INTERNATIONAL AGENCY FOR RESEARCH ON CANCER

IARC MONOGRAPHS

ON THE

EVALUATION OF THE CARCINOGENIC RISK OF CHEMICALS TO HUMANS

Tobacco Habits Other than Smoking; Betel-Quid and Areca-Nut Chewing; and Some Related Nitrosamines

VOLUME 37

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September 1985

WORLD HEALTH ORGANIZATION

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This publication represents the views and expert opinions
of an IARC Working Group on the
Evaluation of the Carcinogenic Risk of Chemicals to Humans
which met in Lyon,

23-30 October 1984

September 1985

IARC MONOGRAPHS

In 1969, the International Agency for Research on Cancer (IARC) initiated a programme on the evaluation of the carcinogenic risk of chemicals to humans involving the production of critically evaluated monographs on individual chemicals. In 1980, the programme was expanded to include the evaluation of the carcinogenic risk associated with exposures to complex mixtures.

The objective of the programme is to elaborate and publish in the form of monographs critical reviews of data on carcinogenicity for chemicals and complex mixtures to which humans are known to be exposed, and on specific occupational exposures, to evaluate these data in terms of human risk with the help of international working groups of experts in chemical carcinogenesis and related fields, and to indicate where additional research efforts are needed.

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NOTE TO THE READER

The term 'carcinogenic risk' in the *IARC Monographs* series is taken to mean the probability that exposure to the chemical will lead to cancer in humans.

Inclusion of a chemical in the monographs does not imply that it is a carcinogen, only that the published data have been examined. Equally, the fact that a chemical has not yet been evaluated in a monograph does not mean that it is not carcinogenic.

Anyone who is aware of published data that may alter the evaluation of the carcinogenic risk of a chemical to humans is encouraged to make this information available to the Unit of Carcinogen Identification and Evaluation, Division of Environmental Carcinogenesis, International Agency for Research on Cancer, 150 cours Albert Thomas, 69372 Lyon Cedex 08, France, in order that the chemical may be considered for re-evaluation by a future Working Group.

Although every effort is made to prepare the monographs as accurately as possible, mistakes may occur. Readers are requested to communicate any errors to the Unit of Carcinogen Identification and Evaluation, so that corrections can be reported in future volumes.

IARC WORKING GROUP ON THE EVALUATION OF THE CARCINOGENIC RISK OF CHEMICALS TO HUMANS: TOBACCO HABITS OTHER THAN SMOKING; BETEL-QUID AND ARECA-NUT CHEWING; AND SOME RELATED NITROSAMINES

Lyon, 23-30 October 1984

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IARC MONOGRAPHS PROGRAMME ON THE EVALUATION OF THE CARCINOGENIC RISK OF CHEMICALS TO HUMANS[1]

PREAMBLE

1. BACKGROUND

In 1969, the International Agency for Research on Cancer (IARC) initiated a programme to evaluate the carcinogenic risk of chemicals to humans and to produce monographs on individual chemicals. Following the recommendations of an ad-hoc Working Group, which met in Lyon in 1979 to prepare criteria to select chemicals for *IARC Monographs* (1), the *Monographs* programme was expanded to include consideration of exposures to complex mixtures which may occur, for example, in many occupations or as a result of human habits.

The criteria established in 1971 to evaluate carcinogenic risk to humans were adopted by all the working groups whose deliberations resulted in the first 16 volumes of the *IARC Monographs* series. This preamble reflects subsequent re-evaluation of those criteria by working groups which met in 1977(2), 1978(3), 1982(4) and 1983(5).

2. OBJECTIVE AND SCOPE

The objective of the programme is to elaborate and publish in the form of monographs critical reviews of data on carcinogenicity for chemicals, groups of chemicals, industrial processes and other complex mixtures to which humans are known to be exposed, to evaluate the data in terms of human risk with the help of international working groups of experts, and to indicate where additional research efforts are needed. These evaluations are intended to assist national and international authorities in formulating decisions concerning preventive measures. No recommendation is given concerning legislation, since this depends on risk-benefit evaluations, which seem best made by individual governments and/or other international agencies.

The *IARC Monographs* are recognized as an authoritative source of information on the carcinogenicity of environmental and other chemicals. A users' survey, made in 1984, indicated that the monographs are consulted by various agencies in 45 countries. As of July

[1]This project is supported by PHS Grant No. 1 U01 CA33193-03 awarded by the US National Cancer Institute, Department of Health and Human Services.

1985, 37 volumes of the *Monographs* had been published or were in press. Four supplements have been published: two summaries of evaluations of chemicals associated with human cancer, an evaluation of screening assays for carcinogens, and a cross index of synonyms and trade names of chemicals evaluated in the series(6).

3. SELECTION OF CHEMICALS AND COMPLEX EXPOSURES FOR MONOGRAPHS

The chemicals (natural and synthetic, including those which occur as mixtures and in manufacturing processes) and complex exposures are selected for evaluation on the basis of two main criteria: (a) there is evidence of human exposure, and (b) there is some experimental evidence of carcinogenicity and/or there is some evidence or suspicion of a risk to humans. In certain instances, chemical analogues are also considered. The scientific literature is surveyed for published data relevant to the *Monographs* programme; and the IARC *Survey of Chemicals Being Tested for Carcinogenicity*(7) often indicates those chemicals that may be scheduled for future meetings.

As new data on chemicals for which monographs have already been prepared become available, re-evaluations are made at subsequent meetings, and revised monographs are published.

4. WORKING PROCEDURES

Approximately one year in advance of a meeting of a working group, a list of the substances or complex exposures to be considered is prepared by IARC staff in consultation with other experts. Subsequently, all relevant biological data are collected by IARC; recognized sources of information on chemical carcinogenesis and systems such as CANCERLINE, MEDLINE and TOXLINE are used in conjunction with US Public Health Service Publication No. 149(8). The major collection of data and the preparation of first drafts for the sections on chemical and physical properties, on production and use, on occurrence, and on analysis are carried out by SRI International, Menlo Park, CA, USA, under a separate contract with the US National Cancer Institute. Most of the data so obtained refer to the USA and Japan; IARC supplements this information with that from other sources in Europe. Representatives from industrial associations may assist in the preparation of sections describing industrial processes. Bibliographical sources for data on mutagenicity and teratogenicity are the Environmental Mutagen Information Center and the Environmental Teratology Information Center, both located at the Oak Ridge National Laboratory, Oak Ridge, TN, USA.

Six months before the meeting, reprints of articles containing relevant biological data are sent to an expert(s), or are used by IARC staff, to prepare first drafts of monographs. These drafts are then compiled by IARC staff and sent, prior to the meeting, to all participants of the Working Group for their comments.

The Working Group then meets in Lyon for seven to eight days to discuss and finalize the texts of the monographs and to formulate the evaluations. After the meeting, the master copy of each monograph is verified by consulting the original literature, then edited by a professional editor and prepared for reproduction. The aim is to publish monographs within nine months of the Working Group meeting. Each volume of monographs is printed in 4000 copies for distribution to governments, regulatory agencies and interested scientists. The monographs are also available *via* the WHO Distribution and Sales Service.

5. DATA FOR EVALUATIONS

With regard to biological data, only reports that have been published or accepted for publication are reviewed by the working groups, although a few exceptions have been made: in certain instances, reports from government agencies that have undergone peer review and are widely available are considered. The monographs do not cite all of the literature on a particular chemical or complex exposure: only those data considered by the Working Group to be relevant to the evaluation of carcinogenic risk to humans are included.

Anyone who is aware of data that have been published or are in press which are relevant to the evaluations of the carcinogenic risk to humans of chemicals or complex exposures for which monographs have appeared is asked to make them available to the Unit of Carcinogen Identification and Evaluation, Division of Environmental Carcinogenesis, International Agency for Research on Cancer, Lyon, France.

6. THE WORKING GROUP

The tasks of the Working Group are five-fold: (a) to ascertain that all data have been collected; (b) to select the data relevant for evaluation; (c) to ensure that the summaries of the data enable the reader to follow the reasoning of the Working Group; (d) to judge the significance of the results of experimental and epidemiological studies; and (e) to make an evaluation of the carcinogenicity of the chemical or complex exposure.

Working Group participants who contributed to the consideration and evaluation of chemicals or complex exposures within a particular volume are listed, with their addresses, at the beginning of each publication. Each member serves as an individual scientist and not as a representative of any organization or government. In addition, observers are often invited from national and international agencies and industrial associations.

7. GENERAL PRINCIPLES APPLIED BY THE WORKING GROUP IN EVALUATING CARCINOGENIC RISK OF CHEMICALS OR COMPLEX MIXTURES

The widely accepted meaning of the term 'chemical carcinogenesis', and that used in these monographs, is the induction by chemicals (or complex mixtures of chemicals) of neoplasms that are not usually observed, the earlier induction of neoplasms that are commonly observed, and/or the induction of more neoplasms than are usually found - although fundamentally different mechanisms may be involved in these three situations. Etymologically, the term 'carcinogenesis' means the induction of cancer, that is, of malignant neoplasms; however, the commonly accepted meaning is the induction of various types of neoplasms or of a combination of malignant and benign tumours. In the monographs, the words 'tumour' and 'neoplasm' are used interchangeably. (In the scientific literature, the terms 'tumorigen', 'oncogen' and 'blastomogen' have all been used synonymously with 'carcinogen', although occasionally 'tumorigen' has been used specifically to denote a substance that induces benign tumours.)

(*a*) *Experimental Evidence*

(i) *Evidence for carcinogenicity in experimental animals*

The Working Group considers various aspects of the experimental evidence reported in the literature and formulates an evaluation of that evidence.

Qualitative aspects: Both the interpretation and evaluation of a particular study as well as the overall assessment of the carcinogenic activity of a chemical (or complex mixture) involve several considerations of qualitative importance, including: (a) the experimental parameters under which the chemical was tested, including route of administration and exposure, species, strain, sex, age, etc.; (b) the consistency with which the chemical has been shown to be carcinogenic, e.g., in how many species and at which target organ(s); (c) the spectrum of neoplastic response, from benign neoplasm to multiple malignant tumours; (d) the stage of tumour formation in which a chemical may be involved: some chemicals act as complete carcinogens and have initiating and promoting activity, while others may have promoting activity only; and (e) the possible role of modifying factors.

There are problems not only of differential survival but of differential toxicity, which may be manifested by unequal growth and weight gain in treated and control animals. These complexities are also considered in the interpretation of data.

Many chemicals induce both benign and malignant tumours. Among chemicals that have been studied extensively, there are few instances in which the neoplasms induced are only benign. Benign tumours may represent a stage in the evolution of a malignant neoplasm or they may be 'end-points' that do not readily undergo transition to malignancy. If a substance is found to induce only benign tumours in experimental animals, it should nevertheless be suspected of being a carcinogen, and it requires further investigation.

Hormonal carcinogenesis: Hormonal carcinogenesis presents certain distinctive features: the chemicals involved occur both endogenously and exogenously; in many instances, long exposure is required; and tumours occur in the target tissue in association with a stimulation of non-neoplastic growth, although in some cases hormones promote the proliferation of tumour cells in a target organ. For hormones that occur in excessive amounts, for hormone-mimetic agents and for agents that cause hyperactivity or imbalance in the endocrine system, evaluative methods comparable with those used to identify chemical carcinogens may be required; particular emphasis must be laid on quantitative aspects and duration of exposure. Some chemical carcinogens have significant side effects on the endocrine system, which may also result in hormonal carcinogenesis. Synthetic hormones and anti-hormones can be expected to possess other pharmacological and toxicological actions in addition to those on the endocrine system, and in this respect they must be treated like any other chemical with regard to intrinsic carcinogenic potential.

Complex mixtures: There is an increasing amount of data from long-term carcinogenicity studies on complex mixtures and on crude materials obtained by sampling in occupational environments. The representativity of such samples must be considered carefully.

Quantitative aspects: Dose-response studies are important in the evaluation of carcinogenesis: the confidence with which a carcinogenic effect can be established is strengthened by the observation of an increasing incidence of neoplasms with increasing exposure.

The assessment of carcinogenicity in animals is frequently complicated by recognized differences among the test animals (species, strain, sex, age), and route and schedule of administration; often, the target organ at which a cancer occurs and its histological type may vary with these parameters. Nevertheless, indices of carcinogenic potency in particular experimental systems (for instance, the dose-rate required under continuous exposure to halve the probability of the animals remaining tumourless(9)) have been formulated in the hope that, at least among categories of fairly similar agents, such indices may be of some predictive value in other species, including humans.

Chemical carcinogens share many common biological properties, which include metabolism to reactive (electrophilic(10-11)) intermediates capable of interacting with DNA. However, they may differ widely in the dose required to produce a given level of tumour induction. The reason for this variation in dose-response is not understood, but it may be due to differences in metabolic activation and detoxification processes, in different DNA repair capacities among various organs and species or to the operation of qualitatively distinct mechanisms.

Statistical analysis of animal studies: It is possible that an animal may die prematurely from unrelated causes, so that tumours that would have arisen had the animal lived longer may not be observed; this possibility must be allowed for. Various analytical techniques have been developed which use the assumption of independence of competing risks to allow for the effects of intercurrent mortality on the final numbers of tumour-bearing animals in particular treatment groups.

For externally visible tumours and for neoplasms that cause death, methods such as Kaplan-Meier (i.e., 'life-table', 'product-limit' or 'actuarial') estimates(9), with associated significance tests(12,13), have been recommended. For internal neoplasms that are discovered 'incidentally'(12) at autopsy but that did not cause the death of the host, different estimates(14) and significance tests(12,13) may be necessary for the unbiased study of the numbers of tumour-bearing animals.

The design and statistical analysis of long-term carcinogenicity experiments were reviewed in Supplement 2 to the *Monographs* series(15). That review outlined the way in which the context of observation of a given tumour (fatal or incidental) could be included in an analysis yielding a single combined result. This method requires information on time to death for each animal and is therefore comparable to only a limited extent with analyses which include global proportions of tumour-bearing animals.

Evaluation of carcinogenicity studies in experimental animals: The evidence of carcinogenicity in experimental animals is assessed by the Working Group and judged to fall into one of four groups, defined as follows:

(1) *Sufficient evidence* of carcinogenicity is provided when there is an increased incidence of malignant tumours: (a) in multiple species or strains; or (b) in multiple experiments (preferably with different routes of administration or using different dose levels); or (c) to an unusual degree with regard to incidence, site or type of tumour, or age at onset. Additional evidence may be provided by data on dose-response effects.

(2) *Limited evidence* of carcinogenicity is available when the data suggest a carcinogenic effect but are limited because: (a) the studies involve a single species, strain or experiment; or (b) the experiments are restricted by inadequate dosage levels,

inadequate duration of exposure to the agent, inadequate period of follow-up, poor survival, too few animals, or inadequate reporting; or (c) the neoplasms produced often occur spontaneously and, in the past, have been difficult to classify as malignant by histological criteria alone (e.g., lung adenomas and adenocarcinomas, and liver tumours in certain strains of mice).

(3) *Inadequate evidence* is available when, because of major qualitative or quantitative limitations, the studies cannot be interpreted as showing either the presence or absence of a carcinogenic effect.

(4) *No evidence* applies when several adequate studies are available which show that, within the limits of the tests used, the chemical or complex mixture is not carcinogenic.

It should be noted that the categories *sufficient evidence* and *limited evidence* refer only to the strength of the experimental evidence that these chemicals or complex mixtures are carcinogenic and not to the extent of their carcinogenic activity nor to the mechanism involved. The classification of any chemical may change as new information becomes available.

(ii) *Evidence for activity in short-term tests*[1]

Many short-term tests bearing on postulated mechanisms of carcinogenesis or on the properties of known carcinogens have been developed in recent years. The induction of cancer is thought to proceed by a series of steps, some of which have been distinguished experimentally(16-20). The first step — initiation — is thought to involve damage to DNA, resulting in heritable alterations in or rearrangements of genetic information. Most short-term tests in common use today are designed to evaluate the genetic activity of a substance. Data from these assays are useful for identifying potential carcinogenic hazards, in identifying active metabolites of known carcinogens in human or animal body fluids, and in helping to elucidate mechanisms of carcinogenesis. Short-term tests to detect agents with tumour-promoting activity are, at this time, insufficiently developed.

Because of the large number of short-term tests, it is difficult to establish rigid criteria for adequacy that would be applicable to all studies. General considerations relevant to all tests, however, include (a) that the test system be valid with respect to known animal carcinogens and noncarcinogens; (b) that the experimental parameters under which the chemical (or complex mixture) is tested include a sufficiently wide dose range and duration of exposure to the agent and an appropriate metabolic system; (c) that appropriate controls be used; and (d) that the purity of the compound or, in the case of complex mixtures, the source and representativity of the sample being tested be specified. Confidence in positive results is increased if a dose-response relationship is demonstrated and if this effect has been reported in two or more independent studies.

Most established short-term tests employ as end-points well-defined genetic markers in prokaryotes and lower eukaryotes and in mammalian cell lines. The tests can be grouped according to the end-point detected:

Tests of *DNA damage*. These include tests for covalent binding to DNA, induction of DNA breakage or repair, induction of prophage in bacteria and differential survival of DNA repair-proficient/-deficient strains of bacteria.

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[1]Based on the recommendations of a Working Group which met in 1983(5).

Tests of *mutation* (measurement of heritable alterations in phenotype and/or genotype). These include tests for detection of the loss or alteration of a gene product, and change of function through forward or reverse mutation, recombination and gene conversion; they may involve the nuclear genome, the mitochondrial genome and resident viral or plasmid genomes.

Tests of *chromosomal effects*. These include tests for detection of changes in chromosome number (aneuploidy), structural chromosomal aberrations, sister chromatid exchanges, micronuclei and dominant-lethal events. This classification does not imply that some chromosomal effects are not mutational events.

Tests for *cell transformation*, which monitor the production of preneoplastic or neoplastic cells in culture, are also of importance because they attempt to simulate essential steps in cellular carcinogenesis. These assays are not grouped with those listed above since the mechanisms by which chemicals induce cell transformation may not necessarily be the result of genetic change.

The selection of specific tests and end-points for consideration remains flexible and should reflect the most advanced state of knowledge in this field.

The data from short-term tests are summarized by the Working Group and the test results tabulated according to the end-points detected and the biological complexities of the test systems. The format of the table used is shown below. In these tables, a '+' indicates that the compound was judged by the Working Group to be significantly positive in one or more assays for the specific end-point and level of biological complexity; '-' indicates that it was judged to be negative in one or more assays; and '?' indicates that there were contradictory results from different laboratories or in different biological systems, or that the result was judged to be equivocal. These judgements reflect the assessment by the Working Group of the quality of the data (including such factors as the purity of the test compound, problems of metabolic activation and appropriateness of the test system) and the relative significance of the component tests.

Overall assessment of data from short-term tests

	Genetic activity			Cell transformation
	DNA damage	Mutation	Chromosomal effects	
Prokaryotes				
Fungi/green plants				
Insects				
Mammalian cells (*in vitro*)				
Mammals (*in vivo*)				
Humans (*in vivo*)				

An overall assessment of the evidence for *genetic activity* is then made on the basis of the entries in the table, and the evidence is judged to fall into one of four categories, defined as follows:

(i) *Sufficient evidence* is provided by at least three positive entries, one of which must involve mammalian cells *in vitro* or *in vivo* and which must include at least two of three end-points — DNA damage, mutation and chromosomal effects.

(ii) *Limited evidence* is provided by at least two positive entries.

(iii) *Inadequate evidence* is available when there is only one positive entry or when there are too few data to permit an evaluation of an absence of genetic activity or when there are unexplained, inconsistent findings in different test systems.

(iv) *No evidence* applies when there are only negative entries; these must include entries for at least two end-points and two levels of biological complexity, one of which must involve mammalian cells *in vitro* or *in vivo*.

It is emphasized that the above definitions are operational, and that the assignment of a chemical or complex mixture into one of these categories is thus arbitrary.

In general, emphasis is placed on positive results; however, in view of the limitations of current knowledge about mechanisms of carcinogenesis, certain cautions should be respected: (i) At present, short-term tests should not be used by themselves to conclude whether or not an agent is carcinogenic, nor can they predict reliably the relative potencies of compounds as carcinogens in intact animals. (ii) Since the currently available tests do not detect all classes of agents that are active in the carcinogenic process (e.g., hormones), one must be cautious in utilizing these tests as the sole criterion for setting priorities in carcinogenesis research and in selecting compounds for animal bioassays. (iii) Negative results from short-term tests cannot be considered as evidence to rule out carcinogenicity, nor does lack of demonstrable genetic activity attribute an epigenetic or any other property to a substance(5).

(b) Evaluation of Carcinogenicity in Humans

Evidence of carcinogenicity can be derived from case reports, descriptive epidemiological studies and analytical epidemiological studies.

An analytical study that shows a positive association between an exposure and a cancer may be interpreted as implying causality to a greater or lesser extent, on the basis of the following criteria: (a) There is no identifiable positive bias. (By 'positive bias' is meant the operation of factors in study design or execution that lead erroneously to a more strongly positive association between an exposure and disease than in fact exists. Examples of positive bias include, in case-control studies, better documentation of the exposure for cases than for controls, and, in cohort studies, the use of better means of detecting cancer in exposed individuals than in individuals not exposed.) (b) The possibility of positive confounding has been considered. (By 'positive confounding' is meant a situation in which the relationship between an exposure and a disease is rendered more strongly positive than it truly is as a result of an association between that exposure and another exposure which either causes or prevents the disease. An example of positive confounding is the association between coffee consumption and lung cancer, which results from their joint association with

cigarette smoking.) (c) The association is unlikely to be due to chance alone. (d) The association is strong. (e) There is a dose-response relationship.

In some instances, a single epidemiological study may be strongly indicative of a cause-effect relationship; however, the most convincing evidence of causality comes when several independent studies done under different circumstances result in 'positive' findings.

Analytical epidemiological studies that show no association between an exposure and a cancer ('negative' studies) should be interpreted according to criteria analogous to those listed above: (a) there is no identifiable negative bias; (b) the possibility of negative confounding has been considered; and (c) the possible effects of misclassification of exposure or outcome have been weighed. In addition, it must be recognized that the probability that a given study can detect a certain effect is limited by its size. This can be perceived from the confidence limits around the estimate of association or relative risk. In a study regarded as 'negative', the upper confidence limit may indicate a relative risk substantially greater than unity; in that case, the study excludes only relative risks that are above the upper limit. This usually means that a 'negative' study must be large to be convincing. Confidence in a 'negative' result is increased when several independent studies carried out under different circumstances are in agreement. Finally, a 'negative' study may be considered to be relevant only to dose levels within or below the range of those observed in the study and is pertinent only if sufficient time has elapsed since first human exposure to the agent. Experience with human cancers of known etiology suggests that the period from first exposure to a chemical carcinogen to development of clinically observed cancer is usually measured in decades and may be in excess of 30 years.

The evidence for carcinogenicity from studies in humans is assessed by the Working Group and judged to fall into one of four groups, defined as follows:

1. *Sufficient evidence* of carcinogenicity indicates that there is a causal relationship between the exposure and human cancer.

2. *Limited evidence* of carcinogenicity indicates that a causal interpretation is credible, but that alternative explanations, such as chance, bias or confounding, could not adequately be excluded.

3. *Inadequate evidence*, which applies to both positive and negative evidence, indicates that one of two conditions prevailed: (a) there are few pertinent data; or (b) the available studies, while showing evidence of association, do not exclude chance, bias or confounding.

4. *No evidence* applies when several adequate studies are available which do not show evidence of carcinogenicity.

(c) Relevance of Experimental Data to the Evaluation of Carcinogenic Risk to Humans

Information compiled from the first 29 volumes of the *IARC Monographs*(4,21,22) shows that, of the chemicals or groups of chemicals now generally accepted to cause or probably to cause cancer in humans, all (with the possible exception of arsenic) of those that have been tested appropriately produce cancer in at least one animal species. For several of the chemicals (e.g., aflatoxins, 4-aminobiphenyl, diethylstilboestrol, melphalan, mustard gas and

vinyl chloride), evidence of carcinogenicity in experimental animals preceded evidence obtained from epidemiological studies or case reports.

For many of the chemicals (or complex mixtures) evaluated in the *IARC Monographs* for which there is *sufficient evidence* of carcinogenicity in animals, data relating to carcinogenicity for humans are either insufficient or nonexistent. **In the absence of adequate data on humans, it is reasonable, for practical purposes, to regard chemicals for which there is sufficient evidence of carcinogenicity in animals as if they presented a carcinogenic risk to humans**. The use of the expressions 'for practical purposes' and 'as if they presented a carcinogenic risk' indicates that, at the present time, a correlation between carcinogenicity in animals and possible human risk cannot be made on a purely scientific basis, but only pragmatically. Such a pragmatic correlation may be useful to regulatory agencies in making decisions related to the primary prevention of cancer.

In the present state of knowledge, it would be difficult to define a predictable relationship between the dose (mg/kg bw per day) of a particular chemical required to produce cancer in test animals and the dose that would produce a similar incidence of cancer in humans. Some data, however, suggest that such a relationship may exist(23,24), at least for certain classes of carcinogenic chemicals, although no acceptable method is currently available for quantifying the possible errors that may be involved in such an extrapolation procedure.

8. EXPLANATORY NOTES ON THE CONTENTS OF MONOGRAPHS ON CHEMICALS AND COMPLEX MIXTURES

The sections 1 and 2, as outlined below, are those used in monographs on individual chemicals. When relevant, similar information is included in monographs on complex mixtures; additional information is provided as considered necessary.

(*a*) *Chemical and Physical Data (Section 1)*

The Chemical Abstracts Services Registry Number, the latest Chemical Abstracts Primary Name (Ninth Collective Index)(25) and the IUPAC Systematic Name(26) are recorded in section 1. Other synonyms and trade names are given, but no comprehensive list is provided. Some of the trade names are those of mixtures in which the compound being evaluated is only one of the ingredients.

The structural and molecular formulae, molecular weight and chemical and physical properties are given. The properties listed refer to the pure substance, unless otherwise specified, and include, in particular, data that might be relevant to carcinogenicity (e.g., lipid solubility) and those that concern identification.

A separate description of the composition of technical products includes available information on impurities and formulated products.

(*b*) *Production, Use, Occurrence and Analysis (Section 2)*

The purpose of section 2 is to provide indications of the extent of past and present human exposure to the chemical.

Monographs on occupational exposures to complex mixtures or exposures to complex mixtures resulting from human habits include sections on: historical perspectives; description of the industry or habit; manufacturing processes and use patterns; exposures in the workplace; and chemistry of the complex mixture.

(i) *Synthesis*

Since cancer is a delayed toxic effect, the dates of first synthesis and of first commercial production of the chemical are provided. This information allows a reasonable estimate to be made of the date before which no human exposure could have occurred. In addition, methods of synthesis used in past and present commercial production are described.

(ii) *Production*

Since Europe, Japan and the USA are reasonably representative industrialized areas of the world, most data on production, foreign trade and uses are obtained from those countries. It should not, however, be inferred that those areas or nations are the sole or even the major sources or users of any individual chemical.

Production and foreign-trade data are obtained from both governmental and trade publications by chemical economists in the three geographical areas. In some cases, separate production data on organic chemicals manufactured in the USA are not available because their publication could disclose confidential information. In such cases, an indication of the minimum quantity produced can be inferred from the number of companies reporting commercial production. Each company is required to report on individual chemicals if the sales value or the weight of the annual production exceeds a specified minimum level. These levels vary for chemicals classified for different uses, e.g., medicinals and plastics; in fact, the minimal annual sales value is between $1000 and $50 000, and the minimal annual weight of production is between 450 and 22 700 kg. Data on production in some European countries are obtained by means of general questionnaires sent to companies thought to produce the compounds being evaluated. Information from the completed questionnaires is compiled, by country, and the resulting estimates of production are included in the individual monographs.

(iii) *Use*

Information on uses is meant to serve as a guide only and is not complete. It is usually obtained from published data but is often complemented by direct contact with manufacturers of a chemical. In the case of drugs, mention of their therapeutic uses does not necessarily represent current practice nor does it imply judgement as to their clinical efficacy.

Statements concerning regulations and standards (e.g., pesticide registrations, maximum levels permitted in foods, occupational standards and allowable limits) in specific countries are mentioned as examples only. They may not reflect the most recent situation, since such legislation is in a constant state of change; nor should it be taken to imply that other countries do not have similar regulations.

(iv) *Occurrence*

Information on the occurrence of a chemical in the environment is obtained from published data, including that derived from the monitoring and surveillance of levels of the chemical in occupational environments, air, water, soil, foods and tissues of animals and humans. When

no published data are available to the Working Group, unpublished reports, deemed appropriate, may be considered. When available, data on the generation, persistence and bioaccumulation of a chemical are also included.

(v) *Analysis*

The purpose of the section on analysis is to give the reader an indication, rather than a complete review, of methods cited in the literature. No attempt is made to evaluate critically or to recommend any of the methods.

(c) Biological Data Relevant to the Evaluation of Carcinogenic Risk to Humans (Section 3)

In general, the data recorded in section 3 are summarized as given by the author; however, comments made by the Working Group on certain shortcomings of reporting, of statistical analysis or of experimental design are given in square brackets. The nature and extent of impurities/contaminants in the chemicals being tested are given when available.

(i) *Carcinogenicity studies in animals*

The monographs are not intended to cover all reported studies. Some studies are purposely omitted (a) because they are inadequate, as judged from previously described criteria(27-30) (e.g., too short a duration, too few animals, poor survival); (b) because they only confirm findings that have already been fully described; or (c) because they are judged irrelevant for the purpose of the evaluation. In certain cases, however, such studies are mentioned briefly, particularly when the information is considered to be a useful supplement to other reports or when it is the only data available. Their inclusion does not, however, imply acceptance of the adequacy of their experimental design or of the analysis and interpretation of their results.

Mention is made of all routes of administration by which the test material has been adequately tested and of all species in which relevant tests have been done(30). In most cases, animal strains are given. Quantitative data are given to indicate the order of magnitude of the effective carcinogenic doses. In general, the doses and schedules are indicated as they appear in the original; sometimes units have been converted for easier comparison. Experiments in which the compound was administered in conjunction with known carcinogens and experiments on factors that modify the carcinogenic effect are also reported. Experiments on the carcinogenicity of known metabolites and derivatives are also included.

(ii) *Other relevant biological data*

LD_{50} data are given when available, and other data on toxicity are included when considered relevant.

Data on effects on reproduction, on teratogenicity and embryo- and fetotoxicity, and on placental transfer that derive from studies in experimental animals and from observations in humans are included when considered relevant.

Information is given on absorption, distribution and excretion. Data on metabolism are usually restricted to studies that show the metabolic fate of the chemical in experimental animals and humans, and comparisons of data from animals and humans are made when possible.

Data from short-term tests are also included. In addition to the tests for genetic activity and cell transformation described previously (see pages 16-18), data from studies of related effects, but for which the relevance to the carcinogenic process is less well established, may also be mentioned.

The criteria used for considering short-term tests and for evaluating their results have been described (see pages 16-18). In general, the authors' results are given as reported. An assessment of the data by the Working Group which differs from that of the authors, and comments concerning aspects of the study that might affect its interpretation are given in square brackets. Reports of studies in which few or no experimental details are given, or in which the data on which a reported positive or negative result is based are not available for examination, are cited, but are identified as 'abstract' or 'details not given' and are not considered in the summary tables or in making the overall assessment of genetic activity.

For several recent reviews on short-term tests, see IARC(30), Montesano *et al.*(31), de Serres and Ashby(32), Sugimura *et al.*(33), Bartsch *et al.*(34) and Hollstein *et al.*(35).

(iii) *Case reports and epidemiological studies of carcinogenicity to humans*

Observations in humans are summarized in this section. These include case reports, descriptive epidemiological studies (which correlate cancer incidence in space or time to an exposure) and analytical epidemiological studies of the case-control or cohort type. In principle, a comprehensive coverage is made of observations in humans; however, reports are excluded when judged to be clearly not pertinent. This applies in particular to case reports, in which either the clinico-pathological description of the tumours or the exposure history, or both, are poorly described; and to published routine statistics, for example, of cancer mortality by occupational category, when the categories are so broadly defined as to contribute virtually no specific information on the possible relation between cancer occurrence and a given exposure. Results of studies are assessed on the basis of the data and analyses that are presented in the published papers. Some additional analyses of the published data may be performed by the Working Group to gain better insight into the relation between cancer occurrence and the exposure under consideration. The Working Group may use these analyses in its assessment of the evidence or may actually include them in the text to summarize a study; in such cases, the results of the supplementary analyses are given in square brackets. Any comments by the Working Group are also reported in square brackets; however, these are kept to a minimum, being restricted to those instances in which it is felt that an important aspect of a study, directly impinging on its interpretation, should be brought to the attention of the reader.

(d) Summary of Data Reported and Evaluation (Section 4)

Section 4 summarizes the relevant data from animals and humans and gives the critical views of the Working Group on those data.

(i) *Exposures*

Human exposure to the chemical or complex mixture is summarized on the basis of data on production, use and occurrence.

(ii) *Experimental data*

Data relevant to the evaluation of the carcinogenicity of the test material in animals are summarized in this section. The animal species mentioned are those in which the carcinogenicity of the substance was clearly demonstrated. Tumour sites are also indicated. If the substance has produced tumours after prenatal exposure or in single-dose experiments, this is indicated. Dose-response data are given when available.

Significant findings on effects on reproduction and prenatal toxicity, and results from short-term tests for genetic activity and cell transformation assays are summarized, and the latter are presented in tables. An overall assessment is made of the degree of evidence for genetic activity in short-term tests.

(iii) *Human data*

Case reports and epidemiological studies that are considered to be pertinent to an assessment of human carcinogenicity are described. Other biological data that are considered to be relevant are also mentioned.

(iv) *Evaluation*

This section comprises evaluations by the Working Group of the degrees of evidence for carcinogenicity of the exposure to experimental animals and to humans. An overall evaluation is then made of the carcinogenic risk of the chemical or complex mixture to humans. This section should be read in conjunction with pages 15-16 and 18 of this Preamble for definitions of degrees of evidence.

When no data are available from epidemiological studies but there is *sufficient evidence* that the exposure is carcinogenic to animals, a footnote is included, reading: 'In the absence of adequate data on humans, it is reasonable, for practical purposes, to regard chemicals for which there is *sufficient evidence* of carcinogenicity in animals as if they presented a carcinogenic risk to humans.'

References

1. IARC (1979) Criteria to select chemicals for *IARC Monographs. IARC intern. tech. Rep. No. 79/003*

2. IARC (1977) IARC Monograph Programme on the Evaluation of the Carcinogenic Risk of Chemicals to Humans. Preamble. *IARC intern. tech. Rep. No. 77/002*

3. IARC (1978) Chemicals with *sufficient evidence* of carcinogenicity in experimental animals - *IARC Monographs* volumes 1-17. *IARC intern. tech. Rep. No. 78/003*

4. IARC (1982) *IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans*, Supplement 4, *Chemicals, Industrial Processes and Industries Associated with Cancer in Humans* (IARC Monographs Volumes 1 to 29)

5. IARC (1983) Approaches to classifying chemical carcinogens according to mechanism of action. *IARC intern. tech. Rep. No. 83/001*

6. IARC (1972-1983) *IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans*, Volumes 1-37, Lyon, France

Volume 1 (1972) Some Inorganic Substances, Chlorinated Hydrocarbons, Aromatic Amines, *N*-Nitroso Compounds and Natural Products (19 monographs), 184 pages

Volume 2 (1973) Some Inorganic and Organometallic Compounds (7 monographs), 181 pages

Volume 3 (1973) Certain Polycyclic Aromatic Hydrocarbons and Heterocyclic Compounds (17 monographs), 271 pages

Volume 4 (1974) Some Aromatic Amines, Hydrazine and Related Substances, *N*-Nitroso Compounds and Miscellaneous Alkylating Agents (28 monographs), 286 pages

Volume 5 (1974) Some Organochlorine Pesticides (12 monographs), 241 pages

Volume 6 (1974) Sex Hormones (15 monographs), 243 pages

Volume 7 (1974) Some Anti-thyroid and Related Substances, Nitrofurans and Industrial Chemicals (23 monographs), 326 pages

Volume 8 (1975) Some Aromatic Azo Compounds (32 monographs), 357 pages

Volume 9 (1975) Some Aziridines, *N*-, *S*- and *O*-Mustards and Selenium (24 monographs), 268 pages

Volume 10 (1976) Some Naturally Occurring Substances (22 monographs), 353 pages

Volume 11 (1976) Cadmium, Nickel, Some Epoxides, Miscellaneous Industrial Chemicals and General Considerations on Volatile Anaesthetics (24 monographs), 306 pages

Volume 12 (1976) Some Carbamates, Thiocarbamates and Carbazides (24 monographs), 282 pages

Volume 13 (1977) Some Miscellaneous Pharmaceutical Substances (17 monographs), 255 pages

Volume 14 (1977) Asbestos (1 monograph), 106 pages

Volume 15 (1977) Some Fumigants, the Herbicides, 2,4-D and 2,4,5-T, Chlorinated Dibenzodioxins and Miscellaneous Industrial Chemicals (18 monographs), 354 pages

Volume 16 (1978) Some Aromatic Amines and Related Nitro Compounds — Hair Dyes, Colouring Agents, and Miscellaneous Industrial Chemicals (32 monographs), 400 pages

Volume 17 (1978) Some *N*-Nitroso Compounds (17 monographs), 365 pages

Volume 18 (1978) Polychlorinated Biphenyls and Polybrominated Biphenyls (2 monographs), 140 pages

Volume 19 (1979) Some Monomers, Plastics and Synthetic Elastomers, and Acrolein (17 monographs), 513 pages

Volume 20 (1979) Some Halogenated Hydrocarbons (25 monographs), 609 pages

Volume 21 (1979) Sex Hormones (II) (22 monographs), 583 pages

Volume 22 (1980) Some Non-Nutritive Sweetening Agents (2 monographs), 208 pages

Volume 23 (1980) Some Metals and Metallic Compounds (4 monographs), 438 pages

Volume 24 (1980) Some Pharmaceutical Drugs (16 monographs), 337 pages

Volume 25 (1981) Wood, Leather and Some Associated Industries (7 monographs), 412 pages

Volume 26 (1981) Some Antineoplastic and Immunosuppressive Agents (18 monographs), 411 pages

Volume 27 (1981) Some Aromatic Amines, Anthraquinones and Nitroso Compounds, and Inorganic Fluorides Used in Drinking-Water and Dental Preparations (18 monographs), 344 pages

Volume 28 (1982) The Rubber Industry (1 monograph), 486 pages

Volume 29 (1982) Some Industrial Chemicals and Dyestuffs (18 monographs), 416 pages

Volume 30 (1982) Miscellaneous Pesticides (18 monographs), 424 pages

Volume 31 (1983) Some Food Additives, Feed Additives and Naturally Occurring Substances (21 monographs), 314 pages

Volume 32 (1983) Polynuclear Aromatic Compounds, Part 1, Chemical, Environmental and Experimental Data (42 monographs), 477 pages

Volume 33 (1984) Polynuclear Aromatic Compounds, Part 2, Carbon Blacks, Mineral Oils and Some Nitroarenes (8 monographs), 245 pages

Volume 34 (1984) Polynuclear Aromatic Compounds, Part 3, Industrial Exposures in Aluminium Production, Coal Gasification, Coke Production and Iron and Steel Founding (4 monographs), 219 pages

Volume 35 (1984) Polynuclear Aromatic Compounds, Part 4, Bitumens, Coal-Tars and Derived Products, Shale-Oils and Soots (4 monographs), 271 pages

Volume 36 (1985) Allyl Compounds, Aldehydes, Epoxides and Peroxides (15 monographs), 369 pages

Volume 37 (1985) Tobacco Habits Other Than Smoking; Betel-Quid and Areca-Nut Chewing; and Some Related Nitrosamines (8 monographs), 291 pages

Supplement No. 1 (1979) Chemicals and Industrial Processes Associated with Cancer in Humans (IARC Monographs, Volumes 1 to 20), 71 pages

Supplement No. 2 (1980) Long-term and Short-term Screening Assays for Carcinogens: A Critical Appraisal, 426 pages

Supplement No. 3 (1982) Cross Index of Synonyms and Trade Names in Volumes 1 to 26, 199 pages

Supplement No. 4 (1982) Chemicals, Industrial Processes and Industries Associated with Cancer in Humans (IARC Monographs, Volumes 1 to 29), 292 pages

7. IARC (1973-1983) *Information Bulletin on the Survey of Chemicals Being Tested for Carcinogenicity*, Numbers 1-11, Lyon, France

Number 1 (1973) 52 pages
Number 2 (1973) 77 pages
Number 3 (1974) 67 pages
Number 4 (1974) 97 pages
Number 5 (1975) 88 pages
Number 6 (1976) 360 pages
Number 7 (1978) 460 pages
Number 8 (1979) 604 pages
Number 9 (1981) 294 pages
Number 10 (1983) 326 pages
Number 11 (1984) 370 pages

8. PHS 149 (1951-1983) Public Health Service Publication No. 149, *Survey of Compounds which have been Tested for Carcinogenic Activity*, Washington DC, US Government Printing Office

1951 Hartwell, J.L., 2nd ed., Literature up to 1947 on 1329 compounds, 583 pages
1957 Shubik, P. & Hartwell, J.L., Supplement 1, Literature for the years 1948-1953 on 981 compounds, 388 pages
1969 Shubik, P. & Hartwell, J.L., edited by Peters, J.A., Supplement 2, Literature for the years 1954-1960 on 1048 compounds, 655 pages
1971 National Cancer Institute, Literature for the years 1968-1969 on 882 compounds, 653 pages
1973 National Cancer Institute, Literature for the years 1961-1967 on 1632 compounds, 2343 pages
1974 National Cancer Institute, Literature for the years 1970-1971 on 750 compounds, 1667 pages
1976 National Cancer Institute, Literature for the years 1972-1973 on 966 compounds, 1638 pages
1980 National Cancer Institute, Literature for the year 1978 on 664 compounds, 1331 pages
1983 National Cancer Institute, Literature for years 1974-1975 on 575 compounds, 1043 pages

9. Pike, M.C. & Roe, F.J.C. (1963) An actuarial method of analysis of an experiment in two-stage carcinogenesis. *Br. J. Cancer*, *17*, 605-610

10. Miller, E.C. (1978) Some current perspectives on chemical carcinogenesis in humans and experimental animals: Presidential address. *Cancer Res.*, *38*, 1479-1496

11. Miller, E.C. & Miller, J.A. (1981) Searches for ultimate chemical carcinogens and their reactions with cellular macromolecules. *Cancer*, *47*, 2327-2345

12. Peto, R. (1974) Guidelines on the analysis of tumour rates and death rates in experimental animals. *Br. J. Cancer*, *29*, 101-105

13. Peto, R. (1975) Letter to the editor. *Br. J. Cancer*, *31*, 697-699

14. Hoel, D.G. & Walburg, H.E., Jr (1972) Statistical analysis of survival experiments. *J. natl Cancer Inst.*, *49*, 361-372

15. Peto, R., Pike, M.C., Day, N.E., Gray, R.G., Lee, P.N., Parish, S., Peto, J., Richards, S. & Wahrendorf, J. (1980) *Guidelines for simple sensitive significance tests for carcinogenic effects in long-term animal experiments*. In: *IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans, Supplement 2, Long-term and Short-term Screening Assays for Carcinogens: A Critical Appraisal*, Lyon, pp. 311-426

16. Berenblum, I. (1975) *Sequential aspects of chemical carcinogenesis: Skin*. In: Becker, F.F., ed., *Cancer. A Comprehensive Treatise*, Vol. 1, New York, Plenum Press, pp. 323-344

17. Foulds, L. (1969) *Neoplastic Development*, Vol. 2, London, Academic Press

18. Farber, E. & Cameron, R. (1980) The sequential analysis of cancer development. *Adv. Cancer Res.*, *31*, 125-126

19. Weinstein, I.B. (1981) The scientific basis for carcinogen detection and primary cancer prevention. *Cancer*, *47*, 1133-1141

20. Slaga, T.J., Sivak, A. & Boutwell, R.K., eds (1978) *Mechanisms of Tumor Promotion and Cocarcinogenesis*, Vol. 2, New York, Raven Press

21. IARC Working Group (1980) An evaluation of chemicals and industrial processes associated with cancer in humans based on human and animal data: *IARC Monographs* Volumes 1 to 20. *Cancer Res.*, *40*, 1-12

22. IARC (1979) *IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans*, Supplement 1, *Chemicals and Industrial Processes Associated with Cancer in Humans*, Lyon

23. Rall, D.P. (1977) *Species differences in carcinogenesis testing*. In: Hiatt, H.H., Watson, J.D. & Winsten, J.A., eds, *Origins of Human Cancer*, Book C, Cold Spring Harbor, NY, Cold Spring Harbor Laboratory, pp. 1383-1390

24. National Academy of Sciences (NAS) (1975) *Contemporary Pest Control Practices and Prospects: The Report of the Executive Committee*, Washington DC

25. Chemical Abstracts Services (1978) *Chemical Abstracts Ninth Collective Index (9CI), 1972-1976*, Vols 76-85, Columbus, OH

26. International Union of Pure & Applied Chemistry (1965) *Nomenclature of Organic Chemistry*, Section C, London, Butterworths

27. WHO (1958) Second Report of the Joint FAO/WHO Expert Committee on Food Additives. Procedures for the testing of intentional food additives to establish their safety and use. *WHO tech. Rep. Ser., No. 144*

28. WHO (1967) Scientific Group. Procedures for investigating intentional and unintentional food additives. *WHO tech. Rep. Ser., No. 348*

29. Sontag, J.M., Page, N.P. & Saffiotti, U. (1976) Guidelines for carcinogen bioassay in small rodents. *Natl Cancer Inst. Carcinog. tech. Rep. Ser., No.1*

30. IARC (1980) *IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans*, Supplement 2, *Long-term and Short-term Screening Assays for Carcinogens: A Critical Appraisal*, Lyon

31. Montesano, R., Bartsch, H. & Tomatis, L., eds (1980) *Molecular and Cellular Aspects of Carcinogen Screening Tests* (*IARC Scientific Publications No. 27*), Lyon

32. de Serres, F.J. & Ashby, J., eds (1981) *Evaluation of Short-Term Tests for Carcinogens. Report of the International Collaborative Program*, Amsterdam, Elsevier/North-Holland Biomedical Press

33. Sugimura, T., Sato, S., Nagao, M., Yahagi, T., Matsushima, T., Seino, Y., Takeuchi, M. & Kawachi, T. (1976) *Overlapping of carcinogens and mutagens*. In: Magee, P.N., Takayama, S., Sugimura, T. & Matsushima, T., eds, *Fundamentals in Cancer Prevention*, Tokyo/Baltimore, University of Tokyo/University Park Press, pp. 191-215

34. Bartsch, H., Tomatis, L. & Malaveille, C. (1982) *Qualitative and quantitative comparison between mutagenic and carcinogenic activities of chemicals*. In: Heddle, J.A., ed., *Mutagenicity: New Horizons in Genetic Toxicology*, New York, Academic Press, pp. 35-72

35. Hollstein, M., McCann, J., Angelosanto, F.A. & Nichols, W.W. (1979) Short-term tests for carcinogens and mutagens. *Mutat. Res., 65*, 133-226

GENERAL REMARKS ON THE HABITS AND SUBSTANCES CONSIDERED

In this thirty-seventh volume of the *IARC Monographs*, the carcinogenic risks of tobacco chewing, snuff taking and related habits and of chewing betel quid and areca nut with and without tobacco are considered. Monographs are also included on some nitrosamines derived from areca nut and some tobacco-specific nitrosamines; one of the latter, *N'*-nitrosonornicotine, was evaluated earlier, in Volume 17 of the *Monographs* (IARC, 1978), and information gathered since that time has been summarized and evaluated. A group that is to meet in February 1985 will consider the habit of tobacco smoking.

In this volume, the term 'smokeless tobacco' is used to describe any of a variety of products and mixtures that contain tobacco as the principal constituent, and are utilized without combustion. This expression was chosen in preference to several alternatives, including 'non-smoking tobacco', 'non-smoked tobacco', 'unsmoked tobacco' and 'uncombusted tobacco', mainly because of its growing use in the USA among tobacco experts. This consideration outweighed the imprecision of this term in the English language and the potential difficulty in its translation.

The oral and nasal use of tobacco, either finely powdered as snuff or in leaf form for chewing, is as old as its use for smoking in pipes, cigars and cigarettes. In the first half of the twentieth century, the use of chewing tobacco and snuff in the western hemisphere was overtaken by a huge increase in the use of smoking tobacco. In some parts of the world, however, smokeless tobacco is still widely used. The various preparations are called by a wide variety of names, and some are discussed in this volume. Additionally, there has been a renaissance in the use of chewing tobacco and snuff in western countries during recent years. In this volume, only snuff that contains tobacco (and sometimes other materials) is considered, and not snuffs made from other plants.

In South-East Asia, betel leaf or tobacco is mixed with a wide variety of other substances, including one another, and chewing habits vary greatly from one region to another. The most common additives are slaked lime and areca nut, and the possible role of these individual components in the development of cancer is still in dispute. Studies on some of the combinations are evaluated in the first two monographs of this volume.

In India, as in many parts of South-East Asia, oral cancer is a leading type of malignancy. A comparison of the frequency of this cancer in North America and in Middle-South Asia (United Nations Region 15) is given in Table 1.

Oral leukoplakia, assumed to be a precursor stage of oral cancer, is also prevalent in South-East Asia; this precancerous state and other precancerous conditions are also discussed.

In most previous volumes in this *Monographs* series, the epidemiological data evaluated have come predominantly from studies of exposure to single substances, or at least single categories of chemicals. In this volume, however, cancer risks are addressed which are

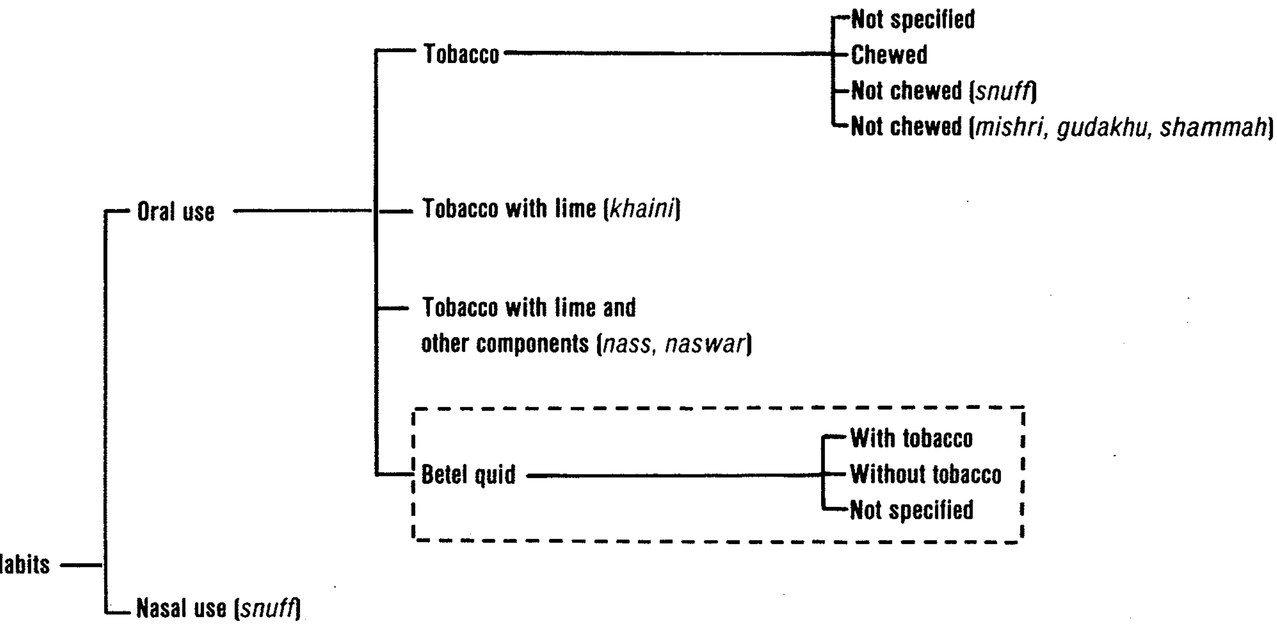
Table 1. Relative importance of oropharyngeal cancer in North America and Middle-South Asia[a]; estimated numbers of new cases, 1975, by sex[b]

Ranking[c]	North America		Middle south Asia	
	Site	No. (thousands)	Site	No. (thousands)
Males				
1	Lung	78	Mouth/pharynx	79
2	Prostate	63	Lung	26
3	Colon/rectum	55	Oesophagus	26
4	Bladder	25	Stomach	20
(6)	(Mouth/pharynx)	(18)		
Females				
1	Breast	105	Cervix	98
2	Colon/rectum	56	Breast	61
3	Lung	25	Mouth/pharynx	39
4	Lymphatic system	17	Oesophagus	18
(9)	(Mouth/pharynx)	(8)		

[a]United Nations Region 15: India, Pakistan, Bangladesh, Nepal, Bhutan, Afghanistan, Iran
[b]From Parkin *et al.* (1984)
[c]Among twelve selected cancer sites

associated with a wide range of complex human behaviours and a variety of complex mixtures of chemicals. The behaviours fall into two major categories: first, habits in which tobacco is used orally or nasally, without combustion and in conjunction with a variety of other additives; second, habits in which betel leaf and areca nut are used with or without the addition of tobacco. Evaluation of the epidemiological data in the first two monographs in this volume has therefore sought to identify distinguishable categories of behaviour and, accordingly, has been formulated in reference to the spectrum shown in Figure 1.

Fig. 1. Categories of behaviour evaluated on the basis of epidemiological data in the first and (for categories within the dotted lines) second monographs in this volume



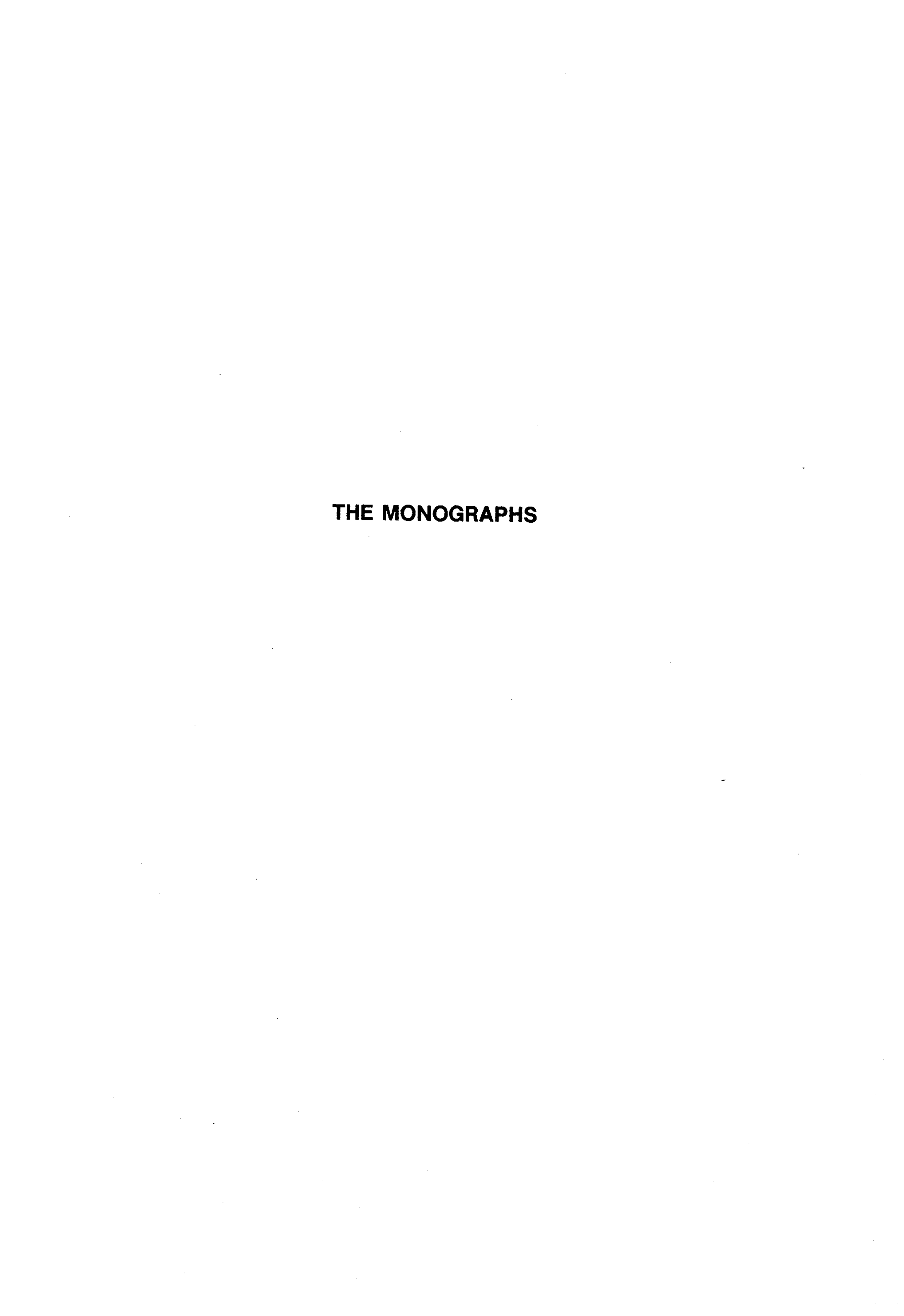
Although some of the exposure categories shown in Figure 1 equate clearly to certain specific, culturally-based habits (e.g., oral use of tobacco-plus-lime equates to the use of *khaini*), the generic approach of Figure 1 is preferred to ensure that the classification is conceptually comprehensive. This approach also makes explicit the desirability for epidemiological and experimental research in the future to measure and report on the specific components of the exposure and the habit in order to allow apportionment of carcinogenic risk between those components.

Tobacco-specific *N*-nitroso compounds have been detected at high concentrations in snuff and chewing tobacco. Thus, an evaluation of the possible carcinogenicity of those *N*-nitroso compounds was considered to be of importance and was undertaken in the five monographs at the end of this volume.

References

IARC (1978) *IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans*, Vol. 17, *Some N-Nitroso Compounds*, Lyon, pp. 281-286

Parkin, D.M., Stjernswärd, J. & Muir, C.S. (1984) Estimates of the worldwide frequency of twelve major cancers. *Bull. World Health Organ.*, *62*, 163-182



THE MONOGRAPHS

TOBACCO HABITS OTHER THAN SMOKING

1. Description of the Habits

1.1 Introduction

Habits associated with the use of smokeless tobacco are found worldwide, with countless variations in the nature of the product used, as well as in the customs associated with its use. The tobacco is often processed and treated with additives and flavouring agents. It may be taken alone or in combination with one or a variety of other ingredients. The predominant use of smokeless tobacco is oral, although it may be placed in or inhaled into the nasal cavity. The saliva produced during oral use may be swallowed or expectorated, according to custom or personal preference.

Tobacco grown for the manufacture of smokeless products is of two species within the genus *Nicotiana* (Solanaceae), *N. tabacum* and *N. rustica*. It is believed that the former originated in Brazil and the latter in Mexico. The vast majority of smokeless-tobacco products are made from *N. tabacum*; all of those manufactured in North America and western Europe are made from this species. *N. rustica* is used in the USSR and, to a limited extent, in India. The alkaloid, nicotine, is the factor that creates dependence in the continued use of tobacco.

In Europe and the USA, the smokeless-tobacco products used are predominantly chewing tobacco and snuff. Within these groups are several types, differentiated by formulation and treatment of the tobacco. During the past few years in the USA, there has been a reclassification of products within the two major categories, and some types of fine-cut smokeless tobacco that were classified as 'chewing tobacco' prior to 1981 are now categorized as 'moist/fine-cut snuff'. In general, *chewing tobaccos*, as the name implies, require the consumer to take a portion of the tobacco product and chew it and/or place it between the buccal mucosa and gum for varying periods of time.

Snuff is available in Europe and the USA as products with different particle sizes and moisture contents. The majority of snuff used today has a relatively high moisture content and is finely cut rather than pulverized; as with chewing tobacco, it is used orally and is placed between the buccal mucosa and gum. The other type of snuff, which is dry and pulverized, is for oral or nasal use. The major use involves placing a suitable amount between the lower lips and gum or between the gum and buccal mucosa; although dry snuff may also be sniffed through the nasal cavities, this technique is a minor use pattern. In some countries, snuff has become available in small packets wrapped in porous paper (like 'tea-bags') which are placed between the buccal mucosa and gum; such preparations appear to appeal to young adult users in Scandinavia and the USA.

In many Asian countries, tobacco is commonly added to betel quid, as described elsewhere in this volume. Tobacco may also be used alone, with lime and in various other combinations.

Nasal use of snuff is widely practised among the Bantu population in South Africa.

Although many of these habits are practised by millions of people, most of the available information is from North America, Europe and South-East Asia. Published estimates of the total number of persons practising the habits do not exist or are of variable reliability.

1.2 Historical overview

(*a*) *Tobacco chewing*

The tobacco plant is thought to have originated on the mainland between North and South America and had already spread throughout the two continents at the time of its discovery. However, cultivation of the plant probably dates back at least 7000 years; tobacco seeds were discovered during archaeological excavations in both Mexico and Peru, and findings in remains of permanent settlements built around 3500 BC show that tobacco was an article of established value to the inhabitants. It would appear that people who frequently lacked sufficient food alleviated their hunger pangs by chewing tobacco (Voges, 1984).

American Indians were probably the first people to smoke, chew and snuff tobacco, as early as the 1400s (Christen *et al.*, 1982). In 1499, Amerigo Vespucci found Indians on Margarita Island, a small island off the coast of Venezuela, chewing a green herb that was carried in a gourd around their necks. Since there was a scarcity of water on the island, Vespucci assumed that the green herb, known as tobacco, was chewed to quench thirst, since it produced an increase in salivary flow; he also reported that the Indians chewed these leaves to whiten their teeth (Heimann, 1960; Stewart, 1967).

According to a report written in the late 1500s by Garcilasso de la Vega, the practice of tobacco chewing was widespread in parts of South America (Voges, 1984); Samuel de Champlain, the founder of Québec, reported the use of plug tobacco in Santo Domingo during the sixteenth century, and Columbus, in 1571, observed men in Veragua, later known as Costa Rica, putting a dry herb in their mouths and chewing it (Heimann, 1960). Tobacco chewing seems to have been common, especially when long distances had to be covered, and it has been reported that an Indian could trek for two or three days with no other support against hunger, thirst and fatigue than tobacco. Several American tribes mixed either lime or finely-powdered and burned, fresh- or salt-water molluscs with their chewing tobacco (Curtis, 1935).

Among native Americans, chewing tobacco was thought to have several medicinal uses, some of which included alleviating toothache and disinfecting cuts by spitting the tobacco juice and saliva mixture onto the wound; it was also thought to relieve the effects of snake, spider and insect bites (Axton, 1975).

By 1531, the Spaniards were growing tobacco commercially in the West Indies and maintained a monopoly over the European markets until 1575, at which time the Portuguese began to grow large quantities of the commodity. Tobacco was soon grown in Europe as both a decorative and medicinal plant. In 1559, Jean Nicot, in whose honour the genus *Nicotiana* was named, was ambassador to Sebastian, King of Portugal. He grew tobacco and promoted the product in Europe for its magic 'cure-all' properties. By 1613, tobacco had become one of the major exports of the American colonies. In the 1600s in southern Africa, people sold land and slaves for tobacco (Christen *et al.*, 1982).

Tobacco arrived in Turkey in 1605, Russia in 1634 and Arabia in 1663; Spaniards transported tobacco seeds to the Philippines, from whence it was shipped to China, on to Siberia and across the Bering Sea back to Alaskan Eskimos. Reports indicate that the leaf reached the Australian aborigines and even the Andaman Islanders. On the western coast of Africa, tobacco became a commercial enterprise (Axton, 1975).

Along the eastern coast of the USA, no evidence of tobacco chewing was found until 1704; it became popular only during the first half of the nineteenth century (Gottsegen, 1940), when chewing tobacco was known in the Connecticut Valley as 'fudgeon' (Brooks, 1952).

Tobacco chewing was not confined to the Americas. When smoking was forbidden on British naval vessels because of the fire hazard, sailors turned to chewing tobacco and snuff. In Europe, tobacco was regarded as an excellent prophylactic during the plague and, for those who did not like smoking, chewing was an alternative. General George Monck, Duke of Albemarle, who was reponsible for aiding the return of the Stuarts to the British monarchy, was a tobacco chewer, as were many of his troops. Charles II chewed tobacco, as did Queen Caroline of England. Tobacco chewing was recommended for cleaning the teeth of women and children (Brooks, 1952).

In 1797, Adam Clarke, a famous Methodist minister, begged all tobacco consumers and especially religious followers, for the sake of their health and their souls, to avoid tobacco. He was particularly disturbed by the fact that it had become unsafe to kneel when praying because chewers had made the floors unsanitary for the devout (Brooks, 1952).

By the mid 1850s, tobacco chewing had been accepted by North Americans, following two centuries of pipe smoking and snuff use, for two reasons: firstly, Americans rejected European habits in general, and British habits in particular, that entailed snuff boxes and formality; and secondly, tobacco chewing was more convenient for Americans trekking westward in their wagons to build new homesites and develop the land (Heimann, 1960).

During the 1860s, tobacco was chewed in the form of either a plug or a twist. Of the 348 tobacco factories listed in the 1860 Census for Virginia and North Carolina, only seven manufactured smoking products (Heimann, 1960). American pioneers resorted to the use of a home-made sweet plug, so-named because the leaf was wadded into a hole in a log and laced with a sweetening agent, usually brandy or cane sugar, which, after removal of the fermented leaf, resulted in a tasty chew (Axton, 1975).

During the latter part of the nineteenth century, the 'germ theory of infection' changed the course of chewing in America, and it was felt that expectorating on the floor and into a brass cuspidor could be a source of contamination and spreading of disease. By the 1890s, public outcry made tobacco chewing socially unacceptable behaviour and unlawful in most public places (Christen *et al.*, 1982). Anti-spitting laws were passed in New York and Philadelphia in 1896 and in Toronto, Canada, in 1904 (Kozlowski, 1981).

In 1945, cuspidors were removed from all federal buildings by order of the US District Court in Washington DC (Brooks, 1952). The apparent decline in tobacco chewing is exemplified by a memorandum of 14 September 1955 to the American Tobacco Company, stating, 'It has become impossible to hire persons in the New York area to clean and maintain cuspidors ... it will be necessary to remove them promptly from the premises' (Heimann, 1960).

The market for chewing tobacco passed its peak in 1890, when some three pounds (about 1.5 kg) of plug, twist or fine-cut chewing tobacco were chewed annually per capita in the

USA (Heimann, 1960). Chewing remained the dominant form of tobacco usage in America until the expansion of the cigarette industry in 1918 (Maxwell, 1980).

During the second half of the 1960s and continuing through the 1970s, however, a resurgence in tobacco chewing occurred in the USA. Chewing tobacco has now become popular among young adult males. Some persons may have selected chewing as an alternative to smoking since it does not, as yet, have the stigma of being linked to health issues. Chewing can be performed in areas where smoking is prohibited, and it is purported to be more economical: advertisements claim that chewers can keep a wad of tobacco 'alive' for several hours, and it is claimed that a three-ounce (85-g) pouch of loose-leaf chewing tobacco can last a week or more for the average chewer (Christen & Glover, 1981).

The tobacco industry is promoting tobacco chewing as a recreational activity, with spitting contests, shirts and clubs. The trend toward the 'western cowboy' image of masculinity is being promoted in advertising by connecting tobacco chewing with western clothes. On one college campus, a chewing club claims the membership of athletes, the president of the student body, and a number of intellectual students; free samples of chewing tobacco are being handed out at other colleges. Commercial advertisements use public figures for extolling the virtues of chewing tobacco (Christen & Glover, 1981).

(*b*) *Snuff taking*

This topic has been reviewed recently (Christen *et al.*, 1982).

The Indians of Brazil were the first people known to use snuff. Using a cup made from a block of rosewood and a pestle of the same wood, the tobacco leaves were ground into a powder and acquired the delicate aroma of the wood. The resulting snuff was placed in ornately decorated bone tubes, one end of which was plugged to preserve the fragrance (Curtis, 1935).

Friar Ramón Pané, a Franciscan monk who travelled with Christopher Columbus on his second voyage to the New World in 1493, reported that the Carib Indians of the lesser Antilles used snuff (Christen *et al.*, 1982). In Haiti, snuff powder was used by medicine men for clearing nasal passages and as an analgesic (Stewart, 1967). Friar Pané's return to Spain with snuff signalled the arrival in Europe of a habit that was to last for several centuries.

In 1519, Ocaranza found that Mexican Indians used tobacco powder to heal burns and wounds, and in 1525 Herrera observed Mexican Indians holding tobacco powder in their mouth to send them to sleep and reduce pain (Stewart, 1967). The Indians inhaled powdered tobacco through a hollow Y-shaped piece of cane or pipe by placing the forked ends into each nostril and the other end near the powdered tobacco. This instrument was called a '*tobago*' or '*tobaca*'. The word was later changed by the Spaniards to 'tobacco' (Christen *et al.*, 1982).

Jean Nicot is credited with introducing snuff to Catherine de Medici, Queen of France, to cure her headaches (Christen *et al.*, 1982).

The Dutch, who named the powdered tobacco 'snuff', were also using the product by 1560 (Christen *et al.*, 1982).

By the early 1600s, snuff had become an expensive commodity and its use had spread throughout South America, China, Japan and Africa. The origin of the process terms 'carotte'

and 'rappee' goes back to the 1600s when tobacco for snuff was prepared in the form of a carrot to be rasped in the quantity desired for use (Curtis, 1935). In 1620, the Royal Snuff Factory was established in Seville, and this became the centre of manufacturing and development of this product (Voges, 1984). Snuff use expanded through Japan to China (Ching Dynasty) in the 1650s: palace artisans produced exquisitely-carved, inlaid enamelled or painted snuff bottles with a tiny spoon attached to the bottle stopper; a small portion of snuff was placed on the left thumbnail and inhaled through the nose. The Chinese believed that snuff cured pains in the eyes and teeth, alleviated throat ailments, constipation and cold symptoms, and promoted sweating (Christen *et al.*, 1982).

By 1650, snuff use had also spread from France to England, Scotland and Ireland. The Irish called snuff 'powder' or 'smutchin'; the Scots called it 'sneeshin' (Harrison, 1964).

Snuff use reached a peak in England during the reign of Queen Anne (1702-1714), and was called the 'final reason for the human nose'. It was at this time that ready-made snuff became available in England. It continued to be popular during the reign of George III, and his wife, Charlotte (1760-1820), referred to as 'Snuffy Charlotte', had an entire room in Windsor Castle devoted to her snuff stock. Lord Nelson, the Duke of Wellington, Marie Antoinette, Disraeli, Pope and Samuel Johnson all used snuff (Harrison, 1964). In diplomatic intrigue, poisons were sometimes placed in snuff. The aristocratic popularity of snuff led to a minor art form, in that snuff boxes became symbols reflecting the wealth and rank of their owner. The dandy, Lord Petersham, was said to own an annual set of 365 snuff boxes (Christen *et al.*, 1982).

The leading snuff supplier of the time, whose shop still exists in The Haymarket, London, provided King George IV with his own special blends, King's Morning Mix, King's Plain, and King's Carotte (Ryan, 1980). Home-made snuff was common. The tightly-rolled tobacco leaves (carotte) were often soaked in cinnamon, lavender, or almond oils; tobacco was dried and ground by means of an iron hand-grater that resembled a modern cheese-grater. The proper manner of inhaling snuff was to place a small quantity on the back of the hand and sniff it up the nostrils to induce a sneeze (Christen *et al.*, 1982).

Although hundreds of varieties of snuff existed in Europe by the 1800s, these consisted of three basic types: Scotch snuff, which was a dry, strong, unflavoured and finely-ground powder; Maccaboy, a moist and highly-scented snuff; and Rapee, also known as Swedish snuff, a coarsely-grated snuff (Heimann, 1960).

Snuff was introduced into Sweden in the middle of the seventeenth century, but its popularity among aristocrats reached a height during the eighteenth century, when use of nasal snuff became the highest fashion at the court of King Gustav III, among both men and women. The habit subsequently spread to the general Swedish population.

In many Swedish cities, snuff has been manufactured since the beginning of the eighteenth century. In Gothenburg, which is considered to be the centre of snuff production, manufacture started in about 1650 (Loewe, 1981). In 1795, Samuel Fiedler established a snuff mill in Gothenburg and began a small business, which later developed into three separate companies. At the end of the nineteenth century, the leading producer was Jacob Ljunglöf in Stockholm; his leading brand 'Ettan' became well known throughout Europe (Loewe, 1981). In 1914, the production of snuff in Sweden was taken over by the Swedish tobacco monopoly, which restored Gothenburg as the centre of snuff production in Sweden. A large factory was built around 1920, and expanded in 1979, for the production of snuff and chewing tobacco.

Since the beginning of the twentieth century, snuff has mainly been used orally in Sweden.

Snuff made its way to North America in 1611 by way of John Rolfe, husband of Pocahontas. Rolfe introduced the better Spanish variety of tobacco to ensure the survival of the Jamestown Colony in Virginia. Although most of the colonists in America never fully accepted the English style of snuff use, American aristocrats used snuff, and Dolly Madison was known to distribute samples of snuff to White House guests. During the 1800s until the mid 1930s, a communal snuff box was installed for members of the US Congress. The colonists also found it more to their taste to place snuff in their mouths rather than to sniff it (Christen *et al.*, 1982).

The first snuff mills in America were constructed in Virginia in about 1730 (Heimann, 1960). The snuff was made from New England tobacco and its quality was said to equal that of the native Scottish varieties (Robert, 1949). Pierre Lorillard, a Huguenot, established a snuff mill in New York in 1760 and carefully guarded the secret of his ingredients and blends (Christen *et al.*, 1982).

Between 1880 and 1930, the production of snuff in the USA increased from four million pounds (1.8 million kg) to more than 40 million pounds (18 million kg) per year (Garner, 1951). By 1945, the American Snuff Company in Memphis, Tennessee, claimed to be the largest snuff manufacturer in the world (Christen *et al.*, 1982). Snuff was made predominantly from dark, air- and fire-cured leaves. Stems and leaves were aged in hogsheads and conditioned before being cut into strips of one to two inches (2.5-5 cm) in width. The chopped leaves underwent further fermentation for about two months, during which time the tobacco lost its creosote-like odour and became more aromatic. It was next dried by passing through steam-heated containers and then ground to a fine powder in a revolving steel drum. The powder was passed over silk cloth containing as many as 96 threads per inch (38/cm). The coarse residue was returned to the mill for additional grinding before being packed into 100-pound (45-kg) bags for storage prior to repacking in smaller containers for retail sale. The dry and moist snuffs were used for dipping and placing in the mouth. Rappee or French snuff was used for inhaling, and Maccaboy snuff was both sucked and inhaled (Garner, 1951).

(*c*) *Tobacco and health*

In the past, tobacco use was considered by some to be beneficial. During the nineteenth and early twentieth centuries in America, dental snuff was advertised to relieve toothache, to cure neuralgia, bleeding gums and scurvy, and to preserve and whiten teeth and prevent decay (Christen *et al.*, 1982).

The use of tobacco, including smokeless tobacco, has been controversial since its introduction. Therefore, a history of smokeless-tobacco use is not complete without a discussion of the attacks on tobacco by various groups. In 1590 in Japan, tobacco was prohibited, and users lost their property or were jailed. King James VI of Scotland, who took over the British throne in 1604, was a strong anti-smoking advocate and increased taxes on tobacco by 4000% in an attempt to reduce the quantity imported into England. In 1633, the Sultan Murad IV of Turkey made any use of tobacco a capital offence, punishable by death from hanging, beheading or starvation, maintaining that tobacco caused infertility and reduced the fighting capabilities of his soldiers. The Russian Czar Michael Fedorovich, the first Romanov (1613-1645), prohibited the sale of tobacco, stating that users would be subjected to physical punishment; persistent users would be killed. A Chinese law in 1638 threatened that anyone possessing tobacco would be beheaded (Christen *et al.*, 1982).

During the mid 1600s, Pope Urban VIII banned the use of snuff in churches, and Pope Innocent X attacked its use by priests in the Catholic Church. Other religious groups banned snuff use: John Wesley (1703-1791), the founder of Methodism, attacked its use in Ireland; similarly, the Mormons, Seventh-Day Adventists, Parsees and Sikhs of India, Buddhist monks of Korea, members of the Tsai Li sect of China, and some Ethiopian Christian sects forbade the use of tobacco (Christen *et al.*, 1982).

In Germany (Bavaria) in 1652, tobacco was available only on a doctor's prescription; Frederick the Great, King of Prussia, prevented his mother, the Dowager Queen of Prussia, from using snuff at his coronation in 1790. Louis XV, ruler of France from 1723-1774, banned snuff use from the Court of France (Christen *et al.*, 1982).

In 1761, John Hill, a London physician and botanist, concluded that nasal cancer could develop as a consequence of tobacco snuff use. He reported five cases of 'polypusses, a swelling in the nostril that was hard, black and adherent with the symptoms of an open cancer' (Redmond, 1970).

1.3 Current practices

(*a*) *Tobacco chewing*

In the USA and Europe, the three main types of chewing tobacco are firm plug/moist plug, loose-leaf and twist/roll (Rizio, 1984). Each of these types is produced by using different tobaccos and additives, and by using different manufacturing processes.

Plug or pressed-leaf tobacco is made from enriched tobacco leaves or leaf fragments (spinner) wrapped in fine tobacco and pressed into flat bars or rolls before being packed in clear cellophane (Rizio, 1984). Plug can be either firm or moist: firm plug is more common and has a moisture content of less than 15%; moist plug is kept in a pouch or 'soft' and has a moisture content of 15% or more (US Department of Agriculture, 1982).

Loose-leaf tobacco, formerly called 'scrap', is made of fermented cigar leaf tobacco. Some brands are lightly sweetened, while others carry large amounts of sugars, syrups, liquorice and other flavouring materials. The treated tobacco is not compressed, but is packaged as a batch of loose pieces or cut strips. It is sold in a 3-oz (about 85-g) foil-lined pouch (Rizio, 1984).

Twist or roll tobacco is made of cured leaf which has been treated with flavouring materials. The processed leaves are then twisted into strands and allowed to dry.

Fine-cut tobacco: Some types of fine-cut smokeless tobacco, which had been classified as chewing tobacco prior to 1982, have been recategorized as moist/fine-cut snuff (see below).

In other regions of the world where tobacco is chewed, the sun-cured leaf is cut into strips of various sizes, followed by a short period of fermentation. For example, in some parts of India, the leaf strips may be over 5 mm in width, while in Indonesia the strips are finely cut.

The habit of chewing tobacco alone is practised predominantly in the USA and South-East Asia with some use in Europe and other Asian countries. Specific information on tobacco

chewing practices was limited for countries other than the USA, the UK, the USSR and India. Sales and production figures reported in section 1.5 give an indication of the extent of usage in other countries.

(i) *USA*

Data for the period 1880 to 1960 show a peak in the consumption of chewing tobacco in the USA between 1910 and 1920 (Heimann, 1960). More recent figures from the US Department of Agriculture (1984a) show a gradual decline in production from 1931 (the earliest year listed in the report) to the 1960s, at which time production began to increase again; this trend continued through the 1970s, and recent figures show an apparent plateau in the early 1980s. Since the upward trend began, production has increased by approximately 60%.

Total US consumption of smokeless-tobacco products was approximately 132 million pounds (60 million kg) per year during the period 1980-1982 (Tobacco Institute, 1981, 1982, 1983). On 1 January 1982, some types of chewing-tobacco products were reclassified as snuff (US Department of Agriculture, 1983). Under this classification, consumption of chewing tobacco in 1982 was 88 million pounds (40 million kg) (Tobacco Institute, 1983).

Part of the recent increase in the use of smokeless-tobacco products may have occurred among adolescents (Christen *et al.*, 1982; Glover *et al.*, 1983). It has been estimated that there are now 22 million users of smokeless-tobacco products (Greer & Poulson, 1983). Holleb (1984) indicates that the increase in chewing was from 2-4% per year from 1972 to 1980 for adults, with a slight decrease from 1981 to 1983; he estimates that there are seven million smokeless-tobacco users in the USA. The 1979 Report of the Surgeon General (US Department of Health, Education, and Welfare, 1979) found the prevalence of tobacco chewing in the USA in 1970 to be 5.6% among men and 0.6% among women. The figure for men decreased to 4.9% in 1975 but remained the same for women.

Chewing usually consists of placing a 'chaw' or 'quid' of leaf or plug tobacco in the gingival buccal area where it is held or chewed. A 'chaw' is a wad of chewing tobacco the size of a golf-ball, and a 'quid' is a much smaller portion, which is usually held in the mouth rather than chewed. Many persons chew during most of their working hours, and some keep a quid in place for 24 h a day (Christen, 1980a). Tobacco chewers habitually spit out the liquid extract produced by chewing.

Some measure of how long a tobacco chewer exposes himself to chewing can be determined from studies on selected populations. Moderate chewers have been defined as individuals for whom the total length of exposure of the buccal mucosa to tobacco was up to 200 min per day, while, for heavy chewers, it was more than 200 min per day (Wahi *et al.*, 1970). Men in Texas reported that they used two to eight chaws per day (Christen *et al.*, 1979a).

A market research bureau undertook a study in 1981 to determine a profile of the smokeless-tobacco user. Those men most likely to chew tobacco were between the ages of 18 and 34 years, were married and/or a parent, and had a high-school education or less (Maxwell, 1982).

There are several regional and demographic variations in chewing-tobacco patterns. According to Rizio (1984), twist or roll chewing tobacco is sold mainly in the southern and southern border states of the USA. Chewing tobacco is preferred by many workers in heavy industries, such as steel, coal and petroleum, where smoking is prohibited due to inflam-

mable work environments. There is also an increase in its use by persons who enjoy leisure-time activities involving their hands, and who prefer to chew rather than smoke cigarettes.

The areas in the USA where chewing is practised and identification of the populations who chew are described in several recent studies, which give indications of the age at which the habit is acquired and the dosages for various groups.

In 1976-1977, as part of the Bogalusa Heart Study in Louisiana, USA, 3147 children aged 8-17 years were asked various questions concerning chewing tobacco. White boys far exceeded those of other races in tobacco usage other than tobacco smoking. Approximately 25% of white boys aged 12-15 years had tried chewing tobacco (Hunter *et al.*, 1980).

A teen-aged population of smokeless-tobacco users in Denver, CO, USA, was identified; of 1119 students, 117 used snuff or chewing tobacco (113 boys and 4 girls). The length of time tobacco was kept in the oral cavity ranged from 53 to 177 min per day, and duration of use of these products ranged from 2.19 and 3.25 years (Greer & Poulson, 1983).

In 1980, a sample population of 2616 students in Nebraska, USA, aged 11-17 years, were asked about their use of chewing tobacco. Only eight girls admitted to chewing tobacco, whereas 90 boys (7.1%) reported practising the habit. Approximately one-third of the tobacco chewers were also cigarette smokers. The product was used daily by 36% of the chewers, 32% chewed weekly and 32% said they chewed only once a month; 23% had chewed for more than three years, 47% for two to three years and only 30% had chewed for one year or less (Newman & Duryea, 1981).

A study of 500 boys aged 10-16 years in Atlanta, GA, USA, showed widespread use of smokeless tobacco. Several boys aged 8-10 mixed chewing tobacco with bubble gum; 15% reported regular use of chewing tobacco (Anon., 1983).

A survey of college athletes in Texas showed that many began chewing between the ages of 10 and 12 years (Christen *et al.*, 1979a).

(ii) *Canada*

A survey was conducted in 1982 in the Northwest Territories to determine the extent of use of chewing tobacco among 7300 school children, 5-19 years old. Approximately 17% (boys, 26%; girls, 8%) of the student population reported that they had used chewing tobacco, and 9% (boys, 13%; girls, 2.9%) were current users. Use was more prevalent among the Indian/Métis and Inuit youths than among the non-native youths (Millar & van Rensberg, 1984).

(iii) *UK*

Tobacco chewing is uncommon in the UK. However, a study of five coal mines in south Lancashire revealed different chewing habits based on the actual location of work at the pit. Among 1490 miners, only 1.7% of surface workers chewed tobacco, as compared to 34.3% of those who worked underground. A further analysis of 858 of these miners found that 91.2% of the 195 tobacco chewers also smoked cigarettes. The quantity of tobacco chewed was 3-15 g per day, with an average daily use of 4.5 g (Tyldesley, 1971).

(iv) *India*

Chewing tobacco in India is made from sun-dried, coarsely cut leaves. About 85% of the tobacco used is sold loosely and is consumed raw. The dried leaf is broken into smaller pieces before being consumed. It may be used alone, in combination with lime (*khaini*), as a component of betel quid (see this volume) or in other forms such as *mishri*, *zarda*, *kiwam* and *pills* (described in section 1.3(*c*)).

Tobacco chewing is prevalent among people of all castes, creeds and religions, except Sikhs and Parsees. Overall, more men than women chew tobacco. In rural and urban low-income groups, the habit of chewing begins at 10-12 years of age and continues until old age.

In the one available study on the prevalence of pure tobacco chewing, Pindborg *et al.* (1967) reported that 2.1% of a population sample of 10 000 chewed tobacco alone.

(*b*) *Snuff taking*

The types of tobacco and ingredients used in snuff differ in the countries where the habit is practised. Prior to the nineteenth century, in Europe and the USA, the most popular means of consuming tobacco was sniffing the finely-ground leaf tobacco through the nose. Presently, the major use of snuff is to place it in the mouth between the lip or buccal mucosa and gum. In South-East Asia and Africa, snuff is taken both orally and nasally.

In the USA and Europe, snuff is currently described either as moist snuff, which is placed in the oral cavity and has a relatively high moisture content (up to 50%), or as dry (Scotch) snuff, which is placed in the oral cavity or administered through the nasal passage. Dry snuff usually has a moisture-content of less than 10%. Originally, in the USA, 'fine-cut' was used to describe chewing tobacco; however, at one point it was also used to describe snuff which may have been made from fine-cut chewing tobacco. It is a term that still may be used by some persons to describe snuff, but has little meaning in terms of a comparison of tobacco particle size. Frequently, the moist snuff category is listed as moist/fine-cut snuff.

Snuff used in Asia and Africa differs in its preparation and constituents from that used in the USA and Europe, as described in subsequent sections.

Although production and usage data, as reported in section 1.5, suggest that snuff is widely used in a number of countries, little or no information was available on the products used and number of persons practising the habit in areas other than Europe (particularly in Scandinavia) and North America.

Since snuff, its tobacco content, flavour components, manufacturing processes, and the customs affecting its use vary throughout the world (Axéll *et al.*, 1976), habits associated with its use are described by country or area.

(i) *Scandinavia*

During the eighteenth century in Denmark and Sweden, snuff was used as a fine, dry powder that was inhaled through the nose (Zacho *et al.*, 1968). Since the end of the nineteenth century, snuff has been used orally in Sweden and is usually placed behind the upper lip (Frithiof *et al.*, 1983); in Denmark it is usually placed behind the lower lip (Pindborg

& Poulsen, 1962; Pindborg & Renstrup, 1963). Currently, wet snuff, which is highly alkaline (pH 8-9), is the preferred type in the Scandinavian countries (Pindborg & Axelsen, 1980; Hirsch *et al.*, 1982).

It is estimated that in 1978, there were 700 000 to 800 000 snuff users in Sweden (Frithiof *et al.*, 1983). Data from clinical studies in Sweden and Denmark indicate that the average snuff user is approximately 50 years old (mean of three investigations: Axéll *et al.*, 1976; Pindborg *et al.*, 1980; Hirsch *et al.*, 1982), uses snuff 10 h per day (mean of three investigations: Roed-Petersen & Pindborg, 1973; Axéll *et al.*, 1976; Hirsch *et al.*, 1982) and has been using snuff for 22 years (mean of two investigations: Axéll *et al.*, 1976; Hirsch *et al.*, 1982). The estimated consumption per day for an average user is about 15 g (mean of two investigations: Axéll *et al.*, 1976; Hirsch *et al.*, 1982). Average consumption is estimated to be approximately 100 g per week and 5.4 kg per year per user (Österdahl & Slorach, 1983).

Almost all snuff users in Sweden are men, and they constitute 17% of the population. The prevalence is 19% in the age group 15-30 years and 10% among those 65 years and older. Only 1% of Swedish women use snuff regularly (Axéll, 1979; Barroll & Ramström, 1979; Hirsch, 1983). Among school children aged 13-16 years, 11-15% of boys but no girls used snuff regularly (Modéer *et al.*, 1980; Hibell & Jonsson, 1982; Hirsch, 1983). The average consumption of snuff among school children aged 13 and 14 years was five pinches per day; the snuff was kept in the oral cavity for an average of 3.5 h (Modéer *et al.*, 1980).

There are geographical differences in the use of snuff in Sweden. The habit is more widespread in the northern parts of the country, where approximately 25% of the male population over the age of 15 years use snuff daily. Snuff usage is also more common in rural areas (20%) and in small towns (13%) than in large towns (7%). It was found that people with high consumption of snuff usually smoked fewer cigarettes than did those people who used moderate amounts of snuff. Of male snuff users, 43% did not smoke, 22% smoked occasionally and 35% were regular smokers (Hirsch, 1983); two-thirds of snuff users were social drinkers. Many different brands of snuff are available, but the majority of snuff takers use only one or two brands (Hirsch *et al.*, 1982).

(ii) *USA*

This section should be read in conjunction with that on p. 55, which describes trends in the production and use of smokeless-tobacco and chewing-tobacco products in the USA.

Data for the period 1880 to 1940 show a peak in snuff consumption in the USA between 1910 and 1920 (Schuman, 1977). More recent figures from the US Department of Agriculture (1984a) show that production was relatively stable from 1931 (the earliest year listed in the report) to the mid-1950s, at which time there was a slow decline in production that continued until the early 1980s.

On 1 January 1982, some chewing-tobacco products were reclassified as snuff (US Department of Agriculture, 1983); under this classification, 43.9 million pounds (20 million kg) of snuff were consumed in the USA in 1982 (Tobacco Institute, 1983). In 1983, sales of moist snuff had increased by 7.1% over the 1982 figures (Shelton *et al.*, 1984).

In 1970, 2.9% of men and 1.4% of women used snuff; by 1975, only 2.5% of men and 1.3% of women used snuff (US Department of Health, Education, and Welfare, 1979); a 1983 market research bureau reported that smokeless tobacco is used in 3.2% of US households in which a male is living (Maxwell, 1984). Christen and Glover (1981) reported that

approximately 30% of women in central North Carolina were snuff users as compared to 1.3% of women in the USA as a whole. A 1983 market research bureau report indicated that US snuff users were more likely to live in a rural area of eastern-central or southern USA, and to be in a lower-income, part-time employment category. In contrast to chewing-tobacco users, a significant number of snuff users had attended college (Anon., 1984).

In the USA, dry snuff is used mostly by middle-aged and older people, whereas moist snuff is used mainly by young adults. The average user of moist snuff consumes 1.5 tins (1.2 oz or 34 g) per week (Maxwell, 1980).

The usual method of taking snuff in the USA is to place a pinch in the gingival buccal area, between the buccal mucosa or lip and the gums, or beneath the tongue and to leave it in position for a few minutes or as long as desired. This is in contrast to the eighteenth-century practice of inhaling the snuff into the nasal cavity. Oral use of snuff is colloquially referred to as 'snuff dipping' in the USA, from the habit of dipping into snuff a stick that had previously been chewed to flatten the end, and applying the snuff to the gums. Some users of dry snuff measure out a small quantity in the lid of the container or into a special spoon and then place the snuff between the lower lip and the gum. Moist snuff is used by taking a 'pinch' of the slightly damp tobacco between the thumb and forefinger and tucking it between the lower lip and gum. Snuff has recently become available in 'tea-bag'-like packets, which are placed in the mouth.

In a study of snuff usage among middle and lower socioeconomic groups in south-eastern USA, it was found that many used snuff most of their waking hours and some slept with the quid in their mouths. In a study of 290 patients with oral-cavity lesions, it was not unusual to find that the persons had used snuff regularly before the age of 10 years, and 75% of the patients had used snuff for 40 years (McGuirt, 1983a).

In a study in south-eastern USA, of 255 women with oral and pharyngeal cancer, it was found that whites had used snuff for an average of 47.6 years *versus* 36.1 years for blacks, and that they used more tins (1.15 oz or 36 g) per week than did blacks (3.0 *versus* 2.4). The average duration of snuff use was less among cigarette smokers (33.0 years) than among nonsmokers (47.4 years) (Winn *et al.*, 1981a).

Of a population of 15 000 snuff users who resided primarily in one state in south-eastern USA, the average age of the subjects who were examined for 'snuff-dippers' lesions' was 55 years; 75% were women. This elevated number of users probably represents a regional population sample bias (Smith *et al.*, 1970; Smith, 1975).

In another south-eastern US state, 11% of 500 boys, 10-16 years of age, reported using snuff regularly (Anon., 1983).

In an urban school population of 565 boys (average age, 13.8 years), 200 were selected for a follow-up dental study. Of this selected group, 11% used snuff regularly (Weather & Offenbacher, 1983). In another study of college men in a southern state, as many as one-third were either tobacco chewers, snuff users, or both (Christen, 1980b).

(iii) *Canada*

A survey was conducted in 1982 in the Northwest Territories to determine the extent of snuff use among 7300 school children aged five to 19 years. Approximately 10% (boys, 16%; girls, 5%) were current users of snuff. Use was more prevalent among the Indian/Métis and Inuit youths than among non-native youths (Millar & van Rensberg, 1984).

(iv) *South-East Asia and Africa*

Unscented snuff is used in many parts of *India* as a dentifrice, while scented snuff is used for sniffing. The habit of sniffing is on the decline, but use of snuff as a dentifrice by men, women and children in the poorer sections of society is widely prevalent. It is used from twice to eight to ten times per day (Anon., 1953). In a study from Ahmedabad, of 57 518 industrial workers examined, 1316 (2.3%) used tobacco only by inhaling snuff (Smith *et al.*, 1975).

In *Thailand*, snuff ('a tan, dry powder') is taken with the aid of a U-shaped metal tube: one end of the tube is placed in the mouth and the other in the nasal passage. Air blown from the mouth scatters the powder into the nose, through which it is inhaled (Harrison, 1964).

In *South Africa*, snuff inhalation is widely practised by Bantu men and women, for whom its use has an important cultural and ritual history. The product typically contains tobacco leaves and ash from aloe plants or other species, with the occasional addition of oil, lemon juice and herbs; use is often one teaspoonful per day (Keen *et al.*, 1955; Harrison, 1964; Baumslag *et al.*, 1971). In a small survey in one town, 19% of Bantu men and 30% of the women used snuff orally or nasally (Higginson & Oettlé, 1960).

Among the Fingo and Xhosa tribes of *South Africa*, snuff is placed between the gingiva and lower lip or buccal area (Harrison, 1964). Among elderly Bantu men and women, snuff is usually retained in the front of the mouth. The saliva mixes with the snuff and is then swallowed. After the snuff has lost its flavour, the procedure is repeated (van Wyk, 1966).

(*c*) *Other smokeless-tobacco habits*

(i) *Tobacco and lime* (khaini)

Tobacco is sometimes chewed in the presence of lime. In certain parts of India, this is referred to as *khaini*. A pinch of raw powdered tobacco is taken in the palm and a small amount of slaked lime paste is added; the mixture is then rubbed thoroughly with the thumb and placed in the mouth — generally in one or both cheeks, or in the mandibular groove. The mixture is retained for 10-15 min, after which time it becomes bland; occasionally it is left in the groove during sleep (Bhonsle *et al.*, 1979). Pieces of areca nut are sometimes chewed with *khaini*.

Use of *khaini* is prevalent among the Munda and Santal tribes of Bihar, India; it is usually placed in the inner side of the lower lip in the gingivolabial groove. Among those examined, approximately 42% of *khaini* users held the mixture in the front of the oral cavity, whereas others placed it either in the left or right side, where it was generally retained with very little chewing (Stich *et al.*, 1982).

Pattiwala tobacco is sun-cured tobacco leaf and is used with or without lime (Wahi, 1968). Mainpuri tobacco is a mixture with slaked lime, areca nut and spices; its use is discussed in the monograph on betel-quid and areca-nut chewing, p. 141.

(ii) Mishri (misheri, masheri)

Mishri is a form of tobacco used in India as a substitute for chewing tobacco. It is a 'roasted or half-burnt' tobacco, prepared by baking tobacco on a hot metal plate until it becomes uniformly black. It is then powdered and used primarily for cleaning teeth (Mehta *et*

al., 1972). However, its use frequently becomes habitual, and a user may apply and retain *mishri* in the mouth (usually along the teeth and in the sulcus) several times a day. Rural Indian women place it in the oral cavity between the gum and buccal mucosa instead of chewing tobacco (Murdia *et al.*, 1982).

Mishri usage has been reported from a house-to-house survey of 101 761 individuals in the state of Maharashtra, where 38.9% of the women and only 0.8% of the men used it (Mehta *et al.*, 1972). In a house-to-house survey of 10 071 individuals in Gujarat (the Bhavnagar District), 7.1% (mostly women) used *mishri* (Mehta *et al.*, 1971). The use of burnt tobacco for cleaning teeth was reported by 0.24% of the population in the Mainpuri District (Wahi, 1968).

(iii) Zarda, kiwam *and* pills

Zarda, which is produced and used in India, is also exported to a number of Arab countries (Sinha, 1984). During the manufacture of *zarda*, tobacco leaf is first broken into small pieces and boiled in water with lime and spices until evaporation. The residual particles of tobacco are then dried and coloured with vegetable dyes. *Zarda* is usually chewed mixed with finely-cut areca nut and spices.

For *kiwam*, the stalks, midribs and veins of tobacco leaves are removed, and the remaining matter is soaked and boiled in water with added rose water and powdered spices, such as saffron, cardamom, aniseed and musk. The mixture is stirred and allowed to macerate. The pulp is strained to remove any stalk or rib remnants and allowed to dry. The product has the consistency of a thick, rough paste. Granules or *pills* are prepared using the same process, but the paste is dried further and pelleted (Anon., 1953).

(iv) Gudakhu

Gudakhu is a paste consisting of powdered tobacco, molasses and some other ingredients. It is used for cleaning teeth by populations in central and eastern states of India. In a house-to-house survey of a random sample in Singhbhum (Bihar district), 8.3% of the population were reported to use this product (Mehta *et al.*, 1971).

(v) Shammah

Shammah is the native name for a tobacco mixture used in some parts of southern Saudi Arabia. It is described by Yousef and Hashash (1983) as a quid of powdered tobacco leaf, carbonate of lime, and other substances, including ash. Salem *et al.* (1984), however, describe *shammah* as a snuff prepared by mixing powdered tobacco leaves with sodium carbonate. The material, a greenish-yellow powder, is placed in the buccal or lower labial vestibule of the mouth. Periodically, the user spits out insoluble debris that is freed from the *shammah* bolus. In a survey made on 661 individuals in various geographical locations of the Gizan district, it was reported that 24% practised this chewing habit (Salem *et al.*, 1984).

(vi) Nass

The habit of using *nass* is practised by the native populations of Iran and the Soviet Central Asian Republics (the Uzbek, Turkmenian, Kirghiz, Tadzhik and Kazakh SSRs) (Shilovtsev, 1941; Joint Iran-International Agency for Research on Cancer Study Group, 1977; Paches & Milievskaya, 1980). It is usually made with local tobacco (which is sometimes only partially cured), ash, cotton oil or sesame oil and lime (Paches & Milievskaya, 1980). How-

ever, the composition of *nass* varies in the different regions in which it is used, the primary difference being the content of lime (Table 1). *Nass* prepared in the Tadzhik SSR and most types of *nass* prepared in the Kazakh SSR do not contain lime (Paches & Djuliev, 1965; Aleksandrova, 1970), whereas that in the Bukhara, Samarkand and Kashka-darya regions of the Uzbek SSR contains the highest amounts of lime (Paches & Milievskaya, 1980).

Table 1. Variations in the constituents of *nass* in five regions of the USSR[a]

Region	Constituents of *nass* (%)						
	Tobacco	Ash	Cotton oil	Sesame oil	Gum	Water	Lime
Bukhara	55	18	-	20	-	2	10
Kashka-darya	50	30	10	-	-	3	7
Samarkand	50	25	15	-	-	3	7
Tashkent	50	20	-	-	2	17	3
Fergana	55	20	-	-	3	18	4

[a]From Paches and Milievskaya (1980)

In addition to variations in composition, there are regional differences in the anatomical site in the mouth where *nass* is put (Aleksandrova, 1970; Khasanov & Fasiev, 1970; Zaridze *et al.,* 1985a). Nugmanov and Baimakanov (1970) reported that 60% of *nass* users in the Chimkent region of the Kazakh SSR put *nass* under the tongue, and 40% between the gum and lower lip. According to Aleksandrova (1970), 96% of *nass* users in the Djambul region of the Kazakh SSR place the *nass* against the inside of the lower lip. In a survey of 1569 men carried out in the Samarkand region of the Uzbek SSR, all but one of the *nass* users reported placing the *nass* under the tongue (Zaridze *et al.,* 1985a).

The daily frequency of *nass* chewing also varies considerably. Surveys indicate that most users take *nass* about 10-15 times per day (Aleksandrova, 1970; Khasanov & Fasiev, 1970; Zaridze *et al.*, 1985b,c). The saliva produced during chewing is expectorated and the mouth rinsed with water when the chew is removed.

According to various surveys, 4-49% of the adult population in *nass*-using areas practise this habit. In a survey of 15 672 persons living in urban (5135) and rural (10 537) areas of the Chimkent region (Oblast) of the Kazakh SSR, 7.5% of the total population and 49% of the native Kazakhs used *nass*. The proportional age distribution and proportion of *nass* users in each age group are given in Table 2; 67.8% of the *nass* users were men and 32.2% women (Nugmanov & Baimakanov, 1970).

According to another survey (Aleksandrova, 1970), the proportion of *nass* users in the Chimkent region is 4.5% (364 out of 8123 persons surveyed). A survey of 2012 persons in

Table 2. Age distribution of *nass* users in the Chimkent region of the Kazakh SSR[a]

Age (years)	% surveyed	% *nass* users
35-39	20.5	6.9
40-49	28.5	17.3
50-59	21.7	21.6
60-69	17.1	31.3
70+	12.2	22.9

[a]From Nugmanov and Baimakanov (1970)

the Djambul region of the Kazakh SSR revealed that 14.3% (289) used *nass*. In the Chimkent region, 22% of *nass* users were women, while in the Djambul region the proportion was 52%.

A survey of 988 persons aged 25 years and above in a rural area of the Mary region of the Turkmenian SSR revealed that 14% of persons of both sexes and 36% of men were *nass* users (Saparov, 1965).

A survey carried out in three regions (Vakhsh, Zeravshan and Gorno-Badakhshan) of Tadzhikistan revealed that 20%, 27% and 17%, respectively, of the population over the age of 20 years were *nass* users. In Tadzhikistan as a whole, the proportion of *nass* users was estimated to be 20% (Paches & Milievskaya, 1980). Of 6520 men and women residents of the Bukhara region of the Uzbek SSR, 1479 (22.6%) chewed *nass* (Khazanov & Fasiev, 1970).

The proportion of *nass* users in surveys carried out in Kirghiz was estimated as 5% (Paches & Djuliev, 1965).

A survey of 1569 men aged between 55 and 69 years, performed in the Samarkand region of the Uzbek SSR revealed that 636 (41%) of the men interviewed were *nass* users (Zaridze *et al.*, 1985a) and 3% both used *nass* and smoked (Zaridze et *al.*, 1985b,c).

(vii) Naswar

Naswar is widely used in Afghanistan and Pakistan. In Afghanistan, it is described as a mixture of powdered tobacco, slaked lime and indigo, and is available in ready-made form which is sold under licence from the government. Most people place it on the floor of the mouth; some put it in the labial groove behind the lower lip; and in rare cases it is placed on the dorsum of the tongue. It is kept in the mouth for about three to 10 min and then spat out; most people rinse their mouth with water after use (Mehta & Pindborg, 1968). To prepare *naswar*, water is poured into a cement-lined cavity; lime is added, followed by air-cured, dried, powdered tobacco and a colouring material, indigo. The ingredients are then thoroughly pounded and mixed. A large, heavy, wooden mallet (an ingenious contrivance raises the wooden mallet-head and brings it down heavily on the mixture) is used for thorough mixing. A number of people in rural areas make their own *naswar* in a manner similar to that used in commerce (Mehta & Pindborg, 1968).

In Pakistan, *naswar* is manufactured by mashing and blending high-nicotine, sun-cured *Nicotiana rustica* tobaccos with ashes of plants, slaked lime, water and flavouring essences, usually cardamom oil and menthol, and the product is formed into semi-dried pellets and powder. It is placed in the mouth behind the lower lip and chewed slowly. Its use dates back to the introduction of tobacco into this region (Ahmad, 1976).

Consumption in Pakistan in 1975 was estimated to be 5.0 million pounds (2.3 million kg) (Ahmad, 1976).

1.4 Manufacturing processes

(a) *Chewing tobacco*

Types of chewing tobacco produced by local techniques and those for which limited information was available on the manufacturing processes are described in the previous sections.

Complete details of current US manufacturing processes for plug, twist and loose-leaf tobacco were not available to the Working Group; however, a general description can be given on the basis of published reports. Usually, tobacco is aged for one to four years, during which time natural fermentation occurs (Shapiro, 1981; Rizio, 1984). Beyond this step, the techniques differ for specific products.

Loose-leaf chewing tobaccos are made with cigar leaf from Pennsylvania and Wisconsin. Following removal of the stem, the tobacco is cut into uniform strips and flavourings are added, which may include honey, liquorice and rum. The combination of ingredients and quantities vary with the brand. One sweet type contained 56% tobacco materials and 44% combined flavouring additives and moisture (Akehurst, 1981).

Plug chewing tobaccos are made with the same aged, cut and blended tobacco used in loose-leaf as well as with air- and fire-cured types (Rizio, 1984). Leaf strips may be immersed in a mixture of liquorice and some form of sugar before being pressed into the plug form. The pressed leaf blends are then wrapped in fine tobacco and moulded into flat bars. The consumer bites or cuts off a small portion of the plug and places it in the mouth.

Twist chewing tobaccos are made with air-cured types such as burley, as well as with fire-cured types; flavouring ingredients and sugar may be added. The twist is created by twisting the tobacco leaves into a shape that resembles a rope (Rizio, 1984).

In the UK, chewing tobacco is made only in plug form. It is unsweetened and usually made of fire-cured tobacco. The wrapper is a particularly fine, thin, well-textured leaf, and the moisture content of the manufactured plug is approximately 30% (Akehurst, 1981).

Chewing tobacco in India is made from *Nicotiana rustica* and *N. tabacum*. The tobacco leaves are harvested when they turn yellow and brown spots start appearing; the leaves remain in the field and are turned over occasionally to achieve uniform drying. They are then tied in bundles and moistened by sprinkling with water; the bundles are stacked for fermentation for a couple of weeks, separated and dried again. The leaves are cut into various sizes. About 85% of the tobacco harvested are consumed raw without further processing.

Nass is not produced commercially in the USSR but is produced and sold at local markets by peasants.

(*b*) *Snuff*

The quality of tobacco is dependent upon the soil and climate where it is grown, as well as the variety. The final snuff product is also influenced by such factors as harvesting and curing; and the drying process is critical to the ultimate chemical composition of the tobacco. Manufacturers may vary the composition of the final product through the use of additives, the identities of which are protected by rules of proprietary information.

(i) *USA*

In the USA, snuff is made primarily from Kentucky, Tennessee, and Virginia air- and fire-cured tobaccos. After the dark tobacco has been stalk-harvested, it may be air- or fire-cured, a process that requires several weeks. In the fire-curing process, the leaf undergoes a yellowing stage, after which brown spots start to appear; a small amount of heat is then created by lighting small fires, thus slowly reducing the humidity. When the stems have darkened, the tobacco is exposed to large volumes of heavy smoke for two to three weeks.

The number of fires is reduced, and the sawdust and floor are kept wet continuously. As soon as the firing is completed, the tobacco is gathered into piles to preserve the finish and flavour (Everette, 1958).

In general, the whole tobacco leaf is used. The leaves are aged for one to four years; snuff tobacco may be allowed to age for a greater number of years than chewing tobacco (Shapiro, 1981; Rizio, 1984). Following ageing, the tobacco is cut into strips, the size of which depends upon the product, and then undergoes fermentation for several weeks. The moisture is adjusted, and the tobacco is cut to meet product specifications. Casings (hygroscopic agents and flavouring constituents, including synthetic sweetening agents (see IARC, 1980)) may be added; however, the composition is proprietary information (Akehurst, 1981). Some products undergo a second fermentation, the temperature and duration of which are carefully monitored; this second fermentation may take several years. After these curing processes, the tobacco is ground into coarse-, medium- or fine-grained powder. The powdered tobacco is then moistened again with additives (Pöschl, 1983).

(ii) *Europe*

In Sweden, snuff is manufactured from dark Kentucky and Virginia tobaccos that are stronger than those used for cigarettes. Water and salt are added to keep the product fresh before it is heated. Various ingredients are then added, the exact composition remaining a trade secret (Hirsch, 1983).

In addition to the method used in the USA, three further processes are used in the manufacture of snuff in Europe.

In the 'Rapid' method, tobacco leaves and stems are pulverized in high-speed, blower-type crushers, sieved and then moistened with a brine solution. The tobacco is then fermented rapidly in hot rooms for six to eight weeks. It is sieved again and mixed with 5-8% fine salt and then fermented for a longer period of time. The Rapid method is the most widely used process today and produces the so-called 'green' snuffs known as *Kovno*, 'refreshment' tobacco, and Danzig types, as well as the modern English type of snuff. Menthol, peppermint oil, camphor and other aromatic additives, such as attar of roses and oil of cloves (see IARC, 1985), are blended with the tobacco. The grain size is small, much like powder. An important feature of these rapid-method snuffs is their high concentration of aromatics (Pöschl, 1983).

In the 'Paris' method, Virginia and Kentucky tobaccos are pounded in salt water and left to ferment for several years in cool storage rooms. The tobacco is then compressed into batches and subsequently crushed or pulverized by pounding machines, sieved, and remoistened with salt water. This method produces the so-called 'black' varieties, such as 'Paris' and 'Saarbrücken'. The Paris method is used in the Federal Republic of Germany and France (Pöschl, 1983).

The final type of processing is by the 'Schmalzler' method. The tobacco leaves, which come mainly from Brazil, are cut and moistened with sugar sauces prior to fermentation at high temperatures over a period of a few months. The tobacco is then dried and ground in special machines called 'grinding chairs', sieved, and then moistened with fine oils. At one time, clarified butter was used on Brazilian tobacco, leading to use of the term 'Schmalzler' (*schmalz* is the German word for lard or melted fat). The fragrance of Schmalzler tobacco results from the admixture of 'mangotes', which are ropes of tobacco that have been treated with special sugar sauces, fermented and then pressed and sewn into fresh cowhides (Pöschl, 1983).

Britain's largest snuff producer uses dark-fired tobacco leaves and stems, mostly from Malawi. The dried leaves are ground into a coarse powder in a motor-powered mortar before being reduced to a finer powder in a grinder. After potash, soda ash and pharmaceutical soda have been added to the sieved tobacco, ground menthol is blended in (Anon., 1981).

(iii) *Asia, Africa and other regions*

With the exception of India, Thailand and Turkey, almost no manufacture of snuff is carried out in Asia, eastern Europe or South America (Pöschl, 1983).

Oriental snuff is used in such countries as Thailand and differs in its preparation and constituents from that used in Europe and the USA. It consists of approximately 50% dry tobacco and 50% oriental gum with a small amount of pulverized cuttle bone. The gum is made by heating 'white earth', which contains calcium carbonate and phosphate, at high temperatures in a kiln. After the addition of water, the gum paste is mixed with tobacco and dried in the sun (Harrison, 1964).

In parts of Africa, snuff is prepared by mixing powdered tobacco leaves with ash from various incinerated plants, such as aloe, *Amaranthus spinosum* or *Turbinata oblongata*; oils and lemon juice are sometimes added (Keen *et al.*, 1955; Harrison, 1964; Baumslag *et al.*, 1971).

1.5 Production and use

(a) *Chewing tobacco*

(i) *Production*

US production data for the major categories of chewing-tobacco products have been reported by one source for the period 1931-1980 (US Department of Agriculture, 1984a). Selected data that show the trends of production for each category are shown in Table 3. Maxwell (1984) reported US production data under slightly different categories, as summarized in Table 4, in which certain types of fine-cut smokeless tobacco that had been classified as chewing tobacco prior to 1980 have been recategorized as moist/fine-cut snuff.

Table 3. US production of chewing tobacco by major category[a]

Year	Production (millions of kg)				
	Plug	Twist	Fine-cut	Loose-leaf	Total
1931	34.8	2.9	1.9	27.8	67.4
1942	24.6	2.7	2.3	21.9	51.7
1950	18.2	2.5	1.2	17.7	39.7
1960	12.0	1.5	1.4	14.5	29.5
1962	11.8	1.3	1.5	14.7	29.4
1974	8.3	1.0	2.8	23.3	35.5
1979	7.0	0.9	6.1	31.8	45.8
1980	7.6	0.9	6.7	32.8	48.1

[a]Data from US Department of Agriculture (1984a)

Table 4. US production of chewing tobacco by major category[a]

Year	Production (millions of kg)				
	Plug[b]	Moist plug[b]	Twist/ roll[c]	Loose-leaf	Total[d]
1981	5.1	2.9	0.9	32.0	40.9
1982	4.6	2.3	0.8	32.2	39.9
1983	4.4	2.0	0.8	32.2	39.3

[a]Data from Maxwell (1984)
[b]Data for plug plus moist plug correspond to data for plug in Table 3
[c]Data correspond to data for twist in Table 3
[d]Excludes data for moist/fine-cut tobacco, which is currently classified as snuff

Data on production of chewing tobacco in selected countries during the period 1974-1978 are given in Table 5.

Table 5. Production of chewing tobacco in selected countries[a]

Country	Production (thousands of kg)				
	1974	1975	1976	1977	1978
Algeria			4045.7	4220.2	
Austria			8.9	5.2	
Belgium	7.1	6.0	4.8	3.9	3.3
Canada			230.6	197.8	
Denmark	102.0	99.0	90.0	84.8	80.5
Egypt			52.0	50.0	
Finland			1.3	1.2	
France			133.6	131.0	
Libyan Arab Jamahiriya			80.3	79.3	
Mexico			1000.0	1050.0	
Netherlands	313.0	288.0	253.0	253.0	
Pakistan			2344.0	3954.9	
South Africa	158.5	185.3	181.1	158.7	139.9
Sweden	13.0	12.0	12.0	13.0	13.0
Tunisia			705.3	752.9	

[a]Data from Anòn. (1978, 1979)

Available statistics on production and exports of chewing tobacco for India in recent years are shown in Table 6.

Table 6. Available statistics on production and exports of chewing tobacco for India (millions of kg)[a]

	1977-1978	1978-1979	1979-1980	1980-1981	1981-1982
Production	70.8	70	72	85.3	85
Export	4.0	8.9	9.2	7.0	-

[a]From Sinha (1984)

Although a major portion of the chewing tobacco produced in India is used internally, considerable quantities of scented tobacco and *zarda* are exported to a large number of countries, particularly to Arab countries; Saudi Arabia is the major importer of Indian chewing tobacco. It is also exported to Bahrain, Belgium, Dubai, Egypt, Israel, Kuwait, the Maldives, Nepal, Oman, Qatar, Singapore, Somalia, the UK and the USA (Sinha, 1984).

(ii) *Use*

In 1880, the earliest recorded year, US per-capita consumption of chewing tobacco was 3.15 pounds (1.43 kg) per person aged 14 years or over (Wynder *et al.*, 1957a). The highest US per-capita consumption for persons aged 15 years or over occurred in 1900, at 4.1 pounds (1.9 kg), followed by a gradual decrease to 0.5 pounds (0.23 kg) in 1962 (Schuman, 1977). Per-capita consumption by males 18 years and older was 1.05 pounds in 1966, increasing to 1.34 pounds (0.61 kg) in 1979 (US Department of Agriculture, 1980). In 1982, some chewing-tobacco products were reclassified as snuff. Under this new classification, male per-capita consumption was 1.06 pounds (0.48 kg) in 1983 (US Department of Agriculture, 1984b).

Data on total use of chewing tobacco have been reported for various countries for the period 1920-1973 (International Trade Centre, 1968; Wilson, 1975). Selected data on trends in individual countries are shown in Table 7. Additional data on sales in four countries during 1974-1978 are given in Table 8.

Estimated per-capita consumption of chewing tobacco in selected countries is summarized in Table 9.

(*b*) *Snuff*

(i) *Production*

Data for production of snuff in selected countries during the period 1974-1978 are given in Table 10 (Anon., 1978, 1979).

The production of snuff in the USA increased from 4 million pounds (1.8 million kg) to more than 40 million pounds (18 million kg) between 1880 and 1930 (Garner, 1951). US production of snuff for the period 1931-1980 has been reported by the US Department of Agriculture (1984a). Selected data to show the trends are shown in Table 11. Maxwell (1984) reported US production data under different categories, as summarized in Table 12, in which certain types of fine-cut smokeless tobacco that had been classified as chewing tobacco prior to 1982 have been recategorized as moist/fine-cut snuff.

The Federal Republic of Germany is the largest producer of nasal snuff (about 250 tonnes per year), followed by the UK (about 150 tonnes per year), and France and Italy (less than 100 tonnes per year) (Pöschl, 1980).

In the Federal Republic of Germany, manufacturers of snuff produce many varieties and flavours, of which the best known type is Bavarian 'Schmalzler'. Most of the snuff produced in Germany is flavoured with menthol. Snuff for nasal use is produced chiefly in Bavaria, particularly in Landshut, where some 70% of German snuff manufacturing is located (Pöschl, 1983).

Table 7. Tobacco use in various countries (millions of kg)[a]

Year	Austria	Brazil		Canada	Denmark	France	Germany (Fed. Rep.)[b]	India	Norway	Sweden[c]
	Chewing tobacco	Cut tobacco	Plug tobacco	Plug tobacco	Chewing tobacco	Chewing tobacco	Chewing tobacco	Chewing tobacco	Chewing tobacco	Chewing tobacco
1920	-	-	-	3	1.4	-	-	-	-	0.4
1922	-	-	-	4.6	0.9	-	-	-	-	0.3
1930	0.4	-	-	2.7	0.9	-	-	-	0.9	0.1
1940	0.3	-	-	1.4	0.6	0.5	-	-	0.5	0.1
1945	0.05	1.1	41	1.4	0.7	0.4	-	-	0.2	0.04
1948	0.05	1.1	41.3	1.0	0.4	0.7	-	56.6	0.4	0.04
1950	0.2	1.1	41.4	1.0	0.4	0.6	-	58.5	0.3	0.04
1955	0.1	0.9	41.6	0.7	0.3	0.5	-	53.7	0.2	0.04
1960	0.05	1.3	40.8	0.5	0.3	0.6	-	60.4	0.2	0.04
1962	0.05	1.5	39.2	0.5	0.2	0.5	1.5	64	0.2	0.04
1963	0.05	1.6	38.4	0.4	0.2	0.5	1.4	54.2	0.1	0
1964	0.05	1.7	37.6	0.4	0.2	0.5	1.2	53.8	0.1	0
1965	0.05	1.8	36.8	0.4	0.2	0.5	1.3	53.7	0.1	0
1966	0.05	1.6	35.9	0.4	0.2	0.5	1.3	53.3	0.1	0
1973	0	2.2	29.3	0.3	0.1	0.5	-	43.4	0.1	0

[a]Data from Wilson (1975), unless otherwise specified

[b]Data from International Trade Centre (1968)

[c]Svenska Tobaks Ab (1964, 1973) reported that use in Sweden was less than 50 tonnes in all years from 1947 on, and the Swedish Tobacco Co. (1983) reported that consumption of chewing tobacco in Sweden was 15 tonnes in 1973 and 23.3 tonnes in 1982.

Table 8. Sales of chewing tobacco in selected countries (thousands of kg)[a]

Country	1974	1975	1976	1977	1978
Netherlands	313.0	293.0	256.0	263.0	-
Norway	85.0	63.0	69.0	69.0	59.0
South Africa	152.5	185.3	181.1	158.7	139.9
Sweden	1.6	1.7	2.4	2.0	1.9

[a]Data from Anon. (1979)

Table 9. Estimated per-capita consumption (g) of chewing tobacco in selected countries[a]

Country	1962	1963	1964	1965	1966
Austria	8	8	8	8	8
Canada	41	36	35	32	31
Denmark	68	66	66	51	50
France	16	14	15	14	14
Germany, Federal Republic of	37	34	30	33	32
Norway	67	52	50	50	50
Sweden	8	-	-	-	-

[a]Data from International Trade Centre (1968)

Table 10. Production of snuff in selected countries (thousands of kg)[a]

Country	1974	1975	1976	1977	1978
Algeria	-	-	745.3	992.1	-
Austria	-	-	2.9	2.0	-
Belgium	6.4	5.2	3.6	3.0	2.9
Canada	594.2	553.6	585.5	570.5	565.9
Denmark	241.0	240.0	223.0	215.3	202.9
Egypt	-	-	22.0	9.0	-
Finland	-	-	13.2	14.2	-
France	-	-	80.0	70.0	-
Germany, Federal Republic of	-	-	292.0	294.0	-
Ireland	11.0	10.0	10.0	9.0	9.0
Israel	-	36.5	35.2	35.2	33.0
Italy	145.6	107.2	126.4	150.8	85.0
Libyan Arab Jamahiriya	-	-	11.6	15.0	-
Morocco	-	-	60.6	66.1	-
Pakistan	-	-	4501.0	4082.0	-
South Africa	1391.0	1474.1	1495.0	1363.8	1244.2
Sweden	2831.0	2917.0	3241.0	3523.0	3468.0
Switzerland	-	-	10.0	8.3	-
USA	-	-	11271.0	11164.0	-

[a]Data from Anon. (1978, 1979)

Table 11. US production of snuff[a]

Year	Production[b] (millions of kg)
1931	18.1
1935	16.4
1943	19.6
1945	19.9
1950	18.2
1960	15.7
1965	13.0
1975	11.4
1980	10.9

[a]Data from US Department of Agriculture (1984a)
[b]Prior to 1982, some moist/fine-cut snuff was classified as chewing tobacco.

Table 12. US production of snuff by major category[a]

Year	Production (millions of kg)		
	Dry snuff	Moist/fine-cut[b] snuff	Total
1981	5.3	13.8	19.2
1982	5.1	14.9	20.0
1983	4.9	15.9	20.7

[a]Data from Maxwell (1984)
[b]Prior to 1982, some moist/fine-cut snuff was classified as chewing tobacco.

World production of snuff is estimated to be 20 million kg per year. Production of snuff tobacco for nasal use amounts to only about one million kg per year at the most. The remainder is used for *snus* and *souffi* used in Scandinavia and North Africa, respectively, and 'moist snuff' used in the USA; these are not the same forms of snuff tobacco that are used nasally and are meant for oral use (Pöschl, 1983).

(ii) *Use*

Data on total use of snuff have been reported for various countries for the period 1920-1973 (Wilson, 1975) and for Sweden for the period 1973-1982 (Swedish Tobacco Co., 1980, 1981, 1982, 1983). Selected data on trends in individual countries are shown in Table 13. Additional data on sales in seven countries for the period 1974-1978 are given in Table 14.

In 1880, the earliest recorded year, US consumption of snuff was 0.12 pounds (0.05 kg) per person aged 14 years or over (Wynder *et al.*, 1957a). The highest US per-capita consumption of snuff for persons aged 15 years or over occurred in the period 1910-1920 at 0.50 pounds (0.2 kg). Consumption *per capita* decreased steadily but slowly from 1920 to 1962, to 0.26 pounds (0.12 kg) (Schuman, 1977). Per-capita consumption for persons aged 18 years and over was 0.23 pounds (0.10 kg) in 1966 and decreased to 0.15 pounds (0.07 kg) in 1979 (US Department of Agriculture, 1980). In 1982, some chewing tobacco products were reclassified as snuff. Under this new classification, US per-capita consumption (18 years and over, including overseas forces) of snuff was 0.26 pounds (0.12 kg) in 1982 and 1983 (US Department of Agriculture, 1984b).

Table 13. Snuff use in various countries (millions of kg)[a,b]

Year	Austria	Canada	Denmark	Finland	France	India	Ireland	Italy	Morocco	Norway	South Africa	Sweden[c]	United Kingdom
1920	-	0.3	0.2	0.04	-	-	0.1	-	-	-	0.04	6.5	-
1928	0.1	0.4	0.3	0.1	-	-	0.1	-	-	0.4	0.04	5.0	0.4
1932	0.1	0.4	0.4	0.04	2.5	-	0.1	-	-	0.4	-	4.9	0.4
1940	0.1	0.4	0.5	0.04	1.4	-	0.1	-	0.3	0.5	0.1	3.9	0.4
1945	0	0.4	0.5	0	0.9	-	0.1	-	0.4	0.3	0.1	3.5	0.5
1950	0.04	0.4	0.5	0.04	0.8	2.5	0.04	-	0.4	0.5	0.1	3.1	0.3
1955	0	0.4	0.4	0.04	0.6	4.4	0.04	-	0.4	0.5	0	2.9	0.3
1960	0	0.4	0.4	0	0.4	5.5	0.04	0.4	0.4	0.4	0	2.7	0.3
1965	0	0.4	0.4	0.04	0.3	4.3	0	0.3	0.4	0.4	0.1	2.5	0.2
1970	0	0.4	0.3	0.04	0.2	4.5	0	0.2	0.4	0.3	0.1	2.5	0.2
1973	0	0.4	0.2	0	0.1	4.3	0	0.1	-	0.3	0.1	2.7	0.2

[a]Data from Wilson (1975)

[b]Other data reported by Wilson (1975) include the following (millions of kg): Argentina, 0.1 in 1951, 0.0 in all other years (1940-1973); Barbados, 0.004 from 1964-1972, 0.0 in 1973; Iceland, 0.02-0.04 from 1932-1973; Portugal, 0.04 from 1940-1953 and in 1957, 0.0 in all other years (1954-1956, 1958-1972)

[c]The Swedish Tobacco Co. (1983) reported that snuff use in Sweden increased from 2.7 million kg in 1973 to 3.4 million kg in 1982.

Table 14. Sales of snuff (thousands of kg)[a]

Country	1974	1975	1976	1977	1978
Australia	0.8	1.2	0.8	1.0	1.4
Canada	660.0	579.4	534.3	560.8	570.2
Ireland	10.9	10.1	9.5	8.5	9.6
Italy	142.9	126.5	122.8	110.8	104.9
Norway	283.0	263.0	267.0	283.0	268.0
South Africa	1391.0	1474.1	1495.0	1363.8	1244.2
Sweden	2812.0	2943.0	3189.0	3361.0	3442.0

[a]Data from Anon. (1979)

Use of snuff in Pakistan is declining and amounted to only 0.6 million pounds (0.3 million kg) in 1975. It was estimated that there would be a further decline to 0.4 million pounds (0.18 million kg) by 1980 (Ahmad, 1976).

Estimated per-capita consumption of snuff in selected countries is summarized in Table 15.

Table 15. Estimated per-capita consumption (g) of snuff in selected countries[a]

Country	1962	1963	1964	1965	1966
Canada	33	29	32	32	27
Denmark	12	12	12	10	10
France	10	9	9	9	7
Ireland	21	20	-	-	-
Italy	11	10	9	8	7
Norway	167	167	161	146	146
Sweden	430	420	420	410	400
UK	9	9	9	9	8

[a]Data from International Trade Centre (1968)

Estimated annual usage of nasal snuff in various countries is as follows (thousands of kg): the Federal Republic of Germany, 300; UK, 200; France and Italy, less than 100 each. Other countries in which it is used include Belgium, the German Democratic Republic, Switzerland and South Africa (Pöschl, 1983). Use of snuff in Austria and the eastern European countries is comparatively low, and use is relatively rare in the Near East.

2. Chemical Data Relevant to the Evaluation of Carcinogenic Risk to Humans

There is a great deal of literature on the chemistry of tobacco, most of which refers to *Nicotiana tabacum*, utilized in western Europe and North America.

At least 2549 individual constituents have been identified in tobacco (Dube & Green, 1982). This number includes the tobacco constituents themselves as well as chemicals that

are applied to tobacco during cultivation, harvesting and processing. Major classes of compounds identified in tobacco are aliphatic and aromatic hydrocarbons, aldehydes, ketones, alcohols, phenols, ethers, carboxylic acids, esters, anhydrides, lactones, carbohydrates, amines, amides, imides, nitriles, *N*- and *O*-heterocyclic compounds and chlorinated organic compounds, and at least 35 metal compounds (Wynder & Hoffmann, 1967; Stedman, 1968; Tso, 1972; Enzell *et al.*, 1977; Schmeltz & Hoffmann, 1977; Davis *et al.*, 1981; Dube & Green, 1982).

2.1 Aliphatic hydrocarbons

Waxy leaf coatings are almost universal throughout the plant kingdom. The major wax constituents are alkanes, alkenes, alcohols, carboxylic acids, esters, aldehydes and ketones. In tobacco, the alkanes (non-volatile aliphatic hydrocarbons) consist primarily of compounds with chain lengths of C_{25}-C_{35}. They comprise a homologue series of normal (*n*), iso (*i*, 2-methyl) and ante-iso (*a*, 3-methyl) saturated hydrocarbons (Mold *et al.*, 1963). Table 16 presents the percentages of non-volatile hydrocarbons in various tobaccos.

Table 16. Aliphatic hydrocarbons in tobacco[a]

Hydrocarbon	Kentucky reference cigarette[b]	Flue-cured tobacco[c]	Cigarette tobacco blend[d]	Flue-cured tobacco[e]
n-C_{25}	0.76	0.10	1.71	0.80
n-C_{26}	0.33	0.42	0.83	0.44
n-C_{27}	5.67	6.56	7.73	5.67
a-C_{28}	0.27	0.40	0.13	1.29
n-C_{28}	0.61	0.74	0.89	
i-C_{29}	1.82	2.03	1.24	9.22
n-C_{29}	6.16	5.41	6.72	
a-C_{30}	6.46	7.30	5.65	9.79
n-C_{30}	2.17	2.49	3.16	
i-C_{31}	12.81	14.19	10.92	39.51
n-C_{31}	23.81	21.31	26.30	
a-C_{32}	12.89	15.25	13.02	16.93
n-C_{32}	3.85	4.23	4.88	
i-C_{33}	6.26	6.96	15.62	15.13
n-C_{33}	12.81	10.00	10.77	
a-C_{34}	1.14	1.21	1.15	1.09
n-C_{34}	0.33	0.28	-	
i-C_{35}	-	0.16	-	
n-C_{35}	0.43	0.28	-	

[a]The values in this table represent the percentile composition of the paraffin fraction.

[b]From Chortyk *et al.* (1975). Paraffin fraction, as its percentile composition of the tobacco blend, was not specified

[c]From Chortyk *et al.* (1975). Paraffin fraction represents 0.36% of the flue-cured tobacco

[d]From Mold *et al.* (1963). Paraffin fraction represents 0.20-0.28% of the cigarette blend

[e]From From Severson *et al.* (1981). Total *n*, *i*- and *a*-isomers represent 0.15% of the flue-cured tobacco

2.2 Isoprenoids

The typical aroma of the tobacco leaf is created during post-harvest treatment. The major contributors to the aroma are isoprenoids (Rowland & Roberts, 1963; Demole & Enggist, 1976; Enzell *et al.*, 1977; Enzell & Wahlberg, 1980). The most prevalent acyclic isoprenoids

are solanesol, solanesenes, solanone, phytone, neophytadiene and norphytene. In processed tobacco, the most abundant of these, neophytadiene, which originates from chlorophyll *via* phytol, can occur in amounts of up to 0.2% (Wynder & Hoffmann, 1967; Enzell & Wahlberg, 1980; Severson *et al.*, 1981). Solanesol occurs in tobacco in free form (0.4%) and as fatty acid esters, predominantly palmitate and linoleate (Wynder & Hoffmann, 1967). In addition to the isoprenoids, hundreds of acyclic and cyclic isoprenoids have been identified in processed tobacco (Enzell & Wahlberg, 1980), the most abundant being duvatrienediols, levantenolides and β-carotene (Wynder & Hoffmann, 1967; Enzell & Wahlberg, 1980).

2.3 Phytosterols

The most widely distributed sterols in higher plants are C_{29}-sterols with a 3-hydroxy group-$\Delta^{5,6}$ unsaturation, stigmasterol and sitosterols. The sterols in tobacco are free alcohols, esters and glycosides (Wynder & Hoffmann, 1967; Tso, 1972; Schmeltz *et al.*, 1975; Davis, 1976; Enzell *et al.*, 1977). Table 17 presents data on the four major phytosterols, which amount to 0.15-0.2% (dry weight) of some processed tobacco types.

Table 17. Phytosterols in processed tobacco (μg/g)[a]

Phytosterol	Tobacco type	Free alcohol	Esters and glycosides	Total steroids
Cholesterol	Cigarette blend	129	45	174
	Cigar filler	155	131	286
	Flue-cured L-1	151	25	176
	Flue-cured L-2	165	49	214
Campesterol	Cigarette blend	197	131	328
	Cigar filler	226	213	439
	Flue-cured L-1	192	36	228
	Flue-cured L-2	241	88	329
Stigmasterol	Cigarette blend	480	94	574
	Cigar filler	584	414	998
	Flue-cured L-1	677	43	720
	Flue-cured L-2	715	108	823
Sitosterol	Cigarette blend	288	181	469
	Cigar filler	329	310	639
	Flue-cured L-1	313	71	384
	Flue-cured L-2	662	278	940
Total sterols	Cigarette blend	1094	451	1545
	Cigar filler	1294	1068	2362
	Flue-cured L-1	1333	175	1508
	Flue cured L-2	1783	523	2306

[a]Data for cigarette blend and cigar filler (wet weight, 11.7%) from Schmeltz *et al.* (1975); data for flue-cured tobacco (dry weight) from Davis (1976)

2.4 Polynuclear aromatic hydrocarbons (PAHs)

Although it has been known for a long time that tobacco smoke contains naphthalene and PAHs (IARC, 1983a), it is less well known that traces of these aromatic hydrocarbons are also found in processed tobaccos (Wynder & Hoffmann, 1967). Whereas naphthalene and its alkyl derivatives may be formed from certain cyclic isoprenoids during tobacco processing, especially during flue-curing, the presence of PAHs in processed tobacco at the part-per-billion level is more likely to be due to contamination with ambient air pollutants or pollu-

tants from heating sources used for the curing process. One US cigarette blend contained 170 µg/kg naphthalene, 18 µg/kg 1-methylnaphthalene and 42 µg/kg 2-methylnaphthalene (Schmeltz *et al.*, 1976). Certain types of tobaccos are treated with wood smoke in order to enhance the flavour of the resulting tobacco smoke. For a processed Latakia tobacco, the following data were reported: 1.1 mg/kg naphthalene, 2.8 and 2.6 mg/kg 1- and 2-methylnaphthalene, respectively, 1.4 mg/kg 1- and 2-ethylnaphthalene, 28.6 mg/kg of 10 isomeric dimethylnaphthalenes, 7.8 mg/kg of 11 isomeric ethylmethylnaphthalenes and 24.4 mg/kg of seven isomeric trimethylnaphthalenes (Nicolaus & Elmenhorst, 1982).

Campbell and Lindsey (1956) reported up to 0.3 mg/kg acenaphthalene, 3 mg/kg phenanthrene, 1.1 mg/kg anthracene, 0.8 mg/kg pyrene, 0.15 mg/kg fluoranthene and 0.08 mg/kg benzo[*a*]pyrene in processed tobaccos. Significantly higher levels (6.6 mg/kg phenanthrene) were found in a dark pipe tobacco. Onishi *et al.* (1957) reported levels as high as 5 mg/kg phenanthrene, 4.2 mg/kg anthracene, 1.8 mg/kg pyrene and 1.4 mg/kg fluoranthene in a Japanese burley leaf.

In a UK snuff, Campbell and Lindsey (1977) found 260 µg/kg pyrene, 335 µg/kg fluoranthene and 7.2 µg/kg benzo[*a*]pyrene. Cooper and Campbell (1955) reported the following levels of PAHs in Zulu snuff: 50-70 µg/kg anthracene, 560-580 µg/kg pyrene, 800 µg/kg fluoranthene, 250-270 µg/kg benzo[*a*]pyrene and 140 µg/kg benzo[*ghi*]perylene; and in Vendu snuff, the following values were found: anthracene, 10 µg/kg; pyrene, 80-90 µg/kg; fluoranthene, 120-150 µg/kg; and benzo[*a*]pyrene was present. In Indian snuff and *mishri*, PAHs occurred in amounts similar to those reported in other studies (Bhide *et al.*, 1984a).

2.5 Alcohols

Tobacco contains isoprenoids with one or more alcoholic functional groups, such as solanesol, duvatriene diols and phytol (see p. 00) and a number of sterols. In addition, long-chain alcohols, eicosanol ($C_{20}H_{41}OH$), docosanol ($C_{22}H_{45}OH$) (Tso, 1972), tetracosanol ($C_{24}H_{49}OH$) and octacosanol ($C_{28}H_{57}OH$), were identified (Severson *et al.*, 1978). Levels of alcohols and sterols in four varieties of flue-cured tobacco are given in Table 18 (Higman *et al.*, 1979). Dark air-cured tobaccos, such as those used for snuff, were also found to contain 6-methyl-5-hepten-2-ol, 3,7-dimethyl-1,6-octadien-3-ol (linalool), 2-buten-2-ol, 2-methyl-1-penten-3-ol, 3-methyl-2-buten-1-ol, 3-methyl-1-penten-3-ol, 2,6-dimethylcyclohexanol, benzyl alcohol and phenylethanol (Davis *et al.*, 1981).

Table 18. Alcohols and sterols (mg/kg) in flue-cured varieties of tobacco[a]

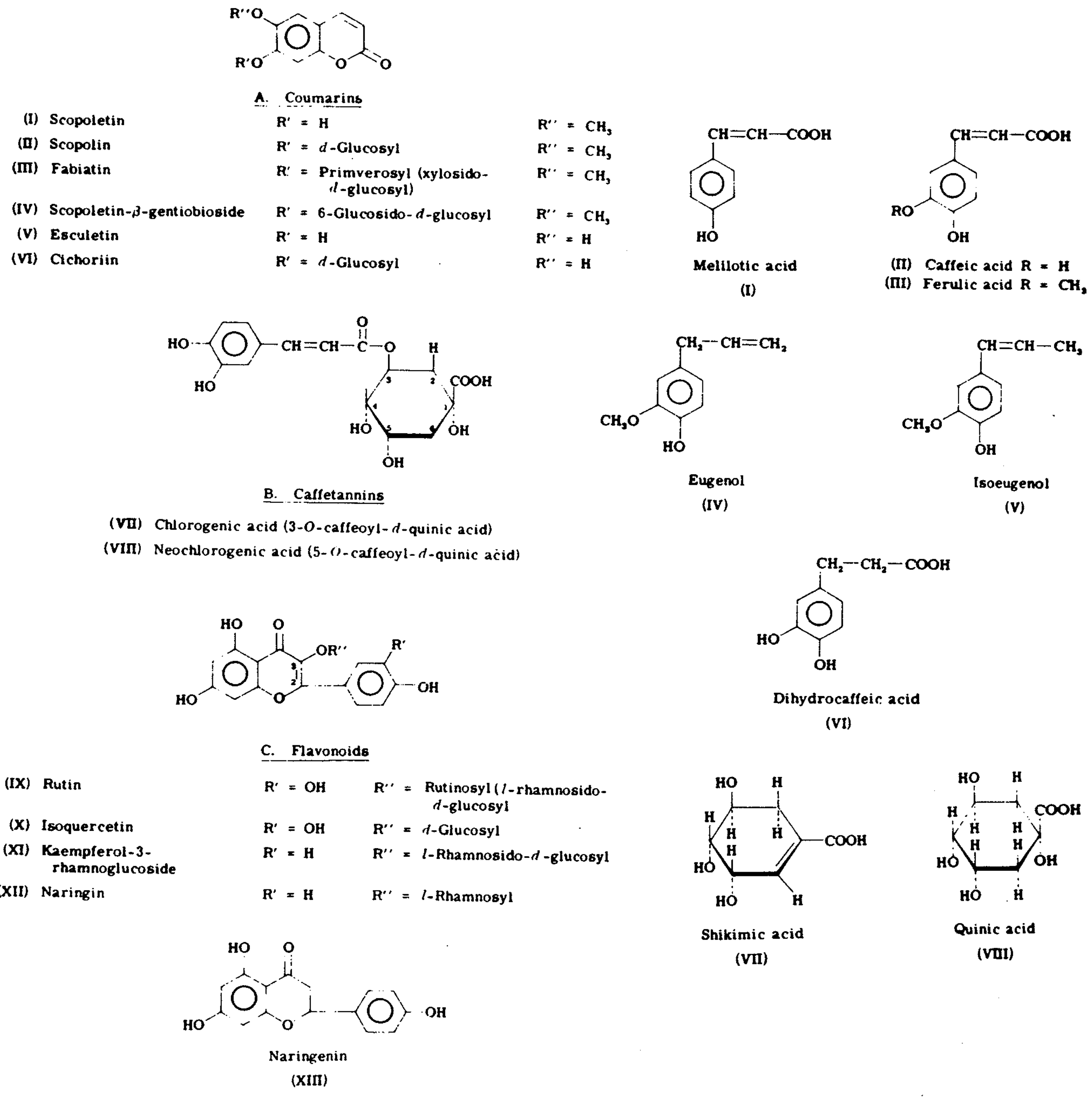
Compound	Concentration
Terpenes and alcohols	
Phytol	149-192
Docosanol	61-123
β-Amyrin	47-242
Cycloartenol	83-277
Solanesol and sterols	
Solanesol	0.88-22.4
Cholesterol	0.09-0.21
Campesterol } Stigmasterol }	0.41-0.98
Sitosterol	0.23-0.71

[a]NC2326, SC1971, Coker 139 and Speight G-28 (Higman *et al.*, 1979)

2.6 Phenols and phenolic acid

Tobacco-leaf phenols comprise coumarins, caffetannins and flavonoids (Fig. 1). The percentage contents of the two major tobacco polyphenols, chlorogenic acid and rutin (for information on the aglycone of rutin, quercetin, see IARC, 1983b) in a number of processed tobaccos are given in Table 19 (Häusermann & Waltz, 1962). In addition, simple phenols, derived partially from hydrolysis of the polyphenols, have been isolated from processed tobacco (Fig. 1). Furthermore, processed tobacco contains phenol, cresols, dimethyl-

Figure 1. Major polyphenols and precursors in tobacco[a]



[a]From Wynder and Hoffmann (1967)

phenols and other volatile phenols (up to 30 μg/g tobacco) (Lipp, 1965). These volatile phenols appear to be present in relatively high concentrations in fire-cured dark tobaccos that are used for snuff (Davis *et al.*, 1981); it is possible that they originate partially from pollution.

Table 19. Chlorogenic acid and rutin (%) in processed tobaccos[a]

Tobacco	Chlorogenic acid	Rutin
Air-cured tobaccos		
Maryland	0.15-0.28	0.08
Burley	0.0-0.46	
Bright and oriental tobaccos		
Virginia - USA	2.32-3.4	0.24
Virginia - Rhodesia	3.20	
Virginia - Italy	3.10	
Oriental	1.60	1.29
Tobacco blends		
Oriental - mixture	1.49-1.58	
Oriental - Virginia	1.85	
Virginia	2.38	
USA - cigarette blend	1.03	

[a]From Häusermann and Waltz (1962)

In addition to melilotic acid, caffeic acid, dihydrocaffeic acid, shikimic acid and quinic acid (Wynder & Hoffmann, 1967; Stedman, 1968; see Fig. 1), tobacco leaf contains 3,4-dihydroxybenzoic acid, 2-hydroxybenzoic acid (salicylic acid), 3,5-dimethoxy-4-hydroxycinnamic acid (sinapic acid) and hydroxynaphthoic acids (Snook *et al.*, 1981). These phenolic acids are genuine tobacco constituents and are, at least partially, biosynthetic intermediates of polyphenols.

2.7 Carboxylic acids

More than 80 organic acids have been identified in tobacco (Wynder & Hoffmann, 1967; Stedman, 1968; Tso, 1972; Spears & Jones, 1981). The concentrations of the major volatile carboxylic acids in bright, oriental (Ismir), burley and Japanese varieties are listed in Table 20 (Wynder & Hoffmann, 1967). The free fatty-acid content amounts to 0.08-0.4% of the leaf tobacco. The specific flavour of oriental tobacco leaves has been attributed to β-methylvaleric acid, which occurs in these tobacco types in significantly higher concentrations than in the others (Kaburaki *et al.*, 1969). Table 21 shows the composition of the fraction of non-volatile fatty acids, which constitute 0.09-0.43% of the leaf (Hoffmann & Woziwodzki, 1968). The major dicarboxylic acids in tobacco are malonic, oxalic, malic and citric acids. After processing, burley tobacco and cigar-type tobaccos, used for chewing tobaccos, have especially high levels of malic and citric acids (Jarboe & Quinn, 1960).

Table 20. Concentration of volatile carboxylic acids in tobacco leaves (mg/kg dry tobacco leaves)[a]

Volatile acid	Bright tobacco		Japanese tobacco types				Burley tobacco	Oriental tobacco	
	Japan	USA	Shifu L.	Matsukawa L.	Daruma L.[c]	Ibusuki L.	Mito No. 3[c]	Turkey	Greece
Formic	208	456	68.9	32.8	107	88.5	161	664	456
Acetic	1378	1552	51.0	36.1	70.1	191	75.5	1423	1075
Propionic	25.2	21.0	1.4	1.3	1.7	2.1	2.4	24.8	21.2
Isobutyric	2.4	2.5	-	-	-	-	-	13.9	11.4
n-Butyric	1.4	2.8	-	0.3	-	-	0.3	8.8	1.8
α-Methylbutyric	9.1	7.8	0.7	0.3	4.0	1.6	2.9	26.2	31.0
Isovaleric	7.7	6.6	0.5	0.3	0.5	5.8	1.2	24.8	33.7
Crotonic	6.2	5.4	-	-	0.6	-	0.4	8.3	6.0
n-Valeric	2.7	4.2	-	-	0.2	-	0.3	2.2	2.1
β-Methylvaleric	8.4	1.2	6.1	0.7	5.7	26.2	-	83.0	115.6
Caproic	1.7	12.2	0.7	0.7	0.7	0.2	0.9	5.3	6.6
α-Furoic	31.7	14.0	-	-	0.5	7.5	-	20.8	16.6
Benzoic	16.2	10.8	10.6	13.3	3.7	3.4	26.0	16.4	22.0
Phenylacetic	7.8	8.1	2.3	2.7	1.3	-	5.1	21.1	16.2
TOTAL	1706.5	2104.6	142.2	88.5	196.0	326.3	276.0	2342.6	1815.2

[a]From Wynder and Hoffmann (1967) unless otherwise specified

[b]From Kaburaki and Sato (1972)

Table 21. Concentration of free non-volatile fatty acids (μg/g) in tobacco[a]

Acid	Tobacco[b]					
	Turkish I	Turkish II	Bright	Maryland	Burley	Blend
Myristic	220	150	65	Trace	-	180
Palmitic	1480	530	930	420	220	530
Palmitoleic	300	160	Trace	-	-	Trace
Stearic	520	480	330	180	110	280
Oleic	480	220	230	110	70	220
Linoleic	880	320	610	250	180	420
Linolenic	2120	870	2130	420	360	1160
TOTAL	6000	2730	4300	1380	940	2790

[a]From Hoffmann and Woziwodzki (1968)

[b]Dried tobacco: moisture content between 0.5-1.0%

2.8 Amines and amides

Processed tobacco contains 27 volatile amines, 11 aromatic amines and more than 50 *N*-heterocyclic compounds, such as pyrroles, pyrrolidines, imidazoles, pyridines and pyrazines (Schmeltz & Hoffmann, 1977). Of special relevance to tobacco carcinogenesis are secondary amines, which can give rise to *N*-nitrosamines during curing, fermentation and ageing. This group includes dimethylamine, di-*n*-butylamine and pyrrolidine (Table 22). Of importance is the observation that nitrogen-containing compounds, including nitrates, amines, amides and proteins, comprise up to 24% of cured and fermented cigar tobaccos, from which many smokeless-tobacco products are made, while they make up only 15.5% of cigarette tobaccos (Wynder & Hoffmann, 1967; Tso, 1972). Some of these nitrogen-containing compounds are known precursors of *N*-nitrosamines.

Table 22. Secondary amines identified in tobacco[a]

Aliphatic amines
n-Butylisobutylamine
Di-*n*-butylamine
Di-*sec*-butylamine
Diethylamine
Dimethylamine
Di-*n*-propylamine
Ethylmethylamine
Methyl-*n*-butylamine
Methylisoamylamine
Methylisopropylamine
Methylpropylamine
Aromatic amines
N-Ethylaniline
N-Methylaniline (*ortho*-toluidine)
N-Methyl-2-toluidine
Tyramine
Pyrrolidines and pyrrolines
2-Methylpyrrolidine-3-carboxaldehyde
Pyrrolidine
Δ^3-Pyrroline

[a]From Schmeltz and Hoffmann (1977)

The acyclic and cyclic amides identified in tobacco include *N*,*N*-dimethylacetamide, maleic imide and *N*-methylnicotinic amide (Schmeltz & Hoffmann, 1977). Although these secondary amides could give rise to *N*-nitrosamides, none of them has as yet been detected in smokeless-tobacco products. It appears that the general instability of the *N*-nitrosamides in moist matrices and the lack of a highly sensitive analytical method for nitrosamides are the major reasons for the scarcity of information on the presence of these compounds in chewing tobacco and snuff.

Tobacco contains many free amino acids, which are listed in Table 23 and Figure 2.

Table 23. Free amino acids identified in tobacco[a]

α-Alanine	6-Hydroxykynurenic acid
β-Alanine	Hydroxyproline
D-Alanyl-*D*-alanine	Isoleucine
α-Aminoadipic acid	Leucine
α-Aminobutyric acid	Lysine
γ-Aminobutyric acid	Methionine
Arginine	Methionine sulphone (methionine *S*-oxide)
Asparagine	1-Methylhistidine
Aspartic acid	Nicotianamine
Azetidine-2-carboxylic acid	Nicotianine
Betaine	Nicotinamide
Choline	Nicotinic acid
Citrulline	Norleucine
Cysteic acid	Ornithine
Cysteine	Phenylalanine
Cystine	Pipecolic acid
Glutamic acid	Proline
Glutamine	Pyrrolidine-2-acetic acid
α-L-Glutamyl-L-glutamic acid	Serine
Glutathione	Taurine
Glycine	Threonine
Histidine	Tryptophan
Homocystine	Tyrosine
Homoserine	Valine

[a]From Schmeltz and Hoffmann (1977)

Figure 2. Free amino acids identified in tobacco[a]

NICOTIANINE

NICOTIANAMINE

AZETIDINE-2-CARBOXYLIC ACID

6-HYDROXYKYNURENIC ACID

[a]From Schmeltz and Hoffmann (1977)

2.9 Alkaloids

Nicotine dependence is now widely held to be the prime factor in the worldwide popularity of tobacco products, including the acceptance of tobacco chewing and oral or nasal use of snuff. Tobaccos contain 0.5-5% of alkaloids (Schmeltz & Hoffmann, 1977), depending on regional customs and preferences for smokeless-tobacco products. At least 85% of the total *Nicotiana* alkaloids are nicotine (Piade & Hoffmann, 1980), almost exclusively present in the L(-) form (Schmeltz & Hoffmann, 1977), which is the pharmacologically active isomer. (The asymmetric centre is the C-2'-carbon of the pyrrolidine ring; Fig. 3.) The remainder of the alkaloid portion of tobacco consists of the minor *Nicotiana* alkaloids, some of which are also presented in Figure 3. A number of studies have shown, in recent years, that the methyl group on the pyrrolidine-ring nitrogen can be replaced by a formyl, acetyl or other acyl group with six or eight carbons (Enzell *et al.*, 1977). Nicotine also occurs as nicotine-*N'*-oxide in chewing tobaccos. Most secondary amines, such as anabasine and anatabine, can be methylated to tertiary amines (Schmeltz & Hoffmann, 1977); however, methylanabasine and methylanatabine rarely amount to more than 0.1% of smokeless-tobacco products. *Nicotiana rustica* differs from *N. tabacum* in the quantitative composition of its alkaloid fraction, in that, generally, nicotine, anabasine and nornicotine are present in higher concentrations (Shmuk, 1953; Sisson & Severson, 1984). The contents of the major alkaloids in four tobacco types in comparison to those in a tobacco blend, the reference cigarette IR1 of the University of Kentucky, are given in Table 24 (Piade & Hoffmann, 1980).

Figure 3. Major tobacco alkaloids[a]

NICOTINE, NORNICOTINE, MYOSMINE, COTININE, ANATABINE

ANABASINE, 3,2'-BIPYRIDYL, OXYNICOTINE, NICOTYRINE

[a]From Schmeltz and Hoffmann (1977)

Table 24. Alkaloid content of various tobacco brands (mg/kg, dry basis)[a]

Alkaloid	Dark commercial tobacco		Burley	Bright	Kentucky ref. (IRI)[b]
	A	B			
Nicotine	11 500	10 000	15 400	12 900	21 100
Nornicotine	550	200	630	210	630
Anatabine	360	380	570	600	930
Anabasine	140	150	90	150	190
Cotinine	195	140	90	40	80
Myosmine	45	50	60	30	85
2,3'-Dipyridyl	100	110	30	10	30
N'-Formyl-nornicotine	175	210	140	40	100

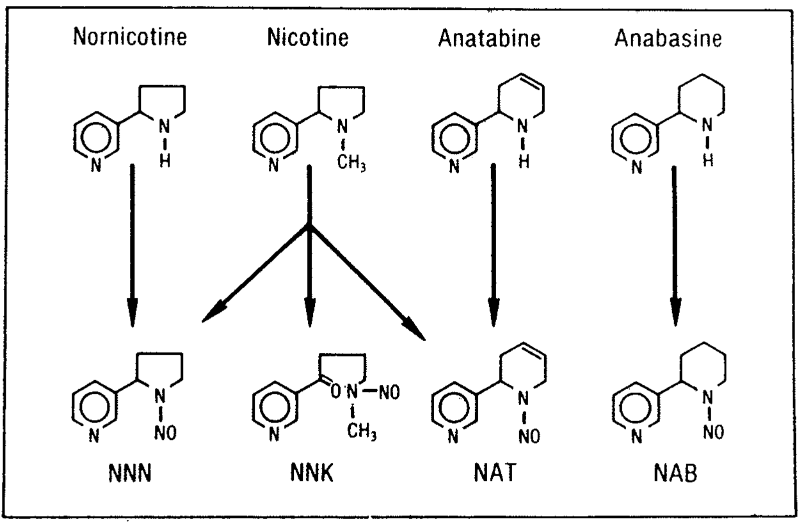
[a]From Piade and Hoffmann (1980)

[b]The tobacco blend used in the reference cigarette IR1 of Kentucky University

2.10 *N*-Nitrosamines

A large number of studies have shown that, during the ageing, curing, fermentation and processing of tobacco, nicotine and other alkaloids give rise to carcinogenic, tobacco-specific *N*-nitrosamines (Hoffmann *et al.*, 1984; Fig. 4; see also monographs on pp. 205-261 of this volume). The concentration of these nitrosamines in tobacco exceeds by at least 100-fold the concentrations found so far in other consumer products (Table 25). It has been calculated (National Research Council, 1981) that, in the USA, cigarette smoking gives rise to at least a 20-fold greater daily exposure to *N*-nitroso compounds than any other consumer product; however, since the relative concentration of tobacco-specific nitrosamines *N'*-nitrosonornicotine (NNN), 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) and *N'*-nitrosoanatabine (NAT) in chewing tobacco is much higher than in cigarette smoke (see monographs pp. 209-223, 233-261) and since the average chewer consumes 10 g of tobacco (Hecht *et al.*, 1983) *versus* <1 g tar inhaled by the smoker, tobacco chewing appears to be the greatest exogenous source of exposure to *N*-nitrosamines (Hoffmann & Hecht, 1985). In certain products marketed in 1980 in the USA and in Sweden, the concentration of nitrosamines was significantly lower than that measured earlier (Table 26). Statistical models for making correlations between tobacco components in commercial tobacco products (Brunnemann *et al.*, 1983) show that this can be achieved by selecting tobaccos with low nitrate levels or by reducing the nitrate content and sealing the smokeless-tobacco products in airtight packages (Brunnemann *et al.*, 1982).

Figure 4. Formation of tobacco-specific nitrosamines (TSNA)[a]



[a]From Hoffmann *et al.* (1984)

Table 25 shows that, in addition to the alkaloid-derived nitrosamines, processed tobacco can also contain volatile nitrosamines, e.g., *N*-nitrosomorpholine (NMOR), *N*-nitrosodiethanolamine (NDELA) and/or *N*-nitrosoproline (NPRO) (Hoffmann *et al.*, 1984; Fig. 5). The morpholine, which is the precursor to NMOR, in the tobacco derives either from the container waxes used in packaging materials or from flavour additives employed in product formulation (Brunnemann *et al.*, 1982). NDELA is formed from residual diethanolamine in those tobaccos that were treated with the sucker-growth inhibitor, maleic hydrazide-diethanolamine (Brunnemann & Hoffmann, 1981). NPRO is formed during the processing of tobacco and can serve as an indicator of the concentration of other non-volatile nitrosamines in tobacco products (Brunnemann *et al.*, 1983).

Table 25. *N*-Nitrosamines in commercial tobacco products (μg/g, dry basis)[a,b]

Tobacco products	Nitrosamines[b]							
	NDMA	NPYR	NMOR	NDELA	NPRO	NNN	NNK	NAT
Cigarette, USA	nd[c]-28	nd-10	0-10	100-200	900-2300	600-6600	100-700	500-1600
Cigarette, UK	nd-10	nd-10	nd	nd-80	600-1000	300	100	200
Cigarette, France	40-180	nd-10	nd-1	nd	1400-1600	600-11900	500-1100	1800-2000
Cigar, little	20	20	nd	420	2000	11200	4500	13000
Cigar	10	10	nd	110	1100	3000-10700	1100-3500	2500-3300
Chewing tobacco, USA	30	20	30	200-300	450	3500-8200	100-3000	500-7000
Chewing tobacco, India	10	10	nd	nd	-	2400	-	-
Snuff, USA	nd-215	nd-360	30-690	29-6900	3500-22000	800-89000	200-8300	200-4000
Snuff, Sweden	nd-60	nd-210	0-44	200-390	-	2000-6700	600-1500	900-2400
Snuff, FRG[d]	10-50	10-300	nd	nd	-	6000-6800	1500-1600	3900-4400
Snuff, Denmark	20-50	20-60	nd	nd	-	4500-8000	1400-7000	2600-6200

[a]From Hoffmann *et al.* (1984)

[b]*N*-Nitrosoanabasine: in cigarette tobacco, ≤ 20 μg/kg; in snuff, 10-1900 μg/kg. NDMA, *N*-nitrosodimethylamine; NPYR, *N*-nitrosopyrrolidine; NMOR, *N*-nitrosomorpholine; NDELA, *N*-nitrosodiethanolamine; NPRO, *N*-nitrosoproline; NNN, *N'*-nitrosonornicotine; NNK, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone; NAT, *N'*-nitrosoanatabine

[c]nd, not detected

[d]FRG, Federal Republic of Germany

Table 26. *N*-Nitrosamines in snuff (μg/kg, dry basis)[a,b]

Brand of snuff		Volatile *N*-nitrosamines[c]				NDELA	Tobacco-specific *N*-nitrosamines				
		NDMA	NPYR	NMOR	Total		NNN	NNK	NAT	NAB	Total
USA	I	215	(-)	24	240	760	2200	600	1700	100	4600
	II	37	120	690	850[d]	1700	19000	2400	19000	800	41200
	III	100	360	690	1150	3300	33000	4600	40000	1900	79500
	IV	92	110	630	830[e]	290	20000	8300	9100	500	37900
	V[f]	(-)	(-)	31	<50	600	830	210	240	10	1290
Sweden	I	22	(-)	44	70	240	5700	1700	900	140	8440
	II	60	(-)	(-)	60	225	6100	1000	2200	80	9380
	III	14	210	(-)	230	390	5300	1400	2400	70	9170
	IV	30	50	10	90	310	4000	610	1400	80	6310
	V	(-)	(-)	(-)	(-)	290	2000	800	1400	40	4240

[a]From Brunnemann *et al.* (1982)

[b]NDMA, *N*-nitrosodimethylamine; NPYR, *N*-nitrosopyrrolidine; NMOR, *N*-nitrosomorpholine; NDELA, *N*-nitrosodiethanolamine; NNN, *N*'-nitrosonornicotine; NNK, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone; NAT, *N*'-nitrosoanatabine; NAB, *N*'-nitrosoanabasine

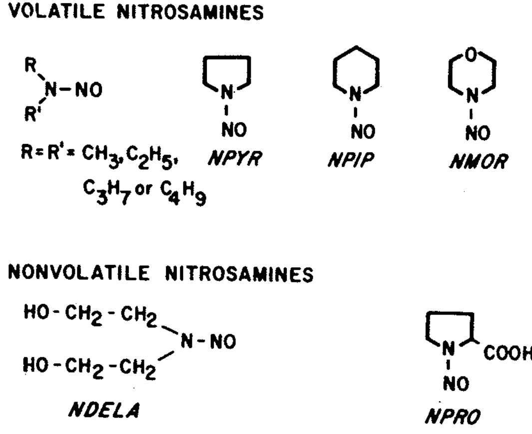
[c](-), below detection limits; values for *N*-nitrosodiethylamine in snuff were below detection limit (<2 μg/kg, except for Sweden I, II, III and IV, which had values of 6, 4, 12 and 5 μg/kg, respectively)

[d]Contained 44 μg/kg *N*-nitrosopiperidine and 21 800 μg/kg *N*-nitrosoproline (Hoffmann *et al.*, 1984)

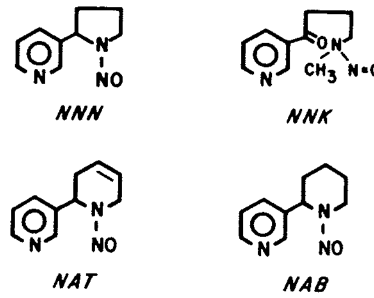
[e]Contained 13 μg/kg *N*-nitrosopiperidine and 350 μg/kg *N*-nitrosoproline

[f]Introduced on the market in 1982

Figure 5. *N*-Nitrosamines in tobacco products



TOBACCO-SPECIFIC NITROSAMINES



[a]From Hoffmann *et al.* (1984)

Four *nass* samples from a local authority district in Samarkand Oblast of the Uzbek SSR, were analysed for volatile *N*-nitrosamines, tobacco-specific *N*-nitrosamines, nitrate and nitrite (Table 27) (Zaridze *et al.*, 1985a,b). The relatively low levels of tobacco-specific nitrosamines in *nass*, compared to US and Swedish snuff brands, is at least partially explained by the short ageing process of the tobacco used.

Table 27. *N*-Nitrosamines[a] in samples of *nass*[b]

N-Nitrosamine (ng/g)	A	B	C	D
N-Nitrosopiperidine	9.0	7.7	8.0	6.0
N-Nitrosopyrrolidine	8.8	1.8	1.7	4.3
N'-Nitrosonornicotine	519	143	119	516
N'-Nitrosoanatabine	289	39	39	167
N-Nitrosoanabasine	34	3.0	4.0	17
4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanone	108	16	29	126
Moisture (%)	7.3	8.6	10.1	6.4

[a]*N*-Nitrosodimethylamine and other volatile nitrosamines were not detected (detection limit, 1 ng/g)
[b]From Zaridze *et al.* (1985a,b)

2.11 Metals

Tobacco leaf contains compounds of at least 35 metallic elements (Tso, 1972; Wynder & Hoffmann, 1977; Fig. 6). The most abundant of these metals in cured tobacco leaf are potassium (300-3500 mg/kg), calcium (5000-90 000 mg/kg), magnesium (500-13 000 mg/kg), sodium (150-8500 mg/kg), iron (80-900 mg/kg), copper (4-100 mg/kg) and zinc (0.8-7 mg/kg). The highest concentrations of potassium and calcium in processed tobacco accumulate in the middle vein of the leaves (Wynder & Hoffmann, 1967; Tso, 1972; Tso *et al.*, 1980).

Figure 6. Metals in tobacco[a]

IA	IIA	IIIB	IVB	VB	VIB	VIIB	—	VIII	—	IB	IIB	IIIA	IVA	VA	VIA	VIIA	VIIIA	*US*
IA	IIA	IIIA	IVA	VA	VIA	VIIA	—	VIII	—	IB	IIB	IIIB	IVB	VB	VIB	VIIB	O	*Europe*
M1	M2	T1	T2	T3	T4	T5	T6	T7	T8	T9	M2′	M3	M4	M5	M6	M7	M8	*Sanderson*
1	2	3d	4d	5d	6d	7d	8d	9d	10d	11d	12d	13	14	15	16	17	18	*ACS*

s block; d block (transition metals); p block

1 H																	2 He
3 Li	4 Be											5 B	6 C	7 N	8 O	9 F	10 Ne
11 Na	12 Mg											13 Al	14 Si	15 P	16 S	17 Cl	18 Ar
19 K	20 Ca	21 Sc	22 Ti	23 V	24 Cr	25 Mn	26 Fe	27 Co	28 Ni	29 Cu	30 Zn	31 Ga	32 Ge	33 As	34 Se	35 Br	36 Kr
37 Rb	38 Sr	39 Y	40 Zr	41 Nb	42 Mo	43 Tc	44 Ru	45 Rh	46 Pd	47 Ag	48 Cd	49 In	50 Sn	51 Sb	52 Te	53 I	54 Xe
55 Cs	56 Ba	57 La	72 Hf	73 Ta	74 W	75 Re	76 Os	77 Ir	78 Pt	79 Au	80 Hg	81 Tl	82 Pb	83 Bi	84 Po	85 At	86 Rn
87 Fr	88 Ra	89 Ac															

f block (lanthanides and actinides)

58 Ce	59 Pr	60 Nd	61 Pm	62 Sm	63 Eu	64 Gd	65 Tb	66 Dy	67 Ho	68 Er	69 Tm	70 Yb	71 Lu
90 Th	91 Pa	92 U	93 Np	94 Pu	95 Am	96 Cm	97 Bk	98 Cf	99 Es	100 Fm	101 Md	102 Na	103 Lr

[a]From Tso (1972); Wynder and Hoffmann (1977)

Of special concern in tobacco carcinogenesis are arsenic, lead, cadmium and nickel. Arsenic (as its trioxide, As_2O_3) has been reported to occur in processed tobacco at concentrations of up to 50 mg/kg. However, since the use of arsenic products as pesticides was suspended in most tobacco-producing countries, the arsenic content of leaf tobacco has decreased drastically during the last decades. The latest available data show a level of 0.5-0.9 mg/kg arsenic trioxide in cured tobacco (Guthrie & Bowery, 1967). Lead has been reported to occur in tobacco at concentrations of 5-80 mg/kg; the earlier levels cited appear to be rather high, and levels of lead do not now generally exceed 10 mg/kg (Cogbill & Hobbs, 1957; Voss & Nicol, 1960; Perinelli & Carugno, 1978). Cadmium levels of between 1-2 mg/kg have been reported (IARC, 1973; Perinelli & Carugno, 1978).

Since a number of nickel compounds are carcinogenic to laboratory animals and since some forms of nickel are probably human carcinogens (IARC, 1982), a large number of studies have been concerned with the nickel content of tobacco products (National Academy of Sciences, 1975; IARC, 1976). While concentrations of nickel in tobacco leaf

generally do not exceed 4 mg/kg, higher concentrations may occur in special settings (National Academy of Sciences, 1975). In Swaziland, a snuff product placed in the nostrils is made of powdered local tobaccos mixed with the ashes of incinerated plants or herbs, such as the plant *Aloe marlothii* (80% of all ashes), the roots of *Turbinata oblongata* and leaves and stems of *Amaranthus spinosum*. Metal analyses of these snuff products, as used by the consumer, revealed, among other metals, the presence of 43, 87 and 25 mg/kg nickel, respectively (Baumslag *et al.*, 1971). As seen in Table 28, the levels of copper, chromium and nickel in Swazi snuff are relatively high in comparison to those in commercial snuffs produced in the USA.

Table 28. Trace metal content of Swazi and commercial US snuffs (mg/kg)[a]

Type of snuff	Copper	Chromium	Lead	Zinc	Cadmium	Nickel
Aloe	25	9	8	65	1.4	43
Ubhoco	63	84	8	50	1.5	87
Amaranthus	16	13	6	47	1.1	25
US Brand I	10	1	4	41	0.8	3
US Brand II	12	1	4	27	0.9	2
US Brand III	9	2	4	40	0.7	2

[a]From Baumslag *et al.* (1971)

2.12 Radioelements

α- and β-Radioactivity have been reported to occur in leaf tobaccos. Naturally-occurring ^{40}K is the major contributor to the minute β-radioactivity in tobacco (Tso, 1972).

It was suggested (Radford & Hunt, 1964) that α-emitting ^{210}Po in tobacco smoke is a contributory factor in bronchogenic carcinoma in cigarette smokers. Since that report, many others have indicated that 1 g tobacco contains between 0.1-1.0 pCi of ^{210}Po. It appears that phosphate fertilizers that contain ^{226}Ra, ^{210}Pb and ^{210}Po and soils derived from rock rich in ^{226}Ra are the major source of the α-radioactivity of tobacco (Harley *et al.*, 1980). A 1-g sample of tobacco was found to contain about 0.021 pCi of ^{238}U (Chakarvarti *et al.*, 1981).

2.13 Agricultural chemicals

During the last three to four decades, many organic chemicals have been marketed for the cultivation and post-harvest treatment of tobacco (Tso, 1972). In the past, the sucker-growth inhibitor, maleic hydrazide, was dissolved in diethanolamine, since it is insoluble in water. More recently, maleic hydrazide has been formulated as its potassium salt (US Environmental Protection Agency, 1981). It has been reported to occur in US tobaccos at concentrations of between 17-178 mg/kg (Liu & Hoffmann, 1973; Chopra *et al.*, 1982). The concentrations of chlorinated hydrocarbon pesticides in a tobacco blend in 1977 are given in Table 29 (Reif & Moser, 1977); these are significantly lower than those reported earlier (Tso, 1972).

Table 29. Organochlorine pesticide residues on one sample of US tobacco analysed in the 1970s[a]

Pesticide	Concentration (mg/kg)
α-Hexachlorobenzene	0.12
γ-Hexachlorobenzene (Lindane)	0.18
β-Hexachlorobenzene	0.22
Heptachlor	0.02
δ-Hexachlorobenzene	0.03
α-Endosulfan	0.03
p,p'-DDE [1,1-dichloro-2,2-bis(4-chlorophenyl)ethylene]	0.15
Dieldrin	0.05
o,p'-TDE [1,1-dichloro-2(2-chlorophenyl)-2(4-chlorophenyl)ethane]	0.05
o,p'-DDT [1,1,1-trichloro-2(2-chlorophenyl)-2(4-chlorophenyl)ethane]	0.38
p,p'-TDE [1,1-dichloro-2,2-bis(4-chlorophenyl)ethane]	0.43
β-Endosulfan	0.19
p,p'-DDT [1,1,1-trichloro-2,2-bis(4-chlorophenyl)ethane]	2.71
Endosulfan sulphate	0.27

[a]From Reif and Moser (1977)

3. Biological Data Relevant to the Evaluation of Carcinogenic Risk to Humans

3.1 Carcinogenicity studies in animals

Tobacco

The Working Group noted that the majority of the published studies evaluated below had various deficiencies, such as lack of quantitative and qualitative information on the nature of tobacco extracts and the degree of extraction, insufficient length of treatment, small group sizes and, in some cases, lack of appropriate controls.

(*a*) *Oral administration*

Mouse: Groups [numbers unspecified] of male Swiss mice, six to eight weeks of age, were administered a tobacco extract (ethanol extract from 50 g tobacco diluted in 10 ml distilled water) from a commercially-available Indian chewing tobacco (*Nicotiana tabacum*) at a dilution of 1:25 or 1:50 [actual dose unspecified] by oral intubation for 15-20 months. A further group of mice was fed a diet containing 10 g of an extract of tobacco per 5 kg diet for up to 25 months. A group of 20 mice received distilled water only by intubation and served as controls. Administration of the 1:25 dilution was terminated at 18 weeks because of high mortality. Tumour incidences at 15-20 months were 0/4, 8/15 and 4/10 in the control, 1:50 dilution and 1:25 dilution groups, respectively. At 21-25 months, 1/20 controls and 8/10 animals fed tobacco extract in the diet had tumours. The types of tumour observed were lung adenocarcinomas or hepatocellular carcinomas (Bhide *et al.*, 1984b). [The Working Group noted the incomplete reporting of the distribution of different types of neoplasm among the various groups.]

(*b*) *Skin application*

Mouse: Groups of 40 CAF_1 (Jackson) and 40 Swiss (Millerton) mice [sex and age unspecified] received topical applications of a 50% methanol extract of unburnt cigarette tobacco on the skin three times a week for 24 months. Groups of 30 CAF_1 and 30 Swiss mice, which received whole-tar extract in the same way for 21 to 24 months, served as controls. Among the CAF_1 mice exposed to the tobacco extract, 11 developed papillomas, and among the Swiss mice, three developed papillomas, compared to 16 papillomas in each of the control groups. One papilloma later developed into cancer in the Swiss mice test group, compared to three in Swiss and eight in CAF_1 controls (Wynder & Wright, 1957). [The Working Group noted that there was no statistical evidence for the carcinogenic effect of this tobacco extract.]

Groups of eight to 17 male and female strain A (Strong) and Swiss mice, two to three months old, received skin applications of five different extracts (petroleum ether, benzene, chloroform, chloroform ether and ethanol) of an Indian chewing tobacco (*N. tabacum*; Vadakkan, Meenampalayam variety) up to 18 months of age; no tumour was observed at the site of application, and no excess incidence was reported at other sites (Mody & Ranadive, 1959). [The Working Group noted the small numbers of animals used.]

A group of 10 male and six female C17 mice, two to three months old, received thrice-weekly applications of a dimethyl sulphoxide extract of an Indian chewing tobacco (Vadakkan variety) on the skin of the interscapular region for life (24 months of age). No skin tumour was observed (Ranadive *et al.*, 1976). [The Working Group noted the small number of animals used.]

Groups of 11-36 inbred Swiss or Paris albino XVII x C_{57} black (hybrid) mice [sex and age unspecified] received twice-weekly skin applications of E8 ('total') plus E9 ('partially alkaloid-free'), E9 or E10 ('totally alkaloid-free') tobacco extracts or acetone for 95 weeks followed by weekly applications of croton oil. Between 61 and 95 weeks after the start of treatment, the incidences of papillomas and of squamous-cell carcinomas at the site of application were 10/21 and 6/21 (E8 plus E9), 9/25 and 2/25 (E9) and 22/35 and 10/35 (E10) in the hybrid mice, respectively. Papillomas occurred in 3/19 acetone/croton oil-treated controls; no carcinoma was observed. [The increases in the incidences of papillomas and carcinomas were statistically significant, except in the E9-treated group.] The incidences of papillomas in the Swiss mice were 2/9, 2/4 and 3/10, respectively; no carcinoma was observed (Ranadive *et al.*, 1963). [The Working Group noted that no control group of Swiss mice was included.]

The cocarcinogenic [promoting] effect of the E10 tobacco extract was tested in a group of 16 Swiss albino and 13 Swiss (Baldy) mice, which received a single topical application of benzo[*a*]pyrene [dose unspecified] followed by twice-weekly applications of E10 for 80 weeks. A group of seven Swiss albino and 10 Swiss (Baldy) mice received the benzo[*a*]pyrene treatment only and served as controls. Two carcinomas and four papillomas were observed in Swiss (Baldy) mice treated with E10 and benzo[*a*]pyrene; no tumour was observed in benzo[*a*]pyrene-treated controls (Ranadive *et al.*, 1963). [The Working Group noted the small number of animals and incomplete information concerning the initiating dose of benzo[*a*]pyrene.]

Groups of 30 female ICR Swiss mice, 57 days old, received a single topical application of 125 μg 7,12-dimethylbenz[*a*]anthracene (DMBA) in 0.25 ml acetone, followed 21 days later by applications of 0.25 ml of an acetone or barium hydroxide extract of unburned commer-

cial tobacco five times a week for 36 weeks. The amount of acetone extract was equivalent to 2.5 cigarettes per day. The barium hydroxide extract was prepared using two different extraction procedures, designated 'concentrated' and 'dilute', according to the yield: the 'concentrated' was equivalent to 0.5 cigarette per day and the 'dilute' was about one quarter as concentrated as the 'concentrated' extract. Two groups of 30 mice received DMBA treatment alone or no treatment and served as controls. The incidences of tumours, all of which were small papillomas, were: acetone extract, 16 tumours in 7/30 (2.3 tumours/mouse); concentrated barium hydroxide extract, 18 tumours in 8/30 (2.2 tumours/mouse); and dilute barium hydroxide extract, six tumours in 2/30 (three tumours/mouse). No tumour was observed in either of the control groups (Bock *et al.*, 1964).

Groups of 30 female ICR Swiss mice, 55-60 days old, received a single topical application of 125 μg DMBA in 0.25 ml acetone, followed three weeks later by applications of different aqueous extracts (crude, acidic, neutral and basic components) of an unprocessed, commercial, flue-cured tobacco five times per week for 26 weeks. A total of 12 papillomas developed in 6/30 mice treated with crude tobacco extract (equivalent to 0.5 g tobacco daily) following DMBA initiation. One mouse developed a papilloma after treatment with the acidic fraction and DMBA. No skin tumour was found in animals treated with DMBA alone or with the various fractions of tobacco alone. With half the concentration (0.25 g tobacco), one mouse developed a papilloma after application of the crude extract and one mouse developed three papillomas with the neutral extract following DMBA initiation. Additional studies demonstrated that the tumour-promoting components of the tobacco extract were stable and non-volatile (Bock *et al.*, 1965).

Groups of 20 female Swiss ICR/Ha mice, eight weeks of age, received a single topical application of 150 μg DMBA in 0.1 ml acetone, followed two to three weeks later by thrice-weekly applications of solvent extracts [ether (25 mg), chloroform (1 mg), methanol (25 mg) or a reconstituted sample (25 mg)] of a flue-cured cigarette variety of tobacco leaf for 52 weeks. Groups of 20 mice receiving DMBA alone or tobacco extracts alone served as controls. Two of 13 survivors in the DMBA/methanol extract group developed 'cancers'. The numbers of mice with papillomas in the various groups were: 4/12 (ether extract), 1/10 (chloroform extract), 2/13 (methanol extract) and 5/14 (reconstituted extract). No tumour was observed in mice treated with DMBA or extracts alone (Van Duuren *et al.*, 1966).

(*c*) *Inhalation*

Mouse: A group of 80 male strain A mice, three months old, were exposed by inhalation to powdered tobacco leaf on alternate days for 30 months. A further group of 80 animals served as controls. The incidences of 'lung cancer', leukaemia and hepatocellular carcinoma in animals surviving to 30 months were 12/75 and 1/80, 11/75 and 2/80, and 3/75 and 0/80 in the treated and control groups, respectively (Hamazaki & Murao, 1969). [The Working Group noted that the incidences of lung and liver tumours in the untreated mice were unusually low.]

(*d*) *Subcutaneous administration*

Mouse: Groups of 17 Paris albino XVII x C_{57} black mice received s.c. injections of 0.1 ml of a 2% solution of 'partially or completely alkaloid-free' extract of tobacco (Vadakkan, Meenampalayam variety) once a month for 41-95 weeks. One squamous-cell carcinoma [site not specified] developed in an animal that received the partially alkaloid-free extract (Ranadive *et al.*, 1963). [The Working Group could not draw any conclusion from this report.]

(e) Application to the oral mucosa or cheek pouch

Mouse: Groups of nine to 16 male and female strain A (Strong) and Swiss mice, two to three months old, were administered different alkaloid-free extracts of an Indian chewing tobacco of the Vadakkan type (*N. tabacum*; Meenampalayam variety). The extracts, a benzene extract and its neutral fraction, a water extract and four successive extracts (petroleum ether, benzene, chloroform and ethanol), were applied by daily application to the oral mucosa for up to 18 months of age. No excess incidence of tumours was observed (Mody & Ranadive, 1959). [The Working Group noted the small number of animals used.]

Rat: A group of 22 Wistar rats, five months of age, were painted on the oral mucosa with a 2% alkaloid-free extract of Vadakkan tobacco of the Meenampalayam variety in acetone twice a week for life; 12 of these animals were also painted with 20% lime in distilled water the day after each treatment. Control groups of 10-14 rats received no treatment or lime only. No tumour was observed at the application site (Gothoskar *et al.*, 1975).

Hamster: A group of 50 young golden hamsters received implantation of a 2-cm^3 plug of chewing tobacco [unspecified] in the cheek pouch. The opening in the cheek pouch was ligated and the animals were followed for up to 30 months. Survival after 13 months was 21/50; and eight were alive at 24 months, but none at 30 months. No tumour was observed in any of the animals (Peacock & Brawley, 1959; Peacock *et al.*, 1960).

Philippine leaf tobacco with 10% lime was mixed with beeswax, and pellets were implanted into the cheek pouch of 34 male and female Syrian golden hamsters, one to two months old. Animals were allowed to live their lifespan and were killed when moribund. No tumour at the implantation site was reported (Dunham & Herrold, 1962).

Groups of 11-12 male Syrian golden hamsters, nine weeks old, received topical applications on the cheek-pouch mucosa of a dimethyl sulphoxide (DMSO) extract of cured Banarsi chewing tobacco or DMSO alone thrice weekly for 21 weeks, at which time all animals were killed. No tumour was seen in treated or control hamsters, but 8/12 treated animals had leukoplakia (Suri *et al.*, 1971).

A group of 12 male inbred Syrian golden hamsters, two to three months old, received topical applications to the cheek-pouch mucosa of DMSO extracts of an Indian chewing tobacco (Vadakkan) thrice weekly for life. A control group of seven animals received applications of DMSO alone. No local tumour but moderate hyperkeratosis was observed (Ranadive *et al.*, 1976).

Groups of 30-41 Syrian golden hamsters [sex unspecified], weighing 40-50 g, received 60 g tobacco (Jada Jarda) alone, in combination with lime, or in combination with lime plus vitamin A in the cheek pouch thrice weekly for 100-110 weeks, at which time 24-32 animals were alive. Moderate to severe keratotic and dysplastic changes in the mucosa developed, but no neoplastic change was observed (Kandarkar *et al.*, 1981).

A group of 20 female Syrian golden hamsters, six to seven weeks of age, received topical applications to the cheek-pouch mucosa of 1 mg lyophilized aqueous tobacco extract in 0.05 ml water twice daily for six months. Animals were observed for a further six months then killed. Squamous-cell papillomas/carcinomas occurred in 3/17 animals, compared to none in 10 untreated and in 10 vehicle (water) controls (Rao, 1984).

(*f*) *Other experimental systems*

Groups of 5-12 male and female hybrid (inbred C_{17}) or Swiss mice, two to three months of age, received a single *intravesicular implantation* of paraffin pellets containing tobacco (Jarda variety of chewing tobacco), a mixture of tobacco and lime or an alkaloid-free tobacco extract or paraffin pellets alone and were observed for 10-30 months of age. Among the hybrid mice receiving the alkaloid-free tobacco implantation, 2/12 developed transitional-cell tumours of the bladder and one female developed a tumour described as a 'myosarcoma of the cervix with metastasis to the kidney'. No tumour was observed in the controls or in the other treated groups (Randeria, 1972). [The Working Group noted the small group size and the potential carcinogenic effect of intravesicular foreign bodies in mice.]

A group of four female hybrid (inbred C_{57}) mice and four female Swiss mice, two to three months of age, received daily *vaginal applications* of a fine mixture of Jarda tobacco dust containing lime derived from sea shells for 10-30 months; no vaginal tumour was observed (Randeria, 1972). [The Working Group noted that no control group was used in this study.]

Snuff

(*a*) *Oral administration*

Hamster: Groups of 50 male BIO 15.16 and BIO 87.20 (carcinogen-susceptible) strain Syrian hamsters, two to three months old, were fed one of the following five experimental diets for two years: 20% damp, fresh US snuff mixed with the diet; cellulose mixed with diet, such that the caloric content was reduced by 20% (negative control); control diet plus 50 treatments with 5 mg 20-methylcholanthrene (MC) per animal by stomach tube (positive control); cellulose diet plus 50 treatments with 0.5 mg MC per animal by stomach tube; and snuff diet plus 50 treatments with 0.5 mg MC per animal by stomach tube. The animals fed snuff diet alone showed a nearly identical tumour spectrum to that of controls. No increased incidence of tumours was noted in animals administered snuff with MC (Homburger *et al.*, 1976).

A total of 13 male and female Syrian golden hamsters, 1.5 months of age, were fed three different test substances for 16 months: group 1 (two males and two females) was fed 0.75 g aromatic snuff [type unspecified] per week; group 2 (two males and two females) was fed 0.75 g aromatic snuff [type unspecified] and 0.75 g calcium hydroxide per week; and group 3 (five animals) [sex distribution not specified] received calcium hydroxide only. One male hamster in group 2, estimated to have consumed 52 g snuff and 52 g calcium hydroxide during the 16-month period, developed a pancreatic carcinoid 4.5 months after the termination of treatment. The tumour incidence in the remaining groups and at other sites was not reported; however, the authors stated that carcinoids had been found previously in only 700 animals in that laboratory (Dunham *et al.,* 1975). [The Working Group noted the relatively small groups used.]

(*b*) *Subcutaneous administration*

Rat: A group of 82 male and female albino (Händler) rats (100 days old) was given s.c. injections of 0.15 ml (50 mg) of an ethanol extract of Swedish snuff (Ettan) in tri-*n*-caprylin once a week for 84 weeks. A group of 81 male and female rats received the same schedule

of injections of ethanol and tri-*n*-caprylin and served as controls. Malignant tumours developed in equal numbers in both test and control rats. These were 'retothelsarcomas' (one in each group), one uterine carcinoma (in a test animal) and one ovarian carcinoma (in a control animal) (Schmähl, 1965).

(*c*) *Application to the oral mucosa or cheek pouch*

Rat: A group of 21 male and 21 female Sprague-Dawley rats, three months of age, was administered snuff into a surgically-created canal in the lower lip. Approximately 0.2 g of a standard Swedish snuff (Röda Lacket), pH 8.3, was injected morning and night on five days per week for up to 22 months. [The calculated daily dose was 1 g/kg bw and the mean retention time after each administration was 6 h (range, 5-8 h) (Hirsch & Thilander, 1981).] The rats were killed at nine, 12, and 18-22 months. A second group of five male and five female rats was treated similarly with the same snuff but at pH 9.3 [produced by addition of 50% more sodium carbonate (1% of the total weight)] and sacrificed between 18 and 22 months. Of 42 animals administered the snuff, one developed a squamous-cell carcinoma of the oral mucosa at 8.5 months. No tumour was seen in rats exposed to the alkaline snuff or in 15 rats with surgically-created canals but not given snuff. Benign tumours outside the oral cavity were observed in roughly equal frequency in control and treated groups in both experiments (Hirsch & Johansson, 1983).

Four groups of 10 female Sprague-Dawley rats with surgically-created canals in the lower lip received the following treatments beginning at three months of age: group 1 was infected with herpes simplex type 1 (HSV-1) virus by scarification and topical application on the inside of the lower lip, followed, ten days later, by administration of a standard Swedish (Röda Lacket) snuff into the canal, morning and night on five days per week; group 2 was infected with virus and received no other treatment; group 3 was sham-infected with sterile saline followed by snuff treatment; and group 4 was given neither virus nor snuff and served as controls. The HSV-1 infection was repeated once after a one-month interval, and snuff was injected 10 days later as before. Snuff treatment was continued for 18 months, after which time all animals were killed. Three animals in each of groups 1 and 2 died from encephalitis shortly after the second infection with HSV-1. Squamous-cell carcinomas of the oral cavity developed in 2/7 rats, and a retroperitoneal sarcoma occurred in 1/7 rats exposed to HSV-1 and snuff. In the group exposed to snuff alone, 1/10 animals developed a squamous-cell carcinoma of the anus and 1/10 a retroperitoneal sarcoma (Hirsch *et al.*, 1984a). [The presence of two oral cancers in animals in group 1 does not constitute a statistically significant result. The Working Group noted, however, that these two tumours were located near the site of application of the snuff.]

Hamster: Groups of 50 young golden hamsters received an instillation into the left cheek pouch of 10 ml of a thick paste of snuff. The opening of the pouch was ligated, and the animals were followed for up to 30 months. The contralateral pouches of 25 of these animals were filled with sand and gum and served as controls. After 13 months, 21/50 were still alive; 10 were alive at 24 months, but none at 30 months. No tumour was observed in control or treated pouches (Peacock & Brawley, 1959; Peacock *et al.*, 1960).

A group of 35 male and female Syrian golden hamsters, one to two months of age, received snuff and lime in the cheek pouch as single depositions. A positive control group of 71 hamsters was exposed to the two carcinogenic hydrocarbons, 7,12-dimethylbenz[*a*]anthracene and 3-methylcholanthrene; and a negative-control group of 36 animals was exposed to beeswax, which was used as a vehicle to prolong the retention time of the test substances. The animals were killed after 15-20 months or when moribund. Two of the 35 animals exposed to 20% snuff and lime and 2/36 exposed to beeswax developed inflam-

matory lesions; among the positive controls, 23/56 developed malignant tumours, including carcinomas (20) and sarcomas (three) (Dunham & Herrold, 1962).

Groups of four to seven male and female weanling Syrian golden hamsters received twice-daily applications of 50 mg of a commercial US 'Scotch' (dry type) snuff, snuff and calcium hydroxide, or calcium hydroxide alone into the cheek pouch on five days per week for up to 99 weeks. No local tumour was observed in any group (Dunham *et al.*, 1966).

A group of 84 male and female Syrian golden hamsters (BIO hamsters of the RB strain), aged three to four months, were exposed to 0.5 g of snuff placed in a stainless-steel webbing cartridge attached to the lower incisors for 30 min daily for 51 weeks. A group of 84 hamsters exposed to dry cotton served as negative controls and further groups, one of 84 animals exposed to benzo[*a*]pyrene and one of 24 animals exposed to 7,12-dimethylbenz[*a*]anthracene, served as positive controls. No tumour was found in the oral mucosa, except in the positive controls (Homburger, 1971). [The Working Group noted the short duration of this study.]

Nass

A series of experiments were reported in two papers (Kiseleva *et al.*, 1976; Milievskaja & Kiseleva, 1976).

(*a*) *Skin application*

Hamster: A group of 19 female and 31 male Syrian hamsters received topical applications of a suspension of *nass* (45% tobacco, 8% lime, 30% ash, 12% plant oil and 5% water) on the dorsal skin. The average lifespan was 44.4 weeks. Three out of nine animals still alive at the time of appearance of the first tumours (53 weeks) developed neoplasms: one liver 'lymphangioendothelioma', one adrenal-gland tumour and one forestomach papilloma. No local tumour occurred. In the control group (either untreated or treated orally with sunflower oil), 2/57 hamsters that survived to the appearance of the first tumour (59 weeks) developed tumours: one adrenal-cortex neoplasm and one forestomach papilloma (Kiseleva *et al.*, 1976).

(*b*) *Application to the cheek pouch*

Hamster: A group of 28 female and 33 male Syrian hamsters, one to three months of age, received applications of *nass* (same composition as described above) as a dry powder into the left cheek pouch for life; another group of 13 females and 24 males received *nass* as a 50% suspension in refined sunflower oil in the cheek pouch (total dose per animal, 6.2-147.5 g, mean 53.8 $\pm$ 2.5 g). The animals were followed until death. No tumour was found at the site of *nass* application. The average lifespan of animals receiving *nass* (50.8 weeks) was slightly shorter than that of untreated animals (57.3 weeks) or that of hamsters receiving sunflower oil alone (57.6 weeks). Of 64 treated hamsters in both groups still alive at the time of appearance of the first tumour (17 and 37 weeks), 13 developed tumours: seven liver-cell tumours and one liver tumour of 'mixed structure', three tumours of the adrenal glands (described as a 'carcinoma of adrenal cortex' and as 'adenoma, chromaffinoma type' or 'carcinoma of adrenal cortex'), one forestomach papilloma, three uterine tumours (leiomyoma and/or fibromyoma and/or cysts), one skin melanoma and one unspecified tumour of the large intestine. Among 110 untreated and 10 animals treated with sunflower oil, 45 survived to the appearance of the first tumour (59 weeks), and two developed tumours (one

adrenal-cortex neoplasm and one forestomach papilloma) (Kiseleva *et al.*, 1976; Milievskaja & Kiseleva, 1976).

In another experiment described in these reports, *nass* was introduced as a suspension in refined sunflower oil into the cheek pouch of male and female hamsters comprising a total of 40 females and 46 males belonging to six generations. *Nass* was administered throughout life, including periods of pregnancy and lactation. No tumour was found at the site of application. Of 36 (36.1%) hamsters that survived to the appearance of the first tumour (54 weeks), 13 developed neoplasms at various sites: two liver-cell tumours, one haemangioendothelioma, one cholangioma and one liver tumour of 'mixed structure', three in the adrenal glands, four papillomas of the forestomach, one of the uterus or ovaries, one benign skin tumour and one pancreatic tumour. The average lifespan of the animals was 51.6 weeks (Kiseleva *et al.*, 1976; Milievskaja & Kiseleva, 1976). [The Working Group noted deficiencies in reporting the results of this series: the number of animals and incidence of tumours in each generation, the number of newborns and neonatal mortality are not indicated, and no multigeneration controls were available.]

[In consideration of the whole study, the Working Group noted that the effective number, i.e., the number of animals surviving to observation of the first tumour, was calculated separately for treated (number of survivors at 17 weeks with the dry powder) and control (59 weeks) animals. Therefore, the effective number of control animals should have been higher in the first experiment. High mortality of animals was noted, even in control groups, in the period preceding observation of the first tumour; average lifespan of untreated control animals was 57.3 weeks. The sex of animals in which liver tumours were found was not indicated.]

A group of 30 Syrian hamsters received a single application of 0.1 mg 7,12-dimethylbenz[*a*]anthracene (DMBA) as a 0.1% solution in benzene into the cheek pouch. Another group of 30 hamsters received the same treatment, followed seven weeks later by daily applications of *nass* (composition as described above) as a dry powder into the cheek pouch; the total dose ranged from 11.2 to 102.5 g (mean, 38.9 $\pm$ 5.2 g). Three out of 11 survivors at the time of appearance of the first tumour (23 weeks) receiving DMBA alone developed tumours: one rhabdomyoblastoma of the cheek pouch, and two papillomas of the forestomach. Six of 11 animals still alive at 50 weeks that received DMBA plus *nass* had tumours: five papillomas of the forestomach and one cystic epithelioma of the skin of the jaw (Milievskaja & Kiseleva, 1976). [The Working Group noted the small number of animals that survived to the time of observation of the first tumour.]

3.2 Other relevant biological data

(*a*) *Experimental systems*

Toxic effects

Application of *nass* to the cheek pouch of Syrian hamsters induced degenerative and proliferative changes in the epithelium, and an inflammatory response and fibrosis in the submucosal layer. The same changes were observed in the oesophageal mucosa of animals receiving *nass* either in the cheek pouch or by gavage. *Nass* administered into the cheek pouch or percutaneously induced foci of hepatocyte proliferation, bile-duct proliferation, cholangiofibrosis and severe vascular disturbances (Kiseleva *et al.*, 1976).

Two to four months after the beginning of daily *nass* administration by gavage to rats, basal-cell proliferation with cell polymorphism and 'signs of invasion' into the submucosa in the oesophagus were observed (Rahmatian *et al.*, 1965).

Snuff (0.2 g commercial brand) was inserted twice daily into a surgically-created canal in the lower lip of rats, on five days a week. Measurement showed that the snuff was retained in the canal after each insertion for 5-8 h. Exposure for nine or 12 months produced mild to severe hyperplasia, hyperorthokeratosis and acanthosis. After longer exposure, vacuolated cells were found in basal layers of the epithelium, with hyperplastic, atrophic, ulcerated lesions, slight dysplastic lesions, and, in a few rats, severe dysplastic changes in the epithelium of the crevice containing the snuff. Squamous-cell hyperplasia was also found in the forestomach of two rats exposed to snuff for 18-22 months (Hirsch & Johansson, 1983). A preliminary study suggests that these lesions, particularly the dysplasia of the squamous epithelium in the canal, may be exacerbated by concurrent herpes simplex virus infection (Hirsch *et al.*, 1984a).

No histological change specifically related to snuff exposure was found in the oral mucosa of Syrian golden hamsters alive at the end of an experiment in which they had been forced to chew on a stainless steel-gauze pouch containing 0.5 g snuff for 30 min per day, on five days a week for 30 (60 animals) or 52 weeks (24 animals). During treatment, mortality occurred in 30% of controls and 34% of the snuff-treated animals in the 30-week group, and 52% of controls and 60% of treated animals in the 52-week group, mostly as a result of trauma (Homburger, 1971). No toxic effect on the cheek pouch or oesophagus was seen in four hamsters fed 6 g of diet containing 2.5% American snuff, on five days per week, for 16 months, or in four hamsters fed diets containing 2.5% snuff and 2.5% calcium hydroxide (Dunham *et al.*, 1974).

Aqueous extracts of snuff inhibit the replication of herpes simplex virus-1 by cultured kidney cells from green monkeys. Greater inhibition was produced by a brand of snuff containing a high concentration of nitrosamines than by a brand with a low nitrosamine content (Hirsch *et al.*, 1984b).

Extracts of chewing tobacco, snuff and tobacco leaf did not inhibit the growth of the oral cariogenic bacteria *Streptococcus mutans* and *S. sanguis* when tested *in vitro* (Lindemeyer *et al.*, 1981).

Effects on reproduction and prenatal toxicity

Anabasine, a tobacco alkaloid, was tested for teratogenicity in pigs. After ingestion by dams of 2.6 mg/kg bw anabasine twice daily between days 43-53 of gestation, defects were induced in all of three litters (21/26 offspring), including cleft palate, fixed, excessive flexure of the front or rear pasterns, fixed, excessive flexure of the carpal joints, and rotation or bowing of limbs. Similar defects were induced by *Nicotiana tabacum* and *N. glauca* (Crowe, 1978; Keeler *et al.*, 1984). Anabasine was teratogenic to chicks (Landauer, 1960; Upshall, 1972). *N. glauca* was also teratogenic to cows (Keeler, 1979).

Nicotine failed to induce defects in pigs, sheep or cows (Keeler, 1979), but it has been shown to be teratogenic in rabbits (Vara & Kinnunen, 1951), mice (Nishimura & Nakai, 1958) and chicks (Landauer, 1960).

Addition of 0.1 mg nicotine/ml to the drinking-water of pregnant mice reduced the weight of 17-day-old foetuses by 12% (Rowell & Clark, 1982).

Absorption, distribution, excretion and metabolism

Nicotine (80 and 250 ng/ml of blood) was detected in two rats 30 min after insertion of snuff (0.2 g) into a surgically-created canal in the lower lip (Hirsch & Thilander, 1981).

Mutagenicity and other short-term tests

Ethanol extracts of a chewing variety of *Nicotiana tabacum*, known locally in India as the Pandharpuri variety, induced mutations in *Salmonella typhimurium* TA98 in the presence but not in the absence of phenobarbital-induced rat-liver 9000 x *g* supernatant (S9). No mutation was induced in *S. typhimurium* TA100, TA1535 or TA1538 in the presence or absence of S9 (Bhide *et al.*, 1984b). Ethanol extracts of this tobacco also induced mutations in Chinese hamster V79 cells; the presence of Aroclor-induced rat-liver S9 enhanced this effect. The same extracts induced micronuclei in bone-marrow cells of Swiss mice (Shirname *et al.*, 1984).

An ethyl acetate extract of Jaffna tobacco (used with betel quid) induced sister chromatid exchanges in cultured human lymphocytes and in a human lymphoblastoid cell line; in the latter case, rat-liver homogenate enhanced the effect. This extract, tested only in the absence of exogenous metabolic activation, did not induce ouabain-resistance in Chinese hamster V79 cells. The same extract, another ethyl acetate extract and an ethanol extract induced cell transformation in Syrian hamster embryo cells (Umezawa *et al.*, 1978; 1981).

Tobacco powder added to the feed of *Drosophila melanogaster* larvae did not induce sex-chromosome loss, sex-linked recessive lethal mutations or autosomal translocations (Abraham *et al.*, 1979).

Aqueous extracts of two *nass* samples induced a dose-related increase in the number of chromosomal aberrations in Chinese hamster ovary (CHO) cells. The frequency of these effects was not altered by the addition of rat-liver S9, catalase or superoxide dismutase (Zaridze *et al.*, 1985a,b). Aqueous extracts of *khaini* also induced chromosomal aberrations in CHO cells (Stich *et al.*, 1982).

The tobacco alkaloids, anatabine, nicotine and nornicotine, induced sister chromatid exchanges in CHO cells in the absence of S9. With anatabine (125-500 μg/ml), the effect was dose-related. The presence of Aroclor-induced rat-liver S9 inhibited the induction of sister chromatid exchanges (Riebe & Westphal, 1983).

(*b*) *Humans*

Toxic and pharmacological effects

Precancerous lesions occurring in users of smokeless-tobacco products are discussed in section 3.3 of this monograph, p. 89.

Few studies were available on the toxicology and pharmacology of smokeless-tobacco products. A significant increase in pulse rate and blood pressure after tobacco chewing has been observed which is presumably due to nicotine (Simon & Iglauer, 1960; Bordia *et al.*, 1977). The pharmacological effects of nicotine have been reviewed extensively (see, for example, Goodman & Gilman, 1982; Balfour *et al.*, 1984).

Studies in Tunisia (Ben Khedher *et al.*, 1984; Ben Miled *et al.*, 1984; Malej *et al.*, 1984) have suggested an increased frequency of bronchitis with the use of snuff (powdered Tunis-

ian tobacco, known as *neffa*). Gingival recession has been associated with the oral use of snuff (Christen *et al.*, 1979a). A case of periodontal bone destruction has been reported in a snuff user (Christen *et al.*, 1979b).

A study of Swedish school children aged 13-14 years, of whom 13 of the boys (11%) used snuff orally, has shown that the use of snuff was associated with an increased intensity of gingivitis (Modéer *et al.*, 1980).

Effects on reproduction and prenatal toxicity

The still-birth rate to Indian women who chewed tobacco was 50 per 1000 live births (11/220) compared with only 17 per 1000 live births (20/1168) in women who did not chew tobacco. The mean birth weight of the offspring of tobacco chewers was approximately 500 g less than that of controls. This change was associated with a decrease in the mean gestation period. The sex ratio (male:female) of the offspring was 80:100 in the chewers in comparison to 108.5:100 in the controls (Krishna, 1978).

The mean weight of the placenta from 48 Indian mothers who took tobacco (in 85% of the cases as a mixture of tobacco and lime) was 15% greater than that from 48 controls (Agrawal *et al.*, 1983). The mean weight of newborn babies of 70 Indian tobacco users (the tobacco was either chewed or ingested alone or mixed with betel leaf or with lime) was 14% less than the weight of the babies of 70 matched controls (Verma *et al.*, 1983).

Absorption, distribution, excretion and metabolism

Gaede (1941) found that during chewing about one-third of the nicotine present in tobacco is extracted each hour. Nicotine is readily absorbed from the mouth (Gritz *et al.*, 1981). The saliva of snuff users contains *N'*-nitrosonornicotine, *N'*-nitrosoanatabine and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (Hoffmann & Adams, 1981; Hoffmann *et al.*, 1982). Three samples of the saliva of two healthy men chewing Indian tobacco contained *N'*-nitrosonornicotine (17-60 ng/ml), *N'*-nitrosoanatabine (14-52 ng/ml), *N*-nitrosoproline (0.5-10 ng/ml), nicotine (120-179 μg/ml), nitrite (10-36 μg/ml) and thiocyanate (9-40 μg/ml). Urine collected from a man from commencement of chewing contained a total of 80 ng *N*-nitrososarcosine per 6-h urine, 860 ng *N*-nitrosoproline, 950 μg nicotine and 795 μg cotinine (Nair *et al.*, 1985).

The concentration of nicotine in the plasma of 11 young adult male users of oral snuff rose from a morning level of 2.9 ng/ml (after overnight abstinence) to 21.6 ng/ml after consuming an average of 11 g snuff over a period of 6-8 h. Plasma cotinine levels rose from a mean level of 137 ng/ml to 197 ng/ml. Subjects fell into two groups, with two-thirds absorbing substantial amounts of nicotine and one-third appearing to have almost no absorption (Gritz *et al.*, 1981).

After inhalation of a single pinch of snuff, blood nicotine concentration rose within minutes to 40 ng/ml — about twice the peak concentration found after smoking a cigar and comparable with the concentration found in heavy cigarette smokers (Russell *et al.*, 1980). The amount of nicotine and the relative proportion of its metabolites in the urine of nasal snuff takers was similar to that in smokers (Temple, 1976).

Nitrite levels were higher in the saliva of *mishri* users from two locations (urban and rural) and of tobacco chewers than in that of control groups. Subjects who chewed tobacco or used *mishri* had higher levels of nitrate reductase activity than controls (Murdia *et al.*, 1982).

Mutagenicity and chromosomal effects

The proportion of exfoliated micronucleated cells from the mucosa of the inner lip of 27 *khaini* users was 2.2%, ranging from 0.8-4.9%. In 15 non-users of *khaini*, the proportion of micronucleated cells was 0.5%, ranging from 0.3-0.8% (Stich *et al.*, 1982; Stich & Rosin, 1984).

The proportion of sublingual exfoliated micronucleated cells in 45 Uzbekis using *nass* was 4.3%, ranging from 1.6-6.3%. In 12 non-users of *nass*, the proportion of micronucleated cells was 0.4%, ranging from 0.0-0.5% (Zaridze *et al.*, 1985b,c).

The saliva collected from subjects during the chewing of Indian tobacco (Parijat Zafrani Patti) enhanced the frequency of chromatid breaks and exchanges in Chinese hamster ovary (CHO) cells. No such increase was observed with saliva produced during the chewing of a western-type tobacco (Stich & Stich, 1982).

3.3 Studies of precancerous lesions and conditions in humans

A precancerous lesion is defined as a morphologically altered tissue in which cancer is more likely to occur than in its apparently normal counterpart. A precancerous condition is a generalized state associated with a significantly increased risk of cancer (WHO Collaborating Centre for Oral Precancerous Lesions, 1978; Axéll *et al.*, 1984). Examples of oral precancerous lesions are leukoplakia and erythroplakia; oral precancerous conditions include sideropenic dysphagia, submucous fibrosis and, possibly, lichen planus.

The concept of leukoplakia as a precancerous lesion is based on the findings: (1) of a significant number of oral carcinomas associated with a pre-existing area of leukoplakia; and (2) that some leukoplakias appear to undergo malignant tranformation (for reviews, see Pindborg *et al.*, 1975; Silverman *et al.*, 1984). However, studies of oral leukoplakia are often difficult to compare owing to lack of uniformity in the histological definition of leukoplakia. This problem was discussed recently (Axéll *et al.*, 1984).

The precancerous conditions submucous fibrosis (Pindborg, 1972) and lichen planus have been observed mainly in Indians with a variety of chewing habits (Pindborg *et al.*, 1972). Lichen planus is a subacute or chronic idiopathic skin disease characterized by small, flat violaceous papules, often combining to form plaques. It is often pruritic and chiefly affects the flexor surface of the wrist, legs, penis and buccal cavity (Gennaro *et al.*, 1979).

Submucous fibrosis is an insidious, chronic disease affecting the oral mucosa and sometimes the pharynx and oesophagus, and occurs almost exclusively among Indians. Subepithelial changes lead to the presence of palpable fibrous bands, especially in the buccal mucosa, palate and labial mucosa. Diffuse blanching of the oral mucosa and especially of the soft palate may be another characteristic sign.

In the majority of studies carried out in Asia on oral precancerous lesions and conditions, the chewing habits of the subjects are not precisely defined, particularly in reference to inclusion of areca nut in the quid. These studies are therefore summarized in the monograph on betel-quid and areca-nut chewing. This section includes only reports of studies conducted in tobacco chewers and oral-snuff takers in North America, Europe and Africa, and on users of *shammah* and *nass*, which do not contain areca nut.

(*a*) *Prevalence of oral leukoplakia*

In a sample of 1490 British coal miners, Tyldesley (1971) found oral leukoplakia in 3.6% of 280 tobacco chewers and in none of 122 non-chewers. Roed-Petersen and Pindborg (1973) reported that of 450 Danish patients with oral leukoplakia, 32 (7.1%) used snuff. Axéll (1976) examined 20 333 Swedes aged 15 years and over: 14.2% of men and fewer than 0.1% of women took snuff. Of the 1444 snuff users, 116 (8%) had 'snuff dipper's lesion' (oral leukoplakia) (15.9% in men and 0.2% in women). The prevalence of oral leukoplakia among the total population examined was 3.6%.

Christen *et al.* (1979a,b) found leukoplakia in nine of 14 US university students who had been chewing tobacco or using snuff or both for two to nine years. Of 1119 US high-school students, 117 (11%) used 'smokeless tobacco' and 43% had oral-mucosal lesions in the labial groove in the form of hyperkeratotic or erythroplakic areas (Greer & Poulson, 1983).

In a study of 585 elderly coloured people resident in homes for the aged in the Cape Peninsula of South Africa, van Wyk *et al.* (1977) found that 119 (20.4%) had oral leukoplakia (excluding the tongue). Of these, eight (6.7%) chewed tobacco and four (3.4%) used snuff orally.

Of 661 individuals examined in Saudi Arabia, 187 used *shammah*; oral leukoplakia was found in 129 (69%) users but was not seen in non-users (Salem *et al.*, 1984).

Khasanov and Fasiev (1970) reported that 217 (14.7%) of 1479 *nass* users had pathological changes of the oral mucosa, such as atrophy, hypertrophy and chronic ulcer, while only 21 (0.5%) of 4674 non-users of *nass* and nonsmokers had the above changes. According to Aleksandrova (1970), 127 (44%) of 289 persons using *nass* and only one (0.4%) non-user had atrophic oral mucosa.

(*b*) *Histology of tobacco-related leukoplakia*

The first histological study of snuff-induced leukoplakia was carried out on seven patients by Pindborg and Poulsen (1962), who reported finding a band of homogeneous, eosinophilic periodic-acid-Schiff (PAS)-positive material in the connective tissue close to the salivary glands in the lower labial mucosa in four of the cases. In 1964, Lyon *et al.* concluded, on the basis of histochemical studies of these same Danish snuff users, that the PAS-positive material was amyloid. With regard to the epithelial changes, Pindborg and Renstrup (1963) studied biopsies from 12 snuff users and found a marked hyperplasia of the epithelium with a thickened surface layer of large and vacuolated cells; the surface epithelium was not keratinized, but streaks of focal parakeratosis were noted. Roed-Petersen and Pindborg (1973) found the streaks of parakeratosis most often in the lower labial mucosa in 31 biopsies; only one biopsy showed signs of epithelial dysplasia. Pindborg *et al.* (1980) compared the snuff-induced epithelial changes with similar changes caused by smoking. Clinically, both types of lesions present with a pumice-like appearance, and histologically they reveal a chevron-like (previously called 'streaks') keratinization of the epithelium. These changes are considered specific for tobacco usage.

Archard and Tarpley (1972) studied three oral biopsies from patients in the USA using snuff and found a homogeneous, eosinophilic deposit in the submucosa similar to that described by Pindborg and Poulsen (1962).

Axéll *et al.* (1976), examining 114 biopsies of snuff users in Sweden, described increased epithelial thickness with a vacuolated surface layer having wavelike, eosinophilic spikes directed toward the surface and a narrow, eosinophilic band demarcating the prickle-cell layer and acanthosis as characteristic features of the leukoplakia lesions associated with use of snuff. They also noted some slight inflammatory reaction.

Hirsch *et al.* (1982) examined clinically, histomorphologically and histochemically lesions of varying severity associated with snuff use. They found a high frequency of keratinized lesions, sialoadenitis and degenerative changes of the salivary glands (42%), and mild epithelial dysplasia (18%). These changes were seen to occur at a higher frequency than had been reported previously by others. Exposure to snuff was shown to be related to superficial as well as to deeply located histopathological cell changes. The most marked degenerative changes in the salivary glands were seen among patients with the most extensive exposure to snuff.

Van Wyk (1965) studied 25 biopsies from Bantus with snuff-induced lesions and characterized a typical snuff lesion as one with a hyperplastic, acanthotic and parakeratotic epithelial layer overlaying a chronically inflamed lamina propria. In four biopsies he found a 'disquiet epithelium'.

(*c*) *Malignant transformation of leukoplakia related to chewing habits*

In specimens from 12 oral-snuff users (Pindborg & Renstrup, 1963) and in 157 biopsies taken from clinically severe cases of leukoplakia among 15 000 oral-snuff users in the USA (Smith, 1975), no epithelial dysplasia was found.

Of 450 patients in Denmark with leukoplakia diagnosed from 1956-1970, 394 were followed for one to 14 years to ascertain oral cancer incidence and mortality (Roed-Petersen & Pindborg, 1973). This group included 32 oral-snuff users, with a mean exposure time of 22 years (exposure-time estimation was based on years of use and the duration of time a quid was retained in the mouth). One of the 32 had dysplasia at first examination, and another individual developed oral cancer during the follow-up period [not specified]. This corresponds to a rate of premalignant or malignant transformation of 6.2% for either dysplasia or carcinoma. In contrast, 19.5% of patients with leukoplakia not associated with snuff use developed carcinoma or showed dysplasia at first or later examination. [The Working Group noted that the follow-up period was not specified; in addition, the sample of snuff users was relatively small.]

In his study of British coal miners, Tyldesley (1976) followed up eight of 22 tobacco chewers with oral leukoplakia for five years. He found one case of malignant transformation to a squamous-cell carcinoma at the site at which the tobacco chew had been held for 30 years. In five other men there was no change, and in two, regression of the lesion was seen. [The Working Group noted the small sample size.]

Brown *et al.* (1965), in an investigation of 394 cases of oral cancer in Georgia, USA, noted that users of snuff had a significantly higher incidence of co-existing leukoplakia (60% *versus* 26% for non-users); 'extensive' leukoplakia was seen in 32% of users *versus* 6% of non-users. Both Landy and White (1961), in a study in Arkansas, USA, and Rosenfeld and Callaway (1963), in Tennessee, USA, noted that in cases of buccogingival cancer either there was concomitant leukoplakia or an evolution from leukoplakia had been observed.

3.4 Case reports and epidemiological studies of carcinogenicity to humans

Oral use

Although most of the studies of the relationship between the use of chewing tobacco or snuff and cancer have focused on risks to the oral cavity and pharynx, some evidence is also available concerning cancer at other anatomical sites. In many of the studies reported, chewing tobacco or snuff use was often only one of many potential risk factors considered.

(*a*) *Tobacco*

(i) *Descriptive studies and case series*

Many reports of case series have emphasized the relatively high frequency of chewing tobacco and snuff use among oral cancer patients (Table 30). [Early reports are not included if chewing tobacco or snuff habits were mentioned only in combination with other tobacco habits from which they were not distinguished; in many studies, neither smoking habits nor alcohol consumption was described.] The clinical characteristics of cancer patients who use smokeless-tobacco products have also been described (Table 31), especially the propensity of these cancers to occur in the presence of leukoplakia, to have often a verrucous appearance, and to be slow-growing, well-differentiated, squamous-cell carcinomas. Patients with cancer and with a chewing-tobacco or snuff habit are frequently described as having cancer at the site or on the side where the quid is most frequently placed.

In a study from Tennessee described more fully below (see Smith *et al.*, 1970; Smith, 1975), no oral cancer was observed in a group of 15 000 oral-snuff users; two carcinomas of the oral cavity were observed in another group of 500 oral-snuff users. [The Working Group noted that there is an absence of documentation on the source and characteristics of the study population.]

(ii) *Case-control studies*

Chewing tobacco or oral-snuff use (not specified) (Tables 32 and 35)

In Minnesota, Moore *et al.* (1952, 1953) studied the tobacco use histories of 40 white male patients, aged 50 years or more, with oral carcinoma (alveolar ridge, floor of mouth and buccal mucosa), 23 oral leukoplakia patients, 72 lip-cancer patients, and 93 with carcinoma of the 'face', in comparison with those of 38 control patients with hernias and other benign diseases. Although there was no difference between the case groups and the benign controls in the frequency of a 20-year history of pipe smoking or of cigar and cigarette smoking, there were statistically significant differences (all $p < 0.05$) for long-term use (more than 20 years) of chewing tobacco or snuff for each of the case groups. Within this population of primarily Scandinavian descent, more than half of the members in each case group and a third of the control group had used chewing tobacco or snuff for a minimum of 20 years. Tobacco histories were obtained by personal interviews conducted by the hospital personnel. [The composition of the case and control groups was not clearly defined. The relative risks associated with exposure of more than 20 years in contrast to none or 20 years or less were estimated by the Working Group from the numbers given. The relative risks were 2.4 for carcinoma of the face, 2.6 for lip cancer, 4.0 for oral carcinoma and 7.8 for oral leukoplakia.]

Peacock *et al.* (1960) identified persons with carcinoma of the buccal mucosa, alveolar ridge and floor of the mouth at a North Carolina, USA, hospital. Cases were included in the study only if information on tobacco use had been ascertained. These cases (staff and private patients) were compared to in-patient (staff and private patients) and out-patient (staff only) controls. It was found that 25/45 (55.6%) of the cases had used snuff or chewing tobacco for more than 20 years, whereas only 49/146 in-patient controls (32.6%) and 94/217 out-patient controls (43.3%) had done so. The association between oral cancer and use of chewing tobacco or snuff reached statistical significance only among older (60 years and over) staff patients in comparison with the in-patient control group. [The Working Group noted that controls whose habits were not known were apparently labelled non-users. Any such misclassification bias would have overestimated the strength of the association.]

The cases in a study by Vincent and Marchetta (1963) were 89 male and 17 female patients admitted successively to a New York State, USA, hospital with cancer of the oral cavity (tongue, floor of mouth, palate, gingiva, buccal mucosa), larynx, pyriform sinus or pharynx seen at the head-and-neck clinic. Controls comprised 100 male and 50 female patients of comparable age seen at the gastrointestinal clinic of the same hospital. Heavy alcohol consumption and smoking were more frequent in each case group than among controls, as determined by systematic history-taking from all subjects. The use of snuff or chewing tobacco (both designated 'snuff' by the authors in their Table 5) was also more common among the cases than in the controls. Only 5/100 male control patients (5%) had a chewing tobacco or snuff habit, whereas 9/33 (27.2%) oral-cavity cases, 3/33 (9.1%) pharynx cases, and 2/23 (8.7%) larynx cases chewed tobacco or took snuff. Cases were more likely to have smoked than controls. [Relative risks associated with use of chewing tobacco or snuff among men, calculated by the Working Group, were 7.1 for oral-cavity cancer, 1.9 for pharyngeal cancer and 1.8 for laryngeal cancer. Smoking was not controlled for.]

Williams and Horm (1977) conducted a population-based case-control study of the etiology of cancer at many different sites based on the interview responses of random-sample patients from the Third National Cancer Survey (1969-1971). Controls for the oral-cancer case group comprised patients with other cancers, excluding lung, larynx and bladder. Among men, use of chewing tobacco and snuff was strongly associated with cancer of the gum or mouth, but not with cancer of the lip or tongue; controlling for age, race and smoking habits, relative risks were 3.9 ($p < 0.01$) for moderate and 6.7 (non-significant) for heavy use of chewing tobacco or snuff. Among women, use of chewing tobacco or snuff was associated with cervical cancer; controls were patients with any other cancer. The relative risks, controlling for smoking, age and race, were 4.7 ($p < 0.05$) for moderate and 3.6 (non-significant) for high use. Suggestive associations (not statistically significant) were also found for laryngeal cancer (in men only, for whom risks were 1.8 and 2.6 for moderate and high use, respectively). [The Working Group noted that in this study multiple comparisons were made of many risk factors and many cancer sites, so that some positive findings may have been due to chance alone.]

Chewing tobacco (Tables 33 and 35)

Wynder *et al.* (1957a) compared 659 cases of lip, oral-cavity and pharyngeal cancer identified in a hospital in New York with 439 hospital-based controls with other benign and malignant conditions. Data on tobacco use and other factors were obtained from personal interviews. Cigarette, cigar and pipe smoking and alcohol use were associated with oral cancer in this study. A total of 17% of male cases had chewed tobacco in contrast to 8% of the controls, indicating a moderate association between tobacco chewing and cancer of the lip, oral cavity and pharynx. Some variation in the proportion of tobacco chewers was evident

Table 30. Proportions of smokeless-tobacco users in series of patients with upperdigestive/respiratory cancers[a]

Reference	US state or country	Series description	Habit	Proportion of smokeless tobacco users	Cancer at site where quid placed	Smoking habit
Abbe (1915)	New York	100 (90 men, 10 women) patients with oral cancer	Chew	13% of cases chewed tobacco	Yes, or near site	89/90 men
Ahlbom (1937)	Sweden	545 men with cancer of the oral cavity, pharynx, larynx or oesophagus, 1931-1936	Snuff, chew, NOS	70% of patients with buccal/-gingival/mandibular (outer oral cavity) cancer, 37% with cancer of the lip, 26% with cancer of the inner oral cavity, 16% with cancer of the pharynx/larynx/-oesophagus	NM	7, 6, 39, 64% cigarette + cigar smokers, respectively; 23, 57, 35, 20% pipe smokers, respectively
Ackerman (1948)	Missouri	31 (26 men, 5 women) patients with verrucous carcinoma of the oral cavity	Chew	61% of 18 patients with buccal-mucosal cancer were 'inveterate' tobacco chewers	NM	NM
Wilkins & Vogler (1957)	Georgia	81 (37 men, 44 women) patients with gingival cancer, 1937-1956	Snuff, chew	50% of women used snuff orally, 32% of men chewed tobacco	NM	1/44 women who used snuff orally also smoked, 3 other women smoked only; 9/37 men smoked only, the 12 who chewed also smoked
Dunn & Farrior (1962)	Florida	112 patients with cancer of the oral cavity, larynx or nasopharynx	Chew, snuff	3% of cases chewed tobacco (2/4 with buccal-mucosal cancer, 1/11 with tonsillar cancer). 5% of cases used snuff orally (1/3 with cancer in palate and upper alveolar ridge, 5/16 with cancer in floor of mouth and lower alveolar ridge)	NM	2 chewers smoked; oral snuff users did not smoke; 90% of cases smoked
Goethals *et al.* (1963)	Minnesota	55 (45 men, 10 women) patients with histologically-confirmed verrucous cancer of oral cavity, 1935-1957	Chew	13% of cases chewed tobacco	NM	21/55 smoked
Coleman (1965)	Virginia	23 patients with buccal-mucosal or gingival cancer, 1956-1964	Snuff, chew, NOS	100% used snuff orally or chewed tobacco	Yes	NM
Leffall & White (1965)	Washington DC	107 (77 men, 20 women) black patients with oral-cavity cancers, 1948-1963	Chew	4/7 (57%) patients with buccal-mucosal cancer chewed tobacco	NM	NM
Kraus & Perez-Mesa (1966)	Missouri	77 (68 men, 9 women) patients with verrucous carcinoma of the oral cavity, 1942-1962	Chew, snuff	47% chewed tobacco, 4% used snuff orally, 22% denied tobacco use (5/9 women chewed or used snuff)	Often	9/68 men and 1/9 women were heavy smokers

Dunn & Dykstra (1967)	Florida	427 patients with cancer of the upper respiratory tract	Chew, snuff	4/45 (9%) of patients with cancer of the floor of mouth and lower alveolar-ridge, 2/18 (11%) with cancer of palate and alveolar ridge and 4/26 (15%) with buccal-mucosal cancer used snuff orally; 7/26 (27%) with buccal-mucosal cancer chewed tobacco; 0/388 patients with other cancers chewed tobacco or used snuff		40/45, 15/18 and 13/26 smoked, respectively
Zacho *et al.* (1968)	Denmark	535 (385 men, 150 women) patients with gastric cancer and medical record notation on tobacco use	Chew, snuff, NOS	25% of men and no women used smokeless tobacco. Use especially common in patients with cancer of the pylorus	NA	87% of smokeless tobacco users were also smokers
Fonts *et al.* (1969)	Kentucky	10 (5 men, 5 women) patients with verrucous carcinoma of the oral cavity, 1961-1966	Chew, snuff	60% were tobacco chewers, 10% oral snuff users, 20% chewers and oral snuff users	NM	NM
Zacho *et al.* (1975)	Denmark	953 patients with gastric cancer (493 with tobacco-use data available in records), 1948-1973	Chew, snuff, NOS	No women used smokeless tobacco and 30 and 34% male smokers and nonsmokers used smokeless tobacco, respectively. Use common in patients with cancer of the body of the stomach	NA	In men, 36% smoked pipes, 29% cheroots, 13% cigars, 22% cigarettes
Hartselle (1977)	Alabama	289 patients with squamous-cell carcinoma of the oral cavity (39 indicated use of tobacco) compared with 248 cases in tumour registry (206 indicated use of tobacco), 1955-1975	Snuff, chew	Of the 39 pathology patients with tobacco use mentioned, 51% used snuff orally, 5% chewed tobacco. Of 206 patients from tumour-registry records, 31% used snuff orally, 6% chewed tobacco	NM	44% of 39 pathology patients smoked. 63% of 206 tumour registry patients smoked
McGuirt (1983b)	North Carolina	169 women with 'mucosal head and neck cancer', 1975-1980	Snuff, chew, NOS	33% used snuff orally or chewed tobacco	NM	40% smoked
Brown *et al.* (1965)	Georgia	394 (231 men, 163 women) patients with cancer in the mucous membrane of the mouth from the anterior tonsillar pillar anteriorly to the lips, but not including lip or tonsillar pillar, 1937-1957	Snuff, chew	In women, 44% used snuff orally, 2% chewed tobacco; in men, 3% used snuff orally, 9% chewed tobacco	NM	NM
Rosenfeld & Callaway (1963); Rosenfeld & Green (1969)	Tennessee	300 (125 men, 175 women) patients with oral or gingival cancer; 225 patients (154 men, 71 women) with cancer of the tongue or floor of mouth (accurate data regarding usage of snuff were obtained from 214 of the women)	Snuff	Among 159 women with buccal/gingival cancer, 90% used snuff orally; among 55 women with cancer of the tongue or floor of mouth, 22 used snuff orally	NM	NM

[a]NOS, not otherwise specified; NM, not mentioned; NA, not applicable

Table 31. Case reports and case series of smokeless-tobacco users with cancer

Reference	US state or country	Cancer site (no. of cases)	Special clinical/histological characteristics, other comments	Cancer at site of quid placement	Years of smokeless tobacco use	Smoking	Alcohol	Leukoplakia
Chewing tobacco								
Wynder (1976) [Warren (1837)]	Massachusetts	Tongue (1)	Men	Yes	NM	NM	Yes, 'ardent spirits'	NM
Friedell & Rosenthal (1941)	Illinois	Mouth (8)	All men, cancers generally well-differentiated, slow-growing	6/8 yes, 1 no, 1 NM	One for 12 years; the others, 35-65 years	4/8	NM	7/8
Moertel & Foss (1958)	Minnesota	Buccal mucosa (1)	Men, 2 separate squamous-cell carcinomas in the buccal mucosa	Yes	'Entire adult life'	NM	NM	Yes
Sorger & Myrden (1960)	Canada	Buccal mucosa (4)	Men with verrucous carcinoma of buccal mucosa. All over 70 years old	Yes for at least 3/4	One for 10 years; one, 'always'; 2, since teens	2/4	NM	2/4
Snuff								
Root *et al.* (1960)	Minnesota	Ear (1)	Farmer who placed snuff in ear	Yes	42 years	NM	NM	NM
Landy & White (1961)	Arkansas	Buccogingival (25)	Women, average age 68 years. Usually well-differentiated squamous-cell carcinoma. Metastases rare	Most	20->50 years	NM	NM	Concomitant
Stecker *et al.* (1964)	Minnesota	Gum (1)	71-year-old man who used snuff 10-12 h daily, verrucous carcinoma	Yes	40 years	NM	NM	Yes
Axéll *et al.* (1978)	Sweden	Oral cavity (49)	Older than other oral cancer cases, more likely to come from northern Sweden	67%	NM	NM for snuff users	NM	NM
Sundström *et al.* (1982)	Sweden	Anterior oral vestibule (23)	Men; *Candida* (58% of 19 cases), multiple carcinomas (26%). Verrucous carcinoma in some. Average age 76 years	Yes, by design	NM	NM	NM	At least 5
McGuirt (1983a)	North Carolina	Oral cavity (57)	13 men, 44 women. Persons with snuff use. Sites: 47% buccal mucosa, 32% alveolar ridge, 11% tonsillar trigone, 10% elsewhere in oral cavity. 24 verrucous-appearing, only 4 verrucous carcinomas, 58% well-differentiated histologically	89%	75% for >40 years	No, by design	No, by design	61%

[a]NM, not mentioned

Table 32. Case-control studies of smokeless-tobacco use (unspecified) and oral cancer

Reference	Source of patients	Cases	Controls	Smokeless tobacco use in cases and controls	RR (95 % confidence limit)[a]	Distribution of smoking habits
Moore *et al.* (1952, 1953)	Minneapolis, MN hospital, since 1951	40 men with oral cancer	38 patients with benign diseases	65% of cases, 32% of controls for >20 years	[4.0]	38% of cases, 53% of controls smoked cigarettes
Peacock *et al.* (1960)	Chapel Hill, NC hospital, 1952-1958	45 patients with oral cancer and known tobacco habits	146 patients over 40 years old, frequency matched on race, sex, economic status; 217 'randomly' sampled outpatients	55.6% of cases; 32.6% of inpatient controls; 43.3% of outpatient controls for >20 years	Significant among older staff cases	Not stated
Vincent & Marchetta (1963)	Buffalo, NY hospital	89 men and 17 women with cancer of the oral cavity, pharynx or larynx	100 men and 50 women, successive patients in same age group at gastrointestinal clinic	16% of male cases, 5% of male controls	Men (2.4-20.7) [7.1]	Among men, 85% of cases and 54% of controls smoked cigarettes
Williams & Horm (1977)	Third National Cancer Survey, 1969-1971	57 men and 27 women with cancer of the gum-mouth	2102 men and 3464 women with cancer at sites unrelated to smoking	Of cases, 11 men and 2 women; of controls, 164 men and 53 women	Men 3.9 ($p < 0.01$)	Smoking, age and race controlled for in analysis

[a]RR, relative risk; figures in square brackets were calculated by the Working Group.

by case type: patients with lip, buccal-mucosal and palate cancer were most likely to chew tobacco. However, all tobacco-chewing cases drank alcohol and all but one smoked.

Wynder and Bross (1961) reported that 21% of 150 male patients with squamous-cell carcinoma of the oesophagus were tobacco chewers, compared with 10% of the 150 male controls with other malignant cancers and benign conditions. Cases and controls were ascertained from hospitals in New York City and Brooklyn, USA, in 1956-1959, and tobacco use was ascertained through interviews with cases and controls. Smoking and alcohol consumption were associated with an increased risk of oesophageal cancer in this population and were more common habits than tobacco chewing, but were not controlled for in analyses related to tobacco chewing. [The Working Group noted that the actual number of tobacco chewers among cases could not be estimated.]

A case-control interview study in Atlanta, Georgia, USA, by Vogler *et al.* (1962) included four groups seen over a 19-month period (1956-1957): 333 patients with cancers of the oral cavity, pharynx and larynx, 214 patients with other diseases of the mouth, 584 patients with other cancers, and 787 patients without cancer whose mouths were not examined. Among rural men, the percentage of tobacco chewers was significantly higher in the oral, pharyngeal and laryngeal cancer group and in the mouth-disease controls (36%) than in other cancer and non-cancer controls (15% or less chewed). This association was also found for urban men: 17% of oral-cavity cancer patients chewed, compared to less than 10% in the other two groups. However, approximately 50% of rural male cases smoked cigarettes and approximately 70% of urban cases smoked (which was more common than in controls). Patients with cancer of the oral cavity were more likely to chew tobacco than patients with cancer at other oral and pharyngeal sites. [Percentages preceded by the word 'approximately' are derived from diagrams in the text. Smoking was not controlled for in the analysis.]

In a case-control study of bladder cancer (Wynder *et al.*, 1963), tobacco chewing was reported by interview in 33/300 male cases (11%) and 24/300 male controls (8%). Study subjects were ascertained over a five-year period (1957-1961) in hospitals in New York City, USA.

In Puerto Rico, Martinez (1969) conducted a population-based case-control study of oral, pharyngeal and oesophageal cancer to examine environmental, tobacco and dietary factors. Each of the 400 histologically-confirmed carcinoma cases was matched with three controls for age and sex: one from the hospital where the case was diagnosed and the other two from the neighbourhood in which the case lived. Overall, 3.7% of the cases (15 persons) chewed tobacco only, compared to 4.0% of controls (48 persons); however, the percentages varied considerably by cancer site and sex. For each of the three cancers studied, the percentage of male cases who chewed only exceeded that of the controls; the same was true for female cases of oesophageal cancer and controls. However, few women with oral (none) or pharyngeal (two in controls) cancer had this habit. The chewing tobacco was typically mixed with molasses. [The Working Group noted that if relative risks for those with only a chewing habit compared to those with no habit are calculated on the basis of the figures given, the risks for men are 11.9 for oral cavity cancer, 8.7 for cancer of the pharynx and 1.2 for cancer of the oesophagus. The relative risk for oesophageal cancer in women was 2.7. However, it was noted that the numbers of tobacco chewers in the site-specific tables do not add up to the total numbers of chewers in the study, and therefore these calculated relative risks may not be accurate.]

Cole *et al.* (1971) found no difference between the observed number of male lower-urinary-tract cancer patients who chewed tobacco (46) and that expected to have the habit (42.3), derived from the distribution of habits in the controls. This population-based study included 470 interviewed cases (men and women) from 111 hospitals in the Boston and

Brockton, Massachusetts, USA, statistical area over an 18-month period (1967-1968) and 500 controls drawn from 'residents lists', which enumerate almost all persons residing in the study area.

Browne *et al.* (1977) compared 75 cases of oral cavity cancer identified through a cancer registry between 1957-1971 with 150 controls matched for age, sex, primary occupation and residence drawn from a clinical practice in the UK in 1974-1976. The subjects or, in the event of death, next-of-kin were interviewed using a structured questionnaire. The authors found that controls were more likely to have used chewing tobacco (36/150) than cases (16/75). No difference between cases and controls was observed with regard to duration of tobacco chewing, and none of the subjects used snuff. Since there was matching in the design on primary occupation (but not on secondary occupation), it appears that some residual confounding by employment remained; indeed, the negative association with chewing tobacco disappeared when the data were stratified by occupation. [The Working Group noted that the discordance in time of ascertainment of the cases (1957-1971) and of controls (1974-1976), resulting in matching of attained age at different time intervals, and the necessity of interviewing primarily the next-of-kin of deceased cases raises concern about bias, especially from secular changes in tobacco habits and differential recall of habits. Overmatching may also have occurred by neighbourhood and occupation.]

Patients with cancers of the lung (1048 cases), oral cavity (591), larynx (387), oesophagus (183) and bladder (586) in 20 hospitals in eight major US cities were compared with 2560 matched hospital controls with diseases unrelated to tobacco use during 1969-1975 (Wynder & Stellman, 1977). Among men, 233 controls (9.0%) had used chewing tobacco at some time, whereas 61 (10.3%) of patients with oral-cavity cancer, 21% with lung cancer, 12% with laryngeal cancer, 11% with oesophageal cancer and 8% with bladder cancer chewed. Less than 0.5% of women chewed tobacco. [The authors estimated that the relative risks for cancer at each of these five sites in men who chewed tobacco included 1.0 within 99% confidence limits, and none attained statistical significance.] The smoking habits of chewers and non-chewers were similar. In this population, smoking was strongly related to cancer at each site studied, while alcohol consumption was linked to cancers of the oral cavity, larynx and oesophagus.

No association between chewing tobacco and bladder cancer was observed in a study of 480 male and 152 female pair-matched bladder-cancer cases and controls in Canada (Howe *et al.*, 1980); the estimated relative risk was 0.9, based on 61 discordant pairs. The relative risk estimate was unchanged after controlling for smoking.

In a study of etiological factors for oesophageal cancer, Pottern *et al.* (1981) noted that the proportion of tobacco chewers was slightly higher among matched controls than among oesophageal-cancer cases in their interview study with the next-of-kin of 120 black, male oesophageal-cancer decedents in Washington DC, USA, and 250 black men who had died of other causes. However, the authors commented that the number of subjects with a chewing habit was small: only 3.3% of subjects chewed.

In an examination of potential etiological factors related to the risk of cancer of the nasal cavity and paranasal sinuses, Brinton *et al.* (1984) found a relative risk of 0.7 associated with chewing tobacco; this was lower than those associated with oral-snuff use (1.5), cigarette smoking (1.2) or pipe smoking (1.2), and equal to that for cigar smoking (0.7), all of which were non-significant. Confidence intervals for all of these relative risks included the null value. This case-control study included 160 hospital-ascertained cases in North Carolina and Virginia, USA, and 290 hospital controls and controls ascertained from death certificate. Subjects or their next-of-kin were interviewed by telephone.

Table 33. Case-control studies of chewing tobacco use and oral cancer

Reference	Source of patients	Cases	Controls	Chewing tobacco use	Distribution of habits
Wynder *et al.* (1957a)	New York, NY hospital	659 (543 men, 116 women) white patients with cancer of the lip, oral cavity or pharynx	439 white patients with benign head and neck tumours, thoracic diseases, lymphomas or gastrointestinal cancers	17% of cases and 8% of controls	All except 1 chewer smoked as well
Vogler *et al.* (1962)	Atlanta, GA hospital, 1956-1957	333 (235 men, 98 women) patients with cancer of the lip, oral cavity, pharynx or larynx	3 groups: 214 with other mouth diseases, 584 with other cancers, 787 non-cancer patients	In urban men, 17% of cases and <10% of controls; in rural men, 36% of cases and other-mouth-disease controls, about 15% and 10% of other controls	At least 45% of male urban and rural cases and controls smoked
Martinez (1969)	Cancer Registry, Puerto Rico, 1966	400 (290 men, 110 women) histologically-confirmed cases of cancer of the lip and oral cavity, pharynx or oesophagus	1 hospital/clinic control per case, 2 neighbourhood controls matched for age and sex per case	3.7% of cases and 4.0% of controls [Among men, RR for oral cavity cancer, 11.9.][a] Few women chewed.	The most common mixed use of tobacco was cigarettes and cigars (50%), followed by cigarettes and chewing tobacco (15.2%), and cigars and chewing tobacco (11.4%)
Browne *et al.* (1977)	English regional cancer registry, 1957-1971	75 patients with cancer of the oral cavity	150 living residents matched for age, sex, residence and primary occupation	9% of cases and 13% controls	All cases smoked. The proportion of controls with no tobacco habit not clear
Wynder & Stellman (1977)	20 hospitals in 8 US cities, 1969-1975	591 patients with cancer or the oral cavity, 1048 of the lung, 387 of the larynx, 183 of the oesophagus and 586 of the bladder	2560 patients without smoking-related diseases matched for age, sex, race and city	Among men, 10.3% of oral-cavity cases and 9.0% of controls; <0.5% of women	Smoking habits did not differ in chewers and non-chewers

[a]RR, relative risk; calculated by the Working Group

Hartge *et al.* (1985) reported that use of chewing tobacco was unrelated to bladder-cancer risk. Their study included 2982 patients with bladder cancer who were identified from records of 10 large population-based case registries throughout the USA (1977-1978) and who were interviewed for information about tobacco use and other factors. There was a total of 5782 population-based controls: controls 65 years of age and older were selected from records of the Health Care Financing Administration, and controls aged under 65 years were chosen by a random digit-dialling method. The analysis was restricted to men. Among men who never smoked cigarettes, the relative risk for bladder cancer was 1.0 for chewing tobacco, controlling for age, race, residence and other tobacco habits. The authors cautioned that the relative risk estimates were somewhat unstable in view of the small number of users (40 in cases and 133 in controls).

Snuff (Tables 34 and 35)

Wynder *et al.* (1957b) compared 477 [misprinted as 472 in the table in the original paper] patients with cancers of the lip, oral cavity, maxillary sinus, nasopharynx, hypopharynx, oesophagus and larynx to 333 patients with other malignancies seen in a hospital in Stockholm, Sweden, from 1952-1955. Interviews with patients and a review of the medical history were undertaken for all study subjects. More of the buccal- and gum-cancer patients used snuff than did controls (no women practised the habit). There was suggestive evidence by ridit analyses that snuff use was related to buccal-mucosal cancer in men, and the majority of cases with gum and buccal-mucosal cancers had their tumours in the area of the mouth where the quid was held. [The Working Group could not extract the number of snuff users.]

In the case-control interview study in Atlanta, Georgia, USA, by Vogler *et al.* (1962) described on p. 98, among 642 female urban subjects, 40% of the 38 oral-cavity cases, but only 2%, 3% and 1% of the 57 other-mouth-disease, 170 other-cancer and 377 non-cancer controls, respectively, had used snuff. Similar findings were observed for the 371 rural females: 75% of the 55 cases had used snuff orally in contrast to 11% of 37 other-mouth-disease patients, 20% of 129 other-cancer patients, and 11% of 150 non-cancer patients. Only 7% of female rural patients smoked. About 30-40% of urban women smoked cigarettes, but smoking habits were similar in each study group. The differences in snuff use between cases and controls were statistically significant for most of the age strata studied. One of 3 (33%) female lip-cancer patients used snuff, in contrast to 53/72 (74%) women with oral-cavity cancer and 2/18 (11%) patients with pharyngeal of laryngeal cancer. [The Working Group noted that the reportedly similar proportions of smoking habits among urban women and the low proportion of smokers in the rural sample indicate that the association between snuff and oral/pharyngeal/laryngeal cancer was not confounded by smoking.]

In a case-control study of bladder cancer (Wynder *et al.*, 1963), study subjects were ascertained over a five-year period (1957-1961) in hospitals in New York City, USA. Snuff use was reported by interview in 6/300 male cases (2%) and 9/300 controls (3%).

In the study described on p. 98, Cole *et al.* (1971) found no difference in the observed number of male lower-urinary-tract cancer patients who used snuff (3) compared to the number expected (2.9), derived from distribution of habits in the controls.

In the study by Wynder and Stellman (1977) described on p. 99, 8 patients with oesophageal cancer, 11 with bladder cancer, 15 with laryngeal cancer, 10 with oral-cavity cancer and 35 with lung cancer used snuff. The highest relative risk was 1.7 for oesophageal

cancer associated with snuff use [but none of the risks attained statistical significance]. Smoking was strongly related to the development of cancer at each site studied, while alcohol consumption was linked to cancers of the oral cavity, larynx and oesophagus.

Westbrook *et al.* (1980) identified 55 female patients with cancer of the alveolar ridge or buccal mucosa from 1955 to 1975 at a university clinic in Arkansas, USA. A random sample of 55 female controls of comparable age seen at the institution over the same time period constituted the comparison group. Of the 55 female patients, 50 (91%) were oral-snuff users whereas only one (2%) member of the control group was a user (RR = 540, highly significant). Only three cases smoked cigarettes and one chewed tobacco. The average duration of snuff use was 52 years. The snuff users, 44 (80%) of whom were white, averaged 66 years of age. In the 15 patients for whom sufficient data were available, 14 were found to have a tumour where the snuff had been typically placed. The cancers in snuff users were all squamous-cell carcinomas, most of them well-differentiated. [The Working Group considered that the apparent use of medical records as a source of information on tobacco and alcohol use may have led to misestimation of snuff use. If controls who used snuff were less likely than cases to be recorded as such, the magnitude of the association would have been overestimated.]

In the study of Pottern *et al.* (1981) reported on p. 99, the proportion of oral-snuff users was slightly lower in matched controls than in oesophageal-cancer cases; 1.7% of oesophageal-cancer cases used snuff.

Winn *et al.* (1981a) conducted a case-control study of oral-cavity and pharyngeal cancers among women in North Carolina in 1975-1978 to examine reasons for the exceptionally high mortality rates from these cancers among white women throughout the south-eastern USA. A total of 232 women hospitalized with or who had died from cancers of the tongue (International Classification of Diseases, 8th revision, code 141), gum (code 143), floor of mouth (code 144), other mouth (code 145), oropharynx (code 146), hypopharynx (code 148), and pharynx unspecified (code 149) were included in the case group. Two age-, race- and region of residence-matched controls were obtained for each case; interview was completed for 410. Subjects or their next-of-kin were interviewed in their homes. Tobacco-related risks were estimated by using a common reference group: women with no tobacco habit. The relative risk for white women who used only oral snuff was 4.2 (95% confidence limits, 2.6-6.7), while the relative risk associated with cigarette smoking among non-users of snuff was 2.9 (1.8-4.7). Among whites, the relative risk in those with both habits was 3.3 (1.4-7.8); these women had smoked fewer cigarettes and used snuff for fewer years than women with only one habit. Risks for black women were somewhat lower, but they had used snuff for fewer years and used fewer tins per week. Although 37 women had chewed tobacco, all except three were also oral-snuff users. One-third of all oral snuff users had developed the habit by the age of 10 years, and the average duration of use was 45 years. For cases of cancer of the gum and buccal mucosa, oral-snuff use among nonsmokers was related to years of use, with relative risks ranging from 13.8 (1.9-98.0) for 1-24 years, 12.6 (2.7-58.3) for 25-49 years, and 47.5 (9.1-249.5) for 50 or more years of use. For cases of cancer at other mouth sites and of the pharynx, the corresponding relative risks were 1.7, 3.8 and 1.3. The findings relating to oral-snuff use could not be explained by poor dentition (Winn *et al.*, 1981b) or by use of mouthwashes (Blot *et al.*, 1983). The consumption of fruits and vegetables was associated with a reduction in risk in the study population, primarily evident in cigarette smokers and in women without tobacco habits, and not among oral-snuff users (Winn *et al.*, 1984).

In the study of Brinton *et al.* (1984) on cancer of the nose and paranasal sinuses, described on p. 99, a slightly elevated, but not statistically significant, relative risk of 1.5 was

attributable to oral-snuff use. This was higher than any smoking-associated risk and higher than the relative risk for chewing tobacco. When analysed by histological type, it was found that both squamous-cell carcinomas and adenocarcinomas were related to snuff use; relative risks were 1.9 and 3.1, respectively, controlling for sex. Snuff use was more strongly related to squamous-cell nasal-cancer risk in men (3.7) than in women (1.4). When data were analysed by site of cancer, it was found that the relative risk for maxillary-sinus cancer was 2.8 (95% confidence interval, 1.2-6.3).

Hartge *et al.* (1985), in the study described on p. 101, reported that snuff use was unrelated to bladder-cancer risk. Among men who never smoked cigarettes, the relative risk for bladder cancer was 0.77 for snuff use, controlling for age, race, residence and other tobacco habits. The authors cautioned that the relative risk estimates were somewhat unstable in view of the small number of users (11 in cases, 50 in controls).

(iii) *Cohort studies*

Chewing tobacco or oral-snuff use (not specified) (Table 36)

Bjelke and Schuman (abstract, 1982) and Schuman *et al.* (1982) described results from a cohort study of 12 945 men in Norway who had been followed for more than 10 years (1967-1978). Relative risks for regular users of oral tobacco were 2.8 for buccal-cavity and pharyngeal cancer and 3.1 for oesophageal cancer; these were statistically significant. In addition, users experienced a relative risk of 2.2 for histologically-confirmed cases of pancreatic cancer (reported to be 'significant'). Prostatic-cancer risk was unrelated to tobacco chewing or snuff use.

Bjelke and Schuman (abstract 1982) and Schuman *et al.* (1982) described cancer risk in relation to use of chewing tobacco and use of snuff in a study of 16 930 US men, who had been policy holders of an insurance association, and were followed for more than 10 years for vital status (1966-1981). Tobacco use was assessed by postal questionnaire. Former snuff users/tobacco chewers had a relative risk of 3.3 (statistically significant) for pancreatic cancer (based on 33 total deaths and 7 deaths in former users), controlling for age and urban/rural residence. The relative risk associated with regular snuff use/chewing was elevated, but was not as high (2.1, based on 5 deaths in regular users) as for former chewers and was not statistically significant. Regular snuff use/chewing (but not former or occasional use) was linked to a 2.2 relative risk for prostatic cancer (91 total deaths, 21 deaths in regular users), which was statistically significant, adjusting for age and urban-rural residence. The authors also noted that tobacco chewing and snuff use were positively related to oesophageal cancer (relative risk, 2.6, non-significant), and that a multiplicative effect was associated with use of chewing tobacco and snuff and of alcohol.

Heuch *et al.* (1983) examined pancreatic cancer etiology using data from a cohort study of 11 959 men and 2519 women who responded to a questionnaire on lifestyle factors. The group consisted of adult residents in the 1960 Norwegian census, brothers living in Norway of migrants to the USA, and the spouses and siblings of subjects from a case-control study of gastrointestinal cancer. Cancer incidence and mortality were ascertained from record linkage with the Norwegian Cancer Registry and death files. Adjusting for region, urban/rural residence, age, sex, and cigarette and alcohol consumption, a marginally significant trend of increasing risk of pancreatic cancer with increasing use of chewing tobacco or snuff was evident. The relative risk of developing cancer in histologically-verified cases was 2.9 in regular users compared to persons who had never used the products.

Table 34. Case-control studies of oral use of snuff and oral-cavity cancer

Reference	Source of patients	Cases	Controls	Snuff use. RR (95 % confidence limit)[a]	Distribution of smoking habits
Wynder *et al.* (1957b)	Swedish hospital, 1952-1955	477 (265 men, 212 women) patients with cancer of lip, oral cavity, maxillary sinus, pharynx, larynx or oesophagus	333 patients with other cancers	In men, snuff use associated by ridit analysis with buccal and gum cancer. Cancers often where quid placed	Smoking more common in cases
Vogler *et al.* (1962)	Atlanta, GA hospital, 1956-1957	333 (235 men, 98 women) patients with cancer of the lip, oral cavity, pharynx or larynx	3 groups: 214 with other mouth diseases, 584 with other cancers, 787 non-cancer patients	Among women, 40% of urban cases, and 2%, 3% and 1% of controls, respectively; 75% of rural cases, and 11%, 20% and 11% of controls, respectively	40% of urban and 7% of rural women smoked
Wynder & Stellman (1977)	New York, NY hospital	591 patients with cancer of the oral cavity, 1047 of the lung, 587 of the larynx, 183 of the oesophagus, 587 of the bladder	2560 patients without smoking-related diseases matched for age, sex, race and city	Among men, 1.7% of oral-cavity cancer cases and 2.7% of controls; only 1% of women	Smoking habits of snuff users not stated
Westbrook *et al.* (1980)	Little Rock, AR hospital, 1955-1975	55 women with cancer of buccal mucosa or alveolar ridge	55 random age-stratified samples of women patients	91% of cases and 1.8% of controls [RR, 540 (143.9-2026.0)]	5% of cases smoked. Controls said not to have different smoking habits
Winn *et al.* (1981a)	5 NC hospitals and death certificates, 1975-1978	232 women with cancer of the oral cavity and pharynx	410 controls with other diseases/causes of death matched for age, race and residence	46% of cases and 30% of controls. RR in white nonsmokers, 4.2	43% of cases and 33% of controls smoked, but controlled for in analysis. Association with snuff remained strong with control of other factors (Winn *et al.*, 1981b; Blot *et al.*, 1983; Winn *et al.*, 1984)

[a]RR, relative risk; RR in square brackets calculated by the Working Group

Table 35. Case-control studies of non-oral cancer sites in which smokeless-tobacco use is described

Reference	Source of patients	Cases	Controls	Smokeless tobacco use. RR (95 % confidence limit)[a]	Distribution of smoking habits
Nasal cancer					
Brinton *et al.* (1984)	VA and NC hospitals and death certificates	160 patients with cancer of nasal cavity or paranasal sinuses	290 matched controls from hospitals or death certificates	In oral snuff users, RR, 1.9 for squamous-cell carcinoma, 3.1 for adenocarcinoma. Maxillary sinus RR, 2.8 (1.2-6.3)	Mentioned but not controlled
Oesophageal cancer					
Wynder & Bross (1961)	NY hospitals, 1956-1959	150 men and 37 women with carcinoma of the oesophagus	150 men and 37 women with other cancers and benign conditions	In men, 21% of cases and 10% of controls chewed tobacco. RR, 2.4 (statistically significant)	Smoking more common in cases
Martinez (1969)	Cancer Registry, Puerto Rico, 1966	400 histologically-confirmed cases of cancer of lip, oral cavity, pharynx and oesophagus	1 hospital/clinic control per case, 2 neighbourhood controls matched for age and sex per case	[Estimated RR for oesophageal cancer, 1.2 for men and 2.7 for women]	The majority of men smoked but tobacco chewers only examined separately
Wynder & Stellman (1977)	20 hospitals in 8 US cities, 1969-1975	183 oesophageal cancers	2560 matched hospital controls	In men, 11% of cases and 9% of controls chewed tobacco	Smoking habits similar in cases and controls
Pottern *et al.* (1981)	Washington DC	120 black men who died of oesophageal cancer	250 black men who died of other causes	3.3% of subjects chewed tobacco, 1.7% used snuff orally. Too few users for firm conclusions	Smoking more common in cases
Bladder cancer					
Wynder *et al.* (1963)	NY hospitals, 1957-1961	300 men and 70 women with bladder cancer	370 (300 men, 70 women) matched patients with other diseases	In men, 11% of cases and 8% of controls chewed tobacco; 2% of cases and 3% of controls used snuff orally. Too few users for firm conclusions	Smoking more common in cases
Cole (1971)	Boston/Brockton, MA, 1967-1968	470 population-based cases with bladder cancer	500 population-based controls	46 observed, 42.3 expected tobacco chewers; 3 observed, 2.9 expected oral snuff users. No difference	Smoking more common in cases
Howe *et al.* (1980)	3 Canadian provinces, 1974-1976	480 men and 152 women with bladder cancer	Pair-matched controls	RR, 0.9 for tobacco chewing (not significant)	Unchanged with adjustment for smoking
Hartge *et al.* (1985)	10 US population-based cancer registries, 1977-1978	2982 population-based cases with bladder cancer	5782 population-based controls	In men, RR, 0.77 for snuff and 1.0 for chewing tobacco (estimates unstable)	RRs controlled for age, race, residence and other tobacco habits
Other					
Williams & Horm (1977)	Third National Cancer Survey, 1969-1971	7518 cancer patients from population-based cancer registries	Persons with other cancers	Elevated risks for cervical cancer, RR, 4.7 (statistically significant) for moderate, 3.6 for heavy use. Excess of laryngeal cancer suggestive in smokeless-tobacco users	RRs adjusted for smoking, age, race

[a]RR, relative risk; estimated RR in square brackets calculated by the Working Group

Snuff

In Tennessee, USA, Smith *et al.* (1970) found that, among the 20 000 persons they examined in an unspecified number of clinics over an unspecified time interval, 15 000 were snuff users. This population included 1751 persons (1240 of them female) with mucosal changes warranting further study. Of the 1751, 157 were thought to require biopsy, but none were found to have a dyskeratotic or malignant lesion. Only 237 of the 1751 had cytological findings consistent with benign hyperkeratosis. [The Working Group noted that it is unclear whether patients with these 237 cytological abnormalities were in the group biopsied.] Repeated biopsies were made on over 75% of the 1751 with 'mucous membrane changes' at six-month intervals for 5.5 years. No cancer occurred.

In a subsequent follow-up of 1550 of the original study population of 1751 persons, including 128 of the original 157 biopsied patients (Smith, 1975), an additional 4.5 years of observation yielded no carcinoma or dyskeratosis. An additional group of 400 snuff users, apparently followed during the same 4.5 years, included 78 patients with mucosal change identified by biopsy. None of these patients developed dyskeratosis or carcinoma.

[The Working Group considered that, in these two papers, the consistent lack of clear specification as to which subset of the study group reference is being made makes it difficult to determine who was examined or followed up. While 15 000 persons appear to have been followed, only 1550 persons, selected on the basis of mucosal change present at outset and available for follow-up, received the 5.5-year follow-up. Perhaps as few as 128 received the full 10-year follow-up. The period of time during which the initial 20 000 persons were accrued is not specified, although at one point the authors state that the paper is a 'report of a 20-year study'. Since no tracing method is described, it is not clear whether the authors would have learned of any hospitalizations for oral cancer or deaths from all cancers in their study population.]

Table 36. Prevalence and follow-up studies of populations using smokeless tobacco

Reference	US state or country	Cohort description	Results[a]
Smith *et al.* (1970); Smith (1975)	Tennessee	128 oral snuff users with oral lesions (not dyskeratotic or malignant)	10-year follow-up yielded no malignancy. Not clear that follow-up methods would have detected new oral cancer patients
Bjelke & Schuman (1982); Schuman *et al.* (1982)	USA	16 930 men followed for 15 years	RR, 3.3 for pancreatic cancer for former snuff users/tobacco chewers (statistically significant). Also elevated RR for regular users (not significant). RR, 2.2 for prostatic cancer (statistically significant). Also association with oesophageal cancer
	Norway	12 945 men followed for 11 years	RR, 2.2 for pancreatic cancer, reported as significant. RR, 2.8 and 3.1 for cancer of the buccal cavity and pharynx and of the oesophagus (statistically significant)
Heuch *et al.* (1983)	Norway	11 959 men and 2519 women from several sources of Norwegians	RR, 2.9 for pancreatic cancer in smokeless-tobacco users, adjusted for cigarette smoking and alcohol consumption

[a]RR, relative risk

(*b*) Mishri, gudakhu *and* shammah

No oral cancer was observed in 22 606 persons using *mishri* in a prevalence study of 101 761 Indian villagers. In contrast, eight oral cancers were noted in 28 638 Indians with a chewing habit which included use of tobacco in the quid; and two cancers occurred in 1073 mostly male users with mixed habits. No oral cancer occurred in persons with no tobacco habit. *Mishri* use was much more common among women (38.9%) than among men (22.2%) (Mehta *et al.*, 1972). [The Working Group noted that women might be expected to have lower cancer rates regardless of tobacco use.]

In a house-to-house survey of a random sample in Singhbhum district, India, 8.3% of the population were reported to use *gudakhu*. No oral cancer or precancerous lesion was found among them (Mehta *et al.*, 1971).

In a survey of 661 persons from Saudi Arabia, of whom 28% used *shammah*, Salem *et al.* (1984) described seven patients with squamous-cell carcinoma of the mouth. Leukoplakia was present, and all had used *shammah* for 'many' years. Smoking and alcohol consumption were not mentioned.

(*c*) *Tobacco plus lime*

Jafarey *et al.* (1977) reported a hospital-based case-control study in Pakistan. The cases were 1192 histologically-diagnosed oral-cavity and oropharyngeal cancers. Controls (3562) were matched for age, sex and place of birth. Among men, 4% (27/683) of cases and 3% (60/1978) of controls, and among women, 7.7% (39/509) of cases and 3% (48/1584) of controls chewed tobacco, giving relative risks of 10.4 and 13.7, respectively, compared to those who neither chewed nor smoked. For further details of this study see p. 108. [The Working Group considered that although the habit in this study is reported as 'tobacco' chewing, in view of other publications by the same authors, it is likely to have been chewing of tobacco and lime.]

Chandra (1962) selected 450 cases of cancer of the buccal mucosa registered in a hospital in Calcutta, India, during 1955-1959, and used 500 of the friends or relatives who came to hospital with the patients as controls. Cases and controls were approximately age matched. Tobacco chewing was reported by 6.3% (18/287) of cases and 4.2% (17/410) of controls among men and 3.1% (5/163) of cases and 2.2% (2/90) of controls among women. For further details see the monograph on betel-quid and areca-nut chewing (p. 177). [Relative risks calculated by the Working Group from the data for tobacco chewing and for no chewing or smoking were 2.7 for males and 2.5 for females. The author did not clarify whether the chewing habit was tobacco only or tobacco plus lime.]

[In addition to these two studies, which directly examined the habit of 'tobacco-and-lime' chewing, some indirect epidemiological evidence is available from various studies detailed in the monograph on betel-quid and areca-nut chewing. In those studies (Wahi *et al.*, 1965; Wahi, 1968; Jussawalla & Deshpande, 1971; see also Tables 17-20, 23, 24 of the monograph on betel-quid and areca-nut chewing, pp. 175-176, 180) in which cancer risks were studied in relation to unspecified habits of betel-tobacco-lime chewing, it is almost certain that the predominant habit within the study populations was tobacco-lime chewing *without* betel. Therefore, at least part of the increased cancer risk reported in those studies is reasonably attributable to tobacco-lime chewing *per se*.]

(*d*) *Tobacco plus lime plus other components*

The first mention in the literature of a possible association between the use of *nass* and oral cancer goes back to 1910, by Petrov (quoted in Shilovtsev, 1941). In 1929, the cancer-notification form developed for the Samarkand region included a question on the *nass* habit, and in 1936 a campaign against the use of *nass* was organized (Shilovtsev, 1941).

(i) *Descriptive studies and case series*

Early reports on a possible association between the use of *nass* and oral cancer are based on clinical observations. Four cases of oral cancer were observed by Borovsky in 1924 among 11 cases of cancer in the Uzbek SSR; 50 cases of oral cancer in *nass* users were described by Kasansky in 1935 in the Turkmenian SSR (quoted in Shilovtsev, 1941). Of 59 cancer cases diagnosed in 1935-1938 in Samarkand among the native population, 39 (66%) were cancers of the mouth; none of the 139 cancer patients of other ethnic groups had oral cancer. *Nass* use is frequent among the native population in Samarkand (Shilovtsev, 1941).

Sharipov (1965) reported that, out of 250 patients with oral cancer diagnosed in Samarkand, 233 were Uzbeks, of whom 203 (87%) used *nass*. Khasanov (1965) reported that of 133 Uzbek patients with oral cancer, 94 (71%) used *nass*.

According to Khasanov (1965), the most frequent sites of oral cancer in people in the Samarkand region are the floor of the mouth (25%) and tongue (48%), sites which are in direct contact with *nass*. In people in the Kazakh SSR, where *nass* is more frequently placed in the lower lip groove, the sites found chiefly to be affected are the gum, buccal-mucous membrane, lip and anterior tongue (Paches & Milievskaya, 1980).

The age-standardized incidence rate for oral cancer in the Uzbek SSR (an area where *nass* use is common) is 2.3 per 100 000, whereas the same figures for two republics where *nass* is not used are 0.9 and 0.4, respectively (Paches & Milievskaya, 1980).

In Afghanistan, only 2.0% of all cancers were of the oral cavity, although the habit of chewing *naswar* is frequent (Sobin, 1969).

(ii) *Analytical studies*

One case of oral cancer was reported among 289 *nass* users in the Kazakh SSR who underwent oral examination; no oral cancer was seen in 243 smokers or in 1480 persons who neither smoked nor used *nass* (Aleksandrova, 1970).

Nugmanov and Bainakanov (1970) carried out a study in the Kazakh SSR in which the habits of oral-cancer patients were compared with the habits of controls in relation to use of *nass*. Of 93 oral-cancer patients, 30.1% used *nass* while only 6.7% of 247 controls did so. Further comparisons, involving 28 *nass* users with oral cancer and 19 *nass*-using controls, revealed that patients with oral cancer used *nass* more frequently and kept it in the mouth longer than controls (Table 37). [The Working Group noted that the sources of cases and controls were not reported; confounding due to other tobacco-related habits was not adjusted for; and no adequate statistical analysis was performed.]

Jafarey *et al.* (1977) found that 35 oral-cancer patients and 33 controls in Pakistan used *nass*, 84 patients and 114 controls used *naswar*, and 88 patients and 1690 controls had no tobacco habit. The relative risk for oral cancer associated with *nass* use was thus 20.4,

Table 37. Oral cancer in *nass* users[a]

Frequency of daily use of nass	Cases (28) %	Controls (19) %
<2 times	3.6	21.0
3-5 times	28.6	57.4
6-10 times	21.4	15.8
>10 times	46.4	15.8

[a]From Nugmanov and Bainakanov (1970)

and that associated with *naswar* use was 14.2. [The Working Group noted that confounding due to other tobacco-related habits was not adjusted for.]

Nasal use

The first reference to nasal use of tobacco as a cause of cancer comes from Hill in 1761, who described nasal cancer in two of his patients in London, which he ascribed to heavy snuff inhaling (Redmond, 1970).

(*a*) *Descriptive studies and case series*

Of the 86 Bantu patients with respiratory cancer seen from 1949 to 1954 in a radiation department in Johannesburg, South Africa, 46 (54%) had nasal-cavity and sinus cancers, which were predominantly well-differentiated squamous-cell carcinomas of the maxillary antrum. The cases were drawn from a population of about 2 000 000, and the authors estimated that about 25% of cases reached a hospital. The authors compared this proportion with the 5% frequency of nasal cancer in previously published series of Europeans with respiratory-tract cancer. [The Working Group noted that the rate of 86/2 000 000 or 4.3/100 000 compares with the age-adjusted incidence rates for 1956-1959 of 1.3/100 000 in male Bantus, 1.1/100 000 in whites and 2.0/100 000 in cape coloureds (Doll *et al*., 1970.]. The authors noted that cigarette smoking is uncommon in the area, but snuff inhalation is widely practised by both Bantu men and women for whom its use has an important cultural and ritual history. The product typically contains tobacco leaves and an ash from aloe plants or other species, with the occasional addition of oil, lemon juice and herbs (Keen *et al*., 1955); use is often 'one teaspoonful' per day (Baumslag *et al*., 1971).

Higginson and Oettlé (1960) conducted a large incidence and mortality survey of cancer and a case-control study among South African Bantus. Cases were found through hospital records, death certificates and private doctors, and census data provided the denominators for the rates. They observed that the incidence of oral cavity cancer is similar for Bantus in South Africa and for US blacks, but that paranasal-sinus cancer occurs at far greater frequency in Africa than in the USA (31 observed, compared with <1 expected for US blacks or whites). Use of snuff both orally and nasally is common among Bantus (19% in men, 30% in women), on the basis of a small survey in one town. In rural hospitals, 21% of men and 37% of women used it nasally. In sinus cancer patients, 43% of seven men used snuff, a proportion higher than that found for patients with cancer of the mouth (0), lung (9%) or oesophagus (7%), or for hospital controls (6% young, 21% older men) and the surveyed population (4% young, 15% older men). [The Working Group noted that it is not always clear whether the snuff was taken nasally or orally.]

Hou-Jensen (1964) described 97 cases of cancer of the postnasal space (nasopharynx) ascertained from hospital and medical laboratory records in Kenya during 1957-1962. On the basis of these figures and census data, one tribe in particular, the Nandi, was found to have a moderately raised incidence of nasopharyngeal cancer, 1.43 per 100 000 per year, in contrast to an incidence of 0.52/100 000 or less for other tribes. The total incidence of other oral, nasal, pharyngeal and laryngeal cancers taken together did not show a high rate of occurrence in the Nandi. Six of 12 patients still alive had been snuff users and one had chewed tobacco. The author noted that snuff inhaling is common among many tribes in East Africa, but that the Nandis use 'liquid' snuff, which is not further described. [The Working Group noted that although the incidences in this report are high, they are based on small numbers and might have been due to over-representation of cases from the Nandi tribe.]

(*b*) *Analytical studies*

In a case-control study, Shapiro *et al.* (1955) reported that cancer of the paranasal sinuses (22 in men, five in women) accounts for a high proportion of respiratory-tract cancer (31 in men, six in women) (71% for men, 83% for women) in Bantu Africans, on the basis of radiation therapy department records from 1949-1951 of 37 Bantu cases from a group of hospitals in Johannesburg, South Africa. This was in sharp contrast to European cases seen in the Transvaal, where only 5% of respiratory-tract cancers occurred in the nasal sinuses. Most of the cancers were in the maxillary antrum (28/34 studied) and were described typically as well-differentiated squamous-cell 'epitheliomata'. The authors noted that 80% (22/28) of antral cancer cases reported 'prolonged and heavy' use of snuff [probably of the same composition as that described by Keen *et al.*, 1955] in contrast to only 34% in Bantu men with cancer at other sites. The authors stated that 'there was no obvious correlation between antral cancer and cigarette, pipe or *dagga* [marijuana] smoking.' [The Working Group noted that the source and nature of the control group is not described. There may be some overlap in the cases in this article with those in the study of Keen *et al.*, 1955.]

A case-control study of oral, pharyngeal and oesophageal cancer in South India was conducted by Shanta and Krishnamurthi (1963). Controls were drawn from a 'non-tumorous population' attending fairs and general health clinics. None of the controls used snuff, but some patients with cancers under study did (probably intranasally). This was especially evident for cancer of the oesophagus: 12.2% of male and 11.1% of female patients practised the habit; for those with cancer of the hypopharynx, 11.1% of men and 8.3% of women used snuff; and for cancer of the oropharynx, tonsils and epilarynx, 9% and 4.4% of men and women, respectively, took snuff. [The Working Group noted that this habit may have been spuriously related to cancer risk because of correlations with other risk factors for the diseases studied, namely, areca-nut and tobacco chewing, and smoking.]

Snuff inhalation was reported in a study from Ahmedabad, India. Out of 57 518 industrial workers examined, 1316 or (2.3%) used tobacco only in the form of snuff inhalation. No oral cancer was found in either a cross-sectional (Smith *et al.*, 1975) or follow-up study of this population (Bhargava *et al.*, 1975).

[The Working Group noted that the composition of the snuff used was not described in any of these studies.]

In three studies on wood-dust exposure (see IARC, 1981) and tumours of the nasal cavity and sinuses, enquiry was made into the use of snuff in order to assess its possible confounding role. Acheson *et al.* (1968) reported that three out of 11 furniture workers who had developed nasal adenocarcinomas and for whom the appropriate history could be elicited

had ever taken snuff, probably by inhalation. Andersen *et al.* (1977) reported that none of the cases of tumours of the nasal cavity and sinuses treated in a major Danish hospital during a 10-year period had used snuff.

Engzell *et al.* (1978) reported that the smoking and snuff habits of cases of carcinoma of the nose and paranasal sinuses reported to the cancer registry of the National Board of Health and Welfare in Sweden between 1961 and 1971 were no different from those found in a general survey. The report does not distinguish between the different ways of administering snuff.

4. Summary of Data Reported and Evaluation

4.1 Exposure data

Smokeless-tobacco habits are practised by many millions of people, principally in Africa, Asia, Europe and North America, utilizing several techniques, products and dosage levels. In some countries, average consumption by users is estimated to be about 5 kg per year.

Among the thousands of compounds present in tobacco, the tobacco-specific nitrosamines are the only identified carcinogens that occur in mg/kg concentrations. Low levels (μg/kg) of carcinogenic polynuclear aromatic hydrocarbons and metals and of the α-emitting ^{210}Po (0.1-1.0 pCi/g) have also been detected. Use of smokeless tobacco entails extensive exposure to relatively high levels of tobacco-specific nitrosamines.

4.2 Experimental data

Various chewing tobaccos and unburnt cigarette tobaccos and their extracts were tested by oral administration in mice, by topical application to the oral mucosa of mice, rats and hamsters, and by subcutaneous administration, skin application, inhalation, intravesicular implantation and intravaginal application to mice. All of these studies suffered from certain deficiencies.

In a two-stage, mouse-skin assay, applications of tobacco extract followed by promotion by croton oil induced papillomas and squamous-cell carcinomas of the skin. In further two-stage, mouse-skin assays, application of tobacco extracts following initiation by 7,12-dimethylbenz[*a*]anthracene resulted in papillomas.

A commercial Swedish snuff was tested for carcinogenicity in rats, by topical administration in a surgically-created oral canal, alone or in combination with herpes simplex virus type 1 infection. Two squamous-cell carcinomas of the oral cavity were observed in the group receiving both treatments, but this result was not statistically significant.

Snuff was tested by oral administration in hamsters, alone and in combination with calcium hydroxide, but the data were insufficient for evaluation. Several studies in hamsters in which snuff was administered as single or repeated applications into the cheek pouch or fed in the diet yielded insufficient data for evaluation.

Subcutaneous injection of ethanol extracts of snuff to rats did not produce an increase in tumour incidence.

Nass was tested for carcinogenicity in hamsters by administration into the cheek pouch or by skin application. No tumour was found at the site of application. Although *nass* was associated with an apparent excess of liver tumours in various groups receiving cheek-pouch administrations, which may be indicative of carcinogenic activity, deficiencies in reporting do not allow an evaluation to be made.

Ethanol extracts of chewing tobacco (*Nicotiana tabacum*) induce mutations in *Salmonella typhimurium* and in Chinese hamster V79 cells. They also induce micronuclei in bone-marrow cells of Swiss mice.

Ethyl acetate extracts of a chewing tobacco induce sister chromatid exhanges in cultured human lymphocytes and in a human lymphoblastoid cell line. Ethyl acetate and ethanol extracts of this tobacco induce transformation in Syrian hamster embryo cells.

Aqueous extracts of *nass* and *khaini* induce chromosomal aberrations in Chinese hamster ovary cells.

Saliva collected during the chewing of an Indian tobacco induced chromosomal aberrations in Chinese hamster ovary cells.

An increased proportion of micronucleated cells was found in exfoliated oral-mucosa cells from users of *khaini* and *nass*.

Sister chromatid exchanges are induced in Chinese hamster ovary cells by anatabine, nicotine and nornicotine.

Overall assessment of data from short-term tests: Ethanol extracts of (*Nicotiana tabacum*) chewing tobacco[a]

	Genetic activity			Cell transformation
	DNA damage	Mutation	Chromosomal effects	
Prokaryotes		+		
Fungi/green plants				
Insects				
Mammalian cells (*in vitro*)		+		
Mammals (*in vivo*)			+	
Humans (*in vivo*)				
Degree of evidence in short-term tests for genetic activity: *Sufficient*				Cell transformation: No data

[a]The groups into which the table is divided and the symbols used are defined on pp. 16-17 of the Preamble; the degrees of evidence are defined on p. 18.

Overall assessment of data from short-term tests: Ethyl acetate extracts of a chewing tobacco[a]

	Genetic activity			Cell transformation
	DNA damage	Mutation	Chromosomal effects	
Prokaryotes				
Fungi/green plants				
Insects				
Mammalian cells (*in vitro*)			+	+[b]
Mammals (*in vivo*)				
Humans (*in vivo*)				
Degree of evidence in short-term tests for genetic activity: *Inadequate*				Cell transformation: Positive

[a]The groups into which the table is divided and the symbols used are defined on pp. 16-17 of the Preamble; the degrees of evidence are defined on p. 18.

[b]An ethanol extract of this chewing tobacco also gave positive results.

4.3 Human data

Oral leukoplakia, a precancerous lesion, has been associated with oral-snuff use in a number of studies. One study of *shammah* users and several studies of *nass* users showed the same association.

Epidemiological studies of cancer and the oral use of smokeless tobacco in western populations have often not distinguished between tobacco chewing and snuff usage. Studies that have are summarized first.

Chewing tobacco

Reports of series of oral-cancer patients indicate that a high proportion were tobacco chewers and that the cancer often developed at the site at which the quid was placed habitually. However, data on chewing tobacco often came only from medical records; coexistent smoking habits often were not mentioned.

In two of five case-control studies in which data on tobacco use were appropriately obtained, the proportion of tobacco chewers among patients with cancer of the oral cavity, pharynx or larynx was two to three times higher than in control subjects; however, confounding by tobacco smoking or alcohol consumption could not be excluded. A large study of oral, pharyngeal and oesophageal cancer reported no difference in chewing-tobacco use between cases and controls; although the relative risk of having cancer of the oral cavity or pharynx was increased in tobacco chewers, this study is not convincing because of major

discrepancies in the tabulated data. Data on dose-response are lacking in all three studies. The other two case-control studies provide no clear evidence that tobacco chewing is associated with oral cancer: one study was very large but did not control for smoking, and one had serious methodological limitations.

Results from the four case-control studies of chewing-tobacco use and cancer of the oesophagus tend to show a slight increase in incidence. Nose and nasal-sinus cancers were found to be unrelated to tobacco chewing in one case-control study. No association between chewing tobacco and bladder cancer was observed in five case-control studies.

No cohort study of chewing tobacco alone and cancer has been reported.

Oral snuff

Reports of case series indicate that a high proportion of oral-cancer patients took snuff orally, and that the cancer frequently developed at the site of snuff application.

Four case-control studies, three from the south-eastern USA and one from Scandinavia, have implicated snuff use in the etiology of cancer of the oral cavity and, to a lesser extent, of the pharynx. In three of these studies, relative risks could not be computed; however, the differences in snuff usage between cases and controls were substantial, and confounding by cigarette smoking could be largely excluded. In the fourth study, in the south-eastern USA, the relative risk of oral and pharyngeal cancer for white women who used snuff but did not smoke was four times that for women with no tobacco habit; a strong dose-response relationship was observed; adjustment for other risk factors did not substantially reduce the relative risks.

In a cohort study of snuff users with non-malignant oral lesions, none developed cancer; however, the study was inadequately reported, had methodological limitations, and therefore could not be satisfactorily interpreted.

One case-control study has suggested that oral use of snuff may be associated with certain types of nasal-sinus cancer; in other case-control studies, no association was evident between snuff use and bladder cancer or between snuff use and cancer of the oesophagus.

Smokeless tobacco, unspecified

Studies that have not distinguished snuff from chewing tobacco are informative for four reasons when considered in conjunction with the habit-specific studies summarized above. First, reports of three case series confirm the high relative frequency of smokeless-tobacco use in oral-cancer patients. Four case-control studies have reported smokeless-tobacco use to be moderately to strongly associated with oral cancer, although smoking habits were not controlled for in three of the studies.

Second, a dose-response relationship was found in one large case-control study. The relative risks for oral cancer in men, after adjustment for other risk factors, ranged from four-fold for moderate smokeless-tobacco use to more than six-fold for heavy use.

Third, two cohort mortality studies, in which large numbers of persons with and without unspecified smokeless-tobacco habits were followed, provide evidence of a positive association with cancer. There was a two- to three-fold increased risk of death from oral, pharyngeal and oesophageal cancer in one study and from oesophageal cancer in the second.

Fourth, studies of unspecified smokeless-tobacco use provide some evidence of an increased risk of cancers at sites outside the upper digestive and respiratory tracts.

Whereas the data summmarized above all come from studies in North America and western Europe, the data below refer to studies of oral use of tobacco and nasal use of snuff in South-East Asia and in Africa.

Mishri/gudakhu

Oral cancer in users of *mishri* and *gudakhu* has been studied only in prevalence surveys; no case was found.

Shammah

Oral cancers were seen in users of *shammah*.

Tobacco plus lime (khaini)

Two large case control-studies, from Pakistan and India, reported two-fold to 14-fold increases in the risk of oral-cancer occurrence in tobacco (presumably tobacco-lime) users relative to non-users, in smokers and nonsmokers considered separately. Indirect evidence, deducible from various other studies of chewing and oral cancer in which the predominant habit entailed use of tobacco and lime without areca nut, corroborates the existence of this increased cancer risk.

Tobacco plus lime plus other components

In two case series, the majority of oral-cancer patients used *nass*; in another, the cancers were found to develop at the site at which the quid was placed habitually. Two case-control studies showed five-fold to 20-fold increases in the risk of oral cancer in association with *nass* use in the USSR; however, adjustment was not made for smoking habits and other potential confounders.

Use of *naswar*, examined in one case-control study in Pakistan, was associated with a marked increase in oral-cancer risk; however, positive confounding by tobacco smoking and betel-quid chewing could not be eliminated.

Nasal snuff

Two case-control studies among Bantu subpopulations in Africa, among whom nasal and oral use of indigenous snuff (containing tobacco and other ingredients, including aloe) are common, showed a moderately elevated risk of nasal-sinus cancer in relation to this habit; however, the studies had severe methodological limitations.

In India, two studies (one cross-sectional, one prospective) of oral cancer found no association between oral cancer and snuff inhaling. A case-control study reported snuff inhaling to be more common among patients with cancers of the oesophagus, hypopharynx or oropharynx than among controls; however, adjustment was not made for other risk factors for these cancers.

No study was available that specifically addressed the possible carcinogenicity of nasal use of snuff formulated in North America or western Europe.

4.4 Evaluation[1]

There is *sufficient evidence* that oral use of snuffs of the types commonly used in North America and western Europe is carcinogenic to humans. There is *limited evidence* that chewing tobacco of the types commonly used in these areas is carcinogenic.

Epidemiological studies that did not distinguish between chewing tobacco and snuff provide *sufficient evidence* for the carcinogenicity of oral use of smokeless-tobacco products, as reported in these studies.

In aggregate, there is *sufficient evidence* that oral use of smokeless tobacco of the above types is carcinogenic to humans.

There is *sufficient evidence* that oral use of tobacco mixed with lime (*khaini*) is carcinogenic to humans.

There is *inadequate evidence* that oral use of the other smokeless-tobacco preparations considered (*nass*, *naswar*, *mishri*, *gudakhu* and *shammah*) is carcinogenic to humans.

There is *inadequate evidence* that nasal use of snuff is carcinogenic to humans.

There is *inadequate evidence* to evaluate the carcinogenicity of chewing tobacco, snuff or *nass* to experimental animals.

5. References

Abbe, R. (1915) Cancer of the mouth: The case against tobacco. *N.Y. med. J.*, *102*, 1-2

Abraham, S.K., Goswami, V. & Kesavan, P.C. (1979) A preliminary assessment of possible mutagenicity of betel nut and ingredients of the betel quid when administered alone or in combinations to larvae of *Drosophila melanogaster*. *Mutat. Res.*, *66*, 261-266

Acheson, E.D., Cowdell, R.H., Hadfield, E. & Macbeth, R.G. (1968) Nasal cancer in woodworkers in the furniture industry. *Br. med. J.*, *ii*, 587-596

Ackerman, L.V. (1948) Verrucous carcinoma of the oral cavity. *Surgery*, *23*, 670-678

Agrawal, P., Chansoriya, M. & Kaul, K.K. (1983) Effect of tobacco chewing by mothers on placental morphology. *Indian Ped.*, *20*, 561-565

Ahlbom, H.E. (1937) Predisposing factors for squamous-cell epitheliomas of the oral cavity, larynx, pharynx and oesophagus. A statistical examination of data of the Radiumhemmet, Stockholm (Ger.). *Acta radiol.*, *18*, 163-185

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[1]For definitions of the italicized terms, see Preamble, pp. 15-16 and 19.

Ahmad, S.M. (1976) Tobacco production and consumption trends in Pakistan. *Tabac J. int.*, *4*, 325

Akehurst, B.C. (1981) *Tobacco*, 2nd ed., New York, Longman, pp. 641-642

Aleksandrova, H.M. (1970) *Role of nass in the development of pathological changes in the mouth* (Russ.). In: *Epidemiology of Malignant Tumours*, Alma-Ata, Nauka Publishing House, pp. 243-246

Andersen, H.C., Andersen, I. & Solgaard, J. (1977) Nasal cancers, symptoms and upper airway function in woodworkers. *Br. J. ind. Med.*, *34*, 201-207

Anon. (1953) *Report on Marketing of Tobacco in India, Agricultural Marketing in India*, pp. 236-237

Anon. (1978) Statistics. *Tabak-J. int.*, *6*, 421-424

Anon. (1979) Statistics. Production of smoking tobacco in kg — for certain selected countries. *Tabak-J. int.*, *6*, 471-472

Anon. (1981) Snuff-making blends the new and the traditional. *World Tobacco*, December, pp. 57-58

Anon. (1983) Boys in Atlanta go for snuff and chewing tobacco. *Chem. Eng. News*, *61*, 56

Anon. (1984) *Progressive Grocer*, September, p. 124

Archard, H.O. & Tarpley, T.M., Jr (1972) Clinicopathologic and histochemical characterization of submucosal deposits in snuff dipper's keratosis. *J. oral Pathol.*, *1*, 3-11

Axéll, T. (1976) A prevalence study of oral mucosal lesions in an adult Swedish population. *Odontol. Rev.*, *27*, Suppl. 36

Axéll, T. (1979) *Sniffing and its adverse effects*. In: *Tobacco Smoking or Health. Adverse Medical Effects of Tobacco Smoking*, Stockholm, Liber Förlag, pp. 72-79

Axéll, T., Mörnstad, H. & Sundström, B. (1976) The relation of the clinical picture to the histopathology of snuff dipper's lesions in a Swedish population. *J. oral Pathol.*, *5*, 229-236

Axéll, T., Mörnstad, H. & Sundström, B. (1978) Snuff and cancer of the oral cavity — A retrospective study (Swed.). *Läkartidningen*, *75*, 2224-2226

Axéll, T., Holmstrup, P., Kramer, I.R.A., Pindborg, J.J. & Shear, M. (1984) International seminar on oral leukoplakia and associated lesions related to tobacco habits. *Commun. Dent. oral Epidemiol.*, *12*, 146-154

Axton, W.F. (1975) *Tobacco and Kentucky*, Lexington, KE, University Press of Kentucky, pp. 8, 25, 58-59

Balfour, D.J.K., ed. (1984) *International Encyclopedia of Pharmacology and Therapeutics*, Section 114, *Nicotine and the Tobacco Smoking Habit*, Oxford, Pergamon Press

Barroll, A. & Ramström, L.M. (1979) *Tobacco habits in Sweden*. In: *Tobacco Smoking and Health. Adverse Effects of Tobacco Smoking*, Stockholm, Liber Förlag, pp. 12-29

Baumslag, N., Keen, P. & Petering, H.G. (1971) Carcinoma of the maxillary antrum and its relationship to trace metal content of snuff. *Arch. environ. Health*, *23*, 1-5

Bhargava, K., Smith, L.W., Mani, N.J., Silverman, S., Jr, Malaowalla, A.M. & Bilimoria, K.F. (1975) A follow up study of oral cancer and precancerous lesions in 57,518 industrial workers of Gujarat India. *Indian J. Cancer*, *12*, 124-129

Bhide, S.V., Murdia, U.S. & Nair, J. (1984a) Polycyclic aromatic hydrocarbon profiles of pyrolysed tobacco products commonly used in India. *Cancer Lett.*, *24*, 89-94

Bhide, S.V., Shah, A.S., Nair, J. & Nagarajrao, D. (1984b) *Epidemiological and experimental studies on tobacco related oral cancer in India*. In: O'Neill, I.K., von Borstel, R.C., Miller, C.T., Long, J. & Bartsch, H., eds, N-*Nitroso Compounds: Occurrence, Biological Effects and Relevance to Human Cancer* (*IARC Scientific Publications No. 57*), Lyon, International Agency for Research on Cancer, pp. 851-857

Bhonsle, R.B., Murti, P.R., Daftary, D.K. & Mehta, F.S. (1979) An oral lesion in tobacco-lime users in Maharashtra, India. *Indian J. oral Pathol.*, *8*, 47-52

Bjelke, E. & Schuman, L.M. (1982) *Chewing of tobacco and use of snuff: Relationship to cancer of the pancreas and other sites in two prospective studies*. In: *Proceedings of the XIIIth International Cancer Congress, Seattle, Washington* (Abstract No. 1172), p. 207

Blot, W.J., Winn, D.M. & Fraumeni, J.F., Jr (1983) Oral cancer and mouthwash. *J. natl Cancer Inst.*, *70*, 251-253

Bock, F.G., Moore, G.E. & Crouch, S.K. (1964) Tumor-promoting activity of extracts of unburned tobacco. *Science*, *145*, 831-833

Bock, F.G., Shamberger, R.J. & Myers, H.K. (1965) Tumour-promoting agents in unburned cigarette tobacco. *Nature*, *208*, 584-585

Bordia, A., Purbiya, S.L., Khabya, B.L., Arora, S.K., Hatimi, I.H. & Singh, S.V. (1977) A comparative study of common modes of tobacco use on pulse, blood pressure, electrocardiogram and blood coaguability in patients with coronary artery disease. *J. Assoc. Phys. India*, *25*, 395-401

Brinton, L.A., Blot, W.J., Becker, J.A., Winn, D.M., Browder, J.P., Farmer, J.C., Jr & Fraumeni, J.F., Jr (1984) A case-control study of cancers of the nasal cavity and paranasal sinuses. *Am. J. Epidemiol.*, *119*, 896-906

Brooks, J.E. (1952) *The Mighty Leaf. Tobacco Through the Centuries*, Boston, MA, Little, Brown & Co., pp. 109, 213, 277

Brown, R.L., Suh, J.M., Scarborough, J.E., Wilkins, S.A., Jr & Smith, R.R. (1965) Snuff dippers' intraoral cancer: Clinical characteristics and response to therapy. *Cancer*, *18*, 2-13

Browne, R.M., Camsey, M.C., Waterhouse, J.A.H. & Manning, G.L. (1977) Etiological factors in oral squamous cell carcinoma. *Commun. Dent. oral Epidemiol.*, *5*, 301-306

Brunnemann, K.D. & Hoffmann, D. (1981) Assessment of carcinogenic *N*-nitrosodiethanolamine in tobacco products and tobacco smoke. *Carcinogenesis*, *2*, 1123-1127

Brunnemann, K.D., Scott, J.D. & Hoffmann, D. (1982) *N*-Nitrosomorpholine and other volatile *N*-nitrosamines in snuff tobacco. *Carcinogenesis*, *3*, 693-696

Brunnemann, K.D., Scott, J.C. & Hoffmann, D. (1983) *N*-Nitrosoproline an indicator for *N*-nitrosation of amines in processed tobacco. *J. agric. Food Chem.*, *31*, 905-909

Campbell, J.M. & Lindsey, A.J. (1956) Polycyclic hydrocarbons extracted from tobacco: The effect upon total quantities found in smoking. *Br. J. Cancer*, *10*, 649-652

Campbell, J.M. & Lindsey, A.J. (1957) Polycyclic aromatic hydrocarbons in snuff. *Chem. Ind.*, 6 July, p. 951

Chakarvarti, S.H., Dhiman, J. & Nagpaul, K.K. (1981) Uranium trace analysis of a chewable betel-leaf preparation and tea leaves. *Health Phys.*, *40*, 78-81

Chandra, A. (1962) Different habits and their relation with cancer cheek. *Chittaranjan Cancer Hosp. Calcutta natl Cancer Res. Centre Bull.*, 33-36

Chopra, N.M., Verma, M.M. & Zuniga, T.H. (1982) On the fate of maleic hydrazide in tobacco smoke. *J. agric. Food Chem.*, *30*, 672-676

Chortyk, O.T., Severson, R.F. & Higman, H.C. (1975) Chromatographic determination of hydrocarbon waxes in tobacco leaf and smoke. *Beitr. Tabakforsch.*, *8*, 204-210

Christen, A.G. (1980a) The case against smokeless tobacco: Five facts for the health professional to consider. *J. Am. dent. Assoc.*, *101*, 464-469

Christen, A.G. (1980b) Tobacco chewing and snuff dipping. *New Engl. J. Med.*, *302*, 818

Christen, A.G. & Glover, E.D. (1981) Smokeless tobacco: Seduction of youth. *World Smoking Health*, *6*, 20-24

Christen, A.G., McDaniel, R.K. & Doran, J.E. (1979a) Snuff dipping and tobacco chewing in a group of Texas college athletes. *Texas dent. J.*, *97*, 6-10

Christen, A.G., Armstrong, W.R. & McDaniel, R.K. (1979b) Intraoral leukoplakia, abrasion, periodontal breakdown, and tooth loss in a snuff dipper. *J. Am. dent. Assoc.*, *98*, 584-586

Christen, A.G., Swanson, B.Z., Glover, E.D. & Henderson, A.H. (1982) Smokeless tobacco: The folklore and social history of snuffing, sneezing, dipping, and chewing. *J. Am. dent. Assoc.*, *105*, 821-829

Cogbill, E.C. & Hobbs, M.E. (1967) Transfer of metallic constituents of cigarettes to the main-stream smoke. *Tobacco Sci.*, *1*, 68-73

Cole, P., Monson, R.R., Haning, H. & Friedell, G.H. (1971) Smoking and cancer of the lower urinary tract. *New Engl. J. Med.*, *284*, 129-134

Coleman, C.C., Jr (1965) Surgical treatment of extensive cancers of the mouth and pharynx. *Ann. Surg.*, *161*, 634-644

Cooper, R.L. & Campbell, J.M. (1955) The aetiology of respiratory tract cancer in the South African Bantu. Part II. Chemical identification of carcinogens. *Br. J. Cancer*, *9*, 533-538

Crowe, M.W. (1978) *Tobacco — A cause of congenital arthrogryposis*. In: Keeler, R.F., Van Kampen, K.R. & James, L.F., eds, *Effects of Poisonous Plants on Livestock*, New York, Academic Press, pp. 419-427

Curtis, M.M. (1935) *The Book of Snuff and Snuff Boxes*, New York, Bramhall House, pp. 20-27, 67

Davis, D.L. (1976) Waxes and lipids in leaf and their relationship to smoking quality and aroma. *Rec. Adv. Tobacco Sci.*, *2*, 80-111

Davis, D.L., Song, B.H. & Weeks, W.W. (1981) *Chemical composition of dark air-cured and fire-cured tobacco*. In: *35th Tobacco Chemists Research Conference, Winston Salem, NC, USA, October 7-9, 1981*, p. 48

Demole, E. & Enggist, P. (1976) A chemical study of *Virginia* tobacco flavour (*Nicotiana tabacum* L.) 1. Isolation and synthesis of two bicyclodamascenones. *Helv. chim. Acta*, *59*, 1938-1943

Doll, R., Muir, C. & Waterhouse, J., eds (1970) *Cancer Incidence in Five Continents*, Vol. II, New York, Springer-Verlag, p. 354

Dube, M.F. & Green, C.R. (1982) Methods of collection of smoke for analytical purposes. *Rec. Adv. Tobacco Sci.*, *8*, 42-102

Dunham, L.J. & Herrold, K.M. (1962) Failure to produce tumors in the hamster cheek pouch by exposure to ingredients of betel quid; histopathologic changes in the pouch and other organs by exposure to known carcinogens. *J. natl Cancer Inst.*, *29*, 1047-1067

Dunham, L.J., Muir, C.S. & Hamner, J.E., III (1966) Epithelial atypia in hamster cheek pouches treated repeatedly with calcium hydroxide. *Br. J. Cancer*, *20*, 588-593

Dunham, L.J., Sheets, R.H. & Morton, J.F. (1974) Proliferative lesions in cheek pouch and esophagus of hamsters treated with plants from Curacao, Netherland Antilles. *J. natl Cancer Inst.*, *53*, 1259-1269

Dunham, L.J., Snell, K.C. & Stewart, H.L. (1975) Argyrophilic carcinoids in two Syrian hamsters (*Mesocricetus auratus*). *J. natl Cancer Inst.*, *54*, 507-513

Dunn, W.I. & Dykstra, C.L. (1967) Tobacco habits in carcinoma of the upper respiratory tract. *Arch. Otolaryngol., 86*, 105-107

Dunn, W.I. & Farrior, R.T. (1962) Tobacco habits in oral and upper respiratory carcinoma: A review of 112 cases. *Arch. Otolaryngol., 75*, 245-247

Engzell, U., Englund, A. & Westerholm, P. (1978) Nasal cancer associated with occupational exposure to organic dust. *Acta otolaryngol., 86*, 437-442

Enzell, C.R. & Wahlberg, I. (1980) Leaf composition in relation to smoking quality and aroma. *Rec. Adv. Tobacco Sci., 6*, 64-122

Enzell, C.R., Wahlberg, I. & Aasen, A.J. (1977) *Isoprenoids and alkaloids of tobacco*. In: Herz, W., Grisebach, H. & Kirby, G.W., eds, *Progress in Chemistry of Organic Natural Products*, Vol. 34, Vienna, Springer-Verlag, pp. 1-79

Everette, G. (1958) *Harvesting, Curing and Preparing Dark-Fired Tobacco for Market*, Lexington, KY, University of Kentucky

Fonts, E.A., Greenlaw, R.H., Rush, B.F. & Rovin, S. (1969) Verrucous squamous cell carcinoma of the oral cavity. *Cancer, 23*, 152-160

Friedell, H.L. & Rosenthal, L.M. (1941) The etiologic role of chewing tobacco in cancer of the mouth. *J. Am. med. Assoc., 116*, 2130-2135

Frithiof, L., Anneroth, G., Lasson, U. & Sederholm, C. (1983) The snuff-induced lesion. A clinical and morphological study of a Swedish material. *Acta odontol. scand., 1*, 53-64

Gaede, D. (1941) Nicotine absorption from chewing tobacco (Ger.). *Naunyn-Schmiedebergs Arch. Pathol. Pharmakol., 197*, 72-77

Garner, W.W. (1951) *The Production of Tobacco*, New York, The Blakiston Company, pp. 477-478

Gennaro, A.R., Nora, A.H., Nora, J.J., Stander, R.W. & Weiss, L., eds (1979) *Blakiston's Gould Medical Dictionary*, 4th ed., New York, McGraw-Hill, p. 760

Glover, E.D., Steven, W.E. & Christen, A.G. (1983) *Smokeless tobacco: A merging concern*. In: *Proc. 5th World Conference on Smoking and Health, Winnipeg, Canada, July 10-15*, pp. 60-61

Goethals, P.L., Harrison, E.G., Jr & Devine, K.D. (1963) Verrucous squamous carcinoma of the oral cavity. *Am. J. Surg., 106*, 845-851

Goodman, A., Goodman, L.S. & Gilman, A., eds (1980) *Goodman and Gilman's The Pharmacological Basis of Therapeutics*, 6th ed., New York, McMillan

Gothoskar, S.V., Sant, S.M. & Ranadive, K.J. (1975) Effect of tobacco and lime on oral mucosa of rats fed on vitamin B deficient diet. *Indian J. Cancer, 12*, 424-429

Gottsegen, J.J. (1940) *Tobacco. A Study of Its Consumption in the United States*, New York, Pitman, p. 3

Greer, R.O., Jr & Poulson, T.C. (1983) Oral tissue alterations associated with the use of smokeless tobacco by teen-agers. Part I. Clinical findings. *Oral Surg.*, *56*, 275-284

Gritz, E.R., Baer-Weiss, V., Benowitz, N.L., Van Vunakis, H. & Jarvik, M.E. (1981) Plasma nicotine and cotinine concentrations in habitual smokeless tobacco users. *Clin. Pharmacol. Ther.*, *30*, 201-209

Guthrie, F.E. & Bowery, T.G. (1967) Pesticide residues on tobacco. *Res. Rev.*, *19*, 31-56

Hamazaki, Y. & Murao, T. (1969) On the carcinogenic factors of tobacco leaf powder. I. Inhalation of tobacco leaf powder. *J. Karyopathol. Esp. Tumor Tumorvirus, 12,* 163-178

Harley, N.H., Cohen, B.S. & Tso, T.C. (1980) *Polonium-210: A questionable risk factor in smoking-related carcinogenesis.* In: Gori, G.B. & Bock, F.G., eds, *A Safe Cigarette?* (*Banbury Report No. 3*), Cold Spring Harbor, NY, Cold Spring Harbor Laboratory, pp. 93-104

Harrison, D.F.N. (1964) Snuff - Its use and abuse. *Br. med. J.*, *ii*, 1649-1651

Hartge, P., Hoover, R. & Kantor, A. (1985) Bladder cancer risk and pipes, cigars, and smokeless tobacco. *Cancer*, *55*, 901-906

Hartselle, M.L. (1977) Oral carcinoma as related to the use of tobacco. *Alabama J. med. Sci.*, *14*, 188-194

Häusermann, M. & Waltz, P. (1962) Quick spectrometric method for determination of chlorogenic acid and rutin in tobacco (Ger.). *Beitr. Tabakforsch.*, *1*, 275-284

Hecht, S.S., Castonguay, A., Rivenson, A., Mu, B. & Hoffmann, D. (1983) Tobacco specific nitrosamines: Carcinogenicity, metabolism and possible role in human cancer. *J. environ. Sci. Health*, *1*, 1-54

Heimann, R.K. (1960) *Tobacco and Americans*, New York, McGraw-Hill, pp. 8, 65, 118-119, 236

Heuch, I., Kvåle, G., Jacobsen, B.K. & Bjelke, E. (1983) Use of alcohol, tobacco and coffee, and risk of pancreatic cancer. *Br. J. Cancer*, *48*, 637-643

Hibell, B. & Jonsson, E. (1982) *A Study of School Children's Alcohol, Narcotics, Tobacco and Chewing Habits in Spring 1981*, Stockholm, Swedish Board of School Education

Higginson, J. & Oettlé, A.G. (1960) Cancer incidence in the Bantu and 'Cape Colored' races of South Africa: Report of a cancer survey in the Transvaal (1953-55). *J. natl Cancer Inst.*, *24*, 589-671

Higman, H.C., Severson, R.F., Chortyk, O.T. & Arrendale, R.F. (1979) Pyrolytic evaluation of close-grown tobaccos. *Beitr. Tabakforsch. int.*, *10*, 65-72

Hirsch, J.-M. (1983) *Snuff-induced Lesions. A Clinical and Experimental Study*, Thesis, University of Göteborg

Hirsch, J.-M. & Johansson, S.L. (1983) Effect of long-term application of snuff on the oral mucosa — An experimental study in the rat. *J. oral Pathol.*, *12*, 187-198

Hirsch, J.-M. & Thilander, H. (1981) Snuff-induced lesions of the oral mucosa — An experimental model in the rat. *J. oral Pathol.*, *10*, 342-353

Hirsch, J.-M., Heyden, G. & Thilander, H. (1982) A clinical, histomorphological and histochemical study of snuff-induced lesions of varying severity. *J. oral Pathol.*, *11*, 387-398

Hirsch, J.-M., Johansson, S.L. & Vahlne, A. (1984a) Effect of snuff and herpes simplex virus-1 on rat oral mucosa: Possible association with development of squamous cell carcinoma. *J. oral Pathol.*, *13*, 52-62

Hirsch, J.-M., Svennerholm, B. & Vahlne, A. (1984b) Inhibition of herpes simplex virus replication by tobacco extracts. *Cancer Res.*, *44*, 1991-1997

Hoffmann, D. & Adams, J.D. (1981) Carcinogenic tobacco-specific *N*-nitrosamines in snuff and in the saliva of snuff dippers. *Cancer Res.*, *41*, 4305-4308

Hoffmann, D. & Hecht, S.S. (1985) Nicotine-derived *N*-nitrosamines and tobacco-related cancer: Current status and future directions. *Cancer Res.*, *45*, 935-944

Hoffmann, D. & Woziwodzki, H. (1968) Chemical studies in tobacco smoke. IV. Quantitative determination of free nonvolatile fatty acids in tobacco and tobacco smoke. *Beitr. Tabakforsch.*, *4*, 167-175

Hoffmann, D., Adams, J.D., Brunnemann, K.D., Rivenson, A. & Hecht, S.S. (1982) *Tobacco specific* N-*nitrosamines: Occurrence and bioassays*. In: Bartsch, H., Castegnaro, M., O'Neill, I.K., & Okada, M., eds, N-*Nitroso Compounds: Occurrence and Biological Effects* (*IARC Scientific Publications No. 41*), Lyon, International Agency for Research on Cancer, pp. 309-318

Hoffmann, D., Brunnemann, K.D., Adams, J.D. & Hecht, S.S. (1984) *Formation and analysis of* N-*nitrosamines in tobacco products and their endogenous formation in consumers*. In: O'Neill, I.K., von Borstel, R.C., Miller, C.T., Long, J. & Bartsch, H., eds, N-*Nitroso Compounds: Occurrence, Biological Effects and Relevance to Human Cancer* (*IARC Scientific Publications No. 57*), Lyon, International Agency for Research on Cancer, pp. 743-762

Holleb, A.I., ed. (1984) Smokeless tobacco and oral cancer: A cause for concern? *Can. Cancer J. Clin.*, *34*, 247

Homburger, F. (1971) Mechanical irritation, polycyclic hydrocarbons, and snuff. Effects on facial skin, cheek pouch, and oral mucosa in Syrian hamsters. *Arch. Pathol.*, *91*, 411-417

Homburger, F., Hsueh, S.-S., Russfield, A.B., Laird, C.W. & Van Dongen, C.G. (1976) Absence of carcinogenic effects of chronic feeding of snuff in inbred Syrian hamsters. *Toxicol. appl. Pharmacol.*, *35*, 515-521

Hou-Jensen, K. (1964) On the occurrence of post nasal space tumours in Kenya. *Br. J. Cancer*, *18*, 58-68

Howe, G.R., Burch, J.D., Miller, A.B., Cook, G.M., Esteve, J., Morrison, B., Gordon, P., Chambers, L.W., Fodor, G. & Winsor, G.M. (1980) Tobacco use, occupation, coffee, various nutrients, and bladder cancer. *J. natl Cancer Inst.*, *64*, 701-713

Hunter, S.M., Webber, L.S. & Berenson, G.S. (1980) Cigarette smoking and tobacco usage behavior in children and adolescents: Bogalusa Heart Study. *Prev. Med.*, *9*, 701-712

IARC (1973) *IARC Monographs on the Evaluation of Carcinogenic Risk of Chemicals to Man*, Vol. 2, *Some Inorganic and Organometallic Compounds*, Lyon, p. 80

IARC (1976) *IARC Monographs on the Evaluation of Carcinogenic Risk of Chemicals to Man*, Vol. 11, *Cadmium, Nickel, Some Epoxides, Miscellaneous Industrial Chemicals and General Considerations on Volatile Anaesthetics*, Lyon, pp. 39-74, 75-112

IARC (1980) *IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans*, Vol. 22, *Some Non-nutritive Sweetening Agents*

IARC (1981) *IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans*, Vol. 25, *Wood, Leather and Some Associated Industries*, Lyon, pp. 99-138

IARC (1982) *IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans*, Suppl. 4, *Chemicals, Industrial Processes and Industries Associated with Cancer in Humans (IARC Monographs Volumes 1 to 29)*, Lyon, pp.167-170

IARC (1983a) *IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans*, Vol. 32, *Polynuclear Aromatic Compounds. Part 1. Chemical, Environmental and Experimental Data*, Lyon, pp. 37-38

IARC (1983b) *IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans*, Vol. 31, *Some Food Additives, Feed Additives and Naturally Occurring Substances*, Lyon, pp. 213-229

IARC (1985) *IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans*, Vol. 36, *Allyl Compounds, Aldehydes, Epoxides and Peroxides*, Lyon, pp. 75-97

International Trade Centre (1968) *The Major Markets for Unmanufactured Tobacco* (*ITC Publ. SF-43*), Geneva, pp. 72-76, 548-549

Jafarey, N.A., Mahmood, Z. & Zaidi, S.H.M. (1977) Habits and dietary pattern of cases of carcinoma of the oral cavity and oropharynx. *J. Pakistan med. Assoc.*, *27*, 340-343

Jarboe, C.H. & Quinn, A.D. (1960) Low temperature column chromatography of tobacco acids. *Tobacco Sci.*, *4*, 168-171

Joint Iran-International Agency for Research on Cancer Study Group (1977) Esophageal cancer studies in the Caspian littoral of Iran: Results of population studies. A prodrome. *J. natl Cancer Inst.*, *59*, 1127-1138

Jussawalla, D.J. & Deshpande, V.A. (1971) Evaluation of cancer risk in tobacco chewers and smokers: An epidemiologic assessment. *Cancer*, *28*, 244-252

Kaburaki, Y. & Sato, Y. (1962) Volatile acids in Shibori-chu tobacco leaf. *Sci. Papers Central Res. Inst. Jpn. Monopoly Corp.*, *105*, 285-286

Kaburaki, Y., Mikami, Y. & Nakamura, M. (1969) Studies on the composition of tobacco smoke. XIII. Volatile acids in tobacco smoke. *Sci. Papers Central Res. Inst. Jpn Monopoly Corp.*, *111*, 159-168

Kandarkar, S.V., Hasgekar, N.N. & Sirsat, S.M. (1981) Optical and ultrastructural pathology of vitamin A pretreated hamster cheek pouch exposed to lime ($Ca(OH)_2$) and tobacco over total life span. *Neoplasma*, *28*, 729-737

Keeler, R.F. (1979) Congenital defects in calves from maternal ingestion of *Nicotiana glauca* of high anabasine content. *Clin. Toxicol.*, *15*, 417-426

Keeler, R.F., Crowe, M.W. & Lambert, E.A. (1984) Teratogenicity in swine of the tobacco alkaloid anabasine isolated from *Nicotiana glauca*. *Teratology*, *30*, 61-69

Keen, P., De Moor, N.G., Shapiro, M.P. & Cohen, L. (1955) The aetiology of respiratory tract cancer in the South African Bantu. Part I. Clinical aspects. *Br. J. Cancer*, *9*, 528-533

Khasanov, T.K. (1965) *Cancer of the oral mucosa in relation to use of nass*. In: *Problems of Geographic Pathology of Oropharyngeal Tumours*, Alma-Ata, Kazakhstan Publishing House, pp. 84-87

Khasanov, T.K. & Fasiev, K.T. (1970) *Epidemiological study of oral precancer in the Bukhara region of Uzbekistan*. In: *Epidemiology of Malignant Tumours*, Alma-Ata, Nauka Publishing House, pp. 236-238

ben Khedher, A., Bouacha, H., Azzouz, L., Bourguiba, M., Ghezal, S., Hlioui, R., Mili, F. & El Mekki, L. (1984) *Chronic bronchitis in adults in hospitals (3-year study)* (Fr.). In: El Gharbi, T., ed., *Proceedings of the XIIIth Congres Medical Maghrebin, Alger — Chronic Bronchitis in Tunisia*, Ariana-Tunis, Institut A. Mamo de Pneumophtisiologie, pp. 39-54

Kiseleva, N.S., Milievskaja, I.L. & Čaklin, A.V. (1976) Development of tumours in Syrian hamsters during prolonged experimental exposure to nas. *Bull. World Health Organ.*, *54*, 597-605

Kozlowski, L.T. (1981) The determinants of tobacco use: Cigarette smoking in the context of other forms of tobacco use. *Can. J. publ. Health*, *72*, 396-401

Kraus, F.T. & Perez-Mesa, C. (1966) Verrucous carcinoma: Clinical and pathologic study of 105 cases involving oral cavity, larynx and genitalia. *Cancer*, *19*, 26-38

Krishna, K. (1978) Tobacco chewing in pregnancy. *Br. J. Obstet. Gynaecol.*, *85*, 726-728

Landauer, W. (1960) Nicotine-induced malformations of chicken embryos and their bearing on the phenocopy problem. *J. exp. Zool.*, *143*, 107-122

Landy, J.J. & White, H.J. (1961) Buccogingival carcinoma of snuff dippers. *Am. Surg.*, *27*, 442-447

Leffall, L.D., Jr & White, J.E. (1965) Cancer of the oral cavity in Negroes. *Surg. Gynecol. Obstet.*, *120*, 70-72

Lindemeyer, R.G., Baum, R.H., Hsu, S.C. & Going, R.E. (1981) In vitro effect of tobacco on the growth of oral cariogenic streptococci. *J. Am. dent. Assoc.*, *103*, 719-722

Lipp, G. (1965) Selective action of cigarette filters on nicotine and phenol as well as of tobacco type (Ger.). *Beitr. Tabakforsch.*, *3*, 109-127

Liu, Y.-Y. & Hoffmann, D. (1973) Quantitative chromatographic determination of maleic hydrazide in cigarette smoke. *Anal. Chem.*, *45*, 2270-2273

Loewe, W. (1981) *Snuff in Gothenburg* (Swed.). In: *Svenska Tobaks AB 1981*, Stockholm, pp. 19-22

Lyon, H., Poulsen, H.E. & Pindborg, J.J. (1964) Studies in oral leukoplakias. 6. Deposits of amyloid in the oral submucosa induced by prolonged use of snuff. *Acta pathol. microbiol. scand.*, *60*, 305-310

Malej, M., Bouacha, H., Ben Khedher, A., Ben Miled, H.T., Nacef, T. & El Gharbi, B. (1984) *Epidemiological aspect of chronic bronchitis in Tunisia (Inquiry in different professional areas)* (Fr.). In: El Gharbi, T., ed., *Proceedings of the XIIIth Congres Medical Maghrebin, Alger - Chronic Bronchitis in Tunisia*, Ariana-Tunis, Institut A. Mami de Pneumophtisiologie, pp. 14-16

Martinez, I. (1969) Factors associated with cancer of the esophagus, mouth, and pharynx in Puerto Rico. *J. natl Cancer Inst.*, *42*, 1069-1094

Maxwell, J.C., Jr (1980) Chewing, snuff is growth segment. *Tobacco Rep.*, *107*, 32-35

Maxwell, J.C., Jr (1982) The smokeless tobacco industry in 1982. *Tobacco int.*, *184*, 118, 122, 125, 127

Maxwell, J.C., Jr (1984) Smokeless tobacco market leveling out in the US. *Tobacco Rep.*, *111*, 66, 68

McGuirt, W.F. (1983a) Snuff dipper's carcinoma. *Arch. Otolaryngol.*, *109*, 757-760

McGuirt, W.F. (1983b) Head and neck cancer in women — A changing profile. *Laryngoscope, 93,* 106-107

Mehta, F.J. & Pindborg, J.J. (1968) *Assignment Report on the Need for a Survey on Oral Pre-cancerous Conditions in Afghanistan; Cancer Studies (Oropharyngeal Cancer)* (*WHO Project: Afhanistan 0069*), Geneva, World Health Organization

Mehta, F.S., Pindborg, J.J., Hamner, J.E., Gupta, P.C., Daftary, D.K., Sahiar, B.E., Shroff, B.C., Sanghvi, L.D., Bhonsle, R.B., Choksi, S.K., Dandekar, V.V., Mehta, Y.N., Pitkar, V.K., Sinor, P.N., Shah, N.C., Turner, P.S. & Upadhyay, S.A., eds (1971) *Report on Investigations of Oral Cancer and Precancerous Conditions in Indian Rural Populations, 1966-1969*, Copenhagen, Munksgaard, pp. 48, 107

Mehta, F.S., Gupta, P.C., Daftary, D.K., Pindborg, J.J. & Choksi, S.K. (1972) An epidemiologic study of oral cancer and precancerous conditions among 101,761 villagers in Maharashtra, India. *Int. J. Cancer*, *10*, 134-141

ben Miled, M.T., Tanabene, A., Maalej, M., Chebbi, M.L., Zakhama, B. & El Gharbi, T. (1984) *Chronic bronchopathies in rural women in Tunisia* (Fr.). In: El Gharbi, T., ed., *Proceedings of the XIIIth Congres Medical Maghrebin, Alger — Chronic Bronchitis in Tunisia*, Ariana-Tunis, Institut A. Mami de Pneumophtisiologie, pp. 17-27

Milievskaja, I.L. & Kiseleva, N.S. (1976) Comparative study of the carcinogenic activities of nas and some chemical carcinogens when introduced into the buccal pouch of the Syrian hamster. *Bull. World Health Organ.*, *54*, 607-614

Millar, W.J. & van Rensberg, S. (1984) Use of chewing tobacco and snuff by students in the Northwest Territories, 1982. *Chron. Dis. Canada*, *4*, 54-56

Modéer, T., Lavstedt, S. & Ahlund, C. (1980) Relation between tobacco consumption and oral health in Swedish schoolchildren. *Acta odontol. scand.*, *38*, 223-227

Mody, J.K. & Ranadive, K.J. (1959) Biological study of tobacco in relation to oral cancer. *Indian J. med. Sci.*, *13*, 1023-1037

Moertel, C.G. & Foss, E.L. (1958) Multicentric carcinoma of the oral cavity. *Surg. Gynecol. Obstet.*, *106*, 652-654

Mold, J.D., Stevens, R.K., Means, R.E. & Ruth, J.M. (1963) The paraffin hydrocarbons of tobacco; Normal, iso- and anteiso-homologs. *Biochemistry*, *2*, 605-610

Moore, G.E., Bissinger, L.L. & Proehl, E.C. (1952) Tobacco and intra-oral cancer. *Surg. Forum*, *3*, 685-688

Moore, G.E., Bissinger, L.L. & Proehl, E.C. (1953) Intraoral cancer and the use of chewing tobacco. *J. Am. geriatr. Soc.*, *1*, 497-506

Murdia, U.S., Mehta, F.J. & Bhide, S.V. (1982) Nitrate reductase activity and nitrite levels in the saliva of habitual users of various tobacco products. *Food Chem. Toxicol.*, *20*, 269-271

Nair, J., Ohshima, H., Friesen, M., Croisy, A., Bhide, S.V. & Bartsch, H. (1985) Tobacco-specific and betel-nut-specific *N*-nitroso compounds: Occurrence in saliva and urine of betel quid chewers and formation *in vitro* by nitrosation of betel quid. *Carcinogenesis*, *6*, 295-303

National Academy of Sciences (1975) *Medical and Biologic Effects of Environmental Pollutants — Nickel*, Washington DC, National Academy Press, pp. 178-183

National Research Council (1981) *The Health Effects of Nitrate, Nitrite and* N-*Nitroso Compounds*, Assembly of Life Sciences, National Academy of Sciences, Washington DC, National Academy Press, pp. 7-35, 7-36

Newman, I.M. & Duryea, E.J. (1981) Adolescent cigarette smoking and tobacco chewing in Nebraska. *Nebraska med. J.*, *66*, 243-244

Nicolaus, G. & Elmenhorst, H. (1982) Qualitative and quantitative detection of alkylnaphthalenes in Latakia tobacco (Ger.). *Beitr. Tabakforsch.*, *11*, 133-140

Nishimura, H. & Nakai, K. (1958) Developmental anomalies in offspring of pregnant mice treated with nicotine. *Science*, *127*, 877-878

Nugmanov, S.N. & Baimakanov, S.S. (1970) *The result of an epidemiological study of oropharyngeal tumours in Kazakhstan following the WHO project* (Russ.). In: *Epidemiology of Malignant Tumours*, Alma-Ata, Nauka Publishing House, pp. 227-231.

Onishi, I., Nagasawa, M., Tomita, H. & Fukuzumi, T. (1957) Studies on the essential oils of tobacco leaves. Part XVI. Neutral fraction. (3). Polycyclic aromatic hydrocarbons of Burley tobacco leaf. *Bull. agric. Chem. Soc. Jpn*, *22*, 17-20

Österdahl, B.-G. & Slorach, S.A. (1983) Volatile N-nitrosamines in snuff and chewing tobacco on the Swedish market. *Food Chem. Toxicol.*, *21*, 759-762

Paches, A.I. & Djuliev, S.S. (1965) *Pathological changes in the oral mucosa in relation to the use of nass in Tadzhikistan* (Russ.). In: *Problems of Geographic Pathology of Oropharyngeal Tumours*, Alma-Ata, ^{U}Kazakhstan^{V} Publishing House, pp. 101-106

Paches, A.I. & Milievskaya, I.L. (1980) *Epidemiological study of cancer of the mucous membrane of the oral cavity in the USSR*. In: Levin, D.L., ed., *Cancer Epidemiology in the USA & USSR* (*NIH Publ. No. 80-2044*), Washington DC, US Department of Health and Human Services, pp. 177-184

Peacock, E.E., Jr & Brawley, B.W. (1959) An evaluation of snuff and tobacco in the production of mouth cancer. *Plast. reconstr. Surg.*, *23*, 628-635

Peacock, E.E., Jr, Greenberg, B.G. & Brawley, B.W. (1960) The effect of snuff and tobacco on the production of oral carcinoma: An experimental and epidemiological study. *Ann. Surg.*, *151*, 542-550

Penn, W.A. (1901) *The Soverane Herbe. A History of Tobacco*, London, Grant Richards, pp. 259-277

Perinelli, M.A. & Carugno, N. (1978) Determination of trace metals in cigarette smoke by flameless atomic absorption spectrometry. *Beitr. Tabakforsch. int.*, *9*, 214-217

Piade, J.J. & Hoffmann, D. (1980) Chemical studies on tobacco smoke. LXVII. Quantitative determination of alkaloids in tobacco by liquid chromatography. *J. liq. Chromatogr.*, *3*, 1505-1515

Pindborg, J.J. (1972) Is submucous fibrosis a precancerous condition in the oral cavity? *Int. dent. J.*, *22*, 474-480

Pindborg, J.J. & Axelsen, N.H. (1980) New age for non-smoked tobacco. *Lancet*, *i*, 775

Pindborg, J.J. & Poulsen, H.E. (1962) Studies in oral leukoplakias. I. The influence of snuff upon the connective tissue of the oral mucosa. Preliminary report. *Acta pathol. microbiol. scand.*, *55*, 412-414

Pindborg, J.J. & Renstrup, G. (1963) Studies in oral leukoplakias. II. Effect of snuff on oral epithelium. *Acta dermatol.-venereol.*, *43*, 271-276

Pindborg, J.J., Kiaer, J., Gupta, P.C. & Chawla, T.N. (1967) Studies in oral leukoplakias. Prevalence of leukoplakia among 10 000 persons in Lucknow, India, with special reference to use of tobacco and betel nut. *Bull. World Health Organ.*, *37*, 109-116

Pindborg, J.J., Mehta, F.S., Daftary, D.K., Gupta, P.C. & Bhonsle, R.B. (1972) Prevalence of oral lichen planus among 7 639 Indian villages in Kerala, South India. *Acta dermatol.-venereol.*, *52*, 216-220

Pindborg, J.J., Mehta, F.S. & Daftary, D.K. (1975) Incidence of oral cancer among 30,000 villagers in India in a 7-year follow-up study of oral precancerous lesions. *Commun. Dent. oral Epidemiol.*, *3*, 86-88

Pindborg, J.J., Reibel, J., Roed-Petersen, B. & Mehta, F.S. (1980) Tobacco-induced changes in oral leukoplakic epithelium. *Cancer*, *45*, 2330-2336

Pöschl, A. (1980) The international importance of snuff. *Tabak-J. int.*, *6*, 490-491

Pöschl, A. (1983) Manufacture of snuff. *Tabak-J. int.*, *6*, 539-540

Pottern, L.M., Morris, L.E., Blot, W.J., Ziegler, R.G. & Fraumeni, J.F., Jr (1981) Esophageal cancer among black men in Washington, D.C. I. Alcohol, tobacco, and other risk factors. *J. natl Cancer Inst.*, *67*, 777-783

Radford, E.P., Jr & Hunt, V.R. (1964) Polonium-210: A volatile radioelement in cigarettes. *Science*, *143*, 247-249

Rahmatian, H., Modjtabai, A., Zakarian, B. & Zarrine, K. (1965) Experimental carcinoma of esophagus. Effect of nass in the squamous epithelium of esophagus in rats and mice. *Acta med. iran.*, *8*, 1-18

Ranadive, K.J., Gothoskar, S.V. & Khanolkar, V.R. (1963) Experimental studies on the etiology of cancer types specific to India. a) Oral cancer; b) Kangri cancer. *Acta unio int. contra cancrum*, *19*, 634-639

Ranadive, K.J., Gothoskar, S.V., Rao, A.R., Tezabwalla, B.U. & Ambaye, R.Y. (1976) Experimental studies on betel nut and tobacco carcinogenecity. *Int. J. Cancer*, *17*, 469-476

Randeria, J.D. (1972) Tobacco induced changes in the bladder, buccal and vaginal mucosae. *Indian J. med. Res.*, *60*, 694-698

Rao, A.R. (1984) Modifying influences of betel quid ingredients on B(a)P-induced carcinogenesis in the buccal pouch of hamster. *Int. J. Cancer*, *33*, 581-586

Redmond, D.E. (1970) Tobacco and cancer: The first clinical report, 1761. *New Engl. J. Med.*, *282*, 18-23

Reif, H. & Moser, F. (1977) A method for the determination of organochlorine and organophosphorus pesticide residues on tobacco. *Beitr. Tabakforsch.*, *9*, 168-175

Riebe, M. & Westphal, K. (1983) Studies on the induction of sister-chromatid exchanges in Chinese hamster ovary cells by various tobacco alkaloids. *Mutat. Res.*, *124*, 281-286

Rizio, D. (1984) Smokeless tobacco. *Tabak-J. int.*, *2*, 183-184

Roed-Petersen, B. & Pindborg, J.J. (1973) A study of Danish snuff-induced oral leukoplakias. *J. oral Pathol.*, *2*, 301-313

Root, H.D., Aust, J.B. & Sullivan, A., Jr (1960) Snuff and cancer of the ear. *New Engl. J. Med.*, *262*, 819-820

Rosenfeld, L. & Callaway, J. (1963) Snuff dipper's cancer. *Am. J. Surg.*, *106*, 840-844

Rosenfeld, L. & Green, J. (1969) Carcinoma of the oral cavity. *J. Tennessee med. Assoc.*, *62*, 707-710

Rowell, P.P. & Clark, M.J. (1982) The effect of chronic oral nicotine administration on fetal weight and placental amino acid accumulation in mice. *Toxicol. appl. Pharmacol.*, *19*, 30-38

Rowland, R.L. & Roberts, D.L. (1963) Macrocyclic diterpenes isolated from tobacco, α- and β-4,8,13-duvatriene-1,5-diols. *J. org. Chem.*, *28*, 1165-1169

Russell, M.A.H., Jarvis, M.J. & Feyerabend, C. (1980) A new age for snuff? *Lancet*, *i*, 474-475

Ryan, P. (1980) Snuff? Snus? At least, an alternative to smoke. *Smithsonian*, *10*(11), 176

Salem, G., Juhl, R. & Schiødt, T. (1984) Oral malignant and premalignant changes in 'shammah'-users from the Gizan region, Saudi Arabia. *Acta odontol. scand.*, *42*, 41-45

Saparov B.S. (1965) *Cancer of the mouth in the department of oncology of the republic hospital No. 1 of Turkmenian SSR* (Russ.). In: *Problems of Geographic Pathology of Oropharyngeal Tumours,* Alma-Ata, Kazakhstan Publishing House, pp. 99-101

Saparov, B.S. (1970) *Cancer of the oral cavity in Turkmenii* (Russ.). In: *Epidemiology of Malignant Tumours,* Alma-Ata, Nauka Publishing House pp. 248-250

Schmähl, D. (1965) Investigation of chewing tobacco extracts for carcinogenic effects in rats (Ger.). *Arzneimittelforschung*, *15*, 704-705

Schmeltz, I. & Hoffmann, D. (1977) Nitrogen-containing compounds in tobacco and tobacco smoke. *Chem. Rev.*, *77*, 295-311

Schmeltz, I., de Paolis, A. & Hoffmann, D. (1975) Phytosterols in tobacco: Quantitative analysis and fate in tobacco combustion. *Beitr. Tabakforsch.*, *8*, 211-218

Schmeltz, I., Tosk, J. & Hoffmann, D. (1976) Formation and determination of naphthalenes in cigarette smoke. *Anal. Chem.*, *48*, 645-650

Schuman, L.M. (1977) *Patterns of smoking behavior.* In: *Research on Smoking Behavior* (*NIDA Research Monograph No. 17*), Washington DC, National Institute of Drug Abuse, pp. 36-66

Schuman, L.M., Bjelke, E., Gibson, R.W. & Watt, G.D. (1982) *Cancer among chewers of snuff/tobacco: International cohort comparisons for Norway and the United States*. In: *Proceedings of the XIIIth International Cancer Congress, Seattle, Washington* (Abstract No. 1172), p. 207

Severson, R.F., Arrendale, R.F., Chortyk, O.T. & Snook, M.E. (1978) A method for determining the transfer of lipids from tobacco to smoke. *Tobacco Sci.*, *22*, 130-133

Severson, R.F., McDuffie, K.L., Arrendale, R.F. & Chortyk, O.T. (1981) A modified method for the rapid analysis of long-chain alkanes and neophytadiene from tobacco. *Beitr. Tabakforsch.*, *11*, 27-32

Shanta, V. & Krishnamurthi, S. (1963) Further study in aetiology of carcinomas of the upper alimentary tract. *Br. J. Cancer*, *17*, 8-23

Shapiro, L. (1981) Warning: Chewing tobacco and snuff may be dangerous to your health. *Coal Age*, *86*, 74-79

Shapiro, M.P., Keen, P., Cohen, L. & de Moor, N.G. (1955) Malignant disease in the Transvaal. III. Cancer of the respiratory tract. *S. Afr. med. J.*, *29*, 96-101

Sharipov, R.K. (1965) *Oral cancer and use of nass*. In: *Problems of Geographic Pathology of Oropharyngeal Tumours*, Alma-Ata, Kazakhstan Publishing House, pp. 73-78

Shelton, A. (1984) Smokeless tobacco: Moist snuff leads American market. *Tobacco Rep.*, July, pp. 30-31

Shilovtsev, S.P. (1941) *Cancer of the Floor of the Mouth in Relation to Nass Smoking* (Russ.), Tashkent, Uzgasisdat

Shirname, L.P., Menon, M.M. & Bhide, S.V. (1984) Mutagenicity of betel quid and its ingredients using mammalian test systems. *Carcinogenesis*, *5*, 501-503

Shmuk, A.A. (1953) *The Chemistry and Technology of Tobacco*, Vol. III, Moscow, Pishchepromizdat, p. 53

Silverman, S., Jr, Gorsky, M. & Lozada, F. (1984) Oral leukoplakia and malignant transformation. A follow-up study of 257 patients. *Cancer*, *53*, 563-568

Simon, D.L. & Iglauer, A. (1960) The acute effect of chewing tobacco and smoking in habitual users. *Ann. N.Y. Acad. Sci.*, *90*, 119-132

Sinha, R. (1984) *Development of chewing, hookah and wrapper tobacco in India*. In: *Proceedings of the 5th National Symposium on Tobacco*, pp. 29-33

Sisson, V.A. & Severson, R.F. (1984) *Alkaloid composition of the* Nicotiana *species*. In: *38th Tobacco Chemists' Research Conference, Atlanta, GA, USA, November 5-8, 1984*, Abstract 24

Smith, J.F. (1975) Snuff-dippers lesion. A ten-year follow-up. *Arch. Otolaryngol.*, *101*, 276-277

Smith, J.F., Mincer, H.A., Hopkins, K.P. & Bell, J. (1970) Snuff-dipper's lesion: A cytological and pathological study in a large population. *Arch. Otolaryngol.*, *92*, 450-456

Smith, L.W., Bhargava, K., Mani, N.J., Malaowalla, A.M. & Silverman, S., Jr (1975) Oral cancer and precancerous lesions in 57,518 industrial workers of Gujarat, India. *Indian J. Cancer*, *12*, 118-123

Snook, M.E., Fortson, P.J. & Chortyk, O.T. (1981) Isolation and identification of phenolic acids from tobacco leaf. *Beitr. Tabakforsch. int.*, *11*, 19-26

Sobin, L.H. (1969) Cancer in Afghanistan. *Cancer*, *23*, 678-688

Sorger, K. & Myrden, J.A. (1960) Verrucous carcinoma of the buccal mucosa in tobacco chewers. *Can. med. Assoc. J.*, *83*, 1413-1417

Spears, A.W. & Jones, S.T. (1981) Chemical and physical criteria for tobacco leaf of modern day cigarettes. *Rec. Adv. Tobacco Sci.*, *7*, 19-39

Stecker, R.H., Devine, K.D. & Harrison, E.G., Jr (1964) Verrucose 'snuff dipper's' carcinoma of the oral cavity. *J. Am. med. Assoc.*, *189*, 144-146

Stedman, R.L. (1968) The chemical composition of tobacco and tobacco smoke. *Chem. Rev.*, *68*, 153-207

Stewart, G.G. (1967) A history of the medicinal use of tobacco, 1492-1860. *Med. Hist.*, *11*, 228-268

Stich, H.F. & Rosin, M.P. (1984) Micronuclei in exfoliated human cells as a tool for studies in cancer risk and cancer intervention. *Cancer Lett.*, *22*, 241-253

Stich, H.F. & Stich, W. (1982) Chromosome-damaging activity of saliva of betel nut and tobacco chewers. *Cancer Lett.*, *15*, 193-202

Stich, H.F., Curtis, J.R. & Parida, B.B. (1982) Application of the micronucleus test to exfoliated cells of high cancer risk groups: Tobacco chewers. *Int. J. Cancer*, *30*, 553-559

Sundström, B., Mörnstad, H. & Axéll, T. (1982) Oral carcinomas associated with snuff dipping: Some clinical and histological characteristics of 23 tumours in Swedish males. *J. oral Pathol.*, *11*, 245-251

Suri, K., Goldman, H.M. & Wells, H. (1971) Carcinogenic effect of a dimethyl sulphoxide extract of betel nut on the mucosa of the hamster buccal pouch. *Nature*, *230*, 383-384

Svenska Tobaks Ab (1964) *Tobacco in Sweden. Celebration Publication 1915-64*, Stockholm

Svenska Tobaks Ab (1973) *Statistics on Sales and Markets, Annual Book 1965-73*, Stockholm

Swedish Tobacco Co. (1980) *Annual Report 1980*, Stockholm

Swedish Tobacco Co. (1981) *Annual Report 1981*, Stockholm

Swedish Tobacco Co. (1982) *Annual Report 1982*, Stockholm

Swedish Tobacco Co. (1983) *Chart of the Consumption of Tobacco Products in Sweden from 1973-1982*, Stockholm

Temple, D.J. (1976) The absorption of nicotine from tobacco snuff through the nasal mucosa. *Arch. Pharm.*, *309*, 984-987

Tobacco Institute (1981) *Tobacco Industry Profile 1981*, Washington DC

Tobacco Institute (1982) *Tobacco Industry Profile 1982*, Washington DC

Tobacco Institute (1983) *Tobacco Industry Profile 1983*, Washington DC

Tso, T.C. (1972) *Physiology and Biochemistry of Tobacco Plants*, Stroudsburg, PA, Dowden, Hutchinson & Ross, Inc., pp. 43-66, 92-99, 152-157, 170-171, 202-208, 259-273, 282-283

Tso, T.C., Chaplin, J.F., LeLachenr, K.E. & Sheets, T.J. (1980) Pesticide-treated *vs.* 'pesticide-free' tobacco. I. Tobacco production and leaf analysis. *Beitr. Tabakforsch. int.*, *10*, 114-119

Tyldesley, W.R. (1971) Tobacco chewing in English coal miners. A preliminary report. *Br. J. oral Surg.*, *9*, 21-28

Tyldesley, W.R. (1976) Tobacco chewing in English coalminers. 2. Malignant transformation in a tobacco-induced leukoplakia. *Br. J. oral Surg.*, *14*, 93-94

Umezawa, K., Takayama, S., Fujie, S., Matsushima, T. & Sugimura, T. (1978) In vitro transformation of hamster embryo cells by betel tobacco extracts. *Toxicol. Lett.*, *2*, 243-246

Umezawa, K., Fujie, S., Sawamura, M., Matsushima, T., Katoh, Y., Tanaka, M. & Takayama, S. (1981) Morphological transformation, sister chromatid exchange and mutagenesis assay of betel constituents. *Toxicol. Lett.*, *8*, 17-22

Upshall, D.G. (1972) Correlation of chick embryo teratogenicity with nicotinic activity of a series of tetrahydropyrimidines. *Teratology*, *5*, 287-293

US Department of Agriculture (1976) *Tobacco Situation* (*TS-155*), Washington DC, US Department of Agriculture, Economic Research Service, March, p. 2

US Department of Agriculture (1980) *Tobacco Situation* (*TS-171*), Washington DC, US Department of Agriculture, Economic Research Service, March, p. 5

US Department of Agriculture (1982) *Tobacco Outlook and Situation* (*TS-180, TS-181, TS-182*), Washington DC, US Department of Agriculture, Economic Research Service, June, pp. 2, 6, 7, 31, 32

US Department of Agriculture (1983) *Tobacco Outlook and Situation* (*TS-183*), Washington DC, US Department of Agriculture, Economic Research Service, March, pp. 5-8

US Department of Agriculture (1984a) *Production of Chewing Tobacco, Smoking Tobacco and Snuff*, Washington DC, Internal Revenue Service

US Department of Agriculture (1984b) *Tobacco Outlook and Situation* (*TS-187*), Washington DC, US Department of Agriculture, Economic Research Service, March, pp. 2, 3, 4, 28, 29

US Department of Health, Education, and Welfare (1979) *Smoking and Health. A Report of the Surgeon General*, Washington DC, pp. 13-10, 13-39, 14-13, 14-30

US Environmental Protection Agency (1981) Maleic hydrazide: Notification of issuances of notices of intent to suspend pesticide registrations. *Fed. Regist.*, *46* (No. 179), 45999-46000

Van Duuren, B.L., Sivak, A., Segal, A., Orris, L. & Langseth, L. (1966) The tumor-promoting agents of tobacco leaf and tobacco smoke condensate. *J. natl Cancer Inst.*, *37*, 519-526

Vara, P. & Kinnunen, O. (1951) The effect of nicotine on the female rabbit and developing foetus. *Ann. med. exp. biol. fenn.*, *29*, 202-213

Verma, R.C., Chansoriya, M. & Kaul, K.K. (1983) Effect of tobacco chewing by mothers on fetal outcome. *Indian Pediatr.*, *20*, 105-111

Vincent, R.G. & Marchetta, F. (1963) The relationship of the use of tobacco and alcohol to cancer of the oral cavity, pharynx or larynx. *Am. J. Surg.*, *106*, 501-505

Voges, E. (1984) The pleasures of tobacco — How it all began and the whole story. *Tobacco J. int.*, *1*, 80-82

Vogler, W.R., Lloyd, J.W. & Milmore, B.K. (1962) A retrospective study of etiological factors in cancer of the mouth, pharynx, and larynx. *Cancer*, *15*, 246-258

Voss, R.C. & Nicol, H. (1960) Metallic trace elements in tobacco. *Lancet*, *ii*, 435-436

Wahi, P.N. (1968) The epidemiology of oral and oropharyngeal cancer. A report of the study in Mainpuri district, Uttar Pradesh, India. *Bull. World Health Organ.*, *38*, 495-521

Wahi, P.N., Kehar, U. & Lahiri, B. (1965) Factors influencing oral and oropharyngeal cancers in India. *Br. J. Cancer*, *19*, 642-660

Wahi, P.N., Mital, V.P., Lahiri, B., Luthra, U.K., Seth, R.K. & Arora, G.D. (1970) Epidemiological study of precancerous lesions of the oral cavity: A preliminary report. *Indian J. med. Res.*, *58*, 1361-1391

Weather, D.R. & Offenbacher, S. (1983) Effects of smokeless tobacco on caries prevalence in adolescent males (Abstract No. 313). *J. dent. Res.*, *62*, 684

Westbrook, K.C., Suen, J.Y., Hawkins, J.M. & McKinney, D.C. (1980) *Snuff dipper's carcinoma: Fact or fiction?* In: Nieburgg, H.E., ed., *Prevention and Detection of Cancer, Part II. Detection*, Vol. 2, *Cancer Detection in Specific Sites*, New York, Marcel Dekker, pp. 1367-1371

WHO Collaborating Centre for Oral Precancerous Lesions (1978) Definition of leukoplakia and related lesions: An aid to studies on oral precancer. *Oral Surg.*, *46*, 518-539

Wilkins, S.A., Jr & Vogler, W.R. (1957) Cancer of the gingiva. *Surg. Gynecol. Obstet.*, *105*, 145-152

Williams, R.R. & Horm, J.W. (1977) Association of cancer sites with tobacco and alcohol consumption and socioeconomic status of patients: Interview study from the Third National Cancer Survey. *J. natl Cancer Inst.*, *58*, 525-547

Wilson, M.J. (1975) *Tobacco consumption in various countries*. In: Lee, P.N., ed., *Research Paper 6*, 4th ed., London, Tobacco Research Council, pp. 4-85

Winn, D.M., Blot, W.J., Shy, C.M., Pickle, L.W., Toledo, A. & Fraumeni, J.F., Jr (1981a) Snuff dipping and oral cancer among women in the southern United States. *New Engl. J. Med.*, *304*, 745-749

Winn, D.M., Blot, W.J. & Fraumeni, J.F., Jr (1981b) Snuff dipping and oral cancer. *New Engl. J. Med.*, *305*, 230-231

Winn, D.M., Ziegler, R.G., Pickle, L.W., Gridley, G., Blot, W.J. & Hoover, R.N. (1984) Diet in the etiology of oral and pharyngeal cancer among women from the southern United States. *Cancer Res.*, *44*, 1216-1222

van Wyk, C.W. (1965) The oral lesion caused by snuff. A clinico-pathological study. *Med. Proc.*, *11*, 531-537

van Wyk, C.W. (1966) The oral lesion caused by snuff. A clinico-pathological study. *J. dent. Assoc. S. Afr.* , *21*, 109-116

van Wyk, C.W., Farman, A.G. & Staz, J. (1977) Oral health status of institutionalized elderly Cape Coloreds from the Cape Peninsula of South Africa. *Commun. Dent. oral Epidemiol.*, *5*, 179-184

Wynder, E.L. (1976) A corner of history: John Collins Warren (1778-1856). *Prev. Med.*, *5*, 317-319

Wynder, E.L. & Bross, I.J. (1961) A study of etiological factors in cancer of the esophagus. *Cancer*, *14*, 389-413

Wynder, E.L. & Hoffmann, D. (1967) *Tobacco and Tobacco Smoke. Studies in Experimental Carcinogenesis*, New York, Academic Press

Wynder, E.L. & Stellman, S.D. (1977) Comparative epidemiology of tobacco-related cancers. *Cancer Res.*, *37*, 4608-4622

Wynder, E.L. & Wright, G. (1957) A study of tobacco carcinogenesis. I. The primary fractions. *Cancer*, *10*, 255-271

Wynder, E.L., Bross, I.J. & Feldman, R.M. (1957a) A study of the etiological factors in cancer of the mouth. *Cancer*, *10*, 1300-1323

Wynder, E.L., Hultberg, S., Jacobsson, F. & Bross, I.J. (1957b) Environmental factors in cancer of the upper alimentary tract: A Swedish study with special reference to Plummer-Vinson (Paterson-Kelly) syndrome. *Cancer*, *10*, 470-487

Wynder, E.L., Onderdonk, J. & Mantel, N. (1963) An epidemiological investigation of cancer of the bladder. *Cancer*, *16*, 1388-1407

Yousef, A. & Hashash, M. (1983) Common features and surgical interference in a prevalent oral cancer in Saudi Arabia (a preliminary report). *J. Laryngol. Otolaryngol.*, *97*, 837-843

Zacho, A., Nielsen, J. & Larsen, V. (1968) On the consumption of unburned tobacco in patients with cancer of the stomach. *Acta chir. scand.*, *134*, 272-274

Zacho, A., Nielsen, J. & Cederqvist, C. (1975) Relationship between type of tobacco used and localization of tumour in patients with gastric cancer. *Acta chir. scand.*, *141*, 676-679

Zaridze, D.G., Kuvshinov J.P., Matiakin E., Polakov B.I., Boyle P. & Blettner, M. (1985a) Chemoprevention of oral and esophageal cancer in Uzbekistan, Union of Soviet Socialist Republics. *Natl Cancer Inst. Monogr.* (in press)

Zaridze, D.G., Blettner, M., Matiakin, E.G., Poljakov, B.P., Stich, H.F., Rosin, M.P., Hoffmann, D. & Brunnemann, K.D. (1985b) The effect of nass use and smoking on the risk of oral leukoplakia. *Cancer Detect. Prev.* (in press)

Zaridze, D.G., Blettner, M., Trapeznikov, N.N., Kuvshinov, J.P., Matiakin, E.G., Poljakov, B.P., Poddubni, B.K., Parshikova, S.M., Rottenberg, V.I., Chamrakulov, F.S., Chodjaeva, M.C., Stich, H.F., Rosin, M.P., Thurnham, D.I., Hoffmann, D. & Brunnemann, K.D. (1985c) Survey of a population with a high incidence of oral and oesophageal cancer. *Int. J. Cancer*, *36*, 153-158

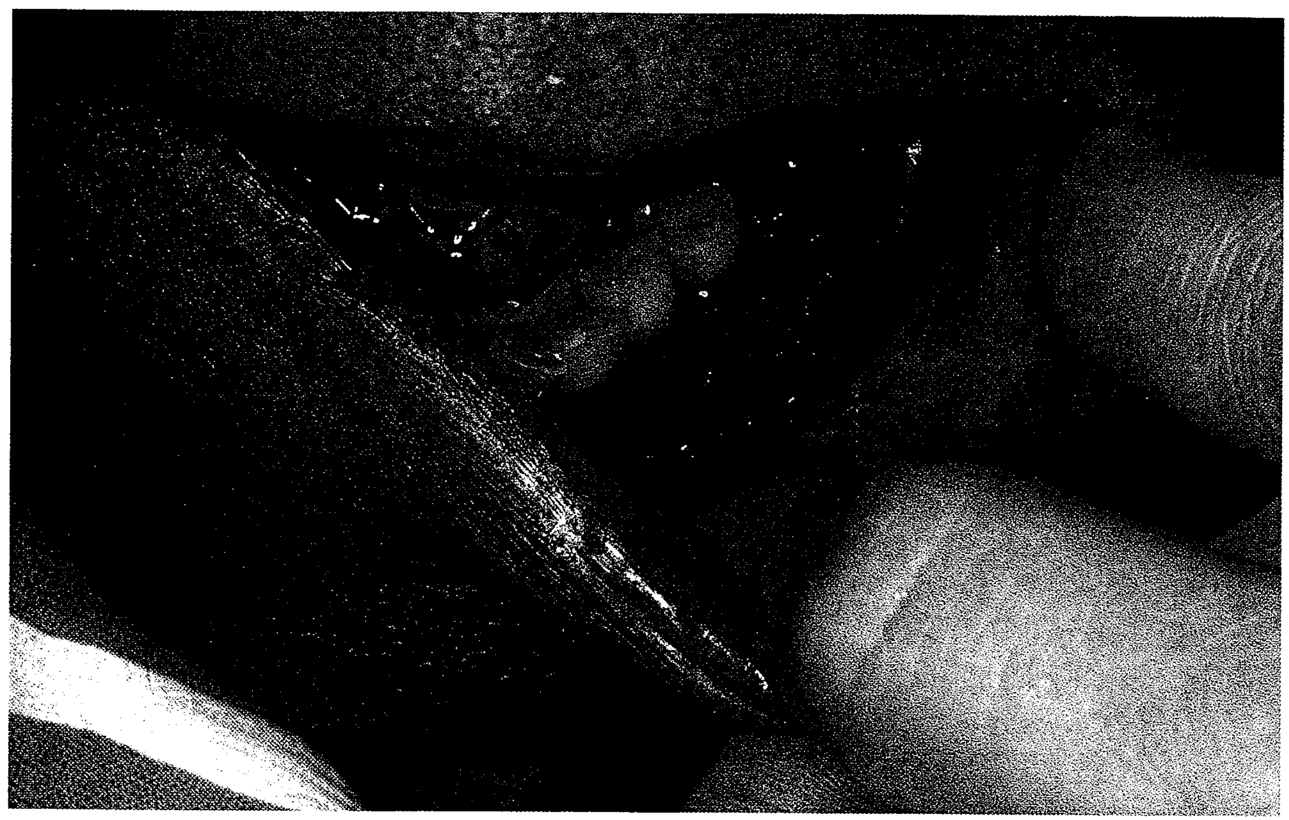

Plate 1. **Snuff placed in the lower buccal groove of a woman in Kentucky, USA**

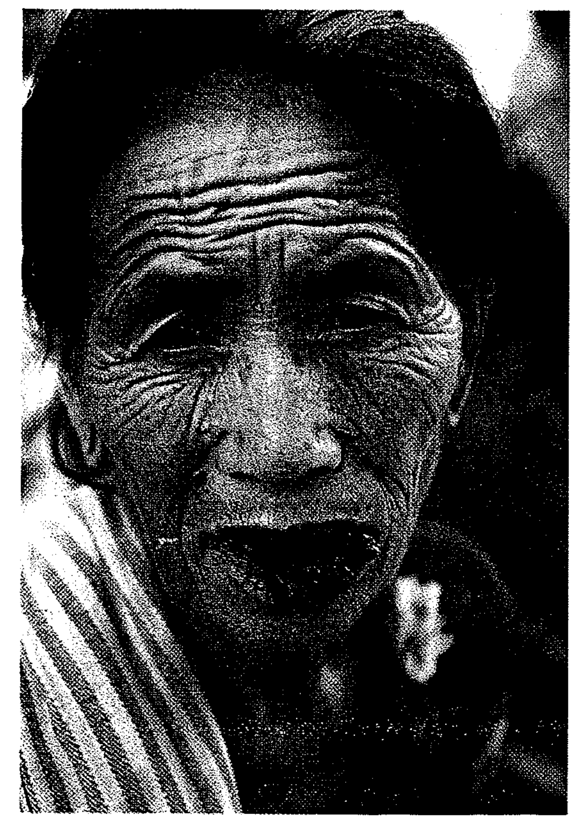

Plate 2. **Indonesian woman with finely-cut tobacco imbibed with juice from betel chewing**

Plate 3. **Danish man with snuff placed in the lower labial groove and on the teeth**

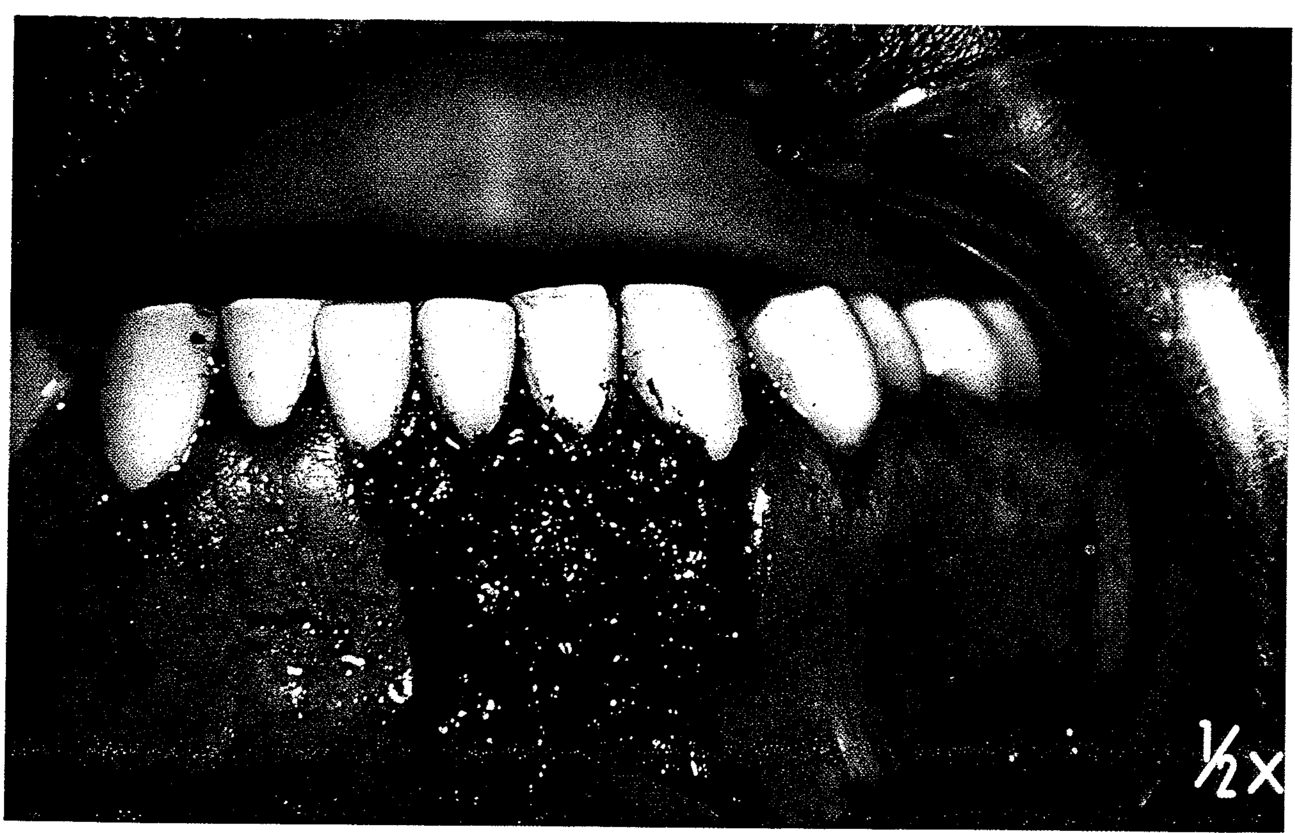

Plate 4. **Bantu with snuff placed in the lower labial groove and on the gingiva**

Plate 5. Afghan man with *naswar* placed on the floor of the mouth

Plate 6. Indonesian women with chunks of tobacco placed in the commissure

Plate 7. **Ingredients of an Indian betel quid: betel leaf on which are placed areca nut, slaked lime and tobacco**

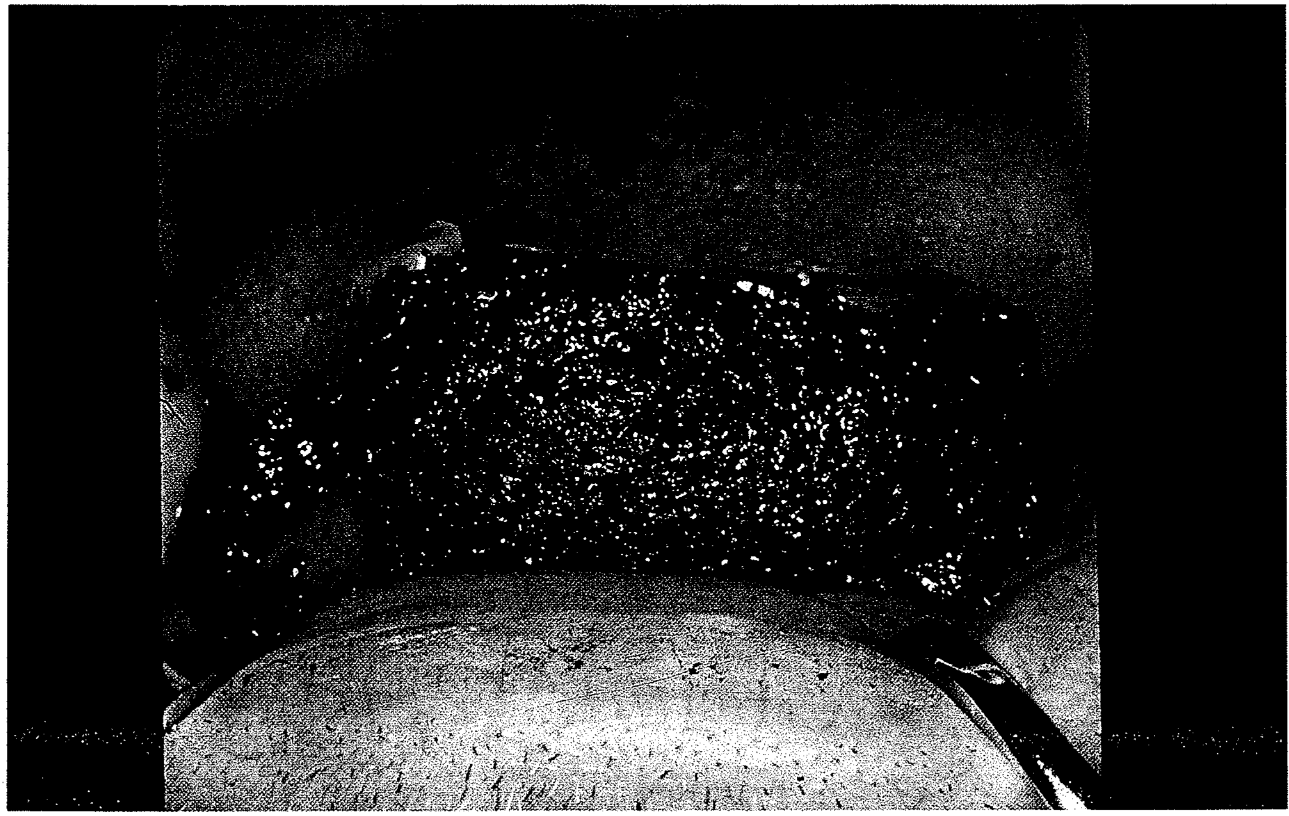

Plate 8. **Danish man with snuff placed in the lower labial and buccal grooves**

BETEL-QUID AND ARECA-NUT CHEWING

1. Description of the Habit

1.1 Historical overview

The chewing of betel quid without tobacco is a habit of great antiquity, which is widespread in the Orient — Bangladesh, Burma, China, Democratic Kampuchea, India, Indonesia, the Lao People's Democratic Republic, Malaysia, Nepal, New Britain, New Ireland, Pakistan, Papua New Guinea, the Philippines, Singapore, Sri Lanka, Taiwan, Thailand and Viet Nam. The habit is also practised in communities of East Indians living in Fiji, Kenya, Mauritius, South Africa, Uganda and the United Republic of Tanzania.

There seems to be general agreement that the first mention of betel quid dates from 504 BC, when it was recorded in the 'Mahawamsa', a register of events in Sri Lanka written in Pali, that a princess made a gift of betel to her nurse (Krenger, 1942). A story is told about the wife of a Singhalese minister who, in about AD 56, learning of a conspiracy against her husband sent his 'betel, etc., for mastication, omitting the chunam (lime) hoping that, in coming to search for this missing ingredient, he might escape his impending fate' (see Tennent, 1860). The chewing of areca nut is mentioned in Sanskrit manuscripts, *Sushruta Samhita*, believed to have been written around 600 BC near Benares. The Sanskrit name for the leaf of the betel vine, 'tambula', persists in modern Hindi (Gode, 1961), as 'tambuli', and is unchanged in Arabic and Persian (Muir & Kirk, 1960). Masudi, the traveller from Baghdad, who wrote an account of his voyages in AD 916, stated that the chewing of betel then prevailed along the southern coast of Arabia, and reached as far as Yemen and Mecca (Krenger, 1942). In 1298, Marco Polo (Raghavan & Baruah, 1958) wrote in his travelogues 'the people of India have a habit of keeping in their mouth a certain leaf called the "tembul"' (Krenger, 1942). The habit is known to have reached the Zanzibar coast between AD 1200 and 1400, and mention is made in Dutch archives of 1664 of a tax on betel leaf imported from India to Malacca (West Malaysia). In 1703, the importation was forbidden, presumably to protect local growers rather than to prevent a well-established habit (Muir & Kirk, 1960).

The habit was accepted as one of the features of the Indian subcontinent, often giving rise to comment from Europeans travelling or resident in these areas on account of the habit of spitting out, both in public and in the home, a red-coloured saliva which stained both the ground and clothing. To quote Tennent (1860): 'The chewing of these nuts with lime and the leaf of the betel-piper supplies to the people of Ceylon the same enjoyment which tobacco affords to the inhabitants of other countries; but its use is, if possible, more offensive, as the three articles, when combined, colour the saliva of so deep a red that the lip and teeth appear as if covered with blood. Yet, in spite of this disgusting accompaniment, men and women, old and young, from morning till night indulge in the expensive luxury.'

Apparently, the first reference to betel chewers' cancer was made by Tennent (1860). He mentions in a footnote that 'Dr Elliot of Colombo observed several cases of cancer in the cheek, which from its peculiar characteristics, he designated the "betel chewer's cancer"'.

Other early references include those of Bala Ram (1902), Niblock (1902) and Boak (1906), writing from Malabar, Madras, the Sulu Archipelago and British Borneo, respectively, about the chewing of betel quid alone or with tobacco.

A number of reasons for chewing betel have been given: betel chewing causes euphoria. It leads to increased salivation, is believed to have anthelmintic properties and to satisfy hunger. Some say it makes the breath smell sweet.

1.2 Major components of the betel quid

Although the chewing of betel quid is practised in several different ways in various countries, the major components are relatively consistent.

Areca nut (*betel nut*) is the fruit of the *Areca catechu* L. tree. *Areca* is a small genus comprising about 20 species of slender palms in the Palmaceae family. The areca palm is native to South Asia and is found throughout South and South-East Asia and in several Pacific islands. It is a handsome palm with a tall slender stem crowned by a tuft of large, elegant leaves. The fruit grows in large bunches at the base of the leaves, and varies in size and shape. The fruit is orange-yellow in colour when ripe and is generally the size of a small egg. The fibrous pericarp of the fruit is separated from the seed or endosperm which is then used fresh or after sun drying or curing. Before curing, the fruits are first shelled; the kernel is then sliced or kept whole and boiled in water, to which is added water left from previous years' curing. Curing results in a uniform colour, softens the nuts and reduces the tannin content (Arjungi, 1976).

Betel leaf (*Piper betle* L.) has been used since ancient times. Betel vines are cultivated in hot and humid climatic conditions in different parts of India, Indonesia, Malaysia and Ceylon.

Lime, known colloquially in India as *chuna* or *chunam*, is prepared either from the calcareous or silicious covering of marine invertebrates (sea shells), harvested along the coastline of India, or from quarried stone in central India. It is manufactured on an industrial scale and is sold as a paste mixed with water in order to release calcium hydroxide (Kandarkar & Sirsat, 1977).

Catechu, colloquially known in India as *kattha* (Dayal *et al.*, 1978), is the resinous extract from the matrix of the *Acacia* tree, usually *A. catechu* or *A. suma* (Muir & Kir, 1960; Millot, 1965).

Tobacco is often added to the above-mentioned ingredients (see also the monograph on tobacco habits other than smoking in this volume).

1.3 Geographic differences and current practices

In *India* where a substantial proportion of the population chew betel quid, the habit is practised by taking a betel leaf on which lime and catechu are smeared. Pieces of areca nut are then placed on the leaf, which is folded, put into the mouth and chewed. Tobacco may be added and, depending on personal taste, spices such as cardamom, cloves (Muir & Kirk, 1960), grated fresh coconut (Schonland & Bradshaw, 1969), ginger (Arjungi, 1976) and sugar (Millot, 1965).

The areca nut is known colloquially in India as *supari* and the quid (betel leaf-lime-areca nut) as *pan* in Hindi (Chopra *et al.*, 1958). Generally, the quid is chewed after meals, but the frequency of chewing by regular quid chewers ranges from two to 15-20 times a day.

The areca nut is also chewed alone, and, in Assam and south-western regions of India, it is chewed raw (Peeters, 1970).

In 1958, it was estimated that there were five to 10 million chewers of betel quid in India (Chopra *et al.*, 1958), and in 1979 it was estimated that there were at least 200 million people world-wide who practised this habit (Burton-Bradley, 1979). The highest consumption of betel quid in India is found in the coastal areas of South India, Assam, Bengal, Uttar Pradesh and Madhya Pradesh (Chopra *et al.*, 1958). Moderate chewing is practised in Maharashtra, Punjab, Gujarat and Rajasthan.

Several studies have investigated the prevalence rate of betel chewing in limited population samples. Table 1 gives information on the prevalence of all chewing habits, with and without areca nut, and with and without tobacco, in house-to-house surveys among villagers in various parts of India. There are marked differences among localities and some differences between the sexes.

Table 1. Prevalence of chewing habits (with and without smoking) in house-to-house surveys among Indian villagers[a]

Location	Size of sample	With tobacco (%)	Without tobacco (%)
Gujarat	10 071	3	1.5
Kerala	10 287	26	0.4
Andhra Pradesh	10 169	2.3	0.5
Bihar, Singhbhum	10 048	13	0.4
Bihar, Darbhanga	10 340	15	1.3
Maharashtra	101 761[b]	28	0.6

[a]From Mehta *et al.* (1971), unless otherwise specified
[b]From Mehta *et al.* (1972a)

A more detailed report on chewing habits in an Indian population has been given by Dayal *et al.* (1978), who studied pure chewing habits (i.e., without a simultaneous smoking habit) among 57 518 textile-mill workers in Ahmedabad, Gujarat. Table 2 shows that the practice of a single chewing habit is rare.

Table 2. Prevalence of various types of current chewing habits among 57 518 textile workers in Ahmedabad, Gujarat[a]

Type of material chewed	Number	% of population sample
Betel quid with lime, catechu and areca nut	737	1.3
Betel quid with lime	2	0.003
Betel quid with areca nut	3	0.005
Areca nut	113	0.2
Betel quid with lime, catechu, areca nut and tobacco	1335	2.3
Others	22	0.04
Total	2212	

[a]From Dayal *et al.* (1978)
Note: Of the entire sample of 57 518 workers, 8710 (15.1%) had no habit, 2212 (3.8%) had a *single* current chewing habit and did not smoke at the time of examination, and 475 others (0.8%) had a non-current chewing habit.

The most detailed account of chewing habits is one of a selected Indian population composed of 10 000 persons admitted to the clinic of the dental school in Lucknow, India. No less than 22 different betel-chewing habits were reported (Pindborg *et al.*, 1967).

The tobacco included in the betel quid varies from region to region. In Uttar Pradesh, 'Mainpuri' tobacco, which is actually a mixture of tobacco with slaked lime, finely cut areca nut and powdered cloves or camphor, is commonly used (Wahi, 1968).

In a study of 434 children, five to 15 years of age, in the *Maldives*, it was found that 26% chewed betel daily. The prevalence rate for the ages 5-6, 10 and 15 years of age was 15, 27 and 51%, respectively (Knudsen *et al.*, 1985).

Betel-quid chewing is widespread in *Indonesia*, including the island of Timor. The habit is practised by chewing betel leaf with areca nut, lime and catechu. When the quid is thoroughly chewed, a piece of finely-cut tobacco is used to remove the remnants of the betel quid adhering to the teeth and buccal grooves. The piece of saturated tobacco is then placed close to the labial commissure until a euphoric state is achieved (Möller *et al.*, 1977).

In a study on dental caries and betel chewing in Java and Bali, Möller *et al.* (1977) found that betel chewing is much more common among women than among men. The number of persons practising the habit appears to be decreasing, since the percentage of betel chewers is much higher among women aged 35 years and older, than among those aged less than 35 years (Table 3). The habit is usually acquired between the ages of 15 and 20 years.

Table 3. Percentage of persons examined, distributed according to betel-chewing habit, age, sex and region[a]

Population	Age in years	Wonosari, Central Java			Kintamani, Bali		
		Sample size	Betel chewers (%)	Non-chewers (%)	Sample size	Betel chewers (%)	Non-chewers (%)
Men	<35	83	0	100	76	0	100
	35	172	2	98	127	30	70
Women	<35	136	35	65	90	3	97
	35	183	80	20	115	59	41
Total		574			408		

[a]From Möller *et al.* (1977)

Betel chewing is practised in a very different way in *Papua New Guinea* as compared to the rest of South-East Asia. The areca nut is chewed when it is ripe but not cured; most often, leaves, seeds and part of the stem from the *Piper betle* are added. When the chewing has lasted a minute or so, slaked lime is added in rather large quantities. The pulverized lime is put on a moistened stick and applied to the buccal mucosa. At the time of withdrawal, the stick is pressed towards the labial commissure and licked clean. Tobacco is never included. The prevalence rate of betel chewing in Papua New Guinea has been studied: of a population sample of 1226, 57.7% were betel chewers, and 73% were smokers. A marked difference was encountered in the three regions studied — in two coastal areas (622 persons), the frequency of betel chewing was 87.8% and of smoking 70.4%, whereas the corresponding figures for the highlands (604 persons) were 26.8 and 76.2%, respectively (Pindborg *et al.*, 1968).

In a subsequent study on dental caries and betel chewing among 301 subjects, aged 12-24 years, Schamschula *et al.* (1977) noted that the betel-chewing habit in Papua New Guinea is usually acquired between the ages of eight and 12 years, although lactating mothers were sometimes observed prechewing boluses for their infants. Among the population sample, 90% practised the habit, with individual frequencies ranging from less than one to 25 areca nuts per day. The proportion of men and women who practised the habit was similar.

Betel-quid chewing is widespread on the island of Hainan in *China*. A betel leaf is smeared with slaked lime and placed in the mouth with pieces of areca nut that have been peeled and sliced in millimetre sections. Tobacco is never added. Of 100 betel-quid chewers in the Linshui Districts of Hainan, 42 used betel quid alone, 31 used betel quid and cigarettes, 21 used betel quid and a water pipe and one used betel quid, cigarettes and a water pipe (Pindborg *et al.*, 1984).

2. Chemical Data Relevant to the Evaluation of Carcinogenic Risk to Humans

Betel quid may be composed of areca nut, betel leaf, catechu and lime and in some areas is often also mixed with crushed leaves from flue-cured and sun-cured tobacco, *Nicotiana tabacum*, and from *N. rustica*; 0.5-1 g of tobacco may be added to the quid (Shirname *et al.*, 1983).

2.1 Areca nut

In general, areca nut is 'refined' or 'cured' before use. Table 4 lists the major chemical ingredients in the areca nut before curing. It should be noted that wide variations can

Table 4. Constituents of areca-nut endosperm before curing[a]

Constituent	Quantity
Tannins	11.4-26.0%
Gallotannic acid	18.03%
Gallic acid	-
D-Catechol	3 g/800 g (0.4%)
Phiobatannin	-
Alkaloids	0.15-0.67%
Arecoline	0.07-0.50%
Arecaidine	Small quantity
Guvacine	Small quantity
Isoguvacine	Trace quantity
Arecolidine	Minute quantity
Guvacoline	Minute quantity
Fats	1.3-17.0%
Sitosterol	Trace quantity
Carbohydrates (saccharose, reducing sugars, galactan, mannan)	47.2-84.5%
Protein	4.9-9.3%
Non-protein nitrogen	0.22-1.6%
Saponins	-
Gums	-
Carotene	5 International Vitamin A units/100 g
Mineral matter	
Calcium	0.018-0.05%
Phosphorus	0.13-2.35%
Iron	1.5-11.6 mg/100 g (0.002-0.01%)

[a]From Raghavan and Baruah (1958)

occur in the composition of the 'cured' areca nut, as shown in Table 5 for polyphenols, arecoline, fat, crude fibre and total polysaccharides (Shivashankar *et al.*, 1969). Among the polyphenols identified in areca nut are leucocyanidins, catechin, 3,4-flavandiols and hexahydroxyflavan. The major areca-nut alkaloids are arecoline, arecaidine, arecolidine, guvacoline and guvacine (Arjungi, 1976) (see Fig. 1).

Table 5. Range of variation in chemical constituents in cured areca nuts[a]

Type/trade name	No. of samples analysed	Range of variation in content (%)				
		Polyphenols	Arecoline	Fat	Crude fibre	Total polysaccharides
Chali	65	7.3-34.9	0.1-0.7	4.9-24.4	7.1-17.4	14.3-26.3
Parcha	18	11.7-25.0	0.1-0.5	12.3-18.1	8.0-14.3	13.0-27.3
Iylon	25	19.6-45.9	0.1-0.7	6.8-18.1	5.4-13.3	13.5-28.2
Api	54	15.2-41.3	0.2-0.9	5.3-18.5	5.4-18.5	9.2-28.2
Batlu	31	22.4-55.2	0.1-0.9	4.3-17.9	3.1-12.3	14.2-27.0
Choor	33	24.9-43.7	0.1-0.9	5.9-17.8	5.1-15.2	11.1-28.1
Erazel	9	16.9-38.0	0.2-0.8	5.5-12.3	5.9-8.7	13.1-26.6
Chalakudi	3	32.0-39.3	0.4-0.9	7.1-10.5	5.3-14.9	22.1-26.9
Nuli	6	39.0-47.9	0.6-0.9	3.7-13.8	3.8-6.0	16.4-22.7

[a]From Shivashankar *et al.* (1969)

Figure 1. Major areca-nut alkaloids[a]

[a]From Mujumdar *et al.* (1982)

In-vitro experiments with arecoline and nitrite have shown that this areca-nut alkaloid can give rise to at least four *N*-nitrosamines: *N*-nitrosoguvacoline, 3-(methylnitrosamino)propionitrile, 3-(methylnitrosamino)propionaldehyde (Wenke & Hoffmann, 1983) and *N*-nitrosoguvacine (Nair *et al.*, 1985) (see Fig. 2). *N*-Nitrosoguvacoline has been detected in the saliva of betel-quid chewers, together with tobacco-specific *N*-nitrosamines when the quid contains

Figure 2. Nitrosation of arecoline

tobacco (Wenke *et al.*, 1984). Recently, *N*-nitrosoguvacoline and *N*-nitrosoguvacine have been found in the saliva of betel-quid chewers (Nair *et al.*, 1985). (See also monographs at the end of this volume.)

2.2 Betel leaf

The mature green leaves of *Piper betle* L. (Piperaceae) contain volatile oils [eugenol (see IARC, 1985) and terpenes], nitrate and small quantities of sugar, starch and tannin (Schonland & Bradshaw, 1969). The dry leaves contain 0.2-1.0% volatile oils, chavibetol, chavicol (Deshpande *et al.*, 1970), cadinene (Balendra, 1949) and allyl pyrocatechol (Ueda & Sasaki, 1951). The following have also been found in betel leaves: sitosterol, stigmasterol, stearic acid, pentatriacontane, *n*-triacontanol and hentriacontane (Deshpande *et al.*, 1970; Ganguly & Choudhury, 1975).

2.3 Catechu

Catechu is the residue of a hot-water extraction of the heart-wood of *Acacia catechu* (Leguminosae). The main constituents are tannin and polyphenols; 25-35% catechutannic acid, 2-10% catechin, catechu red, quercitin (see IARC, 1983) and gum (Windholz, 1983) have also been identified. A number of individual polyphenols, such as kaempferol (see IARC, 1983), dihydroxykaempferol, taxifolin, isorhamnetin, (+)afzelchin and dimeric procyanidin were also isolated in addition to (-)epicatechin (Deshpande & Patil, 1981).

2.4 Lime

Two different types of lime are used in India in the betel-quid mix. They are prepared either from limestone or from shells.

One lime sample was analysed for emission of α-particles. It was reported that in this case 1 g emitted 0.35 pCi of α-activity, which originated from 0.53 mg/kg of uranium-238 (Chakarvarti *et al.*, 1981).

3. Biological Data Relevant to the Evaluation of Carcinogenic Risk to Humans

3.1 Carcinogenicity studies in animals (see also Table 8, at the end of this section)

(*a*) *Oral administration*

Mouse: Groups of 15-21 male Swiss mice, eight to 10 weeks of age, were administered by gastric intubation 0.1 ml of aqueous extracts of areca nut (containing 1.5 mg arecoline and 1.9 mg polyphenol), betel leaf or a polyphenol fraction of areca nut (containing 1.9 mg tannic acid) on five days a week for life. A group of 30 male C17 mice received 0.1 ml

of an aqueous extract of areca nut by gavage. Groups of 20 male Swiss and 20 male C17 mice served as untreated controls. Of the animals treated with aqueous areca-nut extract, 12/21 Swiss mice developed tumours: five hepatocellular carcinomas, two haemangiomas of the liver, two adenocarcinomas of the lung, one adenocarcinoma and one squamous-cell carcinoma of the stomach, and one leukaemia; and 8/30 C17 mice developed tumours: three squamous-cell carcinomas and two adenocarcinomas of the stomach, two leukaemias and one adenocarcinoma of the lung. In mice fed the polyphenol fraction, two developed tumours of the salivary gland and one a haemangioma of the liver. No tumour was observed in either of the control groups or in the mice fed aqueous betel-leaf extract (Bhide *et al.*, 1979). [The Working Group noted the absence of survival data for the treated and control mice.]

A group of 14 male and 18 female C17 mice, 10-12 weeks old, was fed a diet containing 10% (w/w) areca nut coated with saccharin [concentration not specified] for 40 weeks, and the animals were followed for life. A group of 12 males and 12 females was fed the same diet for 40 weeks and also received 0.2 ml of a 0.1% solution of 1,4-dinitrosopiperazine per day by gastric intubation. A further group of 12 males and 22 females served as untreated controls. No statistically significant increase in tumour incidence was observed in animals treated with areca nut compared to controls; treatment with areca nut did not potentiate the carcinogenicity of 1,4-dinitrosopiperazine (Pai *et al.*, 1981). [The Working Group noted the short duration of treatment].

Groups of 20 male Swiss mice, eight to 10 weeks of age, were administered by gastric intubation 0.1 ml of an aqueous extract of betel quid, betel quid and tobacco, areca nut, betel leaf, or areca nut and betel leaf five times per week for life or served as untreated controls. The incidences of lung adenocarcinomas in the various groups are given in Table 6 (Shirname *et al.*, 1983). [The Working Group noted the absence of lung adenomas.]

Table 6. Design and results of experiments in Swiss mice given aqueous extracts of betel quid and its components by gavage[a]

Group	No. at start	No. of mice alive between 10-24 months	No. of mice with lung tumours (%)
Control	20	20	1 (5)
Betel quid	20	15	4 (26)
Betel quid and tobacco	20	18	4 (22)
Areca nut	20	19	9 (47)[b]
Betel leaf	20	14	1 (7)
Areca nut and betel leaf	20	16	6 (38)[b]

[a]From Shirname *et al.* (1983)
[b]Statistically significant as compared to controls ($p < 0.05$)

Groups of eight to 20 female and 16-35 male Swiss mice, six weeks of age, were given 1 mg arecoline hydrochloride daily by gavage on five days a week (in 0.1 ml distilled water), either alone or in combination with potassium nitrate (KNO_3) (1 mg daily), or KNO_3 with slaked lime (1 mg daily); controls were either untreated or received KNO_3 and lime. Treatment was continued for up to 25 months. A total of 15/35 (43%) males given arecoline alone developed tumours (8/18 between 12-18 months and 7/17 between 19-25 months) as compared to 1/20 untreated males. Of the 15 tumours in the arecoline group, eight were liver haemangiomas, four lung adenocarcinomas and three squamous-cell carcinomas of the stomach. No tumour was reported in any of the 18 arecoline-treated females or 20 control females. The incidence of tumours in male mice given arecoline in combination with either

KNO_3 (3/19) or KNO_3 + lime (1/16) did not differ from that in corresponding control males given KNO_3 + lime (2/17). No tumour was found in females treated similarly (Bhide *et al.*, 1984). [The Working Group noted the lack of information on the time of appearance of specific neoplasms and inadequate reporting of the pathological findings. The relevance of the liver haemangiomas is difficult to ascertain.]

Rat: Groups of eight to nine male and eight female ACI rats, 1.5 months old, were fed diets containing either 20% Indonesian areca nut or 20% Formosan betel-leaf powder for 480 and 300-327 days, respectively. A group of 11 males and eight females received a diet containing 20% Indonesian areca nut and 1% calcium hydroxide for 480 days. A group of nine males and 10 females served as untreated controls. At the end of the treatment periods, the animals were fed a normal diet and observed for life. No statistically significant difference in tumour incidence was observed between the treated and control groups (Mori *et al.*, 1979). [The Working Group noted that addition of 20% (w/w) areca-nut powder to the diet results in severe caloric and nutritional imbalances, which may have affected the results.]

Groups of 20-39 male and 21-28 female ACI rats, 1-1.5 months old, were fed marginally vitamin A-deficient or vitamin A-sufficient diets with or without 20% areca nut and 1% calcium hydroxide for up to 647 days. No statistically significant difference in tumour incidence was observed between the treated and control groups (Tanaka *et al.*, 1983). [The Working Group noted the high percentage of areca nut in the diet.]

Hamster: Of a group of two males and two females, one male Syrian golden hamster, 25.5 months of age, developed a carcinoid tumour of the glandular stomach after being fed a diet containing 0.1% arecoline and 2.5% calcium hydroxide for 12 months from the age of 1.5 months (Dunham *et al.*, 1975). [The Working Group noted the small number of animals used.]

(*b*) *Skin application*

Mouse: A group of 12 Swiss mice [sex unspecified] received daily topical applications of an aqueous extract of betel quid plus tobacco on the ears for two years; two animals developed squamous-cell carcinomas and one a benign squamous papilloma at the site of application (Muir & Kirk, 1960). [The Working Group noted that no controls were reported.]

Groups of 16-23 male and female C17 mice, two to three months old, received thrice-weekly topical applications of 0.1 ml dimethyl sulphoxide (DMSO) extracts of tobacco (5 g ground tobacco in 20 ml DMSO), areca nut (30 g ground areca nut in 20 ml DMSO), or tobacco and areca nut, or applications of 0.1 ml DMSO alone on their backs for life. Skin papillomas and epidermoid carcinomas were observed in 1/23 and 2/23 animals, respectively, that received applications of combined tobacco and areca-nut extracts; no local tumour was observed in the other treatment groups (Ranadive *et al.*, 1976). [Although this increased incidence is not statistically significant, it raises a suspicion and is consistent with findings from other experiments.]

(*c*) *Subcutaneous administration*

Mouse: Groups of 10 male and 10 female Swiss mice, two to three months old, were given s.c. injections of 0.2 ml hot or cold aqueous areca-nut extracts once a week for six weeks. Fibrosarcomas at the site of injection were observed in 14/20 and 10/20 mice treated with hot and cold areca-nut extracts, respectively; the first tumour appeared after eight months. No local tumour was seen in a group of 13 male and 12 female controls receiving injections of distilled water for 10 weeks (Ranadive *et al.*, 1976). [No further detail on follow-up and survival was available.]

Groups of 10-20 male Swiss mice [age unspecified] were given weekly s.c. injections of 0.1-0.2 ml of an areca-nut polyphenol fraction (total dose, 24.7 mg/mouse) or a polyphenol-free fraction, aqueous extracts of areca nut (total dose, 24.7 mg polyphenol + 18.5 mg alkaloid/mouse), areca nut plus betel leaf (total dose, 24.7 mg polyphenol + 18.5 mg alkaloid + leaf extract/mouse), betel quid (total dose, 46 mg polyphenol + 38.4 mg alkaloid/mouse), or betel quid plus tobacco (total dose, 45.6 mg polyphenol + 50 mg alkaloid + tobacco extract/mouse) for 13 weeks and were observed for life. A negative-control group of 20 animals received injections of distilled water, and a positive-control group of 10 animals received injections of arecoline (total dose, 18.5 mg/mouse). The incidence of local fibrosarcomas was 2/12 in mice receiving aqueous extract of areca nut, 16/20 in mice receiving the polyphenol fraction of areca nut, 7/20 in mice receiving aqueous extract of betel quid and 2/20 receiving the aqueous extract of betel quid plus tobacco. Tumour incidence in the polyphenol fraction-treated groups was 100%: one animal had a hepatoma and three had adenocarcinomas of the lung. Tumour incidence in other treated groups did not differ from that in controls (Shivapurkar *et al.*, 1980a).

Rat: A group of 15 male and 15 female outbred NIH Black rats, one to two months old, received weekly s.c. injections of 0.5 ml of a tannin-rich aqueous extract of areca nut for up to 56 weeks and were observed for a further 12 weeks. Injections were discontinued at tumour appearance. All treated animals developed malignant mesenchymal tumours at the injection site. No local tumour occurred in 15 male and 15 female saline-injected controls (Kapadia *et al.*, 1978). [The Working Group was concerned by the inadequate description given of the histopathology of the lesions and the statement by the authors that '... induced tumors showed characteristics of malignant mesenchymal tumors. These were similar to those of human malignant fibrous histiocytoma.' It was unclear from this description whether valid malignant tumours were indeed present. Furthermore, the Working Group considered that, if these were malignant tumours, it would be unusual and remarkable that *all* were of this one, specific, uncommon histological type and none of the more common mesenchymal type.]

(*d*) *Intraperitoneal administration*

Mouse: Groups of seven to 10 male Swiss mice [age unspecified] were given weekly i.p. injections of 0.1 ml aqueous extract of areca nut, betel leaf or the polyphenol fraction of areca nut for 13 weeks. Additional groups were injected with commercial tannin, arecoline (1.5 mg) or distilled water. All animals were observed for their entire lifespan. No tumour was observed in any of the groups (Shivapurkar *et al.*, 1980a). [The Working Group noted the small number of animals used and the short period of treatment.]

(*e*) *Administration to the oral mucosa or cheek pouch*

Hamster: Groups of eight to 50 male and female Syrian golden hamsters, one to two months old, received an implantation in the cheek pouch of a single beeswax pellet containing 7-50% betel quid or its various components (areca nut, betel leaf, tobacco). Animals were allowed to live their normal lifespan or were killed when moribund. No malignant tumour was observed at the implantation site in any of the groups (Dunham & Herrold, 1962). [The Working Group noted that only a single administration was given.]

Groups of 11-21 male Syrian golden hamsters, nine weeks old, received topical applications on the cheek-pouch mucosa of dimethyl sulphoxide extracts of areca nut, tobacco, areca nut plus tobacco or dimethyl sulphoxide alone thrice weekly for 21 weeks, at which time all animals were killed. Local squamous-cell carcinomas and leukoplakia were seen in

8/21 and 19/21 of the animals treated with areca-nut extract, and in 16/21 and 18/21 of the groups treated with areca-nut and tobacco extract, respectively; no local tumour was seen in hamsters treated with tobacco extract alone, but 8/12 had leukoplakia. No local tumour was observed in dimethyl sulphoxide-treated controls (Suri *et al.*, 1971). [The Working Group noted that development of cheek-pouch tumours between seven and 21 weeks was extremely unusual and would imply an exceedingly powerful carcinogenic effect.]

A group of nine Syrian golden hamsters, six to seven weeks of age, received applications to the cheek-pouch mucosa of 1.5% arecoline in water five times per week for life; about 30 mg of 0.5% calcium hydroxide was applied to the cheek-pouch mucosa before treatment with arecoline. A papilloma developed in the upper third of the oesophagus in one 15-month-old female (Dunham *et al.*, 1974). [The Working Group noted the small number of animals used.]

Groups of 12-14 male Syrian golden hamsters, two to three months old, received applications to the cheek-pouch mucosa of dimethyl sulphoxide extracts of tobacco, areca nut, or tobacco plus areca nut thrice weekly for life. A solvent-control group of seven animals received applications of dimethyl sulphoxide alone. The combination of tobacco and areca-nut extracts resulted in lesions in the mucous membrane diagnosed as 'early malignant changes' in 3/12 animals and a stomach tumour. One stomach tumour was found in the group treated with tobacco extract, but none were found in animals treated with areca-nut extract alone (Ranadive *et al.*, 1976). [The Working Group noted the small group size and the ambiguous description of the principal lesions reported.]

Groups of Syrian golden and white mutant hamsters [sex and distribution of strains in each group unspecified], two to three months old, were administered betel-quid ingredients separately or in various combinations in the cheek pouch, using the following modes: (1) thrice-weekly applications with aqueous extracts of the test materials; (2) deposition of replaceable wax pellets containing the test materials; (3) introduction of gelatin capsules containing the powdered materials; or (4) insertion of the natural components for direct exposure. Animals were killed at 6-12 or 13-14 months. A group of 64 animals served as controls: 25 received a placebo wax pellet, nine a gelatin capsule, and 30 were untreated. The incidences of cheek-pouch and forestomach carcinomas in animals receiving topical applications of the aqueous extracts of test materials are given in Table 7. Of the group receiving implants of wax pellets containing betel quid, 4/18 and 8/18 developed cancers of the cheek pouch and forestomach, respectively. Cheek-pouch and forestomach carcinomas were observed in 3/21 and 6/21 animals receiving implants of wax pellets containing betel quid plus tobacco. The incidence of tumours in hamsters given betel-quid ingredients in their natural form was not markedly different from that seen after other modes of administration. Cheek-pouch and forestomach carcinomas developed in 5/16 hamsters given cheek-pouch implantations of capsules containing areca-nut powder, tobacco and lime. Cheek-pouch (4/19) and forestomach carcinomas (6/19) occurred in hamsters given capsules containing areca-nut powder (Ranadive *et al.*, 1979). [The Working Group noted the lack of information on sex and strain distribution and the lack of data on the number of tumours per animal.]

Groups of 20 female Syrian golden hamsters, six to seven weeks old, were treated with topical applications to the cheek-pouch mucosa of aqueous extracts of tobacco (1 mg/pouch), areca nut (1 mg/pouch) or betel leaf (5 mg/pouch) twice a day for six months and killed six months later. Squamous-cell papillomas/carcinomas of the cheek pouch developed in 3/20 animals in the tobacco-treated group and in 2/20 in the areca-nut group. No cheek-pouch tumour was observed in 20 animals given the betel leaf or in 10 untreated or 10 vehicle controls (Rao, 1984).

Table 7. Design and results of experiments in hamsters given topical applications of aqueous extracts of betel-quid ingredients on the cheek-pouch mucosa[a]

Group	No. of hamsters at start	Age (months)	Cheek pouch carcinoma		Forestomach carcinoma	
			No.	%	No.	%
Control	19	6-12	-	-	-	-
	11	13-21	-	-	-	-
Areca-nut extract	6	6-12	-	-	1	19
	15	13-21	1	6.6	3	
Polyphenol fraction of areca nut	4	6-12	-	-	-	-
	16	13-21	1	6.2	4	25
Areca-nut and tobacco (Zarda) extract	6	6-12	-	-	ND[b]	-
	12	13-21	2	16.6	3	25
Betel-quid extract	16	6-12	-	-	4	25
	4	13-21	-	-	1	
Betel-quid and tobacco extract	7	6-12	-	-	1	30.7
	6	13-21	-	-	3	

[a]From Ranadive *et al.* (1979)
[b]ND, not done

(*f*) *Other experimental systems*

Intra-vaginal instillation: A group of 60 female Swiss *mice*, about 40 days old, received daily instillation of betel-quid mixture (shell lime and areca nut) with tobacco into the vagina for up to 380 days, at which time 13 animals were still alive. A group of 10 females received instillations of isotonic saline and served as controls. Of the 40 animals that survived for periods ranging from 324-380 days, seven developed 'carcinomatous changes' in the vagina; no tumour was found in controls (Reddy & Anguli, 1967). [The Working Group noted the ambiguous description of the lesions.]

Table 8. Summary of carcinogenicity studies in animals

Species	Route	Extraction	Effect[a]	Reference
1. *Betel quid with tobacco*				
Mouse	Oral intubation	Aqueous	Non-statistically significant increase in lung tumours	Shirname *et al.* (1983)
Hamster	Cheek pouch, beeswax pellet	None	No local malignant tumour [Inadequate]	Dunham & Herrold (1962)
Hamster	Cheek-pouch application	Aqueous	Forestomach carcinomas, 4/13	Ranadive *et al.* (1979)
Hamster	Cheek pouch, wax pellets	None	Cheek-pouch carcinomas, 3/21; forestomach carcinomas, 6/21	Ranadive *et al.* (1979)
Mouse	S.c.	Aqueous	Local sarcomas in 2/20	Shivapurkar *et al.* (1980a)
Mouse	Skin application	Aqueous	Skin carcinomas, 2/12; skin papillomas, 1/12; no control reported	Muir & Kirk (1960)
2. *Betel quid without tobacco*				
Mouse	Oral intubation	Aqueous	Non-statistically significant increase in lung tumours	Shirname *et al.* (1983)

Species	Route	Extraction	Effect[a]	Reference
Hamster	Cheek-pouch application	Aqueous	Forestomach carcinomas, 5/20	Ranadive *et al.* (1979)
Hamster	Cheek pouch, wax pellets	None	Cheek-pouch carcinomas, 4/18; forestomach carcinomas, 8/18	Ranadive *et al.* (1979)
Mouse	S.c.	Aqueous	Local sarcomas, 7/20	Shivapurkar *et al.* (1980a)
3. *Areca nut with tobacco*				
Hamster	Cheek pouch, beeswax pellet	None	No local malignant tumour [Inadequate]	Dunham & Herrold (1962)
Hamster	Cheek-pouch application	DMSO	Local squamous-cell carcinomas, 16/21	Suri *et al.* (1971)
Hamster	Cheek-pouch application	DMSO	Early malignant changes in cheek pouch, 3/12	Ranadive *et al.* (1976)
Hamster	Cheek-pouch application	Aqueous	Cheek-pouch carcinomas, 2/12; forestomach carcinomas, 3/12	Ranadive *et al.* (1979)
Hamster	Cheek pouch, gelatine capsules, with lime	None	Cheek-pouch carcinomas, 5/16; forestomach carcinomas, 5/16	Ranadive *et al.* (1979)
Mouse	Skin application	DMSO	Skin papilloma, 1/23; skin carcinomas, 2/23	Ranadive *et al.* (1976)
Mouse	Intra-vaginal, with lime	None	[Inadequate]	Reddy & Anguli (1967)
4. *Areca nut alone*				
Mouse	Oral intubation	Aqueous	Liver, lung and stomach tumours (polyphenolic fraction not active) [Inadequate]	Bhide *et al.* (1979)
Mouse	Mixed with diet (saccharin-coated nuts)	None	No increased incidence of stomach tumours [Inadequate]	Pai *et al.* (1981)
Mouse	Oral intubation	Aqueous	Reported increased incidence of lung tumours	Shirname *et al.* (1983)
Rat	Mixed with diet, with and without lime	None	No increased tumour incidence [Inadequate]	Mori *et al.* (1979)
Rat	Mixed with vitamin A-deficient and -sufficient diets	None	No increased tumour incidence [Inadequate]	Tanaka *et al.* (1983)
Hamster	Cheek pouch, beeswax pellet	None	No local malignant tumour [Inadequate]	Dunham & Herrold (1962)
Hamster	Cheek-pouch application	DMSO	Local squamous-cell carcinomas, 8/21	Suri *et al.* (1971)
Hamster	Cheek-pouch application	DMSO	No malignant tumour	Ranadive *et al.* (1976)
Hamster	Cheek-pouch application	Aqueous	Cheek-pouch carcinoma, 1/15; forestomach carcinomas, 4/21	Ranadive *et al.* (1979)
Hamster	Cheek pouch, gelatine capsules	None	Cheek-pouch carcinomas, 4/19; forestomach carcinomas, 6/19	Ranadive *et al.* (1979)
Hamster	Cheek-pouch application	Aqueous	Cheek-pouch papillomas/-carcinomas, 2/20	Rao (1984)
Mouse	S.c.	Aqueous	Local tumours, 24/40	Ranadive *et al.* (1976)
Mouse	S.c.	Aqueous	Local sarcomas, 2/12	Shivapurkar *et al.* (1980a)
Mouse	S.c.	Polyphenolic fraction	Local sarcomas, 16/20	Shivapurkar *et al.* (1980a)
Rat	S.c.	Aqueous	Local tumours, 30/30 [Inadequate]	Kapadia *et al.* (1978)
Mouse	Skin application	DMSO	No skin tumour	Ranadive *et al.* (1976)

Species	Route	Extraction	Effect[a]	Reference
Mouse	I.p.	Aqueous	No tumour [Inadequate]	Shivapurkar *et al.* (1980a)
Mouse	I.p.	Polyphenolic fraction	No tumour [Inadequate]	Shivapurkar *et al.* (1980a)
5. *Betel leaf*				
Mouse	Oral intubation	Aqueous	No tumour [Inadequate]	Bhide *et al.* (1979)
Mouse	Oral intubation	Aqueous	No increased incidence of lung tumours	Shirname *et al.* (1983)
Rat	Mixed with diet	None	No increased tumour incidence [Inadequate]	Mori *et al.* (1979)
Hamster	Cheek pouch, beeswax pellet	None	No malignant local tumour [Inadequate]	Dunham & Herrold (1962)
Hamster	Cheek-pouch application	Aqueous	No local tumour	Rao (1984)
Mouse	I.p.	Aqueous	No tumour [Inadequate]	Shivapurkar *et al.* (1980a)
6. *Arecoline*				
Mouse	Oral intubation	None	Liver, lung and stomach tumours in males [Inadequate]	Bhide *et al.* (1984)
Mouse	Oral intubation with potassium nitrate alone or with lime	None	No increase in tumour incidence [Inadequate]	Bhide *et al.* (1984)
Hamster	Mixed with diet, with calcium hydroxide	None	Carcinoma of glandular stomach, 1 [Inadequate]	Dunham *et al.* (1975)
Hamster	Cheek-pouch application, with lime	None	Papilloma of oesophagus, 1 [Inadequate]	Dunham *et al.* (1974)
Mouse	S.c.	None	No local tumour	Shivapurkar *et al.* (1980a)
Mouse	I.p.	None	No tumour [Inadequate]	Shivapurkar *et al.* (1980a)

[a][Inadequate] means that carcinogenicity could not be established by the Working Group from evaluation of the data reported in the study (see also text).

3.2 Other relevant biological data

(*a*) *Experimental systems*

Toxic and pharmacological effects

Areca nut

The toxic and pharmacological effects of areca nut have been reviewed by Arjungi (1976) and Mujumdar *et al.* (1982).

Intraperitoneal injection of an aqueous extract of areca nut (containing 1.5 mg arecoline) increased glutathione content and decreased protein-SH groups in liver, kidney and muscle of Swiss mice (Shivapurkar & Bhide, 1978). Both an aqueous areca-nut extract and arecoline, when injected i.p. into Swiss mice, increased hepatic DNA and RNA content and stimulated DNA and RNA synthesis in the liver (Shivapurkar *et al.*, 1978; Shivapurkar & Bhide, 1979).

Aqueous and ethanolic extracts of areca nut are bactericidal. This antimicrobial activity was associated with the tannins rather than with the alkaloids (Lalithakumari *et al.*, 1965; Mujumdar *et al.*, 1982).

At least five alkaloids occur in areca nut: arecoline, arecaidine, arecolidine, guvacine and guvacoline (Arjungi, 1976). The i.p. LD_{50} of arecoline in rats is approximately 40 mg/kg bw, and that of arecaidine, approximately 800 mg/kg bw (Boyland & Nery, 1969).

The most abundant alkaloid in areca nut is arecoline (Arjungi, 1976), which is cholinomimetic (i.e., mimics the action of acetylcholine) and has a pharmacological action similar to that of muscarine and pilocarpine. In the periphery it selectively stimulates the parasympathetic nervous system by binding to muscarinic and, to some extent, nicotinic receptors (Euler & Domeij, 1945; Gilman *et al.*, 1980) (see also section 3.2(*b*)). Its binding and pharmacological action can be blocked by atropine.

Arecoline also affects the central nervous system. The first evidence found was tremors produced by relatively large doses (e.g., 25 mg/kg bw arecoline i.p. to rats; Holmstedt & Lundgren, 1967); however, more sensitive tests, for example, measurement of the electroencephalogram and studies of behaviour, have shown that very much smaller doses of arecoline can still have measureable effects on the central nervous system (Leslie, 1965; Domino, 1967; Meltzer & Rosecrans, 1982). Since the effects of arecoline on the central nervous system are prevented by atropine, they are presumably caused by an influence on the muscarinic receptors (McKinney & Richelson, 1984).

Areca nut also contains a small amount of arecaidine. Arecaidine is also produced from arecoline by treatment with lime, a component of betel quid (Nieschulz & Schmersahl, 1968), and by enzymes in rat liver (Boyland & Nery, 1969). Arecaidine and the related alkaloid, guvacine, which is present in areca nut and is also produced by the de-esterification of guvacoline by hydrolysis, inhibit the uptake of the neurotransmitter γ-aminobutyric acid in brain slices and when introduced directly into the brain (Johnston *et al.*, 1975; Lodge *et al.*, 1977; Krogsgaard-Larsen, 1980). Arecaidine produced behavioural changes in mice (Nieschulz, 1968), but very large doses (10-50 mg/kg bw) were required, probably because arecaidine and guvacine do not easily cross the blood-brain barrier (Lodge *et al.*, 1977) The possible role of arecaidine and guvacine in the psychopharmacological effect of betel quid is discussed in section 3.2(*b*).

Crude extracts of areca nut stimulate collagen synthesis in human fibroblast cultures (Canniff & Harvey, 1981). Tannins from chewed areca nuts reduced the susceptibility of collagen to degradation by collagenase *in vitro* (Meghji *et al.*, 1982).

Slaked lime

Epithelial atypia were observed in the cheek pouches of hamsters after repeated applications of calcium hydroxide (Dunham *et al.*, 1966). Slaked lime was painted on the palate and buccal mucosa of Wistar rats, and five treated and one control animals were killed at two-month intervals over a period of 12 months. Moderate to severe hyperplasia was seen in all treated animals, and most animals showed hyperkeratosis, cytoplasmic vacuolation and invagination of the rete pegs into the papillary layer (Sirsat & Kandarkar, 1968).

Effects on reproduction and prenatal toxicity

No data were available to the Working Group.

Absorption, distribution, excretion and metabolism

In rats, arecoline is de-esterified in the liver, and both arecoline and arecaidine are excreted as the mercapturic acid *N*-acetyl-*S*-(3-carboxy-1-methylpiperid-4-yl)-*L*-cysteine (Boyland & Nery, 1969).

Arecaidine reacts slowly *in vitro* with cysteine; however, there was no detectable reaction *in vitro* with nucleic acid bases (Boyland & Nery, 1969).

Total aqueous extracts and extracted tannins from areca nut were administered to rats dosed with proline and nitrite. As measured by the amount of *N*-nitrosoproline excreted in the urine, these extracts either catalysed or inhibited endogenous nitrosation, depending on the pH and the relative concentrations of nitrite and phenolic extracts (Stich *et al.*, 1984a).

Mutagenicity and other short-term tests

The results of the different short-term tests vary with the source and methods of storage and preparation of samples of betel quid, areca nut and betel leaf, as well as the extraction procedures used, and the results may therefore not necessarily be comparable.

Betel quid

An aqueous extract of betel quid without tobacco was mutagenic to *Salmonella typhimurium* TA1535 [details not given] and TA100, in the presence and absence of an Aroclor-induced rat-liver metabolic system (S9), but not to strains TA98 or TA1538. A dose-dependent increase in the number of revertants was observed in strain TA100 in the presence of S9 (Shirname *et al.*, 1983). An aqueous extract of betel quid without tobacco was not mutagenic to Chinese hamster V79 cells, with or without S9, and did not induce micronuclei in bone-marrow cells of Swiss mice (Shirname *et al.*, 1984).

An aqueous extract of betel quid with tobacco was mutagenic to *S. typhimurium* TA1535 [details not given] and TA100 in the presence and absence of Aroclor-induced S9; and there was a dose-dependent increase in the number of revertants in strain TA100 (Shirname *et al.*, 1983). An aqueous extract of betel quid with tobacco induced 8-azaguanine resistance in Chinese hamster V79 cells, with or without S9; the effect was slightly greater in the presence of S9. The same extract also induced micronuclei in bone-marrow cells of Swiss mice (Shirname *et al.*, 1984).

Areca nut

Aqueous extracts of areca nut induced a dose-dependent increase in the number of mutations in *S. typhimurium* TA1535 [details not given] and TA100 (Shirname *et al.*, 1983) and in Chinese hamster V79 cells (Shirname *et al.*, 1984). The presence of S9 enhanced the effect. The extract induced micronuclei in bone-marrow cells of Swiss mice (Shirname *et al.*, 1984).

An increase in the frequency of chromatid breaks and exchanges was observed in cultured Chinese hamster ovary cells following exposure to aqueous, ethyl acetate or *n*-butanol extracts of dried areca nut in the absence of an exogenous metabolic system. The chromo-

somal aberration activity of all three areca-nut extracts was enhanced by the addition of 10^{-4}M (5.5 μg/ml) Mn^{++} (Stich *et al.*, 1983a).

An effect of pH on the mutagenic activity of areca-nut preparations was demonstrated by Rosin (1984). An aqueous extract and a 'tannin'-containing fraction of areca nut induced mitotic gene conversion in *Saccharomyces cerevisiae* D7 at alkaline but not at acid pH, in the absence of an exogenous metabolic system.

An ethyl acetate extract of areca nut did not induce ouabain-resistant mutants in Chinese hamster V79 cells or sister chromatid exchanges in a human lymphoblastoid cell line, with or without rat-liver homogenate. The same extract did not induce transformation of Syrian hamster embryo cells (Umezawa *et al.*, 1981).

Areca-nut powder or paste mixed into the feed of *Drosophila melanogaster* larvae did not induce sex chromosome loss, sex-linked recessive lethal mutations or autosomal translocations (Abraham *et al.*, 1979). [The Working Group noted the small sample size used.]

Betel leaf

An aqueous extract of betel leaf was not mutagenic to *S. typhimurium* TA100, TA98, TA1535 or TA1538 with or without an exogenous metabolic system. This extract reduced the mutagenicity of an aqueous extract of areca nut in strain TA100 in the presence of rat-liver S9 (Shirname *et al.*, 1983).

Aqueous, *n*-butanol and ethyl acetate extracts of betel leaf induced chromosomal aberrations in Chinese hamster ovary cells; the latter extract was active only in the presence of 10^{-4}M (5.5 μg/ml) Mn^{++} (Stich *et al.*, 1983a).

An aqueous extract of betel leaf produced chromosomal aberrations in human lymphocytes in the absence of an exogenous metabolic system (Sadasivan *et al.*, 1978).

An ethyl acetate extract of betel leaf did not induce ouabain-resistant mutants in Chinese hamster V79 cells or sister chromatid exchanges in a human lymphoblastoid cell line, with or without rat-liver homogenate. The same extract did not induce transformation of Syrian hamster embryo cells (Umezawa *et al.*, 1981).

Betel-leaf powder or paste in the feed of *D. melanogaster* larvae did not induce sex chromosome loss, sex-linked recessive lethal mutations or autosomal translocations (Abraham *et al.*, 1979). [The Working Group noted the small sample size used.]

Areca-nut alkaloids

Arecoline induced mutations in *S. typhimurium* TA100, TA98, TA1535 and TA1538 [details given only for TA100] (Shirname *et al.*, 1983) and in Chinese hamster V79 cells (Shirname *et al.*, 1984), in the presence and absence of S9, and chromatid breaks and exchanges in Chinese hamster ovary cells in the absence of an exogenous metabolic system (Stich *et al.*, 1981). It also induced sister chromatid exchanges (Panigrahi & Rao, 1983), chromosomal aberrations (Panigrahi & Rao, 1982) and micronuclei (Shirname *et al.*, 1984) in bone-marrow cells of Swiss mice.

Arecaidine was mutagenic to *S. typhimurium* TA100, TA98, TA1535 and TA1538 [details given only for TA100] in the presence of S9, although it was less mutagenic than arecoline

(Shirname *et al.*, 1983). Arecaidine induced 8-azaguanine resistance in Chinese hamster V79 cells in the presence of S9 (Shirname *et al.*, 1984). A dose-dependent increase in the frequency of sister chromatid exchanges was observed in bone-marrow cells of Swiss mice injected intraperitoneally with arecaidine (Panigrahi & Rao, 1984). No elevation in the frequency of micronuclei was found in bone-marrow cells of Swiss mice (Shirname *et al.*, 1984).

(*b*) *Humans*

Toxic and pharmacological effects

Precancerous lesions occurring in betel-quid and areca-nut chewers are discussed on pp. 161-169.

(i) *General and psychopharmacological effects*

The toxic and pharmacological effects of betel quid have been reviewed by Arjungi (1976) and Mujumdar *et al.* (1982). Persons who are not accustomed to betel quid experience a disagreeable, acrid, burning taste, followed by a feeling of constriction in the throat. It causes roughness and slight ulceration of the tongue and buccal mucosa. With continued practice of the habit, these effects gradually decrease and are replaced by agreeable sensations and a feeling of well-being. The perception of taste becomes dulled due to the presence of an essential oil in the betel leaf and to the astringent action of the lime. Betel quid also stimulates salivary secretion and gives the saliva a red colour. In a study of 400 human subjects, caries, deposition of black tartar, regression of gums and partial or complete loss of sensibility of buccal mucosa were observed in 15-54% of habitual betel-quid chewers. Palpitation and dyspepsia were observed in 40-45% of the subjects, neurosis in 9%, and giddiness in 5% (Chopra *et al.*, 1958).

Chewing a quid of tobacco, areca nut and lime increased chemically-induced (10% citric acid) but not mechanically-induced (forced spitting) salivary flow (Reddy *et al.*, 1980). Betel-quid chewing increased nitrite levels in saliva after 1 h; however, no such increase was observed when betel quid was chewed with tobacco. In one study, chewing of betel quid with or without tobacco slightly increased salivary pH. Betel-quid chewing did not alter thiocyanate levels in saliva, but the presence of tobacco increased these levels significantly (Shivapurkar *et al.*, 1980b).

The immediate effects of betel-quid chewing appear to be the results of stimulation of the parasympathetic nervous system by arecoline, which are manifested in contraction of the sphincter of the iris, depression of heart muscle tonus, dilation of blood vessels, contraction of the bronchiolar smooth muscle, contraction of the walls of the gut but relaxation of the sphincters and contraction of the bladder. In addition, secretion of gastric juices, and of sweat, tears and saliva is increased (Katzung, 1982).

The greater interest, however, is in the mechanism of the psychopharmacological effects of betel. On the basis of experiments in animals (see section 3.2(*a*)), these have tentatively been ascribed either to the effect of arecoline on cholinergic neurons of the central nervous system or to the effect of arecaidine on the uptake of the neurotransmitter γ-aminobutyric acid (GABA). Only a few studies have been carried out on the effect of arecoline in humans, but these suggest that very small doses (2-4 mg) affect the human central nervous system in a way similar to that in animals (Sitaram & Weingartner, 1971; Christie *et al.*, 1981). The suggestion that the psychopharmacological effect of areca nut is the result of inhibition of

GABA uptake by arecaidine or guvacine (Nieschulz, 1968; Johnston *et al.* 1975) should be examined in the context of the suggestion that changes in the metabolism of GABA may play a role in some acute psychoses and in the anticonvulsant action of benzodiazepines (Gilman *et al.*, 1980). However, it seems unlikely that such inhibition plays a major role in the psychopharmacology of betel quid, because arecaidine and guvacine do not easily cross the blood-brain barrier (Lodge *et al.*, 1977), and, in experimental animals, large doses were required to produce behavioural changes (see section 3.2(*a*)).

Betel leaf produces a volatile oil containing the phenol chavicol, which has powerful antiseptic properties and antioxidant activity (Sethi & Aggarwal, 1956).

(ii) *Effects on periodontal tissues and teeth*

The use of betel quid, with or without tobacco, results in a series of local lesions affecting the periodontal tissues, the dental hard tissues (teeth) and the oral mucosa.

The first study demonstrating a detrimental influence of betel chewing upon the periodontal tissues was carried out by Mehta *et al.* (1955), who found a higher prevalence of periodontal disease among betel chewers than among non-chewers in a group of 1023 individuals from Bombay. Gupta (1964), who examined 1673 persons in Trivandrum, Kerala, in South India, found that the mean periodontal index (PI) for those who chewed betel was consistently greater than for those who did not chew, suggesting worse periodontal status in chewers.

In 1960, Waerhaug (1967) carried out a comprehensive survey among 8217 persons in Sri Lanka aged 13-60 years and over, 30% of whom had the habit of betel chewing. It was found that betel chewers over the age of 20 years had a very much higher PI (indicating periodontal breakdown) than non-chewers, even when subgroups of equivalent oral hygiene were compared.

In contrast, Howden (1982) found no difference in periodontal status between betel chewers and non-chewers in a sample of 75 persons on Aua Island, Papua New Guinea.

The teeth of habitual betel-quid chewers are stained black and show excessive attrition. The structural composition of the black layer, studied in North Thai hill tribes, was similar to that of subgingival calculus stained with areca-nut polyphenols and covered by a pellicle-like layer (Reichart *et al.*, 1985).

In Bombay, India, Chandra and Desai (1970) found an inverse association between prevalence of caries and both the duration and daily frequency of betel chewing in 930 adolescents and adults. Similar findings were reported by Möller *et al.* (1977) in Java and Bali, and by Schamschula *et al.* (1977) and Howden (1982) in Papua New Guinea.

Effects on reproduction and prenatal toxicity

Verma *et al.* (1983) studied the outcome of pregnancies in India in 70 chewers (45 used tobacco with betel quid, 25 tobacco alone) and in 70 non-chewing controls matched for several potential confounding variables. A statistically significantly lower birth weight, by an average of 395.3 g, was observed in the offspring of the tobacco-consuming group. [The Working Group noted that the matching procedure was not described, nor were the results of the matching presented.]

Absorption, distribution, excretion and metabolism

The nitrosated areca-nut alkaloid *N*-nitrosoguvacoline was detected (mean, 5.6 ng/ml) in the saliva of five chewers of betel quid without tobacco. This nitrosated alkaloid (mean, 75 ng/ml), together with *N'*-nitrosonornicotine (mean, 13 ng/ml), *N'*-nitrosoanatabine (mean, 15.6 ng/ml) and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (mean, 1.5 ng/ml), were detected in the saliva of six chewers of betel quid with tobacco. The salivary thiocyanate concentration was similar in the two groups (0.17 and 0.22 μmol/ml, respectively). No *N*-nitrosoguvacoline was detected in the saliva of four people who neither chewed betel nor smoked (Wenke *et al.*, 1984).

Similar results have been obtained by Nair *et al.* (1985). The nitrosated areca-nut alkaloids, *N*-nitrosoguvacoline (mean, 0.9 ng/ml) and *N*-nitrosoguvacine (mean, 3.2 ng/ml) were found in the saliva of 12 men chewing betel quid without tobacco, together with a great increase in the content of *N*-nitrosoproline (3.2 ng/ml *versus* 1.0 ng/ml in the control, no-habit group). In urine collected over 6 h, betel chewers excreted 60 ng *N*-nitrososarcosine, 440 ng *N*-nitrosoproline and 470 ng *N*-nitrosothiazolidine-4-carboxylic acid, compared with excretion of only 43 ng, 220 ng and 0 ng, respectively, of each of these nitrosamines by the control, no-habit group. However, in two subjects who ingested proline and nitrate together with an aqueous, ethereal or *n*-butanol extract of areca nut, endogenous nitrosation, as measured by *N*-nitrosoproline excreted in urine, was inhibited (Stich *et al.*, 1983b). The increased excretion of *N*-nitrosothiazolidine-4-carboxylic acid and of *N*-nitrosoproline (Stich *et al.*, 1983b) suggests an increase in endogenous nitrosation.

The saliva of 12 men chewing betel quid with tobacco contained *N*-nitrosoguvacoline (0.9 ng/ml), *N*-nitrosoguvacine (4 ng/ml), and *N*-nitrosoproline (5 ng/ml); *N'*-nitrosonornicotine (7.5 ng/ml), *N'*-nitrosoanatabine (4.8 ng/ml) and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (0.3 ng/ml) were also detected. In a 6-h urine sample, these subjects excreted 100 ng *N*-nitrososarcosine and 260 ng *N*-nitrosoproline, and they also excreted 280 ng *N*-nitrosothiazolidine-4-carboxylic acid, which suggests that there is an increase in endogenous nitrosation in these chewers also. The subjects excreted an average of 960 ng nicotine and 430 ng cotinine in the 6-h urine sample, indicating substantial absorption of nicotine from the buccal cavity. In-vitro nitrosation of three samples of betel quid without tobacco for 1 h at pH 7.4 with nitrite (50 mg/kg) in the presence of thiocyanate (100 mg/kg) yielded *N*-nitrosoguvacoline and *N*-nitrosoguvacine; this finding may explain the relatively large amounts of these compounds in the saliva of betel chewers (Nair *et al.*, 1985).

Mutagenicity and chromosomal effects

The proportion of exfoliated micronucleated cells from the buccal mucosa of 17 Khasi (India) chewers of areca nut, betel leaf and lime was 4.7% (range, 2.5-7.5%); in 20 Indian (Hindi) chewers of betel quid with tobacco, it was 7.3% (range, 3.3-13.1%); and in 51 Ifugao (Philippines) chewers of areca nut, betel leaf, tobacco and lime, it was 3.7%. The proportion of exfoliated cells in the buccal mucosa was 0.5% (range, 0.0-0.8%) in 14 non-chewing Khasis, 0.4% (0.0-0.9%) in 34 non-chewing Indians (Hindi) and 0.5% (0.0-0.8%) in 17 non-chewing Ifugaos (Stich *et al.*, 1982; Stich & Rosin, 1984; Stich *et al.*, 1985).

The frequency of micronucleated buccal-mucosa cells is influenced by several factors. It is greatest at the site within the oral cavity where the betel quid is in close contact with the mucosa, and depends on the number of betel quids chewed: 1.5% in chewers of less than four quids per day, compared to 7.05% among users of 15 or more quids per day (Stich *et al.*, 1983c). The frequency of micronucleated buccal-mucosa cells was reduced following a

two- or three-month administration of vitamin A or β-carotene (Stich *et al.*, 1985), or a mixture of the two (Stich *et al.*, 1984b).

The frequency of sister chromatid exchanges was determined in peripheral lymphocytes of 20 non-chewing men, 18 men who chewed betel quid without tobacco and 15 men who chewed betel quid with tobacco (Ghosh & Ghosh, 1984). The average number per cell was elevated in chewers of betel quid with or without tobacco.

Urine samples from chewers of betel quid with tobacco were concentrated on an XAD column and tested for mutagenicity in *Salmonella typhimurium* TA1538 in the presence of a metabolic system (S9). Samples from six out of 14 subjects were mutagenic; 15 samples from subjects with no tobacco habit were not (Menon & Bhide, 1984).

3.3 Studies of precancerous lesions and conditions in humans

Descriptions of oral leukoplakia, a precancerous lesion, and of the precancerous conditions submucous fibrosis and, possibly, lichen planus are given in the monograph on tobacco habits other than smoking, on p. 89 of this volume. The studies summarized here include those carried out in Asia in which the chewing habits of the subjects are not precisely defined, particularly in reference to the inclusion of areca nut in the quid.

Leukoplakia

(*a*) *Prevalence*

The prevalence of oral leukoplakia among chewers of betel and/or tobacco in selected population samples in India, Malaysia and the Pacific area is shown in Table 9. In the studies by Mehta *et al.* (1961, 1971) and Pindborg *et al.* (1967, 1968), strict criteria were applied in the diagnosis of leukoplakia, in contrast to the studies by Forlen *et al.* (1965), Chin and Lee (1970) and Smith *et al.* (1975). [In particular, the figures of Chin and Lee (1970) appear to be overestimated substantially.] Higher prevalences of leukoplakia were found in two studies in subjects who included tobacco in the betel quid. However, owing to the variety of habits in countries in Asia, it is often difficult to find sufficiently large groups with a single habit: Pindborg *et al.* (1967), who surveyed customs in several countries, found no less than 30 different ways of chewing tobacco and/or betel with or without other types of tobacco usage.

Mehta *et al.* (1969a, 1972a) carried out house-to-house surveys in Indian villages. Chewing of betel quid with tobacco was usually associated with a higher prevalence of leukoplakia than the chewing of betel quid alone or than no chewing habit (Table 10). Wahi *et al.* (1970), in another such survey, showed that the habit of chewing, especially of Mainpuri tobacco, was associated with a higher prevalence of oral leukoplakia than no chewing. [The Working Group noted that no distinction was made between those chewing betel alone and those chewing betel with tobacco.]

(*b*) *Incidence and spontaneous-regression rates*

Incidence rates for leukoplakia are available only from India.

Table 9. Prevalence of oral leukoplakia among chewers and controls in selected studies in Asia and the Pacific

Reference	Location	Chewing habit	Size of sample	Prevalence	
				No.	%
Gerry *et al.* (1952)	Guam	Betel quid	822	4	0.5
Mehta *et al.* (1961)	Bombay (police)	Betel quid with tobacco	1898	80	4.2
		Betel quid and smoking	595	42	7.1
		No habit	1112	1	0.001
Forlen *et al.* (1965)	Papua New Guinea	Areca nut and smoking	610	-	9.7-36.3
Pindborg *et al.* (1967)	Lucknow, India (out-patient clinic)	Tobacco alone	206	15	7.3
		Betel quid with tobacco	672	30	4.5
		Betel quid without tobacco	181	6	3.3
		No habit	6699	2	0.03
Pindborg *et al.* (1968)	Papua New Guinea	Areca nut	162	2	1.2
		Areca nut and smoking	767	29	3.8
		No habit	165	-	-
Chin & Lee (1970)	Perak, West Malaysia	Betel quid with tobacco	167	67	40.1
		Betel quid without tobacco	45	9	20.0
		Gambir[a]	45	5	11.1
Mehta *et al.* (1971)	Ernakulam (Kerala), India	Betel quid with tobacco	2661	47	1.8
		Betel quid without tobacco	38	-	-
		Chewing and smoking	1106	67	6.1
		No habit	4210	8	0.2
	Srikakulam (Andhra Pradesh), India	Betel quid with tobacco	281	-	-
		Betel quid without tobacco	56	-	-
		Chewing and smoking	803	23	2.9
		No habit	2620	3	0.1
	Bhavnagar (Gujarat), India	Betel quid with tobacco	299	3	1.0
		Betel quid without tobacco	157	1	0.6
		Mishri	714	2	0.3
		Chewing and smoking	320	19	5.9
		No habit	5647	-	-
	Darbhanga (Bihar), India	Betel quid with tobacco	1572	6	0.4
		Betel quid without tobacco	138	2	1.4
		Chewing and smoking	1485	6	0.4
		No habit	3719	-	-
	Singhbhum (Bihar), India	Betel quid with tobacco	1293	5	0.4
		Betel quid without tobacco	41	-	-
		Gudakhu	832	-	-
		Chewing and smoking	730	2	0.3
		No habit	4454	1	0.02
Smith *et al.* (1975)	Ahmedabad (Gujarat), India (mainly textile-mill workers)	Tobacco chewing	1515	193	12.7
		Smoking and tobacco chewing	2319	300	12.9
		Betel quid/areca nut without	2687	144	5.4
		tobacco	12907	2264	17.5
		Smoking and betel quid	8710	112	1.3
		No habit			
Lin *et al.* (1983) (cited in Pindborg *et al.*, 1984a)	Hainan Island, China	Betel quid	954	-	2.5

[a]*Gambir,* a quid consisting of mature betel leaf, *gehta gambir,* fresh areca nut and lime. *Gehta gambir* is an extract of the shrub *Uncaria gambir,* which contains catechin.

In a 10-year follow-up study of 3674 Bombay policemen, Mehta *et al.* (1972b) reported highest five-year and 10-year incidence rates of leukoplakia among those who chewed betel quid, usually with tobacco, and smoked (5.2% and 8.9%, respectively), and lowest rates among those who neither chewed nor smoked (0.6%). Regression rates were 3.5% for those who chewed only and 9.1% for those with a mixed habit. No regression of lesions was noted in those individuals without a smoking or chewing habit.

Table 10. Prevalence of oral leukoplakia among chewers and controls in various locations in India[a]

Location	Chewing habit	Size of sample	Prevalence (%)
Gujarat	Betel quid with tobacco	299	1.0
	Betel quid without tobacco	157	0.6
	No habit	6361	-
Kerala	Betel quid with tobacco	2661	1.8
	Betel quid without tobacco	38	-
	No habit	4210	0.2
Andhra Pradesh	Betel quid with tobacco	281	-
	Betel quid without tobacco	56	-
	No habit	2620	0.1
Bihar, Singbhum	Betel quid with tobacco	1293	0.4
	Betel quid without tobacco	41	-
	No habit	5286	0.02
Bihar, Darbhanga	Betel quid with tobacco	1572	0.4
	Betel quid without tobacco	138	0.7
	No habit	3719	-
Maharashtra[b]	Tobacco with/without betel quid	28638	1.4
	Betel quid without tobacco	591	0.3

[a]From Mehta *et al.* (1969a), unless otherwise specified
[b]From Mehta *et al.* (1972a)

In a two-year follow-up study of 57 518 industrial workers in Gujarat, Bhargava *et al.* (1975) found most new leukoplakias (585) in the 9506 (6.2%) persons who chewed betel quid with areca nut and smoked; 80 (3.8%) occurred in those who chewed betel quid with areca nut only and 130 (11.2%) in those who chewed tobacco only. In those with no habit, there were 82 (1.2%) new leukoplakias. An overall spontaneous-regression rate of 31.6% was observed. [The Working Group noted that it was not specified if tobacco was included in the quid.]

Silverman *et al.* (1976), who examined 4762 of 6718 of the same workers with oral leukoplakia in Gujarat after two years, found a yearly incidence of new lesions of 2.6%. The spontaneous-regression rate was 31.6%. Although 1.7% of leukoplakias were seen in people with no habit in the original group, no new leukoplakia was seen two years later.

Gupta *et al.* (1980) carried out a 10-year follow-up survey of oral lesions in three areas of India: Ernakulam (Kerala), Bhavnagar (Gujarat) and Srikakulam (Andhra Pradesh). The age-adjusted incidences of leukoplakia (non-palatal) are given in Table 11. The highest incidence was observed in Ernakulam in men who chewed betel quid with tobacco and smoked (six per 1000), and no new case was found among those who did not chew or smoke. Regression rates were calculable only for Ernakulam, where a high annual regression rate (10.4%) was seen among women with a chewing habit; men who chewed had a regression rate of only 3.3%, and that in men with a mixed habit was 3.6%. A rate of 33.3% was seen in men (from the baseline study) with no current chewing habit. No regression was seen in women with no habit or mixed habits.

(*c*) *Histology*

A brief histological description of leukoplakia induced by chewing betel quid with tobacco was given by Orr in 1933. Wahi *et al.* (1961) showed three photomicrographs of leukoplakia in smokers and chewers, but they did not correlate the histological findings with the different habits.

Table 11. Age-adjusted incidence of oral leukoplakia per 1000 per year in a 10-year follow-up study in India[a]

	Chewing habits	Mixed habits
Ernakulam		
Men	2.5	6.0
Women	3.0	-
Bhavnagar		
Men	0.7	-
Women	2.5	-
Srikakulam		
Men	1.4	-
Women	0.8	-

[a]From Gupta *et al.* (1980)

Pindborg *et al.* (1964) studied 39 biopsies from Indian patients with leukoplakia; 16 were from 15 patients who chewed tobacco alone, 10 from patients who smoked *bidis* and chewed betel quid with tobacco, seven from *bidi* smokers and six from five patients who smoked cigarettes. In tobacco chewers, the labial mucosa exhibited epithelial hyperplasia with an unusual hyperparakeratosis, sometimes associated with increased mitotic activity, whereas the buccal mucosa showed atrophy and hyperorthokeratosis. In patients who chewed betel quid with tobacco and smoked *bidis*, the epithelium showed either atrophy or hyperplasia, with hyperorthokeratosis as the most common hyperkeratinization.

Meyer *et al.* (1967) studied biopsies from 16 Indian patients with leukoplakia who chewed betel quid with tobacco. All leukoplakias showed keratinization, and in every keratinized specimen, both para- and orthokeratin were present. Virtually no dividing cell was present in specimens with orthokeratin layers representing 30% or more of the total epithelial thickness. Inflammation was always present in the lamina propria.

Sirsat and Doctor (1967) studied biopsies from 60 Indian tobacco chewers, the tobacco most often being included in a betel quid. Leukoplakia was present in 22 patients, and 30 had an oral carcinoma; in the latter group, biopsies were taken adjacent to the tumour and from the opposite side. The commonest change observed was hyperplasia of the epithelium; parakeratosis was also a common finding, whereas hyperkeratosis [hyperorthokeratosis] was less commonly observed. Dyskeratosis (epithelial dysplasia) was observed in 20% in the leukoplakia group, and in 37% in the group with intraoral carcinomas. Inflammation was often present in both groups.

Tennekoon and Bartlett (1969) studied buccal biopsies from 108 chewers of betel quid plus tobacco and from 42 non-chewers in Sri Lanka. The epithelial changes in the buccal mucosa of betel-quid chewers and controls were similar; generally, a slight degree of chronic inflammatory cell reaction was observed. The authors suggested that other factors, such as dietary habits or deficiencies and poor oral hygiene, may play a role in these changes.

In Malaysia, Lee and Chin (1970) studied 77 biopsies of buccal leukoplakias from 52 chewers; 67 of the biopsies were from chewers of betel quid with tobacco and 10 from chewers of betel quid without tobacco. Of the total, 68% showed the presence of an amorphous brown-staining von Kossa-positive layer on the surface of the keratin. Tobacco did not appear to be essential for its formation, but it appeared to be significantly associated with parakeratosis. Parakeratosis was the most common form of keratinization, and the mitotic activity in parakeratinized leukoplakias was significantly greater than that in orthokeratin-

ized leukoplakias. Epithelial atrophy appeared to be significantly related to the duration of the habit of chewing quids containing tobacco, but not to the 'intensity' of the habit.

During an epidemiological survey on oral habits and associated oral lesions in Thailand, Reichart *et al.* (1984) took 18 biopsies from the oral mucosa of chewers of betel quid with tobacco for examination by transmission and scanning electron microscopy. Among the 18 patients, three had preleukoplakia, two, leukoplakia, one, leukoedema and one, submucous fibrosis. Histologically, epithelial atrophy with marked reduction of the rete pegs, hyperorthokeratosis and/or parakeratosis, and subepithelial oedema and inflammatory changes were the most prominent findings. At the ultrastructural level, cytoplasmic projections of the basal cells into the subepithelial stroma were seen. The basal membrane frequently revealed gaps, the interepithelial space was widened and unusual microvilli were observed on cell surfaces. Intercellularly, crystalloid material of unknown origin was also seen. The subepithelial connective tissue was characterized by bundles of collagen fibres, adjacent to which masses of amorphous material were located. While some of the ultrastructural findings in the epithelium of chewers are indicative of early dysplastic changes, the nature of the juxta-epithelial stromal alterations is still unknown.

Forlen *et al.* (1965) studied 10 biopsies from leukoplakic lesions in Papua New Guineans, all of whom were betel chewers and smokers. They found a peculiar vacuolization of the basal epithelial cells, similar to the ballooning cells observed in the upper part of the spinal cell layer. The type of keratinization was para- and/or orthokeratotic.

Pindborg *et al.* (1968) examined biopsies from four patients with leukoedema, six with preleukoplakia and 16 with leukoplakia in Papua New Guinea. They found a vacuolated cell layer on the surface of two biopsies from preleukoplakia areas and a surface layer of 'ballooning' cells in biopsies from leukoedema patients. Previously, these cells had been considered to be caused by tobacco. In this study, they were demonstrated among betel chewers who did not include tobacco in the quid. However, almost all the betel chewers smoked heavily. Epithelial atypia [dysplasia], carcinoma *in situ* or carcinoma were not seen among the 26 biopsies.

(*d*) *Malignant transformation*

In many of the earlier histological studies of oral cancer, e.g., that of Paymaster (1956), leukoplakia was seen concomitantly with the cancer.

In the 10-year follow-up study of Bombay policemen (Mehta *et al.*, 1961, 1969b, 1972b), one oral cancer developed among 117 cases of leukoplakia in an individual who chewed betel quid (presumably with tobacco) and who also smoked *bidis*.

In the follow-up of Bhargava *et al.* (1975) in Gujarat, India, 22 histologically-confirmed cases of oral cancer were seen among 43 654 persons re-examined after two years. The authors stated that seven (0.13%) of the cases had developed from leukoplakia. Of the 4762 persons with leukoplakia re-examined after two years by Silverman *et al.* (1976), six had developed oral carcinoma, giving an annual incidence of 63/100 000. One man chewed tobacco plus betel quid only, two both chewed (one tobacco, the other tobacco plus betel quid) and smoked *bidis*, two smoked *bidis* only, and the one female case took nasal snuff only.

In a 10-year follow-up in Ernakulam (Kerala), South India, of 410 leukoplakia patients, all of whom were tobacco chewers, nine (six men and three women) developed oral carcinoma

(Gupta *et al.*, 1980). The crude annual rate of malignant transformation was 3.9 per 1000 in men and 6.0 per 1000 in women. Four other oral cancers were observed: two in patients who had been diagnosed with 'preleukoplakia', one in a patient with submucous fibrosis and the other in a case of lichen planus. No oral cancer was seen in subjects who had been normal at the previous examination.

(*e*) *Effects of intervention*

Mehta *et al.* (1982) reported on an intervention study of oral cancer and precancer undertaken in the same areas of India where the 10-year follow-up survey described above had been carried out (Gupta *et al.*, 1980). In each of the three study areas, 12 000 adults with chewing and/or smoking habits were selected, interviewed and examined once a year, and information on the ill effects of tobacco use was given. The results after one year, with regard to chewing habits, are given in Table 12. In Ernakulam, 76 patients with leukoplakia had reduced or stopped their habit; of these, four (5.3%) had regression of their lesion; of 263 who did not reduce or stop their habit, three (1.1%) had regression. In Bhavnagar, only nine of 529 patients with leukoplakia had reduced or stopped their habit; of these, five (55.6%) had regression, while 67 of the 520 (12.9%) of those who did not change their habit had regression.

Table 12. Changes in chewing habits and mixed habits (smoking and chewing) in three areas of India one year after intervention[a]

	No. of subjects	Reduced		Stopped	
		No.	%	No.	%
Ernakulam					
Chewing	4428	288	6.5	120	2.7
Mixed habits	1646	176	10.7	7	0.4
Bhavnagar					
Chewing	748	4	0.5	39	5.2
Mixed habits	170	0	-	0	-
Srikakulam					
Chewing	401	107	26.7	40	10
Mixed habits	1319	286	21.7	44	3.3

[a]From Mehta *et al.* (1982)

Submucous fibrosis

In recent years, the hypothesis has been put forward that the chewing of areca nut has an etiological role in the development of submucous fibrosis (Mehta *et al.*, 1972a; Shiau & Kwan, 1979; Gupta *et al.*, 1980).

(*a*) *Prevalence*

In a survey of villagers in five areas of India, Mehta *et al.* (1971) found submucous fibrosis in people with various chewing and smoking habits. The prevalences are shown in Table 13.

Table 13. Prevalences of submucous fibrosis and lichen planus in five areas of India[a]

Area	Chewing habit[b]	Size of sample	Prevalence of submucous fibrosis		Prevalence of lichen planus	
			No.	%	No.	%
Ernakulam (Kerala)	Betel quid with tobacco	2661	29	1.1	50	1.9
	Betel quid without tobacco	38	-	-	-	-
	Chewing and smoking	1106	5	0.4	41	3.7
	No habit	4210	2	0.05	3	0.07
Srikakulam (Andhra Pradesh)	Betel quid with tobacco	281	1	0.4	1	0.4
	Betel quid without tobacco	56	-	-	-	-
	Chewing and smoking	803	-	-	7	0.9
	No habit	2620	-	-	1	0.04
Bhavnagar (Gujarat)	Betel quid with tobacco	299	-	-	1	0.3
	Betel quid without tobacco	157	-	-	-	-
	Mishri	714	-	-	-	-
	Chewing and smoking	320	-	-	1	0.3
	No habit	5647	16	0.3	-	-
Darbhanga (Bihar)	Betel quid with tobacco	1572	-	-	5	0.3
	Betel quid without tobacco	138	2	1.4	-	-
	Chewing and smoking	1485	3	0.2	3	0.2
	No habit	3719	-	-	-	-
Singhbhum (Bihar)	Betel quid with tobacco	1293	-	-	4	0.3
	Betel quid without tobacco	41	-	-	-	-
	Gudakhu	832	-	-	-	-
	Chewing and smoking	730	-	-	-	-
	No habit	4454	-	-	2	0.04

[a]From Mehta *et al.* (1971)

(b) Incidence and spontaneous-regression rates

In a two-year follow-up study of 43 654 industrial workers in Gujarat, India, Bhargava *et al.* (1975) found seven new cases of submucous fibrosis among 2105 (0.3%) people who chewed betel quid with areca nut; six among 9506 (0.1%) who both chewed and smoked; three among 1161 (0.3%) who chewed tobacco alone; and 10 among 7065 (0.1%) with no such habit.

In the 10-year follow-up survey of Gupta *et al.* (1980), the age-adjusted incidences per 100 000 for submucous fibrosis were seven for men and 17 for women in Ernakulam; the annual incidences per 100 000 were 2.6 for men and and 8.5 for women in Bhavnagar. In Ernakulam, all 11 new cases (out of 39 828 villagers) occurred among chewers of tobacco or of tobacco and betel quid or those with a mixed habit (including smoking). In Bhavnagar, of the four new cases seen in 38 818 persons, two had no tobacco habit, one chewed and one smoked.

(c) Histology

The precancerous nature of submucous fibrosis was first mentioned by Paymaster (1956), who observed the development of a slow-growing, squamous-cell carcinoma in one-third of patients with submucous fibrosis.

The histological features of submucous fibrosis were described in detail by Pindborg (1972), on the basis of examination of 220 biopsies from patients with this condition. The finding of epithelial atypia in 13.2% of biopsies suggested that it is a precancerous condition.

(*d*) *Malignant transformation*

Gupta *et al.* (1980) reported malignant transformation in one out of 44 cases of oral submucous fibrosis in Ernakulam (Kerala); none were found among five cases in Srikakulam.

A follow-up study over four to 15 years of 66 patients with submucous fibrosis was carried out in Ernakulam. Malignant transformation was observed in three patients three, four and seven years after initial examination, giving an overall rate of 4.5% (Pindborg *et al.*, 1984b).

McGurk and Craig (1984) have reported malignant transformation of submucous fibrosis in two Indian women living in the UK. Only one of the women had the habit of chewing areca nut, but both had latent iron deficiency.

Lichen planus

(*a*) *Prevalence*

The prevalence of lichen planus in five areas of India, as reported by Mehta *et al.* (1971), is given in Table 13.

In a house-to-house survey in Ernakulam (Kerala) of 7639 villagers, oral lichen planus was found in 1.5% of men and 1.6% of women. The prevalence in various habit groups is given in Table 14; the highest prevalence was found in chewers of betel quid with tobacco (Pindborg *et al.*, 1972).

Table 14. Prevalence of lichen planus in chewers and controls in Kerala, India[a]

Habit	Number	Lichen planus	
		Number	%
Chewing			
Tobacco and lime	212	3	1.4
Betel quid without tobacco	24	-	-
Betel quid with tobacco	1925	61	3.2
Smoking			
Bidis	1334	10	0.7
Others	388	3	0.8
Chewing and smoking	845	31	3.7
None	2911	10	0.3

[a]From Pindborg *et al.* (1972)

A lichen planus-like lesion was observed among chewers in Kerala exclusively at the site of placement of the quid. Among 1170 chewers of both betel quid and tobacco, 2.6% had the lesion, as did 0.8% of a group of 500 persons who chewed betel quid with tobacco and smoked. None were seen in 36 persons who chewed betel quid without tobacco (Daftary *et al.*, 1980).

(*b*) *Incidence and spontaneous-regression rate*

In a two-year follow-up study of 43 654 industrial workers in Gujarat, India, Bhargava *et al.* (1975) found 13 new cases of lichen planus among 2105 (0.6%) people who chewed betel quid with areca nut; 24 among 9506 (0.3%) who both chewed and smoked; eight among 1161 (0.7%) who chewed tobacco alone; and 29 among 7065 (0.4%) with no such habit.

In the 10-year follow-up survey of Gupta *et al.* (1980), age-adjusted incidences of lichen planus per 1000 per year in Ernakulam were 8.2 for men with mixed habits (including smoking) and 3.7 for men who chewed tobacco or betel quid plus tobacco only; nil for women with mixed habits and 4.5 for those who chewed; the rates were 0.6 and 0.9 for men and women, respectively, with no such habit. The regression rates per year were 5.5% and nil in men and women with mixed habits; 7.5% and 11.5% for those who chewed only; and 16.7% and 19.2% for those with no such habit, respectively.

(*c*) *Histology*

Daftary *et al.* (1980) studied 30 biopsies from patients with oral lichen planus in Ernakulam (Kerala), of which 22 showed epithelial atrophy, one hyperplasia, four normal thickness, and three a combination of epithelial atrophy and hyperplasia. Hyperparakeratosis was a predominant histological characteristic. Six patients showed a mild or moderate epithelial dysplasia.

(*d*) *Malignant transformation*

Gupta *et al.* (1980) observed one oral cancer case among 332 individuals seen with lichen planus.

3.4 Case reports and epidemiological studies of carcinogenicity in humans

Some of the material that follows was reviewed by Jayant (1977), who examined the causality of the relationship between tobacco chewing and oral cancer; by Gupta *et al.* (1982), who compared the carcinogenicity of betel quid with and without tobacco; and by a meeting of the World Health Organization (WHO, 1984) on the control of oral cancer in developing countries.

(*a*) *Descriptive studies and case series*

Oral cancer was described in the *Sushruta Samhita*, a treatise on Indian surgery written in Sanskrit around 600 BC. Especially well-detailed descriptions are given of cancer of the lip, commissure, alveolus, tongue, palate, pharynx and larynx, indicating that these cancers were well known to physicians at that time and therefore probably quite common (Suraiya, 1973). Literary references to the habit of chewing betel quid (betel leaf, areca nut and lime) in India are also at least 2000 years old (Gode, 1961). Tobacco, of course, was introduced into India much later, as everywhere else — around the sixteenth century.

Reports on the association of oral cancer with the habit of chewing betel quid started to appear in the medical literature during the late nineteenth century. In all the reports summarized in Table 15 (where the type of oral cancer was not always specified), the percentage of oral cancer among all cancers diagnosed in hospitals or groups of hospitals in Asia was always much higher than that usually found in western countries. The authors often pointed out that, since the oral cavity is an accessible site, oral cancers are likely to be over-represented in comparison to other cancers; however, the habit of chewing betel quid, with or without tobacco, is very common among people of these regions, and this habit is virtually unknown in western countries. [The Working Group noted that evidence of this nature is inherently weak.]

In Papua New Guinea, oral cancer is generally the most common form of cancer, and the predominant chewing habit is that of betel leaf, areca nut and lime without the addition of tobacco. Two studies (Atkinson *et al.*, 1964; Henderson & Aiken, 1979) were based on a cancer survey and continuing cancer-registration system. Atkinson *et al.* (1964) concluded that, since the occurrence of oral cancer correlated very well with the known distribution of

Table 15. Frequency of oral cancer in betel-quid users

Location	Habit	All cancers (years)	Oral cancer	Reference
Papua New Guinea	Betel quid without tobacco	1175 (1958-1963)	209 (17.8%)	Atkinson *et al.* (1964)
Papua New Guinea	Betel quid without tobacco	2300 (1958-1965)	(17.1%); 29 (9%) oral cancers were verrucous carcinoma	Cooke (1969)
Papua New Guinea	Betel quid without tobacco	6186 (1958-1973)	890 (14.4%)	Henderson & Aiken (1979)
Travancore, South India	Betel quid with tobacco	1700 (five years)	989 (58%)[a]	Bentall (1908)
Neyoor, South India	Betel quid with tobacco	377 epithelial cancers (two years)	346 (91.5%)[b]	Fells (1908)
Bombay, India	Betel quid with tobacco	2880 carcinomas (1941-1943)	1000 (34.7%)[c]	Khanolkar (1944)
Bombay, India (Parsees)	Betel-quid chewing very rare	1705 (1941-1965)	160 (9.4%)[d]	Paymaster & Gangadharan (1970)
Sri Lanka	Betel quid	2344 (1928-1948)	1130 (48.2%)[e]	Balendra (1949)
Thailand	Betel quid	1100	155 (14.1%)[f]	Piyaratn (1959)
Malaysia (Indians)	Betel quid with tobacco	-	219[g]	Marsden (1960)
Singapore	Betel quid with tobacco	7131	(8%)[h]	Muir (1962)
Philippines	Betel leaf, tobacco chewing, reverse cigarette smoking	- (1957-1961)	186	Tolentino *et al.* (1963)
Malaysia	Betel quid with and without tobacco	4369 (1961-1963)	476 (10.9%)[i]	Ahluwalia & Duguid (1966)
Indians	Betel quid with tobacco	912	306 (33.6%)	Ahluwalia & Duguid (1966)
Malays	Betel quid without tobacco	777	74 (9.5%)	Ahluwalia & Duguid (1966)
Bangladesh	Betel quid	3650	672 (18.4%)[j]	Huq (1965)
Pakistan	Betel quid with tobacco, cigarette smoking	14350 (1960-1971)	2608 (18.2%)	Zaidi *et al.* (1974)

[a]Lip, tongue, buccal mucosa
[b]Epithelial cancers of the buccal cavity
[c]Lip, buccal mucosa, alveolus, tongue, palate
[d]Lip, tongue, alveolus, floor of mouth, buccal mucosa, palate
[e]Cheek, tongue, palate and tonsil, jaw, floor of mouth, pharynx and larynx, lip
[f]Lip, tongue, oral cavity
[g]'Betel cancers'
[h]Buccal cavity, pharynx
[i]Lip, tongue, floor of mouth, cheek, palate
[j]Buccal cavity

the betel-chewing habit, areca nut and lime had a definite carcinogenic effect even when chewed without tobacco. [The Working Group noted that the authors did not take into consideration cigarette smoking, which was reported to be common.] Henderson and Aiken (1979) observed that the site distribution of their oral cancer cases was consistent with the reported site distribution of oral cancer among betel chewers from other parts of the world. Cooke (1969) observed that only 5% of all verrucous carcinomas of the oral mucosa occurred in people in the highlands [where 50% of the population lived, but where areca nut does not grow and betel chewing is less popular (Henderson & Aiken, 1979)]. Cigarette smoking was reported to be common in both the highlands and lowlands.

Two of the earliest studies from India were those by Fells (1908) and Bentall (1908). Fells reported that 91.5% of 377 epithelial cancers seen at Neyoor were oral cancers; and Bentall, who compiled data from 15 mission hospitals and 34 other hospitals in Travancore, including Neyoor, reported 58% of all cancers (1700) to be of the oral cavity. They noted that the habit of chewing betel quid was very common. Khanolkar (1944), reviewing the distribution of oral cancer and chewing habits, concluded that the habitual chewing of betel leaf and areca nut had no etiological role in the development of mouth cancer but that the inclusion of tobacco in the quid made it carcinogenic. Paymaster and Gangadharan (1970) enumerated, from hospital records, cancers occurring among Parsees, who are followers of the Zoroastrian religion. They found that 9.4% of Parsee cancer patients had oral cancer and that this percentage was much lower than that previously observed among other religious groups; Parsees do not generally smoke and very rarely chew betel, with or without tobacco.

In Sri Lanka, Thailand, Malaysia, Singapore, the Philippines, Bangladesh and Pakistan, the predominant habit is chewing of betel with tobacco. Balendra (1949) expressed the opinion that oral cancer in Sri Lanka was due not to the betel-chewing habit but to sharp teeth surfaces resulting from the chewing action. In Malaysia (Marsden, 1960; Ahluwalia & Duguid, 1966), oral cancer is common in the Indian community (33.6%) but is rare among Malays (9.5%). It was pointed out that Indians and Malays both chew betel quid, the only difference being that Indians add tobacco to their quid, whereas Malays usually do not. In Pakistan (Zaidi *et al.*, 1974), the time trends of proportions of various cancers recorded during 1960-1971 in a department of radiotherapy were compared. The proportion of oral cancer appeared to decrease very rapidly among women and less rapidly among men, and the decrease was attributed to a reduced availability of betel leaf and areca nut, the principal ingredients for chewing, and *bidis* for smoking, after the partition of India and Pakistan. [The Working Group considered that changing patterns of referral or treatment may also have influenced the proportion of oral to other cancers seen at radiotherapy.]

Additional data showing an elevated frequency of oral cancer in several countries [Thailand, South Viet Nam, Malaysia, Indonesia, China (including Taiwan) and India] where chewing of betel is common were compiled by Pindborg (1965), partially on the basis of personal communications and unpublished reports.

In many descriptive studies, investigators have obtained histories of chewing of betel with tobacco from series of oral-cancer patients (Table 16). In most of these studies, the percentage of oral-cancer patients who practise chewing habits is extremely large. Several authors also commented that the tumour generally develops at the place where the chewing quid is kept.

Table 16. Descriptive studies of oral cancer in case series

Location	Habit	All cancers (years)	Oral cancer	Reference
South-west Pacific Islands - New Britain	Betel quid without tobacco	60 (1921-1940)	7 (11.7%)	Eisen (1946)
Papua New Guinea	Betel quid without tobacco (98%)	-	110	Farago (1963a)
Papua New Guinea	Betel quid without tobacco (129/130)	1160 (1960-1961)	210 (18.1%)	Farago (1963b)
Bombay, India	Tobacco and betel-quid chewing (excessive in 35%)	3627 intra-oral malignant tumours (1941-1947)	650 (buccal mucosa)	Paymaster (1956)

Location	Habit	All cancers (years)	Oral cancer (%)	Reference
Guntur, India	Betel-quid chewers: 9 (3.6%) Betel-quid + tobacco chewers: 29 (12%) Tobacco chewers: 20 (8%)	- (1957-1959)	250 (17.4%) (oral + pharyngeal)	Padmavathy & Reddy (1960)
Bombay, India	36.5% chewers (tobacco + betel) 21.9% chewers and smokers 23.2% smokers 18.4% no habit (among oral-cavity tumour patients)	30 219 carcinomas (1941-1955)	14 162 (46.9%) (oral + pharyngeal)	Paymaster (1962)
Bhopal, India	100% tobacco + betel-quid chewers 55.7% chewers and smokers	519	210 (40.5%) (oropharyngeal)	Agarwal & Arora (1964)
Madras, India	76.7% chewers with tobacco 18.6% without tobacco 4.7% non-chewers	13 626 (1950-1959)	6728 (49.4%) (oral cavity)	Sidiq *et al.* (1964)
Madras, India	95% betel-quid chewers (with tobacco: 83%) 34% smokers	3529 (1962-1963)	362 (10%) (buccal mucosa)	Singh & Von Essen (1966)
Mainpuri, India	26.6% tobacco with lime 15.6% smokers 53.9% both 3.9% no habit 2% betel quid	- (1950-1962)	154 (oral + oropharyngeal)	Wahi *et al.* (1966)
Agra, India	32.5% tobacco with lime 30.1% smokers 18.1% both 19.3% no habit 12% betel quid	-	83 (oral + oropharyngeal)	Wahi *et al.* (1966)
Agra, India	85% betel quid with tobacco 51% smokers (85 gingival cancer patients)	6790 (1957-1965)	3173 (46.7%) (intra-oral), 85 (gingival)	Srivastava & Sharma (1968)
Jabalpur, India	84% (100 oral cancers) tobacco chewers 28% smokers	(1958-1967)	814 (oral + pharyngeal) (33.8%)	Gandagule & Agarwal (1969)
Kanpur, India	14.8% betel quid without tobacco 22% betel quid with tobacco 49% tobacco + lime 5.4% smoking 5% smoking and chewing	2332 (1958-1966)	630 (27%) (oral)	Samuel *et al.* (1969)
Philippines	52 *buyo*[a] chewers 2 non-chewers 21 uncertain	-	75 (49 of the cheek)	Davis (1915)
Thailand	100% betel quid + tobacco	53 (1922-1923)	25 (47%) (oral)	Mendelson & Ellis (1924)
Taiwan	59% betel-quid chewers 82% smokers	- 1953-1963	89	Chang (1964)
Sri Lanka	Only 3 (1.5%) betel-quid chewers among cases 38 smokers	- (1945 on)	508 (buccal mucosa)	Balendra (1965)
		400 new cases seen during 3 months in 1960	214 (53.5%) (buccal mucosa)	Balendra (1965)

[a]*Buyo* can consist of betel leaves, areca nut, slaked lime and tobacco or any combination of these constituents.

In Papua New Guinea, where the predominant habit is chewing betel quid without tobacco, the earliest study (Eisen, 1946) concluded that betel chewing does not appear to cause cancer of the buccal cavity. [The Working Group noted that this conclusion appeared to be based on the finding of no oral cancer in only a cross-section of subjects.] In two reports of Farago (1963a,b), 98% and 99% of oral-cancer patients were chewers of betel quid. Smoking was also reported to be common.

In India, the predominant habits are chewing betel quid with tobacco and chewing tobacco with other ingredients, such as lime and areca nut. In one study (Padmavathy & Reddy, 1960), however, the predominant habit was smoking. Several authors commented on the fact that the site of origin of the growth corresponded to the site at which the tobacco quid was habitually kept.

In the Philippines, 52/75 (69%) patients questioned were *buyo* (betel quid) chewers, and for 21 the information was questionable (Davis, 1915). In Thailand, all of 25 patients were chewers of betel quid with tobacco (Mendelson & Ellis, 1924). A higher frequency of betel-quid chewing was seen in oral-cancer cases, except in the study of Balendra (1965), which reported a much lower frequency of chewers among cases (1.5%) than in the local population. [The Working Group considered that this unusual result may be due to incomplete reporting of the chewing habit in clinical records.]

The incidences of oral cancer were compared in different population groups with different chewing habits in several studies. The incidences of cancers within the upper alimentary tract among women of Indian and English origin in South Africa were compared (Schonland & Bradshaw, 1969). Among Indian and English women, the annual incidences per 100 000 for cancer of the mouth and pharynx were 13.6 and 4.0, for cancer of the oesophagus 12.9 and 2.4, and for cancer of the stomach 30.0 and 12.4, respectively. In a separate population-based survey within the same study, 31% of the Indian women were found to be betel chewers, a habit completely absent among English women.

A study from Bombay in 1964-1966 compared the incidence rates of oral cancer among Parsees and other communities (Jussawalla *et al.*, 1970). Parsees form a very small subgroup (about 1.7%) of the population of the city of Bombay; few smoke and very few chew, whereas chewing and smoking are common in the population of Bombay as a whole. The annual age-adjusted incidences (per 100 000) of cancers at several sites were lower among Parsee men than among the male population of Bombay as a whole: tongue, 2.1 *versus* 14.0; other oral cancers, 1.6 *versus* 6.5; pharynx, 2.2 *versus* 16.0; oesophagus, 3.7 *versus* 13.0; and larynx, 4.5 *versus* 13.8.

In a study in 1971-1978 from Papua New Guinea, the age-adjusted incidence rates of oral cancer were compared for different geographical areas (Atkinson *et al.*, 1982). In the highlands, where very few people chew areca nut with lime, the age-adjusted incidence of oral cancer per 100 000 compared to that in the lowlands, where a very high percentage of people have this habit, was 1.01 *versus* 6.83 for men and 0.41 *versus* 3.03 for women. It was observed that in a part of lowland western Papua, inhabited by a specific tribe among whom very few chew, the incidence of oral cancer was very low; and the authors, while pointing out that the numbers were very small, noted that the finding had been consistent for 21 years.

In another study from Papua New Guinea (Scrimgeour & Jolley, 1983), the changes in the incidence of oral cancer were compared with the changes in smoking-tobacco consumption

during the periods 1965-1969 and 1975-1979. It was found that the incidence of oral cancer had increased among men as well as women; the increase for men was not significant ($p > 0.05$), but that for women was highly statistically significant ($p < 0.01$). It was pointed out that, during the same period, the proportion of adult women in a specific area of Papua who smoked commercial cigarettes had increased from 34% to 76%, although their betel-chewing habits had not changed very much. Smoking habits among men had not changed significantly.

(*b*) *Analytical studies*

In most of the studies described below, chewing habits were not precisely defined, nor was a distinction made between chewing of betel quid with tobacco and without tobacco.

(i) *Case-control studies*

In reviewing case-control studies, relative risks were calculated from the data given in the papers, unless provided by the authors. Wherever separate data were given for men and women, these were combined. Table 17 summarizes the studies that give relative risks for oral and other cancers associated with chewing habits. [The Working Group noted that the way in which cases and controls were selected was not adequately described in most of these studies.] The derived relative risk estimates for use of betel quid (not including those studies that specifically stated use without tobacco) ranged from 4 to 39 in different studies. [The Working Group noted that the anatomical sites considered varied considerably, and it was not always clear which specific sites were included.] The dominant habit in all these areas was chewing betel quid with tobacco. The study by Orr (1933) is remarkable in that it was the first case-control study on oral cancer, and probably one of the first case-control studies on any type of disease. The study by Shanta and Krishnamurthi (1959) provided enough data to calculate a relative risk only for 'cheek' cancer. In the study by Notani and Sanghvi (1976) controls were matched for age and community; in the study of Wahi (1965), controls were matched for age, sex, religion and socioeconomic status.

Kwan (1976) reported a case-control study in Taiwan of oral cancer in which, out of 103 cases, 20 were betel chewers and 35 were betel chewers with other habits. No control chewed betel. [Therefore, it was not possible to estimate the relative risk.]

A comprehensive evaluation of cancer risk among betel-quid chewers and smokers in a case-control study was reported by Jussawalla and Deshpande (1971) in Bombay. They selected 2005 histologically-confirmed cancer patients comprising cancers of the oral cavity, pharynx, larynx and oesophagus. Equal numbers of controls were selected from the population using the voters' list, and the controls were matched for age, sex and religion. Information was collected by interviewing patients and controls. Table 18 shows the assessment of risk for cancer at each site in chewers and non-chewers. The relative risks were highly significant for all cancers and for cancers of the tongue, alveolus, buccal mucosa, hard palate, oral cavity as a whole, base of the tongue, tonsils, oropharynx, hypopharynx, larynx and oesophagus. Table 19 shows the relative risks for cancers at different sites of chewers only, chewers and smokers, and smokers only. The relative risks were highly significant for all the cancers, except cancer of the nasopharynx, in all habit groups. The risks for different types of cancer of those chewing betel quid with tobacco and those chewing betel quid without tobacco are shown in Table 20; they were highly significant for both groups for all cancers except that of the nasopharynx. [The Working Group noted that smoking was not controlled for in this analysis.]

Table 17. Case-control studies of oral[a] and other cancers associated with chewing of betel quid

Location (years)	Cancer site[b]	No. of cases[c]	Habit[d]	No. of controls	Habit[d]	Crude relative risk[e]	Remarks	Reference
Travancore, India	Lip	100	Q 98%	100	Q 66%	25.2		Orr (1933)
Bombay, India (1952-1954)	Base of tongue, oropharynx, hypopharynx, oesophagus		Q 12% Q+S 39% S 47% (M)		Q 9% Q+S 24% S 50% (M)			Sanghvi *et al.* (1955)
		289 M+F (oral) 551 M+F (oesophagus, hypopharynx, base of tongue and oropharynx)		400 400		Q 10.2 Q 4.0		
Assam, India (1954-1955)	Lip, pharynx, oesophagus, larynx	238 (108 larynx)	Q 97%	3678	Q 79%	7.6		Sarma (1958)
Bombay, India (1952-1954)	Base of tongue, oropharynx, lip	371	Q 12% Q+S 38% S 48%		Q 9% Q+S 24% S 50%		Some *khaini* use	Khanolkar (1959)
		95 (oral)	Q 28% Q+S 42% S 18%	288		Q 8.0		
		276 (oropharynx and base of tongue)	Q 5% Q+S 36% S 58%	288		Q 10.0		
Madras, India	Only cheek and floor of the mouth	206	BQ 9% BQ+T 85% S 26%	278	BQ 52% BQ+T 13% S 47%	BQ 0.1 BQ+T 39		Shanta & Krishnamurthi (1959)
Madras, India	Lip, oropharynx, hypopharynx, oesophagus, tongue	882	BQ 20% (M) BQ+T 64% (M) BQ 50% (F) BQ+T 71% (F)	400	BQ 40% (M) BQ+T 9% (M) BQ 56% (F) BQ+T 11% (F)	BQ 0.3 (M) BQ 0.8 (F) BQ+T 17.2 (M) BQ+T 20.1 (F)		Shanta & Krishnamurthi (1963)
Agra, India (1950-1962)	Lip, tongue, tonsil	821	T 73% T+S 38% S 55%	1916	T 12% T+S 6% S 28%	T 41.2	High proportion of *khaini* use	Wahi *et al.* (1965)
Sri Lanka	Oesophagus only	111	Q 81%	1088	Q 30%	9.9		Stephen & Uragoda (1970)
Varanasi, India (1966-1970)	-	206	BQ+T 39% T 50%	100	Q 25%	27.0	50% *khaini* use	Khanna *et al.* (1975)
Bombay, India	Anterior two-thirds of tongue, lip	214 M	Q 29% Q+S 32% S 31%	230	Q 15% Q+S 20% S 48%	Q 4.2	Considerable proportion of *khaini* use	Notani & Sanghvi (1976)

[a]Usually comprises gum, floor of mouth, buccal mucosa and palate; tongue may be included.
[b]In addition to oral cancer
[c]Only those with known chewing habits are included; M, men; F, women
[d]Q, betel quid with or without tobacco; S, smoking only; BQ, betel quid without tobacco; T, tobacco
[e]Calculated by the Working Group for men and women combined

Table 18. Relative risks for oral and other cancers among betel-quid chewers, assuming the risk among non-chewers to be unity[a]

Group	Habit		Relative risk[b]
	None (no.)	Chewing (no.)	
Controls	1340	665	
Cancer patients	853	1152	2.7***
Oral cavity	129	282	4.4***
Base of tongue	175	187	2.2***
Soft palate	35	18	1.0 NS
Tonsils	99	128	2.6***
Lip	8	6	1.5 NS
Anterior two-thirds of tongue	36	54	3.0***
Floor of mouth	10	4	0.8 NS
Alveolus	26	44	3.4***
Buccal mucosa	42	160	7.7***
Hard palate	7	14	4.0**
Oropharynx	309	333	2.2***
Nasopharynx	10	7	1.4 NS
Hypopharynx	21	49	4.7***
Larynx	246	314	2.6***
Oesophagus	138	167	2.4***

[a]From Jussawalla & Deshpande (1971)
[b]**, $p < 0.01$; ***, $p < 0.001$; NS, $p > 0.05$

Table 19. Relative risks for oral and other cancers by habit, assuming the risk among persons with no habit to be unity[a]

Group	No habit (no.)	Chewing only (no.)	Relative risk[b]	Chewing and smoking (no.)	Relative risk[b]	Smoking only (no.)	Relative risk[b]
Controls	925	521		144		415	
Cancer patients	243	557	4.1***	595	15.7***	610	5.6***
Oral cavity	57	192	6.0***	90	10.1***	72	2.8***
Oropharynx	49	91	3.3***	242	31.7***	260	11.8***
Nasopharynx	4	4	1.8 NS	3	4.8 NS	6	3.3 NS
Hypopharynx	8	28	6.2***	21	16.9***	13	3.6**
Larynx	55	142	4.6***	172	20.1***	191	7.7***
Oesophagus	70	100	2.5***	67	6.2***	68	2.2***

[a]From Jussawalla & Deshpande (1971)
[b]**, $p < 0.01$; ***, $p < 0.001$; NS, $p > 0.05$

Table 20. Relative risks for oral and other cancers for chewers of betel quid with and without tobacco, assuming the risk among non-chewers to be unity[a]

Group	No habit	Chewing without tobacco	Relative risk[b]	Chewing with tobacco	Relative risk[b]
Controls	1340	152		513	
Cancer patients	853	291	3.0***	861	2.6***
Oral cavity	129	44	3.0***	238	4.8***
Oropharynx	309	106	3.0***	227	1.9***
Nasopharynx	10	4	3.5 NS	3	0.8 NS
Hypopharynx	21	13	5.5***	36	4.5***
Larynx	246	70	2.5***	244	2.6***
Oesophagus	138	54	3.5***	113	2.1***

[a]From Jussawalla & Deshpande (1971)
[b]***, $p < 0.001$; NS, $p > 0.05$

Jayant *et al.* (1977) examined the possibility of interaction between chewing and smoking habits in the etiology of cancer of the upper alimentary tract using the data of Jussawalla and Deshpande (1971). It was found that chewing and smoking habits interacted synergistically for cancers of the oral cavity, oropharynx, hypopharynx, larynx and oesophagus. The percentage of oral cancer that could be attributed to chewing or smoking habits was estimated to be between 70-80% for cancers of the oral cavity, oropharynx, hypopharynx and larynx, and 50% for cancer of the oesophagus.

In three case-control studies, sufficient information was available to categorize risk in relation to various combinations of chewing and smoking habits. Jafarey *et al.* (1977) included cancers of all sites within the oral cavity; the study by Chandra (1962) was limited to cancers of the buccal mucosa. These studies are described in detail in the monograph on tobacco habits other than smoking, p. 107 in this volume. Hirayama (1966) reported separately on oral- and oropharyngeal-cancer patients and on controls (who were patients with other diseases [unspecified] matched for age and sex). Information was obtained by interview in hospitals in Sri Lanka and India; hospital records were also used, if considered reliable. [The Working Group noted that it was not clear whether hospital records were used for cases only or for controls as well. There is no explanation of the large discrepancy between the number of cases (595) and controls (277) in men in contrast to women (232 cases and 163 controls). Relative risks for various combinations were calculated by the Working Group from the data adjusted for sex and tested for significance, and are summarized in Table 21.]

Table 21. Relative risks, calculated by the Working Group, for oral, oropharyngeal and buccal-mucosal cancer by chewing and smoking habits from case-control studies

Habit	Pakistan[a]		India and Sri Lanka[b]				Calcutta, India[c]	
	Relative risk for oral cancer		Relative risk for oral cancer		Relative risk for oropharyngeal cancer		Relative risk for buccal-mucosa cancer	
	Men	Women	Men	Women	Men	Women	Men	Women
Betel quid	4.2	3.2	1.9	1.2	11.8[e]	-	0.7	2.0
Tobacco	10.4	13.7	-	-	-	-	2.7	2.5
Betel quid and tobacco	8.1	14.1	7.6	11.3	6.0	5.5	3.9	4.1
Betel quid and smoking	25.0	29.9	2.7	4.6	21.5	-	-	-
Tobacco and smoking	15.4	23.6	-	-	-	-	-	-
Betel quid, tobacco and smoking	23.0	35.9	11.9	19.9	12.9	27.5	5.8	-
Smoking	5.7	12.9	2.1	11.5	28.5	27.5	1.2	-
Total no. of cases	683	509	514	211	81	21	287	163
Total no. of controls	1978	1584	277	163	-	-	420	90

[a]From Jafarey *et al.* (1977)
[b]From Hirayama (1966)
[c]From Chandra (1962)
[d]Includes buccal mucosa, anterior tongue and palate
[e]Not statistically significant

The habit of chewing betel quid without tobacco did not incur a statistically significantly increased relative risk for oral cancer in the studies of Hirayama (1966) or Chandra (1962); and the risk for oropharyngeal cancers was also non-significant. The study from Pakistan (Jafarey *et al.*, 1977) demonstrated a significant, but the lowest, relative risk for oral cancer associated with the habit of chewing betel quid without tobacco; for all combinations of

chewing and smoking habits, the risks were three to six times higher. The relative risk of smoking alone was not significantly raised in the Chandra study; the relative risks for oral and oropharyngeal cancers of all other exposure categories in the three studies were in the range of 2.7-20.3 and were statistically highly significant.

Multivariate regression analysis was reported in a case-control study in Thailand (Simarak *et al.*, 1977). Over a period of 16 months (1971-1972), patients coming to a hospital in Chiang Mai with a confirmed diagnosis of cancer of the oral cavity and oropharynx, of the larynx and hypopharnyx, or of the lung were selected as cases (88, 96 and 115, respectively). Controls (1113) were selected from among the patients attending a radiology clinic, mainly with urogenital, respiratory and locomotor disorders; a small proportion of controls (7% men, 15% women) had cancers at sites other than those under study. The variables that showed a significant relationship with cancer, after adjusting for age (three categories) and province of residence, which were included in the analysis were: agricultural employment, rural residence and betel chewing for patients of both sexes, lack of formal schooling, and cigarette and cigar smoking for men. After adjusting for the effects of covariables, the relative risk estimates of the betel-chewing habit were 2.3 for men and 3.2 for women for oral and oropharyngeal cancers and 2.4 for men for cancer of the larynx and hypopharynx. All these adjusted relative risks remained statistically significant.

Only two of the case-control studies described above allow examination of a dose-response relationship between relative risk for oral cancer and frequency of chewing (Orr, 1933; Hirayama, 1966) (Table 22). Both studies showed a positive dose-response relationship, the highest relative risk being that of retaining the quid during sleep.

Table 22. Dose-response relationships between chewing of betel quid with tobacco and oral cancer

Frequency of chewing	Relative risk[a]	
	Hirayama (1966)	Orr (1933)
None	1.0	1.0
<2 times a day	8.4	4.9
3-5 times a day	14.2	17.7
6 times or more	17.6	68
Retaining quid in sleep	63	212.5

[a]Calculated by the Working Group

(ii) *Cross-sectional surveys*

Pindborg *et al.* (1965) examined 10 000 individuals who came consecutively to the outpatient department of a dental clinic in Lucknow, India, 33% of whom were reported to practise betel-quid chewing with or without tobacco and/or smoking. A total of 24 oral cancers were detected among these individuals, giving a prevalence of 73 per 10 000 [calculated by the Working Group]. No oral cancer was found among those who did not chew or smoke.

Mehta *et al.* (1971) examined over 10 000 individuals in each of five districts of India, employing the technique of random sampling. In one district, no oral cancer was found. In Kerala (Ernakulam district), 26.2% reported practising chewing habits only (25.9% betel chewers with tobacco and 0.4% betel chewers without tobacco), 10.8% chewing and smoking

habits, 22.1% smoking habits only, and 41% practised neither chewing nor smoking. Six oral cancers were detected among chewers who did not smoke and six among chewers who smoked as well, giving prevalences of 2.2 and 5.4 per 1000 in these two habit categories. In Gujarat (Bhavnagar district), 7.1% reported using *mishri*, 3% chewed tobacco (with either betel quid or lime) and 1.6% chewed betel quid without tobacco; 3.2% chewed and smoked, 29.1% smoked only, and 56% did not practise any chewing or smoking habit. Oral cancer was found in one individual who chewed and smoked, and in two individuals who smoked only, giving prevalences of 3.1 and 0.7 per 1000, respectively. In Bihar (Darbhanga district), 15.2% practised chewing with tobacco (mostly tobacco and lime), and 1.3% chewed without tobacco; chewing and smoking were practised by 14.3%, smoking alone by 33.2%, and 36.1% did not practise any habit. Only one case of oral cancer was found among those who chewed and smoked, giving a prevalence of 0.7 per 1000. In Andhra Pradesh (Srikakulam district), 2.8% chewed with tobacco and 0.6% chewed without tobacco; chewing and smoking were practised by 7.9%, smoking only by 63% (mostly smoking of cheroots with the lighted end inside the mouth), and 25.8% did not practise any habit. One case of oral cancer was found among those who chewed and smoked and nine among those who smoked only, giving prevalences of 0.8 and 1.9 per 1000, respectively. [Prevalence rates calculated by the Working Group.]

Mehta *et al* (1972a) examined 101 761 villagers in Maharashtra (Poona [Pune] district). Among men, 52.1% and 0.7%, and among women, 9.5% and 0.5%, practised a chewing habit with tobacco (mostly tobacco and lime) and a chewing habit without tobacco, respectively. *Mishri* usage was reported by 0.8% of men and 38.9% of women. Smoking and smoking plus chewing were practised almost exclusively by men: 5.6 and 2.4%, respectively. A total of 12 cases of oral cancer was found and the age-adjusted prevalences among chewers were 0.2 per 1000 for men and 0.3 per 1000 for women. The age-adjusted prevalence was 0.6 per 1000 among smokers (men only) and 1.1 per 1000 among smokers and chewers (men only).

Smith *et al* (1975) examined 57 518 industrial workers (95% from textile mills) in Gujarat (Ahmedabad). Betel quid [presumably without tobacco] was chewed by 4.7%, and betel-quid chewing with tobacco smoking was practised by 23%. Oral cancer was found among workers who smoked only [prevalence, 0.6 per 1000], those who smoked and chewed betel [prevalence, 0.9 per 1000] and among individuals without any habit [prevalence, 0.2 per 1000], but not among individuals who chewed only.

(iii) *Prospective studies*

A population-based prospective study was reported by Wahi (1968) from a temporary cancer-registration system established in Uttar Pradesh (Mainpuri district). Over a period of 30 months (1964-1966), a total of 346 oral- and oropharyngeal-cancer cases were detected and confirmed. Exposure data were obtained by questioning these patients, and a house-to-house interview survey was conducted on a 10% cluster sample of the district population. The numbers in various exposure categories were then extrapolated to the population as a whole and used as denominators for calculating oral cancer 'period prevalence rate'. Chewing of Mainpuri tobacco, a pre-prepared mixture which contains areca nut and lime, was distinguished from other chewing habits. Prevalence rates for the two kinds of chewing habit and for combinations of alcohol and smoking habits are summarized in Table 23. Prevalence rates were highest among users of Mainpuri tobacco and higher for all other chewing habits than for no chewing habit, after controlling for smoking and drinking. The strength of the association between chewing and oral cancer was studied in many ways [frequently intercorrelated] (Table 24) and was reported to be positive by every criterion. [The

Working Group noted that differences in age between cancer patients and the population sample do not seem to have been taken into account; and it is possible that the prevalence of habits within the population was age-dependent.]

Table 23. Numbers of oral cancers and prevalence per 1000 population in a study in Mainpuri district, India[a]

Habit	No tobacco		Mainpuri tobacco		Other kind of tobacco	
	Oral cancer cases	Prevalence	Oral cancer cases	Prevalence	Oral cancer cases	Prevalence
No habit	27	0.18	59	4.51	32	0.80
Alcohol drinking	0	0	6	6.59	2	1.08
Smoking	54	0.57	78	8.12	47	1.76
Drinking and smoking	9	1.56	30	11.45	2	0.58
Total	90	0.36	173	6.60	83	1.15

[a]From Wahi (1968)

Table 24. Strength of the relationship between tobacco chewing and oral cancer[a]

Criterion	Estimated population	No. of cases	Prevalence per 1000
Total	349 710	346	0.99
Non-chewers of tobacco	251 330	90	0.36
Frequency of tobacco chewing			
Occasionally	11 680	5	0.43
Daily	86 700	251	2.90
Age chewing started (years)			
30 and over	38 290	69	1.80
25-29	15 000	28	1.87
20-24	22 230	61	2.74
15-19	16 030	58	3.62
5-14	6 870	40	5.92
Retention of each quid (min)			
1-20	69 030	133	1.93
21-30	18 680	69	3.69
31 and over	9 650	53	5.49
Period of exposure (min) per day			
Up to 99	53 720	123	2.29
100-299	33 670	90	2.67
300-499	9 400	31	3.30
500 and over	2 230	12	5.38
Sleeping with quid in mouth			
Never	85 790	175	2.04
Occasionally	10 790	58	5.38
Daily	1 740	23	13.22
Type of tobacco chewed			
Mainpuri	17 160	134	7.81
Pattiwala	71 610	84	1.17
Mainpuri and Pattiwala	8 950	37	4.13
Other (Kapuri, Rampuri, Moradabadi)	760	1	1.32
Money (paisa) spent on tobacco per day			
0-6	67 240	161	2.39
7-37	19 710	77	3.91
38-74	680	4	5.88
75-100	260	9	34.62

[a]From Wahi (1968)

Mehta *et al.* (1972b) examined a cohort of 4734 policemen in Bombay for oral lesions, at intake in 1959, and five and 10 years later. Of the 3674 followed successfully, 49% chewed (mostly betel quid with tobacco) and 12% chewed and smoked. Oral cancer was found in one man who chewed and smoked.

Of 57 518 industrial workers examined in the first phase of a study (1967-1971), Bhargava *et al.* (1975) re-examined 43 654 after two years. They diagnosed 13 new oral cancer cases, all of which had developed among individuals with tobacco/betel-chewing and/or smoking habits (Table 25).

Table 25. Summary of two prospective studies from India

Habit	Ahmedabad[a]			Ernakulam[b]		
	Number re-examined[c]	New oral cancers	Incidence per 100 000	Person-years	New oral cancers	Age-adjusted incidence per 100 000
Chewing	3 266	1	31	23 416	9	23
Chewing and smoking	16 881	6	36	8 476	4	32
Smoking	15 378	6	39	20 222	0	0
None	7 065	0	0	30 962	0	0

[a]Industrial workers aged 35 years and over; data from Bhargava *et al.* (1975)
[b]House-to-house survey of individuals aged 15 years and over; data from Gupta *et al.* (1980)
[c]Approximately two years after the first examination

Gupta *et al.* (1980) followed up a random sample of 10 287 individuals in Kerala (Ernakulam district) for a period of 10 years (1966-1977) in house-to-house surveys, with a follow-up of 87%. Chewing of betel quid with tobacco was a common habit in that area, and all 13 new oral cancers were diagnosed among chewers only and chewers who smoked as well. The person-years method was used for the data analysis and incidence rates were age-adjusted (Table 25).

4. Summary of Data Reported and Evaluation

4.1 Exposure data

The habit of chewing betel quid is widespread in South-East Asia and the South Pacific islands and in people of Indian origin elsewhere in the world. Betel quid usually contains areca nut, lime and catechu wrapped in a betel leaf. Tobacco is often added.

These habits result in exposure *inter alia* to areca-nut alkaloids, *N*-nitroso compounds derived from these alkaloids, polyphenols, and, when the habit includes tobacco, tobacco-specific nitrosamines.

4.2 Experimental data

Aqueous extracts of *betel quid containing tobacco* were tested for carcinogenicity in mice by gastric intubation, skin painting and subcutaneous injection; some malignant tumours occurred at the site of skin or subcutaneous administration. In hamsters, forestomach carcinomas occurred after painting of the cheek-pouch mucosa with aqueous extracts or implantation of a wax pellet containing powdered betel quid with tobacco into the cheek pouch; carcinomas occurred in the cheek pouch following implantation of the wax pellets.

Aqueous extracts of *betel quid without tobacco* were tested in mice by gastric intubation and by subcutaneous administration; an increased incidence of local tumours was observed after subcutaneous injection. In hamsters, painting of the cheek-pouch mucosa or implantation of wax pellets into the cheek pouch resulted in the induction of forestomach carcinomas; carcinomas occurred in the cheek pouch following implantation of the wax pellets.

Aqueous or dimethyl sulphoxide extracts of *areca nut with tobacco* were tested in mice by skin application. A low incidence of skin tumours was reported in a study lacking controls. In hamsters, applications of such extracts to cheek-pouch mucosa produced squamous-cell carcinomas of the cheek pouch and forestomach carcinomas.

Areca nut or aqueous extracts of areca nut were tested in mice by oral intubation, dietary administration, skin application, and intraperitoneal and subcutaneous injection. Local tumours were produced following subcutaneous injection. In rats, areca nut was tested by oral administration, and aqueous extracts were tested by subcutaneous injection. Studies involving dietary administration were inadequate, but local mesenchymal tumours occurred after subcutaneous injection. In hamsters, administration of areca nut and application of aqueous or dimethyl sulphoxide extracts to the cheek-pouch mucosa resulted in squamous-cell carcinomas of the cheek pouch and carcinomas of the forestomach.

Aqueous extracts of *betel leaf* were tested in mice by oral intubation or intraperitoneal injection and in hamsters by application to the cheek-pouch mucosa. Betel leaf was tested in rats by dietary administration and in hamsters by implantation in beeswax pellets into the cheek pouch. All these studies were inadequate for evaluation.

Arecoline (an alkaloid of areca nut) was tested in mice by oral intubation and by intraperitoneal and subcutaneous injection, and in hamsters by feeding or application to the cheek-pouch mucosa in combination with lime. The data are inadequate for evaluation.

Aqueous extracts of betel quid without tobacco induce mutations in *Salmonella typhimurium* but not in Chinese hamster V79 cells. They do not induce micronuclei in bone-marrow cells of Swiss mice.

Aqueous extracts of betel quid with tobacco induce mutations in *Salmonella typhimurium* and in Chinese hamster V79 cells. They also induce micronuclei in bone-marrow cells of Swiss mice.

Aqueous extracts of areca nut induce mutations in *Salmonella typhimurium* and in Chinese hamster V79 cells, gene conversion in *Saccharomyces cerevisiae*, as well as chromosomal aberrations in Chinese hamster ovary cells. They induce micronuclei in bone-marrow cells of Swiss mice. A tannin fraction of areca nut induces gene conversion in *Saccharomyces cerevisiae*.

Ethyl acetate and *n*-butanol extracts of areca nut induce chromosomal aberrations in Chinese hamster ovary cells. Ethyl acetate extracts do not induce mutations in Chinese hamster V79 cells, sister chromatid exchanges in human lymphoblastoid cells or transformation in Syrian hamster embryo cells.

Aqueous extracts of betel leaf are not mutagenic to *Salmonella typhimurium*. They induce chromosomal aberrations in human lymphocytes and in Chinese hamster ovary cells.

n-Butanol and ethyl acetate extracts of betel leaf induce chromosomal aberrations in Chinese hamster ovary cells. Ethyl acetate extracts of betel leaf do not induce mutations in Chinese hamster V79 cells, sister chromatid exchanges in human lymphoblastoid cells or transformation in Syrian hamster embryo cells.

Arecoline induces mutations in *Salmonella typhimurium* and Chinese hamster V79 cells, and chromosomal aberrations in Chinese hamster ovary cells. It also induces micronuclei, chromosomal aberrations and sister chromatid exchanges in bone-marrow cells of Swiss mice.

Arecaidine induces mutations in *Salmonella typhimurium* and Chinese hamster V79 cells. It induces sister chromatid exchanges but not micronuclei in bone-marrow cells of Swiss mice.

Overall assessment of data from short-term tests: Aqueous extract of betel quid without tobacco[a]

	Genetic activity			Cell transformation
	DNA damage	Mutation	Chromosomal effects	
Prokaryotes		+		
Fungi/green plants				
Insects				
Mammalian cells (*in vitro*)		–		
Mammals (*in vivo*)			–	
Humans[b] (*in vivo*)			+	
Degree of evidence in short-term tests for genetic activity: *Inadequate*				Cell transformation: No data

[a]The groups into which the table is divided and the symbols used are defined on pp. 16-17 of the Preamble; the degrees of evidence are defined on p. 18.

[b]Chewers of betel quid without tobacco

Overall assessment of data from short-term tests: Aqueous extracts of betel quid with tobacco[a]

	Genetic activity			Cell transformation
	DNA damage	Mutation	Chromosomal effects	
Prokaryotes		+		
Fungi/green plants				
Insects				
Mammalian cells (*in vitro*)		+		
Mammals (*in vivo*)			+	
Humans[b] (*in vivo*)		+	+	
Degree of evidence in short-term tests for genetic activity: *Sufficient*				Cell transformation: No data

[a]The groups into which the table is divided and the symbols used are defined on pp. 16-17 of the Preamble; the degrees of evidence are defined on p. 18.

[b]Chewers of betel quid with tobacco

Overall assessment of data from short-term tests: Aqueous extracts of areca nut[a]

	Genetic activity			Cell transformation
	DNA damage	Mutation	Chromosomal effects	
Prokaryotes		+		
Fungi/green plants		+		
Insects				
Mammalian cells (*in vitro*)		+	+	
Mammals (*in vivo*)			+	
Humans (*in vivo*)				
Degree of evidence in short-term tests for genetic activity: *Sufficient*				Cell transformation: No data

[a]The groups into which the table is divided and the symbols used are defined on pp. 16-17 of the Preamble; the degrees of evidence are defined on p. 18.

Overall assessment of data from short-term tests: Ethyl acetate extract of areca nut[a]

	Genetic activity			Cell transformation
	DNA damage	Mutation	Chromosomal effects	
Prokaryotes				
Fungi/green plants				
Insects				
Mammalian cells (*in vitro*)		–	?[b]	–
Mammals (*in vivo*)				
Humans (*in vivo*)				
Degree of evidence in short-term tests for genetic activity: *Inadequate*				Cell transformation: Negative

[a]The groups into which the table is divided and the symbols used are defined on pp. 16-17 of the Preamble; the degrees of evidence are defined on p. 18.

[b]A butanol extract of areca nut gave positive results in Chinese hamster ovary cells.

Overall assessment of data from short-term tests: Aqueous extracts of betel leaf[a]

	Genetic activity			Cell transformation
	DNA damage	Mutation	Chromosomal effects	
Prokaryotes		–		
Fungi/green plants				
Insects				
Mammalian cells (*in vitro*)			+	
Mammals (*in vivo*)				
Humans (*in vivo*)				
Degree of evidence in short-term tests for genetic activity: *Inadequate*				Cell transformation: No data

[a]The groups into which the table is divided and the symbols used are defined on pp. 16-17 of the Preamble; the degrees of evidence are defined on p. 18.

Overall assessment of data from short-term tests: Ethyl acetate extracts of betel leaf[a]

	Genetic activity			Cell transformation
	DNA damage	Mutation	Chromosomal effects	
Prokaryotes				
Fungi/green plants				
Insects				
Mammalian cells (*in vitro*)		–	?[b]	–
Mammals (*in vivo*)				
Humans (*in vivo*)				
Degree of evidence in short-term tests for genetic activity: *Inadequate*				Cell transformation: Negative

[a]The groups into which the table is divided and the symbols used are defined on pp. 16-17 of the Preamble; the degrees of evidence are defined on p. 18.

[b]A butanol extract of betel leaf gave positive results in Chinese hamster ovary cells.

4.3 Human data

Chewers of betel quid in India and the Philippines had elevated frequencies of micronucleated cells in their buccal mucosa. The proportion of micronucleated exfoliated cells is related to the site within the oral cavity where the betel quid is kept habitually and to the number of betel quids chewed per day. The proportion can be reduced by administration for two to three months of vitamin A or β-carotene or a mixture of the two.

Oral leukoplakia shows a strong association with habits of betel-quid chewing in India. Some follow-up studies have shown malignant transformation of a proportion of leukoplakias. Oral submucous fibrosis and lichen planus, which are generally accepted to be precancerous conditions, appear to be related to the habit of chewing betel quid.

Many descriptive studies and reports of case series have identified an association between the habit of chewing betel quid with tobacco and oral cancer. The association has been consistent across many countries [Bangladesh, China (Taiwan), India, Malaysia, Pakistan, the Philippines, Singapore, Sri Lanka and Thailand]. Further, in case-control studies of oral cancer, whether smoking was controlled for (five studies) or not (five studies), the relative risks were high and statistically significant. However, the results are not directly comparable owing to the inclusion of different anatomical sites; most of these studies did not clearly describe the chewing habits of subjects, and some probably included a large proportion of users of tobacco-lime with or without areca nut. A significant association between the chewing of betel quid with tobacco and oesophageal cancer was reported from a case-control study in Sri Lanka.

Two case-control studies of oral cancer and one of oral and oropharyngeal cancer distinguished between different types of chewing and smoking habits. Controls were age-matched, and relative risks were statistically significant, in the range of 4 to 14, for chewing of betel quid with tobacco.

A positive dose-response relationship was found in two case-control studies.

In a population-based case-control study from Bombay, controls selected from the population were matched for age, sex and religion. Chewing habits were not precisely defined. After controlling for smoking habits, the relative risks were statistically significant for cancers of the oral cavity, oropharynx, hypopharynx, larynx and oeosophagus, but not for cancer of the nasopharynx. When habits were categorized into chewing with and without tobacco, but smoking habits were not taken into account, the relative risks remained statistically significant for all sites except the nasopharynx.

In a case-control study from Thailand, relative risks associated with chewing of betel quid with tobacco were statistically significant for oral and oropharyngeal cancers for men and women, and for laryngeal and hypopharyngeal cancer for men, after adjusting for several possible confounding variables, including smoking.

In cross-sectional surveys from India, although the number of oral cancer cases was generally small, samples were large (10 000 to 100 000). Oral cancer was consistently found much more frequently among chewers and/or smokers than among those who did not chew or smoke. Two of these cross-sectional samples were subsequently followed up for two and 10 years. New oral-cancer cases were seen only among those who chewed and/or smoked.

In a large-scale prospective study, two types of chewing habit were distinguished: of Mainpuri tobacco (which contains tobacco, lime and areca nut) and 'other' tobacco usages (which very often included lime and, frequently, areca nut). After controlling for smoking and alcohol drinking (although not for age), prevalence rates of oral cancer were highest for Mainpuri-tobacco usage and generally in second rank for the 'other'-tobacco usage category, in comparison to no chewing habit. The association with oral cancer for both types combined was examined in many different ways and was found to be positive.

Several descriptive studies from Papua New Guinea indicated an association between the habit of chewing betel quid without tobacco and oral cancer. The association is consistent for different areas and different communities (without controlling for smoking); however, analysis of time trends of incidence, chewing and smoking, by sex, suggests that smoking is an important risk determinant.

One case-control study from Pakistan suggests that chewing of betel without tobacco increases the risk of oral cancer when practised alone or in combination with smoking. Another case-control study from India and Sri Lanka provides different results, showing a clear effect of chewing betel with tobacco but not of chewing betel without tobacco, taking into account smoking. A case-control study from Bombay which categorized chewing habits (presumably betel) without tobacco, but did not control for smoking, showed statistically significantly increased relative risks for cancers of the oral cavity, oropharynx, hypopharynx, larynx and oesophagus, in the range of 3 to 5.

Cross-sectional surveys in India show that the percentage of people who chew betel quid without tobacco is small. In one case-control study from India and Sri Lanka, after controlling for smoking, the relative risk for oropharyngeal cancer of the habit of chewing betel quid without tobacco was increased but not significantly so.

4.4 Evaluation[1]

There is *sufficient evidence* that the habit of chewing betel quid containing tobacco is carcinogenic to humans.

There is *inadequate evidence* that the habit of chewing betel quid without tobacco is carcinogenic to humans.

The Working Group also concluded that, while there is *sufficient evidence* that the combined habits of smoking tobacco and chewing betel quid without tobacco cause oral and pharyngeal cancer, the evidence considered here does not allow an assessment of the possible contribution of betel quid without tobacco to this carcinogenic risk.

There is *limited evidence* that aqueous extracts of betel quid with and without tobacco are carcinogenic to experimental animals.

There is *limited evidence* that areca nut with and without tobacco is carcinogenic to experimental animals.

The data are *inadequate* to allow an evaluation of the carcinogenicity of betel leaf or arecoline to experimental animals.

5. References

Abraham, S.K., Goswami, V. & Kesavan, P.C. (1979) A preliminary assessment of possible mutagenicity of betel nut and ingredients of the betel quid when administered alone or in combinations to larvae of *Drosophila melanogaster*. *Mutat. Res.*, *66*, 261-266

Agarwal, V. & Arora, M.M. (1964) Oropharyngeal cancer in Bhopal. *J. Indian med. Assoc.*, *42*, 519-521

Ahluwalia, H.S. & Duguid, J.B. (1966) Malignant tumours in Malaya. *Br. J. Cancer*, *20*, 12-15

Arjungi, K.N. (1976) Areca nut. A review. *Arzneimittelforschung*, *26*, 951-956

Atkinson, L., Chester, I.C., Smyth, F.G. & Ten Seldam, R.E.J. (1964) Oral cancer in New Guinea: A study in demography and etiology. *Cancer*, *17*, 1289-1298

Atkinson, L., Purohit, R., Reay-Young, P. & Scott, G.C. (1982) Cancer reporting in Papua New Guinea: 1958-70 & 1971-78. *Natl Cancer Inst. Monogr.*, *62*, 65-71

Bala Ram, A.P. (1902) The use of betelnut as a cause of cancer in Malabar. *Indian med. Gaz.*, *37*, 414

Balendra, W. (1949) Symposium on oral diseases in tropical countries. I. The effect of betel chewing on the dental and oral tissues and its possible relationship to buccal carcinoma. *Br. dent. J.*, *87*, 83-87

[1]For definitions of the italicized terms, see Preamble pp. 15-16 and 19.

Balendra, W. (1965) The incidence of oral carcinoma in Ceylon. *Br. J. oral Surg.*, *3*, 101-105

Bentall, W.C. (1908) Cancer in Travancore, South India. A summary of 1700 cases. *Br. med. J.*, *ii*, 1428-1431

Bhargava, K., Smith, L.W., Mani, N.J., Silverman, S., Jr, Malaowalla, A.M. & Bilimoria, K.F. (1975) A follow up study of oral cancer and precancerous lesions in 57,518 industrial workers of Gujarat India. *Indian J. Cancer*, *12*, 124-129

Bhide, S.V., Shivapurkar, N.M., Gothoskar, S.V. & Ranadive, K.J. (1979) Carcinogenicity of betel quid ingredients: Feeding mice with aqueous extract and the polyphenol fraction of betel nut. *Br. J. Cancer*, *40*, 922-926

Bhide, S.V., Gothoskar, S.V. & Shivapurkar, N.M. (1984) Arecoline tumorigenicity in Swiss strain mice on normal and vitamin B deficient diet. *J. Cancer Res. clin. Oncol.*, *107*, 169-171

Boak, S.D. (1906) Betel-nut chewing. *J. dent. Sci.*, *49*, 1006-1010

Boyland, E. & Nery, R. (1969) Mercapturic acid formation during the metabolism of arecoline and arecaidine in the rat. *Biochem. J.*, *113*, 123-130

Burton-Bradley, B.G. (1979) Is 'betel chewing' carcinogenic? *Lancet*, *ii*, 903

Canniff, J.P. & Harvey, W. (1981) The aetiology of oral submucous fibrosis: The stimulation of collagen synthesis by extracts of areca nut. *Int. J. oral Surg.*, *10* (Suppl. 1), 163-167

Chakarvarti, S.K., Dhiman, J. & Nagpaul, K.K. (1981) Uranium trace analysis of a chewable betel-leaf preparation and tea leaves. *Health Phys.*, *40*, 78-81

Chandra, A. (1962) Different habits and their relation with cancer cheek. *Chittaranjan Cancer Hosp. Calcutta natl Cancer Res. Centre Bull.*, 33-36

Chandra, S. & Desai, V.M. (1970) Relationship of betel chewing and dental caries. *J. Indian dent. Assoc.*, *42*, 269-276

Chang, K.-M. (1964) Betel nut chewing and mouth cancer in Taiwan. First report: Survey of disposition of mouth cancer in Taiwan. *J. Formosa med. Assoc.*, *63*, 437-448

Chin, C.T. & Lee, K.W. (1970) The effects of betel-nut chewing on the buccal mucosa of 296 Indians and Malays in West Malaysia. A clinical study. *Br. J. Cancer*, *24*, 427-432

Chopra, R.N., Chopra, I.C., Handa, K.L. & Kapur, L.D., eds (1958) *Chopra's Indigenous Drugs of India*, Dhur & Sons, pp. 371-377

Christie, J.E., Shering, A., Ferguson, J. & Glen, A.I.M. (1981) Physostigmine and arecoline: Effects of intravenous infusions in Alzheimer presenile dementia. *Br. J. Psychiatr.*, *138*, 46-50

Cooke, R.A. (1969) Verrucous carcinoma of the oral mucosa in Papua New Guinea. *Cancer*, *24*, 397-402

Daftary, D.K., Bhonsle, R.B., Murti, R.B., Pindborg, J.J. & Mehta, F.S. (1980) An oral lichen planus-like lesion in Indian betel-tobacco chewers. *Scand. J. dent. Res.*, *88*, 244-249

Davis, G.G. (1915) Buyo cheek cancer, with special reference to etiology. *J. Am. med. Assoc.*, *64*, 711-718

Dayal, P.K., Mani, N.J. & Bhargava, K. (1978) Prevalence of oral cancer and precancerous lesions in 'pan'/'supari' chewers. *Indian J. publ. Health*, *22*, 234-245

Deshpande, S.M., Upadhyay, R.R. & Singh, R.P. (1970) Chemical study of *Piper betel* leaves. *Curr. Sci.*, *39*, 372

Deshpande, V.H. & Patil, A.D. (1981) Flavanoids of *Acacia catechu* heartwood. *Indian J. Chem.*, Sect. B., *20*, 628

Domino, E.F. (1967) Electroencephelographic and behavioral causal effects of small doses of nicotine: A neuropsychopharmacological study. *Ann. N.Y. Acad. Sci.*, *142*, 216-244

Dunham, L.J. & Herrold, K.M. (1962) Failure to produce tumors in the hamster cheek pouch by exposure to ingredients of betel quid; Histopathologic changes in the pouch and other organs by exposure to known carcinogens. *J. natl Cancer Inst.*, *29*, 1047-1067

Dunham, L.J., Muir, C.S. & Hamner, J.E., III (1966) Epithelial atypia in hamster cheek pouches treated repeatedly with calcium hydroxide. *Br. J. Cancer*, *20*, 588-593

Dunham, L.J., Sheets, R.H. & Morton, J.F. (1974) Proliferative lesions in cheek pouch and esophagus of hamsters treated with plants from Curaçao, Netherland Antilles. *J. natl Cancer Inst.*, *53*, 1259-1269

Dunham, L.J., Snell, K.C. & Stewart, H.L. (1975) Argyrophilic carcinoids in two Syrian hamsters (*Mesocricetus auratus*). *J. natl Cancer Inst.*, *54*, 507-513

Eisen, M.J. (1946) Betel chewing among natives of the Southwest Pacific Islands. *Cancer Res.*, *6*, 139-141

Euler, U.S. & Domeij, B. (1945) Nicotine-like actions of arecoline. *Acta pharmacol.*, *1*, 263-269

Farago, C. (1963a) Review of 110 cases of cancer of oral cavity in Papua and New Guinea. *Br. med. J.*, *i*, 1264-1266

Farago, C. (1963b) Report of 1,160 registered tumor cases in Papua and New Guinea. *Cancer*, *16*, 670-680

Fells, A. (1908) Cancer of the mouth in southern India, with an analysis of 209 operations. *Br. med. J.*, *ii*, 1357-1358

Forlen, H.P., Hornstein, O. & Stüttgen, G. (1965) Betel quid and leukoplakia (Ger.). *Arch. klin. exp. Dermatol.*, *221*, 463-480

Gandagule, V.N. & Agarwal, S. (1969) Oral and pharyngeal cancer in Madhya Pradesh. *J. Indian med. Assoc.*, *53*, 582-585

Ganguly, P. & Choudhury, M. (1975) Photochemical studies on Bangla variety of betel leaf. *Indian Agric.*, *19*, 199-200 [*Chem. Abstr.*, *86*, 68387n]

Gerry, R.G., Smith, S.T. & Calton, M.L. (1952) The oral characteristics of Guamanians including the effects of betel chewing on the oral tissues. Part I. Part II. Diseases of the oral mucous membranes and dental supporting tissues in Guamanians. Part III. *Oral Surg.*, *5*, 762-781, 884-894, 1004-1011

Ghosh, R. & Ghosh, P.K. (1984) Sister chromatid exchanges in betel and tobacco chewers. *Mutat. Res.*, *139*, 79-81

Gilman, G.A., Goodman, L.S. & Gilman, A., eds (1980) *Goodman and Gilman's The Pharmacological Basis of Therapeutics*, 6th ed., New York, McMillan, pp. 96-97

Gode, P.K. (1961) *Studies in Indian Cultural History*, Vol. 1, Hoshiarpur, India, Vishveshvaranand Vedic Research Institute, pp. 113-114

Gupta, O.P. (1964) An epidemiological study of periodontal disease in Trivandrum, India (Abstract No. 314). *J. dent. Res.*, *43*, 876

Gupta, P.C., Mehta, F.S., Daftary, D.K., Pindborg, J.J., Bhonsle, R.B., Jalnawalla, P.N., Sinor, P.N., Pitkar, V.K., Murti, P.R., Irani, R.R., Shah, H.T., Kadam, P.M., Iyer, K.S.S., Iyer, H.M., Hedge, A.K., Chandrashekar, G.K., Shroff, B.C., Sahiar, B.E. & Mehta, M.N. (1980) Incidence rates of oral cancer and natural history of oral precancerous lesions in a 10-year follow-up study of Indian villagers. *Commun. Dent. oral Epidemiol.*, *8*, 287-333

Gupta, P.C., Pindborg, J.J. & Mehta, F.S. (1982) Comparison of carcinogenicity of betel quid with and without tobacco: An epidemiological review. *Ecol. Dis.*, *1*, 213-219

Henderson, B.E. & Aiken, G.H. (1979) Cancer in Papua New Guinea. *Natl Cancer Inst. Monogr.*, *53*, 67-72

Hirayama, T. (1966) An epidemiological study of oral and pharyngeal cancer in Central and South-East Asia. *Bull. World Health Organ.*, *34*, 41-69

Holmstedt, B. & Lundgren, G. (1967) Arecoline, nicotine and related compounds. Tremorgenic activity and effect upon brain acetylcholine. *Ann. N.Y. Acad. Sci.*, *142*, 126-142

Howden, G.F. (1982) Betel-nut chewing and dental health in Aua Island Papua New Guinea (Abstract No. 23). *J. dent. Res.*, *61*, 526

Huq, S.F. (1965) Some aspect of site distribution of cancer in East Pakistan. *J. Pakistan med. Assoc.*, *15*, 237-245

IARC (1983) *IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans*, Vol. 31, *Some Food Additives, Feed Additives and Naturally Occurring Substances*, Lyon, pp. 171-178, 213-229

IARC (1985) *IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans*, Vol. 36, *Allyl Compounds, Aldehydes, Epoxides and Peroxides*, Lyon, pp. 75-97

Jafarey, N.A., Mahmood, Z. & Zaidi, S.H.M. (1977) Habits and dietary pattern of cases of carcinoma of the oral cavity and oropharynx. *J. Pakistan med. Assoc.*, *27*, 340-343

Jayant, K. (1977) Statistical appraisal of the association of smoking and chewing habits to oral and pharyngeal cancers. *Indian J. Cancer*, *14*, 293-299

Jayant, K., Balakrishnan, V., Sanghvi, L.D. & Jussawalla, D.J. (1977) Quantification of the role of smoking and chewing tobacco in oral, pharyngeal, and esophageal cancers. *Br. J. Cancer*, *35*, 232-235

Johnston, G.A.R., Krogsgaard-Larsen, P. & Stephanson, A. (1975) Betel nut constituents as inhibitors of γ-aminobutyric acid uptake. *Nature*, *258*, 627-628

Jussawalla, D.J. & Deshpande, V.A. (1971) Evaluation of cancer risk in tobacco chewers and smokers: An epidemiologic assessment. *Cancer*, *28*, 244-252

Jussawalla, D.J., Deshpande, V.A., Haenszel, W. & Natekar, M.V. (1970) Differences observed in the site incidence of cancer, between the Parsi community and the total population of Greater Bombay: A critical appraisal. *Br. J. Cancer*, *24*, 56-66

Kandarkar, S.V. & Sirsat, S.M. (1977) Changes in vitamin A conditioned hamster cheek pouch epithelium on exposure to commercial shell lime (calcium hydroxide) and tobacco. I. Optical histopathology. *J. oral Pathol.*, *6*, 191-202

Kapadia, G.J., Chung, E.B., Ghosh, B., Shukla, Y.N., Basak, S.P., Morton, J.F. & Pradhan, S.N. (1978) Carcinogenicity of some folk medicinal herbs in rats. *J. natl Cancer Inst.*, *60*, 683-686

Katzung, B.G., ed. (1984) *Basic and Clinical Pharmacology*, 2nd ed., Los Altos, CA, Lange Medical Publications, Ch. 4, pp. 42-55

Khanna, N.N., Pant, G.C., Tripathi, F.M., Sanyal, B. & Gupta, S. (1975) Some observations on the etiology of oral cancer. *Indian J. Cancer*, *12*, 77-83

Khanolkar, V.R. (1944) Oral cancer in Bombay, India. A review of 1,000 consecutive cases. *Cancer Res.*, *4*, 313-319

Khanolkar, V.R. (1959) Oral cancer in India. *Unio int. contra cancrum Acta*, *15*, 67-77

Knudsen, B., Knudsen, B. & Saleem, M. (1985) Dental caries, oral hygiene and betel chewing in 434 Maldivian school children (Dan.). *Dan. dent. J.* (*Tandlagebladed*) (in press)

Krenger, W. (1942) History and culture of betel chewing. Synthesis and preparation of betel (Ger.). *Ciba Z.*, *84*, 2922-2941

Krogsgaard-Larsen, P. (1980) Inhibitors of the GABA uptake systems. *Mol. cell. Biochem.*, *31*, 105-121

Kwan, H.-W. (1976) A statistical study on oral carcinomas in Taiwan with emphasis on the relationship with betel nut chewing: A preliminary report. *J. Formosa med. Assoc.*, *75*, 497-505

Lalithakumari, H., Sirsi, M. & Govindarajan, V.S. (1965) Antibacterial & antifungal activities of *Areca catechu* Linn. *Indian J. exp. Biol.*, *3*, 66-67

Lee, K.W. & Chin, C.T. (1970) The effects of betel-nut chewing on the buccal mucosa: A histological study. *Br. J. Cancer*, *24*, 433-441

Leslie, G.B. (1965) Central stimulant properties of compounds with peripheral muscarinic properties. *Nature*, *208*, 1291-1293

Lodge, D., Johnston, G.A.R., Curtis, D.R. & Brand, S.J. (1977) Effects of the *areca* nut constituents arecaidine and guvacine on the action of GABA in the cat central nervous system. *Brain Res.*, *136*, 513-522

Marsden, A.T.H. (1960) Betel cancer in Malaya. *Med. J. Malaya*, *14*, 162-165

McGurk, M. & Craig, G.T. (1984) Oral submucous fibrosis: Two cases of malignant transformation in Asian immigrants to the United Kingdom. *Br. J. oral maxillofac. Surg.*, *22*, 56-64

McKinney, M. & Richelson, E. (1984) The coupling of the neuronal muscarinic receptors to responses. *Ann. Rev. pharmacol. Toxicol.*, *24*, 121-146

Meghji, S., Canniff, J.P., Harvey, W., Scott, A. & Phillipson, D. (1982) Inhibition of collagenase activity by areca nut tannins: A mechanism of collagen accumulation in oral submucous fibrosis (OSF)? (Abstract No. 89). *J. dent. Res.*, *61*, 545

Mehta, F.S., Sanjana, M.K., Barretto, M.A. & Doctor, R. (1955) Relation of betel leaf chewing to periodontal disease. *J. Am. dent. Assoc.*, *50*, 531-536

Mehta, F.S., Sanjana, M.K., Shroff, B.C. & Doctor, R.H. (1961) Incidence of leukoplakia among 'pan' (betel leaf) chewers and 'bidi' smokers: A study of a sample survey. *Indian J. med. Res.*, *49*, 393-399

Mehta, F.S., Pindborg, J.J., Gupta, P.C. & Daftary, D.K. (1969a) Epidemiologic and histologic study of oral cancer and leukoplakia among 50,915 villagers in India. *Cancer*, *24*, 832-849

Mehta, F.S., Daftary, D.K., Shroff, B.C. & Sanghvi, L.D. (1969b) Clinical and histologic study of oral leukoplakia in relation to habits. *Oral Pathol.*, *28*, 372-388

Mehta, F.S., Pindborg, J.J., Hamner, J.E., Gupta, P.C., Daftary, D.K., Sahiar, B.E., Shroff, B.C., Sanghvi, L.D., Bhonsle, R.B., Choksi, S.K., Dandekar, V.V., Mehta, Y.N., Pitkar, V.K., Sinor, P.N., Shah, N.C., Turner, P.S. & Upadhyay, S.A. (1971) *Report on Investigations of Oral Cancer and Precancerous Conditions in Indian Rural Populations, 1966-1969*, Copenhagen, Munksgaard, pp. 48, 68, 89, 107, 120

Mehta, F.S., Gupta, P.C., Daftary, D.K., Pindborg, J.J. & Choksi, S.K. (1972a) An epidemiologic study of oral cancer and precancerous conditions among 101,761 villagers in Maharashtra, India. *Int. J. Cancer*, *10*, 134-141

Mehta, F.S., Shroff, B.C., Gupta, P.C. & Daftary, D.K. (1972b) Oral leukoplakia in relation to tobacco habits. A ten-year follow-up study of Bombay policemen. *Oral Surg.*, *34*, 426-433

Mehta, F.S., Gupta, M.B., Pindborg, J.J., Bhonsle, R.B., Jalnawalla, P.N. & Sinor, P.N. (1982) An intervention study of oral cancer and precancer in rural Indian populations: A preliminary report. *Bull. World Health Organ.*, *60*, 441-446

Meltzer, L.T. & Rosecrans, J.A. (1982) Tolerance to the disruptive effects of arecoline on schedule-controlled behavior. *Psychopharmacology*, *77*, 85-93

Mendelson, R.W. & Ellis, A.G. (1924) Cancer as a public health problem in Siam. *J. trop. Med. Hyg.*, *27*, 274-278

Menon, M.M. & Bhide, S.V. (1984) Mutagenicity of urine in bidi and cigarette smokers and tobacco chewers. *Carcinogenesis*, *5*, 1523-1524

Meyer, J., Daftary, D.K. & Pindborg, J.J. (1967) Studies in oral leukoplakias. XI. Histopathology of leukoplakias in Indians chewing 'pan' with tobacco. *Acta odontol. scand.*, *25*, 397-435

Millot J. (1965) India and betel (Fr.). *Objets Mondes*, *5*, 73-88

Möller, I.J., Pindborg, J.J. & Effendi, I. (1977) The relation between betel chewing and dental caries. *Scand. J. dent. Res.*, *85*, 64-70

Mori, H., Matsubara, N., Ushimaru, Y. & Hirono, I. (1979) Carcinogenicity examination of betel nuts and piper betel leaves. *Experientia*, *35*, 384-385

Muir, C.S. (1962) Cancer of the buccal cavity and nasopharynx in Singapore. *Br. J. Cancer*, *16*, 583-591

Muir, C.S. & Kirk, R. (1960) Betel, tobacco, and cancer of the mouth. *Br. J. Cancer*, *14*, 597-608

Mujumdar, A.M., Kapadi, A.H. & Pendse, G.S. (1982) *Pharmacological properties*. In: Bavappa, K.V.A., ed., *The Areca Nut Palm*, Central Plant Crop Research Institute Publication, pp. 245-261

Nair, J., Ohshima, H., Friesen, M., Croisy, A., Bhide, S.V. & Bartsch, H. (1985) Tobacco-specific and betel nut-specific *N*-nitroso compounds: Occurrence in saliva and urine of betel quid chewers and formation *in vitro* by nitrosation of betel quid. *Carcinogenesis*, *6*, 295-303

Niblock, W.J. (1902) Cancer in India. *Indian med. Gaz.*, *37*, 161-165

Nieschulz, O. (1968) On betel and its reactions (Ger.). *Med. Monatsz.*, *22*, 386-389

Nieschulz, O. & Schmersahl, P. (1968) On the pharmacology of the active principles of betel. 2. Transformation of arecoline to arecaidine (Ger.). *Arzneimittelforschung*, *18*, 222-225

Notani, P.N. & Sanghvi, L.D. (1976) Role of diet in the cancers of the oral cavity. *Indian J. Cancer*, *13*, 156-160

Orr, I.M. (1933) Oral cancer in betel nut chewers in Travancore. Its aetiology, pathology, and treatment. *Lancet*, *ii*, 575-580

Padmavathy, G. & Reddy, D.J. (1960) Aetiological factors of carcinoma of the mouth and pharynx in Guntur. *J. Indian med. Assoc.*, *34*, 84-88

Pai, S.R., Shirke, A.J. & Gothoskar, S.V. (1981) Long-term feeding study in C17 mice administered saccharin coated betel nut and 1,4-dinitrosopiperazine in combination. *Carcinogenesis*, *2*, 175-177

Panigrahi, G.B. & Rao, A.R. (1982) Chromosome-breaking ability of arecoline, a major betel-nut alkaloid, in mouse bone-marrow cells *in vivo*. *Mutat. Res.*, *103*, 197-204

Panigrahi, G.B. & Rao, A.R. (1983) Influence of caffeine on arecoline-induced SCE in mouse bone-marrow cells *in vivo*. *Mutat. Res.*, *122*, 347-353

Panigrahi, G.B. & Rao, A.R. (1984) Induction of in vivo sister chromatid exchanges by arecaidine, a betel nut alkaloid, in mouse bone-marrow cells. *Cancer Lett.*, *23*, 189-192

Paymaster, J.C. (1956) Cancer of the buccal mucosa. A clinical study of 650 cases in Indian patients. *Cancer*, *9*, 431-435

Paymaster, J.C. (1962) Some observations on oral and pharyngeal carcinomas in the State of Bombay. *Cancer*, *15*, 578-583

Paymaster, J.C. & Gangadharan, P. (1970) Cancer in the Parsi community of Bombay. *Int. J. Cancer*, *5*, 426-431

Peeters, A. (1970) *La Chique de Betel. Etude Ethnobotanique* (Betel quid. Ethnobotanical study), Vol. 1, Thesis, Paris, Faculté des Lettres et des Sciences Humaines, p. 67

Pindborg, J.J. (1965) Oral precancerous conditions in South East Asia. *Int. dent. J.*, *15*, 190-199

Pindborg, J.J. (1972) Is submucous fibrosis a precancerous condition in the oral cavity? *Int. dent. J.*, *22*, 474-480

Pindborg, J.J., Srivastava, A.N. & Gupta, D. (1964) Studies in oral leukoplakias. VIII. Epithelial changes in tobacco-induced leukoplakias in India. *Acta odontol. scand.*, *22*, 499-512

Pindborg, J.J., Chawla, T.N., Misra, R.K., Nagpaul, R.K. & Gupta, V.K. (1965) Frequency of oral carcinoma, leukoplakia, leukokeratosis, leukoedema, submucous fibrosis, and lichen planus in 10,000 Indians in Lucknow, Uttar Pradesh, India. *J. dent. Res.*, *44*, 615

Pindborg, J.J., Kiaer, J., Gupta, P.C. & Chawla, T.N. (1967) Studies in oral leukoplakias. Prevalence of leukoplakia among 10 000 persons in Lucknow, India, with special reference to use of tobacco and betel nut. *Bull. World Health Organ.*, *37*, 109-116

Pindborg, J.J., Barmes, D. & Roed-Petersen, B. (1968) Epidemiology and histology of oral leukoplakia and leukoedema among Papuans and New Guineans. *Cancer*, *22*, 379-384

Pindborg, J.J., Mehta, F.S., Daftary, D.K., Gupta, P.C. & Bhonsle, R.B. (1972) Prevalence of oral lichen planus among 7639 Indian villagers in Kerala, South India. *Acta dermato-venereol.*, *52*, 216-220

Pindborg, J.J., Zheng, K.-H., Kong, C.-R. & Lin, F.-X. (1984a) Pilot survey of oral mucosa in areca (betel) nut chewers on Hainan Island of the People's Republic of China. *Commun. Dent. oral Epidemiol.*, *12*, 195-196

Pindborg, J.J., Murti, P.R., Bhonsle, R.B., Gupta, P.C., Daftary, D.K. & Mehta, F.S. (1984b) Oral submucous fibrosis as a precancerous condition. *Scand. J. dent. Res.*, *92*, 224-229

Piyaratn, P. (1959) Relative incidence of malignant neoplasms in Thailand. *Cancer*, *12*, 693-696

Raghavan, V. & Baruah, H.K. (1958) Arecanut: India's popular masticatory — History, chemistry and utilization. *Econ. Bot.*, *12*, 315-325

Ranadive, K.J., Gothoskar, S.V., Rao, A.R., Tezabwalla, B.U. & Ambaye, R.Y. (1976) Experimental studies on betel nut and tobacco carcinogenicity. *Int. J. Cancer*, *17*, 469-476

Ranadive, K.J., Ranadive, S.N., Shivapurkar, N.M. & Gothoskar, S.V. (1979) Betel quid chewing and oral cancer: Experimental studies on hamsters. *Int. J. Cancer*, *24*, 835-843

Rao, A.R. (1984) Modifying influences of betel quid ingredients on B(a)P-induced carcinogenesis in the buccal pouch of hamster. *Int. J. Cancer*, *33*, 581-586

Reddy, D.G. & Anguli, V.C. (1967) Experimental production of cancer with betel nut, tobacco and slaked lime mixture. *J. Indian med. Assoc.*, *49*, 315-318

Reddy, M.S., Naik, S.R., Bagga, O.P. & Chuttani, H.K. (1980) Effect of chronic tobacco-betel-lime 'quid' chewing on human salivary secretions. *Am. J. clin. Nutr.*, *33*, 77-80

Reichart, P., Böning, W., Srisuwan, S., Theetranont, C. & Mohr, U. (1984) Ultrastructural findings in the oral mucosa of betel chewers. *J. oral Pathol.*, *13*, 166-177

Reichart, P.A., Lenz, H., König, H., Becker, J. & Mohr, U. (1985) The black layer on the teeth of betel chewers. *J. oral Pathol.* (in press)

Rosin, M.P. (1984) The influence of pH on the convertogenic activity of plant phenolics. *Mutat. Res.*, *135*, 109-113

Sadasivan, G., Rani, G. & Kumari, C.K. (1978) Chromosome-damaging effect of betel leaf. *Mutat. Res.*, *57*, 183-185

Samuel, K.C., Navani, H. & Logani, K.B. (1969) Epidemiology of oral carcinoma in eastern districts of Uttar Pradesh. *J. Indian med. Assoc.*, *53*, 179-186

Sanghvi, L.D., Rao, K.C.M. & Khanolkar, V.R. (1955) Smoking and chewing of tobacco in relation to cancer of the upper alimentary tract. *Br. med. J.*, *i*, 1111-1114

Sarma, S.N. (1958) A study into the incidence and etiology of cancer of the larynx and adjacent parts in Assam. *Indian J. med. Res.*, *46*, 525-533

Schamschula, R.G., Adkins, B.L., Barmes, D.E. & Charlton, G. (1977) Betel chewing and caries experience in New Guinea. *Commun. Dent. oral Epidemiol.*, *5*, 284-286

Schonland, M. & Bradshaw, E. (1969) Upper alimentary tract cancer in Natal Indians with special reference to the betel-chewing habit. *Br. J. Cancer*, *23*, 670-682

Scrimgeour, E.M. & Jolley, D. (1983) Trends in tobacco consumption and incidences of associated neoplasms in Papua New Guinea. *Br. med. J.*, *286*, 1414-1416

Sethi, S.C. & Aggarwal, J.S. (1956) Stabilization of edible fats by spices. Part II. A new antioxidant from betel leaf. *J. Sci. ind. Res.*, *15B*, 34-36

Shanta, V. & Krishnamurthi, S. (1959) A study of aetiological factors in oral squamous cell carcinoma. *Br. J. Cancer*, *13*, 381-388

Shanta, V. & Krishnamurthi S. (1963) Further study in aetiology of carcinomas of the upper alimentary tract. *Br. J. Cancer*, *17*, 8-23

Shiau, Y.-Y. & Kwan, H.-W. (1979) Submucous fibrosis in Taiwan. *Oral Surg.*, *47*, 453-457

Shirname, L.P., Menon, M.M., Nair, J. & Bhide, S.V. (1983) Correlation of mutagenicity and tumorigenicity of betel quid and its ingredients. *Nutr. Cancer*, *5*, 87-91

Shirname, L.P., Menon, M.M. & Bhide, S.V. (1984) Mutagenicity of betel quid and its ingredients using mammalian test systems. *Carcinogenesis*, *5*, 501-503

Shivapurkar, N.M. & Bhide, S.V. (1978) Effect of betel nut constituents on sulfhydryl metabolism. *Indian J. Pharmacol.*, *10*, 257-264

Shivapurkar, N.M. & Bhide, S.V. (1979) Effect of betel nut constituents on nucleic acid metabolism. *Indian J. exp. Biol.*, *17*, 1141-1144

Shivapurkar, N.M., Bhide, S.V. & Ranadive, K.J. (1978) Biochemical studies on betel-nut constituents. *Indian J. Pharmacol.*, *10*, 191-200

Shivapurkar, N.M., Ranadive, S.N., Gothoskar, S.V., Bhide, S.V. & Ranadive, K.J. (1980a) Tumorigenic effect of aqueous and polyphenolic fractions of betel nut in Swiss strain mice. *Indian J. exp. Biol.*, *18*, 1159-1161

Shivapurkar, N.M., D'Souza, A.V. & Bhide, S.V. (1980b) Effect of betel-quid chewing on nitrite levels in saliva. *Food Cosmet. Toxicol.*, *18*, 277-281

Shivashankar, S., Dhanaraj, S., Mathew, A.G., Srinivasa Murthy, S., Vyasamurthy, M.N. & Govindarajan, V.S. (1969) Physical and chemical characteristics of processed arecanuts. *J. Food Sci. Technol.*, *41*, 113-116

Sidiq, Y., Rajagopalan, K.R. & Krishnamurthy, M.S. (1964) Aetiology and epidemiology of oral cavity cancers. *J. Indian med. Assoc.*, *43*, 526-529

Silverman, S., Jr, Bhargava, K., Mani, N.J., Smith, L.W. & Malaowalla, A.M. (1976) Malignant transformation and natural history of oral leukoplakia in 57,518 industrial workers of Gujarat, India. *Cancer*, *38*, 1790-1795

Simarak, S., De Jong, U.W., Breslow, N., Dahl, C.J., Ruckphaopunt, K., Scheelings, P. & MacLennan, R. (1977) Cancer of the oral cavity, pharynx/larynx and lung in North Thailand: Case-control study and analysis of cigar smoke. *Br. J. Cancer*, *36*, 130-140

Singh, A.D. & Von Essen, C.F. (1966) Buccal mucosa cancer in South India. Etiologic and clinical aspects. *Am. J. Roentgenol.*, *96*, 6-14

Sirsat, M.V. & Doctor, V.M. (1967) A histopathologic study on the effect of tobacco chewing on the buccal mucosa in Indians and its relationships to cancer. *Br. J. Cancer*, *21*, 277-284

Sirsat, S.M. & Kandarkar, S.V. (1968) Histological changes in the oral mucosa of the Wistar rat treated with commercial lime (calcium hydroxide) — An optical and submicroscopic study. *Br. J. Cancer*, *22*, 303-315

Sitaram, N. & Weingartner H. (1978) Human serial learning: Enhancement with arecholine and choline and impairment with scopolamine. *Science*, *201*, 274-276

Smith, L.W., Bhargava, K., Mani, N.J., Malaowalla, A.M. & Silverman, S., Jr (1975) Oral cancer and precancerous lesions in 57,518 industrial workers of Gujarat, India. *Indian J. Cancer*, *12*, 118-123

Srivastava, S.P. & Sharma, S.C. (1968) Gingival cancer. *Indian J. Cancer*, *5*, 89-97

Stephen, S.J. & Uragoda, C.G. (1970) Some observations on esophageal carcinoma in Ceylon, including its relationship to betel chewing. *Br. J. Cancer*, *24*, 11-15

Stich, H.F. & Rosin, M.P. (1984) Micronuclei in exfoliated human cells as a tool for studies in cancer risk and cancer intervention. *Cancer Lett.*, *22*, 241-253

Stich, H.F., Stich, W. & Lam, P.P.S. (1981) Potentiation of genotoxicity by concurrent application of compounds found in betel quid: Arecoline, eugenol, quercetin, chlorogenic acid and Mn^{2+}. *Mutat. Res.*, *90*, 355-363

Stich, H.F., Stich, W. & Parida, B.B. (1982) Elevated frequency of micronucleated cells in the buccal mucosa of individuals at high risk from oral cancer: Betel quid chewers. *Cancer Lett.*, *17*, 125-134

Stich, H.F., Bohm, B.A., Chatterjee, K. & Sailo, J. (1983a) *The role of saliva-borne mutagens and carcinogens in the etiology of oral and esophageal carcinomas of betel nut and tobacco chewers*. In: Stich, H.F., ed., *Carcinogens and Mutagens in the Environment*, Vol. 3, *Naturally Occurring Compounds: Epidemiology and Distribution*, Boca Raton, FL, CRC Press, pp. 43-58

Stich, H.F., Ohshima, H., Pignatelli, B., Michelon, J. & Bartsch, H. (1983b) Inhibitory effect of betel nut extracts on endogenous nitrosation in humans. *J. natl Cancer Inst.*, *70*, 1047-1050

Stich, H.F., San, R.H.C. & Rosin, M.P. (1983c) Adaptation of the DNA repair and micronucleus tests to human cell suspensions and exfoliated cells. *Ann. N.Y. Acad. Sci.*, *407*, 93-105

Stich, H.F., Dunn, B.P., Pignatelli, B., Ohshima, H. & Bartsch, H. (1984a) *Dietary phenolics and betel nut extracts as modifiers of* N-*nitrosation in rat and man*. In: O'Neill, I.K., von Borstel, R.C., Miller, C.T., Long, J. & Bartsch, H., eds, N-*Nitroso Compounds: Occurrence, Biological Effects and Relevance to Human Cancer (IARC Scientific Publications No. 57)*, Lyon, International Agency for Research on Cancer, pp. 213-222

Stich, H.F., Rosin, M.P. & Vallejera, M.O. (1984b) Reduction with vitamin A and beta-carotene administration of proportion of micronucleated buccal mucosal cells in Asian betel nut and tobacco chewers. *Lancet*, *i*, 1204-1206

Stich, H.F., Stich, W., Rosin, M.P. & Vallejera, M.O. (1985) Use of the micronucleus test to monitor the effect of vitamin A, beta-carotene and canthaxanthin on the buccal mucosa of betel nut/tobacco chewers. *Int. J. Cancer* (in press)

Suraiya, J.N. (1973) Medicine in ancient India with special reference to cancer. *Indian J. Cancer*, *10*, 391-402

Suri, K., Goldman, H.M. & Wells, H. (1971) Carcinogenic effect of a dimethyl sulphoxide extract of betel nut on the mucosa of the hamster buccal pouch. *Nature*, *230*, 383-384

Tanaka, T., Mori, H., Fujii, M., Takahashi, M. & Hirono, I. (1983) Carcinogenicity examination of betel quid. II. Effect of vitamin A deficiency on rats fed semipurified diet containing betel nut and calcium hydroxide. *Nutr. Cancer*, *4*, 260-266

Tennekoon, G.E. & Bartlett, G.C. (1969) Effect of betel chewing on the oral mucosa. *Br. J. Cancer*, *23*, 39-43

Tennent, J.E. (1980) *Ceylon. An Account on the Island, Physical, Historical, and Topographical .../with Notices of its Natural History, Antiquities and Productions*, Vol. 1, 5th rev., London, Longman, Green, Longman & Roberts, pp. 112-115, 438-439

Tolentino, A.D., Jr, Erese, B.C. & Soriano, O.L. (1963) Malignant and pre-malignant lesions of the oral cavity. *Philippine J. Cancer*, *5*, 406-416

Ueda, E. & Sasaki, T. (1951) Chemical studies of Formosan plants. I. Chemical constituents of the leaves of *Piper betle*. *J. pharm. Soc. Jpn*, *71*, 559-560

Umezawa, K., Fujie, S., Sawamura, M., Matsushima, T., Katoh, Y., Tanaka, M. & Takayama, S. (1981) Morphological transformation, sister chromatid exchange and mutagenesis assay of betel constituents. *Toxicol. Lett.*, *8*, 17-22

Verma, R.C., Chansoriya, M. & Kaul, K.K. (1983) Effect of tobacco chewing by mothers on fetal outcome. *Indian Pediatr.*, *20*, 105-111

Waerhaug, J. (1967) Prevalence of periodontal disease in Ceylon. Association with age, sex, oral hygiene, socioeconomic factors, vitamin deficiencies, malnutrition, betel and tobacco consumption and ethnic group. Final report. *Acta odontol. scand.*, *25*, 205-231

Wahi, P.N. (1968) The epidemiology of oral and oropharyngeal cancer. A report of the study in Mainpuri district, Uttar Pradesh, India. *Bull. World Health Organ.*, *38*, 495-521

Wahi, P.N., Arora, S., Srivastava, M.C., Kehar, U. & Bodkhe, R.R. (1961) Leukoplakia of oral cavity. *Indian J. Pathol. Bacteriol.*, *4*, 189-202

Wahi, P.N., Kehar, U. & Lahiri, B. (1965) Factors influencing oral and oropharyngeal cancers in India. *Br. J. Cancer*, *19*, 642-660

Wahi, P.N., Lahiri, B. & Kehar, U. (1966) Epidemiology of oral and oropharyngeal cancer. A study of regional factors in Uttar Pradesh. *J. Indian med. Assoc.*, *46*, 175-181

Wahi, P.N., Mital, V.P., Lahiri, B., Luthra, U.K., Seth, R.K. & Arora, G.D. (1970) Epidemiological study of precancerous lesions of the oral cavity: A preliminary report. *Indian J. med. Res.*, *58*, 1361-1391

Wenke, G. & Hoffmann, D. (1983) A study of betel quid carcinogenesis. I. On the in vitro *N*-nitrosation of arecoline. *Carcinogenesis*, *4*, 169-172

Wenke, G., Brunnemann, K.D., Hoffmann, D. & Bhide, S.V. (1984) A study of betel quid carcinogenesis. IV. Analysis of the saliva of betel chewers: A preliminary report. *J. Cancer Res. clin. Oncol.*, *108*, 110-113

WHO (1984) Control of oral cancer in developing countries. *Bull. World Health Organ.*, *62*, 817-830

Windholz, M., ed. (1983) *The Merck Index*, 10th ed., Rahway, NJ, Merck & Co., p. 266

Zaidi, S.H.M., Jafarey, N.A. & Aijaz Ali, S. (1974) Cancer trends in Karachi. *J. Pakistan med. Assoc.*, *24*, 87-93

ARECA NUT - fruit of the *Areca catechu* L. (Palmaceae) tree, a palm native to South Asia. The fruit is orange-yellow in colour when ripe and is the size of a small egg. The seed is separated from the fibrous pericarp and used fresh or dried. It is chewed either alone or as a component of mixtures usually including BETEL LEAF. Also known as BETEL NUT and *SUPARI*

BETEL LEAF - leaf of the vine *Piper betle* L. (Piperaceae), cultivated in hot, humid climates in South Asia. Serves as the wrapping for the BETEL QUID

BETEL NUT - see ARECA NUT

BETEL QUID - prepared usually by smearing a BETEL LEAF with SLAKED LIME and *CATECHU*, to which small pieces of ARECA NUT are added. Crushed leaves of cured tobacco may be added. Flavouring agents may be added. The ingredients are folded in the betel leaf and chewed. Also known as *PĀN*, in Hindi

BIDI - Indian cigarette that varies in length from 5-8 cm; it is made up of 0.25-0.5 g of locally-grown tobacco, usually sun-dried and cured, then flaked and hand-rolled in a rectangular piece of dried temburni leaf (*Diospyros melanoxylon*), and tied with cotton thread. Also known as *biri*

CATECHU - substance extracted from the heart-wood of the *Acacia catechu* or *A. suma* (Leguminoseae) tree, which grows in India and Burma, by steeping in boiling water. On cooling, catechin crystallizes out, leaving the more soluble catechutannic acid solution. In Malaysia, catechu is prepared as an aqueous extract of the twigs and leaves of the shrub *Uncaria gambir* (Rubiaceae). Also known as *kattha*

CHEWING TOBACCO - a tobacco product that is placed in the gingivo-buccal area and chewed from time to time. These products are of three main types: PLUG, TWIST/ROLL and LOOSE-LEAF (q.v.).

DIPPING - twigs are chewed to produce a flattened end, dipped into SNUFF and either chewed or used to apply the snuff to the gums. The term has now come to be used to refer to all oral use of snuff, especially in south-eastern USA.

FINE-CUT TOBACCO - type of smokeless tobacco, classified in the USA as chewing tobacco prior to 1980 and now categorized as moist fine-cut SNUFF

GUDAKHU - paste consisting of powdered tobacco, molasses and other ingredients; used for cleaning teeth in central and eastern states of India

KHAINI - a mixture of tobacco and LIME mixed in the hand, formed into a ball and placed in the mouth, usually along the mandibular groove

KIWAM - paste prepared from processed tobacco leaves, from which the stalks and stems have been removed, that are soaked and boiled in water with flavourings and spices, macerated and strained. The paste is chewed.

LIME - powder prepared from sea shells ('shell lime') or from quarried limestone; when mixed with water, SLAKED LIME (calcium hydroxide) is produced. Also known in India as *chuna* or *chunam*

LOOSE-LEAF TOBACCO - fermented cigar leaves, sweetened with sugars, syrups, liquorice and other flavouring materials, packaged as batches of loose pieces or cut strips

MAINPURI TOBACCO - a mixture of tobacco with SLAKED LIME, ARECA NUT, camphor and cloves, chewed in India

MISHRI - roasted or half-burnt tobacco prepared by baking on a hot metal plate, powdered and used primarily for cleaning teeth; also sometimes placed in the mouth as a substitute for chewing tobacco. Also known as *masheri* or *misheri*

NASS - a mixture of tobacco, LIME, wood-ash and cottonseed oil, chewed in Iran and the Soviet Socialist Republics of Central Asia

NASWAR - a mixture of powdered tobacco, SLAKED LIME and indigo placed on the floor of the mouth or in the labial groove behind the lower lip, popular in Afghanistan and Pakistan

PĀN - see BETEL QUID

PATTIWALA TOBACCO - sun-cured tobacco leaf chewed with or without lime in India

PILL - dried and pelleted *KIWAM* paste

PLUG TOBACCO - made from enriched tobacco leaves or leaf fragments wrapped in fine tobacco and pressed into flat bars or rolls; firm and moist plugs exist, firm being more common and containing <15% moisture, and moist containing >15% moisture

SHAMMAH - mixture of powdered tobacco leaves with calcium or sodium carbonate and other substances, including ash, placed in the buccal or lower labial vestibule of the mouth, used in southern Saudi Arabia

SLAKED LIME - see LIME

SMOKELESS TOBACCO - general term used to describe any of a variety of products and mixtures containing tobacco as the principal ingredient and utilized without combustion. The major products are SNUFF and CHEWING TOBACCO.

SNUFF - tobacco of variable composition for oral or nasal use. Two forms are used in western countries: moist and dry. Moist snuff consists of finely-cut tobacco with a high moisture content (up to 50%) and flavouring agents and is placed between the gum and buccal mucosa. Dry snuff is powdered tobacco with a moisture content usually <10%, to which flavouring agents are sometimes added; it is either placed between the lower lip and teeth or gum or between the gum and buccal mucosa or sniffed through the nose. Oriental snuff consists of approximately 50% dry tobacco and 50% heated calcium carbonate and phosphate with a small amount of powdered cuttle-fish bone. In southern Africa, snuff is prepared by mixing powdered tobacco leaves, ash of various plants, and sometimes oils, lemon juice and herbs.

SUPARI - see ARECA NUT

TWIST/ROLL TOBACCO - stripped tobacco leaves rolled or twisted into a rope form

ZARDA - tobacco leaf broken into small pieces and boiled in water with LIME and spices until evaporation, then dried and coloured with vegetable dyes; usually chewed mixed with ARECA NUT and spices

SOME RELATED NITROSAMINES

4-(METHYLNITROSAMINO)-4-(3-PYRIDYL)BUTANAL (NNA)[1]

1. Chemical and Physical Data

1.1 Synonyms and trade names

Chem. Abstr. Services Reg. No.: 64091-90-3

Chem. Abstr. Name: 3-Pyridinebutanal, γ-(methylnitrosoamino)-

IUPAC Systematic Name: γ-(Methylnitrosamino)-3-pyridinebutyraldehyde

1.2 Structural and molecular formulae and molecular weight

CHO

N—N=O

CH_3

N

$C_{10}H_{13}N_3O_2$ Mol. wt: 207.2

1.3 Chemical and physical properties of the pure substance

(*a*) *Spectroscopy data*: Ultraviolet, infrared and mass spectroscopy data have been reported (Hecht *et al.*, 1976, 1977, 1981).

(*b*) *Solubility*: Highly soluble in water (Hecht *et al.*, 1976)

(*c*) *Stability*: Relatively stable when stored in dichloromethane under nitrogen and at 0°C for two months, but decomposes slowly in air (Hecht *et al.*, 1976).

(*d*) *Reactivity*: Treatment with sodium nitrite gives 1-methyl-5-(3-pyridyl)pyrazole and other products (Hecht *et al.*, 1978a).

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[1]The abbreviation NNA was selected to emphasize the relationship of this compound to nicotine, and stands for 'nicotine-derived nitrosamino aldehyde'.

1.4 Technical products and impurities

This compound is not produced commercially.

Synthetic NNA is a mixture of 81% E-isomer and 19% Z-isomer (Hecht *et al.*, 1977).

2. Production, Use, Occurrence and Analysis

2.1 Production and use

NNA was prepared by Hecht *et al.* (1976) by the oxidation of 4-(methylnitrosamino)-4-(3-pyridyl)butanol with dimethylsulphoxide and dicyclohexylcarbodiimide.

When nicotine was reacted with 1.4 equivalents of sodium nitrite in aqueous solution under mild conditions (pH 3.4 at 20°C for 17 h), 80-90% of the nicotine was unreacted, but NNA was produced in yields of up to 2.8% of the starting nicotine. After reaction with five equivalents of sodium nitrite at 90°C, no NNA was found in the products (Hecht *et al.*, 1978a).

No evidence was found that NNA has ever been produced in commercial quantities or that it has any use other than as a laboratory chemical.

2.2 Occurrence

Under certain laboratory conditions, formation of NNA from nicotine has been observed, suggesting its presence in tobacco (Hecht *et al.*, 1978b). However, no analytical evidence could be found that NNA occurs in tobacco or in the saliva of snuff users (Hoffmann & Adams, 1981).

2.3 Analysis

NNA formed by reaction of nicotine and sodium nitrite has been detected in laboratory solutions by gas chromatography-mass spectroscopy (Hecht *et al.*, 1978c). However, suitable analytical methods for NNA in matrices such as tobacco have not been developed.

3. Biological Data Relevant to the Evaluation of Carcinogenic Risk to Humans

3.1 Carcinogenicity studies in animals

Intraperitoneal administration

Mouse: In a screening assay for potential carcinogenicity using pulmonary adenomas as an end-point in strain A mice, a group of 25 female strain A/J mice, six to eight weeks old, received thrice-weekly i.p. injections of 0.2 ml of a 0.5% solution of NNA (purity >99%; Hecht *et al.*, 1977) in saline for seven weeks (total of 22 injections, to give a total dose of 22 mg

[0.11 mmol]) and were held without further treatment for an additional 30 weeks. Further groups, consisting of 25 mice each, served as untreated and vehicle controls. Histological examination of lung and other organs that showed macroscopic lesions revealed lung adenomas in 1/25 untreated controls, 3/25 vehicle controls and 9/25 NNA-treated mice [for vehicle controls compared to treated mice, $p = 0.047$]. Neither lung adenocarcinomas nor malignant tumours were observed at other sites in any of the groups (Hecht *et al.*, 1978b).

3.2 Other relevant biological data

No data were available to the Working Group.

3.3 Case reports and epidemiological studies of carcinogenicity to humans

No data were available to the Working Group.

4. Summary of Data Reported and Evaluation

4.1 Exposure data

No analytical evidence was found that 4-(methylnitrosamino)-4-(3-pyridyl)butanal (NNA) occurs in tobacco or in the saliva of snuff users, although, under certain laboratory conditions, its formation from nicotine has been observed.

4.2 Experimental data

NNA was tested by intraperitoneal injection in female mice of one strain at one dose level only. Although the incidence of lung adenomas in treated mice exceeded those in controls, the experiment does not allow an evaluation of the carcinogenicity of NNA.

No data were available on mutagenic or related effects of NNA.

4.3 Human data

No case report or epidemiological study of the carcinogenicity of NNA to humans was available to the Working Group.

4.4 Evaluation[1]

The available data are *inadequate* to evaluate the carcinogenicity of 4-(methylnitrosamino)-4-(3-pyridyl)butanol to experimental animals.

No data on humans were available.

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[1]For descriptions of the italicized term, see Preamble, pp. 15-16.

5. References

Hecht, S.S., Chen, C.-H.B. & Hoffmann, D. (1976) Synthesis of *N*-nitrosamino aldehydes. *Tetrahedron Lett.*, *8*, 593-596

Hecht, S.S., Chen, C.-H.B., Dong, M., Ornaf, R.M. & Hoffmann, D. (1977) Chemical studies on tobacco smoke. LI. Studies on non-volatile nitrosamines in tobacco. *Beitr. Tabakforsch. int.*, *9*, 1-6

Hecht, S.S., Chen, C.-H.B., Ornaf, R.M., Hoffmann, D. & Tso, T.C. (1978a) *Chemical studies on tobacco smoke. LVI. Tobacco specific nitrosoamines: Origins, carcinogenicity and metabolism*. In: Walker, E.A., Castegnaro, M., Griciute, L. & Lyle, R.E., eds, *Environmental Aspects of* N-*Nitroso Compounds* (*IARC Scientific Publications No. 19*), Lyon, International Agency for Research on Cancer, pp. 395-413

Hecht, S.S., Chen, C.-H.B., Hirota, N., Ornaf, R.M., Tso, T.C. & Hoffmann, D. (1978b) Tobacco-specific nitrosamines: Formation from nicotine *in vitro* and during tobacco curing and carcinogenicity in strain A mice. *J. natl Cancer Inst.*, *60*, 819-824

Hecht, S.S., Chen, C.-H.B., Ornaf, R.M., Jacobs, E., Adams, J.D. & Hoffmann, D. (1978c) Reaction of nicotine and sodium nitrite: Formation of nitrosamines and fragmentation of the pyrrolidine ring. *J. org. Chem.*, *43*, 72-76

Hecht, S.S., Chen, C.-H.B., Young, R. & Hoffmann, D. (1981) Mass spectra of tobacco alkaloid-derived nitrosamines, their metabolites, and related compounds. *Beitr. Tabakforsch. int.*, *11*, 57-66

Hoffmann, D. & Adams, J.D. (1981) Carcinogenic tobacco-specific *N*-nitrosamines in snuff and in the saliva of snuff dippers. *Cancer Res.*, *41*, 4305-4308

4-(METHYLNITROSAMINO)-1-(3-PYRIDYL)-1-BUTANONE (NNK)[1]

1. Chemical and Physical Data

1.1 Synonyms and trade names

Chem. Abstr. Services Reg. No.: 64091-91-4

Chem. Abstr. Name: 1-Butanone, 4-(methylnitrosoamino)-1-(3-pyridinyl)-

IUPAC Systematic Name: 4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanone

Synonym: 4-(*N*-Methyl-*N*-nitrosamino)-1-(3-pyridyl)-1-butanone

1.2 Structural and molecular formulae and molecular weight

$C_{10}H_{13}N_3O_2$

Mol. wt: 207.2

1.3 Chemical and physical properties of the pure substance

(*a*) *Description*: Light-yellow crystalline solid

(*b*) *Melting-point*: 63-65°C (Hecht *et al.*, 1977)

(*c*) *Spectroscopy data*: Infrared, nuclear magnetic resonance (Hecht *et al.*, 1977) and mass spectroscopy data (Hecht *et al.*, 1981) have been reported.

(*d*) *Reactivity*: Reacts with isopropyl nitrite in a saturated solution of hydrogen chloride in methanol to form an α-oxime (Hecht *et al.*, 1978a). Can be reduced with sodium borohydride to 4-(methylnitrosamino)-1-(3-pyridyl)butan-1-ol (NNAl)[2]. Can be oxidized with *meta*-chloroperbenzoic acid to give 4-(methylnitrosamino-1-(3-pyridyl-*N*-oxide)-1-butanone (NNK-*N*-oxide) (Hecht *et al.*, 1980a)

[1]The abbreviation NNK was selected to emphasize the relationship of this compound to nicotine, and stands for 'nicotine-derived nitrosamino ketone'.

[2]NNAl, nicotine-derived nitrosamino alcohol

1.4 Technical products and impurities

This compound is not produced commercially.

Synthetic NNK and NNK isolated from tobacco are mixtures of 72.7% E-isomer and 27.3% Z-isomer (Hecht *et al.*, 1977; Hoffmann *et al.*, 1980a).

2. Production, Use, Occurrence and Analysis

2.1 Production and use

NNK was prepared by Hecht *et al.* (1977) by the reaction of sodium hydroxide and sodium nitrite with 4-(*N*-methyl)-1-(3-pyridyl)-1-butanone dihydrochloride.

NNK was obtained by Hecht *et al.* (1978b) as one of several reaction products when nicotine was allowed to react with 1.4 equivalents of sodium nitrite in aqueous solution at 20°C for 17 h. The highest yield of NNK at pH 4.5 was only 0.5% of the theoretical amount. When nicotine was reacted with five equivalents of sodium nitrite at 90°C for 3 h at pH 3.4-4.2, a 2.3% yield of NNK was obtained.

No evidence was found that NNK has ever been produced in commercial quantities or that it has any uses other than as a laboratory chemical.

2.2 Occurrence

(*a*) *Tobacco and tobacco smoke*

NNK is formed by oxidation and nitrosation of nicotine, during the curing, ageing, processing and smoking of tobacco. NNK has been found in tobacco at levels of 0.1-35 mg/kg, in snuff products at 0.2-8.3 mg/kg, and in cigarette smoke at 0.1-0.5 μg/cigarette. Additional quantities of NNK may be formed in the oral cavity during oral use of snuff or tobacco chewing (Hoffmann *et al.*, 1979, 1980b; Hoffmann & Adams, 1981; Hoffmann *et al.*, 1982a; Brunnemann *et al.*, 1983; Hoffmann & Hecht, 1983).

Table 1 summarizes the data obtained from analyses of the smoke of selected commercial cigarettes with and without filter tips and shows the effectiveness of the filters of cigarettes D and E in decreasing the occurrence levels of NNK (Hoffmann *et al.*, 1982b).

Nicotine and NNK concentrations found in cigarette and cigar tobaccos, in both their mainstream (during puff drawing) and sidestream (generated during smouldering of tobacco in between puffs) smoke and in chewing tobacco are presented in Table 2.

Studies using tracer compounds [carbonyl-^{14}C]NNK (Castonguay *et al.*, 1984a) demonstrated that 6.9-11.0% of the NNK formed in tobacco during the curing process transfer into the cigarette mainstream smoke. This constitutes 26-37% of the NNK present in the mainstream smoke. Thus, 63-74% of NNK in the cigarette mainstream smoke are formed during smoking. The cigarette types used in this study were a US commercial non-filter cigarette and three experimental non-filter cigarettes (burley tobacco, flue-cured tobacco and tobacco blend) (Adams *et al.*, 1983b).

Table 1. Reduction of nicotine and NNK concentrations in cigarette smoke by filtration[a]

Cigarette	Length smoked/length of cigarette (mm)	Nicotine (mg/cigarette)[b]	NNK (µg/cigarette)[b]
A NF	50/65	1.82	0.7
F	50/85	1.10 (-40%)	0.4 (-50%)
B NF	50/65	1.84	0.5
F	50/85	1.31 (-29%)	0.3 (-48%)
C NF	50/65	1.66	0.7
F	50/85	1.15 (-30%)	0.4 (-45%)
D NF	50/65	1.60	0.8
F	50/85	1.05 (-34%)	0.2 (-73%)
E[c] NF	50/73	1.63	0.9
F	50/100	1.08 (-34%)	0.3 (-71%)

[a]Data from Hoffmann *et al.* (1982b)
[b]In parentheses, percentage changes in yields on comparing filtered (F) and non-filtered (NF) smoke
[c]Perforated filter tip

Table 2. NNK concentrations in cigarettes, cigars and chewing tobacco, and in cigarette and cigar mainstream and sidestream smoke[a]

Tobacco product[b]	NNK concentration		
	In tobacco (mg/kg)	In mainstream smoke (µg/cig)	In sidestream smoke (µg/cig)
Burley cigarette, NF	ND[c]	0.3	0.7
Bright cigarette, NF	0.4	0.4	0.5
Commercial cigarette, NF	0.7	0.1	0.4
Commercial cigarette, FA	0.7	0.2	0.2
Kentucky 1R1, NF	0.1	0.2	0.2
US cigarette, NF[d]	--	0.8	--
US cigarette, NF[e]	1.4	0.2	--
German (Federal Republic) cigarettes[f]			
Brand A, NF	--	0.08	--
Brand B, NF	--	0.04	--
Brand C, FA	--	0.06	--
Brand D, FA		0.02	--
Brand E, FA	--	0.05	--
Brand F, FA	-	0.06	--
Commercial French cigarette, NF, 70 mm	0.5	0.4	--
Commercial French cigarettes, FA, 70 mm			
NF	0.4	0.4	--
FA	1.1	0.4	--
FP	1.1	0.2	--
	1.1	0.1	--
Little cigar, FA	35.0	4.2	0.8
Cigar (Colombian tobacco) (5.7 g)	1.1	1.9	15.7
Fine-cut chewing tobacco	2.4	NA[g]	NA
Fine-cut chewing tobacco[d]	7.4	NA	NA

[a]Data from Hoffmann *et al.* (1980b), unless otherwise noted
[b]All cigarettes and the little cigar were 85 mm long, unless otherwise noted. Abbreviations: NF, non-filter; FA, cellulose acetate filter; FP, paper filter
[c]ND, not detected
[d]Data from Adams *et al.* (1983a); cigarettes and tobacco used were purchased in Westchester County, NY, in 1981
[e]Data from Adams *et al.* (1983b); cigarettes used were purchased in Westchester County, NY, in 1981
[f]Data from Rühl *et al.* (1980); cigarettes were popular brands purchased in Berlin in 1979
[g]NA, not applicable

NNK concentrations found after analysis of snuff obtained in Sweden, Denmark, the Federal Republic of Germany and the USA were higher than those in cigarette tobacco presented above. The results are summarized in Table 3. NNK concentrations differed not only among snuff brands but also in samples of the same brand bought in different cities. The latter differences were attributed to possible variations in NNK content between batches and/or effects of ageing. The effects of ageing were demonstrated by opening individual portions (packed in aluminium foil) and storing the snuff in the open air: within eight days, the NNK content had increased by 48% and then remained stable (Hoffmann & Adams, 1981; Hoffmann *et al.*, 1982b).

Table 3. Nicotine and NNK concentrations in commercial snuff[a]

Snuff origin	Type of packaging[b]	Nicotine (%)	NNK (mg/kg)[c]
USA			
Brand I New York and Tennessee	A	2.4	2.4
Brand II New York and Tennessee	A	2.3	4.7
Brand III New York and Tennessee	B	1.5	1.3
Federal Republic of Germany			
Brand I Munich	C	0.6	1.5
Brand II Munich	C	0.5	1.5
Sweden			
Brand I Umeå	A	1.5	2.2
Brand I Uppsala	A	1.5	1.4
Brand I Lund	A	1.5	2.3
Brand II Umeå	A	1.8	1.8
Brand II Uppsala	A	1.8	0.6
Brand II Lund	A	1.8	2.1
Brand III Umeå	A	0.6	2.5
Brand III Uppsala	A	0.6	0.9
Brand III Lund	A	0.7	0.9
Brand IV Umeå	A	1.1	2.1
Brand IV Uppsala	A	1.1	3.8
Brand IV Lund	A	1.1	1.6
Brand V Umeå	D	2.2	1.3
Denmark			
Brand I Copenhagen	E	1.1	2.0
Brand II Copenhagen	E	2.1	1.4
Brand III Copenhagen	E	3.1	7.0
Ageing test[d]: 0 time	-	-	1.3
8 days	-	-	1.9

[a]Data from Hoffmann and Adams (1981) and Hoffmann *et al.* (1982b); values are given for dry snuff, moisture content about 50%

[b]Abbreviations: A, waxed-paper container with metallic lid, containing approximately 50 g; B, 25 individual portions of approximately 11 g packaged in paper in a plastic container; C, plastic foil-lined aluminium bags containing 100 g; D, individual snuff portion in a paper bag packaged in a crimped airtight aluminium envelope, with 10 envelopes in a plastic bag, amounting to approximately 10 g; E, hard-plastic container

[c]NNK values are averages of three runs.

[d]The aluminium foil-wrapped package (Swedish brand V) was opened at '0 time'.

(*b*) *Human tissues and secretions*

Saliva was examined from women who had been long-term oral-snuff users and who were employed in two southern US furniture companies. The wide range of NNK concentrations (2.1-201 ng/g) in the saliva of individual users indicated that during oral use of snuff NNK is extracted from the tobacco plug at varying rates (Hoffmann & Adams, 1981).

In another study, saliva of four women who were long-term (>10 years) oral-snuff users was analysed on two different days after they had used a specific brand of snuff with known levels of NNK. NNK and nicotine levels in saliva (Table 4) varied significantly between subjects, as well as between samples from the same individual taken on different days. The variations in NNK values were at least partially explained by differences in the intensity with which individuals practised the habit at different times, and perhaps are also due to varying rates of salivation (Hoffmann & Adams, 1981; Hoffmann *et al.* 1982b).

Table 4. Nicotine and NNK concentrations in snuff and in saliva of women who were long-term oral-snuff users[a]

Subject	Age (years)	Snuff		Saliva[b]		
		Nicotine (mg/g)	NNK (μg/g)	Day of sampling	Nicotine (mg/g)	NNK (μg/g)
1	41	23.4	4.7	1	0.2	0.026
				2	0.5	0.021
2	37	23.4	4.7	1	0.07	0.013
				2	0.4	<0.010
3	44	23.4	4.3	1	1.2	0.096
				2	1.6	0.062
4	52	23.6	5.2	1	0.2	0.01
				2	0.4	0.023

[a]Data from Hoffmann and Adams (1981) and Hoffmann *et al.* (1982b)

[b]Saliva of three women who did not use snuff (controls) was free of nicotine and tobacco-specific *N*-nitrosamines.

NNK was found in the saliva of chewers of betel quid with tobacco at levels of 1.0-2.3 ng/g [mean, 1.5 ng/g] (Wenke *et al.*, 1984) and up to 2.3 ng/ml (mean, 0.34 ng/ml) (Nair *et al.*, 1985).

2.3 Analysis

Standard methods for the analysis of NNK are described in detail in an IARC manual on selected methods of analysis (Egan *et al.*, 1983).

3. Biological Data Relevant to the Evaluation of Carcinogenic Risk to Humans

3.1 Carcinogenicity studies in animals

(a) Subcutaneous and/or intramuscular administration

Rat: Groups of 12 male and 12 female Fischer 344 rats, seven weeks of age, received thrice-weekly s.c. injections of 11.7 mg [0.06 mmol] NNK (purity, >99%) in 0.3 ml trioctanoin for 20 weeks (total dose, 702 mg; 3.5 mmol). Animals were maintained until they died spontaneously or were killed after 12 months. Malignant tumours of the nasal cavity (olfactory neuroblastomas and rhabdomyosarcomas) were observed in 6/12 males and in 4/12 females; malignant tumours of the lung (an adenocarcinoma and adenosquamous-cell carcin-

omas) in 5/12 males; and malignant liver tumours (hepatocellular carcinomas and haemangiosarcomas) in 7/12 males and 12/12 females. No tumour of the nasal cavity, lung or liver was found in 12 vehicle controls that received trioctanoin only (Hecht *et al.*, 1980b).

Groups of 15-27 male and 15-27 female Fischer 344 rats, nine weeks of age, received thrice weekly s.c. injections of NNK (purity, >99%; Hecht *et al.*, 1977) in trioctanoin (total doses, approximately 9.0, 3.0 and 1.0 mmol/kg bw), or trioctanoin alone (vehicle controls; 27 males and 27 females) for 20 weeks (with a two-week interruption after seven weeks of injections). Animals were killed when moribund or when only 20% of rats in a group were still alive (the experiment was ended at 70-120 weeks). No difference in body weight was seen among treated and control animals. By 80 weeks, all animals in the high-dose group had died; all animals in the other two groups had died by 120 and 130 weeks, respectively. The incidences of malignant tumours in the nasal cavity (aesthesioneuroepitheliomas, squamous-cell carcinomas, anaplastic carcinomas, spindle-cell sarcomas) and of benign tumours (squamous- and transitional-cell papillomas and polyps) in males and females of the high-, medium- and low-dose groups were 14/15 and 14/15, 13/15 and 12/15, and 20/27 and 10/27, respectively. Malignant tumours predominated in the high-dose group (21/28), but nasal-cavity tumours in the low-dose group were mostly benign (29/30). Incidences of lung tumours (adenomas, adenocarcinomas, squamous-cell carcinomas) for males and females in the high-, medium- and low-dose groups were 14/15 and 9/15, 13/15 and 7/15, and 23/27 and 8/27, respectively. These incidences all differed significantly from those in vehicle-control rats who received trioctanoin only: none had a nasal-cavity tumour and 1/52 had a lung adenoma. The incidences of benign and malignant liver tumours in treated males and females in the high-, medium- and low-dose groups were 6/15 and 5/15, 4/15 and 4/15, and 3/27 and 4/27, respectively. Of the vehicle-control rats, 3/26 males and 1/26 females had benign liver tumours. Among the 114 NNK-treated rats, only two benign oesophageal tumours were observed; none were observed in controls (Hoffmann *et al.*, 1984). [The Working Group interpreted these results as showing dose-response relationships for induction of tumours of the nasal cavity, lung and liver.]

Hamster: Groups of 15 male and 15 female Syrian golden hamsters, aged eight to 10 weeks, received thrice-weekly s.c. injections of 10 mg (0.048 mmol) NNK (purity, >99%) in 0.3 ml trioctanoin for 6.3 weeks (19 injections; total dose, 190 mg [0.91 mmol]); a second group of 10 males and 10 females, aged eight to 10 weeks, received thrice-weekly s.c. injections of 2.5 mg (0.012 mmol) NNK in 0.3 ml trioctanoin for 25 weeks (75 injections; total dose, 190 mg [0.91 mmol]). Hamsters were maintained until they died or were killed after 16 months (first group and vehicle controls) or 17 months (second group). There was extensive early mortality in the first group, with only 4/15 male and 4/15 female hamsters still alive after 14 weeks; in contrast, the percentages surviving in the second group after seven, 10 and 13 months were 80%, 75% and 30%, respectively. Among 15 male and 15 female vehicle controls, the only tumours observed were a sarcoma at the site of injection and an adrenocortical adenoma. In the first treated group, 22/30 hamsters had respiratory-tract tumours: 10 males with one adenocarcinoma and seven adenomas of the lung and two pleomorphic carcinomas of the nasal cavity; and 12 females with three adenocarcinomas and eight adenomas of the lung and one pleomorphic carcinoma of the nasal cavity. Of these, 14 hamsters with lung adenomas and one with lung adenocarcinoma died within the first 11 weeks of the experiment. In the second group, NNK induced tumours of the lung, trachea or nasal cavity in 18/20 animals (males and females). These included four adenomas and six adenocarcinomas of the lung, five pleomorphic carcinomas and one papilloma of the nasal cavity and three tumours of the trachea in males; and four adenocarcinomas and two adenomas of the lung, five pleomorphic carcinomas of the nasal cavity and four tumours of the trachea in females. In both groups, there were a number of malignant tumours at other sites, and several hamsters had multiple tumours (Hoffmann *et al.*, 1981).

Groups of 10 male and 9-10 female Syrian golden hamsters, eight weeks of age, received single s.c. injections of 1.0 mg (4.8 μmol), 3.3 mg (15.9 μmol) or 10 mg (48.3 μmol) NNK (purity, >99%) in 0.3 ml trioctanoin and were exposed one week later to cigarette smoke or underwent sham exposure twice daily for 69 weeks, at which time surviving hamsters were killed. No tumour of the lung, trachea or nasal mucosa was observed in vehicle-control hamsters exposed to smoke or sham smoking. In contrast, hamsters in all groups treated with NNK had tumours of the respiratory tract (lung, nasal cavity or trachea); and in six (five groups exposed to cigarette smoke and one group sham exposed) of the 12 NNK-treated groups the numbers of animals with tumours significantly exceeded that among controls. Of the hamsters not exposed to smoke, 4/10 males given 10 mg NNK had tumours, including three with lung adenomas and two with papillomas of the nasal mucosa, and 1/9 females (a mucoepidermoid carcinoma of the lung and an olfactory neuroblastoma). Of the hamsters given 3.3 mg NNK, 2/10 males had tumours of the lung (1, an adenocarcinoma) and 1/10 females had a nasal papilloma. Of those given 1 mg NNK, 3/10 males had tumours, including two lung adenomas, one nasal-mucosa papilloma and one tumour of the trachea, and 3/10 females, including two lung adenomas and one nasal-mucosa papilloma (Hecht *et al.*, 1983a).

(*b*) *Intraperitoneal administration*

Mouse: In a screening study for potential carcinogenicity using pulmonary adenomas as an end-point in strain A mice, a group of 25 female strain A/J mice, six to eight weeks old, received thrice-weekly i.p. injections of 0.1 ml of a 1.0% solution of NNK (purity, >99%; Hecht *et al.*, 1977) in trioctanoin over 7.3 weeks (22 injections; total dose, 22 mg; 0.11 mmol) and were killed 30 weeks later. Lung adenomas were observed in 1/25 untreated controls, 5/24 vehicle controls, and 20/23 NNK-treated mice (2.6 lung tumours per animal). No lung adenocarcinoma or malignant tumour at any other site was seen (Hecht *et al.*, 1978c).

A group of 25 female strain A/J mice, six to eight weeks old, received thrice-weekly i.p. injections of 0.1 ml of a solution of NNK (purity, >99%) in 0.9% trioctanoin for 7.3 weeks (22 injections; total dose, 23 mg; 0.11 mmol) and were killed 30 weeks later. All of the 23 surviving, NNK-treated mice had lung tumours, with an average of 37.6 ± 11.8 per mouse. Of the total of 865 lung tumours, 412 were classified as carcinomas. In addition, one mouse had three liver adenomas and two hepatocellular carcinomas and one had a squamous-cell papilloma of the nasal cavity. In comparison, of 25 vehicle controls, four had lung tumours, with a total of five adenomas and no carcinoma. No other tumour was found in this group. In a group of 25 untreated controls, 10 had lung tumours including 16 adenomas and two carcinomas (Castonguay *et al.*, 1983a).

(*c*) *Carcinogenicity of metabolites*

Mouse: In a screening assay for potential carcinogenicity using pulmonary adenomas as an endpoint in strain A mice, a group of 25 female strain A/J mice, six to eight weeks old, received thrice-weekly i.p. injections of NNK-1-*N*-oxide (purity, >99%; Hecht *et al.*, 1980a) or 4-(methylnitrosamino)-1-(3-pyridyl)butan-1-ol (NNAl)[1] (purity, 99.8%) in 0.1 ml trioctanoin for 7.3 weeks (22 injections; total dose, 23 mg; 0.11 mmol) and were killed 30 weeks later. Lung adenomas were observed in 25/25 NNAl-treated mice (with an average of 26.3 ± 11.7 tumours per mouse), 24/25 NNK-1-*N*-oxide-treated mice (3.6 ± 2.7 tumours/mouse), 4/25 trioctanoin-treated controls (0.2 ± 0.5 tumours/mouse) and 10/25 untreated controls (0.6 ± 0.9 tumours per mouse) (Castonguay *et al.*, 1983a).

[1]NNAl, nicotine-derived nitrosamino alcohol; see also Fig. 1, p. 217.

3.2 Other relevant biological data

(a) Experimental systems

Toxic effects

No data were available to the Working Group.

Effects on reproduction and prenatal toxicity

No data were available to the Working Group on effects on reproduction.

Pregnant C57BL mice were given intravenous injections of 7.0 mg/kg bw [carbonyl-^{14}C]NNK (Castonguay & Hecht, 1985) on days 13, 16 or 18 of gestation and killed at intervals from 5 min to 8 h. Whole-body autoradiograms revealed the presence of radioactivity in various tissues of the mother, in foetal kidneys and urinary bladder, and in the amniotic fluid. NNK and its carbonyl-reduced metabolite, 4-(methylnitrosamino)-1-(3-pyridyl)butan-1-ol (NNAl)[1], were present in the placentas and foetal tissues; some NNK metabolites were covalently bound to tissues of the nose, lung and liver of 18-day-old foetuses. This was shown *in vitro* to be due to the capacity of these foetal tissues to activate NNK metabolically. Enzymatic α-hydroxylation of NNK did not occur in 13-day-old foetal tissue but did so in 16- and 18-day-old foetal tissue. These results demonstrate that NNK and NNAl can cross the placental barrier and be activated by foetal tissues (Castonguay *et al.*, 1984a).

Absorption, distribution, excretion and metabolism

Male Fischer 344 rats were administered 3.5 mg/kg bw [carbonyl-^{14}C]NNK intravenously and killed at various intervals for whole-body autoradiographic studies. After one minute, radioactivity was distributed in most tissues at levels similar to that in blood. Radioactivity exceeding background levels was observed in the mucosa of the ethmoturbinates, the lateral nasal gland, liver, bronchial mucosa, adrenal cortex, preputial gland, submaxillary salivary gland and contents of the stomach. Four and 24 h after injection, these tissues still showed relatively high labelling. Non-extractable, tissue-bound radioactivity was present in the nasal tissues, bronchi and liver. After oral administration of 3.5 mg/kg bw [carbonyl-^{14}C]NNK, the distribution of radioactivity was similar to that observed after intravenous administration, except that there was more labelling in the mouth, oesophagus and upper gastrointestinal tract (Castonguay *et al.*, 1983b). Syrian golden hamsters were given intravenous or subcutaneous injections of 4.9 mg/kg bw [carbonyl-^{14}C]NNK and killed at various intervals for whole-body autoradiography; bound radioactivity was present in the tracheo-bronchial mucosa, nasal mucosa, liver and lateral nasal glands (Tjälve & Castonguay, 1983).

Male Fischer 344 rats given 7.1 μg/kg bw [carbonyl-^{14}C]NNK orally excreted 88% of the dose in 48-h urine, 3% in faeces and $<$0.5% in expired air; after oral administration of 5.2 μg/kg bw [$^{14}CH_3$]NNK, 39% of the dose was excreted in 48-h urine, 8% in faeces, and 47% in expired air as $^{14}CO_2$ (Castonguay *et al.*, 1983b). Male Syrian golden hamsters given 10 mg (59 mg/kg bw) [carbonyl-^{14}C]NNK subcutaneously excreted 96-98% of the radioactivity in 48-h urine and the remainder in faeces (Hoffmann *et al.*, 1981).

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[1]NNAl, nicotine-derived nitrosamino alcohol; see also Fig. 1, p. 217.

Fig. 1. Metabolic transformations of NNK. Structures in brackets represent hypothetical intermediates[a]

[a]Adapted from Castonguay *et al.* (1983a) and Hecht *et al.* (1983b); Compounds: *1*, 1-(methylnitrosamino)-1-(3-pyridyl-*N*-oxide)-1-butanone; *2*, 4-(methylnitrosamino)-1-(3-pyridyl-*N*-oxide)butan-1-ol; *9*, 4-oxo-4-(3-pyridyl)butanal; *11*, 2-hydroxy-5-(3-pyridyl)tetrahydrofuran; *13*, myosmine; *14*, 4-hydroxy-1-(3-pyridyl)-1-butanone; *15*, 4-oxo-4-(3-pyridyl)butyric acid; *16*, 4-hydroxy-4-(3-pyridyl)butyric acid; *17*, 4-hydroxy-4-(3-pyridyl)-1-butanol

Metabolic pathways of NNK are summarized in Figure 1. Three types of metabolic reaction have been established; reduction of the carbonyl group to give 4-(methylnitrosamino)-1-(3-pyridyl)butan-1-ol (NNAl), hydroxylation of the methyl and methylene carbons adjacent (α-) to the *N*-nitroso group, to yield the unstable α-hydroxy-*N*-nitrosamines *3* and *4*, and oxidation of the pyridine nitrogen, yielding compound *1*. NNAl also undergoes α-hydroxylation, leading initially to compounds *5* and *6*, and pyridine-*N*-oxidation, giving compound *2*. Each of these pathways is discussed below in further detail.

NNAl, a major hepatic microsomal metabolite, is found in the urine of NNK-treated Fischer 344 rats (10% of the dose) and Syrian golden hamsters (7% of the dose). It is formed rapidly after administration of NNK to rats or hamsters in blood serum, stomach contents and most tissues at levels that exceed those of NNK. It is also formed rapidly in cultured A/J mouse peripheral lung treated with NNK. NNAl can be reoxidized to NNK *in vitro* and *in vivo* (Hecht *et al.*, 1980a; Hoffmann *et al.*, 1981; Castonguay *et al.*, 1983a,b; Adams *et al.*, 1984).

α-Hydroxylation is thought to be the major activation pathway of NNK. The unstable α-hydroxy-*N*-nitrosamine *4* decomposes to the keto-aldehyde *9*, a rat hepatic microsomal metabolite of NNK, and a methylating agent, presumed to be methyldiazohydroxide (*10*). The mutagenic methylating agent *10* is structurally similar or identical to the methylating agents formed from such well-known carcinogens as *N*-nitrosodimethylamine and *N*-methyl-*N*'-nitrosourea. Thus, administration of NNK to experimental animals should result in formation of O^6-methylguanine and 7-methylguanine in DNA (Hecht *et al.*, 1980, 1983b). This has been confirmed in Fischer 344 rats treated intravenously with 85 mg/kg bw NNK; O^6-methylguanine and 7-methylguanine were detected in the DNA of liver and lung — organs susceptible to the carcinogenic effects of NNK (Castonguay *et al.*, 1984b). Nasal mucosa of Fischer 344 rats cultured with NNK was shown to have a high capacity to hydroxylate the α-carbon of NNK (Brittebo *et al.*, 1983).

The unstable α-hydroxy-*N*-nitrosamine *3* spontaneously decomposes to formaldehyde and the electrophilic diazohydroxide *8*, which induces mutations in *Salmonella typhimurium* (Hecht *et al.*, 1983b). The diazohydroxide *8* reacts with water, yielding keto-alcohol *14*, a hepatic microsomal metabolite of NNK (Hecht *et al.*, 1980a). In Fischer 344 rats and Syrian golden hamsters, keto-alcohol *14* and keto-aldehyde *9* are oxidized or reduced to the urinary metabolites *15*, *16* and *17* (Hecht *et al.*, 1980a; Hoffmann *et al.*, 1981). By pathways similar to those discussed above, α-hydroxylation of NNAl leads to the intermediates *5* and *6* and to the metabolites *16* and *17*. α-Hydroxylation of NNAl occurs more slowly than that of NNK (Castonguay *et al.*, 1983a).

The pyridine-*N*-oxide, NNK-1-*N*-oxide (*1*) is a microsomal metabolite of NNK and has been detected in the urine, serum and various tissues of Fischer 344 rats treated with NNK (Hecht *et al.*, 1980a; Castonguay *et al.*, 1983b). The pyridine *N*-oxide *2* has been detected in tissues of C57Bl mice treated with this compound (Castonguay *et al.*, 1984a).

Mutagenicity and other short-term tests

In the presence of a liver microsomal preparation from Aroclor-induced rats, NNK, at 1-4 µmol/plate, caused a dose-dependent increase in mutations in *Salmonella typhimurium* TA1535 and TA100 (Hecht *et al.*, 1983c).

NNK at 10^{-3}M and 10^{-2}M (1 and 10 mmol/ml) induced unscheduled DNA synthesis in freshly isolated hepatocytes from adult rats (Williams & Laspia, 1979).

O^6-Methylguanine and 7-methylguanine were detected in the DNA of liver and lung of Fischer 344 rats treated intravenously with 85 mg/kg bw NNK (Castonguay *et al.*, 1984b).

(*b*) *Humans*

No data were available to the Working Group on toxic effects or on effects on reproduction and prenatal toxicity.

Absorption, distribution, excretion and metabolism

Human tissues obtained at immediate autopsy and cultured for 24 h with [carbonyl-^{14}C]NNK metabolized NNK to NNAl and to compound *16* (see Fig. 1), as follows (values in nmol/100 ug DNA): buccal mucosa, 470 ± 273 and 0.26 ± 0.23; trachea, 315 ± 157 and 0.24 ± 0.13; oesophagus, 210 ± 163 and 0.14 ± 0.12; bronchus, 740 ± 581 and 0.86 ± 0.83; peripheral lung, 705 ± 398 and 0.37 ± 0.33; and urinary bladder, 398 ± 302 and 0.19 ± 0.28. Of the [carbonyl-^{14}C]NNK added to the medium, 50-80% was converted to NNAl. An unidentified metabolite was also formed (Castonguay *et al.*, 1983c).

No data were available to the Working Group on mutagenicity or chromosomal effects.

3.3 Case reports and epidemiological studies of carcinogenicity to humans

No data were available to the Working Group.

4. Summary of Data Reported and Evaluation

4.1 Exposure data

4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) has been found in a variety of tobacco products (chewing tobacco, snuff, cigarettes and cigars), in mainstream and sidestream smoke from cigars and cigarettes, in saliva of chewers of betel quid with tobacco, and in saliva of oral-snuff users. Some of the NNK in saliva appears to be formed endogenously from salivary nitrite and nicotine. Thus, there is widespread exposure to NNK among users of tobacco products and those exposed to sidestream smoke.

4.2 Experimental data

NNK was tested for carcinogenicity in several studies by subcutaneous injection in rats and hamsters and by intraperitoneal injection in mice. In rats, it induced carcinomas of the nasal cavity, lung and liver, with a clear dose-response relationship. In hamsters, it induced benign and malignant tumours of the nasal cavity, trachea and lung, even after a single administration. In mice, NNK and its metabolites 4-(methylnitrosamino)-1-(3-pyridyl-*N*-oxide)-1-butanone and 4-(methylnitrosamino)-1-(3-pyridyl)buan-1-ol induced benign and malignant tumours of the lung.

NNK and its metabolites can cross the placental barrier in mice. NNK can be metabolically activated by mouse foetal tissues.

Administration of NNK to rats results in abnormal DNA methylation in liver and lung. NNK is mutagenic to *Salmonella typhimurium* in the presence of an exogenous metabolic system. It induces unscheduled DNA synthesis in primary cultures of rat hepatocytes.

Overall assessment of data from short-term tests: 4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanone[a]

	Genetic activity			Cell transformation
	DNA damage	Mutation	Chromosomal effects	
Prokaryotes		+		
Fungi/green plants				
Insects				
Mammalian cells (*in vitro*)	+			
Mammals (*in vivo*)	+			
Humans (*in vivo*)				
Degree of evidence in short-term tests for genetic activity: *Sufficient*				Cell transformation: No data

[a]The groups into which the table is divided and '+' are defined on pp. 16-17 of the Preamble; the degrees of evidence are defined on p. 18.

4.3 Human data

No case report or epidemiological study of the carcinogenicity of NNK to humans was available to the Working Group.

4.4 Evaluation[1]

There is *sufficient evidence*[2] for the carcinogenicity of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone to experimental animals.

No data on humans were available.

5. References

Adams, J.D., Brunnemann, K.D. & Hoffmann, D. (1983a) Chemical studies on tobacco smoke. LXXV. Rapid method for the analysis of tobacco-specific *N*-nitrosamines by gas-liquid chromatography with a thermal energy analyzer. *J. Chromatogr.*, *256*, 347-351

[1]For description of the italicized term, see Preamble, pp. 15-16.

[2]In the absence of adequate data on humans, it is reasonable, for practical purposes, to regard chemicals for which there is *sufficient evidence* of carcinogenicity in animals as if they presented a carcinogenic risk to humans.

Adams, J.D., Lee, S.J., Vinchkoski, N., Castonguay, A. & Hoffmann, D. (1983b) On the formation of the tobacco-specific carcinogen 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone during smoking. *Cancer Lett.*, *17*, 339-346

Adams, J.D., LaVoie, E.J., O'Donnell, M. & Hoffmann, D. (1984) *Pharmacokinetics of tobacco-specific* N-*nitrosamines*. In: O'Neill, I.K., Miller, C.T., von Borstel, R.C., Long, J.E. & Bartsch, H., eds, N-*Nitroso Compounds: Occurrence, Biological Effects and Relevance to Human Cancer* (*IARC Scientific Publications No. 57*), Lyon, International Agency for Research on Cancer, pp. 779-785

Brittebo, E.B., Castonguay, A., Furuya, K. & Hecht, S.S. (1983) Metabolism of tobacco-specific nitrosamines by cultured rat nasal mucosa. *Cancer Res.*, *43*, 4343-4348

Brunnemann, K.D., Hecht, S.S. & Hoffmann, D. (1983) *N*-Nitrosamines: Environmental occurrence, in vivo formation and metabolism. *J. toxicol.-clin. Toxicol.*, *19*, 661-688

Castonguay, A. & Hecht, S.S. (1985) Synthesis of carbon-14 labeled 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone. *J. labeled Compd Radio. Pharm.* (in press)

Castonguay, A., Lin, D., Stoner, G.D., Radok, P., Furuya, K., Hecht, S.S., Schut, H.A.J. & Klaunig, J.E. (1983a) Comparative carcinogenicity in A/J mice and metabolism by cultured mouse peripheral lung of *N'*-nitrosonornicotine, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone, and their analogues. *Cancer Res.*, *43*, 1223-1129

Castonguay, A., Tjälve, H. & Hecht, S.S. (1983b) Tissue distribution of the tobacco-specific carcinogen 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone, and its metabolites in F344 rats. *Cancer Res.*, *43*, 630-638

Castonguay, A., Stoner, G.D., Schut, H.A.J. & Hecht, S.S. (1983c) Metabolism of tobacco-specific *N*-nitrosamines by cultured human tissues. *Proc. natl Acad. Sci. USA*, *80*, 6694-6697

Castonguay, A., Tjälve, H., Trushin, N. & Hecht, S.S. (1984a) Perinatal metabolism of the tobacco-specific carcinogen 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone in C57BL mice. *J. natl Cancer Inst.*, *72*, 1117-1126

Castonguay, A., Tharp, R. & Hecht, S.S. (1984b) *Kinetics of DNA methylation by the tobacco-specific carcinogen 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone in F344 rats*. In: O'Neill, K., Miller, C.T., von Borstel, R.C., Long, J.E. & Bartsch, H., eds, N-*Nitroso Compounds: Occurrence, Biological Effects and Relevance to Human Cancer* (*IARC Scientific Publications No. 57*), Lyon, International Agency for Research on Cancer, pp. 805-810

Egan, H., Preussmann, R., O'Neill, I.K., Eisenbrand, G., Spiegelhalder, B. & Bartsch, H., eds (1983) *Environmental Carcinogens. Selected Methods of Analysis*, Vol. 6, N-*Nitroso Compounds* (*IARC Scientific Publications No. 45*), Lyon, International Agency for Research on Cancer

Hecht, S.S., Chen, C.-H.B., Dong, M., Ornaf, R.M. & Hoffmann, D. (1977) Chemical studies on tobacco smoke. LI. Studies on non-volatile nitrosamines in tobacco. *Beitr. Tabakforsch. int.*, *9*, 1-6

Hecht, S.S., Chen, C.-H.B., Ornaf, R.M., Jacobs, E., Adams, J.D. & Hoffmann, D. (1978a) Reaction of nicotine and sodium nitrite: Formation of nitrosamines and fragmentation of the pyrrolidine ring. *J. org. Chem.*, *43*, 72-76

Hecht, S.S., Chen, C.-H.B., Ornaf, R.M., Hoffmann, D. & Tso, T.C. (1978b) *Chemical studies on tobacco smoke. LVI. Tobacco specific nitrosamines: Origins, carcinogenicity and metabolism.* In: Walker, E.A., Castegnaro, M., Griciute, L. & Lyle, R.E., eds, *Environmental Aspects of* N-*Nitroso Compounds* (*IARC Scientific Publications No. 19*), Lyon, International Agency for Research on Cancer, pp. 395-413

Hecht, S.S., Chen, C.-H.B., Hirota, N., Ornaf, R.M., Tso, T.C. & Hoffmann, D. (1978c) Tobacco-specific nitrosamines: Formation from nicotine *in vitro* and during tobacco curing and carcinogenicity in strain A mice. *J. natl Cancer Inst.*, *60*, 819-824

Hecht, S.S., Young, R. & Chen, C.-H.B. (1980a) Metabolism in the F344 rat of 4-(*N*-methyl-*N*-nitrosamino)-1-(3-pyridyl)-1-butanone, a tobacco specific carcinogen. *Cancer Res.*, *40*, 4144-4150

Hecht, S.S., Chen, C.-H.B., Ohmori, T. & Hoffmann, D. (1980b) Comparative carcinogenicity in F344 rats of the tobacco-specific nitrosamines, *N'*-nitrosonornicotine and 4-(*N*-methyl-*N*-nitrosamino)-1-(3-pyridyl)-1-butanone. *Cancer Res.*, *40*, 298-302

Hecht, S.S., Chen, C.-H.B., Young, R. & Hoffmann, D. (1981) Mass spectra of tobacco alkaloid-derived nitrosamines, their metabolites, and related compounds. *Beitr. Tabakforsch. int.*, *11*, 57-66

Hecht, S.S., Adams, J.D., Numoto, S. & Hoffmann, D. (1983a) Induction of respiratory tract tumors in Syrian golden hamsters by a single dose of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) and the effect of smoke inhalation. *Carcinogenesis*, *4*, 1287-1290

Hecht, S.S., Castonguay, A., Rivenson, A., Mu, B. & Hoffmann, D. (1983b) Tobacco specific nitrosamines: Carcinogenicity, metabolism, and possible role in human cancer. *J. environ. Sci. Health*, *C1*, 1-54

Hecht, S.S., Lin, D. & Castonguay, A. (1983c) Effects of α-deuterium substitution on the mutagenicity of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK). *Carcinogenesis*, *4*, 305-310

Hoffmann, D. & Adams, J.D. (1981) Carcinogenic tobacco-specific *N*-nitrosamines in snuff and in the saliva of snuff dippers. *Cancer Res.*, *41*, 4305-4308

Hoffmann, D. & Hecht, S.S. (1983) *Tobacco and tobacco smoke (volatile and tobacco-specific nitrosamines). General aspects.* In: Egan, H., Preussmann, R., O'Neill, I.K., Eisenbrand, G., Spiegelhalder, B. & Bartsch, H., eds, *Environmental Carcinogens. Selected Methods of Analysis*, Vol. 6, N-*Nitroso Compounds* (*IARC Scientific Publications No. 45*), Lyon, International Agency for Research on Cancer, pp. 63-67

Hoffmann, D., Adams, J.D., Brunnemann, K.D. & Hecht, S.S. (1979) Assessment of tobacco-specific *N*-nitrosamines in tobacco products. *Cancer Res.*, *39*, 2505-2509

Hoffmann, D., Adams, J.D., Piade, J.J. & Hecht, S.S. (1980a) *Chemical studies on tobacco smoke. LXVIII. Analysis of volatile and tobacco-specific nitrosamines in tobacco prod-*

ucts. In: Walker, E.A., Castegnaro, M., Griciute, L. & Börzsönyi, M., eds, N-*Nitroso Compounds: Analysis, Formation and Occurrence* (*IARC Scientific Publications No. 31*), Lyon, International Agency for Research on Cancer, pp. 507-516

Hoffmann, D., Chen, C.-H.B. & Hecht, S.S. (1980b) *The role of volatile and nonvolatile* N-*nitrosamines in tobacco carcinogenesis*. In: Gori, G.B. & Bock, F.G., eds, *A Safe Cigarette?* (*Banbury Report 3*), Cold Spring Harbor, NY, Cold Spring Harbor Laboratory, pp. 113-127

Hoffmann, D., Castonguay, A., Rivenson, A. & Hecht, S.S. (1981) Comparative carcinogenicity and metabolism of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone and *N'*-nitrosonornicotine in Syrian golden hamsters. *Cancer Res.*, *41*, 2386-2393

Hoffmann, D., Brunnemann, K.D., Adams, J.D., Rivenson, A. & Hecht, S.S. (1982a) N-*Nitrosamines in tobacco carcinogenesis*. In: Magee, P.N., ed., *Nitrosamines and Human Cancer* (*Banbury Report 12*), Cold Spring Harbor, NY, Cold Spring Harbor Laboratory, pp. 211-225

Hoffmann, D., Adams, J.D., Brunnemann, K.D., Rivenson, A. & Hecht, S.S. (1982b) *Tobacco specific* N-*nitrosamines: Occurrence and bioassays*. In: Bartsch, H., O'Neill, I.K., Castegnaro, M. & Okada, M., eds, N-*Nitroso Compounds: Occurrence and Biological Effects* (*IARC Scientific Publications No. 41*), Lyon, International Agency for Research on Cancer, pp. 309-318

Hoffmann, D., Rivenson, A., Amin, S. & Hecht, S.S. (1984) Dose-response study of the carcinogenicity of tobacco-specific *N*-nitrosamines in F344 rats. *J. Cancer Res. clin. Oncol.*, *108*, 81-86

Nair, J., Ohshima, H., Friesen, M., Croisy, A., Bhide, S.V. & Bartsch, H. (1985) Tobacco-specific and betel-nut-specific *N*-nitroso compounds: Occurrence in saliva and urine of betel quid chewers and formation *in vitro* by nitrosation of betel quid. *Carcinogenesis*, *6*, 295-303

Rühl, C., Adams, J.D. & Hoffmann, D. (1980) Chemical studies on tobacco smoke. LXVI. Comparative assessment of volatile and tobacco-specific *N*-nitrosamines in the smoke of selected cigarettes from the USA, West Germany and France. *J. anal. Toxicol.*, *4*, 255-259

Tjälve, H. & Castonguay, A. (1983) The in vivo tissue disposition and in vitro target-tissue metabolism of the tobacco-specific carcinogen 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone in Syrian golden hamsters. *Carcinogenesis*, *4*, 1259-1265

Wenke, G., Brunnemann, K.D., Hoffmann, D. & Bhide, S.V. (1984) A study of betel quid carcinogenesis. IV. Analysis of the saliva of betel chewers: A preliminary report. *J. Cancer Res. clin. Oncol.*, *108*, 110-113

Williams, G.M. & Laspia, M.F. (1979) The detection of various nitrosamines in the hepatocyte primary culture/DNA repair test. *Cancer Lett.*, *6*, 199-206

N'-NITROSOANABASINE (NAB)

1. Chemical and Physical Data

1.1 Synonyms and trade names

Chem. Abstr. Services Reg. Nos: 37620-20-5; 1133-64-8[1]; 84237-39-8[2]

Chem. Abstr. Names: Pyridine, 3-(1-nitroso-2-piperidinyl)-; pyridine, 3-(1-nitroso-2-piperidinyl)-,(S)-[1]; pyridine, 3-(1-nitroso-2-piperidinyl), (+,-)-[2]

IUPAC Systematic Name: 1-Nitrosoanabasine

Synonym: *N*-Nitrosoanabasine

1.2 Structural and molecular formulae and molecular weight

N
N=O
N

$C_{10}H_{13}N_{3}0$ Mol. wt: 191.2

1.3 Chemical and physical properties of the pure substance

From Hecht *et al.* (1975) unless otherwise specified

(*a*) *Boiling-point*: 162°C at 1 mm Hg

(*b*) *Optical rotation*: The specific rotation of NAB has been reported (Nazrullaeva *et al.*, 1976).

(*c*) *Spectroscopy data:* Infrared, ultraviolet, nuclear magnetic resonance and mass spectra have been reported.

(*d*) *Reactivity*: Can be reduced to the corresponding hydrazine with lithium aluminium hydride (Neurath & Duenger, 1966). Can be oxidized with *meta*-chloroperoxybenzoic acid to give *N*'-nitrosoanabasine-1-*N*-oxide (Hecht & Young, 1982)

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[1]The Chemical Abstracts Services Registry Number and Name refer to a single stereoisomer (S).

[2]The Chemical Abstracts Services Registry Number and Name refer to the racemic mixture that was synthesized and used in the biological studies reported in this monograph.

1.4 Technical products and impurities

NAB is not produced commercially.

2. Production, Use, Occurrence and Analysis

2.1 Production and use

NAB was prepared by Boyland *et al.* (1964) by treating anabasine with sodium nitrite in dilute hydrochloric acid solution.

No evidence was found that NAB has ever been produced in commercial quantities or that it has any uses other than as a laboratory chemical.

2.2 Occurrence

In several studies, NAB was not detected in tobacco, even when its precursor, anabasine, was present in high concentrations. Various 1-kg samples of commercial and experimental tobacco products were reported in 1975 to be free of NAB, using analytical methods with a detection limit of 0.5 μg/kg. Tobacco products studied included *Nicotiana glauca* (high anabasine content), snuff, fine-cut chewing tobacco (high nitrate and nitrite levels) and plug chewing tobacco (a commercial tobacco with a relatively high anabasine content). NAB was not detected in three commercial tobacco products containing 40-80 mg/kg of anabasine nor in *Nicotiana glauca* tobacco, which contained 2800 mg/kg of anabasine (Hecht *et al.*, 1975). It has been suggested that NAB may be formed in tobacco chewers or smokers from anabasine by nitrosation in the mouth or stomach after swallowing saliva that contains nitrite (Mirvish *et al.*, 1977).

More recently (Brunnemann *et al.*, 1982, 1983), NAB has been identified in tobacco at concentrations of up to 1.9 mg/kg. Cigarette smoke was found to contain up to 120 ng (mean value) NAB per cigarette; and a mean value of 120 ng/cigarette was found in the combined mainstream smoke of 500 non-filter 85-mm US cigarettes (Hoffmann *et al.*, 1982a; Adams *et al.*, 1983; Brunnemann *et al.*, 1983; Hoffmann & Hecht, 1983). It was also identified in fine-cut chewing tobacco purchased in 1981 in Westchester County, NY, at a concentration of 1.8 mg/kg (Adams *et al.*, 1983).

Various brands of snuff from the USA and Sweden were analysed for the presence of NAB. The results are summarized in Table 1. A significant reduction in the concentration of nitrosamines (including NAB) was found in brands introduced in the 1980s (American brand V and Swedish brand V) as compared to other (older) brands shown in Table 1 (Hoffmann *et al.*, 1982b).

2.3 Analysis

Typical methods for the analysis of NAB are summarized in Table 2.

Table 1. NAB content of commercial snuff[a]

Snuff brand	Type of packaging[b]	NAB (μg/kg)
USA		
Brand I	A	100
Brand II	A	800
Brand III	A	1900
Brand IV	B	500
Brand V	C	10
Sweden		
Brand I	A	140
Brand II	A	80
Brand III	A	70
Brand IV	A	80
Brand V	D	40

[a]Data from Brunnemann *et al.* (1982, 1983); values are based on dry weight
[b]Abbreviations: A, cardboard box with a metal lid; B, plastic containers with individual snuff portions in porous paper bags; C, plastic container; D, individual snuff portions in aluminium bags

Table 2. Methods for the analysis of NAB

Sample matrix	Sample preparation	Assay procedure[a]	Limit of detection	Reference
Tobacco or tobacco smoke	Extract tobacco with aqueous ascorbic acid solution; collect smoke in aqueous ascorbic acid traps; enrich by extraction with dichloromethane followed by column chromatography	GC/TEA	Approximately 0.1 ng per injection	Adams *et al.* (1983)
Fine-cut chewing tobacco	As above	HPLC/TEA followed by GC/MS	Not given	Adams *et al.* (1983)

[a]Abbreviations: GC/TEA, gas chromatography with thermal-energy analyser; HPLC/TEA, high-performance liquid chromatography with thermal-energy analyser; GC/MS, gas chromatography with mass spectrometry

3. Biological Data Relevant to the Evaluation of Carcinogenic Risk to Humans

3.1 Carcinogenicity studies in animals

(a) *Oral administration*

Rat: Groups of 16 male and 16 female Chester Beatty strain albino rats, approximately seven weeks old, were given drinking-water containing 0.2% NAB [purity not specified] *ad libitum* on six days per week [presumably continuously]. The estimated daily dose was 5 mg/day and animals were killed when moribund or sick, at various intervals between 251 and 550 days of study. A group of 16 males and 16 females served as untreated controls. All but two female rats in the treated group were subjected to post-mortem examination. Of

the 16 treated males, four had oesophageal carcinomas, nine had oesophageal papillomas and three had no tumour; of the 14 treated females, one had an oesophageal carcinoma, 11 had oesophageal papillomas and two had no tumour. No oesophageal tumour was reported in the control rats (Boyland *et al.*, 1964).

A group of 20 male Fischer rats, seven weeks of age, was administered 200 mg/ml NAB (purity, >99%; Hecht *et al.*, 1975) in the drinking-water on five days per week for 30 weeks (estimated total dose, 630 mg). Animals were killed when moribund or after 11 months, and all animals, except those lost by cannibalism or autolysis, were necropsied. Among the 17 rats necropsied from the treated group, one had an oesophageal papilloma and one a pharyngeal papilloma. No tumour was reported in 20 untreated controls (Hoffmann *et al.*, 1975). [The Working Group noted the short duration of treatment and observation, and the low dose used. It was noted, however, that the two observed tumours occurred in the upper digestive tract; this was consistent with the results of the previous study.]

(*b*) *Subcutaneous and/or intramuscular administration*

Hamster: Groups of 10 male and 10 female Syrian golden hamsters, eight to 10 weeks of age, received thrice-weekly s.c. injections of 5 mg NAB (purity, >99%; Hecht *et al.*, 1975) in saline for 25 weeks (total dose, 375 mg). A further group of 10 males and 10 females received injections of saline only and served as vehicle controls. Animals were killed when moribund or at termination of the experiment at 83 weeks. All animals, except one female in the treated group and one male and two females in the control group lost due to cannibalism, were necropsied and examined histologically. No tumour was detected in any of the treated animals or among the males in the control group; one fibrovascular polyp of the uterus was found in a female in the control group (Hilfrich *et al.*, 1977). [The Working Group noted the short duration of treatment and the small number of animals used.]

3.2 Other relevant biological data

(*a*) *Experimental systems*

Toxic effects

The subcutaneous LD_{50} of NAB in Fischer rats was >1000 mg/kg bw (Hoffmann *et al.*, 1975).

Effects on reproduction and prenatal toxicity

No data were available to the Working Group.

Absorption, distribution, excretion and metabolism

Male Fischer 344 rats administered 100 mg/kg bw [2'-^{14}C]-NAB (racemic) by gavage excreted 68.5% of the dose in the urine over 30 h.

Metabolic pathways for NAB are illustrated in Figure 1. 5-Hydroxy-5-(3-pyridyl)pentanoic acid (*7*) (9.8% of the dose administered), resulting from 6'-hydroxylation of NAB, and *N*'-nitrosoanabasine-1-*N*-oxide (*1*) (30% of the dose), resulting from pyridine-*N*-oxidation of NAB, were detected as urinary metabolites. 5-Oxo-5-(3-pyridyl)pentanoic acid (*6*), which could have resulted from 2'-hydroxylation of NAB, was not detected in the urine. Similar results were obtained with the S-enantiomer (Hecht & Young, 1982).

Figure 1. Metabolism of NAB. Structures in brackets represent hypothetical intermediates[a]

[a]From Hecht and Young (1982). Compounds: *1*, *N'*-nitrosoanabasine-1-*N*-oxide; *6*, 5-oxo-5-(3-pyridyl)pentanoic acid; *7*, 5-hydroxy-5-(3-pyridyl)pentanoic acid

In cultured Fischer 344 rat oesophagus, the major metabolite of [2'-^{14}C]-NAB (racemic) was 5-hydroxy-5-(3-pyridyl)pentanoic acid (*7*), resulting from 6'-hydroxylation; small amounts of 5-oxo-5-(3-pyridyl)pentanoic acid (*6*), formed by 2'-hydroxylation, were also observed (Hecht & Young, 1982).

These results, with regard to the metabolism of NAB, can be compared with those obtained under identical conditions in metabolic studies of NNN, which is structurally similar to NAB. After 48 h of incubation of cultured rat oesophagus with NNN or NAB, the ratios of 2'-hydroxylation to 5'- or 6'-hydroxylation were 3.1 or 0.2, respectively. In the metabolites identified in the urine, the ratio of α-hydroxylation to *N*-oxidation was 4.2 for NNN and 0.3 for NAB (Hecht & Young, 1982).

Mutagenicity and other short-term tests

No data were available to the Working Group.

(*b*) *Humans*

No data were available to the Working Group.

3.3 Case reports and epidemiological studies of carcinogenicity to humans

No data were available to the Working Group.

4. Summary of Data Reported and Evaluation

4.1 Exposure data

N'-Nitrosoanabasine (NAB) has been found in tobacco products (chewing tobacco and snuff) and in mainstream smoke from cigarettes. Thus, there is widespread exposure to NAB among users of tobacco products.

4.2 Experimental data

NAB was tested for carcinogenicity by oral administration in two strains of rats and by subcutaneous injection in hamsters. In rats, it induced oesophageal carcinomas and/or papillomas. The study in hamsters was inadequate for evaluation.

No data were available on mutagenic or related effects of NAB.

4.3 Human data

No case report or epidemiological study of the carcinogenicity of NAB to humans was available to the Working Group.

4.4 Evaluation[1]

There is *limited evidence* for the carcinogenicity of *N'*-nitrosoanabasine to experimental animals.

No data on humans were available.

5. References

Adams, J.D., Brunnemann, K.D. & Hoffmann, D. (1983) Chemical studies on tobacco smoke. LXXV. Rapid method for the analysis of tobacco-specific *N*-nitrosamines by gas-liquid chromatography with a thermal energy analyzer. *J. Chromatogr.*, *256*, 347-351

Boyland, E., Roe, F.J.C., Gorrod, J.W. & Mitchley, B.C.V. (1964) The carcinogenicity of nitrosoanabasine, a possible constituent of tobacco smoke. *Br. J. Cancer*, *18*, 265-270

Brunnemann, K.D., Scott, J.C. & Hoffmann, D. (1982) *N*-Nitrosomorpholine and other volatile *N*-nitrosamines in snuff tobacco. *Carcinogenesis*, *3*, 693-696

[1]For description of the italicized term, see Preamble, pp. 15-16.

Brunnemann, K.D., Hecht, S.S. & Hoffmann, D. (1983) *N*-Nitrosamines: Environmental occurrence, in vivo formation and metabolism. *J. toxicol.-clin. Toxicol.*, *19*, 661-688

Hecht, S.S. & Young, R. (1982) Regiospecificity in the metabolism of the homologous cyclic nitrosamines, *N'*-nitrosonornicotine and *N'*-nitrosoanabasine. *Carcinogenesis*, *3*, 1195-1199

Hecht, S.S., Ornaf, R.M. & Hoffmann, D. (1975) Chemical studies on tobacco smoke. XXXIII. *N'*-Nitrosonornicotine in tobacco: Analysis of possible contributing factors and biologic implications. *J. natl Cancer Inst.*, *54*, 1237-1244

Hilfrich, J., Hecht, S.S. & Hoffmann, D. (1977) A study of tobacco carcinogenesis. XV. Effects of *N'*-nitrosonornicotine and *N'*-nitrosoanabasine in Syrian golden hamsters. *Cancer Lett.*, *2*, 169-175

Hoffmann, D. & Hecht, S.S. (1983) *Tobacco and tobacco smoke (volatile and tobacco-specific nitrosamines). General aspects*. In: Egan, H., Preussmann, R., O'Neill, I.K., Eisenbrand, G., Spiegelhalder, B. & Bartsch, H., eds, *Environmental Carcinogens. Selected Methods of Analysis*, Vol. 6, N-*Nitroso Compounds* (*IARC Scientific Publications No. 45*), Lyon, International Agency for Research on Cancer, pp. 63-67

Hoffmann, D., Raineri, R., Hecht, S.S., Maronpot, R. & Wynder, E.L. (1975) A study of tobacco carcinogenesis. XIV. Effects of *N'*-nitrosonornicotine and *N'*-nitrosoanabasine in rats. *J. natl Cancer Inst.*, *55*, 977-981

Hoffmann, D., Adams, J.D., Brunnemann, K.D., Rivenson, A. & Hecht, S.S. (1982a) *Tobacco specific* N-*nitrosamines: Occurrence and bioassays*. In: Bartsch, H., O'Neill, I.K., Castegnaro, M. & Okada, M., eds, N-*Nitroso Compounds: Occurrence and Biological Effects* (*IARC Scientific Publications No. 41*), Lyon, International Agency for Research on Cancer, pp. 309-318

Hoffmann, D., Brunnemann, K.D., Adams, J.D., Rivenson, A. & Hecht, S.S. (1982b) N-*Nitrosamines in tobacco carcinogenesis*. In: Magee, P.N., ed., *Nitrosamines and Human Cancer* (*Banbury Report 12*), Cold Spring Harbor, NY, Cold Spring Harbor Laboratory, pp. 211-225

Mirvish, S.S., Sams, J. & Hecht, S.S. (1977) Kinetics of nornicotine and anabasine nitrosation in relation to *N'*-nitrosonornicotine occurrence in tobacco and to tobacco-induced cancer. *J. natl Cancer Inst.*, *59*, 1211-1213

Nazrullaeva, N.Z., Astanov, K.A., Abduvakhabov, A.A. & Mukhamedzhanov, S.Z. (1976) Specific rotation of anabasine (Russ.). *Khim. Prir. Soedin.*, *4*, 552 [*Chem. Abstr.*, *86*, 106854x]

Neurath, G. & Duenger, M. (1966) *N*-Nitroso compounds from tobacco alkaloids (Ger.). *Beitr. Tabakforsch.*, *3*, 339-345 [*Chem. Abstr.*, *67*, 64605s]

N'-NITROSOANATABINE (NAT)

1. Chemical and Physical Data

1.1 Synonyms and trade names

Chem. Abstr. Services Reg. No.: 71267-22-6

Chem. Abstr. Name: 2,3'-Bipyridine, 1,2,3,6-tetrahydro-1-nitroso-

IUPAC Systematic Name: 1,2,3,6-Tetrahydro-1-nitroso-2,3-bipyridine

Synonym: NAtB

1.2 Structural and molecular formulae and molecular weight

$C_{10}H_{11}N_3O$

Mol. wt: 189.2

1.3 Chemical and physical properties of the pure substance

(*a*) *Boiling-point*: 176°C at 0.5 mm Hg (Hoffmann *et al.*, 1979)

(*b*) *Spectroscopy data*: Infrared, mass and nuclear magnetic resonance spectra have been reported (Hoffmann *et al.*, 1979; Hecht *et al.*, 1981).

1.4 Technical products and impurities

NAT is not produced commercially.

2. Production, Use, Occurrence and Analysis

2.1 Production and use

NAT was prepared by Hoffmann *et al.* (1979) by the reaction of sodium nitrite with a solution of anatabine in hydrochloric acid (Hu *et al.*, 1974).

No evidence was found that NAT has ever been produced in commercial quantities or that it has any uses other than as a laboratory chemical.

2.2 Occurrence

(*a*) *Tobacco and tobacco smoke*

NAT is produced by nitrosation of anatabine during the curing, ageing, processing and smoking of tobacco. The presence of NAT in cigarette smoke was suggested by Klus and Kuhn (1975) on the basis of data on thin-layer chromatography. It has been found in tobacco at levels of 0.4-13 mg/kg, in snuff products at 0.2-44 mg/kg, and in cigarette smoke at 0.1-4.6 μg/cigarette; laboratory evidence has suggested that additional quantities of NAT may be formed in the oral cavity during oral use of snuff and tobacco (Hoffmann *et al.*, 1979, 1980a,b; Hoffmann & Adams, 1981; Brunnemann *et al.*, 1982, 1983; Hoffmann & Hecht, 1983).

Table 1 summarizes the data obtained from analyses of smoke from selected commercial cigarettes with and without filter tips and shows the effectiveness of the filters of cigarettes D and E in decreasing the occurrence levels of NAT (Hoffmann *et al.*, 1982).

NAT concentrations found in cigarette and cigar tobaccos, in both their mainstream (during puff drawing) and sidestream (generated during smouldering of tobacco in between puffs) smoke, and in chewing tobacco are presented in Table 2.

NAT concentrations found after analysis of snuff brands obtained in Sweden, Denmark, the Federal Republic of Germany and the USA far exceeded (levels of up to 44 mg/kg) those levels presented above for cigarette tobacco. The results are summarized in Table 3. NAT concentrations differed not only among snuff brands, but also in samples of the same brand bought in different cities. The latter differences were attributed to possible variations in NAT content between batches and/or effects of ageing. The effects of ageing were demonstrated by opening individual portions (packed in aluminium foil) and storing the snuff in open air: within 14 days, the NAT content had increased by 50% (Hoffmann & Adams, 1981; Hoffmann *et al.*, 1982).

(*b*) *Human tissues and secretions*

Saliva from women who had been long-term oral-snuff users and who were employed in two southern US furniture companies contained NAT. The wide range of NAT concentrations (6.6-147 ng/g) in the saliva of individual users indicated that during oral use of snuff NAT is extracted from the tobacco plug at varying rates (Hoffmann & Adams, 1981).

Table 1. Reduction of NAT concentrations in cigarette smoke by filtration[a]

Cigarette		Length smoked/length of cigarette (mm)	NAT (μg/cigarette)[b]
A	NF	50/65	1.1
	F	50/85	0.6 (-47%)
B	NF	50/65	0.7
	F	50/85	0.4 (-47%)
C	NF	50/65	1.1
	F	50/85	0.7 (-39%)
D	NF	50/65	1.3
	F	50/85	0.4 (-70%)
E[c]	NF	50/73	1.9
	F	50/100	0.6 (-69%)

[a]From Hoffmann *et al.* (1982)
[b]In parentheses, percentage changes in yields on comparing filtered (F) and non-filtered (NF) smoke
[c]Perforated filter tip

Table 2. NAT concentrations in cigarettes, cigars and chewing tobacco, and in cigarette and cigar mainstream and sidestream smoke[a]

Tobacco product[b]	NAT concentration		
	In tobacco (mg/kg)	In mainstream smoke (μg/cig)	In sidestream smoke (μg/cig)
Burley cigarette, NF	3.2	4.6	1.5
Bright cigarette, NF	0.4	0.4	0.4
Commercial cigarette, NF	1.6	0.3	0.3
Commercial cigarette, FA	1.3	0.4	0.2
Kentucky 1R1, NF	0.6	0.5	0.2
US cigarette, NF[c]	--	1.0	--
German (Federal Republic) cigarettes[d]			
Brand A, NF	--	0.2	--
Brand B, NF	--	0.1	--
Brand C, FA	--	0.2	--
Brand D, FA	--	0.04	--
Brand E, FA	--	0.1	--
Brand F, FA	--	0.1	--
Commercial French cigarette, NF, 70 mm	1.8	0.2	--
Commercial French cigarettes, FA, 70 mm	1.5	0.2	--
NF	2.0	0.6	--
FA	2.0	0.2	--
FP	2.0	0.2	--
Little cigar, FA	13.0	1.7	0.6
Cigar (Colombian tobacco) (5.7 g)	3.3	1.9	-[e]
Fine-cut chewing tobacco	44.0	NA[f]	NA
Fine-cut chewing tobacco[c]	47.7	NA	NA

[a]Data from Hoffmann *et al.* (1980a), unless otherwise noted
[b]All cigarettes and the little cigar were 85 mm long, unless otherwise noted. Abbreviations: NF, non-filter; FA, cellulose acetate filter; FP, paper filter
[c]Data from Adams *et al.* (1983); cigarettes and tobacco used were purchased in Westchester County, NY, in 1981
[d]Data from Rühl *et al.* (1980); cigarettes were popular brands purchased in Berlin in 1979
[e]Not determined because of interference by unknowns
[f]NA, not applicable

Table 3. Anatabine and NAT concentrations in commercial snuff[a]

Snuff origin	Type of packaging[b]	Anatabine (mg/kg)	NAT (mg/kg)[c]
USA			
Brand I New York and Tennessee	A	600	44
Brand II New York and Tennessee	A	5400	22.7
Brand III New York and Tennessee	B	400	1.8
Federal Republic of Germany			
Brand I Munich	C	100	4.4
Brand III Munich	C	100	3.9
Sweden			
Brand I Umeå	A	200	5.6
Brand II Uppsala	A	200	4.3
Brand III Lund	A	200	5.6
Brand II Umeå	A	200	3.2
Brand II Uppsala	A	200	3.1
Brand II Lund	A	200	7.0
Brand III Umeå	A	50	3.1
Brand III Uppsala	A	50	1.2
Brand III Lund	A	50	0.8
Brand IV Umeå	A	100	6.0
Brand IV Uppsala	A	100	25.1
Brand IV Lund	A	200	5.0
Brand V Umeå	D	300	1.0
Denmark			
Brand I Copenhagen	E	30	2.7
Brand II Copenhagen	E	700	3.7
Brand III Copenhagen	E	200	6.2
Ageing test[d]: 0 time	-	--	1.0
14 days	-	--	1.5

[a]Data from Hoffmann and Adams (1981) and Hoffmann *et al.* (1982); values are given for dry snuff, moisture content about 50%
[b]Abbreviations: A, waxed-paper container with metallic lid, containing approximately 50 g; B, 25 individual portions of approximately 11 g packaged in paper in a plastic container; C, plastic foil-lined aluminium bags containing 100 g; D, individual snuff portion in a paper bag packaged in a crimped airtight aluminium envelope, with 10 envelopes in a plastic bag, amounting to approximately 10 g; E, hard-plastic container
[c]NAT values are averages of three runs
[d]The aluminium foil-wrapped package (Swedish brand V) was opened at '0 time'.

Table 4. Anatabine and NAT concentrations in snuff and NAT concentrations in saliva of women who were long-term oral-snuff users[a]

Subject	Age (years)	Snuff		Saliva[b]	
		Anatabine (mg/g)	NAT (µg/g)	Day of sampling	NAT (µg/g)
1	41	0.5	22.7	1	0.2
				2	0.5
2	37	0.5	22.7	1	0.02
				2	0.05
3	44	0.5	20.4	1	0.4
				2	0.3
4	52	0.6	22.6	1	0.01
				2	0.05

[a]Data from Hoffmann and Adams (1981)
[b]Saliva of three women who did not use snuff (controls) was free of nicotine and tobacco-specific *N*-nitrosamines.

In another study, saliva of four women who were long-term (>10 years) oral-snuff users was analysed on two different days after they had used a specific brand of snuff with known concentrations of NAT. NAT levels in saliva (Table 4) varied significantly between subjects, as well as between samples from the same individual taken on different days. The variations in these values were at least partially due to the differences in the intensity with which individuals practised the habit at different times, and perhaps also to varying rates of salivation (Hoffmann & Adams, 1981; Hoffmann *et al.*, 1982).

NAT was found in the saliva of chewers of betel quid with tobacco at levels of 3.2-40 ng/g [mean, 15.6 ng/g] (Wenke *et al.*, 1984) and 1.0-10.9 ng/ml (mean, 4.8 ng/ml); it was also detected in the saliva of chewers of tobacco at levels of 13.5-51.7 ng/ml (mean, 29.8 ng/ml) (Nair *et al.*, 1985).

2.3 Analysis

Standard methods for the analysis of NAT are described in detail in an IARC manual on selected methods of analysis (Egan *et al.*, 1983).

3. Biological Data Relevant to the Evaluation of Carcinogenic Risk to Humans

3.1 Carcinogenicity studies in animals

(*a*) *Subcutaneous and/or intramuscular administration*

Rat: Groups of 12-21 male and 12-21 female Fischer 344 rats, nine weeks of age, received thrice-weekly s.c. injections of NAT (purity, >95%; Hoffmann *et al.*, 1979) in trioctanoin (total doses, approximately 1.0, 3.0 or 9.0 mmol/kg bw), or trioctanoin alone (vehicle controls; 27 males and 27 females), for a total of 20 weeks (with a two-week interruption after seven weeks of injections). Animals were killed when moribund or when only 20% of rats in a group were still alive. Survival (70-90% in males and 66-100% in females at 100 weeks; 0-30% and 25-40%, respectively, at 120 weeks) and body weights of the treated groups were comparable to those of vehicle controls. There was no significant difference in the number and distribution of tumours (Hoffmann *et al.*, 1984). [The Working Group noted the short duration of treatment and the limited number of animals per dose level.]

3.2 Other relevant biological data

No data were available to the Working Group.

3.3 Case reports and epidemiological studies of carcinogenicity to humans

No data were available to the Working Group.

4. Summary of Data Reported and Evaluation

4.1 Exposure data

N'-Nitrosoanatabine (NAT) has been found in a variety of tobacco products (snuff, chewing tobacco, cigarettes and cigars), in mainstream and sidestream smoke from cigars and cigarettes, in saliva of chewers of betel quid with tobacco and in saliva of users of chewing tobacco and oral snuff. Some of the NAT in saliva appears to be formed endogenously from nitrite in saliva and tobacco alkaloids. Thus, there is widespread exposure to NAT among users of tobacco products and those exposed to sidestream smoke.

4.2 Experimental data

NAT was tested for carcinogenicity by subcutaneous injection in rats of one strain at three dose levels. There was no increase in tumour incidence.

No data were available on mutagenic or related effects of NAT.

4.3 Human data

No case report or epidemiological study of the carcinogenicity of NAT was available to the Working Group.

4.4 Evaluation[1]

The available data are *inadequate* to evaluate the carcinogenicity of *N'*-nitrosoanatabine to experimental animals.

No data on humans were available.

5. References

Adams, J.D., Brunnemann, K.D. & Hoffmann, D. (1983) Chemical studies on tobacco smoke. LXXV. Rapid method for the analysis of tobacco-specific *N*-nitrosamines by gas-liquid chromatography with a thermal energy analyzer. *J. Chromatogr.*, *256*, 347-351

Brunnemann, K.D., Scott, J.C. & Hoffmann, D. (1982) *N*-Nitrosomorpholine and other volatile *N*-nitrosamines in snuff tobacco. *Carcinogenesis*, *3*, 693-696

Brunnemann, K.D., Hecht, S.S. & Hoffmann, D. (1983) *N*-Nitrosamines: Environmental occurrence, in vivo formation and metabolism. *J. toxicol.-clin. Toxicol.*, *19*, 661-688

[1]For description of the italicized term, see Preamble, pp. 15-16.

Egan, H., Preussmann, R., O'Neill, I.K., Eisenbrand, G., Spiegelhalder, B. & Bartsch, H., eds (1983) *Environmental Carcinogens. Selected Methods of Analysis*, Vol. 6, N-*Nitroso Compounds* (*IARC Scientific Publications No. 45*), Lyon, International Agency for Research on Cancer

Hecht, S.S., Chen, C.-H.B., Young, R. & Hoffmann, D. (1981) Mass spectra of tobacco alkaloid-derived nitrosamines, their metabolites, and related compound. *Beitr. Tabakforsch. int.*, *11*, 57-66

Hoffmann, D. & Adams, J.D. (1981) Carcinogenic tobacco-specific *N*-nitrosamines in snuff and in saliva of snuff dippers. *Cancer Res.*, *41*, 4305-4308

Hoffmann, D. & Hecht, S.S. (1983) *Tobacco and tobacco smoke (volatile and tobacco-specific nitrosamines). General aspects*. In: Egan, H., Preussmann, R., O'Neill, I.K., Eisenbrand, G., Spiegelhalder, B., O'Neill, I.K. & Bartsch, H., eds, *Environmental Carcinogens. Selected Methods of Analysis*, Vol. 6, N-*Nitroso Compounds* (*IARC Scientific Publications No. 45*), Lyon, International Agency for Research on Cancer, pp. 63-67

Hoffmann, D., Adams, J.D., Brunnemann, K.D. & Hecht, S.S. (1979) Assessment of tobacco-specific *N*-nitrosamines in tobacco products. *Cancer Res.*, *39*, 2505-2509

Hoffmann, D., Adams, J.D., Piade, J.J. & Hecht, S.S. (1980a) *Chemical studies on tobacco smoke. LXVIII. Analysis of volatile and tobacco-specific nitrosamines in tobacco products*. In: Walker, E.A., Castegnaro, M., Griciute, L. & Börzsönyi, M., eds, N-*Nitroso Compounds: Analysis, Formation and Occurrence* (*IARC Scientific Publications No. 31*), Lyon, International Agency for Research on Cancer, pp. 507-516

Hoffmann, D., Chen, C.-H.B. & Hecht, S.S. (1980b) *The role of volatile and nonvolatile* N-*nitrosamines in tobacco carcinogenesis*. In: Gori, G.B. & Bock, F.G., eds, *A Safe Cigarette?* (*Banbury Report 3*), Cold Spring Harbor, NY, Cold Spring Harbor Laboratory, pp. 113-127

Hoffmann, D., Adams, J.D., Brunnemann, K.D., Rivenson, A. & Hecht, S.S. (1982) *Tobacco specific* N-*nitrosamines: Occurrence and bioassays*. In: Bartsch, H., O'Neill, I.K., Castegnaro, M. & Okada, M., eds, N-*Nitroso Compounds: Occurrence and Biological Effects* (*IARC Scientific Publications No. 41*), Lyon, International Agency for Research on Cancer, pp. 309-318

Hoffmann, D., Rivenson, A., Amin, S. & Hecht, S.S. (1984) Dose-response study of the carcinogenicity of tobacco-specific *N*-nitrosamines in F344 rats. *J. Cancer Res. clin. Oncol.*, *108*, 81-86

Hu, M.W., Bondinell, W.E. & Hoffmann, D. (1974) Chemical studies on tobacco smoke. XXIII. Synthesis of carbon-14 labelled myosmine, nornicotine and *N'*-nitrosonornicotine. *J. labelled Compd*, *10*, 79-88

Klus, H. & Kuhn, H. (1975) Studies on the nonvolatile *N*-nitrosamines of the tobacco alkaloids (Ger.). *Fachl. Mitt. Austria Tabakwerke*, *16*, 307-317

Nair, J., Ohshima, H., Friesen, M., Croisy, A., Bhide, S.V. & Bartsch, H. (1985) Tobacco-specific and betel-nut-specific *N*-nitroso compounds: Occurrence in saliva and urine of betel quid chewers and formation *in vitro* by nitrosation of betel quid. *Carcinogenesis*, *6*, 295-303

Rühl, C., Adams, J.D. & Hoffmann, D. (1980) Chemical studies on tobacco smoke. LXVI. Comparative assessment of volatile and tobacco-specific *N*-nitrosamines in the smoke of selected cigarettes from the USA, West Germany and France. *J. anal. Toxicol.*, *4*, 255-259

Wenke, G., Brunnemann, K.D., Hoffmann, D. & Bhide, S.V. (1984) A study of betel quid carcinogenesis. IV. Analysis of the saliva of betel chewers: A preliminary report. *J. Cancer Res. clin. Oncol.*, *108*, 110-113

N'-NITROSONORNICOTINE (NNN)

This substance was considered by a previous Working Group, in October 1977 (IARC, 1978). Since that time, new data have become available, and these have been incorporated into the monograph and taken into account in the present evaluation.

1. Chemical and Physical Data

1.1 Synonyms and trade names

Chem. Abstr. Services Reg. Nos: 80508-23-2; 16543-55-8[1]; 84237-38-7[2]

Chem. Abstr. Names: Pyridine, 3-(1-nitroso-2-pyrrolidinyl)-; pyridine, 3-(1-nitroso-2-pyrrolidinyl)-,(S)-[1]; pyridine, 3-(1-nitroso-2-pyrrolidinyl)-, (+,-)-[2]

IUPAC Systematic Name: 1'-Demethyl-1'-nitrosonicotine

Synonyms: 1'-Demethyl-1'-nitrosonicotine; 1'-desmethyl-1'-nitrosonicotine; 1'-nitroso-1'-demethylnicotine; *N*-nitrosonornicotine; 1'-nitrosonornicotine; nitrosonornicotine; 1-nitroso-2-(3-pyridyl)pyrrolidine; 3-(1-nitroso-2-pyrrolidinyl)pyridine

1.2 Structural and molecular formulae and molecular weight

N
N=O
N

$C_9H_{11}N_3O$

Mol. wt: 177.2

1.3 Chemical and physical properties of the pure substance

(*a*) *Description*: Yellow oil that solidifies on standing in the cold

(*b*) *Boiling-point*: 154°C at 0.2 mm (Hu *et al.*, 1974)

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[1]The Chemical Abstracts Services Registry Number and Name refer to a single stereoisomer (S).

[2]The Chemical Abstracts Services Registry Number and Name refer to the racemic mixture that was synthesized and used in the biological studies reported in this monograph.

(c) *Melting-point*: 47°C

(d) *Spectroscopy data*: Mass (Hecht *et al.*, 1981a), ultraviolet, infrared, and nuclear magnetic resonance spectra (Hu *et al.*, 1974) have been reported.

(e) *Reactivity*: Can be reduced to the corresponding hydrazine with lithium aluminium hydride (Neurath & Duenger, 1966). For formation of *N'*-nitrosonornicotine-1-*N*-oxide, see Hecht *et al.* (1980a). For other reactions, see Chen *et al.* (1979) and Hecht *et al.* (1979).

1.4 Technical products and impurities

NNN is not produced commercially.

2. Production, Use, Occurrence and Analysis

2.1 Production and use

NNN was prepared by Boyland *et al.* (1964) by treating nornicotine with sodium nitrite in dilute hydrochloric acid. Using a variation of this procedure, Hu *et al.* (1974) obtained a yield of 93%. It has also been made by the reaction of nicotine-*N'*-oxide with nitrites or nitrogen dioxide (Klimisch & Stadler, 1976).

When nicotine was reacted with five equivalents of sodium nitrite in aqueous solution at 90°C for 3 h at pH 3.4-4.2, NNN was the identified reaction-product formed in the second highest yield (8.8% of the starting nicotine) (Hecht *et al.*, 1978a).

No evidence was found that NNN has ever been produced in commercial quantities or that it has any uses other than as a laboratory chemical.

2.2 Occurrence

(a) *Tobacco and tobacco smoke*

NNN has not been detected in freshly harvested tobacco (Hecht *et al.*, 1978a); however, it was detected in freshly homogenized, leaf-cured tobacco (Andersen *et al.*, 1982).

NNN has been reported to be produced by nitrosation of nicotine, the most prevalent alkaloid in commercial tobacco, and only to a minor extent from the tobacco alkaloid, nornicotine, during the curing, ageing, processing and smoking of tobacco (Hecht *et al.*, 1978b). NNN has been found in tobacco at levels of 0.2-130 mg/kg, in snuff products at 0.8-77 mg/kg, in chewing tobacco at 1.0-90.6 mg/kg, and in cigarette smoke (mainstream) at 0.1-3.7 μg/cigarette (Hoffmann *et al.*, 1974; Klus & Kuhn, 1975; Hoffmann *et al.*, 1976; Munson & Abdine, 1977; Hoffmann *et al.*, 1979; US Department of Health and Human Services, 1981; Andersen *et al.*, 1982; Brunnemann *et al.*, 1983a; Chamberlain & Arrendale, 1983; Hoffmann & Hecht, 1983). Additional quantities of NNN may be formed in the oral cavity during oral use of snuff or tobacco (Hoffmann *et al.*, 1980a; Hoffmann & Adams, 1981; Brunnemann *et al.*, 1983a; Sipahimalani *et al.*, 1984).

Two studies reported no correlation between nicotine content and NNN levels in 12 flue-cured tobacco samples (Brunnemann *et al.*, 1983b; Chamberlain & Arrendale, 1983).

Table 1 summarizes the data obtained from analyses of smoke from selected commercial cigarettes with and without filter tips and shows the effectiveness of the filters of cigarettes D and E in decreasing the occurrence levels of NNN (Hoffmann *et al.*, 1982).

Table 1. Reduction of nicotine and NNN concentrations in cigarette smoke by filtration[a]

Cigarette	Lengthsmoked/ length of cigarette (mm)	Nicotine (mg/cigarette)[b]	NNN (μg/cigarette)[b]
A NF	50/65	1.8	0.8
F	50/85	1.1 (-40%)	0.5 (-44%)
B NF	50/65	1.8	0.5
F	50/85	1.3 (-29%)	0.3 (-39%)
C NF	50/65	1.7	0.8
F	50/85	1.2 (-30%)	0.5 (-38%)
D NF	50/65	1.6	1.1
F	50/85	1.1 (-34%)	0.3 (-69%)
E[c] NF	50/73	1.6	1.8
F	50/100	1.1 (-34%)	0.5 (-71%)

[a]Data from Hoffmann *et al.* (1982)
[b]In parentheses, percentage changes in yields on comparing filtered (F) and non-filtered (NF) smoke
[c]Perforated filter tip

Nicotine and NNN concentrations found in cigarette and cigar tobaccos, in both their mainstream (during puff drawing) and sidestream (generated during smouldering of tobacco in between puffs) smoke, and in pipe and chewing tobacco are presented in Table 2.

In studies with [2'-^{14}C]NNN, the NNN in tobacco smoke was demonstrated to be partially formed during smoking; however, 40-50% were shown to originate from NNN in the tobacco by direct transfer into the mainstream smoke. Approximately 46% of the NNN in the smoke of a US, blended, non-filter cigarette originated from the tobacco, whereas the rest was formed during smoking. In the case of a French, dark-tobacco cigarette with alkaline smoke, the transfer of NNN from the tobacco into the smoke was about 41%; therefore, the balance of 59% was formed during smoking (Hoffmann *et al.*, 1980b; Adams *et al.*, 1983b).

NNN concentrations found after analysis of snuff obtained in Sweden, Denmark, Federal Republic of Germany and the USA were higher than those in cigarette tobacco presented above. The results are summarized in Table 3. NNN concentrations differed not only among snuff brands but also in samples of the same brand bought in different cities. The latter differences were attributed to possible variations in NNN content between batches and/or effects of ageing. The effects of ageing were demonstrated by opening individual portions (packed in aluminium foil) and storing the snuff in the open air: within eight days, the NNN content had increased by 34% and then remained stable. The data presented in Table 3 suggest that part of the nitrate in the snuff (present at about 2%) was reduced to nitrite while standing in the open air, an occurrence that was not observed in a germ-free atmosphere (Hoffmann & Adams, 1981; Hoffmann *et al.*, 1982).

Addition of nitrate to cigarettes increased the yields of nitrosamines in mainstream smoke (Adams *et al.*, 1984). This may be important, because the levels of nitrate in cigarettes have increased from about 0.5% to 1.2-1.5% over the last 20 years (US Department of Health and Human Services, 1982).

Table 2. NNN concentrations in cigarette, cigar, pipe and chewing tobacco, and cigarette and cigar mainstream and sidestream smoke[a]

Tobacco product[b]	NNN concentration		
	In tobacco (mg/kg)[c]	In mainstream smoke (µg/cig)	In sidestream smoke (µg/cig)
Burley cigarette, NF	7.0	3.7	6.1
Bright cigarette, NF	0.2	0.6	1.7
Commercial cigarette, NF	1.7	0.2	1.7
Commercial cigarette, FA	1.4	0.3	0.2
US cigarettes[d]			
F	2.6	--	--
F	2.2	--	--
NF	1.8	--	--
NF, 70 mm	2.0	--	--
F, menthol	1.9	--	--
F, light	4.4	--	--
F, ultra-light	3.2	--	--
US cigarette, NF[e]	--	1.0	--
Kentucky 1R1[d]	0.7	--	--
Kentucky 1R1, NF	0.6	0.4	0.2
German (Federal Republic) cigarettes[f]			
Brand A, NF	--	0.5	--
Brand B, NF	--	0.2	--
Brand C, FA	--	0.2	--
Brand D, FA	--	0.1	--
Brand E, FA	--	0.2	--
Brand F, FA	--	0.2	--
Commercial French cigarette, NF, 70 mm	2.9	0.5	--
Commercial French cigarettes,			
FA, 70 mm	2.7	0.5	--
NF	11.9	3.2	--
FA	11.9	0.0	--
FP	11.9	0.7	--
British cigarette, NF[g]	0.3	--	--
Little cigar, FA	45.0	5.5	0.9
Cigar (Columbian tobacco) (5.7 g)	10.7	3.2	16.6
Cigar[d]	2.9	--	--
Loose-leaf tobacco[d]	1.2	--	--
Fine-cut chewing tobacco	39.0	NA[h]	NA
Fine-cut chewing tobacco[e]	45.6	NA	NA
Japanese tobaccos[i]			
Cigarette 1	1.1	--	--
Cigarette 2	D	--	--
Cigarette 3	D	--	--
Cigarette 4	D	--	--
Cigarette 5	D	--	--
Cigarette 6	ND	--	--
Kiseru tobacco	1.8	--	--
Pipe tobacco 1	D	--	--
Pipe tobacco 2	ND	--	--
Pipe tobacco 3	ND	--	--
Chewing tobacco[g]			
Scrap-leaf A	3.5	NA	NA
Scrap-leaf B	3.9	NA	NA
Scrap-leaf C	8.2	NA	NA
Plug A	3.4	NA	NA
Plug B	4.3	NA	NA
Fine-cut	90.6	NA	NA
Indian tobacco for betel quid	2.4	NA	NA
Pipe tobaccos[j]			
US	1.6	-	-
US	3.1	-	-

[a]Data from Hoffmann *et al.* (1980a), unless otherwise noted

[b]All cigarettes and the little cigar were 85 mm long, unless otherwise noted. Abbreviations: NF, non-filter; FA, cellulose acetate filter; FP, paper filter; F, unspecified filter

[c]Abbreviations: D, presence doubtful; ND, not detected (detection limit, 0.01 mg/kg (Hecht *et al.*, 1975a))

[d]Data from Brunnemann *et al.* (1983b); products (excluding Kentucky 1R1) were purchased in Westchester County, NY, in 1982

[e]Data from Adams *et al.* (1983a); cigarettes and tobacco used were purchased in Westchester County, NY, in 1981

[f]Data from Rühl *et al.* (1980); cigarettes were popular brands purchased in Berlin in 1979

[g]Data from Hoffmann *et al.* (1976)

[h]NA, not applicable

[i]Data from Bharadwaj *et al.* (1975)

[j]Data from Munson and Abdine (1977)

Table 3. Nicotine, nornicotine and NNN concentrations in commercial snuff[a]

Snuff origin	Type of packaging[b]	Nicotine (g/kg)	Nornicotine (g/kg)	NNN (mg/kg)[c]
USA				
Brand I New York and Tennessee	A	23.8	0.6	39
Brand II New York and Tennessee	A	23.4	0.5	26.5
Brand III New York and Tennessee	B	14.5	0.3	3.5
Federal Republic of Germany				
Brand I Munich	C	5.7	0.8	6.7
Brand II Munich	C	5.4	0.6	6.1
Sweden				
Brand I Umeå	A	15.2	0.3	8.6
Brand I Uppsala	A	15.0	0.3	6.1
Brand I Lund	A	15.1	0.3	8.9
Brand II Umeå	A	18.1	0.2	8.4
Brand II Uppsala	A	18.0	0.2	5.2
Brand II Lund	A	18.1	0.2	10.3
Brand III Umeå	A	6.0	0.05	7.8
Brand III Uppsala	A	6.2	0.05	3.5
Brand III Lund	A	6.7	0.05	3.8
Brand IV Umeå	A	11.2	0.07	9.7
Brand IV Uppsala	A	11.2	0.06	77.1
Brand IV Lund	A	11.4	0.08	8.2
Brand V Umeå	D	21.7	0.9	4.7
Denmark (3)				
Brand I Copenhagen	E	11.1	0.8	6.4
Brand II Copenhagen	E	21.2	0.9	4.5
Brand III Copenhagen	E	30.5	0.6	8.0
Ageing test[d]: 0 time	--	--	--	4.7
8 days	--	--	--	6.3
Snuff and smokeless tobacco[e]				
US	--	--	--	3.2
US	--	--	--	9.3

[a]Data from Hoffmann and Adams (1981), unless otherwise noted; values are given for dry snuff, moisture content about 50%

[b]Abbreviations: A, waxed-paper container with metallic lid, containing approximately 50 g; B, 25 individual portions of approximately 11 g packaged in paper in a plastic container; C, plastic foil-lined aluminium bags containing 100 g; D, individual snuff portion in a paper bag packaged in a crimped airtight aluminium envelope, with 10 envelopes in a plastic bag amounting to approximately 10 g; E, hard-plastic container

[c]NNN values are averages of three runs.

[d]Data from Hoffmann *et al.* (1982). The aluminium foil-wrapped package (Swedish brand V) was opened at '0 time'.

[e]Data from Munson and Abdine (1977)

(*b*) *Human tissues and secretions*

Formation of additional quantities of NNN by the reaction of salivary nitrite with nicotine or nornicotine during oral use of snuff or tobacco chewing has been implied from in-vitro studies (Hoffmann & Adams, 1981). Incubation of fine-cut chewing tobacco (for 3 h at 37°C) with saliva resulted in a value for NNN of 127 μg/g, an increase of 44% over the 88.6 μg/g found in the original chewing tobacco. A similar value, 133 μg/g, was obtained when fine-cut chewing tobacco was extracted with saliva for 18 h at 20°C (Hecht *et al.*, 1975a).

Saliva was examined from women who had been long-term oral-snuff users and who were employed in two southern US furniture companies. The wide range in NNN concentrations (5.0-125 ng/g) in the saliva of individual users indicated that during oral use of snuff NNN is extracted from the tobacco plug at varying rates (Hoffmann & Adams, 1981).

In another study, saliva of four women who were long-term (>10 years) oral-snuff users was analysed on two different days after they had used a specific brand of snuff with known concentrations of NNN. The results (Table 4) indicate that the saliva of users contains significant amounts of NNN and that the levels vary significantly between subjects, as well as between samples from the same individual taken on different days. The variations in NNN values were at least partially explained by differences in the intensity with which individuals practised the habit at different times, and perhaps also by varying rates of salivation (Hoffmann & Adams, 1981; Hoffmann *et al.*, 1982).

Table 4. Nicotine, nornicotine and NNN concentrations in snuff and in the saliva of women who were long-term oral-snuff users[a]

Subject	Age (years)	Snuff			Saliva[b]		
		Nicotine (mg/g)	Nor-nicotine (mg/g)	NNN (μg/g)	Day of sampling	Nicotine (mg/g)	NNN (μg/g)
1	41	23.4	0.05	26.5	1	0.2	0.2
					2	0.5	0.1
2	37	23.4	0.05	26.5	1	0.07	0.03
					2	0.4	0.1
3	44	23.4	0.05	23.1	1	1.2	0.4
					2	1.6	0.3
4	52	23.6	0.06	24.8	1	0.2	0.03
					2	0.4	0.06

[a]Data from Hoffmann and Adams (1981)

[b]Saliva of three women who did not use snuff (controls) was free of nicotine and tobacco-specific *N*-nitrosamines.

NNN was not detected in the combined gastric juices of 14 volunteers who each smoked 28 cigarettes per day (Schweinsberg *et al.*, 1975). [The Working Group noted that analytical methods have improved since 1975-1977.]

NNN was found in the saliva of chewers of betel quid with tobacco at levels of 1.2-38 ng/g [mean, 13.1 ng/g] (Wenke *et al.*, 1984) and 1.6-14.7 ng/ml (mean, 7.5 ng/ml); it was also detected in the saliva of chewers of tobacco, at levels of 16.5-59.7 ng/ml (mean, 33.4 ng/ml) (Nair *et al.*, 1985).

2.3 Analysis

Standard methods for the analysis of NNN are described in detail in an IARC manual on selected methods of analysis (Egan *et al.*, 1983).

3. Biological Data Relevant to the Evaluation of Carcinogenic Risk to Humans

3.1 Carcinogenicity studies in animals

(a) Oral administration

Rat: A group of 20 male Fischer rats, seven weeks old, was given 200 mg/l NNN (purity, >99%; Hu *et al.*, 1974) in the drinking-water on five days per week for 30 weeks (esti-

mated total dose, 630 mg). Animals were killed when moribund or after 11 months, and all animals, except those lost by cannibalism or autolysis, were necropsied. All 12 rats necropsied from the treated groups had developed oesophageal tumours (11 papillomas and three carcinomas); in addition, one pharyngeal papilloma and three carcinomas of the nasal cavity with invasion of the brain were observed. No tumour was observed in 19 untreated controls (Hecht *et al.*, 1975b; Hoffmann *et al.*, 1975).

A group of 15 female Sprague-Dawley rats, eight weeks old, was given approximately 7 mg NNN [purification and characterization checked by ultraviolet and mass spectra] per rat per day in the drinking-water (354 mg/l) on five days per week for 44 weeks. All rats died within 46 weeks, and all had nasal-cavity adenocarcinomas. In addition, one squamous-cell papilloma of the oesophagus, one squamous-cell papilloma of the forestomach and one hepatocellular tumour occurred in three rats surviving 43 weeks or longer (Singer & Taylor, 1976). [The Working Group noted that no detail concerning parallel or historical control groups was reported.]

Groups of 12 male and 12 female Fischer 344 rats, six to eight weeks of age, received 0.012% (w/v) NNN (purity, >99%; Hu *et al.*, 1974) in the drinking-water continuously for 36 weeks (total doses, approximately 3.6 and 3.3 mmol, respectively). The combined incidence of oesophageal tumours in animals of both sexes, including squamous-cell carcinomas (six) and papillomas, was 23/24 rats by 11 months; all rats had died by month 12. Nasal-cavity tumours, including 15 benign and 15 malignant tumours, occurred in 21/24 rats. One female rat had a tracheal papilloma. No such tumour was reported in 12 male or 12 female controls (Hecht *et al.*, 1983a).

Two groups of 30 male Fischer 344 rats were pair-fed either a control liquid diet or an isocaloric liquid diet containing ethanol (6% w/v) starting at nine weeks of age. Starting at 13 weeks of age, rats were placed on control or ethanol liquid diets containing 17.5 mg/l NNN (purity, 98%) continuously, seven days per week for the following 27 weeks until each animal had consumed 1 mmol NNN (3.2-3.7 mmol/kg bw). Thereafter, the liquid diets were replaced by a standard diet, and rats were maintained until killed when moribund or when 98 weeks old (mean survival time, 83 weeks). Two control groups consisted of 26 rats receiving liquid diet with or without ethanol and receiving three injections per week of 0.9% saline solution [number of injections not specified, presumably 56-66]. At the end of the series of injections, the control rats were switched to standard diet and maintained and killed in parallel to the treated rats. All of the NNN-treated rats developed head-and-neck tumours (nasal cavity, oesophagus and tongue), whereas none of the controls had a tumour at these sites. In the group receiving NNN and ethanol, there were 20 benign tumours [not histologically classified], one aesthesioneuroepithelioma, three squamous-cell carcinomas and two anaplastic tumours of the nasal cavity; 13 benign tumours [not histologically classified]; seven squamous-cell carcinomas of the oesophagus; three benign tumours of the root of the tongue; and three adenocarcinomas of the lung. In the group receiving NNN but no ethanol, there were 11 benign tumours, two aesthesioneuroepitheliomas and five squamous-cell carcinomas of the nasal cavity; 16 benign tumours and nine squamous-cell carcinomas of the oesophagus; two benign tumours of the root of the tongue; and one squamous-cell carcinoma of the lung. Among 26 control rats given neither ethanol nor NNN there was one lung adenocarcinoma. Among 26 control rats given ethanol, there was one adenoma and one adenocarcinoma of the lung. Each group had a number of other benign or malignant tumours distributed among several tissues and organs, as would be expected in ageing rats (Castonguay *et al.*, 1984).

Hamster: Groups of 10 male and 10 female Syrian golden hamsters, six to seven weeks of age, received 0.016% (w/v) NNN (purity, >99%; Hu *et al.*, 1974) in the drinking-water con-

tinuously for 31 weeks (total doses, approximately 1.9 and 2.8 mmol). The experiment was terminated after 96 weeks. Papillomas of the nasal cavity developed in 4/20 animals and papillomas of the trachea in 2/20; one lymphoma of the caecum and one angiosarcoma of the liver also occurred. No such tumour occurred in 10 male or 10 female controls (Hecht *et al.*, 1983a).

(*b*) *Skin application*

Mouse: A group of 20 female Ha/ICR/mil Swiss mice, seven to eight weeks of age, received thrice-weekly applications of 0.1 ml of a 0.03% solution of NNN (purity, >99%; Hu *et al.*, 1974) in acetone for 50 weeks. No skin tumour developed in this group (Hoffmann *et al.*, 1976). [The Working Group noted that no data were available on tumours in other organs.]

(*c*) *Subcutaneous and/or intramuscular administration*

Rat: Groups of 12 male and 12 female Fischer 344 rats, seven weeks of age, received thrice-weekly s.c. injections of 10 mg [0.06 mmol] NNN (purity, >99%) in 0.3 ml trioctanoin for 20 weeks (total dose, 600 mg; 3.4 mmol). Animals were maintained until they died spontaneously or were killed after 12 months. Malignant tumours of the nasal cavity (15 olfactory neuroblastomas and two rhabdomyosarcomas) developed in 17/24 rats; in addition, 3/24 rats developed benign nasal-cavity tumours [this category included papillomas, adenomatous polyps and villous polyps, but the histological types of these three tumours were not stated explicitly], and another developed a lesion described as a neoplastic nodule of the liver. No such tumour was seen in 24 vehicle controls that received trioctanoin only (Hecht *et al.*, 1980b).

Three groups of 15-27 male and 15-27 female Fischer 344 rats, nine weeks of age, received thrice-weekly s.c. injections of NNN (purity, >99%; Hu *et al.*, 1974) in trioctanoin (total doses, 9.0, 3.0 or 1.0 mmol/kg bw), or trioctanoin alone (vehicle controls; 52 animals) for 20 weeks. Animals were killed when moribund or when only 20% of rats in a group were still alive. No animal was still alive after 60, 120 or 130 weeks in the three groups, respectively; no difference in body weight was observed between treated and control animals. Nasal-cavity tumours were found in 12/14 males and 15/15 females given the high dose; all were malignant aesthesioneuroepitheliomas, squamous-cell carcinomas, anaplastic carcinomas or spindle-cell sarcomas. In the medium-dose group, 8/15 males and 5/15 females had malignant nasal-cavity tumours, and 3/15 males and 4/15 females had benign nasal-cavity tumours (squamous-cell papillomas, transitional-cell papillomas or polyps). In the low-dose group, 4/27 males had malignant, and 11/27 males and 12/27 females had benign nasal-cavity tumours. Benign oesophageal tumours [histological type not reported] were seen in 4/14 males and 3/15 females in the high-dose group, 5/15 males and 2/15 females in the medium-dose group, and 1/27 males and 1/27 females in the low-dose group. In the three groups combined, four rats developed benign liver tumours, four, bladder tumours, one, a lung adenocarcinoma and 11, lung adenomas. The occurrence of nasal-cavity tumours suggests a dose-response relationship. Four benign liver tumours were observed in controls treated with trioctanoin only (Hoffmann *et al.*, 1984).

Two groups of 30 male Fischer 344 rats were pair-fed either a control liquid diet or an isocaloric liquid diet containing ethanol (6% w/v) starting at nine weeks of age. Starting at 13 weeks of age, each animal was injected s.c. with 10 mg/kg bw NNN (purity, 98%) in a 0.9% saline solution, three times per week until a total dose of 1 mmol/rat (3.2-3.7 mmol/kg;

56-66 injections) had been delivered. The liquid diets were replaced by a standard diet 24 h after the last injection. Rats were killed when moribund or when 98 weeks old (mean survival time, 83 weeks). Two control groups maintained on liquid diet with or without ethanol received s.c. injections of 0.9% saline solution without NNN [number of injections not specified, presumably 56-66] and were then switched to standard diet. Of the NNN-treated rats, 22/30 on the diet with ethanol and 26/30 on the diet without ethanol had head-and-neck tumours, whereas none were seen in controls with or without ethanol in the diet. In the group receiving NNN and ethanol, there were two benign tumours [not histologically classified], 17 aesthesioneuroepitheliomas, two squamous-cell carcinomas and one sarcoma of the nasal cavity; two benign tumours [not histologically classified] and one squamous-cell carcinoma of the oesophagus; and one benign tumour of the root of the tongue. No lung tumour was observed. In the group given NNN but not ethanol, there were four benign tumours, 18 aesthesioneuroepitheliomas, one squamous-cell carcinoma and one anaplastic tumour of the nasal cavity; no oesophageal or tongue tumour; and one lung adenoma. Among 26 control rats given neither ethanol nor NNN, there was one lung adenocarcinoma. Among 26 control rats given ethanol, there was one adenoma and one adenocarcinoma of the lung. Each group had a number of other benign or malignant tumours distributed among several tissues and organs, as would be expected in ageing rats (Castonguay *et al.*, 1984).

Groups of 15 male and 15 female Fischer 344 rats, six weeks of age, were given s.c. injections of 10 mg NNN, 2',5',5'-trideutero-NNN or 2'-deutero-NNN (purity, >99%; Chen *et al.*, 1979) in saline three times per week for 14 weeks (total of 41 injections; total dose, 410 mg/rat). All surviving animals were killed at 21 months. Controls received s.c. injections of saline alone. The mean length of survival was 13 months for the animals treated with NNN and 15 months for animals treated with the deuterated compounds. A total of 20 animals treated with NNN (males and females combined) had invasive olfactory tumours [unspecified]; the corresponding numbers for animals treated with 2',5',5'-trideutero-NNN and 2'-deutero-NNN were 15 and 13; no such tumour was seen in controls. No significant increase in the incidence of tumours at other sites was observed (Hecht *et al.*, 1982a).

Hamster: Groups of 10 male and 10 female Syrian golden hamsters, eight to 10 weeks of age, received thrice-weekly s.c. injections of 5 mg NNN (purity, >99%; Hu *et al.*, 1974) in saline for 25 weeks (total dose, 375 mg). A group of 10 males and 10 females received injections of saline only and served as vehicle controls. Animals were killed when moribund, or at termination of the experiment at 83 weeks. Of 19 effective animals, 12 developed single papillary tumours of the trachea within 83 weeks (first tumour after 38 weeks). One animal had an adenocarcinoma of the nasal cavity after 45 weeks. No such tumour was observed in 17 effective controls given saline only (Hilfrich *et al.*, 1977).

A group of 15 male and 15 female Syrian golden hamsters, eight to 10 weeks of age, received 19 s.c. injections of 8.5 mg (0.048 mmol) NNN (purity, >99%) in 0.3 ml trioctanoin on a three-injection-per-week schedule (total dose, 160 mg [0.91 mmol]). The experiment was terminated after 16 months. Survival rates were the same in treated and untreated animals. Among the 28 effective animals in the treated group, five tracheal papillomas, one lung adenoma and one tumour described as an 'undifferentiated carcinoma of the leg' were reported. A further group of 10 males and 10 females received thrice-weekly s.c. injections of 2.5 mg (0.012 mmol) NNN in trioctanoin for 25 weeks. The experiment was terminated after 17 months; after 13 months, 60% of the treated animals were still alive. Among the 18 effective animals, one tracheal papilloma and one adenocarcinoma of the lung were reported. No respiratory-tract tumour was observed in 30 vehicle controls receiving trioctanoin only (Hoffmann *et al.*, 1981).

(*d*) *Intraperitoneal administration*

Mouse: Groups of 20 male and 20 female Chester Beatty stock mice, approximately six weeks old, were injected i.p. once a week with 0.1 ml NNN [purity unspecified] dissolved in arachis oil (2%) for 41 weeks; 14 males and 11 females died during the first seven months with no tumour. Of eight animals that died after the eighth month, seven (five females and two males) had multiple pulmonary adenomas. Groups of 15 male and 15 female mice injected i.p. weekly with arachis oil served as vehicle controls; the only tumour reported among these 30 control mice was a single lung adenoma in a mouse killed at 11 months (Boyland *et al.*, 1964). These results were confirmed in A/HE mice (Hoffmann *et al.*, 1976).

In a screening assay for potential carcinogenicity using pulmonary adenomas as an end-point in strain A mice, a group of 21 female strain A/J mice, six to eight weeks old, received thrice-weekly i.p. injections of 1 mg NNN (purity, >99%; Hu *et al.*, 1974) in 0.2 ml saline for seven weeks (total of 22 injections; total dose, 22 mg [0.13 mmol]) and were held without further treatment for an additional 30 weeks. A further 23 mice received NNN in trioctanoin by the same schedule; and an untreated control group of 25 mice was available. Among the 23 mice that received NNN in trioctanoin, 12 had lung adenomas, one had a lung adenocarcinoma, one had an undifferentiated carcinoma of the salivary glands and one had a malignant lymphoma. Of the 21 mice that received NNN in saline, 16 had lung adenomas and one had an undifferentiated carcinoma of the salivary gland. In untreated, saline and trioctanoin controls, lung adenomas were seen in 1/25, 3/25 and 5/24 mice, respectively (Hecht *et al.*, 1978b).

In another study, female A/J mice, six to eight weeks old, received 22 thrice-weekly i.p. injections of NNN (purity, >99%; Hu *et al.*, 1974) or 2',5',5'-trideutero-NNN (purity, >99%; Chen *et al.*, 1979) in saline over seven weeks (total dose, 0.12 mmol). Animals were killed 30 weeks after the last injection. Of the treated mice, 16/24 animals developed lung tumours, compared with 7/24 controls receiving a saline solution. The total number of lung tumours in NNN-treated animals was 29, 10 of which were malignant, compared with nine (one malignant) in controls. No tumour was found in other organs. Of mice treated with 2',5',5'-trideutero-NNN, 20/25 had 37 lung tumours, 14 of which were malignant (Castonguay *et al.*, 1983a).

Hamster: Groups of 21 male Syrian golden hamsters received thrice-weekly i.p. injections of NNN (purity, >99%; Hu *et al.*, 1974) in saline for 25 weeks beginning at 13 weeks of age [total dose, 1 mmol (low-dose) or 2 mmol (high-dose)]. The animals were maintained on a liquid diet with or without ethanol, beginning at eight weeks of age. Of the 21 animals in the low-dose group receiving an ethanol-free diet, one developed an invasive nasal-cavity tumour (reported to be of olfactory origin but not further classified) and four developed tracheal papillomas. Of the high-dose group receiving an ethanol-free diet, 5/21 developed nasal-cavity tumours (olfactory but not further classified; two invasive), and 9/21 developed tracheal papillomas. A number of other tumours were observed; many of these were similar to those seen in vehicle controls, except for an adenosquamous-cell carcinoma of the lung. Ethanol did not appear to influence the tumorigenicity of NNN in this study (McCoy *et al.*, 1981a).

(*e*) *Carcinogenicity of metabolites*

In a screening assay for potential carcinogenicity using pulmonary adenomas as an end-point in strain A mice, groups of 25 female A/J mice, six to eight weeks old, received thrice-weekly i.p. injections of 0.2 ml 3'-hydroxy-NNN, 4'-hydroxy-NNN or NNN-1-*N*-oxide

(purity, >99%; Hecht *et al.*, 1980a) in saline for seven weeks (22 injections; total dose, 0.12 mmol) and were killed 30 weeks later. Twenty-five animals served as untreated controls, and 24 vehicle controls received saline only by the same schedule. Ten untreated controls had a total of 16 lung tumours, two of which were carcinomas [histological type not specified]; the average multiplicity of lung tumours per mouse was 0.6 $\pm$ 0.9. Seven vehicle controls had a total of nine lung tumours, one of which was malignant; the average multiplicity was 0.4 $\pm$ 0.6 per mouse. Of mice treated with 3'-hydroxy-NNN, 12/25 had a total of 23 lung tumours, including six carcinomas, and 0.9 $\pm$ 1.4 lung tumours per mouse. In mice treated with 4'-hydroxy-NNN, 19/25 had lung tumours, with a total of 41 lung tumours, including 13 carcinomas, and an average multiplicity of 1.6 $\pm$ 1.5 lung tumours per mouse. Of 25 mice treated with NNN-1-*N*-oxide, 16 had a total of 21 lung tumours, of which six were carcinomas; the average multiplicity was 0.8 $\pm$ 0.7 lung tumours per mouse. Tumours of other organs included a gastric papilloma in a mouse treated with 3'-hydroxy-NNN and an angioma of the adrenal medulla in a mouse treated with NNN-1-*N*-oxide (Castonguay *et al.*, 1983a).

Rat: A group of 12 male and 12 female Fischer 344 rats, six to eight weeks of age, was given 0.012% (w/v) NNN-1-*N*-oxide (purity, >99%; Hecht *et al.*, 1980) in the drinking-water (0.012%, w/v) every day for 36 weeks; the experiment was terminated after 104 weeks. Oesophageal papillomas developed in 5/12 males and 0/12 females; squamous-cell carcinomas of the oesophagus developed in 3/12 males and 3/12 females. Nasal-cavity tumours (papillomas and/or carcinomas) were found in 11/12 males and 7/12 females. In addition, two pulmonary adenomas and two papillomas of the tongue developed in treated males. No such tumour was found in 12 male or 12 female control rats (Hecht *et al.*, 1983a).

Hamster: Groups of 10 male and 10 female Syrian golden hamsters, six to seven weeks of age, were given NNN-1-*N*-oxide in the drinking-water (0.016%, w/v) daily for 31 weeks; the experiment was terminated after 96 weeks. No tumour of the nasal cavity or trachea was reported (Hecht *et al.*, 1983a).

3.2 Other relevant biological data

(*a*) *Experimental systems*

Toxic effects

The subcutaneous LD_{50} of NNN in male rats observed for eight days was >1000 mg/kg bw. In rats that died, haemorrhages were observed in the lungs and abdominal organs and epithelial-cell necrosis in the posterior nasal cavities and liver (Hoffmann *et al.*, 1975).

Effects on reproduction and prenatal toxicity

No data were available to the Working Group.

Absorption, distribution, excretion and metabolism

Single intravenous doses of 3.4-7 mg/kg bw [2'-^{14}C]NNN were distributed within 1-5 min throughout the tissues of C57Bl mice, as determined by whole-body autoradiography. After 24 h, non-extractable radioactivity was present in the tracheobronchial and nasal mucosa, liver, submaxillary and sublingual salivary glands, and oesophagus. Binding to the melanin of the eyes and hair was observed *in vivo* and *in vitro* (Brittebo & Tjälve, 1980; Waddell &

Marlowe, 1980, 1983). Single intravenous doses of 4-5 mg/kg bw [2'-^{14}C]NNN were distributed in tissues of Fischer 344 or Sprague-Dawley rats within 5 min of injection. A high uptake of radioactivity was seen in the mucosa of the ethmo-, naso- and maxilloturbinates, in the submaxillary salivary glands, lachrymal glands, Zymbal glands, tarsal glands of the eyelids, preputial glands, oesophagus and tongue, and in the contents of the stomach. After 24 h, non-extractable radioactivity was present in the nasal, tracheobronchial and oesophageal mucosa, and in the liver (Brittebo & Tjälve, 1981).

Male Fischer 344 rats that received a subcutaneous injection of 3-300 mg/kg bw [2'-^{14}C]NNN excreted 73-91% of the dose in urine over 48 h. Less than 1% of the dose was detected in expired air (Chen *et al.*, 1978; Hecht *et al.*, 1981b). Male Syrian golden hamsters that were given subcutaneous injections of 60 mg/kg bw [2'-^{14}C]NNN excreted 62-78% of the dose in urine over 48 h, 10% in faeces and <0.5% in expired air as $^{14}CO_2$ (Hoffmann *et al.*, 1981). Urine was also the major pathway of excretion in male A/J mice after intraperitoneal injection of 50 mg/kg bw [2'-^{14}C]NNN (Hecht *et al.*, 1981b).

Metabolic pathways of NNN are summarized in Figure 1. The metabolites formed initially result from hydroxylation of each position of the pyrrolidine ring, giving compounds *2-5*, and from oxidation of the pyridine nitrogen, yielding compound *1*.

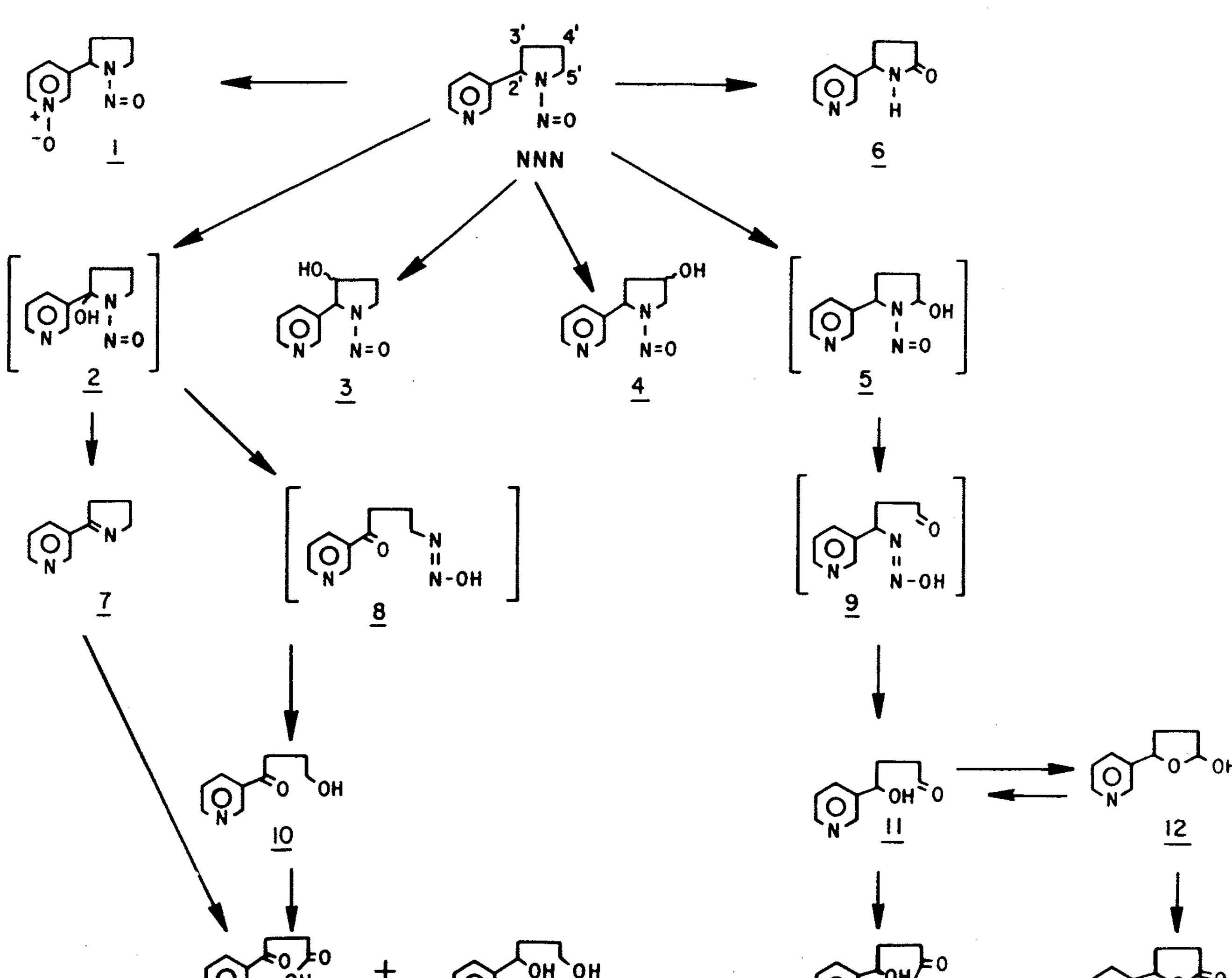
2'-Hydroxylation and 5'-hydroxylation are thought to be the major activation processes in NNN metabolism. 2'-Hydroxylation gives 2'-hydroxy-NNN (*2*), which is unstable and tautomerizes spontaneously to the electrophilic intermediate *8*. This intermediate, which is also formed from 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) (see monograph, p. 209), induces mutations in *Salmonella typhimurium* (Chen *et al.*, 1978; Hecht *et al.*, 1983b). It also reacts with water to give 4-hydroxy-1-(3-pyridyl)-1-butanone (*10*), a major metabolite of NNN formed by rat-liver microsomes (Chen *et al.*, 1978, 1979). Metabolite *10* is further oxidized *in vivo* to give 4-oxo-4-(3-pyridyl)butyric acid (*13*), a major urinary metabolite of NNN in rats, hamsters and mice (Chen *et al.*, 1978). It accounted for 13-31% of a dose of NNN administered to Fischer 344 rats (Hecht *et al.*, 1981b) and 15% of one given to Syrian golden hamsters (Hoffmann *et al.*, 1981).

3'-Hydroxylation and 4'-hydroxylation of NNN lead to the formation of the stable metabolites, 3'- and 4'-hydroxy-NNN (*3* and *4*), which have been detected as minor urinary metabolites in Fischer 344 rats and as minor products of metabolism by Fischer 344 rat-liver microsomes (Hecht *et al.*, 1980a).

5'-Hydroxylation yields 5'-hydroxy-NNN (*5*), which is unstable and tautomerizes spontaneously to the electrophile *9*. Since 5'-hydroxy-NNN is unstable, the corresponding acetate was tested, and was found to induce mutations in *S. typhimurium* (Chen *et al.*, 1978). Reaction of *9* with water gives 2-hydroxy-5-(3-pyridyl)tetrahydrofuran (*12*), a major metabolite of NNN formed by rat-liver microsomes (Chen *et al.*, 1978). Oxidation and ring-opening of this metabolite *in vivo* yield 4-hydroxy-4-(3-pyridyl)butyric acid (*15*), the principal urinary metabolite of NNN in rats (37-53% of the dose administered), hamsters (39% of the dose) and mice (Chen *et al.*, 1978; Hecht *et al.*, 1981b; Hoffmann *et al.*, 1981).

Pyridine-*N*-oxidation of NNN gives NNN-1-*N*-oxide (*1*), a stable metabolite formed by Fischer 344 rat-liver microsomes and excreted in the urine of Fischer 344 rats (7-11% of the dose administered) and Syrian golden hamsters (3% of the dose) (Hecht *et al.*, 1980a, 1981b; Hoffmann *et al.*, 1981). Another urinary metabolite of NNN, norcotinine (*6*), constituted 3-5% of a dose administered to rats (Hecht *et al.*, 1981b).

Figure 1. Metabolism of NNN. Structures in brackets represent hypothetical intermediates[a]



[a]From Hecht *et al.* (1981b). Compounds: *1*, *N'*-nitrosonornicotine-1-*N*-oxide (NNN-1-*N*-oxide); *3*, 3'-hydroxy-*N'*-nitrosonornicotine (3'-hydroxy-NNN); *4*, 4'-hydroxy-*N'*-nitrosonornicotine (4'-hydroxy-NNN); *6*, norcotinine; *7*, myosmine; *10*, 4-hydroxy-1-(3-pyridyl)-1-butanone; *11*, 4-hydroxy-1-(3-pyridyl)butanal; *12*, 2-hydroxy-5-(3-pyridyl)tetrahydrofuran; *13*, 4-oxo-4-(3-pyridyl)butyric acid; *14*, 4-hydroxy-4-(3-pyridyl)-1-butanol; *15*, 4-hydroxy-4-(3-pyridyl)butyric acid; *16*, 5-(3-pyridyl)tetrahydrofuran-2-one

Metabolism of NNN by 2'- and 5'-hydroxylation has been demonstrated in various animal tissues. Cultured Fischer 344 rat oesophagus and nasal mucosa, target tissues in which NNN induces tumours, metabolized NNN extensively, with preferential 2'-hydroxylation; the 2'- to 5'-hydroxylation ratios after 24 h were 3.4 and 1.8, respectively, as compared to a ratio of 0.3 in cultured Syrian golden hamster oesophagus, a non-target tissue (Hecht *et al.*, 1982b; Brittebo *et al.*, 1983). Pieces of rat nasal mucosa converted [2'-^{14}C]NNN to tissue-bound metabolites more efficiently than either rat liver or oesophagus (Brittebo & Tjälve, 1981). In corroboration of these findings, Sprague-Dawley rats that received two intravenous injections of 0.18 mg/kg bw [2'-^{14}C]NNN had greater tissue-bound radioactivity levels

in the nasal mucosa than in any other tissue one, 4 and 24 h after administration. Labelling of DNA was detected in the liver and in nasal mucosa (Löfberg *et al.*, 1982). Cultured A/J mouse peripheral lung principally metabolized NNN by 2'- and 5'-hydroxylation, giving a ratio of 2'- to 5'-hydroxylation of 0.6 24 h after administration (Castonguay *et al.*, 1983a).

The levels of 2'- and 5'-hydroxylation of NNN in various tissues are affected by inducers of the cytochrome-P-450 mixed-function oxidase system. Pretreatment of Fischer 344 rats with 500 mg/kg bw Aroclor 1254 four days prior to killing resulted in a 20-fold induction of 2'-hydroxylation and a 1.9-fold induction of 5'-hydroxylation in hepatic microsomes (Chen *et al.*, 1979). Pretreatment of Fischer 344 rats with 80 mg/kg bw per day phenobarbital intraperitonally for four days prior to killing increased hepatic microsomal 2'-hydroxylation 1.5-fold but had no effect on 5'-hydroxylation; pretreatment of Syrian golden hamsters by the same protocol had no effect on 2'-hydroxylation but increased 5'-hydroxylation 2.5-fold; pretreatment of Fischer 344 rats with 20 mg/kg bw per day 3-methylcholanthrene intraperitoneally for four days prior to killing increased 2'-hydroxylation 2.7-fold but caused a 2.2-fold decrease in 5'-hydroxylation in hepatic microsomes; no effect was observed in hamsters (McCoy *et al.*, 1981b).

Administration of a liquid diet, in which ethanol isocalorically replaced carbohydrate, to Syrian golden hamsters for four weeks resulted in a 1.8-fold increase in liver microsomal 5'-hydroxylation but did not affect 2'-hydroxylation. No effect of ethanol on NNN metabolism was observed in cultured tracheal rings (Chen *et al.*, 1980; McCoy *et al.*, 1982). Treatment of male Fischer 344 rats with a liquid diet containing ethanol for four weeks caused a 1.5-fold increase in 2'-hydroxylation and a 1.7-fold increase in 5'-hydroxylation in cultured nasal mucosa; no effect was observed in cultured lingual mucosa or oesophagus (Castonguay *et al.*, 1984). Pretreatment of male Fischer 344 rats with a variety of isothiocyanates and related compounds, either by gavage 2 h prior to killing or in the diet for two weeks prior to killing, generally caused an inhibition of 2'- and 5'-hydroxylation in cultured rat oesophagus (Chung *et al.*, 1984).

Mutagenicity and other short-term tests

In the presence of a liver microsomal preparation from Aroclor-induced rats, NNN (highest dose tested, 2.5 μmol/plate) caused a dose-dependent increase in mutations in *Salmonella typhimurium* TA100 (Bartsch *et al.*, 1980) and induced mutations in strain TA1530 at 1000 μg/plate (5.7 μmol/plate) (Andrews *et al.*, 1978).

NNN (10^{-3} and 10^{-2}M) induced unscheduled DNA synthesis in freshly isolated hepatocytes from adult rats (Williams & Laspia, 1979).

(*b*) *Humans*

No data were available to the Working Group on toxic effects or on effects on reproduction and prenatal toxicity.

Absorption, distribution, excretion and metabolism

Human liver microsomes obtained from biopsies catalysed 2'- and 5'-hydroxylation of NNN and gave a 2'- to 5'-hydroxylation ratio of 0.6 (Hecht *et al.*, 1979). Human tissues obtained at immediate autopsy and cultured for 24 h with [2'-^{14}C]NNN metabolized NNN to compounds *1* and *15* (see Fig. 1) by *N*-oxidation and 5'-hydroxylation, respectively, as follows (values in nmol/100 μg DNA): buccal mucosa, 0.2 ± 0.2 and 0.03 ± 0.03; trachea, 0.7

± 0.7 and 0.2 ± 0.1; oesophagus, 0.2 ± 0.2 and 0.4 ± 0.9; bronchus, 0.8 ± 1.1 and 0.9 ± 1.7; peripheral lung, 0.5 ± 0.2 and 0.4 ± 0.7; urinary bladder, 2.4 ± 3.4 and 1.1 ± 1.8. Metabolite *13* (see Fig. 1), formed by 2'-hydroxylation, was detected in only a few explants. The ratio of compounds *1* to *15* was different in human tissues from that in the corresponding tissues of animals (Castonguay *et al.*, 1983b).

Mutagenicity and chromosomal effects

No data were available to the Working Group.

3.3 Case reports and epidemiological studies of carcinogenicity to humans

No data were available to the Working Group.

4. Summary of Data Reported and Evaluation

4.1 Exposure data

N'-Nitrosonornicotine (NNN) has been found in a variety of tobacco products (chewing tobacco, snuff, cigarettes and cigars), in mainstream and sidestream smoke from cigars and cigarettes, in saliva of chewers of betel quid with tobacco and in saliva of oral-snuff users. Some of the NNN in saliva appears to be formed endogenously from nitrite in saliva and tobacco alkaloids. Thus, there is widespread exposure to NNN among users of tobacco products and those exposed to sidestream smoke.

4.2 Experimental data

NNN was tested for carcinogenicity in rats, mice and hamsters by different routes of administration in multiple experiments. Following its oral administration, NNN produced carcinomas of the upper digestive tract, mainly the oesophagus, and of the nasal cavity in rats and nasal-cavity tumours in hamsters. Following its subcutaneous administration, NNN produced primarily tumours of the nasal cavity in rats and tumours of the trachea in hamsters. Intraperitoneal injection produced lung tumours in mice and tumours of the nasal cavity and trachea in hamsters. There was evidence of a dose-response relationship after subcutaneous administration of NNN to rats.

Several metabolites of NNN were tested in mice by intraperitoneal injection, producing lung tumours. NNN-1-*N*-oxide was also tested in rats and hamsters by oral administration; it produced nasal-cavity and oesophageal tumours in rats.

NNN is mutagenic to *Salmonella typhimurium* in the presence of an exogenous metabolic system. It induces unscheduled DNA synthesis in primary cultures of rat hepatocytes.

4.3 Human data

No case report or epidemiological study of the carcinogenicity of NNN was available to the Working Group.

Overall assessment of data from short-term tests: *N'*-Nitrosonornicotine[a]

	Genetic activity			Cell transformation
	DNA damage	Mutation	Chromosomal effects	
Prokaryotes		+		
Fungi/green plants				
Insects				
Mammalian cells (*in vitro*)	+			
Mammals (*in vivo*)				
Humans (*in vivo*)				
Degree of evidence in short-term tests for genetic activity: *Limited*				Cell transformation: No data

[a]The groups into which the table is divided and '+' are defined on pp. 16-17 of the Preamble; the degrees of evidence are defined on p. 18.

4.4 Evaluation[1]

There is *sufficient evidence*[2] for the carcinogenicity of *N'*-nitrosonornicotine to experimental animals.

No data on humans were available.

5. References

Adams, J.D., Brunnemann, K.D. & Hoffmann, D. (1983a) Chemical studies on tobacco smoke. LXXV. Rapid method for the analysis of tobacco-specific *N*-nitrosamines by gas-liquid chromatography with a thermal energy analyzer. *J. Chromatogr.*, *256*, 347-351

Adams, J.D., Lee, S.J., Vinchkoski, N., Castonguay, A. & Hoffmann, D. (1983b) On the formation of the tobacco-specific carcinogen 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone during smoking. *Cancer Lett.*, *17*, 339-346

Adams, J.D., Lee, S.J. & Hoffmann, D. (1984) Carcinogenic agents in cigarette smoke and the influence of nitrate on their formation. *Carcinogenesis*, *5*, 221-223

[1]For description of the italicized term, see Preamble, pp. 15-16.

[2]In the absence of adequate data on humans, it is reasonable, for practical purposes, to regard chemicals for which there is *sufficient evidence* of carcinogenicity in animals, as if they presented a carcinogenic risk to humans.

Andersen, R.A., Kasperbauer, M.J., Burton, H.R., Hamilton, J.L. & Yoder, E.E. (1982) Changes in chemical composition of homogenized leaf-cured and air-cured burley tobacco stored in controlled environments. *J. agric. Food Chem.*, *30*, 663-668

Andrews, A.W., Thibault, L.H. & Lijinsky, W. (1978) The relationship between mutagenicity and carcinogenicity of some nitrosamines. *Mutat. Res.*, *51*, 319-326

Bartsch, H., Malaveille, C., Camus, A.-M., Martel-Planche, G., Brun, G., Hautefeuille, A., Sabadie, N., Barbin, A., Kuroki, T., Drevon, C., Piccoli, C. & Montesano, R. (1980) *Bacterial and mammalian mutagenicity tests: Validation and comparative studies on 180 chemicals*. In: Montesano, R., Bartsch, H. & Tomatis, L., eds, *Molecular and Cellular Aspects of Carcinogen Screening Tests* (*IARC Scientific Publications No. 27*), Lyon, International Agency for Research on Cancer, pp. 179-241

Bharadwaj, V.P., Takayama, S., Yamada, T. & Tanimura, A. (1975) *N'*-Nitrosonornicotine in Japanese tobacco products. *Gann*, *66*, 585-586

Boyland, E., Roe, F.J.C. & Gorrod, J.W. (1964) Induction of pulmonary tumours in mice by nitrosonornicotine, a possible constituent of tobacco smoke. *Nature*, *202*, 1126

Brittebo, E. & Tjälve, H. (1980) Autoradiographic observations on the distribution and metabolism of *N'*-/^{14}C/nitrosonornicotine in mice. *J. Cancer Res. clin. Oncol.*, *98*, 233-242

Brittebo, E.B. & Tjälve, H. (1981) Formation of tissue-bound *N'*-nitrosonornicotine metabolites by the target tissues of Sprague-Dawley and Fisher rats. *Carcinogenesis*, *2*, 959-963

Brittebo, E.B., Castonguay, A., Furuya, K. & Hecht, S.S. (1983) Metabolism of tobacco-specific nitrosamines by cultured rat nasal mucosa. *Cancer Res.*, *43*, 4343-4348

Brunnemann, K.D., Hecht, S.S. & Hoffmann, D. (1983a) *N*-Nitrosamines: Environmental occurrence, in vivo formation and metabolism. *J. toxicol.-clin. Toxicol.*, *19*, 661-688

Brunnemann, K.D., Scott, J.C. & Hoffmann, D. (1983b) *N*-Nitrosoproline, an indicator for *N*-nitrosation of amines in processed tobacco. *J. agric. Food Chem.*, *31*, 905-909

Castonguay, A., Lin, D., Stoner, G.D., Radok, P., Furuya, K., Hecht, S.S., Schut, H.A.J. & Klaunig, J.E. (1983a) Comparative carcinogenicity in A/J mice and metabolism by cultured mouse peripheral lung of *N'*-nitrosonornicotine, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone, and their analogues. *Cancer Res.*, *43*, 1223-1129

Castonguay, A., Stoner, G.D., Schut, H.A.J. & Hecht, S.S. (1983b) Metabolism of tobacco-specific *N*-nitrosamines by cultured human tissues. *Proc. natl Acad. Sci. USA*, *80*, 6694-6697

Castonguay, A., Rivenson, A., Trushin, N., Reinhardt, J., Spathopoulos, S., Weiss, C.J., Reiss, B. & Hecht, S.S. (1984) Effects of chronic ethanol consumption on the metabolism and carcinogenicity of *N'*-nitrosonornicotine in F344 rats. *Cancer Res.*, *44*, 2285-2290

Chamberlain, W.J. & Arrendale, R.F. (1983) An alternate method for the analysis of *N*-nitrosonornicotine in tobacco. *J. agric. Food Chem.*, *31*, 909-911

Chen, C.-H.B., Hecht, S.S. & Hoffmann, D. (1978) Metabolic α-hydroxylation of the tobacco-specific carcinogen, *N*'-nitrosonornicotine. *Cancer Res.*, *38*, 3639-3645

Chen, C.-H.B., Fung, P.T. & Hecht, S.S. (1979) Assay for microsomal α-hydroxylation of *N*'-nitrosonornicotine and determination of the deuterium isotope effect for α-hydroxylation. *Cancer Res.*, *39*, 5057-5062

Chen, C.B., Hecht, S.S., McCoy, G.D. & Hoffmann, D. (1980) *Assays for metabolic α-hydroxylation of* N'*-nitrosonornicotine and* N*-nitrosopyrrolidine and the influence of modifying factors*. In: Walker, E.A., Griciute, L., Castegnaro, M. & Börzsönyi, M., eds, N*-Nitroso Compounds: Analysis, Formation and Occurrence* (*IARC Scientific Publications No. 31*), Lyon, International Agency for Research on Cancer, pp. 349-359

Chung, F.-L., Juchatz, A., Vitarius, J. & Hecht, S.S. (1984) Effects of dietary compounds on α-hydroxylation of *N*-nitrosopyrrolidine and *N*'-nitrosonornicotine in rat target tissues. *Cancer Res.*, *44*, 2924-2928

Egan, H., Preussmann, R., O'Neill, I.K., Eisenbrand, G., Spiegelhalder, B. & Bartsch, H., eds (1983) *Environmental Carcinogens. Selected Methods of Analysis*, Vol. 6, N*-Nitroso Compounds* (*IARC Scientific Publications No. 45*), Lyon, International Agency for Research on Cancer

Hecht, S.S., Ornaf, R.M. & Hoffmann, D. (1975a) Chemical studies on tobacco smoke. XXXIII. *N*'-Nitrosonornicotine in tobacco: Analysis of possible contributing factors and biologic implications. *J. natl Cancer Inst.*, *54*, 1237-1244

Hecht, S.S., Raineri, R., Maronpot, R., Hoffmann, D. & Wynder, E.L. (1975b) *N*'-Nitrosonornicotine: Presence and origin in tobacco and carcinogenicity in rats (Abstract No. 605). *Proc. Am. Assoc. Cancer Res.*, *16*, 152

Hecht, S.S., Chen, C.-H.B., Ornaf, R.M., Hoffmann, D. & Tso, T.C. (1978a) *Chemical studies on tobacco smoke. LVI. Tobacco specific nitrosamines: Origins, carcinogenicity and metabolism*. In: Walker, E.A., Castegnaro, M., Gricute, L. & Lyle, R.E., eds, *Environmental Aspects of* N*-Nitroso Compounds* (*IARC Scientific Publications No. 19*), Lyon, International Agency for Research on Cancer, pp. 395-413

Hecht, S.S., Chen, C.-H.B., Hirota, N., Ornaf, R.M., Tso, T.C. & Hoffmann, D. (1978b) Tobacco-specific nitrosamines: Formation from nicotine *in vitro* and during tobacco curing and carcinogenicity in strain A mice. *J. natl Cancer Inst.*, *60*, 819-824

Hecht, S.S., Chen, C.-H.B., McCoy, G.D., Hoffmann, D. & Domellöf, L. (1979) α-Hydroxylation of *N*-nitrosopyrrolidine and *N*'-nitrosonornicotine by human liver microsomes. *Cancer Lett.*, *8*, 35-41

Hecht, S.S., Chen, C.-H.B. & Hoffmann, D. (1980a) Metabolic β-hydroxylation and *N*-oxidation of *N*'-nitrosonornicotine. *J. med. Chem.*, *23*, 1175-1178

Hecht, S.S., Chen, C.-H.B., Ohmori, T. & Hoffmann, D. (1980b) Comparative carcinogenicity in F344 rats of the tobacco-specific nitrosamines, *N*'-nitrosonornicotine and 4-(*N*-methyl-*N*-nitrosamino)-1-(3-pyridyl)-1-butanone. *Cancer Res.*, *40*, 298-302

Hecht, S.S., Chen, C.-H.B., Young, R. & Hoffmann, D. (1981a) Mass spectra of tobacco alkaloid-derived nitrosamines, their metabolites, and related compounds. *Beitr. Tabakforsch. int.*, *11*, 57-66

Hecht, S.S., Lin, D. & Chen, C.-H.B. (1981b) Comprehensive analysis of urinary metabolites of *N'*-nitrosonornicotine. *Carcinogenesis*, *2*, 833-838

Hecht, S.S., Young, R., Rivenson, A. & Hoffmann, D. (1982a) *On the metabolic activation of* N-*nitrosomorpholine and* N'-*nitrosonornicotine: Effects of deuterium substitution.* In: Bartsch, H., O'Neill, I.K., Castegnaro, M. & Okada, M., eds, N-*Nitroso Compounds: Occurrence and Biological Effects* (*IARC Scientific Publications No. 41*), Lyon, International Agency for Research on Cancer, pp. 499-507

Hecht, S.S., Reiss, B., Lin, D. & Williams, G.M. (1982b) Metabolism of *N'*-nitrosonornicotine by cultured rat esophagus. *Carcinogenesis*, *3*, 453-456

Hecht, S.S., Young, R. & Masura, Y. (1983a) Comparative carcinogenicity in F344 rats and Syrian golden hamsters of *N'*-nitrosonornicotine and *N'*-nitrosonornicotine-1-*N*-oxide. *Cancer Lett.*, *20*, 333-340

Hecht, S.S., Lin, D. & Castonguay, A. (1983b) Effects of α-deuterium substitution on the mutagenicity of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK). *Carcinogenesis*, *4*, 305-310

Hilfrich, J., Hecht, S.S. & Hoffmann, D. (1977) A study of tobacco carcinogenesis. XV. Effects of *N'*-nitrosonornicotine and *N'*-nitrosoanabasine in Syrian golden hamsters. *Cancer Lett.*, *2*, 169-175

Hoffmann, D. & Adams, J.D. (1981) Carcinogenic tobacco-specific *N*-nitrosamines in snuff and in the saliva of snuff dippers. *Cancer Res.*, *41*, 4305-4308

Hoffmann, D. & Hecht, S.S. (1983) *Tobacco and tobacco smoke (volatile and tobacco-specific nitrosamines). General aspects.* In: Egan, H., Preussmann, R., O'Neill, I.K., Eisenbrand, G., Spiegelhalder, B. & Bartsch, H., eds, *Environmental Carcinogens. Selected Methods of Analysis,* Vol. 6, N-*Nitroso Compounds (IARC Scientific Publications No. 45*), Lyon, International Agency for Research on Cancer, pp. 63-67

Hoffmann, D., Hecht, S.S., Ornaf, R.M. & Wynder, E.L. (1974) *N'*-Nitrosonornicotine in tobacco. *Science*, *186*, 265-267

Hoffmann, D., Raineri, R., Hecht, S.S., Maronpot, R. & Wynder, E.L. (1975) A study of tobacco carcinogenesis. XIV. Effects of *N'*-nitrosonornicotine and *N'*-nitrosoanabasine in rats. *J. natl Cancer Inst.*, *55*, 977-981

Hoffmann, D., Hecht, S.S., Ornaf, R.M., Wynder, E.L. & Tso, T.C. (1976) *Chemical studies on tobacco smoke. XLII. Nitrosonornicotine: Presence in tobacco, formation and carcinogenicity.* In: Walker, E.A., Bogovski, P. & Griciute, L., eds, *Environmental* N-*Nitroso Compounds. Analysis and Formation* (*IARC Scientific Publications No. 14*), Lyon, International Agency for Research on Cancer, pp. 307-320

Hoffmann, D., Adams, J.D., Brunnemann, K.D. & Hecht, S.S. (1979) Assessment of tobacco-specific *N*-nitrosamines in tobacco products. *Cancer Res.*, *39*, 2505-2509

Hoffmann, D., Chen, C.-H.B. & Hecht, S.S. (1980a) *The role of volatile and nonvolatile* N-*nitrosamines in tobacco carcinogenesis*. In: Gori, G.B. & Bock, F.G., eds, *A Safe Cigarette?* (*Banbury Report No. 3*), Cold Spring Harbor, NY, Cold Spring Harbor Laboratory, pp. 113-127

Hoffmann, D., Adams, J.D., Piade, J.J. & Hecht, S.S. (1980b) *Chemical studies on tobacco smoke. LXVIII. Analysis of volatile and tobacco-specific nitrosamines in tobacco products*. In: Walker, E.A., Castegnaro, M., Griciute, L. & Börzsönyi, M., eds, N-*Nitroso Compounds: Analysis, Formation and Occurrence* (*IARC Scientific Publications No. 31*), Lyon, International Agency for Research on Cancer, pp. 507-516

Hoffmann, D., Castonguay, A., Rivenson, A. & Hecht, S.S. (1981) Comparative carcinogenicity and metabolism of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone and *N*'-nitrosonornicotine in Syrian golden hamsters. *Cancer Res.*, *41*, 2386-2393

Hoffmann, D., Adams, J.D., Brunnemann, K.D., Rivenson, A. & Hecht, S.S. (1982) *Tobacco specific* N-*nitrosamines: Occurrence and bioassays*. In: Bartsch, H., O'Neill, I.K., Castegnaro, M. & Okada, M., eds, N-*Nitroso Compounds: Occurrence and Biological Effects* (*IARC Scientific Publications No. 41*), Lyon, International Agency for Research on Cancer, pp. 309-318

Hoffmann, D., Rivenson, A., Amin, S. & Hecht, S.S. (1984) Dose-response study of the carcinogenicity of tobacco-specific *N*-nitrosamines in F344 rats. *J. Cancer Res. clin. Oncol.*, *108*, 81-86

Hu, M.W., Bondinell, W.E. & Hoffmann, D. (1974) Chemical studies on tobacco smoke. XXIII. Synthesis of carbon-14 labelled myosmine, nornicotine and N'-nitrosonornicotine. *J. labelled Compd*, *10*, 79-88

IARC (1978) *IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans*, Vol. 17, *Some* N-*Nitroso Compounds*, Lyon, pp. 281-286

Klimisch, H.J. & Stadler, L. (1976) The formation of *N*-nitrosonornicotine from nicotine-*N*'-oxide (Ger.). *Talanta*, *23*, 614-616

Klus, H. & Kuhn, H. (1975) Studies on the nonvolatile *N*-nitrosamines of the tobacco alkaloids (Ger.). *Fachl. Mitt. Austria Tabakwerke*, *16*, 307-317

Löfberg, B., Brittebo, E.B. & Tjälve, H. (1982) Localization and binding of *N*'-nitrosonornicotine metabolites in the nasal region and in some other tissues of Sprague-Dawley rats. *Cancer Res.*, *42*, 2877-2883

McCoy, G.D., Hecht, S.S., Katayama, S. & Wynder, E.L. (1981a) Differential effect of chronic ethanol consumption on the carcinogenicity of *N*-nitrosopyrrolidine and *N*'-nitrosonornicotine in male Syrian golden hamsters. *Cancer Res.*, *41*, 2849-2854

McCoy, G.D., Chen, C.-H.B. & Hecht, S.S. (1981b) Influence of mixed-function oxidase inducers on the in vitro metabolism of *N*'-nitrosonornicotine by rat and hamster liver microsomes. *Drug Metab. Disposition*, *9*, 168-169

McCoy, G., Katayama, S., Young, R., Wyatt, M. & Hecht, S.S. (1982) *Influence of chronic ethanol consumption on the metabolism and carcinogenicity of tobacco-related nitros-*

amines. In: Bartsch, H., O'Neill, I.K., Castegnaro, M. & Okada, M., eds, N-*Nitroso Compounds: Occurrence and Biological Effects* (*IARC Scientific Publications No. 41*), Lyon, International Agency for Research on Cancer, pp. 635-642

Munson, J.W. & Abdine, H. (1977) Determination of *N*-nitrosonornicotine in tobacco by gas chromatography/mass spectroscopy. *Anal. Lett.*, *10*, 777-786

Nair, J., Ohshima, H., Friesen, M., Croisy, A., Bhide, S.V. & Bartsch, H. (1985) Tobacco-specific and betel nut-specific *N*-nitroso compounds: Occurrence in saliva and urine of betel quid chewers and formation *in vitro* by nitrosation of betel quid. *Carcinogenesis, 6,* 295-303

Neurath, G. & Duenger, M. (1966) *N*-Nitroso compounds from tobacco alkaloids (Ger.). *Beitr. Tabakforsch. int.*, *3*, 339-345 [*Chem. Abstr.*, *67*, 64605s]

Rühl, C., Adams, J.D. & Hoffmann, D. (1980) Chemical studies on tobacco smoke. LXVI. Comparative assessment of volatile and tobacco-specific *N*-nitrosamines in the smoke of selected cigarettes from the USA, West Germany and France. *J. anal. Toxicol.*, *4*, 255-259

Schweinsberg, F., Sander, J., Schweinsberg, E. & Kollat, P. (1975) Possibility of nitrosation of nicotine and nornicotine and of the formation of *N*-nitrosonornicotine in the stomach of smokers (Ger.). *Z. Krebsforsch.*, *84*, 81-87

Singer, G.M. & Taylor, H.W. (1976) Carcinogenicity of *N'*-nitrosonornicotine in Sprague-Dawley rats. *J. natl Cancer Inst.*, *57*, 1275-1276

Sipahimalani, A.T., Chadha, M.S., Bhide, S.V., Pratap, A.I. & Nair, J. (1984) Detection of *N*-nitrosamines in the saliva of habitual chewers of tobacco. *Food Chem. Toxicol.*, *22*, 261-264

US Department of Health and Human Services (1981) *The Health Consequences of Smoking, The Changing Cigarette, A Report of the Surgeon General* (*DHHS(PHS)81-50156*), Rockville, MD, Public Health Service, Office on Smoking and Health, p. 34

US Department of Health and Human Services (1982) *The Health Consequences of Smoking, Cancer, A Report of the Surgeon General* (*DHHS(PHS)82-50179*), Rockville, MD, Public Health Service, Office on Smoking and Health, p. 208

Waddell, W.J. & Marlowe, C. (1980) Localization of [^{14}C]nitrosonornicotine in tissues of the mouse. *Cancer Res.*, *40*, 3518-3523

Waddell, W.J. & Marlowe, C. (1983) Inhibition by alcohols of the localization of radioactive nitrosonornicotine in sites of tumor formation. *Science*, *221*, 51-53

Wenke, G., Brunnemann, K.D., Hoffmann, D. & Bhide, S.V. (1984) A study of betel quid carcinogenesis. IV. Analysis of the saliva of betel chewers: A preliminary report. *J. Cancer Res. clin. Oncol.*, *108*, 110-113

Williams, G.M. & Laspia, M.F. (1979) The detection of various nitrosamines in the hepatocyte primary culture/DNA repair test. *Cancer Lett.*, *6*, 199-206

SOME *N*-NITROSAMINES DERIVED FROM ARECA-NUT ALKALOIDS

1. Chemical and Physical Data

1.1 Synonyms and structural and molecular formulae

Chemical name [Chem. Abstr. Services Reg. No.]	Chem. Abstr. Name [Synonym] IUPAC Systematic Name	Structural and molecular formulae and molecular weight
3-Methylnitrosaminopro- pionaldehyde [85502-23-4]	Propanal, 3-(methylnitrosoamino)- [MNPA] 3-(Methylnitrosamino)propion- aldehyde	$CH_3-N(N=O)-CH_2CH_2CHO$ $C_4H_8N_2O_2$ Mol. wt: 116.1
3-Methylnitrosaminopropionitrile [60153-49-3]	Propanenitrile, 3-(methylnitroso- amino)-[MNPN] 3-(Methylnitrosamino)propionitrile	$CH_3-N(N=O)-CH_2CH_2C\equiv N$ $C_4H_7N_3O$ Mol. wt: 113.1
N-Nitrosoguvacine [55557-01-2]	3-Pyridinecarboxylic acid, 1,2,5,6-tetrahydro-1-nitroso- [NGC; nitrosoguvacine] 1,2,5,6-Tetrahydro-1-nitroso- nicotinic acid	C(=O)–OH; N–N=O $C_6H_8N_2O_3$ Mol. wt: 156.1
N-Nitrosoguvacoline [55557-02-3]	3-Pyridinecarboxylic acid, 1,2,5,6-tetra-hydro-1-nitroso-, methyl ester [NG; NGL; nitrosoguvacoline] Methyl 1,2,5,6-tetra-hydro-1- nitrosonicotinate	C(=O)–OCH$_3$; N–N=O $C_7H_{10}N_2O_3$ Mol. wt: 170.2

1.2 Chemical and physical properties[a]

Chemical/physical property	MNPA	MNPN	NGC	NGL
(*a*) Description	No data	Light-yellow liquid (Chang *et al.*, 1976)	Colourless, crystalline solid (Lijinsky & Taylor, 1976)	Yellow oil (Lijinsky & Taylor, 1976)
(*b*) Melting-point	No data	No data	175.5-177°C (Lijinsky & Taylor, 1976)	No data
(*c*) Boiling-point	No data	102-103°C (0.04 mm Hg) (Chang *et al.*, 1976); 97°C (0.075 mm Hg) (Wenke & Hoffmann, 1983)	No data	137-178°C (4 mm) (Lijinsky & Taylor, 1976)
(*d*) Spectroscopy data	NMR and MS data have been reported (Wenke & Hoffmann, 1983)	IR, NMR and MS data have been reported (Chang *et al.*, 1976; Wenke & Hoffmann, 1983)	MS data have been reported (Rainey *et al.*, 1978)	MS data have been reported (Rainey *et al.*, 1978)
	Synthetic MNPA is a mixture of E- and Z-isomers in a ratio of 1.4 (Wenke & Hoffmann, 1983)	Synthetic MNPN is a mixture of E- and Z-isomers in a ratio of 1.7 (Wenke & Hoffmann, 1983)		Synthetic NGL is a mixture of E- and Z-isomers in a ratio of 2.5 (Wenke & Hoffmann, 1983)

[a]Abbreviations: MNPA, 3-methylnitrosaminopropionaldehyde; MNPN, 3-methylnitrosaminopropionitrile; NGC, *N*-nitrosoguvacine; NGL, *N*-nitrosoguvacoline; NMR, [1]H-nuclear magnetic resonance; MS, mass spectrometry; MS, mass spectrometry; IR, infrared

2. Production, Use, Occurrence and Analysis

2.1 Production and use

3-Methylnitrosaminopropionaldehyde was prepared by Wenke and Hoffmann (1983) by the reaction of 3-methylaminopropionaldehyde diethyl acetal with nitrite, followed by hydrolysis. 3-Methylnitrosaminopropionitrile was prepared by Chang *et al.* (1976) by the reaction of sodium nitrite with a solution of 3-methylaminopropionitrile hydrochloride. *N*-Nitrosoguvacine was prepared by Lijinsky and Taylor (1976) by nitrosation of guvacine that was synthesized from 3-carbethoxy-4-piperidone by hydrogenation to the piperidonol, followed by dehydration and de-esterification with hydrogen chloride gas at 200°C, followed by esterification with diazomethane. *N*-Nitrosoguvacoline was also prepared by Lijinsky and Taylor (1976) using the same process as that for *N*-nitrosoguvacine.

In in-vitro experiments, *N*-nitrosation of arecoline, the major alkaloid of the areca nut, resulted in the formation of *N*-nitrosoguvacoline, 3-methylnitrosaminopropionitrile and 3-methylnitrosaminopropionaldehyde (Wenke & Hoffmann, 1983).

No evidence was found that any of these compounds has ever been produced in commercial quantities or has any use other than as a laboratory chemical.

2.2 Occurrence

N-Nitrosoguvacoline was found in the saliva of chewers of betel quid with tobacco at levels of 4.3-350 ng/g (mean, 75 ng/g) (Wenke *et al.*, 1984a) and up to 7.1 ng/ml (mean, 0.9 ng/ml) (Nair *et al.*, 1985); it has been found in the saliva of chewers of betel quid alone at levels of 2.2-9.5 ng/g (mean, 5.6 ng/g) (Wenke *et al.*, 1984a) and up to 5.9 ng/ml (mean, 0.9 ng/ml] (Nair *et al.*, 1985) and in the saliva of cigarette smokers at levels of 1.2-7.6 ng/g (Wenke *et al.*, 1984a).

N-Nitrosoguvacine was found in the saliva of chewers of betel quid with tobacco at levels of up to 30.4 ng/ml (mean, 4.0 ng/ml) and in the saliva of chewers of betel quid alone at levels of up to 26.6 ng/ml (mean, 3.2 ng/ml) (Nair *et al.*, 1985).

In-vitro experiments support the hypothesis that, during the chewing of betel quid, nitrosation of arecoline, the major alkaloid of areca nut, produces *N*-nitrosoguvacoline, 3-methylnitrosaminopropionitrile and 3-methylnitrosaminopropionaldehyde. It was concluded that the conditions prevailing in the oral cavity of betel-quid chewers are likely to favour the formation of these three compounds (Wenke & Hoffmann, 1983; Wenke *et al.*, 1984b).

2.3 Analysis

Wenke and Hoffmann (1983) reported a method for analysis of 3-methylnitrosaminopropionitrile, 3-methylnitrosaminopropionaldehyde and *N*-nitrosoguvacoline by a technique using gas chromatography with a thermal-energy analyser.

3. Biological Data Relevant to the Evaluation of Carcinogenic Risk to Humans

3.1 Carcinogenicity studies in animals

(a) 3-Methylnitrosaminopropionaldehyde

No data on the carcinogenicity of 3-methylnitrosaminopropionaldehyde were available to the Working Group.

(b) 3-Methylnitrosaminopropionitrile

Subcutaneous and/or intramuscular administration: A group of 15 male and 15 female Fischer 344 *rats*, seven weeks of age, received thrice-weekly s.c. injections of 2.13 mg 3-methylnitrosaminopropionitrile (MNPN) (purity, >99%, as determined by high-performance liquid chromatography) in 0.3 ml saline for 20 weeks (total dose, 129 mg/rat or 646 mg/kg bw). A group of 12 males and 12 females served as vehicle controls. The experiment was terminated after 24 weeks because of significant weight loss in the treated animals. Statistically significant increases in tumour incidence were observed for the following neoplasms: (i) papillomas of the oesophagus in 12/15 treated males ($p < 0.01$) and 14/15 treated females ($p < 0.01$); 3/15 males and 2/15 females treated with MNPN also developed carcinomas of the oesophagus (significant at $p < 0.05$ for both sexes combined); (ii) papillomas of the nasal cavity in 11/15 treated males ($p < 0.01$) and 9/15 treated females ($p < 0.01$); (iii) papillomas and carcinomas of the tongue in 5/15 treated males ($p < 0.05$) and 6/15 treated females ($p < 0.05$). No tumour was seen in controls (Wenke *et al.*, 1984).

(c) N-*Nitrosoguvacine*

No data on the carcinogenicity of *N*-nitrosoguvacine were available to the Working Group.

(d) N-*Nitrosoguvacoline*

Oral administration: A group of 15 male and 15 female Sprague-Dawley *rats*, eight to 10 weeks of age, were given drinking-water containing 150 mg/l *N*-nitrosoguvacoline (no impurity detected by silica-gel thin-layer chromatography) on five days per week for 50 weeks (total dose, 750 mg/rat). All animals survived to the end of treatment and were observed until death or 133 weeks. At 100 weeks, 12 males and nine females were still alive; the four survivors were killed at 133 weeks. Thirty female and 26 male rats served as untreated [matched or historical unspecified] controls. No statistically significant increase in tumour incidence was found (Lijinsky & Taylor, 1976). [The Working Group noted the short duration of the treatment and the small number of animals used.]

3.2 Other relevant biological data

(a) Experimental systems

No data were available to the Working Group on toxic effects, on effects on reproduction and prenatal toxicity, or on absorption, distribution, excretion and metabolism.

Mutagenicity and other short-term tests

N-*Nitrosoguvacine* was not mutagenic to *Salmonella typhimurium* TA1535, the only strain tested, at doses of up to 600 μg/plate (3.8 μmol/plate) in the presence or absence of a liver microsomal preparation (S9) from non-induced or from Aroclor- or phenobarbital-induced rats (Rao *et al.*, 1977).

N-*Nitrosoguvacoline* caused a dose-dependent increase [at doses of 200-600 μg/plate (1.2-3.6 μmol/plate)] in mutations in *S. typhimurium* TA1535 in the presence of S9 from Aroclor- or phenobarbital-induced rats, the former being more active. No mutagenicity was observed in the absence of S9 or in the presence of S9 from uninduced rats (Rao *et al.*, 1977, 1978).

Concentrations of 10 and 20 mM N-*nitrosoguvacoline* fed to adult *Drosophila melanogaster* for two to three days did not induce sex-linked recessive lethal mutations in mature sperm or spermatids (Nix *et al.*, 1979).

No data were available on the mutagenicity of 3-methylnitrosaminopropionitrile or 3-methylnitrosaminopropionaldehyde.

(*b*) *Humans*

No data were available to the Working Group.

3.3 Case reports and epidemiological studies of carcinogenicity in humans

No data were available to the Working Group.

4. Summary of Data Reported and Evaluation

4.1 Exposure data

N-Nitrosoguvacoline and *N*-nitrosoguvacine have been found in the saliva of betel-quid chewers. Thus, there is some evidence that chewers are exposed to these compounds.

4.2 Experimental data

N-Nitrosoguvacoline was tested in one experiment in rats by administration in drinking-water. Although no increase in the incidence of tumours was detected, the experiment was not adequate to allow an evaluation of its carcinogenicity.

3-Methylnitrosaminopropionitrile was tested in one 24-week experiment in rats by subcutaneous injection. Within this short period, it produced multiple papillomas and carcinomas of the oesophagus and tongue, and papillomas of the nasal cavity.

No data were available to evaluate the carcinogenicity of *N*-nitrosoguvacine or 3-methylnitrosaminopropionaldehyde to experimental animals.

The one available study was inadequate to evaluate the mutagenicity of *N*-nitrosoguvacine to *Salmonella typhimurium*.

N-Nitrosoguvacoline was mutagenic to *Salmonella typhimurium* in the presence of a metabolic system. It did not induce sex-linked recessive lethal mutations in *Drosophila melanogaster*.

No data were available to assess the mutagenic or related effects of 3-methylnitrosaminopropionitrile or 3-methylnitrosaminopropionaldehyde.

Overall assessment of data from short-term tests: *N*-nitrosoguvacoline[a]

	Genetic activity			Cell transformation
	DNA damage	Mutation	Chromosomal effects	
Prokaryotes	+			
Fungi/green plants				
Insects		–		
Mammalian cells (*in vitro*)				
Mammals (*in vivo*)				
Humans (*in vivo*)				
Degree of evidence in short-term tests for genetic activity: *Inadequate*				Cell transformation: No data

[a]The groups into which the table is divided and '+' and '–' are defined on pp. 16-17 of the Preamble; the degrees of evidence are defined on p. 18.

4.3 Human data

No case report or epidemiological study of the carcinogenicity of these compounds to humans was available to the Working Group.

4.4 Evaluation[1]

There is *sufficient evidence*[2] for the carcinogenicity of 3-methylnitrosaminopropionitrile to experimental animals. There is *inadequate* evidence to evaluate the carcinogenicity of *N*-nitrosoguvacoline to experimental animals. No data were available to assess the carcinogenicity of *N*-nitrosoguvacine or 3-methylnitrosaminopropionaldehyde to experimental animals.

No data on humans were available.

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[1]For description of the italicized term, see Preamble, pp. 15-16.

[2]In the absence of adequate data on humans, it is reasonable, for practical purposes, to regard chemicals for which there is *sufficient evidence* of carcinogenicity in animals as if they presented a carcinogenic risk to humans.

5. References

Chang, S.K., Harrington, G.W., Veale, H.S. & Swern, D. (1976) The unusually mild and facile basic hydrolysis of *N*-nitroso-2-(methylamino)acetonitrile. *J. org. Chem.*, *41*, 3752-3755

Lijinsky, W. & Taylor, H.W. (1976) Carcinogenicity test of two unsaturated derivatives of *N*-nitrosopiperidine in Sprague-Dawley rats. *J. natl Cancer Inst.*, *57*, 1315-1317

Nair, J., Ohshima, H., Friesen, M., Croisy, A., Bhide, S.V. & Bartsch, H. (1985) Tobacco-specific and betel-nut-specific *N*-nitroso compounds: Occurrence in saliva and urine of betel quid chewers and formation *in vitro* by nitrosation of betel quid. *Carcinogenesis*, *6*, 295-303

Nix, C.E., Brewen, B., Wilkerson, R., Lijinsky, W. & Epler, J.L. (1979) Effects of *N*-nitrosopiperidine substitutions on mutagenicity in *Drosophila melanogaster*. *Mutat. Res.*, *67*, 27-38

Rainey, W.T., Christie, W.H. & Lijinsky, W. (1978) Mass spectrometry of *N*-nitrosamines. *Biochem. Mass Spectrometry*, *5*, 395-408 [*Chem. Abstr.*, *90*, 86151t]

Rao, T.K., Hardigree, A.A., Young, J.A., Lijinsky, W. & Epler, J.L. (1977) Mutagenicity of *N*-nitrosopiperidines with *Salmonella typhimurium*/microsomal activation system. *Mutat. Res.*, *56*, 131-145

Rao, T.K., Hardigree, A.A., Young, J.A., Winton, W. & Epler, J.L. (1978) Mutagenicity of *N*-nitrosopiperidines: in vitro studies with the *Salmonella typhimurium* system (Abstract No. 2). *Mutat. Res.*, *53*, 66

Wenke, G. & Hoffmann, D. (1983) A study of betel quid carcinogenesis. 1. On the in vitro *N*-nitrosation of arecoline. *Carcinogenesis*, *4*, 169-172

Wenke, G., Brunnemann, K.D., Hoffmann, D. & Bhide, S.V. (1984a) A study of betel quid carcinogenesis. IV. Analysis of the saliva of chewers. A preliminary report. *J. Cancer Res. clin. Oncol.*, *108*, 110-113

Wenke, G., Rivenson, A. & Hoffmann, D. (1984b) A study of betel quid carcinogenesis. III. 3-(Methylnitrosamino)propionitrile, a powerful carcinogen in F344 rats. *Carcinogenesis*, *5*, 1137-1140

SUPPLEMENTARY CORRIGENDA TO VOLUMES 1-36

Corrigenda covering Volumes 1-6 appeared in Volume 7; others appeared in Volumes 8, 10-13 and 15-35.

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p. 94	Table 3	*replace* (mg/kg) *by* (μg/kg)
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Numbers in italics indicate volume, and other numbers indicate page. References to corrigenda are given in parentheses. Compounds marked with an asterisk(*) were considered by the working groups in the year indicated, but monographs were not prepared becaused adequate data on carcinogenicity were not available.

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