The relevance of tobacco-specific nitrosamines to human cancer

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I Introduction

Major prospective studies in North America and in Europe in the 1960s and the 1970s have demonstrated that the risk of lung cancer mortality for smokers is 7.8 to 15.9 times higher than that for non-smokers. These findings were confirmed by more than 100 case–control studies and have also clearly established a dose–response relationship between number of cigarettes smoked and the risk of lung cancer. The studies are summarized in the reports of the Royal College of Physicians of London (1983) and of the Surgeon General of the United States Public Heath Service (US Department of Health and Human Services, 1989). These reports also describe the causal relationship of smoking to cancer of the larynx, pharynx, oral cavity, oesophagus, pancreas, renal pelvis and urinary bladder. In addition, cigarette smoking is

associated with cancer of the nasal cavity and of the cervix and also with leukaemia (IARC, 1986; Kinlen and Rogot, 1988; US Department of Health and Human Services, 1989). Smoking cigars and pipes is causally related to cancer of the respiratory tract, oral cavity and oesophagus, although the causal relationship with lung cancer is in this case not as strong as that of cigarette smoking (IARC, 1986; US Department of Health and Human Services, 1989). More recently, exposure to environmental tobacco smoke (involuntary, or passive smoking) has been incriminated as a risk factor for cancer of the lung in non-smokers (IARC, 1986; US National Research Council, 1986; US Department of Health and Human Services, 1989).

Chewing of tobacco and especially the oral use of snuff are associated with cancer of the oral cavity (IARC, 1985; Department of Health and Human Services, 1986) and possibly with cancer of the nasal cavity, pancreas, kidney and bladder (Brinton *et al*, 1984; Goodman *et al*, 1986; Kabat *et al*, 1986; US Department of Health and Human Services, 1986; World Health Organization, 1988). In India and in other Asian countries, chewers of betel quid with tobacco and chewers of khani, a mixture of tobacco and lime, are at high risk for cancer of the oral cavity, pharynx, larynx and oesophagus (Jussawalla and Deshpande, 1971; IARC, 1985).

Most of the epidemiological observations on tobacco usage and cancer have been supported by evidence of carcinogenicity from bioassays with whole smoke and with the particulate matter ('tar') of the smoke. Such studies in laboratory animals have been summarized in several reviews (Wynder and Hoffmann, 1967; Mohr and Reznick 1978; US Department of Health and Human Services, 1982; IARC, 1986). Bioassays with smokeless tobacco have indicated, though not proved, that these products are carcinogenic in the oral cavity of rats and hamsters (IARC, 1985; Hecht *et al*, 1986a; US Department of Health and Human Services, 1986). Analytical investigations have led to the isolation and identification of approximately 3000 individual components in tobacco and 4000 in tobacco smoke (Roberts, 1988) including various carcinogens in processed tobacco, and a large number of tumour initiators, tumour promoters, cocarcinogens and organ-specific carcinogens in tobacco smoke (Hoffmann and Hecht, 1988, 1989).

It is the purpose of this overview to delineate the contribution of the tobacco-specific nitrosamines (TSNA) to the carcinogenicity of chewing tobacco, snuff and tobacco smoke. Although TSNA as a group represent the most abundant, highly active carcinogens in these products, consideration must be given to the fact that tobacco extracts and tobacco smoke are highly complex mixtures. Consequently, tobacco and its smoke contain not only tumorigenic agents but also tumour inhibitors. The absorption of biologically active agents from mixtures is governed by various factors including the physical and chemical state of the compound and the pH of the mixture. The carcinogenic activities of the TSNA can be influenced by factors such as alcohol and diet. Despite these limitations there is evidence that the TSNA contribute appreciably to the increased risk of tobacco users for cancers of the lung, oral cavity, oesophagus, pancreas and nasal cavity.

II Human exposure to TSNA

Nicotine and the minor *Nicotiana* alkaloids (Fig. 1) represent a major group of pharmacologically active compounds in tobacco products (US Department

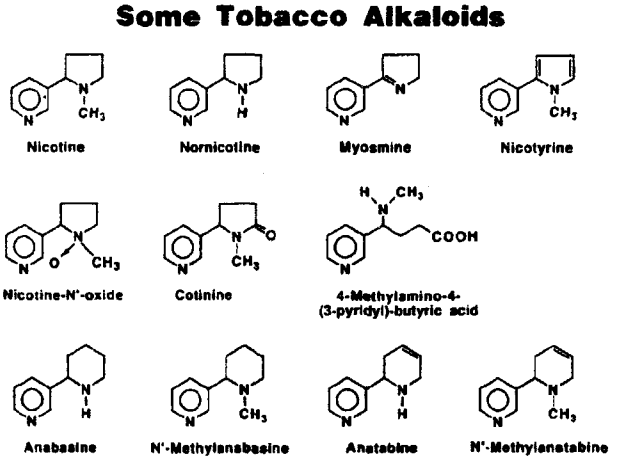

Fig. 1. Structures of nicotine alkaloids

of Health and Human Services, 1988a). Depending on the tobacco type, variety and plant components utilized, processed tobacco contains from 0.5–5% of alkaloids with nicotine as the predominant compound (90–95% of the total alkaloids). Commercial cigarettes smoked under standardized laboratory conditions (Brunnemann *et al*, 1976) deliver 0.1–3.0 mg of nicotine and up to 0.3 mg of minor alkaloids in the mainstream smoke and 1.3–20-fold higher amounts of the alkaloids in the sidestream smoke of cigarettes, the smoke generated between puffs (Adams *et al*, 1987; US Department of Health and Human Services, 1989). Cigars of various shapes, sizes and tobacco types, as well as pipes, can generate up to several milligrams of nicotine in the mainstream smoke (US Department of Health and Human Services, 1982). Indoor environments polluted with tobacco smoke were found to contain 1–13.8 $\mu g/m^3$ of nicotine (US Department of Health and Human Services, 1989).

The alkaloids together with the amino acids and proteins are the most abundant amino compounds in tobacco products. Nicotine is a tertiary amine, while nornicotine, anatabine and anabasine are secondary amines (Fig. 1). Nitrosation of alkaloids yields TSNA; seven have been identified in tobacco products (Fig. 2). Although traces of some of these nitrosamines have also been found in green tobacco leaves before harvesting (Andersen *et al*, 1989; Djordjevic *et al*, 1989a), the largest amounts are formed during the processing of tobacco. The yields of nitrosamines are dependent on the concentrations of

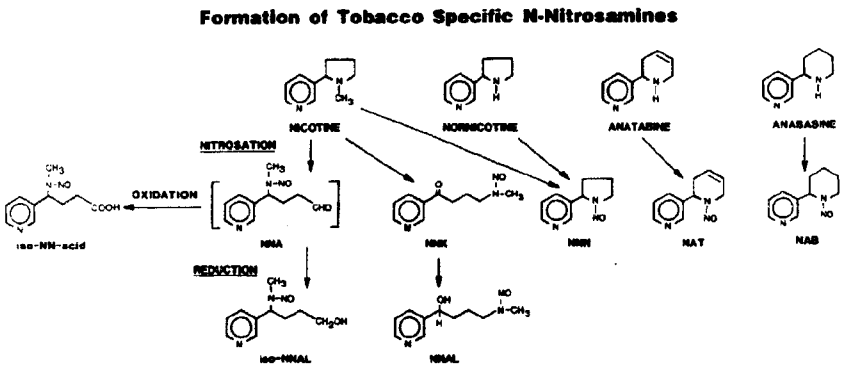

Fig. 2. Formation of tobacco-specific *N*-nitrosamines

alkaloids and nitrate in lamina and ribs and on the tobacco processing methods including curing, fermentation and ageing (Tso, 1972; Brunnemann *et al*, 1983). As seen in Table 1 the highest concentrations of TSNA have been determined in snuff. This is due to the favourable conditions for TSNA formation during fermentation (Hoffmann and Hecht, 1988; Djordjevic *et al*, 1989b; US Department of Health and Human Services, 1989). The sometimes exceptionally high TSNA levels in commercial snuff are a likely consequence of the ageing of products during a long shelf life. But even concentrations of the TSNA in smokeless tobacco products of recent manufacture exceed those of nitrosamines reported in other consumer products by at least two orders of magnitude (US National Research Council, 1981; Preussmann and Eisenbrand, 1984).

Analyses of saliva of tobacco chewers and snuff-dippers have demonstrated that the TSNA are extracted from these tobacco products (Hoffmann and Adams, 1981; Nair *et al*, 1985; Palladino *et al*, 1986; Bhide *et al*, 1986; Brunnemann *et al*, 1987; Oesterdahl and Slorach, 1988). While the individual TSNA in saliva may reach levels up to 400 ppb, unusually high concentrations (up to 2600 ppb *N'*-nitrosonornicotine (NNN) and a mean of 980 ppb) have been measured in the saliva of some Canadian Eskimos who dip snuff (Brunnemann *et al*, 1987). Factors other than high TSNA levels in tobacco which contribute to high TSNA concentrations in the saliva of snuff-dippers include number of years of practising the habit and the frequency of chewing (Palladino *et al*, 1986).

It has been estimated on the basis of using popular American products in 1987-/88 that a snuff-dipper who consumes 10 g/day over a period of 40 years is thus exposed to about 4800 mg of NNN and 260 mg of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK). This exposure to NNN and NNK, the two most carcinogenic TSNA, is not far below the levels which induce tumours in rats. These calculations of human exposure to NNN and NNK may be lower than the real levels of exposure if one considers the likelihood that nitrosation

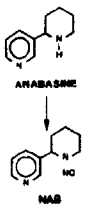

Table 1. TSNA in tobacco

Tobacco product	*Country*	*Tobacco-specific nitrosamines (ppm)*			*Reference*[*]
		NNN	*NNK*	*NAB & NAT*	
Snuff, moist	USA	3.1–135	0.1–13.6	0.64–339	1,2,3,10
	Canada	25–79	1.6–4.5	75–85	2,4,7
	UK	11.8	1.8	3.1	5
	Sweden	2.2–154	0.2–3.0	3.5–21.4	2,3,6,10
	Denmark	2.3–4.0	0.7–3.5	1.4–3.1	2
Snuff, dry	USA	9.0–52	1.8–13	18–200	2
	FRG	6.0–6.7	1.5–1.54	3.9–4.3	2
Chewing tobacco	USA	0.6–8.2	0.38	2.4	2
	UK	0.9	0.3	1.5	3
	Sweden	2.1	0.24	1.7	2
	FRG	0.5–2.3	0.03–0.4	0.4–3.7	2,3
	Belgium	7.4	0.13	0.97	10
	India	0.4–2.5	0.13–0.4	0.3–0.5	2,3
	Thailand	0.5	0.1	0.5	3
Masheri[a]	India	0.3–0.5	0.9–1.1	0.5–1.0	3
Zarda[a]	India	0.4–18.2	0.35–4.48	0.78–99.1	3.9
Nass[a]	USSR	0.12–0.52	0.02–0.13	0.04–0.33	1
Cigarettes	USA	0.6–7.9	0.1–1.3	0.5–5.8	7,10
	UK	0.3	0.1	0.2	7
	France	0.58–18.6	0.13–1.5	0.23–10.0	7,8,10
Little cigars	USA	11.2	4.5	13.0	7
Cigars	USA	3.0–10.7	1.1–3.5	2.5–33	7
	Netherlands	6.8–53.0	2.9–4.3	4.6–20.4	10
Pipe tobacco	UK	3.0	0.6	2.5	10
	France	6.9	1.1	4.9	10
	Netherlands	3.8	n.d.[b]	2.0	10

[a]Specific tobacco products used in certain regions (see WHO, 1988)
[b]n.d.=not detected

*1. Brunnemann *et al* (1985); 2. Hoffmann and Hecht (1988); 3. Tricker *et al* (1988); 4. Brunnemann *et al* (1987); 5. Hoffmann *et al* (1988); 6. Oesterdahl and Slorach (1988); 7. Hoffmann *et al* (1984a); 8. Djordjevic *et al* (1989b); 9. Tricker and Preussmann (1988); 10. Ohshima *et al* (1985)

reactions during chewing will yield additional amounts of TSNA from the alkaloids in the snuff (Nair *et al*, 1987).

TSNA in cigarette smoke originate from tobacco as well as being pyrosynthesized during smoking. Under standard smoking conditions 40–46% of NNN and 26–37% of NNK in cigarette mainstream smoke originate from tobacco by direct transfer, while the remainder is pyrosynthesized during smoking (Hoffmann *et al*, 1980; Adams *et al*, 1983). Table 2 presents quantitative analytical data for TSNA in the smoke of cigarettes and cigars.

The levels of TSNA in tobacco smoke are up to 100-fold higher than those

Table 2. TSNA in mainstream tobacco smoke

Tobacco product	*Country*	*Tobacco-specific nitrosamines (ng/cig)*			*Reference*[*]
		NNN	*NNK*	*NAB & NAT*	
Cigarettes					
F	USA	310	150	370	1
F-VLT	FRG	24–106	6–69	15–128	2
F-LT		38–99	26–55	33–98	2
F-MT		19–179	21–145	35–285	2
F-HT		11–122	27–73	27–108	2
F	France	1000–3200	190–430	190–640	3
NF	USA	120–950	80–770	140–990	1
NF-O	FRG	3–19	n.d.–4	6–20	2
NF-V		16–32	36–91	40–90	2
NF-T		77	59	102	2
NF-B		85–255	70–156	80–225	2
NF-BL		512–625	108–432	266–353	2
NF-BL	France	575–590	127–220	200–350	2
NF-Bu[a]	..	3700	320	4200	1
NF-V[a]	..	620	420	410	1
Little cigars					
F	USA	5500	4200	1700	4
Cigar					
NF	USA	3200	1900	1900	4

[a]Experimental cigarettes

F, filter; NF, non-filter; VLT, very low tar; LT, low tar; MT, medium tar; HT, high tar; O, oriental; V, Virginia; T, Turkish; B, blended tobacco; BL, black tobacco; Bu, Burley tobacco; n.d., not detected

[*]1. Hoffmann *et al* (1984a); 2. Fischer *et al* (1989a); 3. Djordjevic *et al* (1989b); 4. Hoffmann *et al* (1980)

of other nitrosamines in the human environment, except in some specific occupational settings (US National Research Council, 1981; Preussmann and Eisenbrand, 1984). On the basis of TSNA yields such as are present in the smoke of a machine-smoked non-filter cigarette, human exposure estimates at a rate of 40 cigarettes per day over a 40-year span would reach about 590 mg of NNN and 250 mg of NNK. Again, we believe that this is a low estimate since, among other factors, it is arrived at without regard for the possible endogenous formation of TSNA due to alkaloids and nitrosating agents inhaled as constituents of tobacco smoke. Consideration must also be given to the fact that smoke yields obtained under standardized machine-smoking conditions are frequently lower than those generated by cigarette smokers who deviate from 'standard conditions' by drawing puffs far more frequently and inhaling them more intensely. This applies especially to smokers of low-yield cigarettes (US Department of Health and Human Services, 1989). One would, therefore, have to assume additional body burdens of TSNA for exposure estimates among low-yield cigarette smokers (Fischer *et al*, 1989b).

TSNA are also generated during sidestream smoke formation in between puff-taking. Under standardized conditions in the laboratory, the release of TSNA into sidestream smoke exceeds the levels generated in mainstream smoke. This is especially pronounced in the case of cigarettes with perforated filter tips. A low-yield American filter cigarette delivered 66.3 ng NNN and 17.3 ng NNK in mainstream smoke, and 338 ng NNN and 386 ng NNK in sidestream smoke (Adams *et al*, 1987). Attempts to measure TSNA in smoke-polluted indoor environments were until now reported only in one instance. Levels ranged from below detection limit to 3.7 ng/m^3 for NNN and up to 8.6 ng/m^3 for NNK (Klus *et al*, 1987).

III Bioassays of the TSNA

The interest in the TSNA in tobacco and tobacco smoke is primarily due to their carcinogenicity in laboratory animals (Hecht and Hoffmann, 1988; Rivenson *et al*, 1988). Five of the seven TSNA identified thus far have been assayed for carcinogenic activity (Table 3). Three of them, NNN, NNK and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL), are strong carcinogens. Their organospecificities depend in part on the route of administration. For example, in rats the powerful carcinogen NNK causes tumours of the lung, irrespective of the mode of application. When given in drinking water, it elicits tumours of the exocrine pancreas in addition to lung tumours. Upon subcutaneous injection tumours of the nasal cavity, liver and lung are observed. NNK, but not NNN, is also a tumour initiator in mouse skin (LaVoie *et al*, 1987). NNN given in drinking water causes benign and malignant tumours of the oesophagus as well as nasal tumours in rats; when given by subcutaneous injection it induces mainly tumours of the nasal cavity. *N'*-nitrosoanabasine (NAB) is weakly carcinogenic in rats while *N'*-nitrosoanatabine (NAT) is non-carcinogenic in rats at doses up to 9 mmol/kg. NNAL elicits tumours of the lung and pancreas in rats. Iso-NNAL and the recently identified iso-NN-acid are currently being assayed for carcinogenicity.

IV Evidence that TSNA cause cancer in humans

1 Oral cancer in smokeless tobacco users and betel quid chewers

Epidemiological studies have demonstrated that chronic use of smokeless tobacco, in the form of snuff-dipping, causes oral cancer. The relative risk is as high as 50-fold for cancer of the gum and buccal mucosa (Winn *et al*, 1981; IARC, 1985; US Department of Health and Human Services, 1986). Since smokeless tobacco is not a combustion product like tobacco smoke, its composition is simpler and it contains a less complex mixture of carcinogens than does tobacco smoke. The only carcinogens known to be present in the types of smokeless tobacco used for snuff-dipping are nitrosamines, aldehydes, polonium-210 (^{210}Po) and polynuclear aromatic hydrocarbons (Hoffmann *et al*, 1987). NNN and NNK are typically present in amounts ranging

Table 3. Carcinogenicity of TSNA

TSNA	*Animal (strain)*	*Route of application*	*Principal target organ*	*Dose (mmol/animal)*	*Reference*[*]
NNN	Mouse (Sencar)	Topical (TI)[a]	None	0.028	1
	Mouse (A/J)	Intraperitoneal	Lung	0.1	2
	Rat(F344)	Subcutaneous	Nasal cavity, oesophagus	0.2–3.4	2
		Oral	Oesophagus, nasal cavity	1.0–3.6	2
	Rat (Sprague-Dawley)	Oral	Nasal cavity	8.8	2
	SG Hamster	Subcutaneous	Trachea, nasal cavity	0.9–2.1	2
NNK	Mouse (Sencar)	Topical (TI)[a]	Skin	0.028	1
	Mouse (A/J)	Intraperitoneal	Lung	0.02–0.12	2,3
	Rat (F344)	Subcutaneous	Nasal cavity, lung, liver	0.2–2.8	2
		Oral	Lung, pancreas, liver	0.075–0.31	4
	SG Hamster	Subcutaneous	Trachea, lung, nasal cavity	0.005–0.9	2
NNAL	Mouse (A/J)	Intraperitoneal	Lung	0.12	5
	Rat (F344)	Subcutaneous	Lung, pancreas	0.32	4
NAB	Rat (F344)	Oral	Oesophagus	3–12	2
	SG Hamster	Subcutaneous	None	2	2
NAT	Rat (F344)	Subcutaneous	None	2.8	2

[a]TI, tumour initiation assay with TPA as promoter
[*]1. LaVoie *et al* (1987); 2. Hoffmann and Hecht (1985); 3. Hecht *et al* (1988a); 4. Rivenson *et al* (1988); 5. Castonguay *et al* (1983a)
NNN, *N'*-nitrosonornicotine; NNK, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone; NNAL, 4-(methylnitrosoamino)-1-(3-pyridyl)-1-butanol; NAB, *N'*-nitrosoanabasine; NAT, *N'*-nitrosoanatabine

from 1-100 μg/g (Table 1), which is about 1000-fold above the levels of polynuclear aromatic hydrocarbons. Formaldehyde, acetaldehyde and crotonaldehyde are present at levels from 1-10 μg/g, and ^{210}Po from 0.2–1 pCi/g. NNN and NNK are the only carcinogens in smokeless tobacco that have been shown to induce oral cavity tumours in laboratory animals (Hecht *et al*, 1986a). A mixture of NNN and NNK, swabbed daily on the oral tissues of rats in a total dose of 1.6 mmol/kg, induced papillomas of the oral cavity in 8 of 30 rats. The calculated exposure of a snuff-dipper to NNN and NNK, over a 40-year period, is approximately 0.4 mmol/kg, which is similar to the dose which produced tumours in rats. The presence of NNN and NNK in the saliva of snuff-dippers has been confirmed (Hoffman and Adams, 1981). Metabolism studies with human tissues have demonstrated that buccal mucosa can activate NNN and NNK to intermediates that can bind to DNA, as observed with oral tissue of rats (Castonguay *et al*, 1983b, 1984). Taken together, these data provide strong support for the role of NNN and NNK as causative factors in oral cancer induction by smokeless tobacco.

Human exposure to NNN and NNK through snuff-dipping is more intense than that to any other nitrosamine, and possibly that to any other strong carcinogen; it represents an unacceptable risk which should be corrected by legislative action.

Synergisms may be important in oral cancer induction by smokeless tobacco. Extracts of snuff have not been proved to induce tumours in laboratory animals, despite the presence of NNN and NNK (Hecht *et al*, 1986a; US Department of Health and Human Services, 1986). In fact, components in the extracts may inhibit NNN and NNK tumorigenesis (Hecht *et al*, 1986a). Oral cancer can be induced by application of whole snuff to a surgically created canal in the lower lip of the rat (Hirsch and Johansson, 1983; Hecht *et al*, 1986a). Chronic irritation may enhance oral carcinogenesis, and this may play a part in accounting for the fact that whole snuff, but not its extract, can induce oral tumours in the rat (Konstantinidus *et al*, 1982). Snuff in combination with herpes simplex virus type I has been shown to induce tumours in the Syrian golden hamster oral cavity (Park *et al*, 1986). This virus is ubiquitous in man (Nahmias and Roizman, 1972) and may be important as a cocarcinogen with NNN and NNK.

Oral cavity cancer is the leading cancer in males in India. It is caused by chewing betel quid with tobacco. The evidence for cancer causation is strong only when tobacco is included in the quid (IARC, 1985). The presence of NNN and NNK in the tobacco used for preparing the quids, as well as in the saliva of betel quid chewers, has been demonstrated in several studies (Sipahimalani *et al*, 1984; Wenke *et al*, 1984; Nair *et al*, 1985; Prokopczyk *et al*, 1987). As in the case of smokeless tobacco, NNN and NNK are the strongest carcinogens known to be present in the tobacco used for the quids, and the only ones known to induce oral tumours in laboratory animals. Nitrosamines derived from arecoline, the major alkaloid of areca nut, are also present in the quid and in saliva. One of them, 3-(methylnitrosamino)propionitrile, is a powerful carcinogen in the rat; its activity as a locally applied oral carcinogen has not yet been evaluated (Prokopczyk *et al*, 1987).

Thus the available evidence strongly supports the role of NNN and NNK as causative factors in oral cancer induced by tobacco products.

2 Lung cancer in smokers

Since the first large scale epidemiological studies on cigarette smoking and lung cancer (Wynder and Graham, 1950; Doll and Hill, 1952; Hammond and Horn, 1958) it has been well established that smoking cigarettes, cigars and pipes is causally linked with lung cancer (US Department of Health and Human Services, 1989). Smokers of cigarettes made entirely of black tobacco, such as are common in France, North Africa and Cuba, have a higher risk for lung cancer than do smokers of blended cigarettes (Joly *et al*, 1983). Levels of TSNA in the smoke of black cigarettes are higher than in the smoke of other cigarettes (Table 2).

In addition to epidemiological observations there is strong evidence from several laboratory studies which supports the concept that TSNA contribute

appreciably to the risk of lung cancer in smokers. NNK induces benign and malignant lung tumours in mice, rats and hamsters (Table 3). In rats 30 mg NNK/kg, given in the drinking water, induces malignant lung tumours (Rivenson *et al*, 1988), while a single subcutaneous dose of 1 mg NNK induces a significant incidence of respiratory tract tumours in hamsters (Hecht *et al*, 1983a). This latter dose of 1 mg NNK per hamster corresponds to about 6 mg NNK/kg. A smoker of 40 cigarettes per day (425 ng NNK/cigarette) is exposed to about 250 mg of NNK or 3.6 mg/kg over a 40-year period. Hamsters treated with NNK by intratracheal instillation developed preneoplastic cellular changes similar to those observed in the pulmonary epithelium of smokers (Boutet *et al*, 1987). Human respiratory epithelium treated with NNK in vitro, and after transformation transplanted into nude mice, produced undifferentiated carcinoma (Parsa *et al*, 1986).

As we have discussed in earlier review articles, NNK and NNN are metabolically activated by α-hydroxylation to intermediates which bind to DNA in the lung (Hoffmann and Hecht, 1985; Hecht and Hoffmann, 1988). Tissue explants obtained from human bronchi and peripheral lung have the capacity to metabolize NNK and NNN by α-hydroxylation to reactive and DNA-damaging electrophiles (Castonguay *et al*, 1983b). In mice and rats, NNK-derived elctrophiles react with nucleophilic centres in DNA to yield a variety of products including O^6-methylguanine (Hecht and Hoffmann, 1988).

The latter adduct has been shown to cause miscoding (Loechler *et al*, 1984). In strain A mice NNK induces lung adenomas which contain an activated K-*ras* oncogene (Belinsky *et al*, 1988). In lung adenocarcinomas of smokers the K-*ras* oncogene appears to be activated by point mutations in codon 12, which result, in part, from O^6-methylguanine-induced miscoding. It is likely that K-*ras* oncogene activation is an important event in the pathogenesis of adenocarcinomas of the human lung (Rodenhuis *et al*, 1988; Fig. 3).

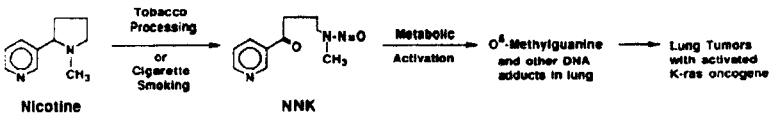

Fig. 3. Scheme linking nicotine to formation of DNA adducts including O^6-methyguanine. The latter leads to DNA miscoding, activation of K-*ras* oncogene and lung tumours

The epidemiological and biochemical data discussed above do not prove that the TSNA cause lung cancer in smokers, but do lead to the conclusion that the TSNA represent an important risk factor for lung cancer in smokers.

3 Oesophageal cancer in smokers

Tobacco smoking is an important cause of oesophageal cancer (IARC, 1986). Nitrosamines are the only known constituents of tobacco smoke that are organospecific for cancer of the oesophagus in laboratory animals. Four

nitrosamines in tobacco smoke—NNN, NAB, *N*-nitrosodiethylamine and *N*-nitrosomethylethylamine—cause oesophageal tumours in rats. Of these, NNN is by far the most prevalent in mainstream cigarette smoke (Table 2). It induces high incidences of oesophageal tumours in F344 rats when given in the drinking water or in a liquid diet (Hoffmann *et al*, 1975; Hecht *et al*, 1983b; Castonguay *et al*, 1984). Only relatively high total doses of approximately 3–9 mmol/kg of NNN have thus far been tested by administration to rats in the drinking water. Lifetime exposure of a smoker to NNN could be estimated as approximately 50 μmol/kg. Metabolic activation of NNN has been observed in cultured rat oesophagus as well as cultured human oesophagus, but the extents of activation by the various pathways are different in the rat and human tissues (Hecht *et al*, 1982; Castonguay *et al*, 1983b). Although further studies will be necessary to more definitely establish the role of NNN as a cause of oesophageal cancer in smokers, the available evidence is suggestive and more compelling than for any other tobacco smoke constituent.

4 Pancreatic cancer in smokers and smokeless tobacco users

Cancer of the pancreas, one of the major cancers in the USA, has increased in both men and women during the last three decades (American Cancer Society, 1988). Both cohort studies and case–control studies have shown that cigarette smoking is causally associated with pancreas cancer in men and women. A dose–response between number of cigarettes smoked per day and the relative risk of developing cancer of the pancreas has been established (IARC, 1986; Department of Health and Human Services, 1989). In a large study in Norway, an increased risk for cancer of the pancreas was indicated for snuff-dippers and tobacco chewers (Heuch *et al*, 1983).

So far, only two agents have been isolated from tobacco products which induce pancreatic tumours in laboratory animals. These are NNK and its enzymatic reduction product NNAL. A dose of 1.0 ppm NNK in the drinking water given to Fischer rats in a lifetime study led to tumours of the exocrine pancreas in 9 of 80 animals, while 8 of 30 rats treated with 5 ppm NNAL in drinking water developed adenoma and adenocarcinoma of the pancreas (Rivenson *et al*, 1988). NNK has been shown to induce early morphological events such as metaplasia and hyperplasia in human pancreas explants but did not produce carcinoma in this in vitro system (Parsa *et al*, 1986). While the available data on the possible contribution of TSNA to pancreatic cancer in tobacco users are limited, they do indicate a need for an in-depth study on the question.

5 Nasal cancer in smokers and snuff-dippers

Although nasal cancer in humans is rare, aside from its occurrence in certain occupational settings, it appears that the risk for squamous cell carcinoma of the nasal passages is significantly elevated in long-term smokers and snuff-dippers (Brinton *et al*, 1984). The observation of nasal cancer in snuff-dippers is suggestive of an organospecific effect. In rats and hamsters both NNN and

NNK induce tumours of the nasal cavity when administered by subcutaneous injection (Hoffmann and Hecht, 1985). NNN and one of its major metabolites, NNN-1-*N*-oxide, also give nasal cavity tumours when administered orally to rats (Hecht *et al*, 1983b; Castonguay *et al*, 1984). The malignant tumours induced by subcutaneous injection of NNN or NNK are found mainly in the olfactory portion of the nose. However, squamous cell carcinoma, like those seen in humans, are the predominant malignant tumours induced in F344 rats by oral administration of NNN and NNN-*N*-oxide (Hecht *et al*, 1980, 1983b; Castonguay *et al*, 1984; Hoffmann *et al*, 1984b). In rats, the nasal mucosa has an exceptionally high capacity for the metabolic activation of NNN and NNK; consequently, extensive methylation of DNA in the nasal mucosa is observed in NNK-treated rats (Brittebo *et al*, 1983; Hecht *et al*, 1986b). The tentative link between TSNA and cancer of the nasal cavity in humans is worthy of further investigation.

V Approaches to quantifying the relationship between TSNA and human cancer

The hundreds of millions of tobacco users in the world represent a unique population that is exposed on a regular basis to TSNA and other carcinogens. The risk of smokers and tobacco chewers for developing lung cancer and/or other tobacco associated cancers is great enough to be measured in epidemiological studies. How can we quantify the role of TSNA in the induction of these cancers? At present, the most promising approach appears to be the measurement of macromolecular adducts of these nitrosamines in tobacco users. In prospective epidemiological studies, it may be possible to relate these adduct levels to risk for cancer development.

Although the levels of TSNA in tobacco products have been extensively investigated and validated (Tables 1 and 2), there remain uncertainties about the extent of individual uptake of these carcinogens. This will depend, for example, on inhalation practices, modes of chewing or dipping and exposure to environmental smoke (IARC, 1985, 1986). These individual variations can be assessed by measuring such parameters as carboxyhaemoglobin or plasma cotinine, but it is not known whether these components or others could be surrogates for uptake of TSNA. The extent to which TSNA may be formed endogenously is also not clear, although the endogenous formation of *N*-nitrosoproline in smokers has been demonstrated (Hoffmann and Brunnemann, 1983; Bartsch and Montesano, 1984; Ladd *et al*, 1984). Major interindividual differences in the extent of metabolic activation of TSNA have been observed in cultured human tissues (Castonguay *et al*, 1983b). These observations demonstrate the need for a method to assess an individual's uptake and metabolic activation of TSNA. Among the various methods that might be considered, haemoglobin adducts and DNA adducts appear to be the most promising at present (Hecht *et al*, 1988b; Wogan, 1988)

As illustrated in Fig. 4, metabolic activation of NNK and NNN lead to a common reactive intermediate, thought to be the diazohydroxide *7*, or a

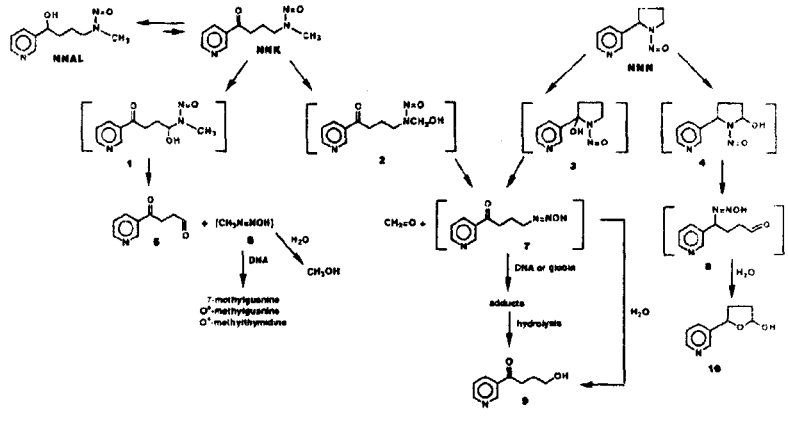

Fig. 4. Metabolic activation of NNK and NNN to hypothetical intermediates which bind to DNA and protein

related carbonium ion. This reacts with both DNA and globin giving adducts of unknown structures. Hydrolysis of DNA with strong acid, or of globin with mild base, releases the keto alcohol *9* (Carmella and Hecht, 1987; Hecht *et al*, 1988a). At present, we are developing mass spectrometric methods for the analysis of *9* released by hydrolysis of DNA or globin obtained from humans. Our continuing research indicates that mass spectrometry is likely to be superior to either ^{32}P-postlabelling or immunoassays for the quantification of *9* or its precursor adducts in human blood or tissue samples. Although NNK is also a methylating agent, and approaches towards measurement of O^6-methylguanine in human DNA are promising, we favour the quantification of *9* or related materials (Foiles *et al*, 1988). There are numerous environmental or endogenous sources of DNA and globin methylation. In contrast, the measurement of compounds such as *9* is more likely to be unambiguously related to TSNA. Because these nicotine derived compounds occur only in tobacco products, these measurements can be specifically related to exposure to tobacco products and may provide a realistic index of susceptibility to tobacco associated cancers.

VI Approaches to prevention of TSNA-induced cancers

It is clear that the most effective way of preventing cancer induction by tobacco products and their constituents is to avoid using tobacco in any form, and to avoid exposure to environmental tobacco smoke. Educational measures and smoking cessation programmes have had a major impact in the US. Among males, the percentage of smokers has dropped from 52 to 33 over the period 1965–87. Among females the corresponding figures are 34% and 28% (US Department of Health and Human Services, 1989). An inverse relationship between the number of years of education and smoking

prevalence has been established (US Department of Health and Human Services, 1988a). Despite these gains and the attendant decrease in lung cancer incidence in young cohorts that has been observed since 1986, there are still more than 50 million smokers in the US and more than 10 million snuff-dippers (IARC, 1986; US Department of Health and Human Services, 1989). There are hundreds of millions of tobacco users worldwide. In developing countries especially, tobacco use is on the rise (Tominaga, 1986). Presently, there are 250 million smokers in mainland China where a major epidemic of lung cancer has been predicted (Peto, 1986; Crofton, 1987). These daunting statistics attest to the potency of nicotine as a habituating agent. There is a very low probability that tobacco use will be eliminated in the near future.

In view of this, one must consider devising methods to reduce exposure to carcinogenic constituents of tobacco smoke as a means of lowering cancer risk in those individuals who continue to use tobacco products. Epidemiological studies have demonstrated that smokers of filter cigarettes have a lower risk for developing cancer of the lung and larynx than those who use non-filter products (Wynder and Stellman, 1979; IARC, 1986). There is an inverse relationship between cancer risk and exposure to cigarette smoke total particulate matter (IARC, 1986). Based on the arguments presented in this paper, we assume that part of this decreased risk is due to reduced exposure to TSNA, the levels of which are decreased with filtration to approximately the same extent as other constituents of total particulate matter (Adams *et al*, 1987). The further reduction of TSNA levels in tobacco and tobacco smoke should be a major priority. Standards for permissible levels of TSNA in tobacco products should be developed by national and international public health agencies. Continuing research on the formation of TSNA has confirmed our initial finding that they are produced primarily during the curing and processing of tobacco (Hecht and Hoffmann, 1988; Andersen *et al*, 1989; Djordjevic *et al*, 1989a). Tobacco blends made with Burley stems, which have a high nitrate content, have higher levels of TSNA than those products with lower nitrate (Brunnemann *et al*, 1983). International comparisons of TSNA levels in products such as snuff have clearly shown that processing techniques can lead to significant reductions in the levels of these carcinogens in tobacco (Brunnemann *et al*, 1985). From 26–46% of the NNN and NNK in tobacco is transferred into mainstream cigarette smoke while the remainder that is found in smoke is formed by the reaction of alkaloid precursors with nitrogen oxides (Hoffmann *et al*, 1980; Adams *et al*, 1983). Nitrate content of tobacco is correlated with TSNA levels in mainstream smoke (Adams *et al*, 1984). Thus, the use of lower nitrate blends would lead to lower levels of TSNA in smoke (Hecht and Hoffmann, 1988). However, it should be noted that this strategy may lead to increased levels of polynuclear aromatic hydrocarbons in mainstream smoke (Hoffmann and Rathkamp, 1968).

Chemopreventive agents which inhibit TSNA-induced cancers are being developed. One promising candidate is phenethyl isothiocyanate (PEITC). In

mice PEITC virtually completely inhibited NNK-induced lung tumours as well as O^6-methylguanine formation in lung DNA (Morse *et al*, 1989a). In rats treated with NNK, PEITC caused a 50% decrease in lung tumour incidence but had no effect on tumour induction in the liver and nasal cavity (Morse *et al*, 1989b). PEITC appears to have minimal toxic effects in rats at doses where inhibition of NNK tumorigenesis is observed. Thus, PEITC is a promising lead compound upon which further structure-activity studies are being based. These studies should lead to new insights on the mechanisms by which TSNA induce cancers in laboratory animals and on methods for inhibiting the carcinogenic process.

The design of intervention studies in tobacco users can be envisaged. Groups of subjects who are using products with lower levels of TSNA, or who are taking non-toxic chemopreventive agents, can be studied. Initially, it should be possible to measure levels of TSNA haemoglobin adducts in order to assess the potential effectiveness of these strategies. Subsequently, it would be desirable to carry out prospective trials, as are presently being done with β-carotene and related compounds (US Department of Health and Human Services, 1988b), in order to determine whether cancer incidence would be decreased.

VII Conclusions

Tobacco use, especially smoking cigarettes, is a major cause of cancer in developed countries. It has been estimated that between 1960 and 1975 30% of all male cancers and 7% of all female cancers in England (Birmingham region) and 28% of all male cancers and 8% of all female cancers in the USA can be attributed to the use of tobacco (Higginson and Muir, 1979; Wynder and Gori, 1977). Doll and Peto (1981) attribute 25–40% of all cancer in the USA to tobacco use. The sites that are most at risk for cancer in tobacco users are lung, upper respiratory and upper digestive tract, pancreas, kidney and urinary bladder. These epidemiological data are supported by chemical and biochemical data and by in vitro and in vivo bioassays.

In this overview we have discussed the concept that the tobacco-specific alkaloid-derived nitrosamines (TSNA) contribute appreciably to cancer of the oral cavity in snuff-dippers and to cancer of the lung and oesophagus in tobacco smokers (Table 4). They also play a part in cancer of the pancreas and nasal cavity in cigarette smokers and possibly in users of smokeless tobacco. The concept that TSNA have a major role in the association of tobacco and cancer is based primarily on the following evidence: NNN, NNK and NNAL are powerful organospecific carcinogens in laboratory animals; their levels in tobacco smoke and smokeless tobacco are comparable to bioassay doses which induce tumours. In laboratory animals, TSNA are metabolically activated to electrophiles which react with nucleophilic centres in DNA. Such DNA adducts can cause miscoding of the DNA which, in turn, causes activation of specific oncogenes. Comparable biochemical processes have been observed in tissue explants from tobacco smokers.

Table 4. The role of TSNA in tobacco-induced cancer in humans

Cancer site	*Cause or association*	*Evidence for TSNA as causative factors*
Oral cavity	Snuff dipping and betel quid chewing	Strong. NNK and NNN are the only tobacco constituents known to cause oral tumours in laboratory animals. Human exposure levels are comparable to levels of NNK and NNN that cause tumours in animals
Lung	Cigarette smoking	Highly suggestive. NNK is a powerful lung carcinogen in all species tested. Human exposure levels are comparable to levels that cause tumours in laboratory animals. Parallel activation mechanisms of NNK in humans and laboratory animals
Oesophagus	Cigarette smoking	Suggestive. Among the constituents of tobacco smoke that cause oesophageal tumours in rats, NNN occurs at the highest levels
Pancreas	Cigarette smoking	Limited but suggestive. NNK and NNAL are the only tobacco constituents known to induce tumours of the exocrine pancreas in laboratory animals
Nasal cavity	Cigarette smoking and snuff dipping	Tentative. Oral administration of NNN to rats causes squamous cell carcinoma of the nasal cavity similar to that seen in humans

In future studies, emphasis should be placed on delineating the endogenous formation of TSNA in chewers and smokers and developing biological markers for the dosimetry of TSNA exposure of humans. Although the only safe way to prevent the occurrence of tobacco-related cancers is cessation of tobacco use, chemopreventive measures are feasible to lower the risk for those who do not cease the tobacco habit.

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(The authors are responsible for the accuracy of the references.)