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**CC/06/18**

**COMMITTEE ON CARCINOGENICITY OF CHEMICALS IN FOOD,  
CONSUMER PRODUCTS AND THE ENVIRONMENT.**

**BETEL QUID, PAN MASALA AND ARECA NUT CHEWING**

Introduction

1. Areca nuts are an ingredient of betel quid or pan masala which is chewed as an aid to digestion and as a stimulant. Areca nuts may have limited use as a food ingredient. The COC last considered the carcinogenicity of areca nut in 1993/4. Since then, a number of new relevant papers have been published. The Food Standards Agency would like the COC to consider whether there is sufficient new information to warrant updating their previous advice, and, whether any conclusions can be drawn about the use of areca nut as a food ingredient.

Background

2. Areca nuts are widely used in Asian immigrant populations in the UK and other countries in Europe (Warnakulasuriya, 2002). Areca nut is primarily used as an ingredient of betel quid, which is made up of areca nut mixed with slaked lime (calcium hydroxide) and catechu<sup>1</sup>, wrapped in a betel leaf. The betel quid is usually chewed for between five minutes up to an hour or maybe more. Traditional use varies between countries, sometimes tobacco is added to the quid and sometimes spices such as cardamom or ginger. Recently, pan masala has become more popular. This is a pre-prepared mixture containing the same ingredients as the betel quid, but is not wrapped in a betel leaf. Betel quid and pan masala are chewed to aid digestion and for their stimulatory effects. The juice is often spat out but sometimes swallowed. Once chewed, the fibrous remains of the quid are also normally spat out.

3. Whole areca nuts can be bought in some supermarkets in the UK and more commonly in shops stocking traditional Asian foods. It is thought that these are generally used to prepare betel quid at home, but it has also been suggested that they may be used to add flavour when cooking, grating or slicing the nut. However, it has not been possible to substantiate this use.

4. Under the terms of The Medicines (Retail Sale or Supply of Herbal Remedies) Order 1977 part 1, areca is considered to be medicinal product and should only be sold on licensed premises (a registered pharmacy or where a pharmacist is present). However betel quid is not considered

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<sup>1</sup> an extract of wood from a variety of *acacia* species but can also be obtained from the leaves and bark of other plants.

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medicinal and would be covered by food law. Advice is being sought on the legal status of the areca nuts sold in normal retail premises.

### *Constituents and metabolism of betel quid ingredients*

5. The constituents of areca nut, betel leaf and catechu are considered in paragraphs 8-24 of paper CC/93/29 attached at Annex 1 and are summarised briefly below.

6. Areca nuts may be used ripe or unripe and may be processed by being sun-dried and/or cured. Uncured areca nuts are reported to contain 11.4- 26% tannins, among the polyphenols identified are leucocyanadins, catechins, 3,4-flavandiols and hexahydroxyflavan. The main pharmacological action of areca nuts is attributed to the alkaloids, arecoline, arecaidine, guvacine, guvacoline and arecolidine which make up 0.15-0.67% of uncured nuts. *In vitro* experiments suggest that nitrosation of arecoline occurs readily, giving rise to at least four N-nitroso compounds: N-nitrosoguvacoline (NGCO), N-nitrosoguvacine (NGCI), 3-(methylnitrosamino)propionitrile (MNPN) and 3-(methylnitrosamino)propionaldehyde (MNPA). Several of these N-nitroso compounds have been detected in the saliva of betel quid chewers.

7. Among their other ingredients, the mature green leaves of *Piper betel* contain volatile oils including eugenol, chavicol, terpenes, and tannins.

8. Catechu is the residue of a hot water-extraction of the heartwood of *Acacia catechu* (also see note 1). It contains mainly tannin and polyphenols, including catechutannic acid, catechin, catechu red, quercetin, kaempferol, dihydroxykaempferol, taxifolin, isorhamnetin, (+) afzechin and dimeric procyanidin.

9. Metabolic studies suggest that arecoline is de-esterified in the liver and both arecoline and arecaidine are excreted in the urine as the mercapturic acid N-acetyl-S-(3-carboxyl-1-methylpiperid-4-yl)-L-cysteine. NGCO and NGCI are metabolised in the liver to N-nitrosonipectoic acid. This is largely excreted in the urine, though faecal excretion also occurs.

### Previous COC advice

10. The COC looked at the use of areca nut in betel quid and pan masala in 1993 and 1994 (CC/93/29 and CC/94/7, these papers are attached as annex 1). The COC considered a range of human epidemiology studies, animal carcinogenicity studies and *in vivo* and *in vitro* mutagenicity studies. These are summarised in the papers attached at Annex 1.

11. On the basis of this evidence, the COC concluded the following:

- There was evidence of 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 had

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been confirmed, and methyl and cyanoethyl adducts had been detected in the DNA of the target tissues in which the tumours developed. There was evidence that endogenous nitrosation of areca nut alkaloids can occur in animals and humans; and areca nut derived nitrosoamines, including MNPN, have been detected in the saliva of betel quid chewers.

- There was very limited data from epidemiological studies on the effect of betel or areca nut products without tobacco, which did not allow any conclusion to be drawn. There was, however, sufficient epidemiological evidence of a link between the chewing of betel quid containing tobacco and cancer in humans.
- The Committee concluded that the use of these products without tobacco was possibly carcinogenic in humans.

### Considerations by other expert committees

12. The International Agency for Research on Cancer (IARC) assessed the use of betel quid in 2003 and concluded that both chewing of betel quid and areca nut should be categorised as group 1 (known) human carcinogens. In their conclusions they stated that there is sufficient evidence in humans to conclude that betel quid chewed without tobacco causes oral cancer. IARC also concluded that there was sufficient evidence in animals to confirm the carcinogenicity of betel quid and areca nut without tobacco (IARC, 2003). The 2003 report followed up a previous review from 1985 in which it was concluded that there was inadequate evidence for the carcinogenicity of betel quid chewed without tobacco.

13. A summary of the IARC monograph with their conclusions can be found at the following web address:

<http://monographs.iarc.fr/ENG/Monographs/vol85/volume85.pdf>

14. A workshop was held in Kuala Lumpur, Malaysia in 1999 to try to standardise the reporting of areca nut and betel quid chewing habits and the diagnosis of betel quid related oral lesions (Zain *et al*, 1999, this paper is included in annex 2 and briefly summarised below).

15. Participants in the workshop defined a quid as “a substance or mixture of substances, placed in the mouth or chewed and remaining in contact with the oral mucosa, usually containing one or both of the two basic ingredients tobacco or areca nut in raw or any manufactured or processed form”.

16. The types of quid were divided into three definite categories.

- Quid with areca nut but without tobacco products (areca nut quid)
- Quid with tobacco products but without areca nut (tobacco quid)
- Quid with areca nut and tobacco products (tobacco and areca nut quid)

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17. Further subdivisions are possible but the participants concluded that a quid- chewer could only be classified under one of the above categories so if they use tobacco quid and areca nut quid at different times they would be classified under group 3. The group concluded that the term “betel nut” should be avoided and areca nut should be used instead, as it is a more accurate descriptor of the nut.

18. The group outlined two different types of lesion. These were:

- Lesions or conditions that are diffusely outlined, involve more than one site, or represent a widespread alteration such as those due to mechanical or chemical trauma. Clinical lesions or conditions such as “Chewer’s mucosa” fall into this category, but transient effects such as stains are excluded.
- Lesions that are localised at the site where the quid is regularly placed. These lesions are equivalent to snuff-induced lesions or to tobacco-lime induced lesions that arise only on the mucosa in contact with the quid.

19. The group also defined various areca and tobacco related oral lesions including oral submucous fibrosis. The descriptions can be found in Annex 2 to this paper.

### Studies published since 1993

20. There follows in annexes 3 and 4, summaries of a number of epidemiology studies published since 1993 that may be useful to the committee when considering whether it needs to update its advice. Annex 3 contains human epidemiology studies considering the effects of betel quid usage and oral cancer or pre-cancerous lesions. Some of these studies compared the risk of developing cancers between swallows and non-swallowers of the areca nut juice. The studies concluded that the cancer risk was higher in those who swallowed the juice. In addition studies, which describe possible relationships between areca nut use and incidence of other forms of cancer, such as liver cancer, as well as some animal and *in vitro* studies can be found in annex 4. It should be noted that whilst a correlation between the use of areca quid and an increase in liver cancer has been observed in some populations, it has also been reported that up to 37.5% of areca nut samples were infected with *Aspergillus flavus*, a fungus capable of producing aflatoxin B1 known for its hepatocarcinogenic properties (Tsai et al, 2001). Annex 5 contains the tables of epidemiology data assessed by IARC in 2003.

21. Much of the data available concerns the use of areca nut in betel quid or pan masala and the link with oral cancers. However, there is some human epidemiology data which links the use of areca nut with other cancers such as liver cancers. These, along with animal studies, may be useful in determining the carcinogenic potential of areca nut in other parts of the body other than the oral cavity, especially where areca nut may be used as a food ingredient.

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The animal studies may be useful in determining a possible mechanism. Summaries of these studies can be found in annex 4.

### Summary and Discussion

21. Areca nut is widely used an ingredient in betel quid or pan masala and may be used to a limited extent as a food ingredient.

22. In 1993/4 the COC concluded that betel quid chewing without tobacco was possibly carcinogenic in humans. This was based on a range of human epidemiology studies, animal carcinogenicity studies and *in vivo* and *in vitro* mutagenicity studies which showed mutagenic and carcinogenic activity of areca nut extracts and limited epidemiological evidence of a link between areca nut use and cancer in humans.

23. Areca nut contains a number of alkaloids, which form N-nitroso products that can be detected in saliva. These have been demonstrated to have mutagenic activity *in vitro*.

24. Animal studies have been carried out and some mechanisms for carcinogenicity have been suggested.

25. Betel quid chewing has been associated with an increased risk of oral cancer and pre-cancerous lesions in humans.

26. Since the previous COC assessment, further studies have been published, including epidemiology, animal carcinogenicity and *in vitro* mutagenicity.

27. Key points of new data include a number of epidemiology studies from various countries where areca nut is traditionally chewed which primarily look at the relationship between oral cancers and areca nut use. Some studies have looked at the relationship with other forms of cancer such as liver cancers however. Also some limited animal studies have been published which have attempted to identify mechanisms for the carcinogenic potential of areca nut extracts. Comparisons between those who swallowed the juice whilst chewing and those who didn't showed an increased risk for cancer development in those who swallowed the juice.

### Questions for the Committee

- 1) Do members consider that there is sufficient evidence of a link between areca nut usage and oral cancers to update its previous advice from 1993 that areca nut chewing was possibly carcinogenic in humans?
- 2) Can any conclusions be drawn about the potential effects of using areca nut as a food ingredient?

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**CC/06/18 Annex 1**

**COMMITTEE ON CARCINOGENICITY OF CHEMICALS IN FOOD, CONSUMER PRODUCTS AND THE ENVIRONMENT.**

**BETEL QUID, PAN MASALA AND ARECA NUT CHEWING**

This Annex contains previous COC papers from their assessments in 1993 and 1994 (CC/93/29 and CC/94/7).

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**CC/06/18 Annex 2**

**COMMITTEE ON CARCINOGENICITY OF CHEMICALS IN FOOD, CONSUMER PRODUCTS AND THE ENVIRONMENT.**

**BETEL QUID, PAN MASALA AND ARECA NUT CHEWING**

This Annex contains a paper from Zain *et al* 1999.

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**CC/06/18 Annex 3**

**COMMITTEE ON CARCINOGENICITY OF CHEMICALS IN FOOD, CONSUMER PRODUCTS AND THE ENVIRONMENT.**

**BETEL QUID, PAN MASALA AND ARECA NUT CHEWING**

The following studies were all assessed by IARC in 2003 and are in chronological order. The summary tables of epidemiology studies used by IARC can be found in annex 5 of this paper.

Human Studies: Epidemiology – oral cancer or pre-cancerous lesions and relationship to betel quid usage.

*A case control investigation of cancer of the oral tongue and the floor of the mouth in Southern India. Sankaranarayanan R., Suffy S.W., Day N.E., Nair K., Padmakumary G. Int J Cancer 44 (1989) 617-621.*

Cases for this study were 228 patients with biopsy proven squamous cell carcinoma of the tongue (188 cases) and floor of the mouth (40 cases). Two controls were selected for each case matched to within 5 years of age, sex and religion from a pool of patients attending the medical centre with non malignant conditions in areas other than the head and neck. 314 males and 139 female controls were selected. A detailed habit history for cases and controls was recorded by social workers during a direct interview. Betel quid chewing, betel quid-tobacco chewing, smoking, alcohol drinking and nasal snuff inhalation were assessed in terms of daily frequency, duration in years and age of initiating the habit. Statistical analysis by conditional logistic regression produced odds ratio (OR) estimates of relative risk and deviance chi-squared tests for effect. Estimates were adjusted for age when necessary and males and females were assessed separately as the only habit indulged in by females in non-negligible numbers was betel quid-tobacco chewing. Only 6 subjects chewed betel quid alone, thus this variable was not included in the analyses.

Excluding occasional users, the use of betel quid and tobacco in males and females had a significant predisposing effect for oral cancer. Although numbers were too small to yield conclusive results, there was a significant positive effect of occasional betel quid-tobacco chewing in both sexes. This may, however, be due to under-reporting of use because of social intolerance of betel quid chewing in some cultures. Relative risks associated with late adoption of habit (at or after age 21) shown a significant reduction in risk associated with late age at starting the betel quid-tobacco habit, in females a similar effect is seen, but this is not statistically significant.

In this study, the authors state that although betel quid-tobacco chewing is a risk factor for cancer of the tongue and floor of the mouth, the strength of association in terms of relative risk estimates seems to be lower than for other intra-oral cancers.

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*Betel quid chewing, cigarette smoking and alcohol consumption related to oral cancer in Taiwan. Ko Y.C., Huang Y.L., Lee C.H., Chen M.J., Lin L.M., Tsai C.C. J Oral Pathol Med (1995) 24: 450-3*

All patients from the Kaohsiung Medical College Hospital that received a confirmed diagnosis of oral cancer during the years 1992 and 1993 were included in this study. The study group consisted of 104 men and 3 women. The control group consisted of non-carcinoma patients treated during the same period in the ophthalmology and physical check-up departments but excluded peptic ulcer patients as betel quid has been shown to damage the gastric mucosa and cause stomach cancer in animals. Each oral cancer patient was randomly matched to control patients of the same age and sex. 93 cases were matched to two controls, 14 cases to one control and some cases could only be matched to within 5 years of age. 194 controls participated in this study. A trained interviewer filled in a structured questionnaire for each individual, collecting information on demographic characteristics, occupation, historical information on use of alcohol, cigarettes and betel quid. A habitual betel quid chewer was defined as someone who has chewed one quid a day for at least a year. A cigarette smoker was defined as someone who has smoked one cigarette or more per day for at least a year and a regular drinker was defined as someone consuming alcohol on more than four days per week. The duration of habits and daily amounts were also recorded. The risks of swallowing betel quid juice were also explored. The types of betel products were classified as areca nut with betel leaf, areca nut with betel fruit or mixed.

Odds ratios (OR's) with 95% confidence intervals were calculated to estimate the risk of developing oral cancer in relation to the risk factors in question.

The estimated odds ratios were found to be lower in better educated patients and white collar workers as compared to lesser educated patients and blue collar workers. The estimated OR's were elevated in cigarette smokers (OR = 8.4, 95% CI 3.5-20.4), alcohol consumers (OR = 3.2, 95% CI 1.8-5.6) and betel quid chewers (OR = 8.5 CI 4.4-16.2) as compared to abstainers. The adjusted OR's for factors such as alcohol drinking, cigarette smoking, and betel quid chewing remained significantly elevated even after logistic regression analysis. Betel quid chewing was shown to be the most potent risk factor for oral cancer. The OR's for patients with more than one habit were significantly higher than for those with only one habit. The incidence of oral cancer amongst individuals who were betel quid chewers, alcohol drinkers and cigarette smokers was 123-fold higher than that amongst abstainers. Swallowers of betel juice were more likely to develop oral cancer than non-swallowers.

*Cancer of mouth, pharynx and nasopharynx in Asians and Chinese immigrants resident in the Thames region. Warnakulasuriya K.A.A.S., Johnson N.W., Linklater K.M., Bell, J. Oral Oncology 35 (1999) 471-475.*

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The Thames Cancer Registry (TCR) covers 25% of the population of England and Wales (13.8 million residents). Registerable cases of cancer are collected from a variety of sources within the Thames Heath region by peripatetic officers who visit designated hospitals and other healthcare facilities within their area. A minimum set of data are recorded for each case and information on ethnicity was not collected until 1993. Diagnoses of lip, mouth, pharynx, nasal cavity, larynx and bronchial cancers were selected for the period 1986-1991 and tabulated by age, sex and cancer site. The stage of cancer was defined using a modified TNM system and a scale of TCR 1-4. Stage 1 is local, stage 2 shows extension beyond the organ of origin, stage 3 shows regional lymph node enlargement and stage 4 has distant metastasis.

Asians were defined as natives from India, Pakistan, Bangladesh, Nepal and Sri Lanka. Chinese were defined as natives from China, Hong Kong and Vietnam. All other nationalities were grouped under the "other nationalities" category.

A total of 7521 cancers were recorded in the database at these sites. Of these 232 were Asians (3.1%) and 67 were Chinese (0.9%). Males accounted for 5072 cases (67.4%) and females 2449 cases (32.6%). The mean age at diagnosis for the total sample was  $64.3 \pm 16.8$  years, for Chinese populations this was  $47.6 \pm 14.8$  years and for Asians this was  $51.6 \pm 34.8$  years. The mean ages of the Asian and Chinese migrant groups were statistically significantly lower than for the "other populations" group. Of the cancer sites recorded, 95/232 Asians (40.9%) had cancer of the mouth or pharynx whilst 45/67 Chinese (67.2%) had nasopharyngeal cancer. A significantly higher proportion of cancers occurred at these sites among ethnic migrant groups compared to other natives. Compared to other groups, Asians had a lower incidence of cancer of the floor of the mouth or palate, whereas incidence for all other types of cancer was higher in Asians than in the general population. The Chinese group showed a lower rate of cancer of the oropharynx or larynx compared to the general population. Asians and Chinese demonstrated a better outcome from oropharyngeal and nasopharyngeal cancers.

Whilst possible causes of these head and neck cancers were not investigated in this study, the authors do refer to the prevalence of betel quid chewing amongst Asian and Chinese populations and note a number of studies that have connected the chewing of betel quid with an increase in head and neck cancers. The Authors acknowledge that the use of betel quid and chewing tobacco is a significant contributory factor in the increased prevalence and earlier onset of these cancers in Asian and Chinese immigrant populations.

*Paan without tobacco: an independent risk factor for oral cancer. Merchant A., Husain S. S. M., Hosain M., Fikree F. F., Pitiphat W., Siddiqui A. R., Hayder S. J., Haider S. M., Ikram M., Chuang S-K., Saeed S. A., Int J Cancer 86 128-131 (2000)*

Paan is another name for betel quid. In this case-control study, 79 cases and 149 controls were recruited. The cases were biopsy proven primary cases of

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oral squamous cell carcinoma from three different hospitals. The cases and controls were matched by age, sex, hospital and time of admittance to hospital. All persons with a history of malignancy were excluded from the study. After obtaining verbal consent, a clinically trained interviewer administered a structured, pre-tested questionnaire in Urdu, examined the mouth and checked for the presence of oral sub-mucous fibrosis (OSF). In addition to socio-economic and demographic data, information on the use of cigarettes, bidis, hookah, naswar, betel quid, areca nut and alcohol. Regular users of naswar, betel quid or areca nut were defined as people who had used these products everyday for a month. Regular smokers were defined as people who had smoked cigarettes, bidis, hookah, cigars or a pipe daily for at least a month. The results from people reporting only areca nut use were combined with those for people reporting use of betel quid without tobacco as the results were found to be very similar.

The results of this study showed that people with OSF were 19.1 times more likely to develop oral cancer than those without OSF after adjusting for smoking, alcohol use and naswar and betel quid use with or without tobacco. Those people who used betel quid without tobacco were 9.9 times more likely to develop oral cancer than non-users after adjustment for other co-variates. The risk of oral cancer increased with higher intakes of betel quid with tobacco and without tobacco after adjusting for other co-variates.

*Chewing Tobacco, Alcohol and the Risk of Erythroplakia. Hasibe M., Mathew B., Kuruvilla B., Thomas G., Sankaranarayanan R., Maxwell Parkin D., Zhang Z.-F., Cancer Epidemiology, biomarkers and Prevention 9 (2000) 639-645*

The tobacco chewing referred to in this study was predominantly found as an ingredient in betel quid and therefore the results may be relevant for this assessment. Erythroplakia is the most advanced form of oral premalignant lesion found and presents as a chronic red mucosal lesion which cannot be attributed to traumatic, vascular or inflammatory causes.

A randomised oral cancer screening trial was carried out in Kerala, India due to the high incidence of this cancer in this area. 51.9% of the population of Kerala either smoke or chew tobacco products, primarily betel quid. A total of 59,894 subjects were recruited aged over 35 years and these subjects received three rounds of screening at 3 year intervals. In the first round of intervention, 49,174 eligible subjects were interviewed and screened in their homes by trained health workers. The 100 cases of erythroplakia and 47,773 controls were identified from this group.

A face to face interview was carried out using a structured questionnaire. Demographic information and a history of tobacco chewing, smoking and alcohol drinking habits were collected from each participant. Tobacco chewers were asked to describe whether or not they kept the tobacco in their mouths overnight and whether they swallowed the tobacco fluid. Consumption of fruit and vegetables and vitamin and iron supplements was also recorded along with blood pressure, body weight and height. Following the interview, the

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health workers carried out a systematic visual inspection of the buccal and labial mucosa, gingivae, bucco alveolar sulci, tongue, palate and floor of the mouth. 3585 subjects were referred for further investigation with a qualified dentist or oncologist. Erythroplakia was diagnosed in 100 subjects and controls were defined as having no oral condition or disease. Three models were used to assess exposure effects: (a) no covariates (crude analysis) (b) statistical adjustment for age, sex, education and BMI and (c) statistical adjustment for additional covariates including chewing tobacco (continuous, duration in years), smoking (continuous, pack-years) and drinking (continuous, duration in years) in the logistic regression model where appropriate.

Current chewing habits were very high among both male cases (74.5%) and female cases (83.7%). The prevalence of current smoking among women was low but was higher in the case group (8.2%) than the controls (1.8%). Smoking habits among men were slightly higher among cases (54.9%) than among controls (50.7%). Overall current smoking habit prevalence was 32.0% among cases and 20.1% among controls. Alcohol drinking amongst women was rare with 2.0% in the case group and 0.2% in the control group. The overall drinking habit prevalence was 21.0% amongst the case group and 5.8% amongst the control group. The adjusted odds ratio (OR) for regular chewers was 19.8 (95% confidence interval 9.8 - 40) after controlling for age, sex, education, BMI, pack-years of smoking and years of alcohol drinking. The adjusted OR was highest for current chewers, followed by the OR for past chewers and then the OR for occasional chewers. A strong dose response relationship was shown for the frequency and duration of tobacco chewing years with the risk of erythroplakia. Chewers who swallowed chewing tobacco fluid had a higher adjusted OR than those who did not swallow. Chewers who kept the tobacco in their mouth overnight also had a higher adjusted OR than those who did not. The study did not show an obvious interaction between alcohol and tobacco habits, but an interaction was found between alcohol and nutrition and the risk of developing erythroplakia.

The authors did point out that this study may be subject to bias as the health workers were aware of the exposure of the subjects to risk factors and therefore may have looked more thoroughly for lesions in the mouths of some individuals with greater exposure to these risk factors. The authors also stated that they did not define the exposure levels sufficiently and so some exposures may have been mis-classified. Also the number of cases was very small and therefore some estimates may not be sufficiently accurate.

*Independent and combined effects of tobacco smoking, chewing and alcohol drinking on the risk of oral, pharyngeal and esophageal cancers in Indian men. Znaor A., Brennan P., Gajalakshmi V., Mathem A., Shanta V., Varghese C. and Boffetta P., Int J Cancer 105 (2003) 681-686*

The study was conducted in 1993 and 1999 at two sites in India. The cases included 1563 oral (lip, tongue and mouth) 636 pharyngeal (oropharynx, hypopharynx and pharynx unspecified) and 566 oesophageal male cancer

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patients. Male patients (1711) with non-tobacco related cancers reported during the same time period were selected as disease controls. Additionally 1927 healthy male hospital visitors were also selected as controls.

Subjects were interviewed by trained social investigators using a structured questionnaire. Subjects were questioned about demographic and socioeconomic parameters, clinical history, tobacco and alcohol habits, diet and occupational exposures. Ever smokers, chewers and drinkers were defined as those who smoked, chewed or consumed alcohol at least once a day for a minimum of 6 months. Former smokers were defined as those who had stopped smoking 2 or more years previously. For pack years and alcohol consumption, tobacco and ethanol levels were standardised so that different products were weighted to reflect their strength e.g. cigars had a greater tobacco content than cigarettes and so had a weighting of twice that of cigarettes. Chewers were asked about their chewing habits and whether they included tobacco in their betel quid.

Odds ratios (ORs) and 95% confidence intervals (CIs) for the sites of interest were estimated according to smoking, chewing and alcohol habits using unconditional multiple logistic regression models. Interactions between the effects of the 3 habits were also assessed. All OR's were adjusted for age, centre, and level of education.

An increased risk for oral cancers of over 2-fold and a 60% increased risk for oesophageal cancers were observed among chewers without tobacco, whereas among chewers with tobacco the increase in risk was 5-fold for oral cancers and about 2-fold for pharyngeal and oesophageal cancers. Chewers with and without tobacco showed higher risks for cancer of the mouth than for cancer of the tongue, when compared to the smoking and alcohol drinking groups.

The authors concluded that the higher incidence of oesophageal cancers in chewers who did not chew tobacco could be due to the swallowing of the liquid extract produced by chewing the betel quid. The authors also stated that chewing betel quid seemed to have a role in the development of oral and oesophageal cancers.

*The precancer risk of betel quid chewing, tobacco use and alcohol consumption in oral leukoplakia and oral submucous fibrosis in southern Taiwan. Lee C.-H., Ko Y.-C., Huang H.-L., Chao Y.-Y., Tsai C.-C., Shieh T.-Y. and Lin L.-M. British Journal of Cancer 88 (2003) 366-372*

Cases were recruited from one area in southern Taiwan and all subjects who visited the hospital dental department during 1994 and 1995 with suspected oral leukoplakia (OL) or oral submucous fibrosis (OSF) were treated as potential cases. Only newly diagnosed patients that were histologically confirmed with either OSF or OL were included in the study. Patients with signs of both were excluded. Among the 219 oral precancerous patients, 125 cases (57.1%) suffered from OL and 94 cases (42.9%) suffered from OSF.

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Controls were selected randomly from the catchment area of the hospital from individuals aged over 15 years old. 876 matched controls participated in the study. Each subject was interviewed face to face about demographic information, occupations, betel quid chewing, smoking history, and alcohol drinking habits with a structured questionnaire. Subjects who had chewed one betel quid or more per day for at least 1 year were defined as ever-chewers. For the ever-chewers, a detailed history of their chewing habits was recorded including daily consumption, age of commencement, and duration of practice. The number of "pack-years" was calculated by multiplying the number of betel quid per pack (10) consumed daily by the years of chewing. Additionally the types of betel materials chewed were recorded (areca nut with inflorescence of Piper betel, areca nut with betel leaf or both mixed). Most betel quid use in this area of India includes tobacco.

Odds ratios (ORs) and 95% confidence intervals (CI) were estimated for the relative risk of development of precancerous lesions for the various lifestyle factors by using conditional logistic regression analyses. OL and OSF were investigated separately. ORs were adjusted for educational level and occupation. The risks for the two oral preneoplastic conditions among current chewers were 22.3-40.7-fold higher than among those who had never chewed betel quid. For ex-chewers, the risk was 7.1-12.1-fold higher than for non-chewers. Chewing betel quid with a piece of Piper betel inflorescence showed the highest risk for both oral diseases. For non-smokers and non-drinkers who did chew betel quid, the risks of oral OSF increased 39.3- and 26.5-fold respectively, compared with those who did not chew betel quid. Risks were increased further with the addition of further habits. Betel quid was found to be the strongest risk factor for OL and OSF. In this study, mainly younger patients were shown to have OSF compared with mainly older patients with OL. Although it was also noted that OSF patients generally started chewing at a younger age and chewed more betel quids per day. Betel quid chewing with no other confounding habits such as drinking or smoking seemed to be related to more cases of OSF than OL.

### Studies not assessed by IARC

The following studies were not assessed by IARC in 2003, but may provided useful additional information:

*Oral submucous fibrosis: a case controlled study in Chennai, South India. Ranganathan K, Uma Devi M, Joshua K, Kirankumar K, Saraswathi T.R. J Oral Pathol Med (2004) 33:274-7*

A hospital based case-controlled study on lifestyle and oral submucous fibrosis (OSF) was performed over a 3-year period in Chennai, India. 185 consecutive patients with OSF were paired with age- and sex-matched controls and their history was recorded in a pre-determined format by qualified dental surgeons. OSF is a pre-cancerous condition and has been closely linked with the chewing of areca nut. OSF is characterised by oral mucosal pallor, stomatopyrosis and mucosal rigidity which leads to restricted mouth

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opening, tongue protrusion and cheek flexibility. All of the OSF patients that took part in this study had a history of chewing betel quid; there is no mention of anyone using it as an ingredient and no mention of other somatic effects which may be of relevance to the use of areca nut as an ingredient.

*Risk factors for esophageal cancer in Coimbatore, Southern India: a hospital-based case-control study. Chitra S., Ashok L., Anand L., Srinivasan V., Jayanthi V., Indian Journal of Gastroenterology (2004) 23, 1, 19-21.*

Ninety patients with squamous cell carcinoma of the oesophagus were recruited with an equal number of age- and sex- matched controls that were undergoing upper GI endoscopy for dyspepsia. Information obtained using demographic data included occupation, religion, literacy and socioeconomic status. Other details noted in a pre-structured pro-forma included smoking, alcohol use, tobacco chewing and use of nasal snuff. Pre-illness dietary habits with special reference to salted pickle and fish, hot beverages and the amount of green vegetables consumed per week were recorded by a single person.

The majority of the cases and controls were of low socio-economic status. 22% of cases and 27% of controls were literate. The odds ratio between cases and controls for areca nut usage was 2.8 with a confidence interval of 1.3 - 5.9. The authors did not describe how they accounted for confounding factors in these results.

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**CC/06/18 Annex 4**

**COMMITTEE ON CARCINOGENICITY OF CHEMICALS IN FOOD, CONSUMER PRODUCTS AND THE ENVIRONMENT.**

**BETEL QUID, PAN MASALA AND ARECA NUT CHEWING**

Human Studies: Epidemiology – other cancers and areca nut usage.

The following studies were all assessed by IARC in 2003 and are in chronological order.

*Liver Cancer in Thailand. II. A case control study of hepatocellular carcinoma. Srivatanakul P. Parkin DM. Khlai M. Chenvidhya D. Chotiwan P. Insiripong S. L'Abbe KA. Wild CP. Int J Cancer 48, 329-332 (1991)*

Incidences of liver cancer in Thailand were reported to be very high. Sixty five case-control pairs consisting of 47 male and 18 female matched pairs fulfilled the diagnostic criteria and were included in this study. Twenty of the cases had undergone confirmatory histology for their condition and the remainder were included in the study on the basis of findings following an ultrasound or angiography. All except 16 of the cases had elevated serum alpha-fetoprotein ( $\geq 10$  ng/ml). This study suggested that regular use of betel quid conferred a higher risk of hepatocellular carcinoma although this was not statistically significant. The Authors noted that, in India where large numbers of people use betel quid, there did not seem to be a greater incidence of hepatocellular carcinoma when compared to other regions of the world such as Thailand.

*Betel quid chewing as a risk factor for hepatocellular carcinoma: a case-control study. Tsai JF, Chuang LY, Jeng JE, Ho MS, Hsieh MY, Lin ZY, Wang LY. British Journal of Cancer 84, 709-713 (2001)*

Pairs of age- and sex- matched hepatocellular carcinoma (HCC) patients and healthy controls (263 pairs) were enrolled for this study. A structured questionnaire was used to assess age, sex, educational level, alcohol consumption (quantity per week and duration and types of drinks consumed), cigarette consumption (number per day and length of use) and betel quid consumption (daily amount consumed, duration of the habit and type of betel quid consumed). All participants in the study were tested for hepatitis B surface antigens and antibodies to hepatitis C virus as well as traditional liver function tests to check enzyme activity.

The results of the study, following statistical analysis, indicated that there was an association between habitual betel quid chewing and risk for HCC complicating cirrhosis even after controlling for the possible confounding effects of cirrhosis. Whilst the effects of habitual quid chewing were less strongly associated with HCC than the effects of hepatitis B and C virus, the

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chewing of betel quid seemed to have a synergistic effect when both factors were found together, although this relationship was thought to be weak.

The authors concluded however, that there was insufficient evidence to conclude that betel quid chewing was a definite risk factor for cirrhotic HCC in subjects without chronic viral hepatitis.

### Studies not assessed by IARC

The following study was not assessed by IARC in 2003, but may provided useful additional information:

*Habitual betel quid chewing and risk for hepatocellular carcinoma complicating cirrhosis. Tsai J-F., Jeng J-E., Chuang L-Y., Ho M-S., Ko Y-C., Lin Z-Y, Hsieh M-Y., Chen S-C., Chuang W-L., Wang L-Y., Yu M-L., Dai C-Y., Medicine 83: 3 (2004)*

Two hundred and ten consecutive newly diagnosed cirrhotic patients with HCC, 210 newly diagnosed patients with cirrhosis alone and 210 healthy controls were recruited. Each healthy control was sex- and age-matched (+/- 5 years) to a patient with HCC. HCC was diagnosed by aspiration cytology or biopsy. Cirrhosis was diagnosed by liver biopsy, abdominal sonography or biochemical evidence of parenchymal damage. The healthy controls had normal serum aminotransferase levels.

A structured questionnaire was designed to obtain information on age, sex, educational level, previous history or surgery and blood transfusion, habits of smoking (number of cigarettes per day and duration of smoking), alcohol drinking (quantity and duration of drinking, types of alcoholic beverages) and betel quid chewing (daily amount consumed, duration of the habit, type of betel quid consumed). A habitual betel quid chewer was defined as a person chewing one quid or more per day for at least a year.

There was no statistical difference between the distribution and median age among the three groups. The prevalence of hepatitis B surface antigen (HbsAg) or antibodies to hepatitis C virus (anti-HCV) in the healthy control group was significantly lower than that in patients with cirrhosis alone or HCC patients. There was no significant difference in the prevalence of HBsAg or anti-HCV between HCC patients and patients with cirrhosis alone. At least one marker for HBsAg or anti-HCV was found in 89% of patients with cirrhosis alone and 94% of HCC patients. The extent of liver damage was measured using the Child-Pugh scale where A is classed as mild, B moderate and C is severe. Compared to patients with cirrhosis alone, HCC patients had a higher prevalence of being classed as Child-Pugh grade B and a lower prevalence of being Child-Pugh grade C. Habitual betel quid chewing was found in 11 healthy controls. This was significantly lower than in the other groups: 34 patients with cirrhosis alone and 52 patients with HCC were classed as habitual betel quid chewers.

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Using healthy controls as a reference group, multivariate analysis indicated that only betel quid chewing, HBsAg positivity and anti-HCV positivity were independent risk factors for HCC. To adjust the possible confounding effects of cirrhosis on risk of HCC, the cirrhotic patients and those with HCC were compared to patients with cirrhosis alone by multivariate analysis. The results indicated that betel quid chewing and HbsAg-positivity were independent risk factors for cirrhotic HCC.

Using betel quid non-users without chronic HBV/HCV infection as a reference group, the risk for HCC increased significantly in subjects with HbsAg alone or in patients co-infected with HBV/HCV infection. Patients in the group of betel quid chewers alone had a significantly higher risk for developing HCC. The risk for developing HCC in habitual betel quid chewers with either HBV or HCV infection was significantly higher than in those without HBV/HCV infection.

The authors concluded that there was an association between habitual betel quid use and risk for HCC complicating cirrhosis. After controlling the possible confounding effects of cirrhosis on the risk of HCC, habitual betel quid chewing was still an independent factor for HCC. However, among betel quid chewers without HBV/HCV infection, the association between betel quid chewing and HCC was not so strong. The evidence from this study did not indicate that betel quid is a risk factor for cirrhotic HCC in subjects without chronic viral hepatitis

### Animal Studies: Long term studies

This study was assessed by IARC in 2003.

*Long-term carcinogenicity of Pan Masala in Swiss mice. Bhisey R. Ramchandani AG. D'Souza AV, Borges AM. Notani PN. Int J Cancer 83, 679-684 (1999)*

Swiss mice were divided into two groups, an intermediate dosing group and a long-term dosing group. Fifty-four animals of each sex were used per group. The intermediate dosing group was sub-divided into three further groups that were dosed for 6, 12 or 18 months. The lifetime dosing group was dosed until the animals were moribund after which they were killed or until the study was terminated at 24 months. The mice were dosed with pan masala which consisted of areca nut mixed with catechu, lime, spices and flavouring agents (but not tobacco). Diets contained either 0%, 2.5% or 5% (equivalent to 3.75 and 7.5 g/kg bw/day) pan masala. The study found a statistically significant dose related increase in lung adenocarcinoma but not in liver and stomach cancers.

### Animal Studies: Short term studies

These studies were all assessed by IARC in 2003.

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*Evaluation of the Modifying Influence of Arecanut on the Garlic-Modulated Hepatic Detoxication System Enzymes, Sulfhydryl Content and Lipid Peroxidation in Mice. Singh A and Rao A.R. Teratogenesis, Carcinogenesis and Mutagenesis 15:127-134 (1995)*

Swiss albino mice of either sex were used in the study (6 animals/sex/dose group with a total of 9 groups). Areca nut was fed in the diet at levels of 0.25%, 0.5% or 1% (equivalent to 0.375, 0.75 and 1.5 g/kg bw/day) for a period of 35 days and then followed by oral administration of garlic for 10 days at either 20mg/kg bw/day or 100mg/kg bw/day. Controls were fed either a normal diet throughout the study or a normal diet until day 35 followed by 10 days of garlic supplementation at either 20 mg/kg bw/day or 100 mg/kg bw/day.

On day 45, the animals were sacrificed and the livers removed for determination of cytosolic glutathione S-transferase (GST) activity, cytochromes P450 and b5 and acid soluble sulphhydryl (-SH) content and malondialdehyde (MDA) content.

No significant differences were found in bodyweight gain, mortality or cytosolic and microsomal proteins between the experimental and control animals. Significant elevation of hepatic GST activity was observed in animals receiving a normal diet followed by both doses of garlic. The induced GST activity by garlic was significantly depressed in animals receiving both areca nut and garlic but this modulation was dose independent. The authors concluded that this effect may indicate a mechanism for the carcinogenicity of areca nut through a reduction in the rate of cellular detoxication by reduced GST levels.

Treatment with the higher doses of areca nut with garlic produced an elevation in cytochrome P450 and b5 enzymes although the lowest dose of areca nut did not produce a significant elevation in these enzymes. The Authors refer to a link between high levels of phase 1 enzymes such as these with the activation of procarcinogens to their reactive carcinogenic metabolites thus increasing the incidence of neoplasia.

The -SH level was increased significantly following administration of the higher doses of garlic, but these levels were suppressed following administration of the areca nut at the two highest levels. The authors conclude that this reduction in non-protein thiols that are thought to be free-radical scavengers may promote carcinogenesis.

No significant effects on MDA level were seen following administration of garlic at either dose, but the introduction of areca nut in the diet showed significant increases in cellular MDA levels. The authors concluded that this increase in MDA may show a significant effect by areca nut on the chemopreventative pathways in the cell.

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*Effect of arecanut on the black mustard (Brassica niger, L.)- modulated detoxication enzymes and sulfhydryl content in the liver of mice. Singh A and Rao AR. Cancer letters 72 (1993) 45-51.*

This study was carried out using a similar protocol to that used in the Singh and Rao study described above and the areca nut containing diets were prepared in exactly the same way.

The black mustard was administered as freshly ground powder mixed in with the diet at levels of 0.5% and 1% (equivalent to 0.75 and 1.5 g/kg bw/day respectively). The diets and areca nut were administered to the same time scales as those stated above and the black mustard was administered in the same way as the garlic used in the study above. The same number and type of animals were used and they were sacrificed and their livers prepared in the same way as the study mentioned above. Equivalent tests were carried out to those above, except the MDA assay was not completed.

The experimental animals did not show any significant change in mortality rate and body weight gain or cytosolic protein and microsomal protein over the control animals. Significant elevation of hepatic GST activity was observed in animals receiving a normal diet followed by both doses of black mustard. The induced GST activity by black mustard was significantly depressed in animals receiving both areca nut and black mustard but this modulation was dose independent. The authors concluded that this effect may indicate a mechanism for the carcinogenicity of areca nut through a reduction in the rate of cellular detoxication by reduced GST levels.

The elevated cytochrome B5 activity was only evident in the control group receiving 1% black mustard. However, both doses of mustard were found to elevate the cytochrome P450 levels. The diet containing 1% areca nut was shown to further augment the elevated levels of cytochrome B5 caused by the black mustard. The lower doses of areca nut, however, had no effect on the black mustard elevated cytochrome B5 levels. The Authors refer to a link between high levels of phase 1 enzymes such as these with the activation of procarcinogens to their reactive carcinogenic metabolites thus increasing the incidence of neoplasia.

The -SH level was increased significantly following administration of both doses of black mustard, but these levels were suppressed following administration of the areca nut at the highest level only. The authors conclude that this reduction in non-protein thiols that are thought to be free-radical scavengers may promote carcinogenesis.

The authors concluded that black mustard could be a potential inhibitor in the process of carcinogenesis. They also concluded that areca nut reverses this effect and could be a potential carcinogen regardless of the level of mustard present in the diet because of its effects on GST, -SH and cytochrome levels.

In vitro studies.

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*Oxidative damage to DNA induced by areca nut extract (1996). Liu T-Y, Chen C-L, Chi C-W, Mutation Research 367 25-31.*

In Taiwan, Betel quid often contains fresh tender areca nut with husk, whereas in most parts of Asia, ripe, fully-grown areca nut without husk is used. The level of oral cancer in Taiwan is low compared to other betel quid chewing countries. This study was designed to quantify the amount of DNA damage caused by reactive oxygen species (ROS) produced by tender fresh areca nut extracts (ANE) and by ripe fully-grown ANE's. The method for producing the extracts was not described. The level of DNA damage was determined by measuring the levels of 8-hydroxy-2'-deoxyguanosine (8-OH-dG).

Herring sperm DNA and exponentially growing CHO-K1 cells were incubated with different levels of ANE and the levels of DNA damage were measured. Both tender and ripe ANE generated 8-OH-dG dose-dependently in this study. Ripe ANE consistently generated higher levels of 8-OH-dG than tender ANE. For example at 1mg/ml tender ANE induced a 1.9-fold increase in the level of 8-OH-dG and similarly prepared ripe ANE generated a 4.3-fold increase in 8-OH-dG. Iron (II) stimulated the formation of 8-OH-dG in DNA incubated with ripe and tender ANE. Ripe ANE (1mg/ml) and Fe<sup>2+</sup> (100 $\mu$ M) in combination produced a 3.8-fold increase in 8-OH-dG levels. Tender ANE (1mg/ml) generated less than a 2.1-fold increase in 8-OH-dG levels under similar conditions. This difference between tender and ripe ANE was significant at  $p < 0.05$ .

Ripe ANE was more cytotoxic than tender ANE to the CHO-K1 cells following incubation. Tender ANE incubated with CHO-K1 cells for 18h generated higher levels of 8-OH-dG than the control at 0.05, 0.1, 0.2 and 0.4 mg/ml, but the results were not statistically significant except at 0.4mg/ml. Ripe ANE incubated with CHO-K1 cells for 18h at 0.05, 0.1, 0.2 and 0.4 mg/ml induced 1.2-, 1.5-, 2.0- and 2.8-fold increases in 8-OH-dG levels, respectively. The cytotoxicity of ANE-treated CHO-K1 cells was positively correlated with the formation of 8-OH-dG in both tender ( $r=0.97$ ) and ripe ( $r=0.91$ ) ANE groups.

As ripe ANE was shown to be a potent cytotoxic agent when compared to tender ANE, the authors decided to look at the effects of iron (II) on ripe ANE-mediated cytotoxicity in CHO-K1. Addition of the iron chelating agent  $\alpha$ -phenanthroline (10 – 20  $\mu$ M) to CHO-K1 cells prior to ripe ANE (100  $\mu$ g/ml) exposure increased the viability of CHO-K1 cells 1.5- and 1.8- fold, respectively.

The authors concluded that the levels of catechin, a phenolic component of ANE, are 20 times higher in ripe ANE than in tender ANE. They state that both ripe and tender ANE generate 8-OH-dG dose dependently when incubated with herring sperm DNA. However ripe ANE consistently induced greater 8-OH-dG formation as compared with tender ANE and furthermore the presence of iron (II) enhanced the 8-OH-dG formation in DNA treated with

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ripe ANE more than that with tender ANE. The authors state that these conclusions support the theory that auto-oxidation of polyphenols by superoxide formation in an alkaline environment may be the mechanism by which areca nut causes oral cancers.

*Genotoxic and non-genotoxic effects of betel quid ingredients on oral mucosal fibroblasts in vitro. Jeng J.H., Kuo M.L., Hahn L.J., Kuo M.Y.P., J Dent Res 73 (5) 1043-1049 (1994)*

The authors carried out a number of genotoxicity studies on betel quid ingredients using oral mucosal fibroblasts.

Thirty grams of each betel quid ingredient (areca nut (AN), inflorescence of *piper betel* (IPB) and lime paste) were individually ground into a thick paste and extracted with de-ionised water. After being filtered, the extracts were lyophilised and redissolved in deionised water. All three extracts were then refiltered, divided into aliquots and frozen.

Normal oral mucosa was used from a healthy patient. Prepared mucosal cells were incubated in the presence or absence of the three extracts of the quid ingredients or with arecoline or catechin, two active components of areca nut.

The results from this test showed that arecoline decreased cell proliferation and cell survival in a dose dependent manner. Catechin and extracts of IPB and AN showed moderate dose dependent cytotoxicity at higher concentrations. Lime extract had no detectable cytotoxicity after incubation at the highest concentration tested (800 µg/mL).

Effects on cell proliferation were measured by monitoring *de novo* DNA synthesis with radio labelled thymidine. The results from this study demonstrated that lime extract stimulated oral mucosal fibroblast proliferation over a 5 day culture period. The cell numbers of the treated groups were 20-40% higher than those of untreated controls. In contrast extracts of AN, IPB, arecoline and catechin were either cytostatic or cytotoxic. These results were in agreement with those obtained by the thymidine incorporation assay. The inhibition concentrations [IC<sub>50</sub>] required to inhibit *de novo* DNA synthesis by extracts of IPB, AN, arecoline and catechin were 132.5, 107.6, 27.7 and 10.2 µg/mL respectively. The effect of inhibition was also dose-dependent. Arecoline at a concentration of 50 µg/mL almost completely inhibited the DNA synthesis of cells, whereas a concentration of 100 µg/mL lime extract increased DNA synthesis by about 23%.

A DNA precipitation assay was also carried out to measure DNA strand breakage. Hydrogen peroxide was used as the positive control.

The results from this study demonstrated that IPB and AN extracts showed evidence of DNA strand breaks at fairly cytotoxic concentrations (3 and 2 mg/mL respectively). Although arecoline and catechin had strong cellular cytotoxicity, no DNA strand breakage was detected, even at concentrations of

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up to 400 and 600  $\mu\text{g/mL}$ . Lime also showed no genotoxicity at high levels (3 mg/mL).

Inhibition of arecoline cytotoxicity by antioxidants was measured using fibroblasts in culture. Different concentrations of antioxidant (superoxide dismutase, catalase, mannitol, glutathione and L-cysteine) were added to each group to test the protective effects of each antioxidant. After incubation, cell numbers were measured.

The results from this experiment showed that glutathione could protect the cells from arecoline cytotoxicity in a dose dependent manner. Cysteine had a similar effect to glutathione, but its protective effect was not as pronounced. Superoxide dismutase, catalase and mannitol did not show any protective effects on arecoline cytotoxicity. Further studies on glutathione determined that glutathione was effective in preventing cytotoxicity caused by IPB extract but not by AN extract.

*Study of unscheduled DNA synthesis following exposure of human cells to arecoline and extracts of betel nut in vitro. Sharan R.N., Wary K.K., Mutation Research 278 (1992) 271-276.*

Extracts of areca nut were prepared (aqueous, acetic acid, hydrochloric acid and ethanol) and used to assess their effects on cell viability and the degree of unscheduled DNA synthesis in Hep 2 cells (derived from a human larynx carcinoma).

Shelled areca nuts (100g) were ground and suspended separately in either distilled water, 1% acetic acid, 0.1 N HCl or ethanol.

The Hep 2 cells were exposed to various concentrations of arecoline and the areca nut extracts.

The results from the cell viability test showed that arecoline toxicity was dose dependent beyond a concentration of  $10\mu\text{g/ml}$ . The aqueous extract and acetic acid extracts of areca nut did not show significant cell death as compared with arecoline. However,  $100\mu\text{g}$  aqueous extract and acetic acid extract showed approximately 40% reduction in viable cell population. Hydrochloric acid and ethanol extracts of areca nut showed dose-dependant cell death where a  $100\mu\text{g}$  dose showed approximately 65% reduction in viable cell number.

Following exposure to the areca nut extracts, UDS was assessed using radio-labelled thymidine and the radioactivity measured using liquid scintillation counting.

Exposure of cells to arecoline showed a dose dependent increase in incorporation of radio-labelled thymidine in the DNA. Other extracts induced UDS at lower levels with the lowest levels being hydrochloric acid and ethanol extracts of areca nut.

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**CC/06/18 Annex 5**

**COMMITTEE ON CARCINOGENICITY OF CHEMICALS IN FOOD, CONSUMER PRODUCTS AND THE ENVIRONMENT.**

**BETEL QUID, PAN MASALA AND ARECA NUT CHEWING**

This Annex contains tables of epidemiology data used by IARC in the assessment from 2003.

Note: For copyright reasons the documents in this annex will not be included when this paper becomes publicly available. The documents will be in the public domain and individuals can obtain it by application to the appropriate sources.

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