TABLE 4.—Volatile N-nitrosamines in tobacco and tobacco products

Nitrosamine	Tobacco ppb	Chewing tobacco or snuff ppb	Cigarette smoke ng/cigarette
Nitrosodimethylamine	7–190 (*33*)	2–56 (*12a,33*)	4–180 (*33,79,130a*)
Nitrosoethylmethylamine			1–40 (*33,130a*)
Nitrosodiethylamine	0–15 (*33*)	8.6 (*12a*)	0.1–28 (*79,130a*)
Nitrosodi-n-propylamine			0–1 (*130a*)
Nitrosodi-n-butylamine			0–3 (*130a*)
Nitrosopyrrolidine		0.05–2.0 (*12a,30*)	0–110 (*33,130a*)
Nitrosopiperidine			0–9 (*30a*)
Nitrosomorpholine		20–700 (*30*)	(*130a*)

SOURCE: Hoffmann and Adams (77).

induced in 29 of 36 Syrian golden hamsters given only 6 mg of NDEA (*138*). The other identified VNA are strong to moderate organ-specific carcinogens (*97*). Although the hydrophilic VNA are primarily found in the vapor phase of fresh cigarette smoke, they are retained by a Cambridge filter. This glass fiber filter has been chosen arbitrarily to separate the gas phase from the smoke particulates and has been utilized for smoke gas phase inhalation studies. The selective retention of hydrophilic VNA from smoke by cellulose acetate filter tips of cigarettes can also be explained by the fact that moisture and the moist smoke particulate act as retainers. This selective retention can remove more than 80 percent of the VNA from mainstream cigarette smoke (*33, 139*).

Recent evidence has incriminated snuff dipping for an increased risk of cancer of the oral cavity (*77, 200*). Since fine cut tobacco and snuff contain high levels of VNA (Table 4) and other nitrosamines, special efforts should be made to reduce these quantities in tobaccos used for snuff dipping. The high concentration of VNA is a consequence of the high nitrate levels in these tobacco varieties, which range from 2 to 5 percent, and of long fermentation times under anaerobic conditions. N-nitrosomorpholine (NMOR) was also detected in relatively high concentrations (*30*) in several snuff samples. Protein and amino acids serve as major precursors for most VNA in processed tobacco and in smoke, but the origin of the precursor for NMOR remains unknown. NMOR is a relatively potent animal carcinogen (*97*), inducing primary liver tumors in mice and rats and tumors of the larynx, trachea, and lung in Syrian golden hamsters.

Metabolic activation of the simplest member of this group, dimethylnitrosamine (DMN), is presumed to involve α-hydroxylation of one methyl group, followed by loss of formaldehyde, to yield a monomethylnitrosamine. In turn, this unstable intermediate loses

OH and nitrogen to form a methylating moiety that reacts with proteins and nucleic acids. In the latter, the N-7 and 0-6 positions are attacked. Both adducts were detected relatively soon after administration of DMN (*151*). The demethylative enzyme is a cytochrome P-450-dependent microsomal mixed-function oxidase that requires NADPH and 0_2 and can be inhibited by CO or by pretreatment of the animal with $CoCl_2$ which inhibits the synthesis of cytochrome P-450. Since ethanol is often consumed in conjunction with smoking, it is pertinent to note that in rats chronic consumption of ethanol enhanced the metabolism of DMN and the formation of mutagenic substances therefrom (*57, 131*). This observation is of special interest in view of human data showing an increased incidence of cancer of the oral cavity and esophagus in smokers who also drink large amounts of alcohol (*189*).

Diethylnitrosamine, the next higher member of the series, is also metabolized by α-oxidation to acetaldehyde and an ethylating species. In contrast, ω-oxidation of the alkyl chain of longer chain dialkylnitrosamines yielded hydroxy, keto, and carboxylic acid derivatives. Some of these metabolites, for example, N-nitroso-n-butyl-(4-hydroxybutylamine), were more active as bladder carcinogens than the parent N-nitrosodi-n-butylamine (*53*).

Like other acyclic and cyclic carcinogenic nitrosamines, NMOR undergoes metabolic α-hydroxylation to electrophilic diazohydroxide intermediates that may act as ultimate carcinogens (*73, 127*).

N-Nitrosodiethanolamine

Among the agricultural chemicals used for the cultivation of tobacco crops are found several amines, amides, and carbamates. These include dimethyldodecylamine (Penar), maleic hydrazide diethanolamine, and carbaryl (Sevin) as a representative of the ethyl urethanes (Figure 3) (*186, 202*). Small residual amounts of these agents were found on harvested tobacco (*169*). Diethanolamine has been studied as a possible precursor for nitrosodiethanolamine (NDELA), a carcinogen found in tobaccos (0.1 to 6.8 ppm) that were treated with the sucker growth inhibitor maleic hydrazide diethanolamine. The smoke of tobaccos thus treated contained 10 to 40 ng per cigarette of NDELA. Snuff contains especially high levels of 3.2 to 6.8 ppm of NDELA (*31*). This nitrosamine induces carcinoma of the kidney and liver of rats (*97, 123*) and carcinoma of the trachea of hamsters following subcutaneous injection, painting the skin, or swabbing the oral cavity (*83, 97*). NDELA penetrates rat (*122*) and human skin (*54*) and is primarily excreted via the urinary tract (*122, 153*).

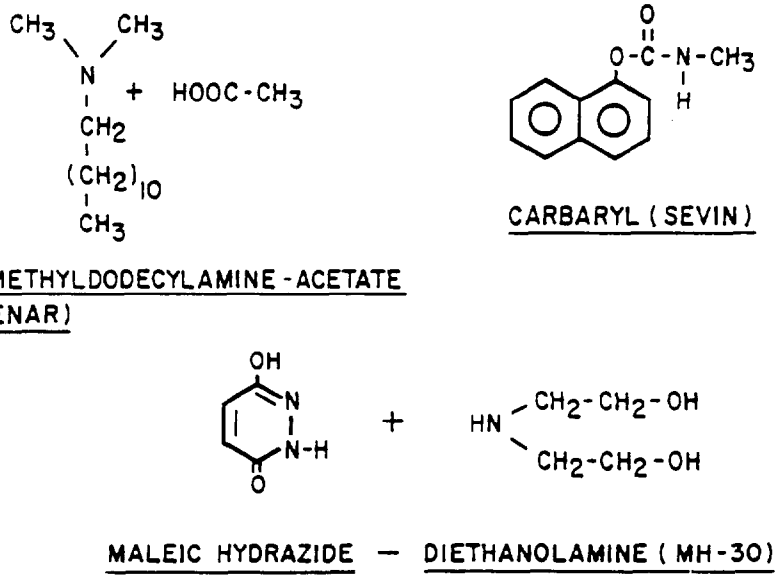

FIGURE 3.—Agricultural chemicals for tobacco cultivation
SOURCE: Tso (*186*), and Wynder and Hoffmann (*202*).

Tobacco-Specific N-Nitrosamines

Commercial tobaccos in the United States contain 0.5 to 2.7 percent alkaloids, 85 to 95 percent of which is nicotine. Important minor alkaloids are nornicotine, anatabine, anabasine, cotinine, and N'-formylnornicotine (Figure 4). Several of these alkaloids are secondary and tertiary amines and, as such, are amenable to N-nitrosation. Tobacco and tobacco smoke were shown to contain N'-nitrosonornicotine (NNN), 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), N'-nitrosoanatabine (NAT), and N'-nitrosoanabasine (NAB). In model experiments, nitrosation of nicotine also yielded 4-(methylnitrosamino)-4-(3-pyridyl)butanal (NNA), which has not as yet been identified in tobacco nor in the smoke (*71, 78*).

In experiments with ^{14}C-labeled nicotine, 0.009 percent of this alkaloid is nitrosated to NNN during the curing of Burley tobacco (*68*). Of the NNN in cigarette smoke, 41 to 46 percent originates from the NNN in tobacco by transfer, and the remainder is pyrosynthesized primarily from nicotine (*80*).

Table 5 presents data for tobacco-specific N-nitrosamines (TSNA) in the tobacco and smoke of cigarettes and cigars (*80*). In addition, it must be noted that cigarette smoke contains traces of NAB (up to 0.015 μg/cig). Recent studies carried out on popular snuff tobaccos from the United States, Denmark, Germany, and Sweden revealed 5.5 to 106 ppm of TSNA in these materials, the highest levels of

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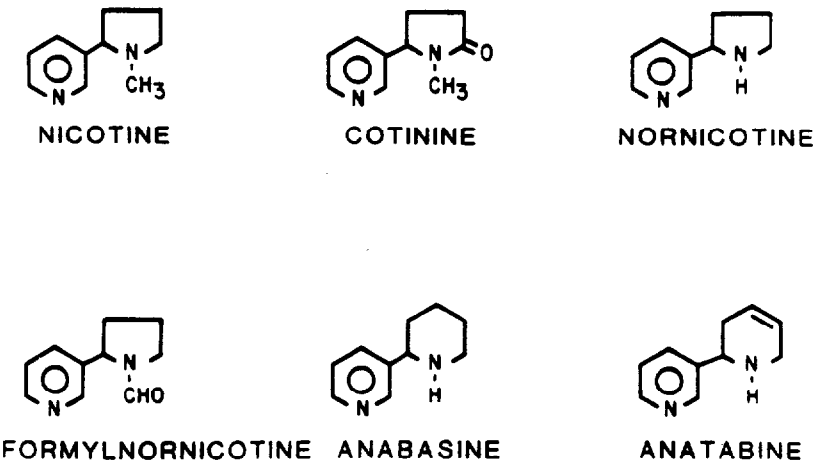


FIGURE 4.—Common tobacco alkaloids in tobacco and tobacco smoke

SOURCE: Hoffmann et al. (*80*).

TABLE 5.—Tobacco specific N-nitrosamines in tobacco products

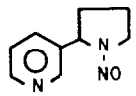
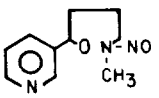
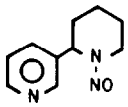
Nitrosamines	Tobacco ppm	Chewing tobacco or snuff ppm	Cigarette smoke μg/cigarette	Cigar smoke μg/cigar
N'-Nitrosonornicotine	0.2 - 45	3.5 - 77	0.2 - 3.7	3.2 - 5.5
NNK[a]	0.1 - 35	0.8 - 4.7	0.12 - 0.44	1.9 - 4.2
N'-Nitrosoanabasine	0.0 - 0.01	0.04 - 1.9	0.0 - 0.15	n.d.[b]
N'-Nitrosoanatabine	0.6 - 13	0.8 - 44	0.15 - 4.6	1.7 - 1.9

[a] NNK = 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone.
[b] n.d. = not determined.
SOURCE: Hoffmann et al. (*78, 79*).

carcinogenic nitrosamines reported in a consumer product that is taken into the body. The saliva of snuff dippers yielded TSNA levels at concentrations of 0.02 to 0.9 ppm (*77*). These observations are of relevance to the epidemiological findings of increased risk for cancer of the oral cavity in snuff dippers (*200*). The importance of the carcinogenic TSNA is underscored in that these compounds can also be formed within the oral cavity during snuff dipping (*68*).

At this time, there is no experimental evidence on the formation of TSNA in the lung upon inhalation of cigarette smoke. However, a smoker of one or two packs of cigarettes daily retains 20 to 60 mg of nicotine, 1 to 4 mg of nornicotine, 1.5 to 6 mg of anatabine, and 0.2 to 0.8 mg of anabasine, and inhales 0.3 to 24 mg of NO_x. Thus, *in vivo* formation of tobacco-specific N-nitrosamines is a real possibility.

TABLE 6.—Carcinogenic activity of tobacco-specific nitrosamines

Compounds	Species	Application	Principal organ affected
 NNN	Mouse	I.P.	Lung (Adenoma, Adenocarcinoma) Salivary glands (?)
	Rat	S.C. P.O. (Water)	Nasal cavity (Carcinoma) Esophagus (Papilloma, Carcinoma) Pharynx (Papilloma) Nasal cavity (Carcinoma)
	Hamster	S.C.	Trachea (Papilloma) Nasal cavity (Carcinoma)
 NNK	Mouse	I.P.	Lung (Adenoma, Adenocarcinoma)
	Rat	S.C.	Nasal cavity (Carcinoma) Liver (Hepatocarcinoma) Lung (Adenoma, Carcinoma)
	Hamster	S.C.	Lung (Adenoma, Adenocarcinoma) Trachea (Papilloma) Nasal cavity (Carcinoma)
 NAB	Rat	P.O. (Water) S.C.	Esophagus (Carcinoma) Esophagus (Papilloma) Pharynx (Papilloma)
	Hamster	S.C.	Inactive (375 mg/hamster)

The data for the carcinogenicity of NNN, NNK, and NAB are summarized in Table 6 (*23, 70, 84*); NAT assay results are not as yet reported. NNK is by far the most potent carcinogen of the TSNA. In the Syrian golden hamster, NNK has about the same carcinogenic potency as N-nitrosomorpholine and about twice the activity of N-nitrosopyrrolidine, but it has only about one-tenth of the activity of N-nitrosodiethylamine, which is the most potent carcinogenic nitrosamine in hamsters.

The influence of alcohol as a dietary component on NNN carcinogenicity was assayed in the Syrian golden hamster at two dose levels. The data did not show an accelerating effect of the alcohol on NNN carcinogenicity in the test animals whose total caloric intake was equal to that of the control animals (*131*). The metabolic pathways of NNN and NNK have been studied in rats and hamsters (*73, 74, 84*). As was seen with other acyclic and cyclic nitrosamines, the metabolic activation of these TSNA involves most likely also *via* α-hydroxylation (*73, 127*). Figures 5 and 6 depict the

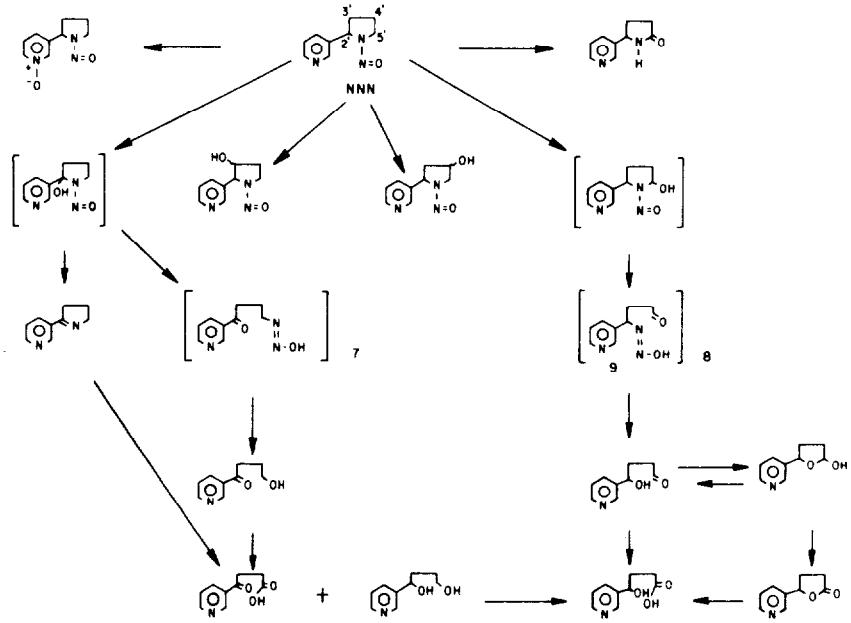


FIGURE 5.—Metabolism of NNN in rats and Syrian golden hamsters

SOURCE: Hecht et al. (*73*).

metabolic pathways of NNN and NNK (*73, 74*). Among the stable metabolites, NNN-N′-oxide and NNK-N′-oxide, as well as the secondary alcohol formed by reduction by NNK (Figure 6, formula 2), are most likely also carcinogens, based on induction of lung adenomas in strain A mice. The electrophilic diazohydroxide intermediates of NNN (Figure 5, formulas 7 and 8) and of NNK (Figure 6, formulas 7 and 9), respectively, or the resulting carbonium ions are probably the ultimate carcinogenic forms of these tobacco-specific nitrosamines. Assays of NNN metabolites obtained by incubation of the carcinogen with human liver microsomes showed that five out of six human liver specimens tested contained the enzymes that effected NNN activation by α-hydroxylation (*69*).

Two autoradiographic studies and one biochemical report on the distribution of [2′-^{14}C]NNN and [1-^{14}C]NNK in mice and hamsters, respectively, have shown that the metabolites of these labeled nitrosamines are bound to macromolecules of the tracheobronchial and nasal mucosa and to kidney, liver, sublingual and submaxillary glands, esophagus, and melanin of the eye (*25, 84, 196*). These data indicate that the binding of metabolites to the tissues of specific organs does not by itself explain the organ-specificity of the TSNA.

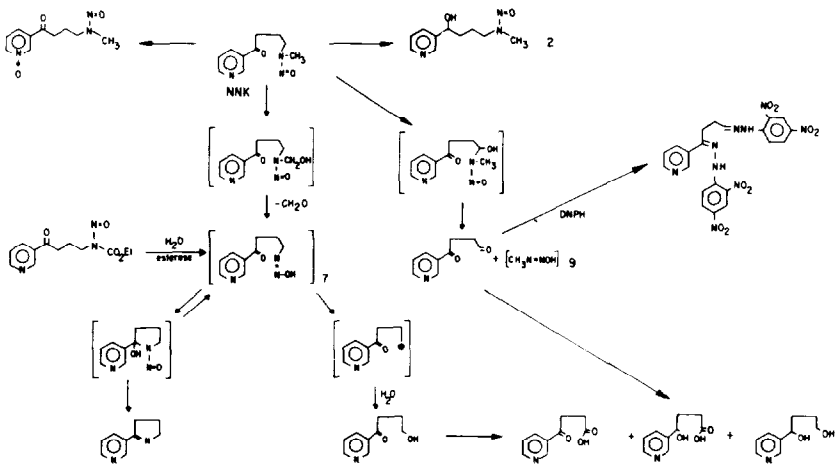


FIGURE 6.—Metabolism of NNK in rats and Syrian golden hamsters

SOURCE: Hecht et al. (*74*).

Other aspects such as the DNA repair of the affected cells must be considered.

Aromatic Amines and Aromatic Nitrohydrocarbons

The incomplete combustion of organic matter yields C,H-radicals, which serve as precursors for benzene, naphthalene, or PAH (*5*). In the burning cone of a cigarette, the aromatic hydrocarbons or their radicals react with nitrogen oxides to form nitrobenzene, nitronaphthalenes, or nitro-PAH (*85, 150*). These can be reduced to aromatic amines in the oxygen deficient zones. Aromatic amines may also be formed directly from proteins and amino acids (*129*). The presence of both aromatic nitrohydrocarbons and aromatic amines and their dependence on the nitrate concentration in the tobacco is thus not surprising (*85, 150*). Tables 7 and 8 list the data available at present on these compounds in cigarette smoke. 4-Nitrocatechol and other nitrophenols are also present in cigarette smoke. The reported values of 200 ng/cigarette of 4-nitrocatechol and also the values for other nitrophenols require verification, since they were obtained without the precautions that prevent artifacts during smoke collection and analysis (*106, 111*).

Epidemiological data from dye workers have documented that certain aromatic amines such as 2-naphthylamine and 4-aminobiphenyl are human bladder carcinogens (*149*). Some *o*-aminotoluenes induce cancer in animals (*39*). On the basis of quantitative data for aromatic amines in cigarette smoke, an etiological significance of these traces of carcinogenic amines in human bladder cancer is

TABLE 7.—Nitroarenes and nitrophenols in cigarette smoke

Nitro compound	μg/cigarette[a]
Nitrobenzene	25.3
2-Nitrotoluene	21.4
3-Nitrotoluene	10.4
4-Nitrotoluene	19.6
2-Nitro-1,4-dimethylbenzene	
4-Nitro-1,2-dimethylbenzene	6.5
4-Nitro-1,3-dimethylbenzene	18.5
4-Nitrocumene	5.3
2-Nitrophenol	35
3-Nitrophenol	+
4-Nitrophenol	20
2-Nitro-3-methylphenol	30
2-Nitro-4-methylphenol	90
4-Nitro-3-methylphenol	+
2-Nitro-5,6-dimethylphenol	+
4-Nitrocatechol	200

[a] + = present
SOURCE: Schmeltz and Hoffmann (*164*).

questionable, even if one were to consider the total of the aromatic amines and their active metabolites, which may be formed *in vivo* from aromatic nitrohydrocarbons of the smoke. However, Doll (*45*) concluded that 2-naphthylamine (together with other aromatic amines) may suffice to explain the increased bladder cancer risk for cigarette smokers working in gasification plants.

Although the importance of traces of aromatic amines in smoke for the increased bladder cancer risk of smokers is disputed, there may be reason for concern about the increasing levels of nitrate in present-day cigarettes (1.2 to 1.5 percent). Twenty years ago, these levels were only about 0.5 percent. The increased potential for formation of aromatic amines and of N-nitrosamines should be studied carefully.

The metabolic detoxification and activation of 2-naphthylamine (2-NA) have been studied intensively (*22, 155*). Many detoxification products have been identified; most are hydroxylated derivatives that can also be excreted as sulfuric acid or glucosiduronic acid conjugates. Premercapturic and mercapturic acids have also been identified. However, the evidence points toward an N-hydroxy derivative of 2-NA as the active carcinogen rather than the parent compound. Furthermore, an N-glucuronide appeared to be the transport form. 2-NA or the N-hydroxy derivative form adducts with guanine in nucleic acids (*103*), and other adducts have also been identified (*105*). By analogy to the situation with 1-hydroxynaphthylamine, the O-6 position of guanine is arylaminated (*104*). The

TABLE 8.—Aromatic amines in cigarette smoke

Aromatic amine	ng/cigarette [a]
Aniline	100 - 1,200
2-Toluidine	32
3-Toluidine	15
4-Toluidine	14
2,3-Dimethylaniline	8
2,4-Dimethylaniline	14
2,5-Dimethylaniline	+
2,6-Dimethylaniline	15
3,4-Dimethylaniline	+
3,5-Dimethylaniline	+
2-Ethylaniline	+
3-Ethylaniline	+
4-Ethylaniline	+
2,4,6-Trimethylaniline	+
2-Methylaniline	+
3-Methylaniline	+
3-Methoxyaniline	+
4-Methoxyaniline	+
Diphenylamine	+
1-Naphthylamine	4.3 - 27
2-Naphthylamine	1.0 - 22
2-Methyl-1-naphthylamine	5.8
2-Aminobiphenyl	1.8
3-Aminobiphenyl	2.7
4-Aminobiphenyl	2.4
2-Aminostilbene	+

[a] + = present
SOURCE: Patrianakos and Hoffmann (*150*) and Schmeltz and Hoffmann (*164*).

biological significance of the different adducts has not been delineated as yet.

Although N-hydroxylation also occurs during metabolism of 2-aminostilbene (*145*), the N-hydroxy group does not participate in formation of nucleic acid adducts. Instead, the ethylenic bond of the stilbene forms adducts at the N-1 and N-6 of adenosine or similar adducts with the nitrogens in other bases (*167, 168*).

A definitive experiment on the metabolism of *o*-toluidine showed that acetylation of the amino group and hydroxylation at the 4-position of the ring were the major pathways during metabolism (*173*). Mainly sulfate and to a lesser extent glucuronide conjugates of the cresols thus formed were also excreted. There was some oxidation of the methyl group to a hydroxymethyl or carboxylic acid. Another minor pathway was oxidation of the amino group, since azoxytoluene and nitrosotoluene were identified. Whether these metabolites were derived from an N-hydroxy-*o*-toluidine was not delineated.

Polonium-210

In 1964, Radford and Hunt (*154*) suggested that bronchogenic carcinoma in cigarette smokers could be induced by the α-particle emitter polonium-210 (^{210}Po). Since then, a number of studies have reported varying quantities of ^{210}Po in the smoke (0.03 to 1.0 pCi per cigarette) (*66, 202*). Harley et al. (*66*) gathered data for ^{210}Po in cigarette tobaccos from many countries and calculated 0.45 pCi of the radioactive element per gram tobacco as a median value. Major sources for ^{210}Po in tobacco are airborne particles, taken up by the glandular hair of the tobacco leaf, as well as lead-210 (^{210}Pb) and ^{210}Po from soil that is fertilized with certain phosphates (*128, 187*). Thirty to fifty percent of ^{210}Po in the cigarette tobacco were reported to be transferred into the mainstream smoke of cigarettes; up to 90 percent of ^{210}Po can be retained by filter tips (*24*).

Upon inhalation, ^{210}Po produces tumors of the lung in rats (*204*). Tests with multiple intratracheal instillations of ^{210}Po in Syrian golden hamsters revealed a dose-response relationship in regard to bronchocarcinoma and adenocarcinoma in the peripheral lung (*108*). Simultaneous multiple instillations of benzo[a]pyrene (total dose 4.5 mg) and ^{210}Po (total dose 50,000 pCi) on the same carrier induced about twice the number of tumors expected from the additive effect of the two carcinogens (*124*).

Lead-210 (^{210}Pb), the grandparent of ^{210}Po, is found in all environmental atmospheres (0.01 pCi ^{210}Pb/m^3 and 0.003 pCi ^{210}Po/m^3). The daily exposure of a cigarette smoker to ^{210}Pb has been estimated to be 2.5 to 3.0 times greater than that of a nonsmoker (*66*). Harley et al. (*66*) reviewed 12 studies that had determined ^{210}Po in the parenchyma of the lungs and in the bronchial tissues of cigarette smokers, ex-smokers, and nonsmokers. The studies showed general agreement that ^{210}Po is stored in the parenchyma of smokers at three times higher levels than in nonsmokers and that it also persists in the bronchial mucosa of smokers in higher concentrations than in nonsmokers.

From comparisons of radon-daughter exposure of underground miners with their relative risk of lung cancer, Harley et al. deduced that ^{210}Po is a questionable risk factor for lung cancer in cigarette smokers. They recommend, nevertheless, that methods for lowering ^{210}Po levels in tobacco should be considered (*66*).

Nickel

A large number of studies from the United States and from other countries have shown that the tobacco of one cigarette contains 2 to 14 μg of nickel (*141, 202*). Analyses have determined that 10 to 20 percent of the nickel in cigarettes is transferred into the mainstream smoke (*141*). In one study, it was found that an average of 84 percent

of the nickel is present in the gas phase (*183*), indicating that cigarette smoke may contain nickel carbonyl.

The possible existence and relative stability of nickel carbonyl in cigarette smoke is indirectly supported by several observations. Sunderman et al. (*181*) found nickel carbonyl in the exhaled air as well as in the blood of man. Stähly (*176*) reported that passing carbon monoxide through an unlit cigarette column removed much of the nickel from the tobacco. Nickel has also been found in pipe tobacco 0.5 to 10 μg/cig), cigars (1.9 to 15 μg/cigar), and in U.S. snuff (2 to 3 μg/g) (*141*).

The presence of nickel in tobacco smoke is an important finding regardless of whether it is in the form of nickel carbonyl or in other forms, because nickel itself and several nickel compounds are carcinogenic in laboratory animals, inducing sarcomas by subcutaneous injection and rhabdomyosarcomas upon intramuscular injection. It appears that nickel subsulfide (Ni_3S_2) is a strongly sarcogenic agent (*96, 141*). Intrarenal injection of a single dose of 5 mg Ni_3S_2 induced a high rate of renal carcinomas in rats (*180*). Exposure of rats for 30 minutes three times weekly for 1 year to an atmosphere containing 30 to 60 μg of nickel carbonyl produced pulmonary carcinoma in two of six animals (*179*).

Workers in nickel refineries in England and Canada were reported to have excessive rates of cancer of the nasal cavity and of the lung. Studies from Japan, the U.S.S.R., and the German Democratic Republic also reported increased incidences of lung cancer among nickel workers. The International Agency for Research on Cancer (*96*) concluded on the basis of epidemiological studies that workers in nickel refineries have an increased risk for cancer of the nasal cavity and of the lung. Although it is not likely that nickel plays a significant role in the etiology of lung cancer in cigarette smokers (*141*), prudence dictates that efforts should be made to reduce the amount of this metal in tobacco and to avoid contamination of tobacco with nickel during cutting and other processes in cigarette manufacture.

Arsenic

Extensive studies have been conducted on paired soil residues in tobacco. From 1932 to 1951, arsenical pesticides were used on tobacco in the United States. During this time, the arsenic content of U.S. cigarettes rose from 12.6 to 42 μg/cigarette (*63*). In 1952, arsenicals were removed from the list of recommended insecticides for control of hornworms on tobacco. Since then, a sharp decrease in the arsenic content of cigarette tobacco has occurred. Guthrie (*62*) concluded in 1968 that arsenic residues in U.S. cigarettes do not exceed 2 ppm and are normally about 1 ppm or less and that tobacco is no greater source of arsenic for consumers than food. The last reported data for

U.S. tobacco range between 0.5 and 0.9 ppm. The arsenic now found in tobacco appears to come primarily from natural sources (*63*). Between 7 and 18 percent of the total arsenic on tobacco leaves is recovered in the mainstream smoke of cigarettes. Studies with ^{74}As-labeled cigarettes have shown that, depending on the individual's smoking pattern, 2.2 to 86 percent of the arsenic in cigarette tobacco is transferred to the respiratory tract. About 50 percent of the inhaled arsenic is eliminated within 10 days, primarily in urine, the remainder is either deposited in tissues, exhaled or otherwise eliminated (*91*).

Skin cancers have been reported to be particularly prevalent among people exposed to arsenicals through drugs, drinking water, or pesticides. The anatomic sites of these tumors suggest that they are causally associated with exposure to arsenic. Lung cancer has been associated with inhalation exposure to arsenicals in copper smelters, workers in pesticide manufacturing plants, Mosel vineyards, and Rhodesian gold mines (*99, 142*). The International Agency for Research on Cancer (*99*) concluded in its review, "There is sufficient evidence that inorganic arsenic compounds are skin and lung carcinogens in humans." The U.S. National Academy of Sciences (*142*) arrived at a similar conclusion, but also mentioned that exposure to arsenicals or other metals and to sulfur dioxide may constitute carcinogenic cofactors for an increased risk for lung cancer of miners and metal workers. The view that inorganic arsenicals cause cancer of the skin and lung has not been widely accepted, since these compounds have not produced cancers in experimental animals (*101, 118, 142, 170*). Ivankovic et al. (*101*) reported in 1979 the induction of lung carcinomas in rats after a single intratracheal instillation of an arsenic-containing pesticide mixture, such as those formerly used by vineyard workers. Of the 15 rats exposed, 7 developed bronchogenic adenocarcinoma and 2 had bronchioalveolar carcinoma following a single instillation of 0.07 mg of arsenic as calcium arsenate.

Cadmium

Several forms of cadmium (Cd) are carcinogenic in experimental animals (*95*). Two studies indicate that occupational exposure to cadmium oxide is associated with an increased risk for prostatic cancer. It has been suggested that a heavy smoker who is exposed by inhalation to 70 to 90 ng Cd per cigarette retains 1.5 μg of Cd per day and may accumulate up to 0.5 mg (*95*).

In Table 9 is summarized the present knowledge of the presence of organ-specific carcinogens in cigarette smoke. Special importance in this group of carcinogens should be given to the tobacco-specific N-nitrosamines, since these are found only in the *Nicotiana* varieties, and appear in high concentrations in tobacco products. They are

TABLE 9.—Organ-specific carcinogens in cigarette smoke

Smoke carcinogen	Amount per cigarette
Nitrosodimethylamine	4 - 180 ng
Nitrosoethylmethylamine	1 - 40 ng
Nitrosodiethylamine	0.1 - 28 ng
Nitrosodi-n-butylamine	0 - 3 ng
Nitrosopyrrolidine	0 - 110 ng
Nitrosopiperidine	0 - 9 ng
Nitrosodiethanolamine	0 - 40 ng
N'-Nitrosonornicotine	0.2 - 3.7 μg
NNK[a]	0.12 - 0.44 μg
N'-Nitrosoanabasine	0 - 0.15 μg
N'-Nitrosoanatabine	0.15 - 4.6 μg
2-Naphthylamine	4.3 - 27 ng
4-Aminobiphenyl	2.4 - 4.6 ng
Polonium-210	0.03 - 1.0 pCi
Nickel	0 - 3 μg

[a]NNK = 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone.
SOURCE: Brunnemann and Hoffmann (*29*), Brunneman et al. (*33*), and Patrianakos and Hoffmann (*150*).

moderately active animal carcinogens or, as in the case of NNK, a potent animal carcinogen.

Sidestream Smoke

The sidestream smoke (SS) is a composite of effluents generated in different ways during the burning and smoking of a tobacco product. While the product smoulders in between puff taking, smoke is freely emitted into the air; during puffing a little smoke escapes from the burning cone, and vapor phase components diffuse partially through the cigarette paper. The SS, generated between puffs, originates from a hydrogen-enriched, strongly reducing atmosphere. It contains, therefore, combustion products formed by thermal cracking and compounds that result from reactions involving nitrates in greater proportions than are found in mainstream smoke (MS). These compounds include nitrogen oxides, nitrosamines, ammonia and amines, and total particulate matter. Table 10 lists the known SS/MS ratios for major toxic and tumorigenic agents.

The SS/MS ratios are especially high for volatile nitrosamines and for the nitrogen oxides, which constitute major precursors for *in vitro* and *in vivo* formation of nitrosamines. The relevance of this finding in regard to the SS exposure of nonsmokers in closed environments has been repeatedly discussed (*26, 29, 158, 189*). The SS components are diluted by air prior to being inhaled and the particulates settle rather quickly on environmental surfaces. Deep and intentional inhalation of MS delivers a far greater burden of

TABLE 10.—Toxic and tumorigenic agents of cigarette smoke; ratio of sidestream smoke (SS) to mainstream smoke (MS)

A. Gas phase	Amount/cigarette				SS/MS
Carbon dioxide	10	-	80	mg	8.1[1]
Carbon monoxide	0.5	-	26	mg	2.5[1]
Nitrogen oxides (NO_x)	16	-	600	µg	4.7 - 5.8
Ammonia	10	-	130	µg	44 - 73
Hydrogen cyanide	280	-	550	µg	0.17 - 0.37
Hydrazine			32	µg	3
Formaldehyde	20	-	90	µg	51
Acetone	100	-	940	µg	2.5 - 3.2
Acrolein	10	-	140	µg	12
Acetonitrile	60	-	160	µg	10
Pyridine			32	µg	10
3-Vinylpyridine			23	µg	28
N-Nitrosodimethylamine	4	-	180	ng	10 - 830
N-Nitrosoethylmethylamine	1.0	-	40	ng	5 - 12
N-Nitrosodiethylamine	0.1	-	28	ng	4 - 25
N-Nitrosopyrrolidine	0	-	110	ng	3 - 76

B. Particulate phase	Amount/cigarette				SS/MS
Total particulate phase	0.1	-	40	mg	1.3 - 1.9[1]
Nicotine	0.06	-	2.3	mg	2.6 - 3.3[1]
Toluene			108	µg	5.6
Phenol	20	-	150	µg	2.6
Catechol	40	-	280	µg	0.7
Stigmasterol			53	µg	0.8
Total phytosterols			130	µg	0.8
Naphthalene			2.8	µg	16
1-Methylnaphthalene			1.2	µg	26
2-Methylnaphthalene			1.0	µg	29
Phenanthrene	2.0	-	80	ng	2.1
Benz(a)anthracene	10	-	70	ng	2.7
Pyrene	15	-	90	ng	1.9 - 3.6
Benzo(a)pyrene	8	-	40	ng	2.7 - 3.4
Quinoline			1.7	µg	11
Methylquinoline			6.7	µg	11
Harmane	1.1	-	3.1	µg	0.7 - 2.7
Norharmane	3.2	-	8.1	µg	1.4 - 4.3
Aniline	100	-	1,200	ng	30
o-Toluidine			32	ng	19
1-Naphthylamine	1.0	-	22	ng	39
2-Naphthylamine	4.3	-	27	ng	39
4-Aminobiphenyl	2.4	-	4.6	ng	31
N'-Nitrosonornicotine	0.2	-	3.7	µg	1 - 5
NNK[2]	0.12	-	0.44	µg	1 - 8
N'-Nitrosoanatabine	0.15	-	4.6	µg	1 - 7
N-Nitrosodiethanolamine	0	-	40	ng	1.2

[1] In cigarettes with perforated filter tips the SS/MS ratio rises with increasing air dilution. In the case of smoke dilution with air to 17 percent, the SS/MS ratios for TPM rise to 2.14, CO_2 36.5, CO 23.5, and nicotine to 13.1

[2] NNK = 4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanone.

SOURCE: Hoffmann et al. (82).

respiratory pollutants to the lungs than does normal breathing

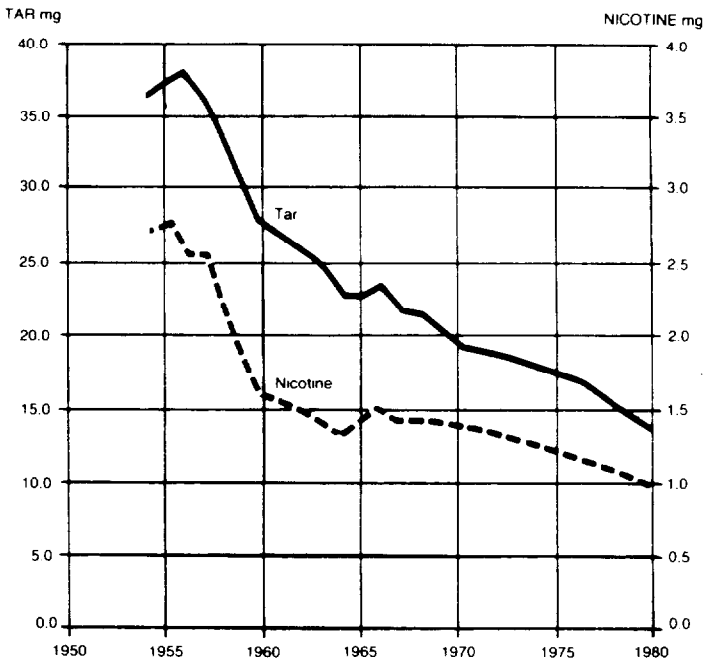


FIGURE 7.—U.S. sales-weighted average tar and nicotine yields

SOURCE: American Cancer Society (*1*).

during regular nonoccupational activities.

Reduction of Tumorigenic Potential

The trends for the sales-weighted average tar and nicotine deliveries of U.S. cigarettes since 1955 (≅37 mg tar, 2.7 mg nicotine) until 1980 (≅14 mg tar, 1.0 mg nicotine) are shown in Figure 7 (*1*). During this time, the percentage of filter-tipped cigarettes in U.S. cigarette production increased from 18.7 to 90 percent.

The agricultural aspects and methods of tobacco processing and product manufacturing leading to changes in smoke composition, toxicity, and carcinogenicity have been discussed in previous Surgeon General's Reports (*188, 189*) and elsewhere (*60, 89*). Table 11 summarizes the average machine-smoked values of selected smoke components for the cigarette before 1960 and during 1978–79, as well as the average values for a leading low-tar U.S. cigarette with a perforated filter tip (*89*).

A significant reduction of carbon monoxide in cigarette smoke did not occur until cigarettes with perforated filter tips were introduced (Table 12; *89*). A recent publication reported that the average

TABLE 11.—Changes in smoke composition of cigarettes manufactured in the United States

Smoke constituent	Average delivery per cigarette		
	Before 1960	1978/79	1978/79 (Low-tar cigarette)
Total particulate matter	43	16	8
Nicotine (mg)	3.0	1.1	0.6
CO (mg)	23	17	8.9
NO_x (μg)	270	280	100
HCN (μg)	410	200	130
Acrolein (μg)	130	80	50
Phenol (μg)	100	60	20
Benzo[a]pyrene (ng)	35	18	10

SOURCE: Hoffmann et al. (*89*).

cigarette sold in the United Kingdom between 1934 and 1940 (>99 percent plain cigarettes) delivered under standard smoking conditions 32.9 mg tar, 2.0 mg nicotine, and 18.6 mg carbon monoxide (*197*). In contrast, in 1979 the average cigarette in the United Kingdom (9 percent plain tobacco, 77 percent unventilated filter brands, and 14 percent ventilated filter cigarettes) delivered 16.8 mg tar, 1.39 mg nicotine, and 16.6 mg carbon monoxide. The authors also point out that there was a sizeable decrease since 1934 in delivery of tar (49 percent) and nicotine (31 percent), but only an 11 percent decrease in carbon monoxide delivery. The average U.K. unventilated filter cigarette of 1979 delivered 18.1 mg carbon monoxide and the average ventilated filter cigarette delivered 12.0 mg carbon monoxide (*197*). This finding and the values of Table 12 support the concept that filter perforation is the most important development for the reduction of carbon monoxide in cigarette smoke.

The reported data are based on measurements obtained by machine smoking of cigarettes under standard conditions. As discussed before, these conditions may have reflected the average smoking habits of individuals 25 years ago, but today they appear to be representative of less than 10 percent of U.S. smokers. Russell and coworkers (*160*), as well as others (*75, 76*), reported that some smokers of lower tar, lower nicotine cigarettes will intensify smoking and inhalation in order to satisfy a physiological need for nicotine and cotinine. A statistical reevaluation (*113*) of the data of Russell et al., however, showed that the nicotine blood serum levels of smokers of cigarettes with perforated filter tips were, in fact, lower than those of other cigarette smokers. On the basis of model studies, it also appears unlikely that a smoker of perforated filter cigarettes can increase his smoking intensity to such a degree that he can fully compensate for the loss in nicotine delivery without significantly

TABLE 12.—Carbon monoxide in smoke of cigarettes

Commercial product	Carbon monoxide (mg/cigarette)		
	Nonfilter	Regular filter	Perforated filter
U.K. (1975)*	9.0–16.0 (N=9)	13.0–18.0 (N=10)	—
U.K. (1979)**	10.9	18.1	12.0
Germany (1975)	16.0–21.0 (N=7)	15.5–22.5 (N=17)	—
Germany (1978)	14.5–19.9 (N=16)	8.6–18.5 (N=15)	2.2–13.8 (N=9)
U.S.A. (90% of av. 1977/78 sales)***	11.0–17.0 (N=8)	14.4–20.0 (N=23)	2.8–12.8 (N=9)
U.S.A. (FTC - 1981)	13.0–22.0 (N=18)	13.0–26.0 (N=87)	0.5–13.0 (N=82)

* Average values for nonfilter cigarettes, 12.5 mg; for filter cigarettes, 16.1 mg.

** Sales-weighted average carbon monoxide yields, average of all U.K. brands, 16.6 mg. Wald et al. (*200*)

*** Average values for nonfilter cigarettes, 14.9 mg; for regular filter cigarettes, 17.1 mg; for perforated filter cigarettes, 8.9 mg.

SOURCE: Hoffmann et al. (*89*).

increasing his daily cigarette consumption (*81*). The increase in smoking intensity by the smoker of perforated filter cigarettes may lead to an increase in the delivery of carcinogenic tar.

In addition to these changes in the pattern of smoking, smokers of lower tar and nicotine products may increase their actual dose of smoke constituents over that predicted by machine measurements through voluntary or involuntary blocking of the ventilation holes in filters. Kozlowski et al. (*112*) examined the effect of partial and total occlusion of perforations on machine measurement of tar, nicotine, and carbon monoxide in one brand of lower tar cigarettes. With full occlusion, he found that the nicotine yield increased 118 percent, the tar yield increased 186 percent, and the carbon monoxide yield increased 293 percent. He reported survey results of from 32 to 69 percent (95 percent confidence limits) of lower tar smokers had blocked holes with fingers, lips, or tape. Further research is necessary to define the actual impact of occlusion of ventilations in filters on actual smoker exposure.

The development of the low-tar cigarette required enrichment of smoke flavors in order to make the product acceptable to the consumer. The flavor is enhanced by the addition of undescribed materials that may include concentrates of flavor precursors obtained from tobacco, licorice, extracts from other plants, or semisynthetic or fully synthetic flavor components. Because these additives

have not been identified, no judgment can be made as to whether they result in new compounds or in higher concentrations of hazardous components in the smoke. The practice of flavor enrichment requires detailed toxicological studies that are not available at present for scientific evaluation of their health impact (*116a, 189*).

Research Needs and Priorities

Tobacco carcinogenesis has been intensively studied for more than 25 years by epidemiologists, chemists, biochemists, toxicologists, and pathologists. As a result, there is a much expanded knowledge of the major factors contributing to the toxicity and carcinogenicity of cigarette smoke. Nonetheless, significant gaps in that knowledge remain.

Benign and malignant tumors have been induced in the larynx of hamsters by long-term exposure to diluted cigarette smoke. Attempts to induce significant numbers of bronchogenic carcinoma in laboratory animals were negative in spite of major efforts with several species and strains. Neither rats nor hamsters nor baboons inhale cigarette smoke as deeply and as intensely as the cigarette smokers who have provided the data with the consequences of their "experiment" in the form of clinical evidence gathered by epidemiologists. In view of this compelling evidence, it appears that the experimental induction of bronchogenic carcinoma should receive limited priority as a research goal.

However, major efforts should be devoted to the elucidation of the steps involved in the formation of lung tumors. Such investigations must attempt to answer the following questions: Does cigarette smoke induce enzymes that activate tumor initiators and carcinogens to their ultimate active forms? Are certain carcinogens, such as tobacco-specific N-nitrosamines, formed from smoke components in the lungs? Can the *in vivo* formation of such carcinogens in the lung be prevented? Is it feasible to inhibit metabolic activation and DNA binding of tobacco smoke carcinogens by chemopreventive measures? Both prospective and retrospective studies have indicated that cigarette smokers with low serum vitamin A levels have an increased risk for lung cancer compared with cigarette smokers with normal or high vitamin A levels (*133, 198*). Evidence from *in vivo* and *in vitro* studies in carcinogenesis has supported the protective role of vitamin A (*115*). Studies of the specific effects of vitamin A and retinoic acid on the induction of lung tumors by tobacco carcinogens are thus needed.

So far, only limited attention has been given to mechanisms of induction of cancer of the esophagus, pancreas, kidney, and urinary bladder by tobacco smoke. Initial experiments support the concept that certain nutritional deficiencies such as those of zinc and

vitamin A may increase the susceptibility of the esophageal epithelium to insults from tobacco smoke constituents. Whether tobacco smoke as an enzyme inducer may be indirectly responsible for increased metabolic activation of organ-specific carcinogens in the esophageal epithelium needs to be determined.

Only a few studies have been concerned with the effect of tobacco smoke and its nicotine level on the biochemistry and function of the pancreas in smokers and in laboratory animals (*7, 140*). It needs to be determined whether nicotine has a direct influence on the induction of pancreatic cancer in cigarette smokers or whether it gives rise to an organ-specific N-nitrosamine or a carcinogenic metabolite of the latter. The elucidation of these questions should have high priority, since pancreatic cancer is associated with cigarette smoking, and since its incidence in the United States has increased steadily between 1950 and 1970.

An earlier Part of this Report dealt with the various concepts on the correlation of cigarette smoking and bladder cancer. Currently, the most valid theory relates to the likelihood that the urine of smokers contains traces of bladder carcinogens that derive from inhaled smoke constituents either directly or via precursors. Whether urine of smokers does in fact contain precursors that lead to the formation of carcinogens in the presence of infectious agents or under the influence of other pathologic conditions or whether the urine of smokers contains cocarcinogens needs to be explored.

The identification of cocarcinogenic agents in the neutral and weakly acidic portions of tobacco smoke will also require much more detailed investigation as to chemical nature, precursors, and biological interactions of such compounds.

In view of repeatedly expressed concerns regarding the possible transplacental effects of cigarette smoke inhalation (*188, 189, 190*), intensive research in this area is urgently needed. The concern is based in part on the observation that the foreskin of newborn infants of smoking mothers contains enzymes that metabolize benzo[a]pyrene (*41, 121*). Furthermore, it is known that nicotine crosses the placenta (*184*) and may thus give rise to formation of carcinogenic nitrosamines in the fetus. The hamster appears to be a suitable model for smoke inhalation studies designed to examine various aspects of transplacental carcinogenesis (*11, 51*).

The ongoing modifications of tobacco products offer constant challenges to the analytical chemists and toxicologists who monitor the characteristics of these products. The increasing nitrate content of cigarettes raises concerns regarding the possibility of higher yields of volatile and tobacco-specific N-nitrosamines in the smoke and regarding possible formation of aromatic nitrohydrocarbons and amines.

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The changes in flavor composition or changes in tobacco that affect the "flavor bouquet" of tobacco products may conceivably be responsible for mutagenic, tumorigenic, or otherwise toxic smoke constituents. Monitoring and identifying such biological activity and associated chemical characteristics remain a constant responsibility of the tobacco health research scientist.

Although the published epidemiologic data regarding a possible effect of sidestream smoke on lung cancer induction in nonsmokers are not in total agreement (see the Part of this Report on involuntary smoking), the release of carcinogens from the burning cigarette into enclosed environments warrants a detailed study of this problem. Subsequent approaches toward a reduction of risks by inhibiting or altering the release of certain sidestream smoke components may need to be developed.

Summary

This overview presents evidence and observations on tobacco carcinogenesis primarily developed since 1978.

1. The biological activity of whole cigarette smoke and its tar and tar fractions can now be measured by improved inhalation assays in addition to tests for tumor-initiating, tumor-promoting, and cocarcinogenic activities on mouse skin.
2. Studies on smoke inhalation with the hamster now appear suitable for estimating the relative tumorigenic potential of whole smoke from commercial and experimental cigarettes. The identification of the smoke constituents that contribute to tumor induction in the respiratory tract is best achieved by fractionations of tar and by assays on mouse epidermis that determine the type and potency of the carcinogens. In combination with biochemical tests, mouse skin assays should also aid in evaluating the possible role of nicotine as a cocarcinogen.
3. The identification, formation, and metabolic activation of organ-specific carcinogens have been studied which help explain the increased risk to cigarette smokers of cancer of the esophagus, pancreas, kidney, and urinary bladder. In addition to certain aromatic amines, tobacco-specific N-nitrosamines appear to be an important group of organ specific carcinogens in tobacco and tobacco smoke. Little is known of the *in vivo* formation of organ-specific carcinogens from nicotine and other *Nicotiana* alkaloids. The modification of their enzymatic activation to ultimate carcinogenic forms needs to be explored by chemopreventive approaches.
4. Transplacental carcinogenesis as it may relate to effects of cigarette smoking should be investigated more fully. It has been known for some time that inhalation of tobacco smoke

activates enzymes in the placenta and fetus and the consequences of such changes need to be studied.

5. The continuing modification of U.S. cigarettes has led to changes in the quantitative and perhaps also the qualitative composition of the smoke. This ongoing development requires continued monitoring of the toxic and carcinogenic potential of the smoke of new cigarettes.
6. The changes in cigarette composition lead generally to reduced emission of major toxic mainstream smoke constituents as measured in analytical laboratories under machine-smoking conditions. Many smokers intensify puff volume and degree of inhalation when smoking a lower-yield cigarette. Therefore, it should be determined what effect different techniques of air dilution and filtration have in counteracting the increased smoke exposure that results from intensified smoking.
7. Snuff tobaccos are increasingly used as an alternative to cigarette smoking. More information is needed regarding the carcinogenic activity of snuff tobaccos and the presence of tumorigenic agents in these products.

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