CHAPTER 3

Advances in Tobacco Carcinogenesis

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A. Introduction

In their first reports on smoking and disease both the ROYAL COLLEGE OF PHYSICIANS OF LONDON (1962) and the US SURGEON GENERAL OF THE PUBLIC HEALTH SERVICE (1964) concluded that cigarette smoking is causally related to lung cancer in humans and is associated with cancer of the oral cavity, larynx and urinary bladder. These conclusions were based on epidemiological data and were supported by laboratory studies. Today, 25 years later and after extensive research, epidemiological reports from more than 20 countries have led the US SURGEON GENERAL (1986a) and the INTERNATIONAL AGENCY FOR RESEARCH ON CANCER (1986) to the conclusion that smoking of cigarettes is causally related to cancer of the respiratory tract, the upper digestive tract, pancreas, renal pelvis and bladder and that cigarette smokers also face an increased risk for cancer of the cervix. Cigar and pipe smoking are also causally related to cancer of the respiratory tract, oral cavity and esophagus, although, in the case of lung cancer, not to the same extent as cigarette smoking (US SURGEON GENERAL 1986a; IARC 1986). In addition to active smoking, involuntary smoking, i.e., the exposure to environmental tobacco smoke, has been incriminated as a risk factor for cancer of the lung in nonsmokers (IARC 1986; US NATIONAL RESEARCH COUNCIL 1986; US SURGEON GENERAL 1986a). Furthermore, chewing of tobacco and especially the oral use of snuff were found to be associated with cancer of the oral cavity (IARC 1985a; US SURGEON GENERAL 1986b) and possibly with cancer of the nasal cavity, kidney and bladder (BRINTON et al. 1984; US SURGEON GENERAL 1986a; KABAT et al. 1986; GOODMAN et al. 1986).

The rise in lung cancer in industrialized countries has been directly correlated with the increase in the manufacture and consumption of cigarettes (DOLL and PETO 1981). In fact, it has been estimated that 85%–90% of all lung cancer deaths in American males in 1978 were caused by tobacco smoking and that about 30% of all cancers in the USA and in the UK can be attributed to tobacco use (WYNDER and GORI 1977; HIGGINSON and MUIR 1979; DOLL and PETO 1981).

In view of the convincing epidemiologic evidence and widespread awareness of the role of tobacco products as causes of cancer, one is sometimes asked why there is a need for further studies in tobacco carcinogenesis. After all, if tobacco use were to cease, the problem would disappear in a few decades. The hazards of tobacco usage are well-known by the public and are taught in many educational institutions. In fact, the Surgeon General of the US Public Health Services deserves the full support of the medical and scientific community in his quest for

a "smoke-free society" by the year 2000 (KOOP 1986). Unfortunately, the recent statistics on tobacco use are not supportive of this goal. In 1985, for example, approximately 600 billion cigarettes were sold in the USA alone and the annual per capita consumption of cigarettes for individuals aged 18 years and older was approximately 3400 (TOBACCO JOURNAL INTERNATIONAL 1987). The consumption of cigarettes in many Asian, African and South American countries has also sharply risen in recent years. For example, between 1976 and 1986 the cigarette production in the People's Republic of China increased by 84.4% to 1.296 billion, in Egypt by 112.9% to 49.5 billion cigarettes, and in Brazil by 44.4% to 168.9 billion cigarettes (IARC 1986; TOBACCO JOURNAL INTERNATIONAL 1987). In the USA and Sweden there has also been a constant rise in the consumption of snuff tobacco, at least until 1985 (MAXWELL 1986; TOBACCO JOURNAL INTERNATIONAL 1988).

This review is intended to document the progress achieved in tobacco toxicology during the past 2 decades (WYNDER and HOFFMANN 1967). New knowledge in this field has contributed much to our understanding of the epidemiologic findings and to tobacco carcinogenesis and environmental carcinogenesis in general. New methods and concepts have been developed in tobacco carcinogenesis and in chemical carcinogenesis and have provided new insights into both fields of research (HOFFMANN and HARRIS 1986).

B. Tobacco and Tobacco Smoke

In most parts of the world, more than 60 species of *Nicotiana* can be found, but only *N. tabacum* is commercially cultivated on a large scale. *N. rustica* is grown in some areas of China, India and the USSR. Tobacco leaves are usually dried, cured, aged, and, in some instances, fermented. The leaves of the bright (Virginia) varieties of *N. tabacum* are flue cured in steam-heated barns, which results in tobacco with high sugar content and relatively low levels of nitrate (<0.1%). Burley leaves, on the other hand, are simply air-cured and are low in sugars and relatively high in nitrate content (< 5%), and oriental leaves which are sun-cured, have medium sugar content and are low in nitrate (<0.6%). For cigars, for some types of pipe tobaccos, and for use as smokeless tobaccos, leaves are not only cured but also fermented (Tso 1972).

Processed, unadulterated tobacco contains at least 2550 known compounds (DUBE and GREEN 1982). The bulk of the tobacco consists of carbohydrates (≃50%) and proteins. Other significant constituents are alkaloids (0.5%–5%) with nicotine as the predominant compound (90%–95% of total alkaloids), terpenes (0.1%–3.0%), polyphenols (0.5%–4.5%), phytosterols (0.1%–2.5%), carboxylic acids (0.1%–0.7%), alkanes (0.1%–0.4%), aromatic hydrocarbons, aldehydes, ketones, amines, nitriles, *N*- and *O*-heterocyclic compounds, pesticides, alkali nitrates (0.01%–5%), and at least 30 metallic compounds (WYNDER and HOFFMANN 1967; IARC 1986).

The burning of tobacco generates mainstream smoke (MS) during puff-drawing, and sidestream smoke (SS) during smouldering between puffs. The physicochemical nature of these smoke types is a function of various fac-

tors. These include the type of tobacco, the temperatures prevailing during puff-drawing (860°–900° C) or smouldering (500°–650° C), the reducing atmosphere characteristic of the burning zone and the physical design of the tobacco product (e.g. length, diameter, paper, wrapper or pipe bowl, variety of the cigarette paper and filter tip).

The composition of the processed tobacco in cigarettes has a profound influence on the chemistry and toxicity of the smoke. Cigarette manufacture in the USA, Japan, and most European countries utilizes blends of bright, burley, and oriental tobaccos, whereas cigarettes sold in the UK and Finland contain exclusively bright tobaccos. Both types of cigarettes deliver a weakly acidic mainstream smoke (MS) (pH 5.5–6.2) in which nicotine occurs in protonated form in the particulate matter. In France and some parts of Italy, North Africa, and South America, a high percentage of the cigarette brands contain only burley tobaccos. In the smoke of these cigarettes which is neutral to weakly alkaline (pH 6.8–7.5), a significant proportion of the nicotine is found in the vapor phase in unprotonated form. The smoke of cigars is neutral to alkaline (pH 6.5–8.0), and, like the smoke of burley cigarettes, it contains unprotonated nicotine in the vapor phase. The pH of sidestream smoke (SS) of cigarettes and cigars ranges between 6.8–8.5; thus, it contains free nicotine (BRUNNEMANN and HOFFMANN 1974). Unprotonated nicotine is more quickly absorbed through the buccal mucosa than protonated nicotine (ARMITAGE and TURNER 1970).

The 400–500 mg of MS freshly emerging from the mouthpiece of a cigarette is an aerosol which contains about 1×10^{10} particles per ml; these range in diameter from 0.1–1.0 μm (mean diameter 0.2 μm) and are dispersed in a vapor phase (INGEBRETHSEN 1986). About 95% of the MS effluent of a non-filter cigarette is comprised of 400–500 individual gaseous components with nitrogen, oxygen, and carbon dioxide as major constituents. As of our state of knowledge, the particulate matter contains at least 3500 individual compounds (Fig. 1; DUBE and GREEN 1982).

All combustion products contain free radicals; in the case of tobacco smoke these are highly reactive oxygen- and carbon-centered types in the vapor phase, and relatively stable radicals in the particulate phase. The principle of the latter appears to be a quinone/hydroquinone complex which is capable of reducing molecular oxygen to superoxide and, eventually, to hydrogen peroxide and hydroxyl radicals (NAKAYAMA et al. 1984; CHURCH and PRYOR 1985).

The generation of MS and SS components follows different pathways. The compounds are either transferred structurally intact from the tobacco into the smoke (e.g. nicotine, phytosterols, long-chain paraffins), or they are completely pyrosynthesized in the hot zones without specific precursors (e.g. carbon monoxide, phenols, benzene, benzo[*a*]pyrene), or they are partially transferred and partially pyrosynthesized (e.g. *N'*-nitrosonornicotine, certain volatile aldehydes). The majority of the smoke components are pyrosynthesized either by partial degradation or by oxidation of specific tobacco precursors (e.g. furans, indoles, flavor components derived from tobacco terpenoids), or they are totally synthesized from specific constituents (e.g. hydrogen cyanide, nitrogen oxides, ammonia, catechols; GREEN 1977; SCHMELTZ and HOFFMANN 1977; JOHNSON 1977; CARMELLA et al. 1984).

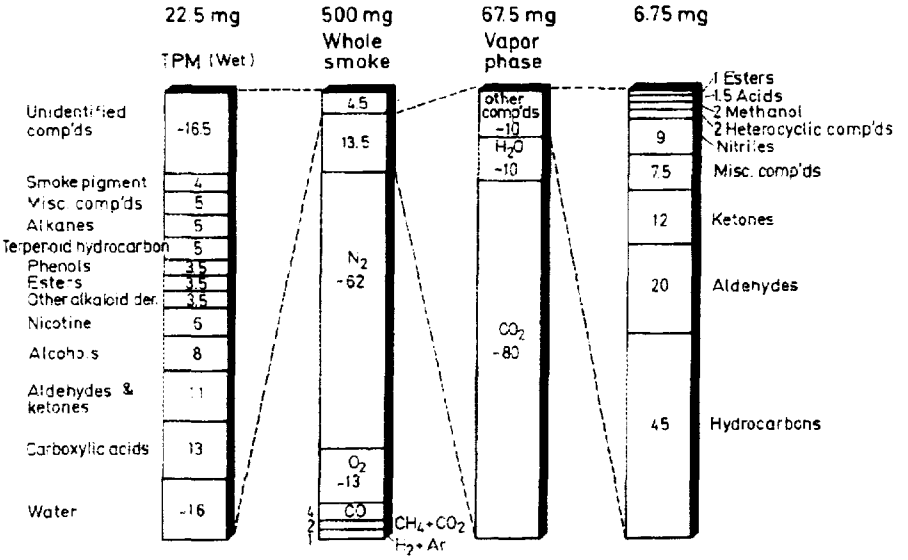

Fig. 1. Total cigarette smoke composition (% w/w) (DUBE and GREEN 1982) *TPM*, total particulate matter

For chemical analysis, the smoke is arbitrarily separated into a vapor phase and a particulate phase. Those individual smoke components of which more than 50% appear in the vapor phase of fresh MS are considered volatile smoke components; all others are particulate phase components (Fig. 1). Tables 1 and 2 list the major types of components identified and their estimated concentration in the smoke of one cigarette (WYNDER and HOFFMANN 1967; TSO 1972; GREEN 1977; ENZELL et al. 1977; US SURGEON GENERAL 1982; IARC 1986; WAHLBERG and ENZELL 1987). These tables present data which are important with regard to bioactivity of smoke constituents but are by no means to be regarded as a complete analysis of cigarette smoke. The quantitative data in this review are derived from cigarettes that were machine-smoked under standardized laboratory conditions (BRUNNEMANN et al. 1976). Therefore, the data do not fully reflect the human setting. This applies especially to smokers of low yield cigarettes who tend to compensate for the low nicotine and low tar delivery by drawing smoke more intensely and inhaling it more deeply (HERNING et al. 1981; HALEY et al. 1985).

Tobacco is known to contain at least 30 metals (NORMAN 1977). For example, the tobacco of one cigarette was found to contain 38 mg of potassium, 22 mg of calcium, and 5.5 mg of magnesium as the major metals. Since less than 1% of the metals is transferred from the tobacco into the smoke (JENKINS et al. 1985), these elements form too minute a proportion to be listed in Table 2.

Tables 1 and 2 also omit information about the chemical nature and concentrations in cigarette smoke of agricultural chemicals and pesticides, which originate from the residues of such compounds on the tobacco (WYNDER and HOFFMANN 1967; IARC 1985a, 1986). We have not included this information be-

Table 1. Major constituents of the vapor phase of the mainstream smoke of nonfilter cigarettes

Compound[a]	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 µg
Nitrogen oxides [NO_x]	100 – 600 µg
Hydrogen cyanide	400 – 500 µg
Hydrogen sulfide	20 – 90 µg
Methane	1.0– 2.0 mg
Other volatile alkanes (20)	1.0– 1.6 mg[b]
Volatile alkenes (16)	0.4– 0.5 mg
Isoprene	0.2– 0.4 mg
Butadiene	25 – 40 µg
Acetylene	20 – 35 µg
Benzene	12 – 50 µg
Toluene	20 – 60 µg
Styrene	10 µg
Other volatile aromatic hydrocarbons (29)	15 – 30 µg
Formic acid	200 – 600 µg
Acetic acid	300 –1700 µg
Propionic acid	100 – 300 µg
Methyl formate	20 – 30 µg
Other volatile acids (6)	5 – 10 µg[b]
Formaldehyde	20 – 100 µg
Acetaldehyde	400 –1400 µg
Acrolein	60 – 140 µg
Other volatile aldehydes (6)	80 – 140 µg
Acetone	100 – 650 µg
Other volatile ketones (3)	50 – 100 µg
Methanol	80 – 180 µg
Other volatile alcohols (7)	10 – 30 µg[b]
Acetonitrile	100 – 150 µg
Other volatile nitriles (10)	50 – 80 µg[b]
Furan	20 – 40 µg
Other volatile furans (4)	45 – 125 µg[b]
Pyridine	20 – 200 µg
Picolines (3)	15 – 80 µg
3-Vinylpyridine	10 – 30 µg
Other volatile pyridines (25)	20 – 50 µg[b]
Pyrrole	0.1– 10 µg
Pyrrolidine	10 – 18 µg
N-Methylpyrrolidine	2.0 – 3.0 µg
Volatile pyrazines (18)	3.0– 8.0 µg
Methylamine	4 – 10 µg
Other aliphatic amines (32)	3 – 10 µg

[a] Numbers in parentheses represent the individual compounds identified in a given group.
[b] Estimate.

Table 2. Major constituents of the particulate matter of the mainstream smoke of nonfilter cigarettes

Compound[a]	µg/cigarette
Nicotine	1000 –3000
Nornicotine	50 – 150
Anatabine	5 – 15
Anabasine	5 – 12
Other tobacco alkaloids (17)	n.a.
Bipyridyls (4)	10 – 30
n-Hentriacontane [n-$C_{31}H_{64}$]	100
Total nonvolatile hydrocarbons (45)[c]	300 – 400[c]
Naphthalene	2 – 4
Naphthalenes (23)	3 – 6[c]
Phenanthrenes (7)	0.2 – 0.4[c]
Anthracenes (5)	0.05– 0.1[c]
Fluorenes (7)	0.6 – 1.0[c]
Pyrenes (6)	0.3 – 0.5[c]
Fluoranthenes (5)	0.3 – 0.45[c]
Carcinogenic polynuclear aromatic hydrocarbons (11)[b]	0.1 – 0.25
Phenol	80 – 160
Other phenols (45)[c]	60 – 180[c]
Catechol	200 – 400
Other catechols (4)	100 – 200[c]
Other dihydroxybenzenes (10)	200 – 400[c]
Scopoletin	15 – 30
Other polyphenols (8)[c]	n.a.
Cyclotenes (10)[c]	40 – 70[c]
Quinones (7)	0.5
Solanesol	600 –1000
Neophytadienes (4)	200 – 350
Limonene	30 – 60
Other terpenes (200–250)[c]	n.a.
Palmitic acid	100 – 150
Stearic acid	50 – 75
Oleic acid	40 – 110
Linoleic acid	60 – 150
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
Other *O*-heterocyclic compounds (42)	n.a.
Stigmasterol	40 – 70
Sitosterol	30 – 40
Campesterol	20 – 30
Cholesterol	10 – 20
Aniline	0.36
Toluidines	0.23
Other aromatic amines (12)	0.25
Tobacco-specific *N*-nitrosamines (4)[b]	0.34– 2.7
Glycerol	120

[a] Numbers in parentheses represent individual compounds identified. [b] For details, see Table 3. [c] Estimate. n.a., Not available.

cause of the many variations in the nature and the amounts of these agents in tobaccos from country to country and from year to year (WITTEKINDT 1985). Nevertheless, it is fairly certain that commercial tobacco contains up to a few parts per million of DDT, DDD, and maleic hydrazide; less than 20% of these amounts are transferred into the MS.

The increasing market share of cigarettes with low smoke yields has only been attained because flavor additives made these products "consumer-acceptable". Flavor compounds are usually derived from extracts of tobacco or other plant products but may also be synthetic in nature (LEFFINGWELL et al. 1972). Except for menthol (0–500 μg in the smoke of a cigarette: PERFETTI and GORDIN 1985) the flavor additives are trade secrets; thus, there is little information in the literature about their presence and levels in commercial tobacco products. However, it is known that manufacturers in many countries have discontinued the use of coumarin (a carcinogen in rats; IARC 1976).

C. The Changing Cigarette

Epidemiological studies have documented a dose-response relationship between the number of cigarettes smoked and the development of cancer of the lung, oral cavity, larynx, esophagus, bladder, and kidney (US SURGEON GENERAL 1982; IARC 1986). Bioassays with whole smoke and with tar have also demonstrated a dose-response relationship (WYNDER and HOFFMANN 1967; DONTENWILL 1974; BERNFELD et al. 1974). Thus, a reduction of tar and nicotine was considered as one step towards the reduction of cancer risk for those smokers who were not willing to give up smoking (US SURGEON GENERAL 1981). In addition to tar and nicotine, several toxic and tumorigenic agents such as carbon monoxide, volatile *N*-nitrosamines, and carcinogenic PAH were also significantly reduced (HOFFMANN et al. 1980, 1984; US SURGEON GENERAL 1981). Although smokers of low yield cigarettes tend to compensate for reduced intake of nicotine (HERNING et al. 1981; HALEY et al. 1985), they do not, in general, compensate fully for low smoke yields. Studies on smokers indicate that prolonged use of low-yield cigarettes reduces the risk for cancer to some extent. However, the reduction in risk is only minor compared with giving up cigarette smoking altogether (US SURGEON GENERAL 1982; IARC 1986).

Figure 2 shows the reduction in sales-weighted tar and nicotine delivery of the average American cigarette. Arrows pinpoint the introduction of technical changes during various years which had a profound influence on the sales-weighted average nicotine and tar deliveries (NORMAN 1982). Since 1981 the tar delivery has varied between 14.0 and 12.7 mg, and the nicotine values have remained stable at 0.9 mg per cigarette. These data indicate that the reduction in nicotine has not occurred to the same extent as the reduction in tar. This trend is even more pronounced for cigarettes in the United Kingdom (Fig. 3; JARVIS and RUSSELL 1985). Since nicotine is the habituating agent in tobacco products, it is of major concern that further reduction of its smoke yield has not been implemented.

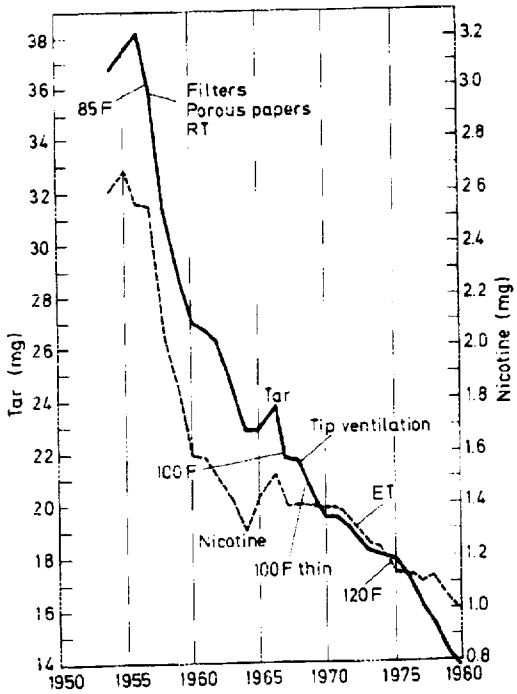

Fig. 2. Sales-weighted average tar and nicotine yields of American cigarettes

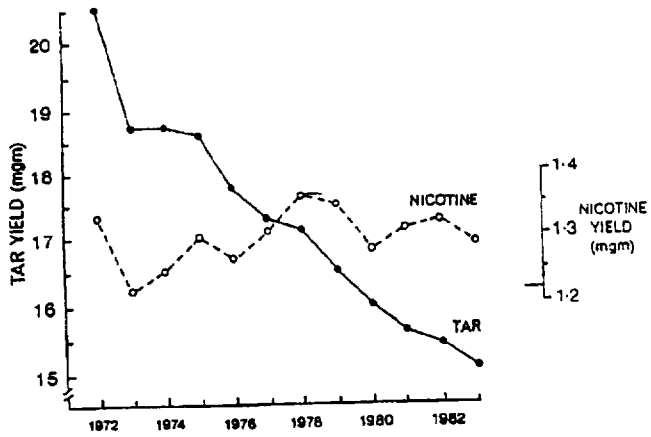

Fig. 3. Sales-weighted tar and nicotine yield of British cigarettes, 1972–1983 (JARVIS and RUSSELL 1985)

Some modifications in the make-up of commercial cigarettes have also led to a selective reduction of certain toxic and tumorigenic agents. Cellulose acetate filters, the most common cigarette filter tips, can selectively reduce phenols and volatile *N*-nitrosamines; perforated filter tips effect a reduction of smoke yields by air dilution and, in addition, a selective reduction of carbon monoxide and hydrogen cyanide. Charcoal filter tips are capable of selectively reducing volatile aldehydes and hydrogen cyanide. The utilization of reconstituted tobacco, expanded tobacco, and tobacco ribs in the manufacture of cigarettes has led to a selective reduction of carcinogenic PAH in the smoke. When tar from such cigarettes is evaluated for tumorigenicity on mouse skin, one observes a reduction of its biological activity by comparison with the same dose of other cigarette tars (WYNDER and HOFFMANN 1967; BERNFELD et al. 1974; US SURGEON GENERAL 1981; HALEY et al. 1985). However, the incorporation of ribs and stems into the cigarette blend and the utilization of more burley varieties as cigarette fillers have caused an increase in the nitrate content of the American blended cigarette from $\simeq$0.5% to 1.2%–1.5%. While this development has led to a reduction of the smoke yields of tar, phenols, and carcinogenic PAH, it has, on the other hand, increased nitrogen oxides (NO_x) and carcinogenic nitrosamines in the smoke (WYNDER and HOFFMANN 1967; US SURGEON GENERAL 1981, 1982; HOFFMANN et al. 1980, 1984). The biological activity of *N*-nitrosamines is not reflected in bioassays on mouse skin and requires evaluation by other assays in mice, rats, or hamsters.

It needs to be stressed that the modified cigarettes have somewhat reduced toxicity and tumorigenic activity in bioassays; however, these reductions are in no way equal to the reduction of cancer risk which can be achieved by cessation of smoking.

D. Carcinogenic Compounds in Tobacco and Tobacco Smoke

The recent IARC monographs on *Tobacco Smoking* and *Tobacco Habits Other Than Smoking* have presented comprehensive reviews of the carcinogenic and toxic components of tobacco and tobacco smoke (IARC 1985a, 1986). Table 3, condensed from these monographs, summarizes the known carcinogens in tobacco and tobacco smoke and gives the ranges of their concentrations along with the evaluations of their carcinogenic activities where available. Structures of representative carcinogens are shown in Fig. 4. The diversity of carcinogenic compounds in tobacco and tobacco smoke may cause ambiguity as to which among them are most important. In the following section we will discuss the likely role of the various types of carcinogens in cancer induction by tobacco and tobacco smoke (see Table 4).

I. Polynuclear Aromatic Hydrocarbons (PAH)

Inhalation studies with laboratory animals have demonstrated that the particulate matter of tobacco smoke induces malignant tumors of the respiratory tract, most notably in the larynx of the Syrian golden hamster (DONTENWILL 1974;

Table 3. Tumorigenic agents in tobacco and tobacco smoke

Compounds	In processed tobacco (per g)	In mainstream smoke (per cigarette)	IARC evaluation of evidence of carcinogenicity[a]	
			In laboratory animals	In humans
PAH				
Benz[*a*]anthracene		20 – 70 ng	Sufficient	
Benzo[*b*]fluoranthene		4 – 22 ng	Sufficient	
Benzo[*j*]fluoranthene		6 – 21 ng	Sufficient	
Benzo[*k*]fluoranthene		6 – 12 ng	Sufficient	
Benzo[*a*]pyrene	0.1 – 90 ng	20 – 40 ng	Sufficient	Probable
Chrysene		40 – 60 ng	Sufficient	
Dibenz[*a,h*]anthracene		4 ng	Sufficient	
Dibenzo[*a,i*]pyrene		1.7 – 3.2 ng	Sufficient	
Dibenzo[*a,l*]pyrene		present	Sufficient	
Indeno[1,2,3-*cd*]pyrene		4 – 20 ng	Sufficient	
5-Methylchrysene		0.6 ng	Sufficient	
Aza-arenes				
Quinoline		1 – 2 µg		
Dibenz[*a,h*]acridine		0.1 ng	Sufficient	
Dibenz[*a,j*]acridine		3 – 10 ng	Sufficient	
7*H*-Dibenzo[*c,g*]-carbazole		0.7 ng	Sufficient	
N-Nitrosamines				
N-Nitrosodimethylamine	ND– 215 ng	0.1 – 180 ng	Sufficient	
N-Nitrosoethylmethyl-amine		3 – 13 ng	Sufficient	
N-Nitrosodiethylamine		ND – 25 ng	Sufficient	
N-Nitrosopyrrolidine	ND– 360 ng	1.5 – 110 ng	Sufficient	
N-Nitrosodiethanolamine	ND–6900 ng	ND – 36 ng	Sufficient	
N'-Nitrosonornicotine	0.3 – 89 µg	0.12– 3.7 µg	Sufficient	
4-(Methylnitrosoamino)-1-(3-pyridyl)-1-butanone	0.2 – 7 µg	0.08– 0.77 µg	Sufficient	
N'-Nitrosoanabasine	0.01– 1.9 µg	0.14– 4.6 µg	Limited	
N-Nitrosomorpholine	ND– 690 ng		Sufficient	
Aromatic amines				
2-Toluidine		30 – 200 ng	Sufficient	Inadequate
2-Naphthylamine		1 – 22 ng	Sufficient	Sufficient
4-Aminobiphenyl		2 – 5 ng	Sufficient	Sufficient
Aldehydes				
Formaldehyde	1.6 – 7.4 µg	70 – 100 µg[b]	Sufficient	
Acetaldehyde	1.4 – 7.4 µg	18 –1400 µg[b]	Sufficient	
Crotonaldehyde	0.2 – 2.4 µg	10 – 20 µg		
Miscellanous organic compounds				
Benzene		12 – 48 µg	Sufficient	Sufficient
Acrylonitrile		3.2 – 15 µg	Sufficient	Limited
1,1-Dimethylhydrazine	60 – 147 µg		Sufficient	
2-Nitropropane		0.73– 1.21 µg	Sufficient	
Ethylcarbamate	310 – 375 ng	20 – 38 ng	Sufficient	
Vinyl chloride		1 – 16 ng	Sufficient	Sufficient

Table 3 (continued)

Compounds	In processed tobacco (per g)	In mainstream smoke (per cigarette)	IARC evaluation of evidence of carcinogenicity[a]	
			In laboratory animals	In humans
Inorganic compounds				
Hydrazine	14 – 51 ng	24 – 43 ng	Sufficient	Inadequate
Arsenic	500 – 900 ng	40 – 120 ng	Inadequate	Sufficient
Nickel	2000 –6000 ng	0 – 600 ng	Sufficient	Limited
Chromium	1000 –2000 ng	4 – 70 ng	Sufficient	Sufficient
Cadmium	1300 –1600 ng	41 – 62 ng	Sufficient	Limited
Lead	8 – 10 µg	35 – 85 ng	Sufficient	Inadequate
Polonium-210	0.2 – 1.2 pCi	0.03– 1.0 pCi		

[a] No designation indicates that an evaluation by IARC has not been carried out.
[b] The 4th report of the independent scientific committee on smoking and health (1988) published values for the 14 leading British cigarettes in 1986 (51.4% of the market) of 20–105 µg/cigarette (mean 59 µg) for formaldehyde and 550–1150 µg/cigarette (mean 910 µg) for acetaldehyde.
PAH, polynuclear aromatic hydrocarbons, ND, not detected.

Table 4. Likely causative agents for tobacco-related cancers

Organ(s)	Initiator or carcinogen	Enhancing agents
	PAH	Catechol (cocarcinogen), weakly acidic tumor promoters
Lung, larynx	NNK Polonium-210 (minor factor), acetaldehyde, formaldehyde	Acrolein, crotonaldehyde(?)
Esophagus	NNN	
Pancreas	NNK	
Bladder	4-Aminobiphenyl 2-Naphthylamine	
Oral cavity (smoking)	PAH NNK, NNN	Ethanol
Oral cavity (snuff dipping)	NNK, NNN Polonium-210	Irritation(?) *Herpes simplex*(?)

PAH, polynuclear aromatic hydrocarbons; NNK, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone; NNN, *N*'-nitrosonornicotine.

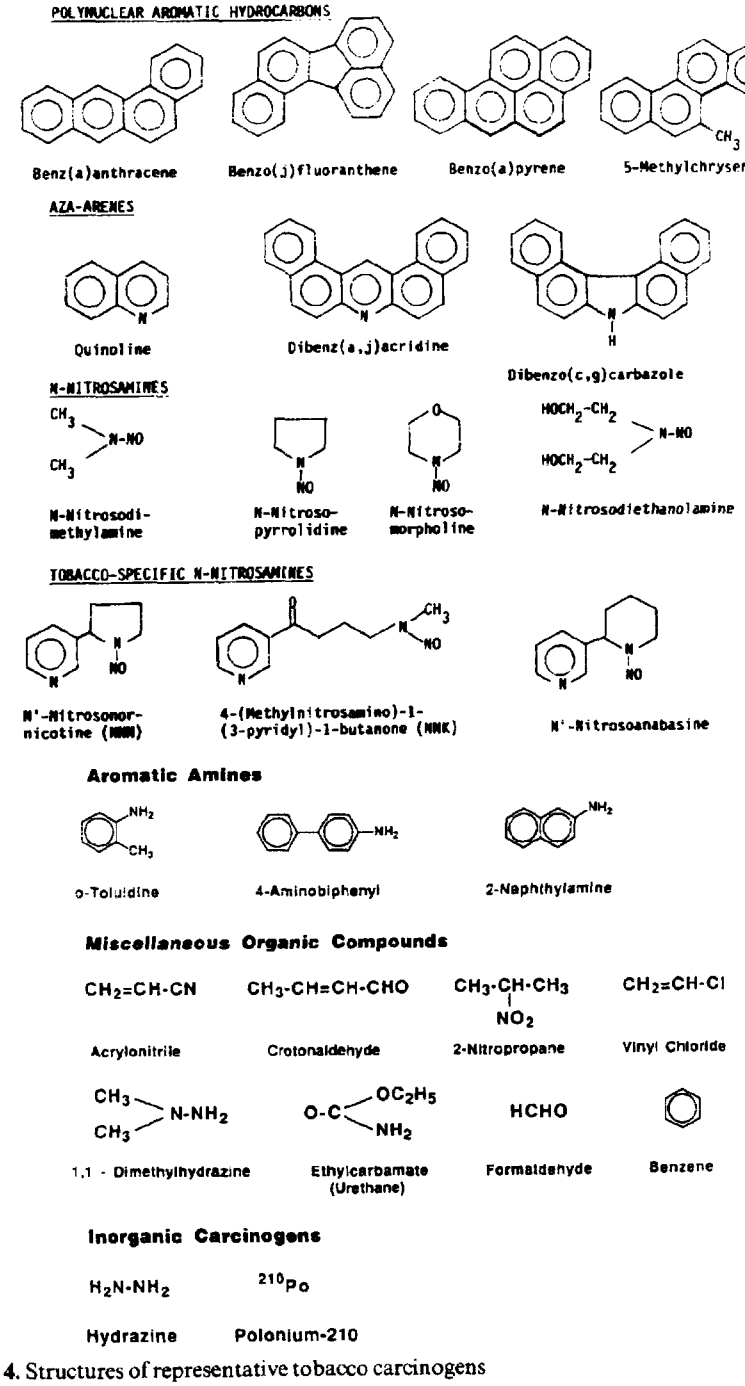

Fig. 4. Structures of representative tobacco carcinogens

BERNFELD et al. 1974; IARC 1986). The particulate matter is more carcinogenic than the gas phase. Fractions and subfractions of the particulate matter have been extensively assayed for tumorigenic activity on mouse skin. It has been clearly demonstrated that the most tumorigenic fractions in these assays are those with highly concentrated PAH (HOFFMANN and WYNDER 1971; HOFFMANN et al. 1978). However, PAH by themselves do not account for the tumorigenic activity on mouse skin induced by the total particulate matter. Most of the skin tumor activity can again be demonstrated when the PAH-enriched fractions are tested together with the weakly acidic fraction in either an initiation-promotion protocol or a cocarcinogenesis protocol (HOFFMANN and WYNDER 1971; HOFFMANN et al. 1978). This approach has indicated that PAH, together with weakly acidic tumor promoters and carcinogens such as catechol, are crucial factors in mouse skin tumorigenesis induced by the particulate matter of tobacco smoke. However, it must be emphasized that mouse skin is particularly responsive to PAH tumorigenesis. It is not equally responsive to other important classes of carcinogens such as *N*-nitrosamines or aromatic amines.

PAH are recognized as contact carcinogens, and in this respect it is likely that the neoplasms obtained in mouse skin would also be observed in other tissues which are in direct contact with tobacco smoke. It is well established that PAH induce tumors in the respiratory tract of hamsters upon intratracheal instillation (SAFFIOTTI et al. 1985). PAH also induce lung tumors upon implantation in the lung with beeswax as a carrier. This protocol has also been successfully employed with tobacco smoke condensates (STANTON et al. 1972; DEUTSCH-WENZEL et al. 1983). The neutral subfraction of cigarette smoke condensate in which the PAH are concentrated is the only fraction which upon repeated intratracheal instillation induces squamous tumors in the lung of rats (DAVIS et al. 1975).

The tumorigenicity of inhaled BaP has also been established in Syrian golden hamsters (THYSSEN et al. 1981). Intratracheal instillation in Syrian golden hamsters of 7,12-dimethylbenz[*a*]anthracene – a highly tumorigenic, synthetic PAH which does not occur in tobacco smoke – followed by tobacco smoke exposure leads to a high incidence of respiratory tract tumors consistent with an initiation : promotion model (KOBAYASHI et al. 1974). These findings, taken together with the results of the bioassays on mouse skin, provide strong evidence for the role of PAH as tumor initiators in tobacco-related respiratory carcinogenesis.

The levels of exposure to PAH as experienced by smokers are not inconsistent with their potential role as causative agents for respiratory tract cancer. BaP, as a representative PAH, typically induces a high incidence of tumors on mouse skin after topical application of 5 μg three times weekly for 60 weeks (HECHT et al. 1976). This corresponds to a total dose of approximately 36 mg/kg body weight. Tumors of the respiratory tract are induced upon intratracheal instillation of a single dose of 5 mg BaP on Fe_2O_3, corresponding to approximately 50 mg/kg body weight (0.2 mmol/kg), or upon chronic administration of a total dose of 7.5 mg BaP on Fe_2O_3 (SAFFIOTTI et al. 1972). A smoker who smokes 40 cigarettes per day for 40 years would be exposed to approximately 12 mg of BaP, or about 0.16 mg/kg (0.61 μmol/kg). Applying these calculations on the basis of mg/kg body weight may be too conservative for a locally acting carcinogen such as BaP.

The calculated doses delivered locally to the target tissue areas may be comparable in the animal models and human systems. These exposure estimates and the determinations of the tumorigenic potential of PAH in bioassays strongly suggest that PAH play a significant role in the induction of respiratory tract cancer in smokers.

II. *N*-Nitrosamines

Among the various carcinogenic *N*-nitrosamines that have been detected in tobacco and tobacco smoke, *N'*-nitrosonornicotine (NNN) and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) are consistently the most prevalent (IARC 1985a, 1986). NNK induces tumors of the lung, nasal cavity, pancreas, and liver in F344 rats, as well as tumors of the lung, trachea, and nasal cavity in Syrian golden hamsters, and lung tumors in mice. NNN gives tumors of the esophagus and nasal cavity in rats, tumors of the trachea and nasal cavity in Syrian golden hamsters, and lung tumors in mice (HOFFMANN and HECHT 1985; HECHT and HOFFMANN 1988; RIVENSON et al. 1988). The carcinogenic potency of NNK is particularly notable. In hamsters, a single dose of 1 mg (5 μmol) induced respiratory tract tumors in 6 of 20 animals (HECHT et al. 1983a). A comparative study of NNK and *N*-nitrosodimethylamine demonstrated that NNK is more tumorigenic, especially in the lung and nasal cavity (HECHT 1986a).

The organospecificity of NNK for the lung is consistent with its role in tobacco smoke-induced respiratory carcinogenesis. The lung is the main target organ for NNK administered either p.o. or s.c. to rats and hamsters (HECHT and HOFFMANN 1988; RIVENSON et al. 1988). Lung tumors have also been induced in mice after topical applications of high doses of NNK (LAVOIE et al. 1987a). It has not been tested by inhalation. In contrast to NNK, NNN seldom induces lung tumors. However, it is the most prevalent nitrosamine in tobacco smoke known to induce tumors of the esophagus (HECHT and HOFFMANN 1988).

Human exposure to NNK in tobacco smoke is consistent with its potential role as a causative agent for lung cancer. In the MS of an American nonfilter cigarette, purchased in 1986, NNK amounted to 425 ng (ADAMS et al. 1987). On the basis of 40 cigarettes per day, cumulative exposure to NNK in 40 years of smoking would be about 250 mg, or approximately 3 mg/kg (0.015 mmol/kg). A single dose of 0.05 mmol/kg of NNK induces a significant incidence of respiratory tract tumors in Syrian golden hamsters. These calculations, which ignore the probable endogenous formation of NNK (HOFFMANN et al. 1984), point to a significant risk for the smoker and strongly support the role of NNK as an important etiologic factor in lung cancer.

NNK and NNN are likely causative agents for oral cancer induced by snuff. Their levels in snuff tobaccos are typically in the range of 1–100 μg/g and are thus generally 1000 times above those of BaP. The only other carcinogens known to be present in snuff are ^{210}Po, formaldehyde, acetaldehyde, and crotonaldehyde (HOFFMANN et al. 1987). A mixture of NNK and NNN applied to the oral mucosa of rats (total dose, 1.6 mmol/kg) induced tumors at or near the site of application in 8 of 30 animals (HECHT et al. 1986b). Snuff dippers who use the most popular products presently marketed in the USA would be exposed to 67 μg/g

tobacco of NNK and NNN (HOFFMANN et al. 1987). A user of 10 g of snuff per day would be exposed to 670 μg or 3.5 μmol. In 40 years of snuff dipping, total estimated exposure would be approximately 9.8 g or 130 mg/kg (about 0.7 mmol/kg). Disregarding the possible endogenous formation of tobacco-specific *N*-nitrosamines (TSNA), this dose fairly approximates the dose used in the animal bioassay, indicating that NNK and NNN are important etiologic factors for oral cancer induction by snuff.

III. Aromatic Amines

Among the aromatic amines identified in cigarette smoke, 4-aminobiphenyl and 2-naphthylamine are the most carcinogenic compounds and are recognized as human bladder carcinogens (IARC 1972, 1974a). Because their concentration in cigarette MS is relatively low, there is uncertainty about their role in human bladder cancer induced by smoking, although DOLL has postulated that they may be involved in the etiology of bladder cancer among cigarette smokers (DOLL 1971). Recent data on the levels of 4-aminobiphenyl-hemoglobin adducts in smokers support DOLL's concept. Levels of 4-aminobiphenyl-hemoglobin adducts correlated with relative risk for bladder cancer among groups of Italians who were either nonsmokers or smoked cigarettes made from bright tobacco or black burley tobacco. Use of black tobacco cigarettes was associated with the highest risk and the highest levels of adducts (BRYANT et al. 1988).

Of the known carcinogenic pyrolysis products of the amino acids, so far only 2-amino-3-methylimidazo(4,5-*f*)quinoline has been detected in trace amounts of 0.26 ng in the smoke of a Japanese filter cigarette (YAMASHITA et al. 1986).

Among the compounds identified in tobacco smoke, only the aromatic amines are associated with bladder cancer in experimental animals and humans. None of the *N*-nitrosamines which are consistently found in tobacco smoke have been shown to be bladder carcinogens in laboratory animals.

IV. Aldehydes

Studies of the chronic inhalation of formaldehyde (14 ppm) and acetaldehyde (1000–3000 ppm) have conclusively demonstrated that these compounds cause significant incidences of nasal cavity tumors in rats (IARC 1982a). Since rats are obligatory nose breathers, these results indicate that the aldehydes are contact carcinogens, which might be expected to affect the lung in humans. In these bioassays the actual exposure of the rat nasal tissues to formaldehyde and acetaldehyde is not known, and it is therefore difficult to compare the dose received to that of a smoker. However, due to the high levels of formaldehyde and acetaldehyde in cigarette smoke, a role in respiratory tract carcinogenesis may be surmised. In 40 years of smoking at a rate of 40 cigarettes/day, exposure to formaldehyde (100 μg/cigarette) and acetaldehyde (1000 μg/cigarette) would amount to about 58 g (26 mmol/kg) and 580 g (177 mmol/kg), respectively. These doses are 1000–10000-fold higher than those of the PAH and *N*-nitrosamines.

A total dose of approximately 17 mmol/kg of crotonaldehyde administered to F344 rats in the drinking water induces liver tumors (CHUNG et al. 1986). Although it is a relatively weak carcinogen, it occurs in cigarette MS in amounts up to 10 μg/cigarette and, consequently, could play a role in tobacco carcinogenesis. High levels of acrolein are also found in cigarette MS. While it has not been shown to be carcinogenic, its ciliatoxic effects are likely to play an indirect role in tobacco smoke-related respiratory carcinogenesis (IARC 1974b).

V. Miscellaneous Organic Compounds

Significant amounts of benzene are found in cigarette MS (up to 50 μg/cigarette). Sufficient evidence exists that this aromatic hydrocarbon causes leukemia in humans (IARC 1974b). On the basis of analytical data obtained for exhaled breath, it has been calculated that a smoker inhales about 2 mg of benzene per day while a nonsmoker inhales only 0.2 mg per day (WALLACE et al. 1987). Former epidemiological studies have not demonstrated a strong association of smoking and leukemia (IARC 1982b). However, a recent prospective study among 248,000 U.S. veterans indicates that cigarette smokers have a significant increase in mortality from leukemia (KINLEN and ROGOT 1988).

On the basis of levels of ethylene oxide-hemoglobin adducts found in smokers, it has recently been estimated that up to 15% of lung cancer caused by smoking could be due to endogenous formation of ethylene oxide from ethylene (TÖRNQVIST et al. 1986). However, there is no evidence that ethylene causes tumors in animals (IARC 1979a). Cigarette smoke contains also traces of ethylene oxide (0.02 μg/cigarette; BINDER and LINDNER 1972), a known animal carcinogen and a probable human carcinogen (IARC 1985b).

Aza-arenes such as dibenz[*a,j*]acridine, dibenz[*a,h*]acridine, and 7*H*-dibenzo[*c,g*]carbazole are recognized as strong carcinogens (IARC 1986), but their levels in cigarette smoke are low. Quinoline, a liver carcinogen in rats (SHINOHARA et al. 1977) and in newborn mice (LA VOIE et al. 1987b), is present in cigarette smoke at a concentration of 1–2 μg/cigarette (DONG et al. 1978). Sufficient evidence exists for the carcinogenicity of acrylonitrile in animals (IARC 1979b). It induces primarily tumors of the CNS in rats. Although it is present in cigarette MS, its potential role in tobacco carcinogenesis is difficult to evaluate due to the lack of data.

Hydrazine causes tumors of the lung and liver upon oral administration to mice and rats and gives nasal tumors in rats upon inhalation (IARC 1974c). Hydrazine in cigarette smoke may originate partly from maleic hydrazide (LIU et al. 1974). Data on hydrazine levels in cigarettes marketed in 1987 are not available.

2-Nitropropane ($\simeq$1.0 μg/cigarette; HOFFMANN and RATHKAMP 1968) induces hepatocellular tumors in rats upon inhalation or oral exposure (IARC 1982c). Its organospecificity for liver suggests that it does not play a major role in tobacco carcinogenesis.

Ethyl carbamate (urethane) is carcinogenic to a variety of tissues including the respiratory tract of mice, rats, and hamsters (IARC 1974d). Its levels in

cigarette smoke are similar to those of hydrazine (0.03 μg/cigarette; SCHMELTZ et al. 1978). Its potential role in tobacco carcinogenesis is difficult to evaluate.

Vinyl chloride, a human carcinogen, causes angiosarcoma of the liver and produces a variety of tumors in rats, mice, and hamsters (IARC 1979c). Its low levels in cigarette MS do not support a major role in tobacco carcinogenesis.

VI. Inorganic Carcinogens

Carcinogenic metals occur in both unburned tobacco and in tobacco MS (WYNDER and HOFFMANN 1967; NORMAN 1977; JENKINS et al. 1985). Evidence for carcinogenicity in humans or experimental animals exists for arsenic, nickel, chromium, cadmium, and lead.

Levels of arsenic in tobacco have decreased since 1952, when its use as a pesticide was discontinued. Arsenic levels in tobacco are between 0.5–0.9 ppm. Some 7%–18% of this amount is found in MS (US SURGEON GENERAL 1982). Arsenic is known to cause skin and lung cancer in humans, but data in laboratory animals are limited (IARC 1980a). However, a simple intratracheal instillation into rats of an arsenical mixture induces bronchiogenic carcinoma (IVANKOVIC et al. 1979).

Levels of nickel in cigarette tobacco range from 2.0–6.2 μg/cigarette. From 10% to 20% of the nickel is transferred into MS (NATIONAL RESEARCH COUNCIL 1975). It has been suggested that part of the nickel in cigarette smoke may exist as nickel carbonyl, but this was not proven experimentally (ALEXANDER et al. 1983). A variety of nickel compounds are carcinogenic in experimental animals, giving local as well as systemic tumors. Nickel subsulfide produces lung cancer in rats upon inhalation. Epidemiologic data have demonstrated that workers in nickel refineries have an excess incidence of cancer of the lung and nasal cavity (IARC 1973). Taken together, these data suggest a possible role for nickel in tobacco carcinogenesis.

Chromium is present in ppm quantities in tobacco and from 4–70 ng/cigarette in MS (IARC 1986). Increased incidences of lung cancer have been observed in workers in the chromate-producing industry. Calcium chromate is carcinogenic to rats after administration by several routes, including intrabronchial instillation. Several other chromium compounds produce local tumors (IARC 1980b).

Levels of cadmium have been determined to be 1.3–1.6 μg/g in tobacco and 41–62 ng/cigarette in tobacco smoke (PERINELLI and CARUGNO 1978). Cadmium chloride, oxide, sulfate, and sulfide cause local tumors in rats upon s.c. injection. Long-term exposure of rats to aerosols of cadmium chloride (12.5, 25, and 50 μg/m^3) produces a dose-dependent incidence of primary lung carcinomas (adenocarcinoma and squamous cell carcinoma; TAKENAKA et al. 1983). Evidence for carcinogenicity in humans is limited (IARC 1980c).

Levels of lead in tobacco range from 8 to 10 μg/g tobacco and 34 to 85 ng/cigarette in cigarette MS. Lead acetate and subacetate produce a variety of tumors in rats and mice, but evidence for human carcinogenicity of lead is considered inadequate (IARC 1980c). The possible roles of chromium, cadmium, and lead in tobacco carcinogenesis are difficult to evaluate given the present data base.

Taken together, the evidence for a major role of these materials as etiologic factors in tobacco carcinogenesis is not compelling.

Polonium-210 exists in unburned tobacco (0.2–1.2 pCi/g) and cigarette MS (0.03–1.0 pCi/cigarette; IARC 1985a, 1986). This α-particle-emitting element is strongly carcinogenic, producing tumors of the lung upon inhalation in rats and upon intratracheal instillation in Syrian golden hamsters (US SURGEON GENERAL 1982). The quantities of polonium-210 found in the lungs of smokers are generally about three times higher than those in nonsmokers. However, the significance of polonium-210 in tobacco-induced lung cancer has been questioned upon comparison of these data with those obtained in miners (HARLEY et al. 1980). In 1987, the US NATIONAL COUNCIL ON RADIATION PROTECTION AND MEASUREMENT ascribed about 1% of the risk of lung cancer after 50 years of cigarette smoking to the role of polonium-210 inhaled from the smoke (NATIONAL COUNCIL ON RADIATION PROTECTION AND MEASUREMENT 1987). The role of polonium-210 (0.2–1.2 pCi/g snuff) as a potential etiologic factor in oral cancer induction by snuff dipping requires further evaluation (HOFFMANN et al. 1987).

E. Smokeless Tobacco

Smokeless tobacco is being used in various forms throughout the world. Its composition varies depending on the regional availability of tobaccos and on local customs. In North America and in Western Europe smokeless tobaccos can be purchased as plug tobacco, loose leaf, twist tobacco, and snuff. Between 1978 and 1985, sales in the USA of plug and twist tobacco, pipe tobacco, cigars, and cigarettes decreased significantly while loose leaf tobacco sales increased by 12.6% to 32500 tons and snuff sales by 35% to 22000 tons per year (MAXWELL 1981, 1986). Of all tobacco products sold in Sweden since 1983, only snuff sales have increased (1987, 4695 tons; TOBACCO JOURNAL INTERNATIONAL 1988).

Tobacco chewing entails placing a "chaw" of loose leaf or a "quid" of plug tobacco in the gingival buccal area, where the material is held. Moderate chewers hold the tobacco up to 200 min per day; heavy chewers are known to hold each "chaw" or "quid" for longer times and to use up to eight "portions" per day.

The steep rise in snuff consumption in the USA is directly correlated with the increasing popularity of snuff dipping, especially among male adolescents and young men. In the USA, snuff dipping is practised by at least 10 million people and in Sweden by 17% of all men (IARC 1985a; US SURGEON GENERAL 1986b). Snuff dipping is the practice of placing a pinch of moist or dry snuff or a "tea bag" (sachet) containing snuff into the gingival fold. Young snuff dippers hold the tobacco 100–250 min per day and consume on the average 10 g of snuff per day (US SURGEON GENERAL 1986b; PALLADINO et al. 1986).

The natives of Iran and of the Soviet Central Asian Republics practise oral use of nass which is usually composed of local tobaccos, ash, cotton oil or sesame oil, and lime. Most nass users consume about 10–15 portions of the tobacco mixture each day (IARC 1985a; ZARIDZE et al. 1985).

Chewing of betel quid with or without tobacco is practised throughout Asia, especially in India, Pakistan, Sri Lanka, Indonesia, and Singapore. Worldwide, at least 200 million people are estimated to be betel quid chewers. Although betel quid is chewed in several different ways in various countries, its composition is relatively consistent. The mixture usually contains pieces of areca nut, catechu, and lime as major ingredients and often also tobacco. The components are wrapped in the betel leaf, often together with spices and/or flavoring agents. The betel leaf is folded over its contents, and it is placed into the mouth and chewed. Generally, the quid is chewed after meals; however, habitual users chew 15–20 quids per day (ARJUNGI 1976; IARC 1985a).

I. Epidemiology

A number of case control studies have shown the proportion of tobacco chewers among patients with cancer of the oral cavity, pharynx, and larynx to be 2–3 times higher than among controls. Although some of these studies did not separate tobacco chewers from snuff dippers, and confounding by smoking and/or alcohol consumption was not always excluded, the epidemiological data as a whole incriminate chewing of tobacco as a significant risk factor for cancer of the upper digestive tract including cancer of the esophagus (IARC 1985a; US SURGEON GENERAL 1986b; CONNOLLY et al. 1986).

Four case-control studies have implicated snuff dipping in the etiology of cancer of the oral cavity and, to a lesser extent, cancer of the pharynx. In a study conducted by WINN et al. in North Carolina and published in 1981, the relative risk of oral and pharyngeal cancer for white women who were snuff dippers was four times that of women who did not use tobacco in any form. In addition, they observed a strong dose-response relationship (IARC 1985a). The US SURGEON GENERAL and the IARC concluded their reviews on the association of snuff dipping and oral cancer by stating that oral use of snuff of the types commonly used in North America and Western Europe is carcinogenic to humans (IARC 1985a; US SURGEON GENERAL 1986b).

There is only limited epidemiologic evidence that chewing of nass alone is associated with an increased risk of cancer of the oral cavity or esophagus (IARC 1985a; ZARIDZE et al. 1985).

A large number of case-control studies, primarily from India, have clearly demonstrated that chewing of tobacco-containing betel quid is causally associated with cancer of the oral cavity, pharynx, and esophagus. The evidence is supported by two dose-response studies and a prospective study from India (HIRAYAMA 1966; GUPTA et al. 1982; IARC 1985a; US SURGEON GENERAL 1986b). Evidence for a role of tobacco-free betel quids in the etiology of human cancer is less clear since most studies have not separated the habits of chewing these quids from smoking and from chewing quids with tobacco (GUPTA et al. 1982; US SURGEON GENERAL 1986b).

II. Bioassays

Gavage feeding of an alcohol extract of tobacco induces tumors of the lung and of the liver in mice (IARC 1985a). Swabbing of the oral cavity of mice, rats, and

hamsters with extracts of chewing tobacco did not lead to a significant number of tumors of the oral cavity in any of these animals (IARC 1985a; US SURGEON GENERAL 1986b).

Most bioassays with snuff and with snuff extracts have failed to elicit significant tumor response in laboratory animals (IARC 1985a; US SURGEON GENERAL 1986b; HECHT et al. 1986b). Insertion of snuff into a surgically created lip canal of rats has induced small, but statistically insignificant, numbers of tumors in the lip canal and oral cavity (HIRSCH and JOHANNSON 1983; HECHT et al. 1986b). However, application of snuff extract to the cheek pouches of hamsters, repeatedly infected with *herpes simplex*, results in a high percentage of invasive squamous cell carcinoma in the animals (PARK et al. 1986).

Treatment of the oral cavity of hamsters with tobacco-containing betel quid, tobacco-free betel quid, areca nut with tobacco, as well as extracts thereof has led to significant numbers of benign and malignant tumors of the cheek pouch and/or the forestomach (RANADIVE et al. 1976).

III. Carcinogens

The composition of processed, unadulterated tobacco has been discussed in Sect. B. In addition, various flavor additives are found in chewing tobacco, snuff, and betel quid preparations (NAIR et al. 1986a; LAVOIE et al. 1989).

It is of special significance that the preparation of smokeless tobacco products, which entails curing, fermenting, and aging, leads to the formation of TSNA from nicotine and other tobacco alkaloids such as nornicotine, anatabine, and anabasine (Fig. 5). So far, six TSNA have been identified in smokeless tobacco. These are the carcinogens NNN, NNK, *N*'-nitrosoanabasine (NAB)

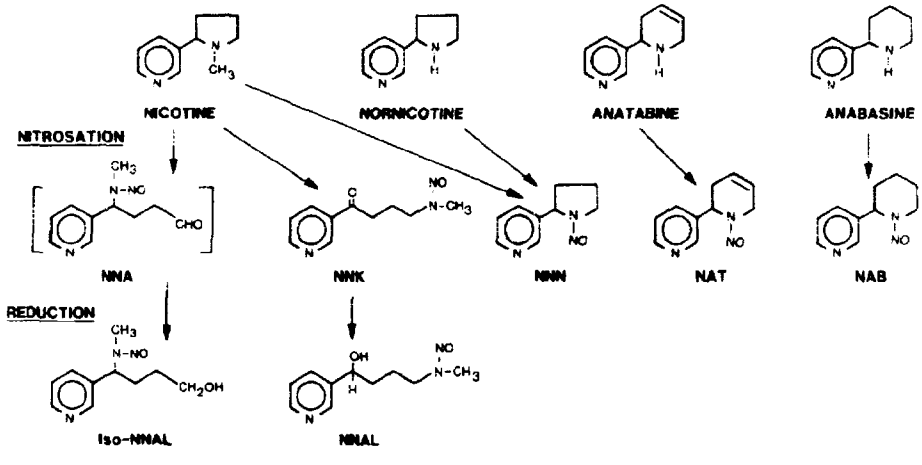

Fig. 5. Formation of tobacco-specific *N*-nitrosamines

Table 5. Tobacco-specific *N*-nitrosamines in smokeless tobacco (ppb)[a]

Product	NNN	NNK	NAT	NAB
USA				
Loose leaf	670– 8200(6)	380(1)	2300(1)	140(1)
Plug tobacco	3400– 4300(3)			
Snuff – moist	3120–135000(26)	100–13600(25)	1340–339000(20)	10– 6700(16)
Snuff – dry	9000– 52000(3)	1800–13000(3)	18000– 38000(3)	600–60000(3)
Sweden				
Snuff	2260–154000(18)	870– 2950(18)	1840–21400(18)	130–150(3)
Plug tobacco	2090(1)	240(1)	1580(1)	100(1)
Canada				
Snuff	50400– 79000(2)	3200– 5800(2)	152000–170000(2)	4000– 4800(2)
Denmark				
Snuff	4460– 8000(3)	1350– 7030(3)	2680– 6170(3)	
Germany				
Plug tobacco	1420– 2130(2)	30– 40(2)	330– 500(2)	30– 50(2)
Snuff	6080– 6700(2)	1500– 1540(2)	3920– 4370(2)	
United Kingdom				
Snuff – moist	11800(1)	1820(1)	3020(1)	86(1)
USSR				
Nass	120– 520(4)	20– 130(4)	32– 300(4)	8– 30(4)
India				
Chewing tobacco	470– 2400(5)	130– 230(4)	300– 450(4)	30– 70(4)
Belgium				
Chewing tobacco	7380(1)	130(1)	970(1)	

[a] Number in parentheses, number of samples analyzed.
NNN, *N*'-nitrosonornicotine; NNK, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone; NAT, *N*'-nitrosoanatabine; NAB, *N*'-nitrosoanabasine.

and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL), the noncarcinogenic *N*'-nitrosoanatabine (NAT), and 4-(methylnitrosamino)-4-(3-pyridyl)-1-butanol, (iso-NNAL), which is now being bioassayed. Swabbing of the oral cavity of rats with a solution containing a mixture of low doses of NNN and NNK induces a significant number of tumors of the mouth and lung, indicating that these TSNA are active contact carcinogens as well as organ-specific systemic carcinogens (HECHT et al. 1986 b).

Table 5 lists the currently available quantitative analytical data on TSNA in various smokeless tobacco products (BRUNNEMANN et al. 1986; HOFFMANN et al. 1987, 1988). The concentrations of TSNA in smokeless tobacco exceed by far the permissible limits of 5 ppb set by US governmental agencies for individual *N*-nitrosamines in beer and bacon (US FDA 1980; USDA 1983). Several studies have shown that the tobacco chewer and snuff dipper do indeed extract TSNA from the tobacco (HOFFMANN and ADAMS 1981; PALLADINO et al. 1986; NAIR et al. 1986 b). It is also very likely that during chewing and snuff dipping additional amounts of TSNA are formed endogenously from the tobacco alkaloids and nitrosating agents (HOFFMANN and HECHT 1985; NAIR et al. 1986 b).

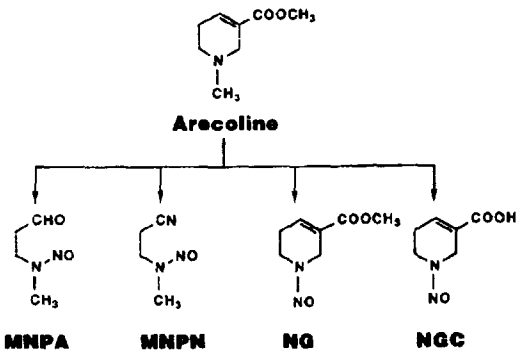

Fig. 6. Formation of *N*-nitrosamines from arecoline

Other carcinogens also identified in smokeless tobacco are volatile *N*-nitrosamines (*N*-nitrosodimethylamine up to 4 ppb in chewing tobacco and up to 215 ppb in snuff), *N*-nitrosomorpholine (up to 40 ppb in snuff), *N*-nitrosodiethanolamine (up to 680 ppb in chewing tobacco and up to 6800 ppb in snuff), formaldehyde (up to 7.4 ppm in snuff), acetaldehyde (up to 7.4 ppm in snuff), crotonaldehyde (up to 2.4 ppm in snuff), benzo[*a*]pyrene (up to 80 ppb in chewing tobacco and 90 ppb in snuff), as well as traces of the radioelement polonium-210 (up to 0.6 pCi/g in snuff) (IARC 1985a; NAIR et al. 1986b; HOFFMANN et al. 1987; LAVOIE et al. 1989).

Chewing of betel quid with tobacco leads not only to the formation of TSNA but also to the formation of arecoline-derived nitrosamines, namely *N*-nitrosoguvacine (NGC) and *N*-nitrosoguvacoline (NG), and the highly carcinogenic 3-(methylnitrosamino)propionitrile (MNPN) (Fig. 6; BRUNNEMANN et al. 1986; NAIR et al. 1986b; PROKOPCZYK et al. 1987).

The chemical and biochemical assays described in the foregoing strongly support the human data which incriminate smokeless tobacco as a human carcinogen. This applies especially to oral snuff and to betel quid with tobacco.

A major obstacle to an appropriate bioassay of chewing tobacco and snuff is the difficulty of repeating the administration of fresh snuff several times each day, assuring its retention in the oral cavity over several hours, and conducting such an assay over a period of up to 2 years. At this time, the surgically created lip canal, or a modification thereof, in the rat which allows for longer retention of the snuff product, appears to be the most approximate simulation of the human habit. A lifetime assay with this model is expected to lead to a significant tumor incidence in the oral cavity of rats.

F. Environmental Tobacco Smoke

Epidemiological studies have incriminated environmental smoke exposure as a risk factor for lung cancer in nonsmokers (IARC 1986; US NATIONAL RESEARCH COUNCIL 1986; US SURGEON GENERAL 1986a). In fact, it has been estimated that nonsmokers living with smoking spouses have an about 30% higher risk for lung cancer than nonsmokers with to nonsmoking spouses (US NATIONAL RESEARCH COUNCIL 1986). However, this conclusion has been challenged by several investigators (IARC 1986; US NATIONAL RESEARCH COUNCIL 1986; LEE 1987). In 1985 IARC considered the available epidemiologic data as inconclusive (IARC 1986). Nevertheless, a biological basis for an association between environmental smoke exposure and lung cancer clearly exists. Compounds resulting from the combustion of tobacco, which are known carcinogens, are inhaled as pollutants of ambient air and are retained by the nonsmoker. IARC concluded that because of its physicochemical nature and in view of known concepts of tobacco carcinogenesis, "passive smoking gives rise to some risk of cancer" (IARC 1986). This judgement emphasizes that epidemiologic methods may not be sufficiently sensitive for establishing a risk factor for cancer from environmental smoke exposure. Therefore, highly sensitive chemical and biochemical methods are needed to assay this exposure and the uptake of tumorigenic agents by nonsmokers.

The smoke generated during smouldering of tobacco products between puff drawing is SS. When it is obtained under standardized laboratory conditions, undiluted SS contains far higher concentrations of toxic and tumorigenic agents than MS, which is drawn puff by puff. Table 6 presents data for those agents in SS that are known to be carcinogens, tumor promoters, or cocarcinogens. The release of volatile *N*-nitrosamines and aromatic amines into SS is remarkably high (IARC 1985a; US NATIONAL RESEARCH COUNCIL 1986; US SURGEON GENERAL 1986a; HOFFMANN and WYNDER 1986; GUERIN 1987). Whereas filter tips, especially perforated filter tips, can significantly reduce the concentration of toxic and tumorigenic agents in MS (see Sect. C), they have no reducing effect on the agents released in SS (ADAMS et al. 1987). Thus, it does not matter whether the source for environmental tobacco smoke is the SS of a nonfilter cigarette, or the SS of a cigarette with a highly active filter tip. SS appears to be slightly more genotoxic in the Ames test than MS (LEWTAS et al. 1987), and on a gram to gram basis the particulate matter of SS is more carcinogenic on mouse skin than the particulate matter of MS (WYNDER and HOFFMANN 1967).

SS represents the major source for environmental tobacco smoke; the smoke diffusing through the cigarette paper, that escaping from the burning cone during active smoking, and that portion of MS which is exhaled are other contributors. Table 7 presents some data for toxic agents in indoor environments (IARC 1986; US NATIONAL RESEARCH COUNCIL 1986; US SURGEON GENERAL 1986a). The concentration of toxic agents in environmental tobacco smoke appears low by comparison with their levels in undiluted MS, but one needs to take into consideration that the active inhalation of tobacco smoke is limited to the time it takes to smoke each cigarette, while the involuntary inhalation of environmental tobacco smoke can occur over several hours each day. This is reflected in

Table 6. Some toxic and tumorigenic agents in undiluted cigarette sidestream smoke

Compound	Type of toxicity	Amount in sidestream smoke per cigarette	Ratio of sidestream: mainstream smoke
Vapor phase			
Carbon monoxide	T	26.8 – 61 mg	2-5 – 14.9
Carbonyl sulfide	T	2 – 3 µg	0.03– 0.13
Benzene	C	240 – 490 µg	8 – 10
Formaldehyde	C	1500 µg	50
3-Vinylpyridine	SC	330 – 450 µg	24 – 34
Hydrogen cyanide	T	14 – 110 µg	0.06– 0.4
Hydrazine	C	90 ng	3
Nitrogen oxides (NO_x)	T	500 –2000 µg	3.7 – 12.8
N-Nitrosodimethylamine	C	200 –1040 ng	20 –130
N-Nitrosopyrrolidine	C	30 – 390 ng	6 –120
Particulate phase			
Tar	C	14 – 30 mg	1.1 – 15.7
Nicotine	T	2.1 – 46 mg	1.3 – 21
Phenol	TP	70 – 250 µg	1.3 – 3.0
Catechol	CoC	58 – 290 µg	0.67– 12.8
o-Toluidine	C	3 µg	18.7
2-Naphthylamine	C	70 ng	39
4-Aminobiphenyl	C	140 ng	31
Benz[*a*]anthracene	C	40 – 200 ng	2 – 4
Benzo[*a*]pyrene	C	40 – 70 ng	2.5 – 20
Quinoline	C	15 – 20 µg	8 – 11
NNN	C	0.15– 1.7 µg	0.5 – 5.0
NNK	C	0.2 – 1.4 µg	1.0 – 22
N-Nitrosodiethanolamine	C	43 ng	1.2
Cadmium	C	0.72 µg	7.2
Nickel	C	0.2 – 2.5 µg	13 – 30
Polonium-210	C	0.5 – 1.6 pCi	1.06– 3.7

C, carcinogenic; CoC, cocarcinogenic; SC, suspected carcinogen; T, toxic; TP, tumor promoter.

comparative measurements of the uptake of nicotine by active and passive smokers. In blood serum and in the urine of passive smokers, the concentration of cotinine, a major metabolite of nicotine, amounts to about 1% of its concentration in the physiologic fluids of an active cigarette smoker (GREENBERG et al. 1984; US NATIONAL RESEARCH COUNCIL 1986; US SURGEON GENERAL 1986a; RUSSELL 1987; SEPKOVIC et al. 1988). Other indicators for the uptake of environmental tobacco smoke constituents such as carboxyhemoglobin, thiocyanate, and 4-aminobiphenyl adducts are not significantly elevated in physiologic fluids of exposed individuals, primarily because levels of these pollutants are low and can be derived from sources other than tobacco combustion (US NATIONAL RESEARCH COUNCIL 1986; US SURGEON GENERAL 1986a). There is a great need for other biological markers as indicators of the uptake of carcinogenic agents from environmental tobacco smoke by nonsmokers. The determination of ad-

Table 7. Some toxic and tumorigenic agents in indoor environments polluted by tobacco smoke[a]

Pollutant	Location	Concentration/m^3
Nitric oxide	Workrooms	50 – 440 µg
	Restaurants	17 – 270 µg
	Bar	80 – 520 µg
	Cafeteria	2.5– 48 µg
Nitrogen dioxide	Workrooms	68 – 410 µg
	Restaurants	40 – 190 µg
	Bar	2 – 116 µg
	Cafeteria	67 – 200 µg
Hydrogen cyanide	Living room	8 – 122 µg
Benzene	Public places	20 – 317 µg
Formaldehyde	Living room	23 – 50 µg
Acrolein	Public places	30 – 120 µg
Acetone	Public places	360 –5800 µg
Phenols (volatile)	Coffee houses	7.4– 11.5 ng
N-Nitrosodimethylamine	Restaurants, public places	0 – 240 ng
N-Nitrosodiethylamine	Restaurants, public places	0 – 200 ng
Nicotine	Public places	1 – 6 µg
	Restaurants	3 – 10 µg
	Workrooms	1 – 13.8 µg
Benzo[*a*]pyrene	Restaurants, public places	3.3– 23.4 ng

[a] References: KLUS and KUHN (1982); IARC (1986): US NATIONAL RESEARCH COUNCIL (1986); KLUS et al. (1987).

ducts of NNN and NNK with globin appears to be one promising approach to this problem of dosimetry (CARMELLA and HECHT 1987).

G. Recent Studies on Mechanisms of Tobacco Carcinogenesis and Their Application to Dosimetry

By understanding the mechanisms involved in cancer causation by tobacco, tobacco smoke, its subfractions, and constituents, one can develop a rational hypothesis applicable to cancer prevention not only in tobacco users but also in the general population as a whole. The epidemiology of tobacco use provides leads for mechanistic studies relevant to carcinogenesis in several tissues. The mechanistic studies, in turn, provide insights for preventive approaches.

One important area that emerges from an understanding of metabolic activation and detoxification of tobacco smoke constituents is human dosimetry. Although measurements of carboxyhemoglobin, thiocyanate, nicotine and cotinine are objective indicators of an individual's uptake of tobacco smoke, and measurements of parameters such as urinary mutagenicity and sister chromatid exchanges in peripheral blood lymphocytes provide an indication of biological response to tobacco smoke, these measures do not, in themselves, delineate the individual's response to these specific environmental carcinogens (IARC 1986). That information is best obtained by assessing levels of macromolecular adducts

with carcinogens or metabolites of carcinogens. Development of such assays is based on examining the mechanisms of metabolic activation and detoxification of tobacco smoke carcinogens. We will briefly summarize some recent studies on the mechanisms of tobacco carcinogenesis, with emphasis on those components that, according to our present knowledge, appear to play major roles in cancer induction – PAH, tobacco-specific *N*-nitrosamines, and aromatic amines – and we will note the applications of these studies to human dosimetry where appropriate.

I. Polynuclear Aromatic Hydrocarbons

The mechanisms by which PAH interact with DNA, activate oncogenes, and initiate the carcinogenic process are described in detail in other chapters in this book. These studies have shown that diol epoxides with one carbon terminus of the epoxide ring in the bay region (bay region diol epoxides) such as (+)7a,8β-dihydroxy-9β,10β-epoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene [(+)-*anti*-BPDE] are major ultimate carcinogens of several of the carcinogenic PAH which occur in tobacco smoke. Similar mechanisms of activation are seen with carcinogenic methylated PAH in tobacco smoke, such as 5-methylchrysene, with the additional requirement that highly tumorigenic bay region diol epoxides have a methyl group and epoxide ring in the same bay region (HECHT et al. 1986c, 1987). These studies are important with respect to tobacco carcinogenesis because they provide a rationale for the high tumor-initiating activity on mouse skin of the PAH-enriched subfractions of tobacco smoke condensate (HOFFMANN and WYNDER 1971). However, an apparent contradiction exists in ascribing the mouse skin tumorigenicity of PAH such as benzo[*a*]pyrene (BaP) to the formation of *anti*-BPDE as an ultimate carcinogen. Assays of *anti*-BPDE on mouse skin consistently show that it is less tumorigenic than BaP. To investigate factors responsible for this apparent contradiction, the disposition, metabolism, and DNA binding in mouse epidermis of *anti*-BPDE and BaP were compared (MELIKIAN et al. 1987). The results indicate that there are remarkable differences in the penetration of *anti*-BPDE and BaP through the epidermis. Whereas BaP removal from the epidermis is slow, 60%–65% of *anti*-BPDE disappears within 3 min of application, and a second, slower phase of removal is observed between 8 min and 2 h after application. During this second phase, *anti*-BPDE is apparently protected from hydrolysis and from reaction with DNA, such that formation of adducts is more efficient from BaP than from *anti*-BPDE. Thus, the disposition and reactivity of *anti*-BPDE that is topically applied to mouse skin differs from that observed with the diol epoxide that is generated intracellularly from topically applied BaP. This difference may account for the relatively low tumorigenicity of *anti*-BPDE on mouse skin.

An important aspect of the role of PAH in tobacco carcinogenesis is their tumor-initiating or cocarcinogenic effect in co-application with the weakly acidic fraction of tobacco smoke condensate (HOFFMANN et al. 1978). Extensive fractionation studies have clearly shown that the acidic fraction has both promoting and cocarcinogenic activities and that the majority of the tumorigenic activity of tobacco smoke condensate, as measured on mouse skin, can be ascribed to the

combined effects of PAH, weakly acidic cocarcinogens, and tumor promoters (HOFFMANN et al. 1978). The cocarcinogenic activity of tobacco smoke condensate on mouse skin can largely be attributed to catechol (VAN DUUREN and GOLDSCHMIDT 1976; HECHT et al. 1981). Recent investigations have focused on the mechanism underlying this observation (MELIKIAN et al. 1986). According to these studies catechol has several major effects on BaP metabolism in mouse epidermis. Important among these is that the ratio of *anti-* to *syn*-BPDE-DNA adducts in mouse epidermis increases in the presence of catechol. Thus, the major effect of catechol as a cocarcinogen is exerted during the terminal activation of (−)BaP-7,8-diol to (+)*anti*-BPDE.

Although subfractions of the weakly acidic fraction of tobacco smoke condensate are known to contain tumor promoters, and a number of components of these subfractions have been identified, none has shown significant activity (HECHT et al. 1975). The characterization of the tumor promoters in tobacco smoke is a continuing challenge. Studies of the effects of cigarette smoke condensate subfractions on normal human bronchial epithelial cells have led to the conclusion that the methanol-extracted neutral fraction is likely to contain compounds with promoting activities similar to those of the phorbol esters, indole alkaloids, and certain polyacetates, since this fraction affects growth, morphology, epidermal growth factor binding, and plasminogen activator activity, and causes single-strand breaks similar to those induced by such promoters (WILLEY et al. 1987).

Antibodies developed against the major BPDE-DNA adduct have been used to assess its presence in surgical specimens of lung tissue, in human placenta, and in peripheral blood lymphocytes (PERERA et al. 1982; HARRIS et al. 1985; EVERSON et al. 1986). Although evidence has been obtained for the presence of such adducts in samples from smokers, significant differences between smokers and nonsmokers have not been observed. These analyses are complicated by the fact that the antibodies crossreact with DNA adducts formed from PAH other than BaP, and by uncertainties in quantitation introduced by the method of raising the antibodies (see Chap. 13).

II. Tobacco-Specific *N*-Nitrosamines

Pathways of NNK and NNN metabolism have been investigated in vivo in laboratory animals and in vitro in subcellular fractions, cultured cells, and cultured tissues from animals and humans (HECHT et al. 1983b; IARC 1985a). These studies have identified a variety of metabolic transformations, among which α-hydroxylation appears to be the most important, leading to the formation of DNA adducts and protein adducts. Figure 7 summarizes these pathways for NNK and NNN. The fact that formation of 7-methylguanine, O^6-methylguanine, and O^4-methylthymidine occurs in the lung, liver, and nasal mucosa of F344 rats treated with NNK but not in the esophagus, spleen, kidney, and brain has been noted in several studies (CASTONGUAY et al. 1985; HECHT et al. 1986a; BELINSKY et al. 1986). It is remarkable and perhaps significant that DNA methylation has been consistently detected only in target tissues of NNK-treated rats. The organospecificity of NNK for the lung of rats, mice, and hamsters has

been noted. Studies of O^6-methylguanine levels in lung DNA during chronic dosing with NNK have shown that this promutagenic base accumulates and persists, in part due to inhibition by high doses of NNK of the repair enzyme, O^6-methylguanine-DNA methyl-transferase (BELINSKY et al. 1986, 1987). It is also significant that the repair enzyme is inhibited by acrolein and other aldehydes which occur in high concentrations in cigarette smoke (KROKAN et al. 1985). The efficiency of O^6-methylguanine formation was particularly high in Clara cells. These studies with NNK, which create a mechanistic link between nicotine exposure and the formation of promutagenic DNA adducts, suggest that smokers should have methylated DNA. This has recently been examined using a monoclonal antibody against O^6-methyldeoxyguanosine. The adduct was detected in 6 of 20 samples of human placental DNA, but no relationship to smoking was observed (FOILES et al. 1988).

A second pathway of NNK α-hydroxylation has been observed, yielding the intermediate 4-(3-pyridyl)-4-oxobutyl diazohydroxide (compound 7 of Fig. 7). This intermediate is also formed by 2′-hydroxylation of NNN. The formation of the keto alcohol (compound 9) upon neutral thermal or acid hydrolysis of DNA from rats treated with NNK or NNN has been demonstrated and is consistent with 4-(3-pyridyl)-4-oxobutylation of DNA by compound 7 (HECHT et al. 1988). Although the biological significance of such adducts is presently unknown, they would appear to have potential for dosimetry studies because of their unique structural relationship to nicotine. Thus, it is significant that 4-(3-pyridyl)-4-oxobutylation of hemoglobin is observed in rats treated with NNK or NNN, as indicated by the release of compound 9 upon base or acid hydrolysis (CARMELLA and HECHT 1987). The development of sensitive analytical methods for compound 9, as released from globin or DNA, is likely to provide an approach to human dosimetry of TSNA.

III. Aromatic Amines

4-Aminobiphenyl and 2-naphthylamine are the most likely cigarette smoke components to be involved in bladder cancer induction in smokers, according to presently available data. The mechanisms by which these compounds are metabolically activated and produce DNA adducts in the bladder epithelium have been extensively studied and are discussed elsewhere (BELAND and KADLUBAR, this volume). These studies have shown that the corresponding hydroxylamines are key intermediates in DNA and protein modification. The hydroxylamines also react with hemoglobin to form, in the case of 4-aminobiphenyl, a sulfonic acid amide of β-cysteine (GREEN et al. 1984; NEUMANN 1984; BRYANT et al. 1987). This adduct readily releases 4-aminobiphenyl upon treatment with dilute acid. A method was developed to analyze the released 4-aminobiphenyl by gas chromatography with detection by negative ion chemical ionization mass spectrometry (BRYANT et al. 1987). Application of this method to smokers shows that adduct levels are higher than in nonsmokers and decrease after quitting. This method should be useful in further assessing the role of aromatic amines in bladder cancer induction by tobacco smoke.

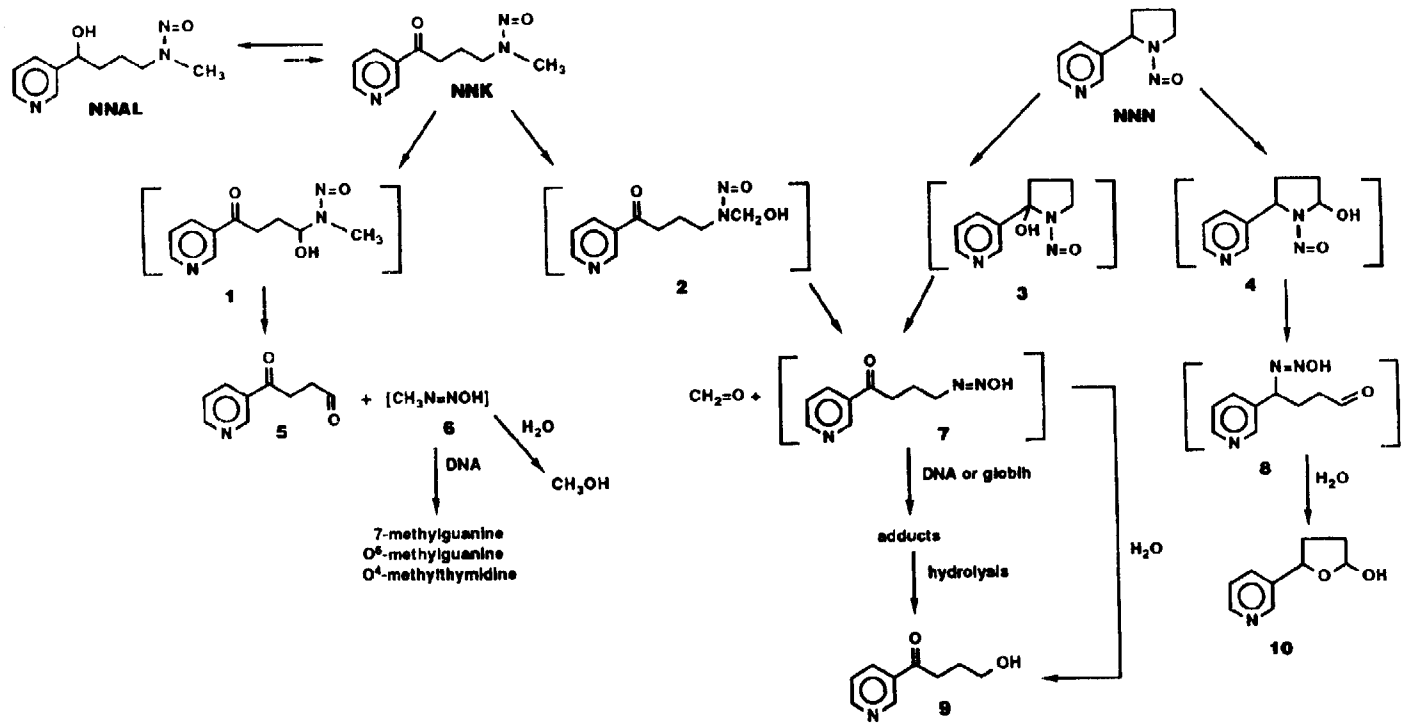

Fig. 7. Metabolic activation of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (*NNK*) and *N'*-nitrosonornicotine (*NNN*) to hypothetical intermediates (shown in *brackets*) which bind to DNA and protein. Structures of aldehydes and alcohols resulting from decomposition or reaction with H_2O of the intermediates are also indicated. Further metabolic transformation of these compounds as well as other metabolic pathways of NNK and NNN are summarized in references: IARC (1985, 1986); CARMELLA and HECHT (1987). *NNAL*, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol

Recent studies have also shown that single ring aromatic amines, including the weak bladder carcinogen *o*-toluidine, are present in human urine (EL-BAYOUMY et al. 1986). The available data do not indicate that there are significant differences between smokers and nonsmokers.

IV. DNA Damage Induced by Unknown Constituents of Tobacco Smoke

The ^{32}P-postlabeling assay for DNA adducts, developed by RANDERATH and coworkers, is described in detail in the chapter in this volume by D. PHILLIPS. This exceedingly sensitive assay has been applied to DNA isolated from the placentas of smoking and nonsmoking mothers and has shown the presence of tobacco smoke-related DNA adducts (EVERSON et al. 1986). The occurrence of such adducts has also been observed in DNA from the bronchus and larynx of smokers (RANDERATH et al. 1986). DNA from mice treated with cigarette smoke condensate shows the presence of a number of adducts, one of which appears to be chromatographically indistinguishable from the major smoking-related adduct seen in human DNA samples (RANDERATH et al. 1986). Although the source of this adduct is not known, it does not appear to be derived from BaP or several other PAH, nor from tobacco-specific *N*-nitrosamines or aromatic amines. This suggests that there are other agents in tobacco smoke leading to DNA damage.

Cigarette smoke generates active oxygen species such as hydrogen peroxide, superoxide radical, and hydroxyl radical (NAKAYAMA et al. 1984; CHURCH and PRYOR 1985; COSGROVE et al. 1985). These agents can induce single-strand breaks in human cells or cell-free systems (BORISH et al. 1985; NAKAYAMA et al. 1986). Cigarette smoke trapped in phosphate buffered saline induces single-strand breaks ten times more efficiently than the hydrogen peroxide-generated ones, indicating that nonvolatile constituents of smoke can also cause single-strand breaks (KAO-SHAN et al. 1987). The identities of these agents are not known nor is there strong evidence that such intermediates are involved in carcinogenesis.

Most investigations on the prevalence of chromosomal aberrations in peripheral blood lymphocytes of cigarette smokers and nonsmokers have found increased levels in smokers (IARC 1986). Similarly, the overall evidence indicates that frequencies of sister chromatid exchanges are higher in smokers than in nonsmokers (IARC 1986). Compared with nonsmokers, smokers were also found to have a higher level of fragile sites, an increased number of metaphases with extensive breakage, and elevated expression of fragile sites at cancer break-points and oncogene sites in chromosomal DNA of peripheral blood lymphocytes (KAO-SHAN et al. 1987). The components of tobacco smoke which are responsible for such changes are not known.

H. Perspectives

The Centers for Disease Control have calculated that 84400 cancer deaths in men and 35900 cancer deaths in women in the USA in 1984 were directly attributable to tobacco smoking (CENTERS FOR DISEASE CONTROL 1987). In other industrial-

ized countries, especially in Europe, the deaths from smoking-related cancers have reached comparable proportions (IARC 1986). This fact alone places continued emphasis not only on smoking withdrawal clinics and health education but also calls for further research into tobacco carcinogenesis. As we see it, high priorities should be assigned to research programs in the areas discussed in the following paragraphs.

I. Inhalation Bioassays

The high toxicity of whole smoke, primarily due to CO, presents a major obstacle for the successful induction of lung carcinoma and lung adenocarcinoma in rats and hamsters in inhalation assays with tobacco smoke. However, the use of markers of deposition of smoke particulates, such as decachlorobiphenyl (HOFFMANN et al. 1979), should lead to the design of inhalation devices and smoking cycles for long-term studies which will enable a higher degree of deposition of tar in the respiratory tracts of the animals. This in turn should elicit a significant tumor response. Reports from recent inhalation bioassays do, in fact, show progress in methodology (HAYES et al. 1988). Improved methodologies and specially designed experimental cigarettes will assist in evaluating the contribution to the carcinogenicity of whole smoke by such agents as PAH, catechols, tobacco-specific *N*-nitrosamines, and volatile aldehydes.

II. Flavor Additives

The increased market share of low yield cigarettes in many countries has led to the design of cigarette filters which deliver a rich "flavor bouquet". This has been achieved partially by selecting tobacco varieties that are rich in flavor and/or by adding natural or synthetic flavoring agents to the cigarette tobaccos. However, the possible contribution of such flavoring materials to the overall toxicity and tumorigenicity of the smoke of low yield cigarettes is not known at this time. Knowledge of the structure of the flavor compounds and their breakdown products in the smoke and the utilization of new inhalation methodologies should allow estimation of their possible contribution to the overall toxicity and carcinogenicity of low yield cigarettes. Presently available in vitro assays and in vivo bioassays other than inhalation studies can be used as screening methods and could provide some meaningful information.

III. Bioassays with Smokeless Tobacco

As was discussed earlier in this review, there is an urgent need for new bioassay methods which will lead to the induction of oral tumors in laboratory animals with smokeless tobacco products. The evaluation of snuff and betel quid tobacco mixtures is particularly important. Such bioassays should define the contribution of tobacco-specific *N*-nitrosamines to the carcinogenicity of smokeless tobaccos and of *Areca*-derived *N*-nitrosamines to betel quid with tobacco mixtures as well

as the extent of endogenous formation of both types of *N*-nitrosamines during chewing and snuff dipping. The chemical nature of the tumor initiators in smokeless tobacco and the tumorigenic effects of the high amounts of flavoring agents added to these tobacco products also need to be determined.

IV. Nutrition and Tobacco Carcinogenesis

A number of laboratory studies support the concept that certain types of cancer are greatly influenced by macro- and micronutrients (REDDY et al. 1980; HAYASHI et al. 1986; IP et al. 1986). Epidemiological data, supported by laboratory studies, have indicated that nutrition can also play a role in tobacco carcinogenesis (ARMSTRONG and DOLL 1975; WYNDER et al. 1987; BIRT and POUR 1983; BEEMS and VAN BEEK 1984). A systematic follow-up of these leads by further laboratory studies is needed.

V. Tobacco Smoke and Indoor Radon Levels

Several studies are envisioned to answer the question whether there is any synergism between the carcinogenic effects of active smoking and/or involuntary smoking and elevated indoor levels of radon (US NATIONAL RESEARCH COUNCIL 1986). Case control studies on lung cancer should be combined with determination of the background α-radiation in the homes of lung cancer patients and with measurements of tobacco smoke exposure by specific markers of uptake. Inhalation assays with concurrent exposure to radon and tobacco smoke lead to a better understanding of the mechanisms involved in the suspected synergistic effects of radon exposure and active and/or passive smoking.

VI. Biochemistry of Tobacco Carcinogenesis

In Sect. G we discussed some recent developments in the metabolic activation and macromolecular binding of tobacco carcinogens. It is expected that at least some of the chemical lesions formed between metabolites of TSNA or *Areca*-derived carcinogens and DNA will be identified. We also need to determine how DNA alkylation by tobacco carcinogens is affected by smoke constituents such as catechols and other tobacco cocarcinogens and tumor promoters. Do such agents enhance DNA binding and/or inhibit the repair of chemical lesions in DNA?

Currently, highly sensitive methods are being developed for assaying the uptake of carcinogenic agents from tobacco smoke and from environmental tobacco smoke. These types of studies should also lead to an estimate of the endogenous formation of carcinogens in smokers or in environmentally exposed persons. It is known that endogenous nitrosation reactions in cigarette smokers lead to higher urinary excretion of nitrosoproline and nitrosothioproline compared with nonsmokers (HOFFMANN and HARRIS 1986). However, little is known about the possible endogenous formation of TSNA, ethylene oxide, and other carcinogens from specific precursors upon smoke inhalation.

It has been hypothesized that smokers detoxify tobacco carcinogens in a more efficient manner than do nonsmokers (REMMER 1987). In this regard, we need methods which will enable us to determine rates of metabolic activation and DNA binding of tobacco carcinogens in humans as a function of nutritional factors, age, and other characteristics which may modify the susceptibility to tobacco carcinogens. It is hoped that our increased awareness of the nature and biochemical fate of major tobacco carcinogens will provide answers to at least some of the questions concerning the genotoxic effects of tobacco smoke exposure in smokers as well as in nonsmokers with prolonged heavy exposure to environmental tobacco smoke.

Acknowledgements. The inspiration and support given to us by Ernst L. Wynder and the extensive contributions of our colleagues John D. Adams, Shantu Amin, Klaus D. Brunnemann, Steven G. Carmella, Fung-Lung Chung, Karam El-Bayoumy, Peter Foiles, Edmond J. LaVoie, Assieh A. Melikian, Bogdan Prokopczyk, Abraham Rivenson, and Neil Trushin are gratefully acknowledged. We thank Ilse Hoffmann, and Bertha Stadler for editorial assistance. Our studies are supported by grants no. CA-29580, CA-32391 and CA-44377 from the National Cancer Institute.

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