

COMMITTEE ON CARCINOGENICITY OF CHEMICALS IN FOOD, CONSUMER PRODUCTS  
AND THE ENVIRONMENT**BETEL QUID, PAN MASALA AND ARECA NUT CHEWING***Introduction*

1. The Ethnic Minorities and Refugees section of the Department's Health Promotion Division has requested the advice of the Committee on Carcinogenicity on the potential carcinogenicity of products containing Areca nut (betel nut). An educational video has been commissioned on the health effects of chewing products containing Areca nut and the Department would like the Committee's views so that any statements or warnings concerning health effects have sound scientific backing.

2. The Areca nut chewing habit is prevalent in a variety of different forms throughout the Asian subcontinent and South-east Asia and in Papua New Guinea and is also carried out by migrant communities of Indians living in South Africa, Uganda and elsewhere. There have been several reviews of regional variations in the habit (1,2,3), including a section in the 1985 IARC Monograph, which is attached at Appendix 1. In most parts of Asia, the major traditional form of the habit is the chewing of betel quid or pan, the main ingredients of which are Areca nuts (betel nut), betel leaf, slaked lime and catechu. Chopped Areca nuts, the fruit of the Areca catechu tree, which may be used fresh, or after sun drying or curing, are wrapped in a betel leaf (Piper betle L.) previously smeared with a paste of catechu (the resinous extract from the matrix of the Acacia tree, usually A. catechu or A. suma) and slaked lime (from sea shells or limestone). Finely cut tobacco is often added and depending on regional custom and personal preferences, spices such as cardamom, cloves, grated fresh coconut, ginger and sugar may also be added. The leaf is folded into a quid, which is put into the mouth and chewed and/or sucked. Later the quid may be swallowed or spat out, particularly if it contains tobacco. The quid is chewed and kept compressed against the buccal mucosa for varying periods of time ranging from 5 to 60 minutes; in some cases the quid is retained in the mouth overnight while sleeping (22). As many as 5 to 15 quids may be consumed per day. Variations on this basic habit occur throughout Asia and South-east Asia. The habit is practised differently in Papua New Guinea, where tobacco is not added. Ripe but uncured Areca nut is chewed, usually with leaves, seed and part of the stem of the Piper betel, and slaked lime added, using a moistened stick or the Piper betel inflorescence, after a minute or so. Ready-made preparations of betel quids are sold in Indian shops and markets, wrapped in aluminium foil and containing a betel leaf together with the various basic ingredients, often together with added colouring agents.

3. The prevalence of betel quid chewing (with or without added tobacco) has been reported to be declining in India in recent years but is being replaced by the use of **pan masala**, a commercial preparation containing all the ingredients of betel quid except betel leaf (4,5,6). Constituents vary depending on the particular brand but typically include 70-80% of chopped or powdered Areca nut by weight, together with lime, catechu, spices and flavourings and may also include tobacco. These products are apparently being aggressively marketed in India, where they have been regarded as a "safe" alternative to other chewing habits, and, unlike betel chewing, have a high degree of social acceptance and are used by women and children as well as men. Pan masala is usually consumed in toto, without spitting.

4. Areca nut may also be consumed on its own, either chewed as raw nuts or after processing by boiling, soaking or roasting. In Taiwan, Areca nut is chewed alone or in combination with slaked lime and in Guam, the Areca nut is chewed on its own or with betel leaf but no lime is added. **Supari**, a processed preparation of Areca nut with or without flavourings, spices and sweeteners is commercially available and may be sucked or chewed on its own or in combination with a quid. The saliva is not usually expectorated during Areca nut chewing, giving exposure to the upper digestive tract as well as the mouth.

5. Following recent legislation, both pan masala and supari on sale in India now carry a statutory health warning and steps are being taken to ban advertisements for these products on the government-controlled television network and to discourage their sale in government run stores, supermarkets and educational institutions (7). Areca-nut containing products on sale in the UK which contain tobacco carry the same warning label as other tobacco products but those preparations not containing tobacco are currently unlabelled.

6. The prevalence of consumption of Areca nut-containing products in the UK is not clear but the number of "pan" shops selling Areca nut products is said to be increasing. A study conducted in 1986 in the major stores in Glasgow catering for the Indian/Pakistani population found that neither specialised smoking equipment and tobacco products nor chewing forms of tobacco were on sale but betel quid constituents were widely available. Based on this survey, estimates of total sales of these products per annum in Scotland were made as follows: betel leaf 800 kg; Areca nut 700 kg; "mixture" (areca nut, fennel seeds, sugar, cucumber seeds, cloves, cardamom, saccharin, menthol and colouring) 450 kg; lime 200 kg; catechu 125 kg. When 150 individuals (75 men and 75 women) visiting these stores were questioned as to their betel chewing habits, 8.7% reported chewing betel quids regularly; all of these individuals had been born abroad.

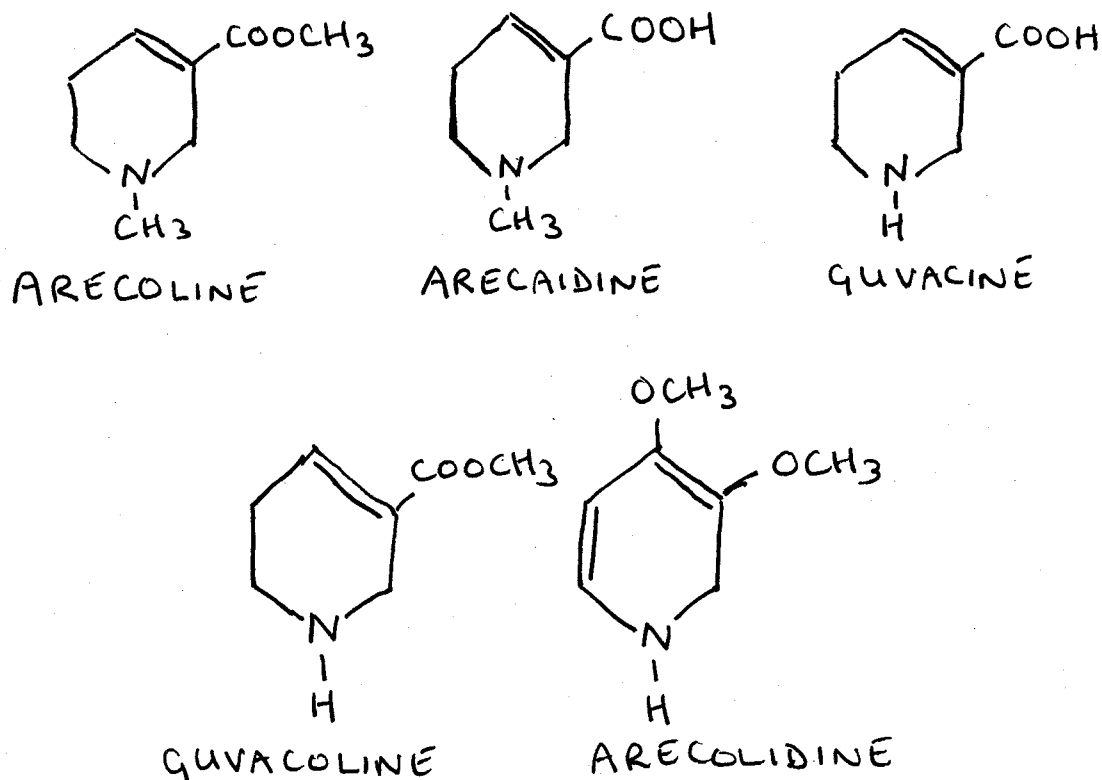
#### *Advice from COC*

7. The Committee on Carcinogenicity has already issued clear advice on the increased risk of oral epithelial cancer associated with the use of non-smoked tobacco products (statement on oral snuff, Annex B to CC/91/1) and no further consideration of tobacco-containing products is considered necessary. The Department would however like the Committee to comment on whether there is sufficient evidence to advise that chewing of betel quid and/or other Areca-nut containing preparations *without tobacco* is likely to result in an increased risk of oral or other cancers.

**ARECA NUT**

8. Unripe or ripe Areca nuts may be used and the nuts may be sundried and/or processed by curing. The curing process usually consists of boiling the sliced or whole shelled nuts in water to which water left from the previous year's curing is added (1). The major constituents of uncured Areca nuts are carbohydrates (47.2-84.5%), lipids (1.3-17%), proteins (4.9-9.3%) and tannins (11.4-26.0%). Among the polyphenols identified are leucocyanidins, catechin, 3,4-flavandiols and hexahydroxyflavan. Fungal spoilage, which may occur to a significant extent, can increase the concentration of polyphenols (10).

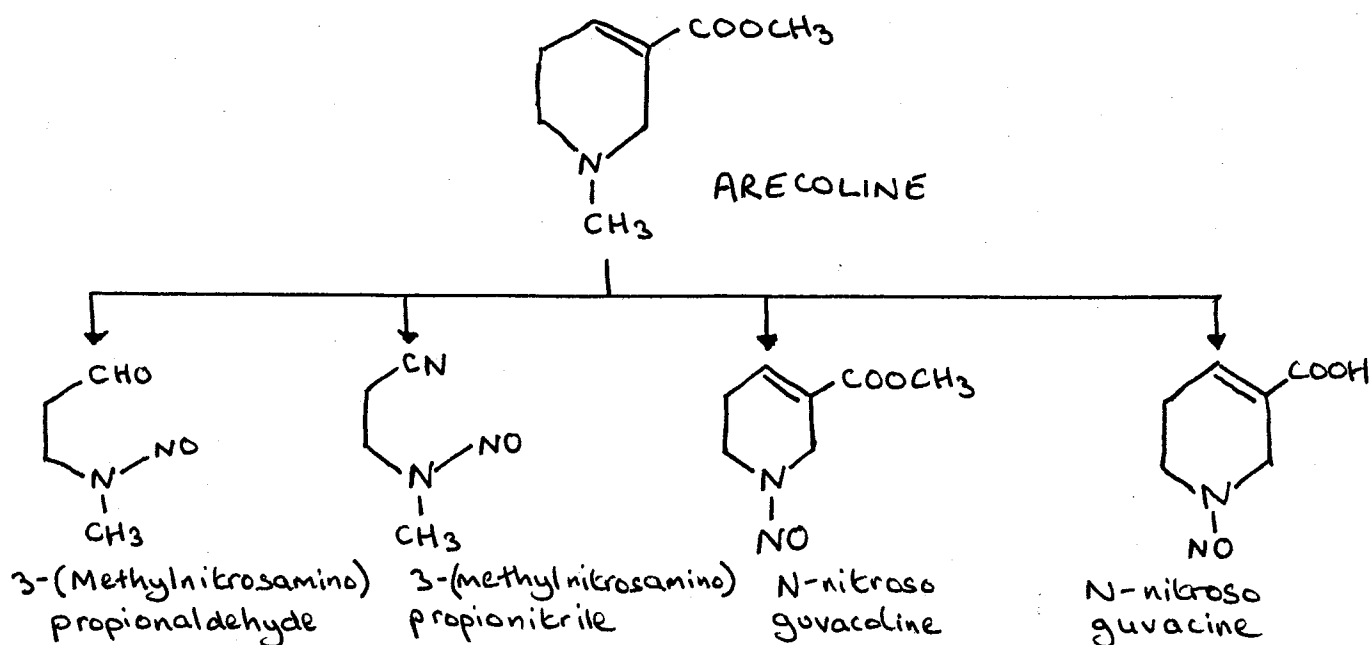
9. The main pharmacological action of Areca nuts is attributed to the areca nut alkaloids arecoline, arecaidine, arecolidine, guvacine and guvacoline, which have the following structures:-



The areca nut alkaloids make up 0.15-0.67% of uncured nuts, with arecoline accounting for 0.07-0.5%. After curing, arecoline content varies from 0.1-0.9%. A study comparing arecoline levels in commercial samples from Bombay detected levels of 0-1.4% (mean 0.5%) in sundried nuts; 0.4-1.3% (mean 0.8%) in boiled nuts; and 0.4-1.3% (mean 0.9%) in roasted nuts (9). Levels of 123 to 200 ug/g arecoline were detected in an aqueous extract of betel quid without tobacco purchased in Bombay (12).

## Nitrosation of Areca nut alkaloids

10. In vitro experiments have shown that nitrosation of arecoline occurs readily, giving rise to at least four N-nitrosocompounds:- N-nitrosoguvacoline (NGCO), N-nitrosoguvacine (NGCI), 3-(methylnitrosamino)propionitrile (MNPN) and 3-(methylnitrosamino)propionaldehyde (MNPA) (11,12,18)). NGCO, MNPN and MNPA were detected following in vitro nitrosation of arecoline under various conditions (ph 3 to 7, for up to 5 days) (11). In vitro nitrosation of an aqueous extract of betel quid under mild conditions designed to simulate those occurring in saliva (pH 7.4 for 1 hour) or the stomach (pH 2.1) revealed the production of NGCO and NGCI, but not MNPN or MNPA (12). N-nitrosoguvacoline can also be formed by nitrosation of guvacoline and N-nitrosoguvacine can result from nitrosation of guvacine or arecaidine (see below).



### BETEL LEAF

11. The mature green leaves of Piper betel L. (Piperaceae) contain volatile oils, including eugenol, chavicol and terpenes, tannins, sugar, starch and nitrate (1). The dry leaves contain 0.2-1.0% volatile oils, chavibetol, chavicol, cadinene and allyl pyrocatechol.

### CATECHU

12. Catechu is the residue of a hot-water extraction of the heart-wood of Acacia catechu (Leguminosae)(1). It contains mainly tannin and polyphenols - 25-35% catechutannic acid, 2-10% catechin-, catechu red, quercetin and gum. Individual polyphenols such as kaempferol, dihydroxykaempferol, taxifolin, isorhamnetin, (+)afzechin and dimeric procyanidin have also been identified. On cooling, catechin crystallises out, leaving the more soluble catechutannic acid in solution. In Malaysia, catechu (also known as gambir) is prepared as an aqueous extract of the twigs and leaves of the shrub Uncaria gambir (Rubiaceae).

## LIME

13. Two types of lime are used; "shell lime" prepared from powdered sea shells and "stone lime" from quarried limestone. In both cases mixture with water produces slaked lime or calcium hydroxide, which can release free alkaloidal bases from their esters and also results in the hydrolysis of arecoline to arecaidine (2).

### Metabolic studies

14. Arecoline is de-esterified in the liver of rats and both arecoline and arecaidine are excreted in urine as the mercapturic acid N-acetyl-S-(3-carboxy-1-methylpiperid-4-yl)-L-cysteine (1). Arecaidine reacts slowly with cysteine but there is no detectable reaction with nucleic acids in vitro (1).

15. N-nitrosoguvacine and N-nitrosoguvacoline are metabolised in the rat to N-nitrosonipecotic acid (NNIP), with 65-85% of an oral dose of 50 or 500ug/rat excreted in the urine as NNIP and 2-8% as N-nitrosoguvacine (13). NNIP was also detected in faeces, at levels of 2-3%.

16. Arecoline has been detected in the saliva of chewers of betel quid without tobacco at levels of 0-89.89 ug/ml (mean 29.69 ug/ml) (12) and 3.4-141.9 ug/ml (18).

17. N-nitrosoguvacoline has been found in the saliva of chewers of Indian betel quid without tobacco, at levels of 2.2-9.5 ng/g (mean 5.6 ng/g) (14). MNPN and MNPA were not detected (limit of detection not stated). In another study, saliva from chewers of betel quid without tobacco contained NGCO at levels of 0.6-8.8 ng/ml (18).

18. N-nitrosoguvacoline and N-nitrosoguvacine were detected in the saliva of chewers of Indian betel quid without tobacco, at levels of up to 5.9 ng/ml (mean 0.91 ng/ml) and up to 26.6 ng/ml (mean 3.2 ng/ml), respectively (12). No NGCO or NGCI were detected in the saliva of controls with no chewing or smoking habit. A large increase in salivary N-nitrosoproline was also seen (3.2 ng/ml compared to 1.0 ng/ml in non-chewing controls) and saliva from betel quid chewers also contained increased amounts of nitrite (16.38 ug/ml compared to 8.52 ug/ml in controls). Thiocyanate levels in chewers were lower than in those with no chewing or smoking habit, at 14.08 ug/ml compared to 26.43 ug/ml).

19. Saliva samples collected during chewing of betel quid without tobacco by individuals residing in the New York area contained detectable levels of MNPN (identity confirmed by GC-MS) ranging from 0.5-11.4 ng/ml (median 1.18 ng/ml)(15).

20. Saliva samples collected from Taiwanese users of betel quid (betel leaf, whole green Areca nut and slaked lime with or without catechu) contained no detectable nitrosoguvacine, MPNA or MNPA but did contain high levels of N-nitrosoguvacine ranging from 0-142.0 ng/ml (mean 70.8 ng/ml)(16). When Areca nut alone was chewed, N-nitrosoguvacine levels in saliva ranged from 6.4-24.5 ng/ml, increasing to 28.5-55.0 ng/ml when slaked lime was added and to 54.0-103.0 ng/ml when betel leaf was also present.

21. Evidence of endogenous nitrosation has been obtained in both experimental animals and man. In hamsters, animals fed 20g powdered Areca nut/kg of diet together with 2 g/kg sodium nitrite excreted N-nitrosonipecitic acid (NNIP, a major urinary metabolite of N-nitrosoguvacoline and N-nitrosoguvacine) at levels of  $1.9 \pm 0.9$  ng/ml of urine (range 0.57-2.85 ng/ml)(17). The urine of hamsters fed Areca nut or nitrite alone contained no detectable levels of NNIP (limit of detection 0.1 ng/ml urine).

22. When aqueous extracts of Areca nut and extracted tannins were administered to rats dosed with proline and nitrite, endogenous nitrosation, measured as N-nitrosoproline urinary excretion, was either enhanced or inhibited, depending on the pH and the relative concentrations of nitrite and phenolic extracts (1,19).

23. Evidence for endogenous nitrosation in the oral cavity of chewers of betel quid without tobacco was obtained in a study in which saliva samples were collected from healthy males after sequential chewing of unmodified betel quid, betel quid to which proline had been added and betel quid to which proline and ascorbic acid were added (18). Levels of N-nitrosoguvacoline in saliva were increased in the presence of proline, from 0.6-8.8 ng/ml to 1.3-12.9 ng/ml, the further addition of ascorbic acid reduced levels slightly, to 2.3-8.6 ng/ml. Nitrosoproline levels, expressed as the ratio of NPRO (ng/ml) to arecoline (ug/ml), were increased after chewing betel quid with added proline. The presence of ascorbic acid reduced the formation of NPRO in half of the samples but increased nitrosation in the remaining half.

24. Increased nitrosamine levels have been detected in urine collected for 6 hours after chewing betel quid without tobacco (12). Betel chewers excreted 58 ng N-nitrososarcosine, 443 ng N-nitrosoproline and 471 ng N-nitrosothiazolidine-4-carboxylic acid, compared to 43 ng, 220 ng and no detectable levels of these nitroso-compounds respectively in non-chewers. N-nitrosonipecotic acid was not detected in urine (limit of detection 0.5 ug/ml). In another study in which two subjects injected proline and nitrate together with an aqueous, ether or n-butanol extract of Areca nut, endogenous nitrosation measured as urinary N-nitrosoproline excretion, was inhibited (1,20).

### *Mutagenicity*

25. The available studies on the mutagenicity of extracts of betel quid, pan masala, Areca nut, Areca nut alkaloids, the alkaloid-derived nitrosamines, betel leaf and other components of the quid are summarised in detail in Appendix 2.

### **BETEL QUID**

26. Aqueous extracts of whole betel quid have undergone limited testing in vitro and there is one reported in vivo study. Aqueous extracts of the combined components of betel quid (Areca nut, betel leaf, lime and catechu) showed mutagenic potential in bacteria in the presence of S9 but did not induce gene mutation in mammalian cells in vitro. No in vitro cytogenetic tests have been reported. An aqueous extract of betel quid was not mutagenic in a micronucleus test in mouse bone marrow.

## PAN MASALA

27. Commercial pan masalas have been tested for mutagenic activity in a variety of tests and have shown mutagenic activity both in vitro and in vivo. Extracts of pan masala showed only weak activity in bacterial tests and no in vitro tests for gene mutation in mammalian cells have been carried out. However, structural chromosome aberrations, micronuclei and SCEs were induced in vitro by aqueous extracts of pan masala, with activity reduced but not abolished in the presence of S9 mix. Pan masala is an in vivo mutagen, with SCEs induced in mouse bone marrow following ip injection and both cytogenetic damage in primary spermatocytes and sperm head abnormalities produced by oral dosing in mice. An aqueous extract of pan masala also induced UDS in mouse stomach.

## ARECA NUT

28. Both aqueous and ethyl acetate extracts of Areca nut have been tested for mutagenic activity in vitro and aqueous extracts have also undergone in vivo testing. Aqueous extracts of Areca nut are mutagenic in vitro in the absence of added metabolic activation, inducing mutation in bacteria and both gene mutation and chromosomal damage in mammalian cells. The in vitro clastogenic activity was reduced by SOD and saliva and abolished by catalase. DNA damage has also been produced in yeast (gene conversion, only at alkaline pH) and mammalian cells (UDS and single strand breaks in DNA) in the absence of S9. Reactive oxygen species were generated from aqueous Areca nut extract at alkaline pH and concomitant production of 8-OH-dG adducts detected in DNA in vitro. An aqueous extract of Areca nut showed promoting activity in an in vitro test which was abolished by the addition of retinol.

29. Powdered Areca nut was not mutagenic in Drosophila assays. In mammalian in vivo tests, studies using ip dosing of aqueous extracts to mice detected the induction of micronuclei and SCEs in the bone marrow, but no chromosome damage was produced in mouse bone marrow in another study using prolonged oral dosing.

30. Ethyl acetate extracts of Areca nut have only been tested in vitro but have shown no evidence of mutagenic activity in tests for gene mutation and for SCE induction in mammalian cells. An in vitro cell transformation test was also negative.

## ARECA NUT ALKALOIDS

### Arecoline

31. The mutagenicity of arecoline has been fairly extensively studied, both in vitro and in vivo. It is a direct-acting in vitro mutagen, causing mutation in bacteria and both gene mutation and chromosome aberrations in mammalian cells. UDS and single strand DNA breakage have also been induced by arecoline in vitro in the absence of metabolic activation. Arecoline is mutagenic in vivo in studies for the induction of chromosome aberrations, SCEs and micronuclei in mouse bone marrow and for micronucleus induction in fetal mouse blood. Arecoline also produced UDS in early spermatids and induced sperm head abnormalities in mice.

## Arecaidine

32. Arecaidine has not been as well studied as arecoline. Studies in bacteria and mammalian cells suggest that arecaidine has some potential to induce gene mutation in the presence of added metabolic activation. It has not been tested for clastogenic potential in vitro. No DNA damage in the form of single strand breaks was detected in one study in which arecoline also produced a negative result. Two in vivo tests using the ip route have given conflicting results. One study reported no increase in micronuclei in mouse bone marrow, but an increased frequency of SCEs in mouse bone marrow was found in the second study. Arecaidine thus appears to have some mutagenic potential but is not as potent a mutagen as arecoline.

## Guvacoline, guvacine and arecolidine

33. Neither guvacoline nor guvacine have been tested for mutagenic activity. Both were inactive in a single in vitro assay for the induction of single strand breaks in DNA. Arecoline and arecaidine were also inactive in this system. There are no studies on arecolidine.

## ARECA NUT ALKALOID-DERIVED NITROSAMINES

### N-nitrosoguvacoline (NGCO)

34. NGCO showed mutagenic potential in bacteria only in the presence of S9. It did not induce single-strand breaks in DNA in vitro in the absence of added metabolic activation and was not mutagenic in a Drosophila assay. It has not been tested in vivo.

### N-nitrosoguvacine (NGCI)

35. NGCI has not shown any evidence of mutagenic activity but has only been tested in bacteria. It did not induce single strand breakage of DNA in vitro in the absence of metabolic activation.

### 3-methylnitrosaminopropionaldehyde (MNPA)

36. The mutagenic potential of MNPA has not been studied. However, it did induce single strand breakage of DNA in vitro in the absence of metabolic activation, suggesting that it does have the potential to damage DNA.

### 3-methylnitrosaminopropionitrile (MNPN)

37. There are no mutagenicity studies on MNPN. It did not induce DNA strand breakage in an in vitro study in the absence of metabolic activation. However, a study in the rat detected the in vivo production of methyl and cyanoethyl adducts in DNA from the liver, oesophagus and nasal mucosa following subcutaneous injection of MNPN; the latter is a primary target organ for the carcinogenic action of MNPN in this species.

## BETEL LEAF

38. The in vitro mutagenic potential of betel leaf has been investigated in bacteria and mammalian cells and there is one in vivo study. Aqueous extracts of betel leaf did not induce gene mutation in bacteria with or without S9 and were not mutagenic to yeast, but aqueous extracts were clastogenic to mammalian cells in vitro in the absence of metabolic activation. Ethyl acetate extracts were clastogenic in vitro only in the presence of  $Mn^{2+}$  and did not induce SCEs, gene mutation or cell transformation. Betel leaf was not mutagenic in Drosophila. The only in vivo study of betel leaf did not report any increase in the frequency of micronuclei in the bone marrow of mice dosed intraperitoneally with an aqueous extract.

## CATECHU

39. Catechu was reported to be mutagenic to bacteria but no details are available. It is clastogenic in vitro in the absence of metabolic activation, with activity reduced by SOD and abolished by catalase or saliva. Catechu is mutagenic in vivo, inducing SCEs in mouse bone marrow on ip dosing and dominant lethal mutations in mice given the compound orally.

## SUMMARY

40. Aqueous extracts of betel quid were mutagenic to bacteria but not in other in vitro or in vivo tests. Extracts of Areca nut have shown evidence of mutagenic activity both in vitro, where at least some of its activity is associated with the production of reactive oxygen species, and in vivo. The Areca nut alkaloid arecoline is a direct-acting mutagen in vitro which is also mutagenic in vivo. Of the other alkaloids, arecaidine has not been so well studied but shows some indications of mutagenic potential; the mutagenic potential of guvacoline and guvacine has not been investigated. The mutagenicity of the Areca-derived nitrosamines has not really been studied. NGCO showed some evidence for mutagenic activity in bacteria but has not been adequately tested. NGCI has not shown any evidence of mutagenic activity but has only been tested in bacteria. The mutagenic potential of MNPA has not been investigated; it did induce DNA strand breaks in one study. There are no mutagenicity studies on MNPN, which has however produced DNA adducts in vivo in the rat. Aqueous extracts of betel leaf are clastogenic but no activity was seen in the only in vivo study. Catechu is clastogenic in vitro; as with Areca nut the activity appears to be associated in part with the production of reactive oxygen species. Pan masala, the commercial preparation containing all the components of betel quid except betel leaf, is clastogenic in vitro and is an in vivo mutagen.

### *Animal studies*

## TOXICITY

41. The toxic and pharmacological effects of Areca nut and its constituents have been reviewed by IARC (Appendix 1). Increased amounts of glutathione and reduced levels of protein-SH groups in liver, kidney and muscle were found following intraperitoneal injection of an aqueous extract of Areca nut in mice. Increases in hepatic RNA and DNA content accompanied by increased synthesis of

nucleic acids occurred in mice given intraperitoneal injections of aqueous extract of Areca nut and of arecoline. The ip LD<sub>50</sub> of arecoline in rats is reported to be about 40 mg/kg and that of arecaidine about about 800 mg/kg.

42. Some indications of possible toxicity to the liver, brain and testes were seen in a limited study in which groups of 10 rats received daily gavage doses of 84, 420 or 840 mg/kg pan masala (powdered and dispersed in water) 5 days/week for 6 months (21). A parallel group was given an acute oral dose of 4.2 g/kg, administered in two portions, 1 hour apart. There were no clinical signs of toxicity and body weight was unaffected. Relative weights of brain and testis (but not liver) were increased in the chronic study; acute dosing had no significant effect on organ weights. At the highest dose level, serum levels of GOT, GPT and ALP were significantly increased. Serum GPT, but not GOT or ALP, was also significantly increased by the low and intermediate dose levels. Liver enzymes were also affected, with significant reductions in liver GPT at doses of 420 and 840 mg/kg. Liver GPT was unaffected at the 84 mg/kg dose and there was no effect on liver GOT at any dose. The only effect on enzyme activity following acute dosing was an increase in serum ALP. There was no pathological examination of any organ.

43. Arecoline is cholinomimetic, with a pharmacological action similar to that of muscarine and pilocarpine. It stimulates the parasympathetic nervous system by binding to muscarinic and nicotinic receptors and its action is blocked by atropine. Behavioral and EEG studies have shown effects on the central nervous system, presumably caused by an effect on muscarinic receptors. Arecoline has also reportedly been shown to inhibit both the humoral and cell-mediated immune responses in mice (2).

44. Arecaidine, which is present in Areca nuts and is formed from arecoline on reaction with lime and by enzymes in rat liver, and guvacine, which is also present in Areca nut and is produced by de-esterification of guvacoline, inhibit the uptake of the neurotransmitter GABA in brain slices and when introduced directly into the brain. Large doses of arecaidine have produced behavioral changes in mice.

## CARCINOGENICITY

45. The available studies on the carcinogenicity of betel quid, pan masala, Areca nut, Areca alkaloids and derived-nitrosamines, and betel leaf in experimental animals are summarised in detail in Appendix 3. The relevant sections of the IARC Monograph are highlighted in Appendix 1.

### Betel quid

46. IARC considered there to be *limited evidence* for a carcinogenic effect of extracts of betel quid without tobacco. Studies reviewed by IARC showed an increased incidence of local sarcomas in mice after subcutaneous injection of aqueous extract of betel quid and the induction of tumours of the forestomach and cheek pouch in hamsters

following painting of the cheek pouch or implantation of wax pellets containing aqueous extracts of betel quid components. The only study of betel quid published since the IARC review reported an increase in DNA content of mouse gastric crypt cells with oral administration of an aqueous extract of combined components of betel quid.

#### **Pan masala**

47. The habit of chewing pan masala as such, rather than as a component of a betel quid, is relatively recent and this habit was not considered by IARC. There are no animal carcinogenicity studies on pan masala. Precancerous changes (mild leukoplakia and submucous collagen deposition) were seen in rats treated orally with a paste of a commercial pan masala.

#### **Areca nut**

48. IARC considered there to be *limited evidence* that Areca nut without tobacco is carcinogenic in experimental animals. The studies reviewed by IARC showed the production of local tumours in mice given subcutaneous injections of aqueous or polyphenolic fractions of Areca nut and in rats given aqueous extract by subcutaneous injection. Dietary studies in mice and rats showed no induction of tumours but were not considered adequate. There was some indication of a carcinogenic effect following oral intubation in the mouse. Tumours of the forestomach and cheek pouch were produced by administration of aqueous extracts or DMSO extracts of Areca nut and by application in gelatine capsules to the hamster cheek pouch.

49. Studies published since the IARC review, although limited, provide additional evidence for a carcinogenic effect of Areca nut. Precancerous changes were seen in the oral, oesophageal and forestomach epithelia of rats in studies of dietary administration of various dried Areca nut preparations and of application of pastes of the same preparations to the oral epithelium. No tumours were seen in animals treated with processed nuts but in both studies oesophageal tumours were induced by unprocessed nut preparations. Increases in the overall incidence of malignant tumours, mainly malignant lymphoma in males, were observed in a dietary study of Areca nut and nitrite in hamsters; Areca nut alone was not carcinogenic in this study. Areca nut in the diet of rats increased the carcinogenic activity of the carcinogens 4-NQO and FAA.

#### **Areca nut alkaloids**

50. Only arecoline has been studied for carcinogenicity in experimental animals; there are no studies on arecaidine, guvacine guvacoline or arecolidine.. Some of the studies reviewed by IARC showed some indications of a possible carcinogenic effect; in mice in an oral intubation study and in hamsters following dietary administration or application to the cheek pouch with lime. Other studies showed no increase in tumour incidence in mice following oral intubation with nitrate alone or with lime nor with subcutaneous or intraperitoneal injection of arecoline. None of these studies were considered adequate and IARC concluded that the data were *inadequate* to allow an evaluation of the carcinogenicity of arecoline. There have been no new studies published since the IARC review.

## Areca-derived nitrosamines

### N-nitrosoguvacoline (NGCO)

51. There are only two studies on the carcinogenicity of NGCO. In one study, reviewed by IARC, NGCO in the drinking water of rats was not carcinogenic. This study was not however considered adequate. A more recent study using a single dose of NGCO in the drinking water of male rats detected an increased incidence of benign pancreatic tumours but no increase was seen in malignant tumours. The carcinogenic potential of NGCO has clearly not been adequately assessed.

### N-nitrosoguvacine (NGCI)

52. There are no animal carcinogenicity studies on NGCI.

### 3-methylnitrosaminopropionaldehyde (MNPA)

53. There is one published study on the carcinogenicity of MNPA, using subcutaneous injection in the rat. Although limited by small numbers of animals, this study showed an increased incidence of lung tumours in treated animals. In addition, nasal and forestomach papillomas, although not significantly increased, were seen in treated animals but not in controls.

### 3-methylnitrosaminopropionitrile (MNPN)

54. On the basis of a subcutaneous injection study in rats which showed increases in the frequency of tumours of the oesophagus, nasal epithelium, and tongue in MNPN-treated animals, IARC considered there to be *sufficient evidence* for the carcinogenicity of MNPN to experimental animals. Subsequent studies have confirmed the carcinogenic potential of MNPN, with malignant nasal tumours produced in rats by subcutaneous administration and tumours of the nasal epithelium, lung, liver and oesophagus induced in rats by application to the oral mucosa. Multiple lung adenomas were also seen following dermal application to Sencar mice.

### **Betel leaf**

55. Studies reviewed by IARC, in which aqueous extracts of betel leaf were tested in mice by oral intubation or intraperitoneal injection and in hamsters by application to the cheek pouch, betel leaf added to the diet of rats, and betel leaf applied to the hamster cheek pouch in a wax pellet, did not show any suggestion of carcinogenic activity, but were all considered inadequate. IARC considered there to be *inadequate* data to allow an evaluation of the carcinogenicity of betel leaf to experimental animals.

56. There have been no new studies on betel leaf *per se* but no indications of carcinogenic potential were seen with betel leaf alone in studies showing a protective effect of betel leaf extract against known carcinogens when given in the drinking water or by topical application in the mouse. Taken together the available studies do not suggest that betel leaf has any carcinogenic potential.

## TOXIC AND PHARMACOLOGICAL EFFECTS

57. The toxic and pharmacological effects of betel quid chewing were reviewed by IARC (Appendix 1) and will be mentioned only very briefly here. When betel quid is first chewed, the experience is disagreeable, with an acrid, burning taste followed by constriction of the throat and the production of roughness and slight ulceration of the tongue. These effects gradually lessen with repeated use and are replaced by feelings of well-being. Betel chewing stimulates secretion of saliva and gives the saliva a red colour. Habitual use can lead to caries, deposition of tartar, regression of gums and partial or complete loss of feeling in the buccal mucosa, as well as local lesions affecting the periodontal tissues, teeth and oral mucosa. Palpitations, dyspepsia, neurosis and giddiness have also been reported in habitual betel quid chewers.

58. The immediate effects of betel quid chewing are due to stimulation of the parasympathetic nervous system by arecoline. On the basis of animal experiments the psychopharmacological effects have been ascribed either to the effect of arecoline on cholinergic neurons or to the effect of arecaidine or guvacine on the uptake of the neurotransmitter GABA. Studies in humans suggest that very small doses of arecoline can affect the human central nervous system in a similar way to that in animals.

## MUTAGENICITY AND CHROMOSOMAL EFFECTS

59. Cytogenetic studies of users of betel quid, pan masala or Areca nuts are summarised in Appendix 2.

60. Saliva collected during chewing of a commercial brand of pan masala (containing dried Areca nut, catechu, cardamom, copra, menthol and slaked lime) was highly clastogenic to mammalian cells in vitro in the absence of added metabolic activation. Saliva collected during chewing of fresh betel leaves was also clastogenic, although to a much less significant extent, while saliva collected during chewing of a commercial preparation of dried Areca nut (supari) was only weakly clastogenic in comparison. In all three cases, the clastogenic activity had disappeared 5 minutes after cessation of chewing.

61. An increased frequency of micronucleated exfoliated buccal mucosa cells has been reported in Khasi Indians chewing betel quids containing fresh Areca nut, betel leaf and lime, and in Taiwanese, who chew betel quids containing a whole fresh green Areca nut, betel leaf and lime, but not in subjects from Guam, who chew fresh green Areca nuts alone or with betel leaf but without the addition of lime. Salivary pH levels during chewing rise considerably with the addition of lime and it has been suggested that the observed clastogenic effects are due to the production of reactive oxygen species in the mouth (44).

62. Small but statistically significant increases in the frequency of micronucleated exfoliated buccal mucosa cells were also observed in Indian chewers of pan masala and Areca nuts.

63. Cytogenetic studies of peripheral lymphocytes from Indian chewers of pan masala or Areca nuts showed increased levels of structural chromosomal aberrations and SCEs compared to matched controls. Statistically significant increases in the frequency of SCEs in peripheral lymphocytes were also seen in habitual users of Indian betel quid without tobacco.

64. There is therefore evidence of clastogenic effects of betel quid, pan masala and Areca nut chewing in exposed human populations.

#### PRECANCEROUS LESIONS AND CONDITIONS

65. The 1985 IARC monograph (Appendix 1) reviewed the available studies on the prevalence of oral leukoplakia, submucous fibrosis and lichen planus in users of betel quid (1). Prevalence studies of leukoplakia in Guam, Papua New Guinea, India, Malaysia and China showed an association between oral leukoplakia and chewing of Areca nut or betel quid without tobacco (Table 9 in Appendix 1). The prevalence of leukoplakia was higher in users of betel quid without tobacco than in individuals with no habit (Table 10 in Appendix 1).

66. The precancerous nature of oral submucous fibrosis, which is characterised by stiffness of the oral mucosa and symptoms due to restricted mobility, trismus, stricturing at angles of the mouth, inability to protrude the tongue, altered pronunciation and intolerance to spicy-hot food, is well established (22). Chewing of Areca nut has been suggested to have an aetiological role in the development of oral submucous fibrosis (1). Arecoline and arecaidine stimulate collagen synthesis and proliferation of human buccal mucosal fibroblasts and the phenolic compounds in Areca nut inhibit collagen degradation (24).

67. Prevalence rates of 0.2 to 1.2% have been reported for oral submucous fibrosis in India. A study of oral submucous fibrosis in 5 areas of India found a prevalence of 1.4% in users of betel quid without tobacco in one area (Table 13 in Appendix 1).

68. A more recent study in South Africa by Seedat and Van Wyk (1988) examined the betel chewing and dietary habits of chewers without any signs of submucous fibrosis (SF), of subjects suffering from SF and of SF sufferers who also had oral cancer (23). A random stratified sample of 2058 subjects older than 10 years revealed 186 betel nut chewers, of whom 71 had symptoms of SF or impending SF. Of these, 162 chewers, 99 with no signs of SF and 63 with impending or established SF, participated in the study, together with a further 124 hospital referred SF cases, 10 of whom also had oral cancer, giving a total of 286 subjects. The majority of the subjects chewed less than 5 nuts or quids per day, with no differences between the groups. Subjects suffering from SF, both survey subjects and hospital cases, were significantly younger than those with no signs of SF, and had practised the habit for a significantly shorter period of time. Tobacco was incorporated into the quid in some cases: 69.7% (69/99) of survey subjects without SF, 85.7% (54/63) of survey subjects with SF and 93.6% (116/124) of hospital cases did not use tobacco, this difference was statistically significant. Areca nut only was chewed by 31.3% (31/99) of survey subjects without SF, by 58.7% (37/63) of survey subjects with SF and by 80.7% (100/124) of the referred SF patients. Areca nut plus lime was chewed by 1/99 survey chewers without SF, by none of the survey subjects with SF and by 4 of the referred cases. The remainder chewed Areca nut in the form of a betel

quid or paan; this form of Areca consumption was significantly higher in survey chewers without SF. Both boiled and baked Areca nuts were used; the proportion of subjects using boiled nuts was significantly greater in the group of referred SF cases than in the survey subjects with or without SF (67.8% compared to 38.0% and 39.4%, respectively). The majority of lime users incorporated it into a betel quid, resulting in significantly higher numbers of lime users in the group of survey subjects with no SF (63.6%, 63/99), than in survey subjects with SF (38.1%, 24/63) and in hospital cases (17.7%, 22/124). The possible effect of chilli consumption was also examined. 98% of the study population consumed chillies, with significantly fewer non-chilli users among the referred SF cases.

69. In summary, the study showed a relationship between the chewing of Areca nut, particularly consumption of the boiled nuts alone ie not in a betel quid, and the development of oral submucous fibrosis. No relationship was established between the development of SF and the use of tobacco or lime in the quid or with consumption of chillies.

## CASE REPORTS AND EPIDEMIOLOGICAL STUDIES

### Studies reviewed by IARC

70. Studies of the association between betel quid use or Areca nut chewing and cancer were reviewed by IARC in 1985. The IARC Monograph is attached at Appendix 1 and sections referring to studies of Areca nut or betel quid without tobacco are highlighted.

71. IARC reviewed studies of the frequency of oral cancer in betel quid users (Table 15 in Appendix 1). Frequencies of 14.4% to 17.8% were reported in users of betel quid without tobacco from Papua New Guinea (Atkinson *et al* 1964; Cooke, 1969; Henderson and Aiken, 1979) and of 10.9% in Malaysia (Ahluwalia and Duguid 1966).

72. Few of the descriptive studies reviewed by IARC dealt with chewing of betel quid without tobacco (Table 16 in Appendix 1). In Papua New Guinea, where tobacco is never added to the betel quid, one early study (Eisen 1946) concluded that betel quid did not cause cancer of the buccal cavity. In two later studies (Farago, 1963), 98% and 99% of oral cancer cases were chewers of betel quid.

73. A study by Atkinson *et al* (1982) comparing age-adjusted rates of oral cancer in different geographical regions of Papua New Guinea for the years 1971-1978 found a higher rate in the lowlands, where a large proportion of the population chewed Areca nut with lime (6.83/100,000 in men and 3.03/100,000 in women) than in the highland areas, where very few people practised the habit (1.01/100,000 in men and 0.41/100,000 in women).

74. Another study in Papua New Guinea, (Scrimgoeur and Jolley (1983)), which compared oral cancer rates with changes in smoking tobacco habits, found a highly significant increase in the incidence of oral cancer in women during a period in which tobacco smoking in women increased dramatically while betel chewing habits showed little change.

75. IARC also reviewed a number of case-control studies of oral and other cancers associated with chewing of betel quid (Table 17 in Appendix 1). Crude relative risks associated with betel quid use without tobacco were calculated by the IARC Working group as 0.1 for

cancer of the cheek and floor of the mouth in one study from India (Shanta and Krishnamurthi, 1959) and 0.3 for men and 0.8 for women for cancers of the lip, oropharynx, hypopharynx, oesophagus and tongue in a later study by the same authors (Shanta and Krishnamurthi, 1963).

76. A large case-control study from Bombay (Jussawalla and Deshpande, 1971), which categorized chewing habits (presumably betel, not precisely defined) without tobacco, showed statistically significant increased relative risks for cancers of the oral cavity, oropharynx, hypopharynx, larynx and oesophagus, in the range of 3 to 5; smoking was not controlled for in this analysis (see Table 20 in Appendix 1).

77. Three case-control studies supplied sufficient information for categorization of risk in relation to various combinations of chewing and smoking habits (Table 21 in Appendix 1). A case-control study from Pakistan (Jafarey *et al*, 1977) suggested that chewing of betel quid alone increased the risk of oral cancer when practised alone or in combination with smoking (relative risks:- for betel quid, 4.2 for men and 3.2 for women; for betel quid and smoking, 25.0 for men and 29.9 for women). Another case-control study from India and Sri Lanka (Hirayama, 1966) did not show a clear effect for the risk of oral cancer associated with chewing betel quid without tobacco (relative risk:- for betel quid, 1.9 in men, 1.2 in women; for betel quid and smoking, 2.7 in men, 4.6 in women). The relative risk for oropharyngeal cancer associated with betel quid use was increased after controlling for smoking (11.8 in men) but not significantly so. In the third study, from Calcutta in India (Chandra, 1962), the relative risk for buccal-mucosal cancer associated with the use of betel quid without tobacco was not significant (0.7 in men, 2.0 in women).

78. IARC concluded that there was *inadequate evidence* that the habit of chewing betel quid without tobacco is carcinogenic to humans and 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 did not allow an assessment of the possible contribution of betel quid without tobacco to this carcinogenic risk (1).

#### **New data**

79. There have been few studies published since the IARC review. A case report from Karachi described the occurrence of poorly differentiated squamous cell carcinoma of the oesophagus in a 30 year old woman with a habit of consuming 8 to 10 packets of a commercially available sweetened Areca nut preparation (raseeli supari) every day for 10 to 12 years (Sheikh *et al*, 1992). No information was provided on other habits associated with oesophageal cancer, such as smoking and drinking.

80. The group of South African oral submucous fibrosos patients chewing Areca nut preparations which was studied by Seedat and Van Wyk (1988) also included 10 patients with oral cancer (23). The patients all had moderately to well-differentiated squamous carcinomas arising in the buccal sulci (8), the soft palate (1) and the upper oesophagus (1). 9 of the 10 cases chewed Areca nut only,

without added lime or tobacco; one subject chewed betel quid which included tobacco, lime and catechu as well as Areca nut.

81. A follow-up study to that of Atkinson in Papua New Guinea confirmed the regional differences in oral cancer rates (26). Age-standardised incidence rates for oral cancer for the years 1979-1983 had increased considerably but remained significantly lower in the highland regions (where Areca nut chewing is rare) than in the lowlands (where it is common). The study identified 458 cases of cancer of the tongue, gum, floor and other parts of the mouth, of whom 11 were excluded from the analysis. All but 9 of the tumours were of epithelial origin, mostly squamous cell carcinoma. Age-standardised incidence rates per 100,000 for the four administrative regions of the country were as follows:-

<u>Region</u>	<u>Males</u>	<u>Females</u>	<u>Total</u>
Highland	2.6	1.4	2.0
Southern	21.1	11.1	16.4
Momase	22.5	12.0	17.3
Island	31.2	15.1	24.0
<u>Total</u>	<u>14.9</u>	<u>7.6</u>	<u>11.4</u>

82. Rates for each sex were significantly lower in the Highland region than elsewhere ( $p < 0.05$ ), except for women from Southern region ( $p < 0.1$ ). Rates for both sexes combined significantly lower in the Highlands than any of the other regions ( $p < 0.01$  compared to Southern region,  $p < 0.001$  compared to Momase and Islands).

83. The marked regional variation was also apparent when comparing cancers at specific sites. Cancer of the angle of the mouth and of the buccal mucosa were considered separately, since both come into repeated contact with the stick or betel inflorescence used to add slaked lime to the quid of partially chewed Areca nut. Crude annual incidence rates per million adults (15 years and over) were as follows:-

<u>Region</u>	<u>Cancer of angle of mouth</u>	<u>Cancer of buccal mucosa</u>	<u>Cancer of rest of mouth</u>
Highlands	-	4.0	10.9
Southern	5.6	24.2	44.0
Momase	8.0	34.1	43.3
Island	9.0	27.9	57.6
<u>Total</u>	<u>4.6</u>	<u>19.7</u>	<u>33.0</u>

84. These differences were not considered to be the result of the relative under supply of dentists and doctors in the Highland region, since the apparent incidence of oral cancer varied 10-fold in the different regions, compared to a 3-fold difference in doctor-population ratios, and crude reporting rates for all cancers were only slightly lower in the Highland region than in other areas. In the absence of detailed data no account could be made for differences in smoking habits. The smoking habit was said to be well established in most areas, but the relatively recent use of manufactured cigarettes, while established in the lowland areas, was said to be still spreading to the Highlands. Detailed information on alcohol consumption were also not available although its introduction into the community was said to have been more recent and more uniform. However, the pattern of regional variation in cancers at specific sites is consistent with a possible association with betel chewing.

85. The possible relationship between site of lime application and development of oral cancer was considered further in another study from Papua New Guinea (27). 169 patients presenting with newly diagnosed oral squamous cell carcinoma over the period 1985-1987 were examined to determine the side and distribution of the tumour and interviewed to determine chewing habits and in particular the site of application of lime. 4 non-chewers and 3 patients with mid-line tumours were excluded. Site distributions were similar in both sexes and were combined. Most of the tumours were distributed from the corner of the mouth, linearly along the buccal mucosa and the lateral border of the anterior tongue. Over half were in the buccal mucosa and 61% involved the corner of the mouth, including 23% that were limited to the corner of the mouth alone. Few tumours were found in the floor of the mouth, the commonest site for oral cancer in Western countries. The site of tumours was strongly correlated with the side of lime application ( $p < 0.0001$ ). Of 86 patients with left-sided tumours, 76% commonly added lime on the left and 62% did so exclusively. Similarly, 79% of patients with right-sided tumours usually added lime to the right side and 70% did so only on this side. A separate survey of normal adults found rapid and substantial changes in mouth pH during chewing; before chewing, mean pH was 6.8, rising to 9.9 one minute after application of lime and falling to 8.2 after 5 minutes.

86. A study from Taiwan found human papilloma virus (HPV-16) in 76.4% (13/17) of oral epidermoid carcinoma samples and in 100% (3/3) oral papillomas, compared to only 1 HPV positive (type unknown) sample in 17 normal oral tissues (28). When HPV infection was compared in relation to specific tumour sites and betel quid and smoking habits, all 7 cases with tumours of the buccal mucosa were found to be betel quid chewers (for longer than 10 years) and were HPV positive; 5/7 also smoked. Betel quid was also used by 1/6 cases with tumour of the palate, 1/3 cases with tumour of the tongue/mouth floor and 1 case with a gingival tumour. Betel quid in Taiwan contains Areca nut, betel leaf and lime without catechu.

87. A case-control study from Thailand recently reported an increased risk of hepatocellular carcinoma associated with weekly or more chewing of Areca nut (30). Details of the habit were not given, but according to other authors a fresh piece of unripe nut with a clove and betel leaf smeared with lime are used; cut tobacco is not included but may be used to wipe the teeth (3). The study looked at 65 cases of hepatocellular carcinoma in comparison with 65 age-matched controls. Univariate matched pair analysis for known or

suspected risk factors for liver cancer revealed infection with hepatitis-B virus as the greatest risk factor (odds ratio for HBSAg positive on matched analysis 12). A small effect was found for regular consumption of alcohol (odds ratio 2.4) but not for recent aflatoxin intake, as measured by consumption of possibly contaminated foods or albumin-bound aflatoxin in serum, nor with regular tobacco smoking. Regular use of Areca nut conferred a high, but not statistically significant, risk for hepatocellular carcinoma on univariate analysis (14 cases compared to 6 controls, odds ratio 5). Multivariate analysis was carried out for the following risk factors:- HBSAg positive, regular alcohol consumption, shrimp paste consumption, powdered peanut consumption, other fresh vegetable consumption and regular use of Areca nut. The highest relative risk was obtained for HBSAg positive (OR 15.2,  $p < 0.001$ ). Areca nut chewing carried the second highest risk (OR 11.0,  $p < 0.05$ ). Regular use of alcohol carried a non-significant elevation of risk (OR 3.4). The association of Areca nut chewing with hepatocellular carcinoma had not been reported previously.

88. It was not possible to separate the effects of Areca nut or betel quid chewing without tobacco in the remaining studies.

89. A study of the prevalence of betel quid chewing, smoking and alcohol consumption in 100 cases of oral squamous cell carcinoma in Malaysia found that betel quid chewing was practiced by 85%, with 34% practising this habit alone ie without smoking or drinking (29). Further analysis was restricted to the 86 Indian subjects. In 63/85 betel quid chewers the tumour was in the buccal sulcus and there was a significant association ( $p < 0.001$ ) between the side where SCC developed and the side where the betel quid was habitually kept. Most chewers preferred to use raw Areca nut in the quid and most added slaked lime; 80% also added tobacco.

90. An increased incidence of cervical dysplasia in Indian women was reported in association with the habit of betel quid chewing (31). 11.4% (226) of a group of 1990 women undergoing cervical smear tests were reported as betel quid chewers (it is not clear whether the quid included tobacco or not), 11.9% (236) were betel quid chewers and "tobacco consumers", 75.5% had no habits. Cervical dysplasia was found in 50.9% (115/226) of betel quid chewers and in 54.7% of betel quid chewers and consumers of tobacco, compared to 4.1% of women with no habits. Intraepithelial carcinoma was detected in 2 women who chewed betel quid. The incidence of cervical dysplasia was not related to the number of quids chewed per day but 100/115 betel quid chewers with cervical dysplasia had persued the habit for 6 or more years.

91. Case-control studies of the risk factors for cancer of the buccal and labial mucosa (32) and for cancer of the oesophagus (33) in Kerala, India, have recently been published. Chewing of betel quid containing tobacco was confirmed as the most important risk factor for buccal and labial cancers, with a relative risk of 13.24 with 31-40 years of use and 37.75 with 40 years of use in males, and corresponding relative risks of 21.30 and 54.93 in females. Bidi smoking (but not cigarette smoking) and alcohol consumption were also significant risk factors. Bidi smoking and alcohol consumption were the most significant risk factors for cancer of the oesophagus; chewing of betel quids containing tobacco did not have a significant effect. In both studies the numbers chewing betel quid without tobacco were too small for inclusion in the analysis.

## SUMMARY

92. Cytogenetic studies have revealed higher frequencies of chromosomal aberrations and SCEs in peripheral lymphocytes of people who chew pan masala, betel quid or Areca nuts and subjects who chew these products have increased frequencies of micronucleated buccal mucosal cells, although one study found no increase when fresh green Areca nuts were chewed without lime.

93. Studies of the association of betel quid chewing with precancerous lesions reviewed by IARC reported increases in the prevalence of oral leukoplakia in populations who chewed Areca nut or betel quid without tobacco. A recent study from South Africa found a higher incidence of Areca nut chewing, particularly chewing of boiled Areca nut alone ie not as part of a quid, in patients suffering from submucous fibrosis than in subjects with no signs of the disease. No relationship of submucous fibrosis was found with the use of tobacco or lime in the quid.

94. The IARC Monograph reviewed a number of studies of the possible association of oral cancer with the use of Areca nut or betel quid without tobacco. The studies reviewed by IARC included surveys showing a higher rate of oral cancer in lowland Papua New Guinea, where betel quid use is common, than in the highland area, where its use is rare; a case-control study from India which described significantly increased relative risks for cancers of the oral cavity, oropharynx, hypopharynx, larynx and oesophagus, but did not control for smoking; and a case-control study from Pakistan which suggested an increased relative risk of oral cancer for betel quid chewing alone or in combination with smoking. However, two other case-control studies from India did not show a clear effect for chewing of betel quid alone independently of smoking; the risk of oesophageal cancer was increased after controlling for smoking in one study, but not significantly so.

95. The relatively few new studies published since the IARC review lend some support to the suggestion of an association between human cancer and chewing of Areca nut-containing products without tobacco.

96. A case report has been published describing oesophageal cancer in a young woman who regularly consumed large quantities of processed Areca nut in the form of supari. However no information was provided on other possible confounding habits.

97. 90% (9/10) submucous fibrosis patients also suffering from oral cancer in the South African study (paragraph 93) regularly chewed Areca nut alone, without the addition of tobacco or lime, compared to 31% of subjects without signs of submucous fibrosis or cancer; the remaining case used a tobacco-containing betel quid.

98. The regional differences in oral cancer rates between lowland and highland areas of Papua New Guinea were confirmed in a follow-up study, which also reported a markedly higher incidence of cancer of the angle of the mouth and buccal sulcus in areas where the use of betel quid is common (it was not possible to take possible differences in smoking habits into account). Another study from Papua New Guinea related the site of lime application to the mouth to the location of the cancer; the association was highly significant. (This is consistent with the study showing an increase in micronuclei in the buccal mucosa only when lime was added, and again suggests the involvement of oxidative free radical generation).

99. A study from Taiwan (where neither tobacco nor catechu are used in the betel quid) reported that 7/7 HPV positive oral cancers occurred in betel quid users; 5/7 also smoked.

100. A possible association between betel quid use and an increased risk of hepatocellular carcinoma was reported in a case-control study from Thailand, the first time such an association has been suggested.

### Conclusions

101. Since the publication of the IARC Monograph in 1985, new data have been published in three main areas:-

a) Additional evidence has been produced for the mutagenic and carcinogenic activity of Areca nut extracts and derived compounds in experimental systems. In particular, the potent carcinogenic activity of the Areca-derived nitrosamine MNPN has been confirmed and methyl and cyanoethyl adducts detected in DNA in the target organs for carcinogenesis. There is evidence that endogenous nitrosation of Areca nut alkaloids can occur in animals and man and Areca-derived nitrosamines, including MNPN, have been detected in the saliva of betel quid chewers.

b) Human studies have provided supporting evidence for a role of Areca nut or betel quid chewing in the induction of cytogenetic damage in the oral mucosa, oral precancerous lesions and oral cancer. Some studies have suggested a particular link with the generation of reactive oxygen species from reactions involving compounds in Areca nut and lime. In addition, a case-control study has described a possible association of Areca nut consumption with human hepatocellular carcinoma. There is also evidence for cytogenetic damage in peripheral lymphocytes of habitual users of products containing Areca nut.

c) Reports from India suggest increasing usage of commercial preparations of pan masala, which contain all the ingredients of traditional betel quid except betel leaf. There are no animal carcinogenicity studies on these preparations but they are mutagenic in both in vitro and in vivo tests and cytogenetic damage has been reported in humans using pan masala.

102. The Committee is asked to comment on whether there is sufficient evidence to regard the use of Areca nut-containing products, ~~whether~~ in the form of betel quid, pan masala or Areca nut alone, as likely to result in an increased risk of human cancer.

Secretariat  
October 1993.

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ANNEX 1 to CC/93/29

IARC monograph

Carcinogenicity studies  
on betel quid etc without  
tobacco marked II in margin

## 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<sup>a</sup>**

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 <sup>b</sup>	28	0.6

<sup>a</sup>From Mehta *et al.* (1971), unless otherwise specified

<sup>b</sup>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<sup>a</sup>**

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	

<sup>a</sup>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\***

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		

\*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).