# TOBACCO AND CANCER

## Perspectives in Preventive Research

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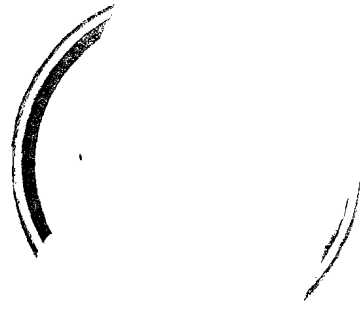

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# PREFORMED NITROSAMINES IN SMOKELESS TOBACCO.

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## INTRODUCTION

The expression 'smokeless tobacco' is used to describe a variety of products and mixtures that contain tobacco as the principal constituent and are used without combustion by placing in the oral cavity or by placing in, or inhaling into the nasal cavity. In 1985, The International Agency for Research on Cancer concluded that 'there is sufficient evidence that oral use of snuffs of the types used in North America and Western Europe is carcinogenic to humans' (1). A view which was independently reached in America one year later (2). Various forms of tobacco smoking have been attributed as causal agents for the occurrence of malignant tumours of the respiratory and digestive tracts as well as malignant tumours of the bladder, renal pelvis and pancreas in cigarette smokers (3). Epidemiological evidence also relates cigarette smoking with cancers of the cervix and nasal cavity (4).

A considerable number of compounds in tobacco have been shown to be carcinogenic in experimental animals including N-nitroso compounds, polycyclic aromatic hydrocarbons and the radioactive element polonium-210. On a quantitative basis, N-nitroso compounds are the most abundant chemical carcinogens present in tobacco (5).

## N-NITROSO COMPOUNDS PRESENT IN TOBACCO

N-Nitroso compounds in smokeless tobacco can be divided into three main classes depending on their physical properties. The so-called tobacco-specific nitrosamines (TSNA) result from the nitrosation of nicotine and minor tobacco alkaloids also present in tobacco. N-Nitrosonornicotine (NNN), 4-(N-nitrosomethylamino)-1-(3-pyridyl)-1-butanone (NNK) and two isomeric alcohols 4-(N-nitrosomethylamino)-4-(3-pyridyl)-1-butanol (iso-NNAL). and 4-(N-nitrosomethylamino)-1(3-pyridyl)-1-butanol (NNAL) result from the nitrosation of nicotine. The nitrosation of secondary tobacco alkaloids produces the corresponding N-nitroso analogues, nornicotine yields NNN, whilst N-nitrosoanabatine (NAB) and N-nitrosoanatabine (NAT) are formed from the nitrosation of anabasine and anatabine as shown in figure 1.

The nitrosation of simple dialkylamines and low molecular weight nitrogen containing heterocyclic compounds produces volatile N-nitroso compounds (VNA). Simple nitrosated

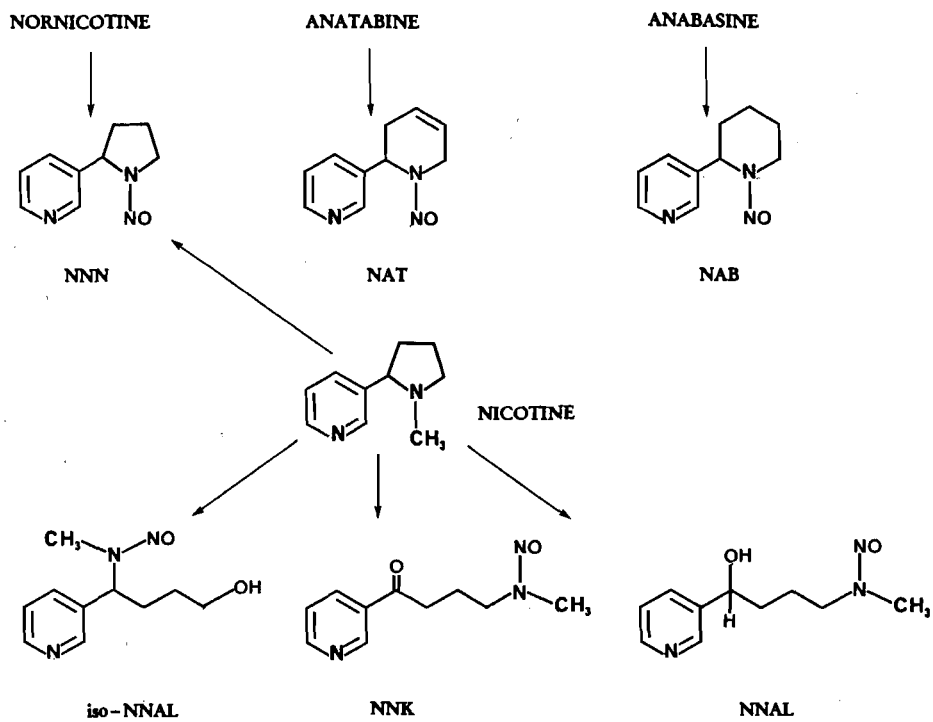


Fig. 1. Tobacco-specific N-nitroso compounds

derivatives of the dialkylamines dimethylamine (NDMA), ethylmethylamine (NEMA), diethylamine (NDEA), dipropylamine (NDPA), dibutylamine (NDBA) as well as the heterocyclic compounds piperidine (NPIP), pyrrolidine (NPYR) and morpholine (NMOR) have been found in cured tobacco. The structures of these compounds are shown in figure 2.

With the exception of NMOR, all the previously mentioned N-nitroso compounds result from the nitrosation of constituents naturally occurring in tobacco. The use of waxed cardboard containers and 'casing solutions', mixtures of hygroscopic agents containing volatile and nonvolatile flavouring components applied to snuff can result in both direct contamination of tobacco products with NMOR as well as a migratory source of morpholine which becomes nitrosated during prolonged contact with tobacco (6).

N-Nitrosodiethanolamine (NDELA) contamination of tobacco products results from the nitrosation of diethanolamine during the fermentation and storage of diethanolamine contaminated tobacco. In this case, contamination of tobacco occurs as a result of the use of maleic hydrazide formulated as the diethanolamine salt as a plant sucker growth when inhibitor (7).

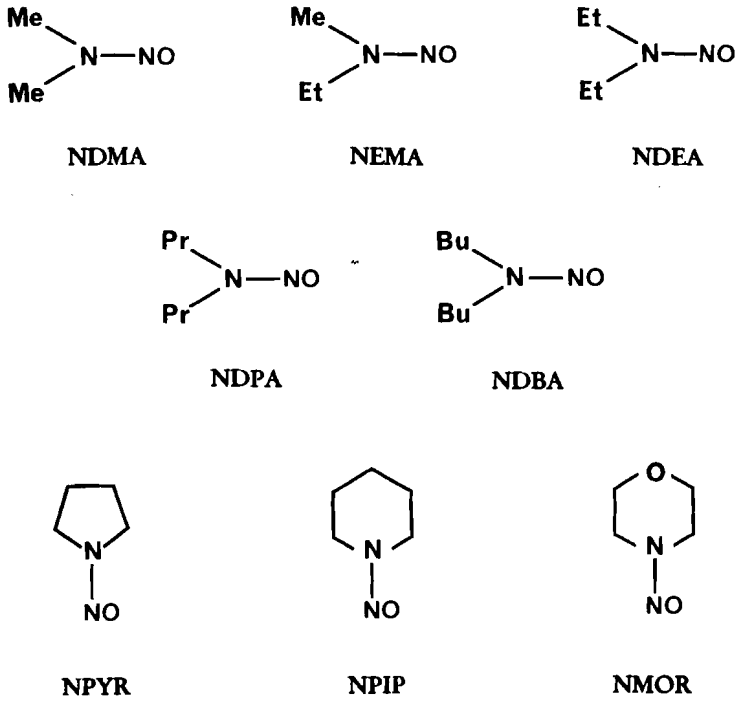


Fig. 2. Volatile N-nitroso compounds in tobacco

In 1981, the use of diethanolamine salts of maleic hydrazide for tobacco management was banned in the United States and in several other tobacco growing countries. As a result, NDELA contamination of tobacco products has steadily decreased from the pre 1981 levels (<6800μg/kg) to almost undetected in most tobacco products available in 1988. In the very few samples of tobacco still containing NDELA, the contamination is probably due to the incorporation of aged tobaccos (harvested prior to 1981) into tobacco products.

A wide range of nonvolatile N-nitroso compounds (NVNA) which result from the nitrosation of amino acids and their derivatives have recently been identified in tobacco. N-Nitrosarcosine (NSAR) is the simplest member of an open chain homologus series which includes 3-(N-nitroso-N-methylamino)propionic acid (NMPA) and 4-(N-nitroso-N-methylamino)butyric acid (NMBA). N-Nitrosoazetadine-2-carboxylic acid (NAzCA) is the simplest member of a homologous series of heterocyclic compounds which includes N-nitrosoproline (NPRO) and N-nitrosopipecolic acid (NPIC). N-Nitroso-pyrrolidine-2-acetic acid (NPYRAC), its higher homolog N-nitrosopiperidine-2-acetic acid

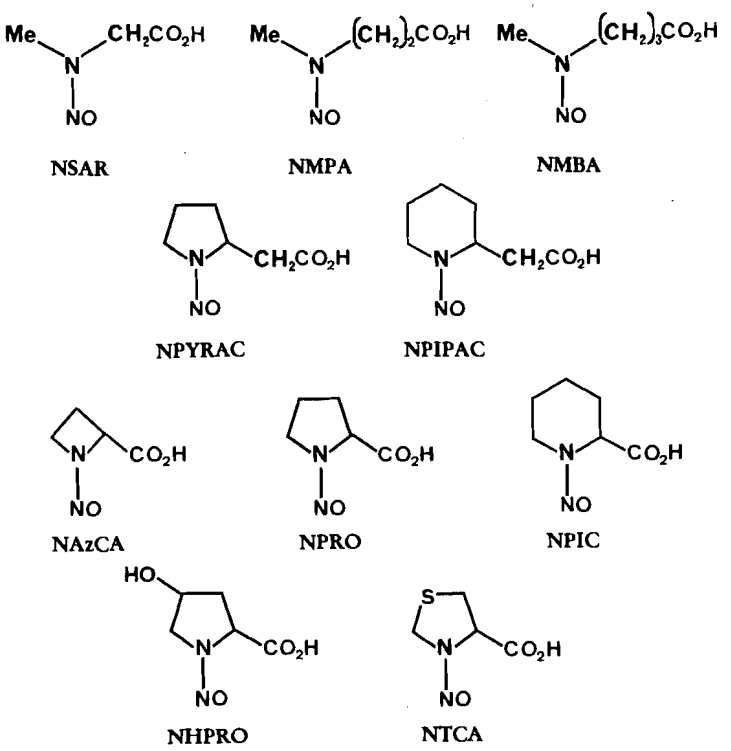


Fig. 3. Nonvolatile N-nitroso compounds in tobacco

(NPIPAC), N-nitrosohydroxyproline (NHPRO) and N-nitrosothiazolidine-4-carboxylic acid (NTCA) are also present in tobacco. The structures of these nonvolatile compounds are shown in figure 3.

The presence of TSNA (8-10), NVNA excluding NDELA (11), NDELA (7) and VNA (6) have been extensively reported in tobacco products. However, data for the presence of more than one group of N-nitroso compounds in tobacco products has been less seldom reported (11-14). Of which, the reviewed data for the presence of 15 different N-nitroso compounds in a variety of tobacco products by Brunnemann et al. (12) provides some of the most extensive data available. We have recently determined the presence of 22 different N-nitroso compounds in a variety of tobacco products including chewing tobacco, dry and moist snuffs (15), zarda (16) and kiwam (17).

The Federal Republic of Germany is the largest producer of nasal snuff (about 250 tonnes per year), followed by the United Kingdom (about 150 tonnes per year) (18). Representative data for the occurrence of N-nitroso compounds in dry nasal snuffs are presented in table I.

TABLE I

N-NITROSO COMPOUNDS IN NASAL SNUFF TOBACCO

| Nitrosamine | Concentration range (μg/kg) | | | |
|---|---|---|---|---|
| | English snuff | | German snuff | |
| | Mean | (Range) | Mean | (Range) |
| NDMA | 25 | (4.5-82) | 16 | (2.0-42) |
| NEMA | 2.0 | (ND[1]-8.5) | 1.0 | (ND-3.0) |
| NDPA | 1.0 | (ND-2.5) | 0.2 | (ND-1.0) |
| NPYR | 58 | (1.5-130) | 31 | (5.0-75) |
| NDELA | 16 | (ND-42) | 10 | (ND-20) |
| NSAR | 14 | (ND-35) | 27 | (ND-85) |
| NMPA | 1630 | (960-2760) | 1620 | (490-4260) |
| NMBA | 170 | (96-280) | 160 | (76-410) |
| NPRO | 5340 | (2710-8730) | 3570 | (770-7500) |
| NPIC | 99 | (ND-310) | 63 | (ND-190) |
| NTCA | 3.0 | (ND-15) | 12 | (ND-46) |
| NHPRO | 120 | (46-270) | 74 | (58-94) |
| Iso-NNAL | 660 | (ND-1590) | 62 | (ND-200) |
| NAB/NAT | 3180 | (1760-2450) | 3060 | (1030-5840) |
| NNN | 7690 | (3000-15700) | 7980 | (2830-18800) |
| NNK | 1860 | (970-4320) | 3010 | (580-6430) |
| TOTAL | 20900 | (11700-41900) | 18700 | (7200-42500) |

[1]ND not detected (<0.5μg/kg for VNA and 0.1μg/kg for NVNA and TSNA). Results based on the analysis of 5 differnt brands of English snuff and 7 different brands of German snuff.

Only one other report is available for dry nasal snuffs in which only the presence of TSNA in American snuffs are reported (10). From the limited data available (analysis of only 3 samples), it would appear that American dry snuffs contain much higher TSNA levels (30.8-111μg/g) than both English and German snuffs. The levels reported for individual TSNA were; 0.66-1.17μg/g NAB, 18.8-40.2μg/g NAT, 9.37-55.3μg/g NNN and 1.98-14.4μg/g NNK.

Representative data for the occurrence of N-nitroso compounds in English and Swedish moist snuffs, and zarda, a scented Indian chewing tobacco are presented in table II. Zarda is a partially fermented tobacco produced by boiling small pieces of tobacco leaves in water with various spices and lime until evaporation. The tobacco residue is coloured with vegetable dyes and perfume is added to produce a scented tobacco which is either chewed alone, or added to betel quid (19).

TABLE II

N-NITROSO COMPOUNDS IN ORAL TOBACCOS

| Nitrosamine | Concentration range (μg/kg) | | | | | |
|---|---|---|---|---|---|---|
| | English moist snuff (7 samples) | | Swedish moist snuff (5 samples) | | Indian zarda (11 samples) | |
| | Mean | (Range) | Mean | (Range) | Mean | (Range) |
| NDMA | 40 | (6.0-82) | 1.5 | (1.0-2.5) | 11 | (2.0-31) |
| NEMA | 1.5 | (ND-3.0) | ND[1] | | 1.0 | (ND-2.0) |
| NPIP | 20 | (ND-40) | ND | | 0.3 | (ND-2.0) |
| NPYR | 270 | (64-860) | 5.0 | (4.5-6.0) | 100 | (6.0-690) |
| NMOR | 0.5 | (ND-1.5) | 1.0 | (ND-1.0) | ND | |
| NDELA | 230 | (ND-740) | 19 | (8-31) | 9.5 | (ND-54) |
| NSAR | 310 | (29-1050) | 19 | (8-31) | 49 | (ND-350) |
| NMPA | 6890 | (1360-18600) | 1340 | (1040-1820) | 2050 | (22-18000) |
| NMBA | 2120 | (62-8030) | 70 | (53-94) | 170 | (ND-2040) |
| NAzCA | ND | | ND | | 18 | (ND-140) |
| NPRO | 2260 | (330-4950) | 1100 | (630-1820) | 2850 | (280-18000) |
| NPIC | 900 | (83-2360) | 36 | (ND-130) | 260 | (ND-2040) |
| NTCA | 19 | (ND-69) | 21 | (ND-69) | 48 | (ND-280) |
| NHPRO | 410 | (92-730) | 140 | (ND-230) | 69 | (ND-190) |
| Iso-NNAL | 76 | (ND-150) | 27 | (ND-80) | 1420 | (120-8100) |
| NAB/NAT | 27620 | (1980-65100) | 2640 | (1650-3250) | 16030 | (780-99100) |
| NNN | 22670 | (1090-51500) | 3360 | (2100-4800) | 13420 | (400-79000) |
| NNK | 4900 | (400-13300) | 790 | (400-1040) | 13420 | (220-24100) |
| TOTAL | 68900 | (6130-163700) | 9570 | (6220-12500) | 40500 | (1670-241000) |

[1]ND, not detected.

## EVALUATION OF NITROSAMINE EXPOSURE

If accurate data is available for the average consumption of a particular tobacco product, it is possible to estimate a theoretical exposure to N-nitroso compounds occurring in different tobacco using populations as presented in table III. Exposure estimates of this kind, based on the average tobacco consumption multiplied by the mean concentrations of N-nitroso compounds found in a particular type of tobacco product assume that two hypothetical criteria are met. It is assumed that (i) quantitative extraction of N-nitroso compounds and (ii) adsorption of the extracted compounds occurs. This is clearly not the case. However, in vitro studies show that tobacco amines including the tobacco alkaloid nornicotine undergo nitrosation in human saliva (21) and under simulated gastric conditions (22), thus increasing the exposure

TABLE III

EXPOSURE[1] TO N-NITROSO COMPOUNDS IN ORAL TOBACCOS

| Nitrosamine | Calculated exposure | | | | | |
|---|---|---|---|---|---|---|
| | English moist snuff | | Swedish moist snuff | | Indian zarda | |
| | [Day] [μg] | [Year] [mg] | [Day] [μg] | [Year] [mg] | [Day] [μg] | [Year] [mg] |
| NDMA | 0.20 | 0.07 | 0.45 | 0.16 | 0.10 | 0.04 |
| NEMA | 0.007 | 0.003 | ND[2] | ND | 0.01 | 0.004 |
| NPIP | 0.09 | 0.03 | ND | ND | ND | ND |
| NPYR | 1.20 | 0.45 | 0.08 | 0.03 | 1.0 | 0.36 |
| NMOR | 0.002 | NC[3] | 0.016 | 0.006 | ND | ND |
| NDELA | 1.04 | 0.40 | 0.45 | 0.16 | 0.10 | 0.04 |
| NSAR | 1.40 | 0.50 | 0.30 | 0.10 | 0.50 | 0.20 |
| NMPA | 31.0 | 11.3 | 21.2 | 7.8 | 20.5 | 7.5 |
| NMBA | 9.5 | 3.5 | 1.1 | 0.4 | 1.7 | 0.6 |
| NAzCA | ND | ND | ND | ND | 0.20 | 0.07 |
| NPRO | 10.2 | 3.7 | 17.5 | 6.4 | 28.5 | 10.4 |
| NPIC | 4.1 | 1.5 | 0.60 | 0.20 | 2.60 | 0.95 |
| NTCA | 0.09 | 0.032 | 0.33 | 0.12 | 0.50 | 0.18 |
| NHPRO | 0.85 | 0.67 | 2.2 | 0.8 | 0.7 | 0.25 |
| Iso-NNAL | 0.34 | 0.13 | 0.43 | 0.16 | 14.2 | 5.2 |
| NAB/NAT | 124.0 | 45.4 | 42.0 | 15.3 | 160.0 | 59.0 |
| NNN | 103.0 | 37.2 | 53.4 | 19.5 | 134.0 | 49.0 |
| NNK | 22.0 | 8.1 | 12.6 | 4.6 | 40.0 | 14.7 |
| TOTAL | 310 | 113 | 152 | 55.5 | 405 | 148 |

[1]Exposures based on the following daily use: English moist snuff, 4.5g/day (20); Swedish moist snuff, 14.3g/day (13) and Indian zarda tobacco, 10g/day.

[2]ND, not detected; [2]NC, exposure not calculated: <0.001μg/day or 1μg/year.

of N-nitroso compounds which may under certain circumstances lead to an even higher exposure than that based on the above exposure estimate.

The analysis of saliva obtained from tobacco chewers shows that TSNA are rapidly extracted from oral tobacco products, even when placed in the mouth between the gum and cheek without chewing. Some recent data has been summarized in table IV. In the study on Inuit Indians (25), large differences were observed for the extraction of TSNA in snuff. Following 15 min snuff dipping of 0.5-1.5g tobacco, 22-fold differences in the amounts of NNN and 37-fold differences in the amounts of NAB/NAT in saliva were found. Unlike

most tobacco chewing populations, Inuit Indians do not spit out saliva during snuff dipping and most of the saliva produced during snuff taking is swallowed. The very high levels of TSNA found in tobacco samples used by Inuit Indians; 2090–79000μg/g NNN, 1580–170000μg/g NAT, 100–4800μg/g NAT and 240–5800μg/g NNK are responsible for the very high salivary TSNA levels as shown by a mean salivary NNN concentration of 980ng/g saliva. Using this mean value, and assuming an average consumption of 6.5 dips/day lasting 25 mins with a salivary flow of 1.17ml/min, then the amount of ingested NNN and NNK are in the order of 185μg/day and 11μg/day respectively. Under extreme conditions, the total exposure to TSNA approaches 440μg/day.

TABLE IV

TSNA LEVELS IN THE SALIVA OF HABITUAL TOBACCO CHEWERS

TSNA concentrations expressed as mean (range) ng/g saliva.

| Tobacco habit | NAT/(NAB)[1] | NNN | NNK | Reference |
|---|---|---|---|---|
| Snuff dipping women (USA) | 187 (12.5–470) | 154 (26–420) | 32.6 (<10–96) | 23 |
| Snuff dipping students (USA) | 204 (48–555) | 99 (37.4–222) | 3.4 (ND[2]–60.6) | 24 |
| Inuit snuff dippers (Canada) | 1318 (123–4560) | 980 (115–2601) | 56 (ND–201) | 25 |
| Snuff dippers (Sweden) | 18.5 (5–37) | 36.5 (6–65) | 2.6 (ND–9) | 26 |
| Betel quid + tobacco (India) | 4.8 (1.0–10.9) | 7.5 (1.6–14.7) | 0.3 (ND–2.3) | 27 |
| Tobacco chewers (India) | 29.8 (13.5–51.7) | 33.4 (16.5–59.7) | ND | 27 |
| Masheri, women (India) | ND | 28.3 (14.3–43.5) | ND | 28 |
| Tobacco + lime (India) | 30.4 (ND–133) | 113 (10–430) | 3.8 (ND–28.5) | 28 |

[1]Refers primarily to NAT, NAB may be present in some cases. [2]ND, not detected.

In a Swedish study, Österdahl and Slorach have also used salivary measurements of TSNA to calculate the average TSNA exposure of Swedish snuff dippers. The average levels of TSNA in the saliva of four snuff dippers during 30 mins snuff dipping ranged between 15–125 ng/g saliva. Assuming a salivary production of 60ml saliva/hour, then an exposure of between 0.9–7.5μg TSNA/hour during snuff dipping occurs. As snuff dippers often maintain snuff in the oral cavity for up to 15 hours per day, an exposure of around 110μg TSNA/day (74μg NNN+NNK/day) was calculated (26). It was also found that after removal of tobacco from the oral cavity, detectable levels of TSNA in saliva persist for about 20 mins. Our results, for the same population (presented in table III) which were calculated using a different method, estimated that the exposure to TSNA would be 108.4μg TSNA/day (66μg NNN+NNK/day).

## CARCINOGENICITY OF TOBACCO AND TOBACCO CONSTITUENTS

Three types of chemical carcinogens have been detected in tobacco: Polycyclic aromatic hydrocarbons, of which one example is benzo[a]pyrene; the radioactive alpha-emitting element $^{210}Po$ and N-nitroso compounds. The presence of benzo[a]pyrene at concentrations up to 63ng/g tobacco (5) is of the same magnitude as benzo[a]pyrene concentrations found in smoked foods (29,30), and at this low concentration is unlikely to present a significant carcinogenic risk. The alpha radiation emitted from 0.9-6.7pCi $^{210}Po$ found in moist snuff tobacco which is constantly held in one place between the cheek and gum by snuff dippers may be an important factor in snuff-induced carcinogenesis (31). N-Nitroso compounds, which are quantitatively the most abundant carcinogens present in tobacco are considered to be an important risk factor for tobacco related cancers. Over 300 different N-nitroso compounds tested in experimental animals have been shown to be carcinogenic causing a wide range of malignant tumours (32,33). The carcinogenicity in rats of N-nitroso compounds found in tobacco products following oral administration has been summarized in table V.

Carcinogenicity bioassays show that NNN and NNK are the strongest carcinogens amongst the tobacco-specific nitrosamines. The carcinogenic potency of NNK is particually notable, a single dose of 1mg (5mmol) is sufficient to induce respiratory tract tumours in 30% of treated Syrian golden hamsters (48). In rats, NNK is a more potent carcinogen that NDMA inducing lung tumours (13/27 rats) and nasal cavity tumours (6/27 rats) in addition to liver tumours produced by both carcinogens when administered at a total dose of 0.33mmol/kg (49). The organ specificity of NNN and NNK for the nasal cavity and lung respectively in experimental animals emphasises their importance in tobacco carcinogenesis. These two compounds are also effective local carcinogens, a property seldom seen for other nitrosamines (33). A mixture of NNN and NNK applied to the oral cavity in rats induces local tumours (8/30 rats), NNN and NNK applied together in an aqueous snuff extract resulted in a lower incidence of tumour induction in 3/30 rats (50). In the same bioassay, snuff extracts containing one tenth of the NNN and NNK content failed to produce local tumours suggesting that NNN and NNK induced oral carcinogenesis are modified by other agents also present in aqueous snuff extracts. Pretreatment of the rat oral cavity with snuff extracts prior to application of NNK results in reduced DNA methylation (51). However, the presence of tumour inhibitors present in aqueous snuff extracts (most probably polyphenols) does not prevent the induction of local oral cavity tumours in rats treated with whole snuff (50,52). Thus it appears that potentiating factors such as irritation, which has been shown to enhance the local induction of tumours by N-nitrosomethylurea in the Syrian golden hamster oral cavity (53), and Herpes simplex virus which enhances the tumourigenicity of snuff but not snuff extracts (54,55), may be important factors in tobacco carcinogenesis of the oral cavity.

TABLE V

CARCINOGENICITY IN RATS OF N-NITROSO COMPOUNDS FOUND IN TOBACCO PRODUCTS FOLLOWING ORAL ADMINISTRATION

| Nitrosamine | Principal target organs | Reference |
|---|---|---|
| NDELA | Liver, nasal cavity | 32,34,35 |
| NDMA | Liver, kidney, (lung) | 36 |
| NEMA | Liver, nasal cavity, (oesophagus) | 37 |
| NDEA | Liver, kidney, (oesophagus) | 36 |
| NDPA | Liver, oesophagus, (tongue) | 32 |
| NDBA | Liver, urinary bladder, (oesophagus, pharynx) | 36 |
| NPIP | Liver, oesophagus | 32 |
| NPYR | Liver | 32 |
| NMOR | Liver, (kidney) | 36 |
| NSAR | Oesophagus | 32 |
| NMPA | Suspected carcinogen | |
| NMBA | Urinary bladder | 38 |
| NAzCA | Unknown | |
| NPRO | Negative | 39-41 |
| NPIC | Negative | 39 |
| NPYRAC | Unknown | |
| NPIPAC | Unknown | |
| NTCA | Unknown | |
| NHPRO | Negative (Spleen ?) | 40 (42) |
| NAB | Oesophagus | 43,44 |
| NAT[1] | Negative | 45 |
| NNN | Oesophagus, nasal cavity | 44 |
| NNK[1] | Liver, lung, nasal cavity | 46 |
| iso-NNAL | Unknown | |

[1]No data for oral administration, tested by s.c. injection.

## ORAL TOBACCO USE AND HUMAN CANCER

The strongest evidence that smokeless tobacco use causes human cancer comes from epidemiological studies on the association of snuff dipping with oral cancer (56). Epidemiological studies (reviewed in ref 1 and 2) are supported by medical case reports that describe oral can-

cers occuring precisely where tobacco is placed in the oral cavity (57–60).

Epidemiological studies are further supported by cancer risk evaluations based on the exposure to TSNA in oral tobacco products. The exposure to NNN and NNK for some tobacco using population are as follows: Swedish snuff dippers; 66μg NNN+NNK/day (table III) and 74μg NNN+NNK/day (26) respectively; Indian zarda chewers, 174μg NNN+NNK/day (table III); Inuit snuff dippers, 196μg NNN+NNK/day (25). In rats, a total dose of 1.6mmol/kg (ca. 295mg/kg) of NNN and NNK applied to the oral cavity is sufficient to induce a significant incidence of oral tumours (50). After 40 years of snuff dipping, the exposure to NNN and NNK in the three different tobacco using populations would be as follows: Swedish snuff dippers, 0.07mmol/kg; Indian zarda chewers, 0.18mmol/kg and Inuit snuff dippers, 0.20mmol/kg. This exposure is approximately 10% of the total dose shown to cause oral cavity cancers in experimental animals.

These calculations are probably underestimates of the actual exposure since the possibility of endogenous TSNA formation, and in particular NNN formation (22), is not taken into consideration. However, both epidemiological and scientific data clearly show that the oral use of tobacco presents an unacceptable cancer risk to the tobacco consumer.

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