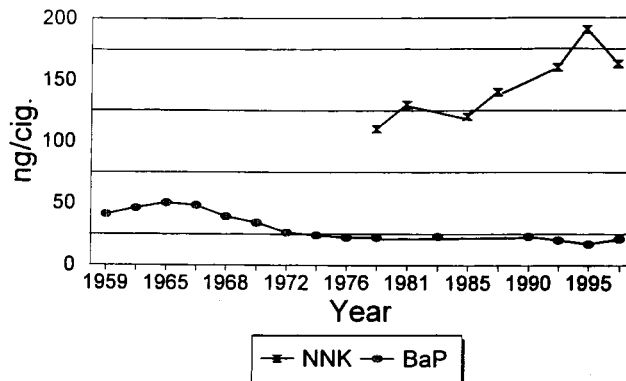


Figure 3. BaP and NNK in mainstream smoke of a leading U.S. nonfilter cigarette, 1959–1997.

in nitrate ($\leq 6.8\%$). Consequently, stems, as components of expanded and reconstituted tobaccos in a cigarette blend, contribute in a major way to NO_x in the smoke (95).

Freshly generated smoke, as it leaves the mouthpiece of a cigarette, contains NO_x virtually only in the form of nitric oxide (NO), and only minor amounts of nitrogen dioxide (NO_2) and of nitrous oxide (N_2O) (92, 93). However, nitrogen dioxide is quickly formed upon aging of the smoke. It has been calculated that, within 500 s half of the NO in undiluted smoke is oxidized to NO_2 (94). Nitrogen dioxide can cause inflammation of the lungs which may lead to edema. Of major importance is the high reactivity of NO_x upon its formation in the burning cone and in the hot zones of a cigarette. The thermally activated nitrogen oxides serve as scavengers of C,H-radicals, whereby they inhibit the pyrosynthesis of carcinogenic PAH (94, 96, 97).

The freshly generated nitrogen oxides also react with secondary and tertiary amines resulting in the formation of volatile *N*-nitrosamines (VNA) and of several *N*-nitrosamines from amino acids, as well as from some additives. Furthermore, NO_x also form tobacco-specific *N*-nitrosamines (TSNA) by *N*-nitrosation of nicotine and of the minor tobacco alkaloids (Figure 2) (44, 98). Figure 3 depicts data on the decline of BaP and the increase of NNK in the smoke of a leading U.S. nonfilter cigarette between 1974 and 1997. Both trends are correlated with the use of tobacco blends with higher nitrate content. Increasing concentrations of nitrate in tobacco have also led to an increase in cigarette smoke of nitroalkanes,

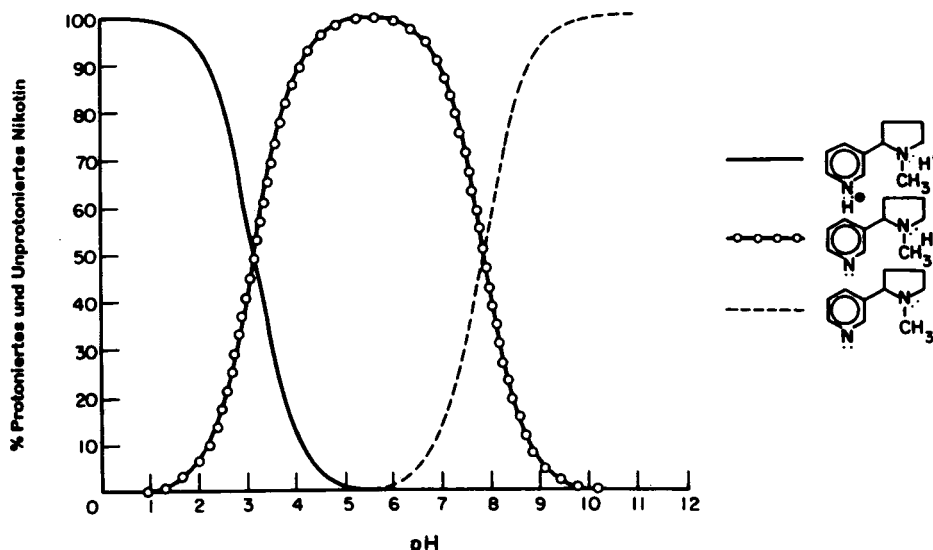


Figure 4. pH of total mainstream smoke of various tobacco products, Brunnemann and Hoffmann, 1974 (101).

Table 7. Smoke Yields and Tumorigenicity of the Tars from the Four Major *N. Tabacum* Varieties^a

factors	flue-cured tobacco	sun-cured tobacco	air-cured tobacco Kentucky ^b	air-cured tobacco Maryland
(A) yields/cigarette				
tar (mg)	33.4	31.5	25.6	21.2
nicotine (mg)	2.4	1.9	1.2	1.1
phenol (μg)	95	120	60	43
benzo(a)pyrene ^c (ng)	53 (1.6)	44 (1.4)	24 (0.94)	18 (0.85)
(B) tumorigenicity ^d				
% of mice with skin tumors ^e	34	36	22	18

^a Wynder and Hoffmann (96). ^b Low nicotine, air-cured tobacco (Kentucky). ^c Number in parentheses (μg of BaP/1 g of tar). ^d Bioassayed on a gram-to-gram basis of tar. ^e Fifty mice in each group.

including the carcinogenic nitromethane, 2-nitropropane, and nitrobenzene, and of aromatic amines, including the human bladder carcinogens 2-naphthylamine and 4-aminobiphenyl (15, 97, 99, 100).

Another important aspect relative to the toxicology of cigarette smoke is the correlation between the nitrate content of tobacco and the pH of cigarette smoke. Even though the different processes used to flue-cure and air-cure tobaccos significantly influence the smoke composition of the major types of tobacco, the amount of nitrate present is also of major importance in determining the pH of the smoke. Whereas flue-cured tobacco and U.S. cigarette tobacco blends deliver weakly acidic smoke (pH 5.8–6.3), cigarettes made from air-cured tobacco deliver neutral to weakly alkaline smoke (pH 6.5–7.5). The major reason for the range of pH values encountered in the smoke of the two major tobacco types is the concentration of ammonia in the smoke, which is primarily tied to the concentration of nitrate in the tobacco. When pH levels of the smoke rise >6.0, the percentage of free, unprotonated nicotine increases to about 30% at pH 7.4 and to about 50% at pH 7.8 (101). Protonated nicotine is relatively slowly absorbed in the oral cavity; yet, unprotonated nicotine, some of which is present in the vapor phase of the smoke, is quickly absorbed through the mucosal membranes of the oral surfaces (102). This is a distinguishing factor in cigar smoking. The pH of cigar smoke rises with increasing puff numbers from pH 6.5 to 8.5; therefore, the rapid oral absorption of the free nicotine in the vapor phase gives the primary cigar smokers instant nicotine stimulation so that there is no need to inhale the smoke (Figure 4). Similarly, the

primary smoker of black, air-cured cigarettes tends not to inhale the smoke or to do so minimally (13, 102, 103).

In 1963, the first comparative study of the tumorigenicity on mouse skin of the tars from the four major types of *Nicotiana tabacum* revealed the highest activity for tars obtained from flue-cured and sun-cured tobaccos and the lowest for tars from two types of air-cured tobacco (Table 7). The concentration of BaP, a surrogate measure for the carcinogenic PAH, was correlated with the tumor initiation potential of the tars (96). Upon topical application to mouse skin and bronchial epithelia, carcinogenic PAH induce papilloma and carcinoma. In inhalation studies with Syrian golden hamsters, the smoke of a cigarette, made with a U.S. tobacco blend, was significantly more active in inducing carcinoma of the larynx than was the smoke of a cigarette with air-cured (black) tobacco (23).

To support the concept that the reduction of carcinogenic PAH in the smoke by means of high levels of nitrate in tobacco leads to diminished mouse skin tumorigenicity of the tar, sodium nitrate (8.3%) was added to the standard tobacco blend. On a gram-to-gram basis, the tar from the cigarette with added nitrate (0.6 μg of BaP/g of tar) induced skin tumors in only 2 out of 50 mice, whereas the tar from the control cigarette (without the addition of nitrate; 1.05 μg of BaP/g of tar) induced skin tumors in 25 of 100 mice (104). In inhalation experiments with Syrian golden hamsters, smoke from the experimental cigarette, made with 8.0% of sodium nitrate, induced laryngeal carcinomas in only 25 of 160 animals compared to this type of neoplasm in 60 of 200 animals in assays

with the control cigarette (23). Thus, mouse skin bioassays with tar and smoke inhalation studies with hamsters support the concept that increased nitrate content of the tobacco inhibits the pyrosynthesis of the carcinogenic PAH and that the tars of these cigarettes, and their smoke as a whole, have a reduced potential for inducing tumors in epithelial tissues of the skin and of the upper aerodigestive tract compared to the tar or whole smoke of cigarettes with low-nitrate tobacco.

E. Reconstituted Tobacco and Expanded Tobacco

In the early 1940s, the technology of making reconstituted tobacco (RT) was developed. It was first applied to the manufacture of cigar wrappers. The RT technology enables the utilization of tobacco fines, ribs, and stems in cigarette tobacco blends (105). Prior to this technology, tobacco fines and stems had been wasted. The utilization of RT as part of the tobacco blend requires less of the top quality tobaccos for cigarette manufacture. Beginning in 1965, laboratory studies have shown that cigarettes made entirely of RT deliver a smoke with significantly reduced levels of tar, nicotine, volatile phenols, and carcinogenic PAHs. The two major technologies for making RT for cigarettes are the slurry process and the paper process; both lead to RT with low density. It permits a high degree of aeration of the tobacco which enhances combustion. Most of the tested tars from cigarettes made with these reconstituted tobaccos had significantly reduced carcinogenic activity on mouse skin (74, 106). In inhalation assays with Syrian golden hamsters, diluted smoke from cigarettes made of reconstituted tobacco induced significantly fewer carcinoma in the larynx (19/160) than the diluted smoke from control cigarettes (60/200). The cigarette with RT, tested in the smoke inhalation assay, gave per cigarette only 7 puffs and yielded 20.8 mg of tar and 16 ng of BaP compared to 10 puffs, 33.7 mg of tar, and 35.4 ng of BaP for the control cigarette (23). Burton, Dye, and Bush analyzed in detail the distribution of nitrite, nitrate, alkaloids, and TSNA in segments of an air-cured, dark tobacco leaf. They reported the highest concentrations of the alkaloids in the tips and the lowest in the base of the leaf, whereas, nitrite and TSNA were present in the highest concentrations in the base of the leaf and decreasing concentrations toward the tip of the leaf. These data indicate a better relationship between nitrite and TSNA than between the alkaloids and TSNA (107). Tobacco ribs and stems, the major components of RT, contain more nitrate and nitrite (and this applies especially to the ribs and stems of air-cured tobaccos) than the laminae of tobacco (95, 107, 108). Therefore, in general, the nitrate content of today's blended U.S. cigarette, which may contain 20–30% RT, is at a level of 1.2–1.5%—significantly higher than the nitrate level in cigarettes during the fifties and sixties when it was $\leq 0.5\%$ (89, 109). Commercial cigarettes with RT emit in their smoke significantly more TSNA than cigarettes of the past. These TSNA include the NNK which induces AC in rodents. NNK is metabolically activated to carcinogenic species in target tissues such as the lung (43). One major U.S. cigarette manufacturer has been granted a patent in December of 1978, presenting a process that reduces more than 90% of the nitrate content of the RT made from ribs and stems (110, 111).

There are at least three methods for expanding tobacco by freeze-drying (75). As a result of freeze-drying, the

expanded tobacco has greater filling power than natural tobacco; thus, less tobacco is needed to fill a cigarette. An 85-mm filter cigarette, filled entirely with expanded tobacco, required 363 mg of tobacco, while a regular filter-tipped cigarette of the same dimensions required 667 mg of tobacco. The tar yields in the smoke of these two types of cigarettes were 12.4 and 22.1 mg, respectively (75, 76). In 1982, incorporation of all possible modifications in the makeup of the cigarette required only 785 mg of leaf tobacco; in contrast, in 1950, making the blended U.S. cigarette required 1230 mg of leaf tobacco (109). Levels of most components measured in the smoke of cigarettes with puffed tobacco, expanded tobacco, or freeze-dried tobacco, were significantly reduced, by comparison to the control cigarette (75, 76).

F. Additives

1. Humectants. Humectants serve to retain moisture and plasticity in cigarette and smoking tobaccos. They delay or prevent the drying of tobacco. Dry tobacco (<8% moisture) gives a harsh tasting smoke. Humectants also preserve those compounds that impart flavor to the smoke. Today, the principal humectants in cigarette tobacco are glycerol (propane-1,2,3-triol) and propylene glycol (PG; propane-1,2-diol); of lesser importance are diethylene glycol (2,2'-di[hydroxyethyl]ether) and sorbitol (112). In the past, ethylene glycol (ethane-1,2-diol) has been used as a humectant for cigarette tobacco. During smoking, this compound leads to the formation of ethylene oxide. Workers exposed to this volatile agent face an increased risk for lymphatic leukemia and non-Hodgkin's lymphoma (113). For this reason, ethylene glycol is no longer used as a humectant for tobacco. In 1972, Binder and Lindner reported the presence of 7 μg of ethylene oxide in the smoke of a cigarette brand filled with tobacco that had not been treated with ethylene glycol (114). The significance of the possible endogenous oxidation of the inhaled ethylene (150–300 $\mu\text{g}/\text{cigarette}$) has not been evaluated (115).

Humectants may comprise up to 5% of the weight of cigarette tobacco. In a 1964 study, 18 U.S. cigarette tobacco blends contained between 1.7 and 3.15% of glycerol. To some extent, glycerol decomposes to the ciliotoxic acrolein; U.S. cigarette tobacco contains also between 0.46 and 2.24% of PG (116). Four American cigarettes contained between 0.34 and 0.96 mg/cigarette of PG (117); during smoking, PG gives rise to the carcinogenic propylene oxide (118). Recently, levels of 12–100 ng of propylene oxide have been determined in the smoke of U.S. cigarettes. Several samples of PG that were intended as humectants for cigarette tobacco already contained traces of propylene oxide (36).

2. Flavor Additives. Natural tobacco contains a wide spectrum of components that, upon heating, release flavorants. These include tobacco-specific terpenoids, pyrroles and pyrazines among others (119–122). The effective reduction of smoke yields by filter tips and by the incorporation of reconstituted tobacco also brought about a reduction of flavor components. In 1993 and 1994, the industry convened an expert panel of toxicologists to screen agents that were in use, or considered for use, as tobacco additives. The panel then released a list of 599 agents that were generally regarded as safe (GRAS), whereby the term "safety" applied only to the additives as such without considering the fate and reactivity of

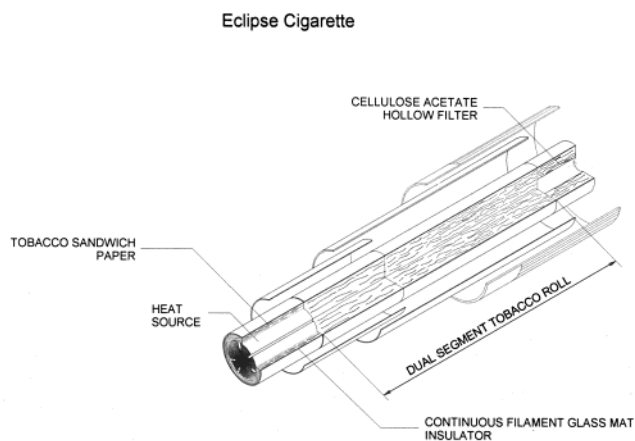


Figure 5. A new type of cigarette: Eclipse, Bombick et al. (130).

these agents during combustion (123). In inhalation assays, the mainstream smoke (MS) of cigarettes with and without flavor additives induced essentially the same responses in the respiratory tracts of rats; specifically hyperplasia and metaplasia in the nose and larynx (124). As this study involved maximally 65 h of exposure, one cannot deduce with certainty that the addition of these flavoring agents to tobacco blends has no additional impact on the development of tumors by cigarette smoke.

G. New Types of Cigarettes

The industry has initiated research toward developing new types of cigarettes. During smoking, such cigarettes were to generate an aerosol with nicotine in the range present in the smoke of conventional filter cigarettes, but low to very low emissions of tar and of toxic and carcinogenic agents. In 1988, the first new type of cigarettes appearing on the test market was called "Premier", a cigarette that "heats rather than burns tobacco" (125–129). This 80-mm cigarette is comprised of three sections. The first 40-mm section is made with compressed, activated charcoal that is linked to an inner aluminum tube containing tobacco, flavor additives, and glycerol. This tube is embedded in tobacco. Section 2 (~10 mm) is a cellulose acetate filter, dusted with charcoal powder. The third section (~30 mm) is a cellulose acetate filter tip. Under standardized smoking conditions (52), Premier delivered a MS with low levels of most of the toxic and genotoxic agents compared to concentrations of these agents in the MS of the University of Kentucky reference filter cigarette. Short-term bioassays indicated that the MS of Premier most likely exhibits reduced toxic and genotoxic activities (125–129). In 1988, Premier was placed on a test market; however, it was not accepted by the consumers.

Undergoing significant changes, the Premier re-emerged as "Eclipse". This product consists of four sections. Section 1, the heat source, is a specially prepared charcoal; section 2 consists of tobacco plus glycerol; section 3 contains finely shredded tobacco; and section 4 is a filter tip (Figure 5). Upon ignition, the special charcoal heats the air stream during puff drawing. The heated air stream enters the tobacco sections and aerosolizes volatile and semivolatile tobacco constituents including nicotine, as well as portions of glycerol. Eclipse is produced in four prototypes; their MS were thoroughly

analyzed. Under FTC smoking conditions, the standard Eclipse delivers 8 mg of CO (low-yield filter cigarette, 6–12 mg), 150 μ g of acetaldehyde (700 μ g), 30 μ g of NO_x (200–300 μ g) and 180 μ g of hydrogen cyanide (300–400 μ g), 5.1 mg of tar (11–12 mg) and 0.2–0.4 mg of nicotine (0.7–1.0 mg). The tar consists of 33% water, 47% glycerol, and 17% of various other compounds. The emissions of the major smoke carcinogens, such as BaP, 2-naphthylamine, 4-aminobiphenyl, and the TSNA, are lowered by 85–95% (126–129). The particulate matter of the aerosol generated by smoking Eclipse, according to the FTC standard method, contains as major a constituent about 47% glycerol (in the low-yielding Eclipse this means 2.4 mg glycerol in 5.1 mg smoke particulates) (129).

A number of short-term tests were completed with whole smoke, the vapor phase, the tar and/or fractions of the tars of one or several prototypes of Eclipse; the University of Kentucky reference filter cigarette 1R4F and, in some cases, also 1R5F, served as a positive controls. The tars of the two reference cigarettes were cytotoxic, while the tars of two types of Eclipse were not (130). Whole smoke of four prototypes of Eclipse had minor cytotoxic activity. However, the activities were significantly less than those of the whole smoke of the Kentucky reference cigarettes (131). The tars of the four Eclipse prototypes were not, or at best weakly, mutagenic (with or without activation by the S9 enzyme fraction from rat liver homogenate), but in each case, they were significantly less active than the tars of the reference cigarette (130). In the sister chromatid exchange test, the tars of the Eclipse induced only a slightly positive response or no response; the tars from the Kentucky reference cigarettes were significantly more mutagenic (130).

The exposure of rats and random-bred Syrian golden hamsters to diluted whole smoke for 5 days a week for 13 weeks led only to moderate changes in the upper respiratory tract of these animals. In each case, the recorded histopathological changes in the short-term assay were less pronounced than the changes observed in the upper respiratory tract of the animals exposed to the diluted smoke of the Kentucky reference cigarette (132). The tars of the reference filter cigarette 1R4F and four types of Eclipse were bioassayed at three doses (10, 20, and 40 mg/application) for their tumor promoting activity on the skin of Sencar mice, initiated with DMBA. With one exception, the tumor promoting activities of the tars from the Eclipse cigarettes were reduced by at least two-thirds compared with the tumor promoting activity of the tar from the reference tobacco cigarette (132). Using the ³²P-postlabeling technique in mice exposed dermally to the tars of four prototypes of Eclipse, resulted in significantly lower DNA-adduct formations in the skin, heart, or lung than DNA adducts in the three organs with the tar from the 1R4F reference cigarette (133).

Regular cigarette smokers were asked to switch for 2 weeks from their regular brand to Eclipse and the smoking parameters for these types of cigarettes were determined. There were four study groups, composed of 26–30 volunteers each, for a total of 109 smokers. On the basis of the main values for the four groups, the smoking of Eclipse resulted in about a 30% larger volume per puff, about 50% more puffs per Eclipse adding up to a total puff volume that was more than twice that of the total volume drawn from the control cigarettes (134). These data indicate that the Eclipse was very intensely

smoked. This is also reflected in the uptake of nicotine (135). The mutagenic activities of the urine of smokers of the four types of Eclipse, assayed on two bacterial strains were reduced by 72% to 100% compared with the mutagenic activities of the urine of the same persons, after smoking their usual cigarette brand (136). On request of the Food & Drug Administration, the Institute of Medicine of the National Academy of Sciences assembled an Expert Committee to assess the scientific basis for a possible reduction of the "harm" of the changing cigarette, including Eclipse. The Committee published the evaluation of the Eclipse independent of the entire report on "harm" reduction of tobacco smoke that is to be released in the summer of 2001. The Eclipse evaluation is summarized as follows: "Eclipse" offers the committed smoker an option that is not currently available." Eclipse does not add to the inherent biological activity of smoke for the range of cigarettes currently on the market. The elevated COHb levels should be regarded as a potential risk factor for cardiovascular diseases. The magnitude of the risk remains to be determined (132).

The high concentration of glycerol in the aerosol generated by the Eclipse cigarette motivated scientists to bioassay glycerol in "nose only" inhalation studies with Sprague-Dawley rats. These 2-week (1.0, 1.93, and 3.91 mg/L) and 13-week (0.033, 0.167, and 0.662 mg/L) assays tested for toxicity and especially for irritating effects. The investigators detected metaplasia of the lining of the epiglottis (132). The 13-week inhalation studies with rats and hamsters had also resulted in some early histopathological changes in the upper respiratory tract in both types of laboratory animals. These findings, the exposure to the smoke of Eclipse, and to glycerol aerosol, should lead to lifetime inhalation assays with the smoke of Eclipse in rats, preferably Fisher 344 rats, or better yet, in Syrian golden hamsters possibly with an inbred strain of hamsters susceptible to carcinogens in the respiratory tract (137). Pauly et al. from the Roswell Park Cancer Institute, Buffalo, NY, caution that harmful glass fibers have been found to migrate into the filter tip of the Eclipse and may be inhaled during puffing (138).

The Massachusetts Department of Health and the Society for Research on Nicotine and Tobacco challenged the claim made for Eclipse as the consumer's "next best choice". They request that the FTC and the FDA formulate regulatory procedures. Such procedures should ensure that insufficiently documented health claims for tobacco products such as Eclipse, or for tobaccos with reduced TSNA-levels ("safer tobacco") cannot be used in advertising (139, 140).

In 1998, a second U.S. tobacco company manufactured another new type of cigarette; in this case, an electrically heated cigarette (EHC). The EHC releases an aerosol which, on the basis of chemical analyses and short-term bioassays, induces significantly lower toxicity and mutagenicity than the smoke of the Kentucky reference filter cigarette, 1R4F. The prototype, containing a tobacco filler wrapped in a tobacco mat, is kept in constant contact with eight electrical heater blades in a microprocessor-controlled lighter (Figure 6) (141). This cigarette contains about half the amount of tobacco of a conventional cigarette. Under FTC-standardized smoking conditions, the cigarette delivered within an average of 8 puffs about 1 mg of nicotine, whereas all other analyzed smoke constituents were significantly lower than those in the smoke of the University of Kentucky reference cigarette,

View of EHC Cigarette with Electrical Lighter

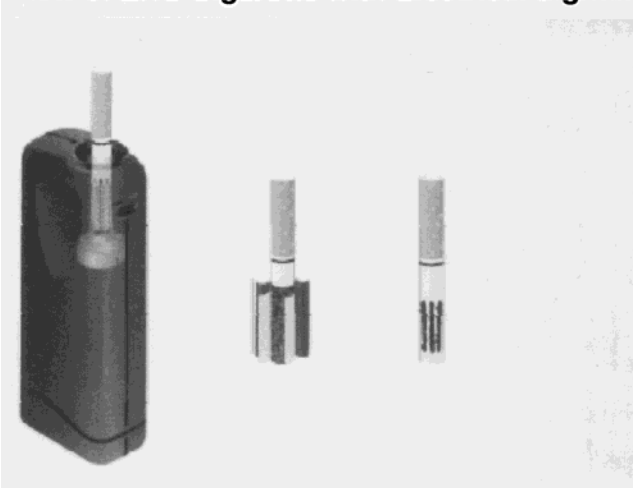


Figure 6. A new type of cigarette: EHC, Terpstra et al. (141).

1R4F. The carcinogenic PAH were below the detection level (141). However, formaldehyde yields were significantly higher in the smoke of the EHC and emissions of glycerol and 2-nitropropane were comparable to those recorded in the smoke of the 1R4F cigarette. Per gram tar, the smoke of the EHC had significantly lower mutagenic activity than the smoke of the 1R4F reference cigarette in TA98, TA100, and TA 1537 tester strains with and without the S9 fraction of rat liver homogenate (141).

H. Summary of the Changing Cigarette

Table 8 summarizes the changes in the composition of cigarette smoke that resulted from alterations in the makeup and in the composition of the tobacco filler of cigarettes since 1950. In examining these modifications, we gained important, new knowledge about the physicochemical nature of tobacco smoke, the toxic and carcinogenic agents in this aerosol, and their precursors in tobacco. We are now aware of methods that will selectively reduce or even remove specific carcinogenic agents from cigarette smoke. With regard to major carcinogenic agents in cigarette smoke, it has been documented that the gradual increase of the nitrate content of the tobacco blend caused lower smoke yields of the PAH, as shown with BaP as a surrogate; however, the smoke yield of the lung carcinogen NNK (and of other TSNA) has increased (Figure 3) (15). Around 1990, the nitrate content of U.S. cigarette tobacco amounted to 0.6–1.5% and that of the Canadian cigarette, composed only of bright varieties was $\leq 0.3\%$. At that time, the leading U.S. cigarette gave smoke yields of $1.04 \pm 0.3 \mu\text{g}$ of BaP/g of tar, while the smoke of the leading Canadian cigarette contained $1.47 \pm 0.36 \mu\text{g}$ of BaP/g of tar; the average smoke yields per cigarette of the leading U.S. cigarette amounted to 150 ng of NNN and 100 ng of NNK; and those of the leading Canadian cigarette were 35 ng of NNN and 75 ng of NNK (142, 143).

All analytical data presented in Section III, the "Changing Cigarette", were based on the standard machine smoking method of the FTC (52). However, smokers create their own patterns of puff drawing and inhaling smoke primarily for satisfying their acquired need for nicotine. When smoking a low-nicotine cigarette, long-term habitual smokers (≥ 1 year) tend to smoke more

Table 8. Reduction of Smoke Components^a

smoke component	charcoal filter	cellulose acetate filter	perforated filter	cigarette paper	reconstituted tobacco	expanded tobacco	flue-cured tobacco ^b	air-cured tobacco ^b
tar	↓	↓↓	↓↓	↓	↓	↓	+	↓
nicotine	↓	↓↓	↓	↓	↓	↓	±	±
CO	↓	±	↓	↓	↓	↓	+	±
HCN	↓	±	↓	↓	±	↓	±	±
acetaldehyde	↓	±	↓	±	±	±	±	±
acrolein	↓	±	↓	±	±	±	±	±
VNA	±	↓↓	↓	↓	±	↓	↓	↑↑
volatile phenols	±	↓↓	↓	±	±	±	↑	↓
BaP	±	±	±	±	↓	↓	↑	↓
TSNA	±	±	±	±	±	↓	↓	↑↑
carcinogenicity of tar	±	±	±	±	↓	↓	↑	↓

^a (±) Insignificant change; (↓) significant increase (≥20%); (↓) significant reduction; (↓) highly significant decrease (>30%); (↑) highly significant increase (>30%). ^b For air-cured and flue-cured tobaccos, values are measured against those in the U.S. blended cigarette.

intensely and to inhale the smoke deeper into their lungs. Doing so, they may partially block the perforations of the filter tip or even completely close them (144–148). These observations were supported by studies in which the smokers used cigarettes that varied from each other in the yields of nicotine but not in other major smoke components. The smokers of the low-nicotine cigarettes compensated for the low dose delivery (low according to smoking by the FTC method) by inhaling a greater volume of smoke than the smokers of (FTC) high-yield cigarettes (149, 150). In the 1980s, the actual puffing parameters of American smokers recorded for the high- and low-nicotine cigarettes substantiated the concept that the smoker of the low-nicotine cigarette takes larger puffs than the smoker of nonfilter cigarettes (149–152).

In summary, the sales-weighted average nicotine yield in the mainstream smoke of U.S. cigarettes changed from 2.7 mg/cigarette in 1953 to 0.85 mg since 1991 (Figure 1) as determined with the Federal Trade Commission standard machine-smoking method (52). Today, reconstituted and expanded tobacco make up 25–30% of the cigarette filler; in addition, the proportion of burley tobacco in the blended U.S. cigarette has increased. Therefore, the nitrate content of the U.S. cigarette has risen, in general, from ≤0.5% to between 1.2 and 1.5%. Other changes pertain to the increased consumption of filter-tipped cigarettes as it rose from 0.5% in 1950 to more than 97% of all U.S. cigarettes since 1997. About two-thirds of all U.S. cigarettes have perforated filter tips that cause air dilution of the smoke to vary between 20 and 45%. In addition, the porosity of the wrapping paper has significantly increased for all manufactured cigarettes.

An important outcome of these changes in the commercial cigarette is the increase in the smoke of the carcinogenic volatile nitrosamines, nitrosamino acids, tobacco-specific *N*-nitrosamines, aromatic amines, and nitroalkanes (43, 98–100).

IV. Observations on Cigarette Smokers

A. Comparison of the Smoke of High- and Low-Yield Cigarettes, 1950–1975

On the basis of the laboratory data generated with the FTC standard machine smoking method for cigarettes, it was assumed that the lung cancer risk among cigarette

smokers would decrease. Three cohort studies and four case-control studies published between 1968 and 1981 had reported that the long-term smoker of low-yield cigarettes had a 20–50% lower risk for lung cancer than the smoker of the conventional high-yield nonfilter cigarettes (1, 153–159). In a longitudinal study that began in 1959, Hammond et al., from the American Cancer Society, followed 1 million men and women over 12 years. Few of the smokers who shifted from high-tar and nicotine to low-tar and nicotine cigarettes increased their daily cigarette consumption. Adjusted for numbers of cigarettes smoked per day, the smokers of low-yield cigarettes showed somewhat reduced total death rates and death rates from coronary heart disease and from lung cancer (155). Wynder and Stellman reported in 1979 that long-term smokers of filter cigarettes had reduced risks for cancer of the lung as well as for cancer of the larynx (158).

Auerbach et al. examined the bronchial tubes of 211 men (including 154 smokers) from autopsy specimens collected in the years 1955–1960 and bronchial tubes from 234 men (187 smokers) from autopsy material obtained in 1970–1977. Auerbach compared the loss of cilia, and the occurrence of bronchial metaplasia and atypical nuclei. The earlier samples presented significantly more pronounced changes in the bronchial tubes than the samples collected in 1970–1977 (160).

A U.S. Surgeon General's Committee on the changing cigarette stated in 1981 that low-tar, low-nicotine cigarettes produce lower rates of lung cancer than the higher tar and higher nicotine predecessors (78). In 1986, an IARC expert panel on the epidemiology of smoking-associated cancers, on tobacco toxicology, and on tobacco carcinogenesis concluded that epidemiological studies suggest that "prolonged use of nonfilter, high-tar cigarettes is associated with a greater risk for lung cancer than the prolonged use of filter and low-tar cigarettes" (1).

B. Comparison of the Smoke from High- and Low-Yield Cigarettes, 1976–1999

These tentative conclusions about the lower toxicity and carcinogenicity of low-yield cigarettes manufactured during the 1970s and early 1980s were questioned as to their applicability to cigarettes produced in the 1980s and

thereafter. Study groups in the U.K., in the U.S., and elsewhere observed that the machine-smoking schedule chosen by the FTC, CORESTA, and other agencies, did not reflect the smoking patterns observed among most of the cigarette smokers in developed countries during the preceding 2–3 decades (144–149, 161, 162).

In 1978, the tobacco industry was well aware of the fact that the standard machine-smoking schedule did not reflect the smoking habits of most of the cigarette smokers. Schultz and Seehofer, determining nicotine in the butts of cigarettes smoked by men and women, found significantly higher levels of it in these butts than in those from identical cigarettes that were machine-smoked, whereby the butt lengths were not different from those left by the smokers (163).

In 1970, Ashton and Watson, and Benowitz et al., had already observed that smokers of low-yield cigarettes took more puffs than smokers of high-nicotine cigarettes (149, 164). This observation was confirmed by Haley et al. in 1985 (150). Kozlowski et al. were the first to report that smokers of low-nicotine cigarettes with perforated filter tips tend to occlude the holes in the filter tip with their lips and/or fingers, thereby increasing the smoke yields of tar, nicotine, and carbon monoxide (148, 165, 166). This was also confirmed by studies in other laboratories (167, 168). Using the ninhydrin color reaction of the saliva-derived residual protein and amino acids on the perforated filter tips, it was determined in a study examining 1229 cigarette butts that 5.2% of the holes were completely or partially closed and 18.9% were partially closed during smoking (169). In a second report, 15% of 300 butts gave evidence of the holes being covered with saliva (170).

Assays on nicotine uptake by smokers demonstrated that there is no significant relationship between plasma cotinine, a major nicotine metabolite, and the nicotine yield of cigarettes smoked according to the FTC method (171). Benowitz et al. reported that the cotinine level in plasma was virtually the same for all cigarettes smoked and inhaled, except for ultra-low-yield cigarettes (172). Gori and Lynch found no correlation between expired carbon monoxide from cigarette smokers and the FTC yields of carbon monoxide (173).

The development of a tobacco smoke inhalation testing system (TSITS) in the late 1980s enabled assays of the smoking intensities of smokers of cigarettes with different nicotine yields (174, 175). This system was utilized for the determination of smoking profiles of long-term smokers (≥ 1 year) of a specific brand of cigarettes that had FTC nicotine yields of 0.6–0.8 mg/cigarette (56 volunteers) and of 0.9–1.2 mg of nicotine/cigarette (76 volunteers). The observed average values were puff volumes of 48.6 and 44.1 mL (FTC, 35 mL), puff duration of 1.5 s (FTC, 2.0 s), and a total puff volume per cigarette between 615 and 523 mL (FTC, 280–350 mL). The average smoke yields for nicotine were 1.74 mg (FTC, 0.7) and 2.39 mg (1.11), for tar 22.3 mg (8.5) and 29 mg (15.4), and for carbon monoxide 17.3 mg (9.7) and 22.5 mg (14.6), respectively. This study revealed also that, compared with FTC data, smokers of cigarettes with low or medium smoke yields actually not only inhaled significantly higher quantities of tar, nicotine and carbon monoxide but also of the major lung carcinogens, PAH (BaP = 1.6–1.8 times higher) and of the TSNA (NNK = 1.7 times higher) (176).

Unfortunately, the public generally assumes that the smoke yields published by the FTC are reflecting the degree of exposure to harmful smoke constituents inherent in smoking a given brand of cigarette. Thus, it is believed that smoking low-yield and ultra-low-yield brands carries less of a risk than smoking high-yield nonfilter cigarettes. However, the suggestion that there is a meaningful quantitative relationship between FTC-measured smoke yields and actual uptake of smoke carcinogens by the cigarette smoker is misleading (171). It has, therefore, been stated that “the time has come for (requiring) meaningful information on the smoke yields of cigarettes” (177, 178). While the tobacco industry has not taken a stand on this issue, the FTC principally agrees that a better and more comprehensive test program for cigarettes is needed (179).

C. Epidemiological Studies

As cited in subsection V. A., seven independent epidemiological studies published between 1969 and 1981 in the U.K. and in the U.S. reported a 20–50% lower risk of lung cancer for long-term smokers of filter cigarettes than for smokers of nonfilter cigarettes (1, 153–159). One may assume, on the basis of previous reports, that during the years 1950–1975, when filter cigarettes gave relatively high yields of nicotine (in 1962, sales weighted average = 2.0 mg), smokers had not significantly increased their smoking intensities, in the way smokers did in later years. Therefore, they may have benefited from the reduction of tar in the smoke of filter cigarettes between 1950 and 1975 as is reflected in a somewhat reduced risk for lung cancer.

The sales-weighted average FTC nicotine yields of the U.S. cigarette decreased since 1980 from 1.0 mg to 0.85 mg and that of filter cigarettes declined from 0.9 mg to 0.80 mg/cigarette (180). Because smokers compensate for nicotine uptake (175, 176), the consumers of low-nicotine cigarettes are likely to smoke these cigarettes more intensely and inhale the smoke more deeply into the lung than do smokers of nonfilter cigarettes.

Since 1983, at least 10 epidemiological studies have reported that the lung cancer risk of smokers of low-tar, low-nicotine cigarettes is comparable to, or only slightly lower than that of smokers of nonfilter cigarettes (181–189). The large prospective study, CPS I (Cancer Prevention Study I), by the American Cancer Society, involving more than one million men and women, compares lung cancer mortality and morbidity rates, CHO, COPD, and stroke during the period 1959–1965 with corresponding data from the CPS II study for the years 1982–1988 (187, 190). The smokers in the CPS II group had a significantly higher lung cancer risk than the smokers of filter cigarettes in the CPS I group, and only a slightly lower risk than the smokers of nonfilter cigarettes in the CPS II study (191). These epidemiological data are supported by laboratory data from smokers of low- and high-yield cigarettes (144–152, 161–176). Over the years, the changing cigarette led to a decline in sales-weighted average FTC-nicotine yields for the U.S. cigarette. In 1970, it was 1.31 mg; since 1990, it declined further from 0.94 to 0.85 mg of nicotine (180). Since 1990, more than 60% of all U.S. cigarettes delivered ≤ 1.2 mg of nicotine under the FTC machine-smoking conditions. During the past 2 decades, most of the cigarette-smoking men and women in the United States smoked their cigarettes

rather intensely. There are many indications, though no actual measurements have been reported that smokers of cigarettes yielding ≤ 1.2 mg of nicotine/cigarette do, in fact, inhale the smoke more deeply than smokers of cigarettes with higher yields.

One indication for differences in depth of smoke inhalation between smokers in earlier and recent decades is the apparent shift in the prevalence of adenocarcinoma in the peripheral lung in both men and women who smoked cigarettes. In the first large-scale epidemiological study on cigarette smoking and lung cancer, the tumors were classified as bronchiogenic carcinoma and adenocarcinoma (AC). Bronchiogenic carcinoma included squamous cell carcinoma (SCC) and small cell carcinoma. Of the 605 men with lung cancer, 566 had bronchiogenic carcinoma and only 39 had AC, i.e., there was a ratio of 15:1. Among the 592 male cigarette smokers with lung tumors in this study, there were 561 cases of bronchiogenic carcinoma and 31 AC (16:1) (19). These assessments were completed before the WHO's acceptance of the classification system by Kreyberg in 1967 (193). Thus, caution is necessary in comparisons of the SCC to AC ratios of the studies conducted in the 1950s and in later years. Nonetheless, there has clearly been a gradual change in that the prevalence of lung tumors leaned more and more toward AC in the peripheral lung while there initially was a preponderance of SCC in the major bronchi. The SCC:AC ratios for lung cancer cases changed from 3.1:1.0 in men and 1.0:1.64 in women in the years 1964 to 1971 to 1.4:1.0 in men and 1.0:1.8 in women in 1984–1986 (185, 194–200). The prevalence of centrally originating bronchiocarcinoma declined from 69.3% in specimens examined before 1978 to 57.3% in specimens collected between 1986 and 1989 (201).

In Section III,D, we discussed that the average nitrate content of the U.S. blended cigarette tobacco increased gradually from less than 0.5% in the 1950s to between 1.2 and 1.5% since the 1980s. Increased nitrate content is a major factor for the increased formation of TSNA during tobacco processing and during smoking (43, 107). NNK, a TSNA, formed by the nitrosation of nicotine, is an organ-specific carcinogen that induces lung adenocarcinoma in mice, rats, and hamsters (43). Literature data support the potential contribution of NNK in the development of human lung cancer in smokers (200). Carbonyl reduction of NNK is the major metabolic pathway. α -Hydroxylation leads to the formation of intermediates that can damage lung DNA (43, 202). These data support the concept that smoking low-yield cigarettes enhances the formation of adenocarcinoma of the lung. It appears that efforts to render cigarettes less harmful have had an impact on changes in the type of lung tumors caused by cigarette smoking but have not led to a reduction of the overall lung cancer risk for smokers.

V. Future Directions

The Surgeon General's report on Smoking and Health for the year 2000, entitled "Reducing Tobacco Use", reviews past achievements and outlines the most promising methods for the treatment of nicotine addiction (203). It is encouraging that the prevalence of cigarette smoking among adults has decreased from 40% in the 1960s to about 25% in the late 1990s. However, the decline of smoking prevalence progressed only at the rate of 0.5%/

year. It is of great concern that, during the last 10 years, cigarette smoking among junior high school students has increased to 9.2% and among high school students to 34.8%. In addition, snuff dipping prevalence in these groups of students increased to 3.6 and 10.0%, respectively (14, 203). It is also of great concern that in certain subgroups in our society the percentage of smokers is now significantly higher than in the rest of the U.S. population. In 1997, among men and women with less than 12 years of education, 35.4% smoked cigarettes compared to 11.6% among college graduates. Among those in the lower income strata (income below the poverty level), cigarette smoking prevalence is 33.3% (196).

The most promising approaches toward reducing tobacco smoking are four types of intervention: (1) education (school curricula and by mass media), (2) clinical approaches (prescription of drugs, including nicotine, and other treatment for behavioral changes); (3) regulatory intervention (product controls, restriction of product sales to minors, smoking bans at the workplace), and (4) economic measures (taxation) (203).

The Surgeon General's report for the year 2000 neither discusses nor mentions the concept of "the less harmful cigarette". As discussed earlier, from a public health standpoint, the only harmless cigarette is the one that is not smoked. Adherence to this view may have been reinforced by the lack of significant progress over the past three decades toward "the less harmful cigarette". However, several scientists outside the industry see it as a mandate that cigarettes with significantly reduced overall toxicity, carcinogenicity and with less addictive potential are made available. After all, smoking control and prevention has not reached the many millions of cigarette smokers who remain dependent on nicotine and are, therefore, at high risk for tobacco-related diseases. These smokers include the large segment of economically disadvantaged, healthcare-underserved men and women in our society.

There are a number of possibilities for changing tobacco products toward overall reduction of toxicity, carcinogenicity and their addictive nature. It needs to be strongly emphasized that there will never be a safe cigarette (16, 203). However, "the less harmful cigarette" is a necessary compromise for those smokers who cannot overcome their nicotine addiction. It is suggested that the first step toward such renewed efforts will be the inception of a Tobacco Study Group (TSG) composed of individual experts in the tobacco-related sciences and disease research areas. This TSG will not be a revival of the Tobacco Working Group (TWG) that was active at the National Cancer Institute between 1968 and 1979. The first step for the proposed TSG will be the establishment and adoption of the conditions that will ensure its credibility. The group will be composed of scientists from academia, tobacco control agencies of state and federal governments, and industry. It will be the primary goal of the TSG to study and to establish the conditions for research strategies that, on the basis of current scientific knowledge, would represent the most promising approaches toward the "less harmful cigarette". In the second step, the smoke of the research cigarettes will be analyzed for tar, nicotine, CO, and those smoke parameters and components that contribute to the toxicity, carcinogenicity, and addictive nature of cigarette smoke. The cigarettes will be smoked under conditions that reflect the average smoking habits of long-term cigarette

smokers. The smoke of these experimental cigarettes will be bioassayed in short-term *in vitro* and *in vivo* tests for their mutagenicity and carcinogenic potential and for their cilia toxicity. These tests would be followed by long-term smoke inhalation assays with Syrian golden hamsters for the indication of tumors in the upper aerodigestive tract. Tars will also be assayed on the skin of strain A mice for their potential for induction of skin tumors and lung adenomas. Those experimental cigarettes that deliver smoke with lower potentials for toxicity, carcinogenicity, and nicotine addiction than current U.S. cigarettes with comparable tar and nicotine yields will be smoked by voluntary long-term cigarette smokers who volunteer to participate in this assay. The serum of these smokers will be analyzed for nicotine, cotinine, thiocyanate, NNAL, 1-hydroxypyrene, and for COHb. The urine will be analyzed for the same metabolites determined in serum and, in addition, for *N*-nitrosoproline and total NNAL after hydrolysis, and for muconic acid.

A. Some Thoughts on Cigarettes with Low Nicotine Delivery

To achieve low nicotine delivery, the cigarette tobaccos will consist of a low-nicotine blend and will have efficient filter tips. These filter tips will be constructed in a manner that precludes smokers' compensation and manipulation of the perforation, thus allowing nicotine delivery in the smoke to be no greater than that deemed the lowest appropriate dose per cigarette according to the TSG. Initial aims will be for a nicotine delivery of no more than 0.7–0.8 mg of nicotine/cigarette. This dose may be gradually reduced to between 0.5 and 0.6 mg/ cigarette.

A survey by the American Cancer Society reported that between 1992 and 1998 among 1.2 million men and women in the United States, 7.8% of all male smokers and 13.9% of female smokers, consumed cigarettes that deliver ≤ 6 mg FTC tar. Of the cigarettes delivering 6 mg of tar (57 cigarette brands), five brands delivered 0.6 mg, 42 brands 0.5 mg, and 10 brands 0.4 mg of nicotine (180, 204).

Benowitz and Henningfield estimated 0.4–0.5 mg of nicotine content in the tobacco of one cigarette to be the upper limit for effective prevention of nicotine addiction in young people (205). It is unlikely that a cigarette with such a low nicotine delivery would be accepted by the consumers. The smoker will rather seek products with higher nicotine yield so that any concept of "a less harmful cigarette" would be self-limiting. However, ultimately, the lowest acceptable level of nicotine emission per cigarette has to be part of "the less harmful cigarette".

B. Major Reduction of TSNA

At a 1962 meeting on *N*-nitrosamines in Hamburg, Germany, sponsored by the West German Cigarette Research Council, H. Druckrey and R. Preussman discussed the possibility that nornicotine and possibly nicotine may give rise during smoking to the suspected carcinogen *N*-nitrosornicotine (NNN) (206). In 1975, Klus and Kuhn, from the Austrian Tobacco Company, determined in the smoke of a cigarette filled with tobacco rich in nornicotine 40 ng of NNN (207). On the basis of the absence of publications in the open literature, it appears that the tobacco industry had only limited

interest in the analytical and chemical aspects of the TSNA and their carcinogenic activities until the early 1990s (208–210). Following a large-scale bioassay for the carcinogenic activity of NNN in the 1970s, the tobacco industry did not publish data on the carcinogenicity of any of the seven TSNA identified in tobacco products (201).

TSNA are the major carcinogens in chewing tobacco and snuff and are associated with cancer of the oral cavity of snuff dippers (43, 211, 212). The nicotine-derived NNK is an organ-specific carcinogen that induces adenocarcinoma in the peripheral lung of mice, rats, and hamsters. In addition, NNK and its enzymatic reduction product NNAL are the only environmental agents known to induce cancer of the exocrine pancreas in laboratory animals (48, 202). NNK and NNN are specifically formed by *N*-nitrosation of nicotine. NNN is also formed from nornicotine (43). The *N*-nitrosation of nicotine and nornicotine occurs during curing, fermentation, and aging of tobacco and involves reduction of nitrate, primarily by bacteria, leading to the formation of nitrite which is a *N*-nitrosating agent (86, 213, 214).

Star Scientific, Inc. has succeeded in reducing the formation of *N*-nitrosamines, and especially that of the highly carcinogenic NNK, during curing and aging of tobacco (215). However, about 30–50% of the TSNA in cigarette smoke result from pyrosynthesis in the burning cone and the hot zones of the cigarettes and are emitted into mainstream smoke together with the preformed TSNA that are aerosolized into the smoke stream (216, 217).

On the basis of our current knowledge, a drastic reduction of TSNA levels in chewing tobacco and snuff is expected to lower the risk for oral cancer; in fact, such low levels of TSNA may be below the threshold level for the induction of tumors in snuff dippers. However, it will also be of importance to investigate the possible endogenous formation of the carcinogenic TSNA in consumers of the snuff brands that contain only traces of TSNA (43, 218–222).

Inhibition of NNN and NNK formation in the hot zones of burning cigarettes may also be achieved by trapping the nitrogen oxide radicals. The trapping by free radicals will at the same time, at least partially, remove these freshly generated, highly reactive and, therefore, undesirable agents from tobacco smoke.

C. Nicotine Analogues

Nicotine is regarded as the major addictive agent in smokeless tobacco and in tobacco smoke. Nicotine is also the primary substrate for the two highly carcinogenic, tobacco-specific *N*-nitrosamines, NNK, and NNN (43, 202, 223). Thus, a possible approach toward "the less harmful cigarette" would be the replacement of nicotine with an analogue that has reduced receptor binding but also a low potential for the formation of NNN- and NNK-analogues and that these *N*-nitrosamines are also only weakly carcinogenic or noncarcinogenic. Preliminary data by several investigators supports this approach (224–227).

VI. Epilogue

The first large-scale epidemiological studies on smoking and disease in 1950 revealed a dose–response

relationship between the number of cigarettes smoked and the risk for lung cancer. These findings were supported by bioassays resulting in a dose-response between the amount of tar applied to mouse skin and the induction of skin tumors. They were further strengthened by inhalation studies with hamsters documenting a dose-response between the amount of cigarette smoke inhaled and the occurrence of tumors in the upper respiratory tract. On the basis of these observations, the initial research toward the less toxic cigarette emphasized the reduction of smoke yields for tar and nicotine and utilized the standard machine smoking method of the FTC to measure such reduction. The emission of tar and nicotine from the U.S. sales-weighted average cigarette was gradually lowered from 38 mg of tar and 2.7 mg of nicotine in 1953 to 18 and 1.2 mg in 1975, and since 1996, to 12 and 0.85 mg, respectively. The tar and nicotine reductions were achieved by using filter tips primarily made from cellulose acetate. The prevalence of filter tipped cigarettes increased from 0.5% of all U.S. cigarettes in 1950 to more than 97% since 1990. Reductions were also achieved by incorporating into the cigarette blend reconstituted and expanded tobacco, by increasing the porosity of the cigarette paper, by changing the tobacco blend, including increasing the portion of air-cured tobacco, and by developing perforated filter tips. It was always recognized that it is highly unlikely that there will ever be a nontoxic cigarette and that there is only one certain way to prevent, respectively to reduce, smoking-related diseases, namely, by not starting the smoking habit or, for smokers, to stop the habit. "The less harmful cigarette" was, and is, only regarded as a compromise for those who cannot or will not give up smoking cigarettes. The group of men and women who continue to smoke includes a large segment of the economically disadvantaged, healthcare-underserved people who also, in general, lack the opportunity to be treated for nicotine addiction.

Between 1969 and 1981, epidemiological studies indicated that the long-term smoker of filter cigarettes has a 20–50% reduced risk for smoking-related diseases compared with the risk of the smoker of nonfilter cigarettes. However, beginning with the 1980s, this difference in risks for cancer, heart disease, chronic obstructive lung disease, and stroke between smokers of low-yielding cigarettes and of smokers of nonfilter cigarettes gradually disappeared. Primarily, three factors are considered to be associated with the disappearance of a reduction in the differences in risks for diseases among smokers of filter cigarettes and among smokers of nonfilter cigarettes. These are the increased smoking intensities and increased depth of inhalation by the smokers of filter cigarettes as a consequence of their acquired need for nicotine. The third major reason is the increased nitrate concentration in the tobacco of the U.S. blended cigarette. The nitrate increase leads to greater concentrations of nitrogen oxides in the smoke and, thereby enhancing formation of carcinogenic *N*-nitrosamines, especially of the nitrosamines formed from nicotine and nornicotine during tobacco processing and during smoking. These TSNA are organ-specific carcinogens that induce adenocarcinoma in the peripheral lung, carcinoma in the upper aerodigestive tract and carcinoma in the pancreas.

For those adults who did not succeed in refraining from smoking, "the less harmful cigarette" has to be developed

as the U.S. cigarette of the future. A number of scientists in tobacco control and tobacco-health research regard "the less harmful cigarette" as a "must" for our society.

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