BETEL QUID AND ARECA NUT

Betel quid and areca nut were considered by previous IARC Working Groups in 1984, 1987 and 2003 (IARC, 1985, 1987, 2004). Since that time, new data have become available, these have been incorporated into the *Monograph*, and taken into consideration in the present evaluation.

1. Exposure Data

1.1 Constituents of betel quid

1.1.1 Definitions

Betel quid chewing is an ancient practice in the Indian subcontinent and many parts of Asia, and is still prevalent today. In modern times the term "betel quid" for most people is synonymous with "*pan*," a chewing item used in India and neighbouring countries.

The term "quid" denotes a substance or a mixture of substances that is placed and retained in the mouth, and often swallowed. Apart from areca nut it may contain a variety of ingredients, including betel leaf and tobacco (Zain *et al.*, 1999; IARC, 2004).

In India and neighbouring countries, dry areca nut pieces or tobacco may be chewed alone, as a mixture of areca nut, tobacco and slaked lime, or tobacco and slaked lime. Dry powdered ready-to-chew mixtures containing areca nut, catechu, lime, unspecified spices without betel leaf and with or without tobacco are sold commercially in India (Ramchandani *et al.*, 1998). The product that does not contain tobacco is called *pan masala* while the term *gutka* is used for the product that contains tobacco in addition to the ingredients of *pan masala* (Nair *et al.*, 2004). In the south-eastern part of China, unprocessed fresh areca nut is treated with maltose and lime. It is cut into pieces and chewed with a few drops of cassia oil (Tang *et al.*, 1997).

A *pan* comprises mainly betel leaf (Piper betel), areca nut (areca catechu), catechu and slaked lime. The basic ingredients may be supplemented with condiments, sweetening agents and tobacco as per individual preference (IARC, 1985). The ingredients are placed on the betel leaf and the leaf is folded into a triangular-shaped object to obtain a betel quid with or without tobacco. Like slaked lime, thick paste of catechu may be smeared on the betel leaf or small bits of dry catechu may be placed on the betel leaf before it is folded to form a *pan*. Three types of betel quid are consumed in Taiwan, China. These are *lao-hwa* quid, betel quid and stem quid (Yang *et al.*, 2001). *Lao-hwa* quid is prepared by inserting a piece of inflorescence of Piper betel L. and red lime into an unripe areca nut. Another variety of Taiwanese betel quid is prepared by wrapping two halves of an unripe areca nut and white slaked lime in a betel leaf. The third variety is similar to the *lao-hwa* quid except that stems

Table 1.1 Composition of the different types of areca-containing chewing substances

	Areca nut[a]	Betel[b]			Catechu[d]	Tobacco[e]	Slaked lime
		Leaf	Inflorescence	Stem[c]			
Areca nut	X						
Betel quid without tobacco	X	X			(X)[f]		X
Betel quid with tobacco	X	X			(X)[f]	X	X
Gutka	X				X	X	X
Pan masala[g]	X				X		X
Mawa	X					X	X
Mainpuri tobacco	X					X	X
Lao-hwa (Taiwan, China)	X[g]		X				X
Betel quid (Taiwan, China)	X[g]	X					X
Stem quid (Taiwan, China)	X[g]			X			X

[a] May be used unripe, raw or processed by baking, roasting or baking with sweetening, flavouring and decorative agents (see Table 1.2)
[b] In place of the leaf, the inflorescence or its stem may also be used (see Table 1.2)
[c] Stem of inflorescence
[d] In powdered or paste form (see Table 1.2)
[e] In flaked, powdered or paste form, with or without processing, with or without sweetening (see Table 1.2)
[f] (X) means optional
[g] Used in unripe form
Adapted from IARC (2004)

of Piper betel L. are used in place of the inflorescence (IARC, 2004). While flavouring agents may be added to the Taiwanese betel quid, it does not contain tobacco. Different types of areca nut-containing chewing products and their ingredients are listed in Table 1.1.

1.1.2 Main ingredients of a quid

Areca nut, the major constituent of a betel quid, is the fruit of the Areca catechu L., a palm tree that grows in South and South-East Asia and the Pacific islands.

The chemical composition of areca nut has been reported in many studies (Raghavan & Baruah, 1958; Shivashankar *et al.*, 1969; Arjungi, 1976; Jayalakshmi & Mathew, 1982). The major constituents are carbohydrates, fats, proteins, crude fibre, polyphenols, alkaloids and minerals. The concentrations of various constituents vary between raw and ripe areca nuts (Jayalakshmi & Mathew, 1982). Arecaidine, arecoline, guvacine and guvacoline are the four alkaloids conclusively identified in areca nut (Raghavan & Baruah, 1958; Huang & McLeish, 1989; Lord *et al.*, 2002). Areca nut also contains sodium, magnesium, calcium, vanadium, manganese and copper (Wei & Chung, 1997; Ridge *et al.*, 2001).

Betel leaf (Piper betel L.) is a vine cultivated in many South-Asian countries including India. It contains betel oil, which includes phenolic compounds such as hydroxychevicol, euginol phenol, and chevicol. Trace elements, vitamin C and carotenes are also present in betel leaf (Wang & Wu, 1996; Zaidi *et al.*, 2002).

Slaked lime is prepared from seashells or quarried from limestone in regions that are far from the sea. Seashells are roasted, finely powdered and water is added to make slaked lime paste. The pH of slaked lime obtained from seashells or limestone is similar (Bhonsle *et al.*, 1992).

Catechu is a common ingredient of betel quid. It is a reddish brown substance derived from the heartwood of the Acacia Catechu tree, which is indigenous to India and Myanmar. It is obtained from the resins extracted from the matrix of Acacia catechu or Acacia suma (Muir

Table 1.2 Forms of different betel quids that contain areca nut and regions where they are used

Some common names and spellings	Major ingredients	Country where used
Betel quid	Areca nut (fresh, unripe) alone or with lime	Southern China, Pacific Islands
	Areca nut (dried, unripe) alone or with lime	Hunan Province in China
	Areca nut (cured, ripe) alone or with lime	South Asia
	Areca nut (fresh, unripe) with lime and betel leaves	Taiwan, China, Hainan Island, Papua New Guinea and Pacific Islands
Lao-hwa quid	Areca nut (fresh, unripe) with lime and betel inflorescence	Taiwan, China (lao-hwa quid), Papua New Guinea
Stem quid	Areca nut (fresh, unripe) with lime and betel stem	Taiwan, China
	Areca nut (fresh, unripe) with betel leaves	Guam
	Areca nut (cured, ripe) with lime and betel leaves	South Asia
Pan or *paan*	Areca nut (cured, ripe) with lime, an additional source of catechins, flavourings and betel leaves	South Asia
Pan or *paan* with tobacco, (the most common form)	Areca nut (cured, ripe) with lime, an additional source of catechins, flavourings, tobacco and betel leaves	South Asia, parts of South-eastern Asia
Pan masala or *chaalia*	Areca nut (cured, ripe) with lime, catechu, flavourings and other chemicals	India (paan masala), Pakistan (chaalia)
Mawa, *kharra*	Areca nut (cured, ripe) with lime, catechu, flavourings and other chemicals and tobacco – a variant of pan masala – usually called gutka; similar products with different proportions and shavings of areca nut	India

& Kirk, 1960). The main constituents of catechu are catechin, catechu tannic acid, quercetin and catechu red (IARC, 2004). Catechu contains a variety of trace elements as well (Zaidi *et al.*, 2002).

The chewing tobacco added to a betel quid is prepared from sun-dried and partly fermented coarse leaves of *Nicotiana tabacum* and *Nicotiana rustica* (IARC 2004).

A list of different forms in which areca nut is used is given in Table 1.2 (Gupta & Warnakulasuriya, 2002; IARC, 2004).

1.2 Prevalence of use

1.2.1 Distribution of betel quid chewing worldwide

It has been estimated that betel quid is used by about 10–20% of the world's population and that globally up to 600 million users chew areca nut (Gupta & Warnakulasuriya, 2002). Users are distributed around the world, but concentrated in South and South-eastern Asia, including South-eastern China, Hainan Island and Taiwan, China, and the Pacific Islands, as well as in areas of immigration of peoples from South Asia, e.g. in the Malay peninsula, eastern and southern Africa, Europe and North America. Concern among health professionals over increasing use of areca nut among South Asians and in Taiwan, China, have led to increasing numbers of prevalence surveys in the past several years.

In South Asia, South-eastern Asia, and parts of the Pacific Islands, the most common way of chewing betel quid is by inserting smokeless tobacco in the quid. Betel quid is chewed exclusively without tobacco in Southern China, Taiwan, China and Papua New Guinea, but in these areas, most chewers are also cigarette smokers. Emigrants from these areas have

carried their betel quid practices to the countries of immigration.

In South Asia, dry mixtures of areca nut and betel quid related ingredients (minus the betel leaf) are prepared industrially and sold in sachets. The most popular form contains tobacco and is usually called *gutka*, a variant of *pan masala*. These forms are now being exported from India to over 50 countries. Surveys on the prevalence of areca nut use across the world are summarized in Table 1.3 (available at http://monographs.iarc.fr/ENG/Monographs/vol100E/100E-05-Table1.3.pdf) and Table 1.4 (available at http://monographs.iarc.fr/ENG/Monographs/vol100E/100E-05-Table1.4.pdf).

1.2.2 Prevalence by country or region

(a) Adults

Information from several countries, especially in South-eastern Asia, has indicated that areca nut usage may be dying out in Cambodia, Indonesia, Thailand, and Viet Nam as it has declined considerably and become confined to the older middle aged and elderly groups. In contrast, rapidly increasing prevalence of areca nut usage has been registered in India and Taiwan, China (IARC, 2004). This corresponds, in India, to the introduction of industrially manufactured areca nut products, especially *pan masala*, *gutka* and *mawa*, while betel quid use has declined; and in Taiwan, China, to changes in marketing of betel quid, where young women sell betel quid and cigarettes on roadsides.

Surveys on prevalence of areca nut use have been conducted in India, Pakistan, Taiwan, China, the People's Republic of China, Thailand, the United Kingdom and the United States.

(i) India

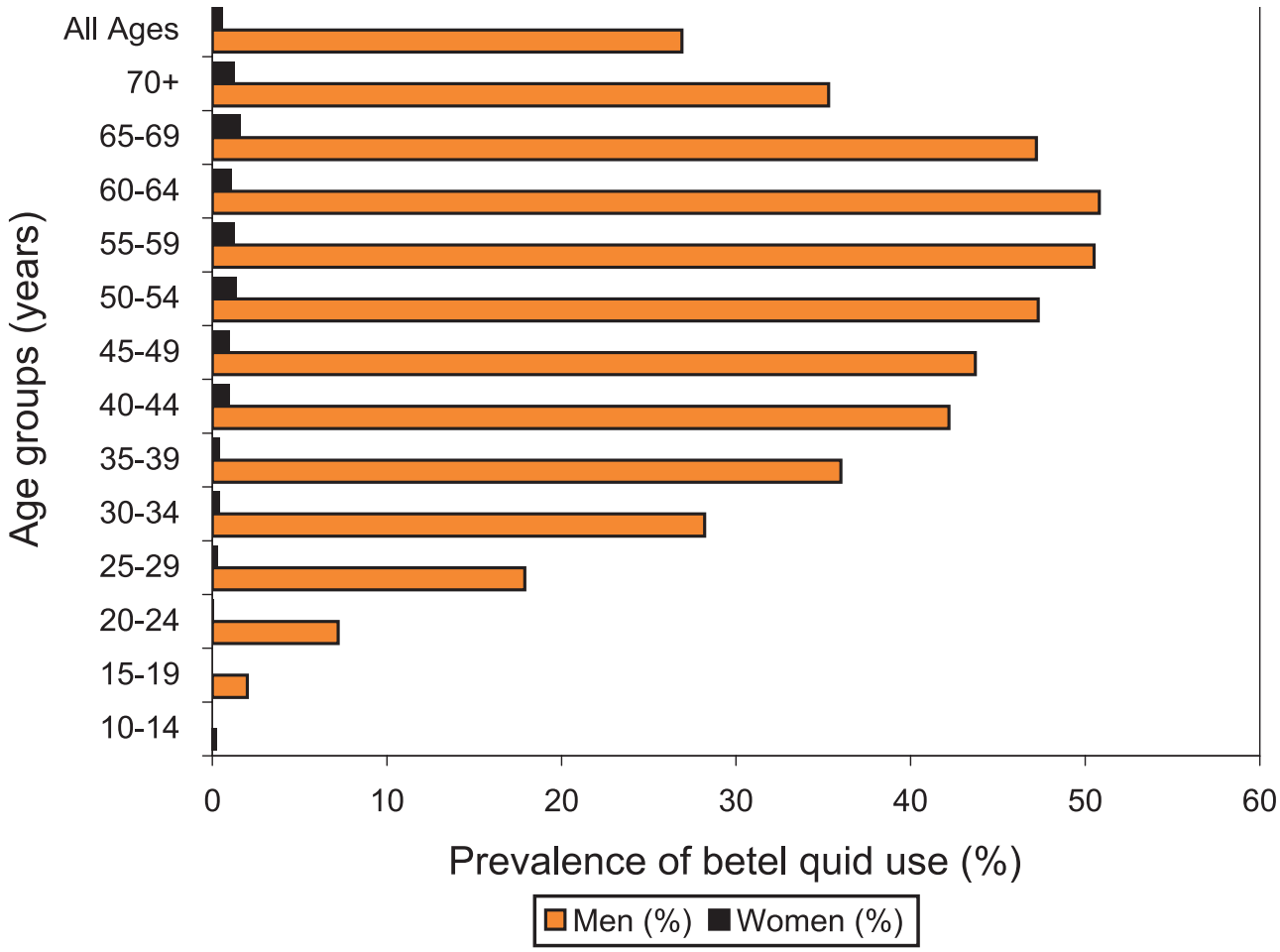
In India, prevalence of areca nut chewing nationwide can be estimated at around 30% of men and 7% of women, since the National Family Health Survey found 36.5% of men and 8.4% of women aged 15–49 years chewing some form of tobacco, "including *pan masala*, *gutka*, and other tobacco" (IIPS, 2007). [The Working Group noted that *pan masala* does not contain tobacco.] Since in many states of India tobacco is mainly chewed in the form of betel quid, and betel quid is mainly chewed with tobacco, the prevalence of "tobacco chewing" is only slightly higher than that of areca nut use. Local surveys have found betel quid use to be as high as 80% among both male and female adult school personnel in Mizoram; *gutka* was used by 44.8% of male school personnel in Sikkim (Sinha *et al.*, 2003). Reasons for use of tobacco products, including those containing areca nut (*gutka, mawa,* and *pan*, i.e. betel quid), among non teaching university personnel in Mumbai included peer pressure, the media (TV, advertisements, films, sports) as well as family influence (Bansode, 2002). In Chitrakoot, Madhya Pradesh, on the border with Uttar Pradesh, 46% of dental outpatients were current *gutka* users (Anwar *et al.*, 2005). In two districts of Uttar Pradesh in 2001, the prevalence of betel quid with tobacco use was only 2.0% (2.3% men, 1.4% women) (Chaudhry *et al.*, 2001). Fig. 1.1 and 1.2 present the age and sex distribution of use of betel quid with tobacco in Karnataka and Uttar Pradesh, respectively (Chaudhry *et al.*, 2001).

A statewide survey in 63 districts of Uttar Pradesh found that among 1209 *pan* [betel quid] and *pan masala* users, 94.4% (1141) used *pan* while 59.1% (n = 714) used both *pan* and *pan masala*, mostly by incorporating *pan masala* into *pan*. Additionally, 5.6% (n = 68) were exclusive *pan masala* users (Tripathi *et al.*, 2006).

(ii) Pakistan

A few recent studies in low-income urban areas of Karachi, Pakistan, have found 30–40% use of areca nut use among adults, as betel quid, areca nut by itself (*chaalia*), *gutka* and packaged *chaalia*, the equivalent of Indian *pan masala* (Mazahir *et al.*, 2006; Nisar *et al.*, 2007; Tanwir *et al.*, 2008). Among the ethnic groups in Karachi,

Fig. 1.1 Current use of betel quid with tobacco by age and sex in Karnataka



From Chaudhry *et al.* (2001)

the Mohajir appear to have a higher prevalence of use of areca nut products (Mazahir *et al.*, 2006). Adolescents prefer *chaalia* (Mazahir *et al.*, 2006), while adults over 30 years prefer betel quid (Tanwir *et al.*, 2008).

(iii) Bangladesh

A rural oral screening study in Bangladesh found that 40% of adult villagers of Kishore Ganj used areca nut with slaked lime and tobacco in various combinations (Eswar, 2002).

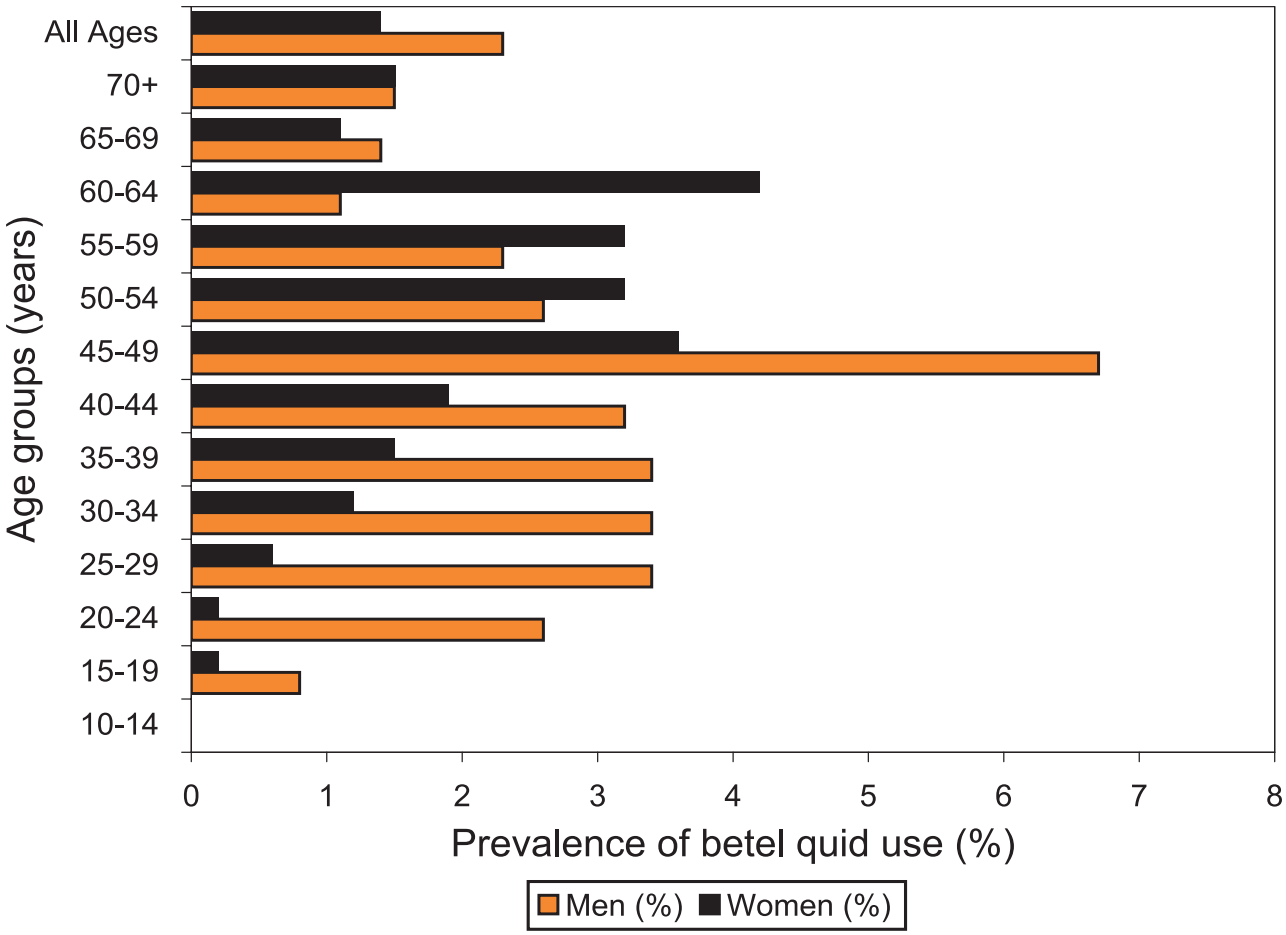
(iv) Thailand

In a survey of 4955 rural adults aged 30–89 years in Thailand, 17% reported using betel quid (Chatrchaiwiwatana, 2007). Betel quid chewing has been reported to be on the decline in Thailand as early as 1955 as a result of educational campaigns, and to be more common in the older population (Reichart, 1995).

(v) China

National surveys in Taiwan, China, indicate that 20.9% of men and 1.2% of women chew betel quid. Prevalence was highest in the

Fig. 1.2 Current use of betel quid with tobacco by age and sex in Uttar Pradesh



The age-wise *pan*-tobacco usage pattern of men and women differs significantly, but prevalence may be too low to be interesting.
From Chaudhry *et al.* (2001)

aboriginal population: 54.3% of men and 33.8% of women (Yap *et al.*, 2008). Betel quid chewing is more common among those who consume alcohol or who smoke. In another study, betel quid was chewed by 34.7% of aboriginal pregnant women (Chou *et al.*, 2009). Almost all betel quid chewers started chewing after they started smoking, particularly so among people over 25 years (Wen *et al.*, 2005a). Two thirds of the increase in betel quid chewing in the past decade has been attributed to the opening of the market to foreign cigarette brands in 1987, after which these cigarettes began to be placed in betel quid stalls. Betel quid sales increased dramatically as smokers turned to betel quid stalls to purchase cigarettes. Notably, 34% of betel quid chewers smoke, while 3% of non-smokers chew. Per capita consumption of betel quid increased 5 fold from 1981 to 1996 (Wen *et al.*, 2005b).

In the People's Republic of China, betel quid chewing is most common in, but not confined to, Yunnan and Hunan provinces and Hainan Island, all located in the south-eastern part of China. In Hunan, a land locked province where areca nut is not grown, the nuts are cut in half with the husk and dried, flavoured and industrially

packaged. Reports on prevalence of betel quid chewing from China are limited; a review of the Chinese literature from the late 1980s and early 1990s showed that prevalence in Hunan at that time was between 64.5% and 82.7% (Zhang & Reichart, 2007).

(vi) Pacific Islands

In the Pacific islands, betel quid chewing is high among adults: 72% of men and 80% of women in Palau (Ysaol *et al.*, 1996); and 76.8% of adults (83.0% men, 68.4% women) in the Solomon Islands (Tovosia *et al.*, 2007) use betel quid.

(vii) Immigrants

In areas of immigration of South Asians, such as the United Kingdom, people of Bangladeshi origin appear to have the highest prevalence of betel quid use (mostly with tobacco) from around 30% to over 90% in both men and women (IARC, 2004). In a recent study of Bangladeshi women in the United Kingdom aged 18–39 years, prevalence of betel quid chewing was 25–35% (Núñez-de la Mora *et al.*, 2007).

Changrani *et al.* (2006) from the United States found 25% current use of betel quid and 6% *gutka* use among people of Bangladeshi origin, but a reverse pattern among people of Indian-Gujarati origin, with 2% current betel quid use and 24% *gutka* use. Areca nut and betel quid chewing without tobacco have been reported from South Africa for many years among the population of Indian origin, but no recent studies are available.

(b) Children and youth

In India *pan masala* use and *gutka* use have increased among children, also in rural areas, as a mouth freshener and a status symbol. Even after an educational intervention and a local ban on *gutka* sales near schools, 46% of 986 rural school-children aged 10–15 years in Madhya Pradesh were using *gutka* regularly (Chaturvedi *et al.*, 2002). In a state-wide survey in Uttar Pradesh in 2002, 9.9% of students in 8th through 10th grades (mostly 13–15 years) were currently using *gutka* (at least once in 30 days) (Sinha & Gupta, 2005). In a survey of 385 rural adolescents (15–19 years) in villages of Wardha, Maharashtra in 2008, 17.1% were using *gutka* (31.7% boys, 4.0% girls) and 26.2% (54.1% boys, 1.0% girls) were using *kharra* [*mawa*] (Dongre *et al.*, 2008). In a very small unpublished survey in a small town in Gujarat in 1999, 16% of boys in 8th and 9th grades were using *gutka* (Gupta & Ray, 2002). In a survey in Delhi, 10.2% of 2387 urban students aged 10–18 years were using betel quid with tobacco (Kapil *et al.*, 2005a).

Male college students (16–23 years) in Karnataka in 1998 who smoked cigarettes said they sometimes substituted *gutka* for a cigarette when and where it was inappropriate to smoke. Though believed it to be very harmful and addictive, some students used *gutka* to help themselves quit smoking and then switched to *pan masala* to wean themselves off *gutka*. Those who believed that *gutka* was more addictive than cigarettes thought this strategy was unwise (Nichter *et al.*, 2004).

Use of areca nut products is prevalent among youth in other South Asian countries. In a deprived area of Karachi, Pakistan, 47.2% of school boys aged 10–16 years were using areca nut by itself; [12.6%] used betel quid without tobacco and 16.1% used *gutka* or other smokeless tobacco products (Rozi & Akhtar, 2007). In Pokhara City, Nepal, ever use of *pan masala* and *gutka* by adolescents aged 13–15 years was found to be 51.4% in boys and 30.3% in girls (Paudel, 2003).

Among adolescent students in Taiwan, China, overall use of betel quid was 3.9% (6.6% boys, 1.5% girls), and ranged from 0.8% in cities to 4.3% in towns and 7.6% in villages (Wang *et al.*, 2003a). The most variance in prevalence of betel quid use is found by type of school, ranging from 10.3% boys and 1.4% girls in general schools to 20.6% in boys and 4.7% in girls in agricultural schools (Wang *et al.*, 2004). It was found that 26.9% of

ex-chewers and 22.3% of current chewers tried betel quid for the first time in elementary school. Peer pressure was the most important influence, followed by fathers giving the nuts to their child (Wang *et al.*, 2003a). A survey of fourth grade elementary students in northern Taiwan, China, found ever chewers to be 10.8% in city schools and 56.6% in mountain schools, reflecting a higher prevalence in aboriginal population in mountainous areas (Huang *et al.*, 2009).

Areca nut and tobacco practices and products from India are also becoming popular among children in countries of immigration, especially but not exclusively among children of Asian origin. A study from United Republic of Tanzania found that *gutka* and other packaged oral products imported from India were beginning to be used by adolescent students there, including those not of Indian origin (Kaduri *et al.*, 2008). In the United Kingdom, betel quid chewing is known to be taken up by students of South Asian origin, and *gutka* is available and has been reportedly used among them (Warnakulasuriya, 2002). In East London, three quarters of the students of Bangladeshi origin in ninth grade had ever tried betel quid [apparently no question was asked about *gutka*] (Jayakody *et al.*, 2006).

2. Cancer in Humans

2.1 *Cancer of the oral cavity*

Studies on betel quid and oral cavity cancers have been conducted in India, Pakistan, Sri Lanka, Thailand, Taiwan, China, South Africa, and Papua New Guinea. These populations differ in their patterns of betel quid use and the products and ingredients added to the quid (Yang *et al.*, 2001; Gupta & Warnakulasuriya, 2002). Betel quid is defined as any chewing substance that contains areca nut. In evaluating betel quid exposure, the main distinction is whether or not tobacco is added to the betel quid. When this was not explicitly stated, tobacco was considered to be absent from the betel quid only if the study was conducted in a region/ethnicity where it is uncommon/unlikely for tobacco to be added to the betel quid (i.e. Taiwan, China). However if there was good background information that the habit of betel quid chewing was very prevalent in a region/ethnicity (i.e. India, Sri Lanka, persons of Indian descent), studies that assessed "tobacco chewing" and mentioned betel quid chewing in the exposure assessment were considered as exposure to betel quid with added tobacco. If this background information was not available, studies that assessed tobacco chewing without mention of betel quid chewing were excluded from both this *Monograph* and the *Monograph* on Smokeless Tobacco in this volume. Studies that evaluated genetic polymorphisms as a main effect and their interaction with betel quid chewing were also excluded even if a crude relative risk for betel quid chewing could be calculated.

2.1.1 *Overview of studies*

When the carcinogenicity of betel quid was first evaluated in 1984 (IARC, 1985), the relationship between betel quid chewing and cancer of the oral cavity had been investigated in four cohort studies (Wahi, 1968; Mehta *et al.*, 1972; Bhargava *et al.*, 1975; Gupta *et al.*, 1980) and many case–control studies (Orr, 1933; Sanghvi *et al.*, 1955; Sarma, 1958; Khanolkar, 1959; Shanta & Krishnamurthi, 1959, 1963; Chandra, 1962; Wahi *et al.*, 1965; Hirayama, 1966; Jussawalla & Deshpande, 1971; Khanna *et al.*, 1975; Kwan, 1976; Notani & Sanghvi, 1976; Simarak *et al.*, 1977; Jafarey *et al.*, 1977). The effect of betel quid without added tobacco was investigated in only a few studies.

When the available evidence was evaluated in 2003 (IARC, 2004), 15 additional case–control studies had been published (Sankaranarayanan *et al.*, 1989a, b, 1990a; Nandakumar *et al.*, 1990; van Wyk *et al.*, 1993; Rao *et al.*, 1994; Ko *et al.*,

1995; Lu *et al.*, 1996; Rao & Desai, 1998; Wasnik *et al.*, 1998; Dikshit & Kanhere, 2000; Merchant *et al.*, 2000; Balaram *et al.*, 2002; Chen *et al.*, 2002; Znaor *et al.*, 2003).

The case–control studies of cancer of the oral cavity that clearly distinguish betel quid without and with added tobacco are summarized in Table 2.1 (available at http://monographs.iarc.fr/ENG/Monographs/vol100E/100E-05-Table2.1.pdf) and Table 2.2 (available at http://monographs.iarc.fr/ENG/Monographs/vol100E/100E-05-Table2.2.pdf) respectively. The derived relative risk estimates ranged from 1.5 to 58.4 for use of betel quid without tobacco and from 0.7 to 45.9 for betel quid with tobacco. Most of these studies adjusted for potential confounders such as tobacco smoking, use of smokeless tobacco, alcohol use, and HPV infection.

Since then there have been several publications assessing the association between betel quid chewing and cancer of the oral cavity (Wen *et al.*, 2005a; Yang *et al.*, 2005a; Subapriya *et al.*, 2007; Thomas *et al.*, 2007; Muwonge *et al.*, 2008; Yen *et al.*, 2008b; Jayalekshmi *et al.*, 2009). The relative risk estimates from the three case–control studies ranged from 2.03 to 5.4 for use of betel quid without tobacco and from 3.19 to 11.8 for betel quid with tobacco.

2.1.2 Risk by type of agent

(a) Betel quid without added tobacco

An increased risk of statistical (or borderline) significance associated with betel quid chewing without tobacco was reported from all case–control studies of cancer of the oral cavity that considered this after adjusting for smoking and/or alcohol intake (Nandakumar *et al.*, 1990; Ko *et al.*, 1995; Lu *et al.*, 1996; Wasnik *et al.*, 1998; Dikshit & Kanhere, 2000; Merchant *et al.*, 2000; Balaram *et al.*, 2002; Chen *et al.*, 2002; Znaor *et al.*, 2003; Subapriya *et al.*, 2007; Thomas *et al.*, 2007; Muwonge *et al.*, 2008; Table 2.1 online). Znaor *et al.* (2003) reported an increased risk for cancer of the oral cavity associated with the use of betel quid without added tobacco in non-smoking and non-drinking men that had no other known risk factors (OR, 3.39; 95%CI: 2.04–5.66) after adjustment for age, centre and education level. In a study in Pakistan (Merchant *et al.*, 2000) an increased risk for oral cancer was associated with the use of betel quid without added tobacco, after adjusting for smoking and alcohol. Data from Taiwan, China and Papua New Guinea, where betel quid is generally used without tobacco, also support this association.

In three cohort studies (Bhargava *et al.*, 1975; Yang *et al.*, 2005a; Yen *et al.*, 2008b) increased risks of cancer of the oral cavity among betel quid chewers were found (IARC, 2004; Table 2.3 available at http://monographs.iarc.fr/ENG/Monographs/vol100E/100E-05-Table2.3.pdf); in two of these studies (Bhargava *et al.*, 1975; Yang *et al.*, 2005a), incident cancers only occurred among betel quid chewers. Yen *et al.* (2008b) reported that the use of betel quid was significantly associated with cancer of the oral cavity in subjects who were neither smokers nor drinkers (OR, 10.97; 95%CI: 3.22–37.34). In a nested case–control study conducted in India, betel quid use without added tobacco was associated with cancer of the oral cavity overall (Muwonge *et al.*, 2008). Among women [with a low prevalence of smoking in this population], the risk was highly significant after adjusting for smoking and drinking.

In a meta-analysis Thomas *et al.* (2007) included 11 independent studies that examined risk of cancer of the oral cavity associated with chewing betel quid without added tobacco (Chandra, 1962; Hirayama, 1966; Jafarey *et al.*, 1977; Ko *et al.*, 1995; Lu *et al.*, 1996; Dikshit & Kanhere, 2000; Merchant *et al.*, 2000; Balaram *et al.*, 2002; Chen *et al.*, 2002; Znaor *et al.*, 2003; Thomas *et al.*, 2007). These studies either excluded smokers or controlled for smoking. The overall odds ratio estimated for betel quid

without tobacco was 2.14 (95%CI: 1.06–4.32) among non-smokers and 3.50 (95%CI: 2.16–5.65) in studies that adjusted for smoking.

(b) Betel quid with added tobacco

Significantly increased risks for cancer of the oral cavity associated with chewing betel quid with added tobacco were observed in all of the case–control studies that considered this (Orr, 1933; Sanghvi *et al.*, 1955; Sarma, 1958; Khanolkar, 1959; Shanta & Krishnamurthi, 1959, 1963; Chandra, 1962; Hirayama, 1966; Wahi *et al.*, 1965; Wahi, 1968; Jussawalla & Deshpande, 1971; IARC, 1985; Sankaranarayanan *et al.*, 1989a, b, 1990a; Nandakumar *et al.*, 1990; van Wyk *et al.*, 1993; Rao *et al.*, 1994; Rao & Desai, 1998; Wasnik *et al.*, 1998; Dikshit & Kanhere, 2000; Merchant *et al.*, 2000; Balaram *et al.*, 2002; Znaor *et al.*, 2003; Subapriya *et al.*, 2007; Muwonge *et al.*, 2008; Table 2.2 online) and in two cohort studies (Wahi, 1968; Gupta *et al.*, 1980). All of the case–control studies adjusted for smoking and some studies additionally adjusted for alcohol use.

(c) Areca nut and betel inflorescence

The risk of chewing areca nut alone without other ingredients (particularly tobacco) was examined in one Indian study (Wasnik *et al.*, 1998), a suggestive increased risk of cancer of the oropharynx was reported (OR, 2.6; 95%CI: 0.9–7.7).

In a study in Taiwan, China, the risk for cancer of the oral cavity was highest among those who chewed only unripe areca nut (OR, 11.6; 95%CI: 3.7–36.9; 41 exposed cases) compared with those who chewed betel leaf alone (OR, 0.1; 95%CI: 0.0–6.3; 1 exposed case) or a mixture of the two (OR, 8.5; 95%CI: 2.7–26.3; 34 exposed cases) after adjustment for education, occupation, smoking and drinking (Ko *et al.*, 1995).

2.1.3 Exposure–response relationship

(a) Intensity and duration

An exposure–response relationship (by various metrics of exposure such as intensity, duration, age at starting or betel quid-years) between betel quid chewing and oral cancer was demonstrated in several studies (Orr, 1933; Sankaranarayanan *et al.*, 1989a, b, 1990a; Nandakumar *et al.*, 1990; Rao *et al.*, 1994; Lu *et al.*, 1996; Rao & Desai, 1998; Wasnik *et al.*, 1998; Dikshit & Kanhere, 2000; Merchant *et al.*, 2000; Balaram *et al.*, 2002; Znaor *et al.*, 2003; Thomas *et al.*, 2007; Muwonge *et al.*, 2008; Jayalekshmi *et al.*, 2009; IARC, 1985; Table 2.4 available at http://monographs.iarc.fr/ENG/Monographs/vol100E/100E-05-Table2.4.pdf). Not all reports distinguished whether or not tobacco was added to the betel quid, though many controlled for smoking, consumption of alcoholic beverages, or both. [Merchant *et al.* (2000) did not present odds ratios and corresponding 95% confidence intervals were not present for the tertiles of paan-years (P for trend = 0.004 for paan-years with tobacco and P for trend = 0.0008 for paan-years without tobacco.]

(b) Cessation

The effect of cessation has not been examined extensively. In one study, having quit chewing betel quid with added tobacco 10 years earlier or within 10 years did not demonstrate a beneficial effect in either sex (Balaram *et al.*, 2002). Znaor *et al.* (2003), however, were able to demonstrate a decrease of risk for cancer of the oral cavity after 10 years or more of quitting. [Znaor *et al.* (2003) did not distinguish whether or not tobacco was added to the quid for this analysis.]

2.1.4 Anatomical subsites of cancer

Some authors reported site-specific (gingiva, tongue, mouth) differences in relative risk (Sanghvi *et al.*, 1955; Khanolkar, 1959; Shanta

& Krishnamurthi, 1959, 1963; Chandra, 1962; Hirayama, 1966; Wahi, 1968; Jussawalla & Deshpande, 1971; Kwan, 1976; Sankaranarayanan *et al.*, 1989b; Rao & Desai, 1998; Znaor *et al.*, 2003). In non-smokers and non-drinkers, Wahi (1968) reported the highest risks for the buccal mucosa, gingiva and lip combined associated with chewing betel quid with tobacco. After adjusting for smoking and alcohol, Znaor *et al.* (2003) reported higher risks for the mouth compared to the tongue, for betel quid use both with or without tobacco.

2.1.5 Population characteristics

In most studies, markedly higher estimates of risk for cancer of the oral cavity were found in women than in men for betel quid chewing, with or without tobacco (Sanghvi *et al.*, 1955; Chandra, 1962; Shanta & Krishnamurthi, 1963; Hirayama, 1966; Wahi, 1968; Notani & Sanghvi, 1976; Jafarey *et al.*, 1977; Simarak *et al.*, 1977; Sankaranarayanan *et al.*, 1989a, b, 1990a; Nandakumar *et al.*, 1990; van Wyk *et al.*, 1993; Rao *et al.*, 1994; Rao & Desai, 1998; Dikshit & Kanhere, 2000; Balaram *et al.*, 2002; Znaor *et al.*, 2003; Muwonge *et al.*, 2008; Yen *et al.*, 2008b; Jayalekshmi *et al.*, 2009).

2.1.6 Interactions

Among the many studies of cancer of the oral cavity that have examined multiple habits with 2- and 3-way combinations among tobacco smoking, alcohol drinking and betel quid chewing, only a few studies formally tested for interaction. Table 2.5 (available at http://monographs.iarc.fr/ENG/Monographs/vol100E/100E-05-Table2.5.pdf) and Table 2.6 (available at http://monographs.iarc.fr/ENG/Monographs/vol100E/100E-05-Table2.6.pdf) provide data from studies reporting combined odds ratios for combination of habits. Findings are not consistent across studies. In general interaction is at an additive level only.

In three studies the interaction between betel quid chewing without added tobacco and tobacco smoking was examined (Ko *et al.*, 1995; Znaor *et al.*, 2003; Thomas *et al.*, 2007) and it was found that risk was highest in those who smoked, drank alcohol and chewed betel quid. For subjects consuming betel quid (with or without added tobacco) there was an interaction with smoking among non-alcohol drinkers by Znaor *et al.* (2003) ($P = 0.00$). However, in another study from India, there was no suggestion of an interaction between betel quid chewing with or without added tobacco and tobacco smoking (Muwonge *et al.*, 2008). For those chewing betel quid with tobacco, interactions with tobacco smoking were found in a few other studies (Sankaranarayanan *et al.*, 1989a, b, 1990a; Dikshit & Kanhere, 2000), and were significant in some (Sankaranarayanan *et al.*, 1989a, b, 1990a). In a study that examined 2-way interactions between betel quid chewing and consumption of alcoholic beverages, evidence suggestive of a synergistic effect was observed in men who chewed betel quid with tobacco (Znaor *et al.*, 2003). However, in another study from India, there was no suggestion of an interaction between betel quid chewing with or without added tobacco and consumption of alcoholic beverages (Muwonge *et al.*, 2008).

The 3-way interaction of betel quid chewing, tobacco smoking and consumption of alcoholic beverages was considered in few studies (Table 2.7 available at http://monographs.iarc.fr/ENG/Monographs/vol100E/100E-05-Table2.7.pdf). While the interactions were found significant in two studies (Sankaranarayanan *et al.*, 1989a; Znaor *et al.*, 2003) and, in 2 other studies from India there was no suggestion of an interaction (Sankaranarayanan *et al.*, 1990a; Muwonge *et al.*, 2008).

2.1.7 Population attributable risk

The population attributable risk fraction of cancer of the oral cavity was observed to be 66% for chewers of betel quid with tobacco in Bhopal, India (Dikshit & Kanhere, 2000). In a study in Trivandrum, India, the adjusted population attributable risk fraction estimated for women for having ever chewed (81.2%) was nearly double that of men (42.6%) (Muwonge *et al.*, 2008).

2.2 Precancerous lesions of the oral cavity

Precancerous lesions or potentially malignant disorders of the oral cavity precede cancer development and largely contribute to the burden of cancer of the oral cavity in South Asia. In the restricted geographic locations where people consume betel quid, the disorders of concern are leukoplakia, erythroplakia, erythroleukoplakia, and oral submucous fibrosis (Warnakulasuriya *et al.*, 2007).

In India, betel quid or areca nut use either alone or in combination with tobacco account for most of the leukoplakia cases (Smith *et al.*, 1975; Gupta *et al.*, 1980). The studies examining the association between betel quid chewing and oral precancerous lesions undertaken before 2004 were reviewed in previous *IARC Monographs* (IARC, 1985, 2004). The relative risk estimates for oral leukoplakia (Hashibe *et al.*, 2000a; Shiu *et al.*, 2000; Yang *et al.*, 2001; Lee *et al.*, 2003), erythroplakia (Hashibe *et al.*, 2000b), oral submucous fibrosis (Sinor *et al.*, 1990; Maher *et al.*, 1994; Gupta *et al.*, 1998; Hazare *et al.*, 1998; Shah & Sharma, 1998; Hashibe *et al.*, 2002) ranged from 7 to around 30. Other studies of oral submucous fibrosis reported high risks associated with betel quid use: RR 32 (95%CI: 6–177) for betel quid without tobacco and RR 154 (95%CI: 34–693) for areca nut alone (Maher *et al.*, 1994); RR 75.6 among users of mawa (a mixture of areca nut, tobacco and slaked lime) (Gupta *et al.*, 1998) and RR 49.2 (95%CI: 24.3–99.6) among betel quid chewers (with and without added tobacco) (Hashibe *et al.*, 2002).

Since then new evidence has accumulated on the association between betel quid and areca nut use and oral pre-cancer. Some of these studies evaluated the risks for combinations of oral mucosal disorders grouped together (oral precancer; oral potentially malignant disorders) or separately for leukoplakia, erythroplakia or oral submucous fibrosis. Data from these new studies are summarized in Table 2.8 (available at http://monographs.iarc.fr/ENG/Monographs/vol100E/100E-05-Table2.8.pdf). In two cross-sectional studies from Sri Lanka and Taiwan, China, where betel quid is used without added tobacco, significant associations for areca quid/betel quid chewing with oral precancerous lesions were found. The risks were 8.40 (95%CI: 5.13–13.75) in Taiwan, China (Chung *et al.*, 2005) and 3.01 (95%CI: 2.25–4.0) in Sri Lanka (betel quid with or without added tobacco) (Ariyawardana *et al.*, 2007). Both studies were adjusted for tobacco smoking and alcohol drinking. In several case–control studies (in India, Sri Lanka, Taiwan, China and Papua New Guinea), use of betel quid without added tobacco in non tobacco smokers and/or non alcohol drinkers was associated with an increased risk in oral precancerous lesions (Jacob *et al.*, 2004; Yang *et al.*, 2005b; Thomas *et al.*, 2008). The risks for oral leukoplakia and erythroplakia were significantly elevated in betel quid chewers with tobacco, as well as in those chewing betel quid without tobacco in an Indian population (Jacob *et al.*, 2004) and among Taiwan, China, Chinese populations, who do not add tobacco to their betel quid (Shiu *et al.*, 2000; Chen *et al.*, 2006; Yen *et al.*, 2007). In some of these studies significant exposure–response relationships were found (Jacob *et al.*, 2004; Yang *et al.*, 2005b; Yen *et al.*, 2008a). Shiu *et al.* (2000) found that betel quid use without added tobacco is a significant factor influencing malignant transformation of oral leukoplakia (OR, 4.59;

95%CI: 1.25–16.86). Ho *et al.* (2009) however found no positive association with betel quid use without added tobacco and malignant transformation of existing premalignant disorders (OR, 0.98; 95%CI: 0.36–2.97). Yang *et al.* (2005b) reported a significant positive association for betel quid chewing without tobacco (among non smokers) with oral submucous fibrosis from a case–control study in Taiwan, China (OR, 4.51; 95%CI: 1.20–16.94). In a further study from Sri Lanka, Ariyawardana *et al.* (2006) found that betel quid chewing with and without tobacco was the only significantly associated risk factor in oral submucous fibrosis (OR, 171.8; 95%CI: 36.35–812.25) and there was no interaction with either tobacco smoking or alcohol use. However, alcohol drinking had a significant effect on the malignant transformation in oral submucous fibrosis, while areca/betel quid chewing showed no association (Ho *et al.*, 2007). In a study from the People's Republic of China, duration of betel quid use without added tobacco was associated with a significantly increased risk (OR for longest duration, 10.15; 95%CI: 2.72–37.79) for malignant transformation of oral submucous fibrosis, (*P* for trend = 0.008) (Zhou *et al.*, 2008). [The Working Group noted that interpretation of these results may be hampered by the use of oral submucous fibrosis controls]. In a further case–control study (Ahmad *et al.*, 2006), *gutka* and other areca nut products had a highly significant association with oral submucous fibrosis (χ^2 = 188.14, $P < 0.001$). [The Working Group noted that oral submucous fibrosis is not associated with tobacco use or alcohol drinking.]

Intervention studies demonstrated that reduction in the use of betel quid with added tobacco resulted in lowering the incidence of precancerous lesions (Gupta *et al.*, 1986, 1992) and cessation resulted in development of no new precancerous lesions (Gupta *et al.*, 1995).

Thomas *et al.* (2008) included 6 studies in a meta-analysis that examined risk of oral precancerous disorders associated with betel quid without tobacco. These studies either excluded smokers or controlled for smoking. Among non-smokers with oral precancerous lesions their overall odds ratio estimated for betel quid without tobacco was 10.13 (95%CI:4.09–25.08) and in studies that adjusted for smoking the combined odds ratio was 5.17 (95%CI: 2.79–9.57).

2.3 Other cancers of the upper aerodigestive tract

2.3.1 Cancers of the pharynx

(a) Nasopharynx

In a cohort study from Taiwan, China, where tobacco is never added to betel quid (Wen *et al.*, 2005a), betel quid chewers who smoked had an increased risk of death from cancer of the nasopharynx (RR, 4.2; 95%CI: 1.5–11.4) after adjusting for age, alcohol use and education (Table 2.9 available at http://monographs.iarc.fr/ENG/Monographs/vol100E/100E-05-Table2.9.pdf). [There has been no publication from Taiwan, China where betel quid chewing was reported separately from smoking, because most betel quid chewers smoke.] Positive associations with 20 or more years of area nut use were found in a case–control study of cancer of the nasopharynx from Taiwan, China (Yang *et al.*, 2005; Table 2.10 available at http://monographs.iarc.fr/ENG/Monographs/vol100E/100E-05-Table2.10.pdf). [The models were adjusted for age and sex but it was unclear if they were further adjusted for other factors such as cigarette smoking, Guangdong salted fish consumption during childhood, and cumulative wood dust exposure]. Two case–control studies of cancer of the nasopharynx from India, where tobacco is commonly added to the betel quid, also found positive associations with betel quid chewing (Jussawalla & Deshpande, 1971; Chelleng *et al.*, 2000).

(b) Oropharynx

Cancer of the oropharynx has been associated with chewing betel quid with added tobacco (Sanghvi *et al.*, 1955; Khanolkar, 1959; Shanta & Krishnamurthi, 1963; Hirayama, 1966; Jussawalla & Deshpande, 1971; Wasnik *et al.*, 1998; Dikshit & Kanhere, 2000; Znaor *et al.*, 2003) and without added tobacco (Shanta & Krishnamurthi, 1963; Hirayama, 1966; Jussawalla & Deshpande, 1971; Wasnik *et al.*, 1998; Znaor *et al.*, 2003) in all of the studies in which it was assessed (IARC (2004) and Table 2.10 online. [The Working Group noted that the title of the study by Dikshit & Kanhere (2000) mentioned 'oropharyngeal' but the authors made occasional references to 'oral cavity' in the article]. None of the studies controlled for HPV, an important risk factor for cancer of the oropharynx. All of the studies were conducted in India, and Hirayama (1966) additionally enrolled subjects from Sri Lanka.

(c) Hypopharynx

Several positive associations between cancer of the hypopharynx and chewing betel quid (with or without added tobacco or unspecified) have been reported (Sanghvi *et al.*, 1955; Shanta & Krishnamurthi, 1963; Jussawalla & Deshpande, 1971; Simarak *et al.*, 1977; Znaor *et al.*, 2003; Sapkota *et al.*, 2007; IARC (2004) and Table 2.10 online. Most analyses accounted for tobacco use and two additionally adjusted for alcohol drinking (Znaor *et al.*, 2003; Sapkota *et al.*, 2007). For users of products containing both tobacco and areca nut (*mawa*, *pan* with tobacco and *gutka*), statistically significant results were seen for each of those behaviours (separately evaluated) for never smokers only, with adjustment for snuff use (nasal or oral), alcohol, drinking and smoking (Sapkota *et al.*, 2007).

(d) Pharynx

In several case–control studies a positive association between chewing betel quid with added tobacco and cancer of the pharynx has been found after controlling for tobacco smoking (Sanghvi *et al.*, 1955; Shanta & Krishnamurthi, 1963; Jussawalla & Deshpande, 1971; Simarak *et al.*, 1977; Wasnik *et al.*, 1998; Dikshit & Kanhere, 2000; Znaor *et al.*, 2003; Sapkota *et al.*, 2007; IARC, 2004; Table 2.10 online). Znaor *et al.* (2003) and Sapkota *et al.* (2007) additionally adjusted for alcohol drinking. Znaor *et al.* (2003) also found dose-dependent increases in risk of combined oro-, hypo- and unspecified pharyngeal cancers by amount used, duration of use and cumulative use of unspecified betel quid (considered to be mostly with added tobacco).

In two studies chewing of betel quid without added tobacco was found to be positively associated with cancer of the pharynx (Znaor *et al.*, 2003; Lee *et al.*, 2005a), after adjusting for tobacco smoking and alcohol drinking. Lee *et al.* (2005a) showed dose-dependent increases in risk of combined hypo- and oro-pharyngeal cancers by age of chewing initiation and amount chewed. The highest odds ratios were for people who used betel inflorescence and for those who swallowed the juice of the quid (Lee *et al.*, 2005a).

2.3.2 Cancer of the oesophagus

The risk of cancer of the oesophagus associated with chewing betel quid with added tobacco has been assessed in several studies (IARC, 2004; Table 2.10 online). These included studies carried out in India that specifically assessed betel quid with added tobacco (Shanta & Krishnamurthi, 1963; Jussawalla & Deshpande, 1971; Jayant *et al.*, 1977; Sankaranarayanan *et al.*, 1991; Znaor *et al.*, 2003); others carried out in India that did not specify as to whether tobacco was added to the betel quid (Sanghvi *et al.*, 1955; Nandakumar *et al.*, 1996; Chitra *et al.*, 2004); and studies in Thailand (Phukan *et al.*, 2001; Boonyaphiphat

et al., 2002) where tobacco is typically added to the quid. The majority of studies reported positive associations but only three (Nandakumar et al., 1996; Boonyaphiphat et al., 2002; Znaor et al., 2003) controlled for both smoking and alcohol use. In a case–control study in Kerala, India no association of cancer of the oesophagus with chewing betel quid with added tobacco was found but there was no control for smoking (Sankaranarayanan et al., 1991). In the case–control study in Thailand odds ratios increased with increasing number of quids chewed from 1.47 (95%CI: 0.9–2.3) with chewing less than 10 quids per day to 5.6 (95%CI: 2.7–11.8) for chewing more than 10 quids per day (Boonyaphiphat et al., 2002).

The association between betel quid without added tobacco and cancer of the oesophagus has been evaluated in eight studies (Shanta & Krishnamurthi, 1963; Jussawalla & Deshpande, 1971; Wu et al., 2001, 2004a, 2006; Znaor et al., 2003; Lee et al., 2005b, 2007); five analyses controlled for tobacco smoking and alcohol drinking (Znaor et al. 2003; Wu et al. 2004a, 2006; Lee et al. 2005b, 2007). Positive associations were found in all studies, of which six (Jussawalla & Deshpande, 1971; Wu et al., 2001, 2004a; Znaor et al., 2003; Lee et al., 2005b, 2007) were statistically significant. Significant dose–response relationships after controlling for smoking and alcohol were observed by Lee et al. (2005b, 2007). In a cohort study based on a national survey and community, Wen et al. (2005a) could not separate the effect of chewing betel quid without added tobacco and tobacco smoking since currently most betel quid chewers smoke in Taiwan, China (Table 2.9 online). The highest relative risks were reported in Taiwan, China among those who chewed betel inflorescence (Wu et al., 2004a, Lee et al., 2005b, 2007; Wu et al., 2006). [Betel inflorescence contains a high concentration of safrole, a possible human carcinogen (IARC Group 2B)].

In two studies risk was evaluated for cancer at subsites of the oesophagus. The highest magnitude of effect associated with chewing betel quid were reported for the upper third of the oesophagus in Taiwan, China (Lee et al., 2007) and for the middle-third of the oesophagus in India (Nandakumar et al., 1996). Both studies controlled for tobacco smoking and alcohol drinking.

2.3.3 Cancer of the larynx

In India, where tobacco is commonly added to the betel quid, positive associations with chewing betel quid were found in two case–control studies of cancer of the larynx (Jussawalla & Deshpande, 1971; Kapil et al., 2005b) while in two other case–control studies there was no association (Sankaranarayanan et al., 1990b; Sapkota et al., 2007; IARC, 2004; Table 2.10 online). [Jussawalla & Deshpande (1971), Sankaranarayanan et al. (1990b) and Kapil et al. (2005b) did not adjust for smoking or drinking habits.] In Taiwan, China (Lee et al., 2005a), chewing betel quid without added tobacco was positively but not significantly associated with the risk of cancer of the larynx, after adjusting for smoking and alcohol (OR, 1.3; 95%CI: 0.7–2.5).

2.3.4 Interactions

Several studies have reported the joint effects of chewing betel quid, adding chewing tobacco, smoking tobacco and/or drinking alcohol. A re-analysis of the data from Jussawalla & Deshpande (1971) found that chewing and smoking practices interacted synergistically for cancers of the oral cavity, oropharynx, hypopharynx, larynx and oesophagus (Jayant et al., 1977). Znaor et al. (2003) also showed a synergistic relationship between betel quid chewing, tobacco smoking and alcohol consumption for cancer of the pharynx. These findings are similar to those on smoking and betel quid chewing from a cohort study in Taiwan, China with nasopharyngeal and oesophageal cancer as reported outcomes (Wen

et al., 2005a). The common occurrence of dual or multiple substance use (chewing betel quid, adding chewing tobacco, smoking tobacco and drinking alcohol) in populations makes these findings important, as the magnitude of effect is highest for those who combine these habits.

2.4 Cancer of the liver

2.4.1 Cohort studies

Three cohort studies conducted in Taiwan, China, investigated the association between betel quid use [without added tobacco] and cancer of the liver (Sun *et al.*, 2003; Wang *et al.*, 2003b; Wen *et al.*, 2005a; Table 2.11 available at http://monographs.iarc.fr/ENG/Monographs/vol100E/100E-05-Table2.11.pdf). Sun *et al.* (2003) found a synergistic association between hepatocellular carcinoma and betel quid chewing without added tobacco in those with hepatitis C virus (HCV) infection. [The number of cases was small (2 cases among betel quid chewers with HCV infection; 8 cases among betel quid chewers without HCV infection) and there was no adjustment for tobacco smoking and alcohol consumption.]

Wang *et al.* (2003b) found high and statistically significant relative risks for hepatocellular carcinoma associated with betel quid chewing without added tobacco. Compared to Hepatitis B surface Antigen (HBsAg) seronegative men who did not chew betel quid, chewing betel quid without added tobacco conferred a relative risk of 3.43 (95%CI: 1.19–9.89), with a dose–response relationship for quantity chewed per day (P trend = 0.007). [The Working Group noted that the authors adjusted for liver function at baseline but did not adjust for tobacco smoking and alcohol consumption.] Wen *et al.* (2005a) conducted a cohort study in Taiwan, China and found a statistically significant positive association with liver cancer and cirrhosis of the liver, after adjusting for HBsAg, for those who both smoked cigarettes and chewed betel quid without added tobacco (RR, 1.8; 95%CI: 1.1–2.8). The magnitude of effect observed was much higher than that observed for those who only smoked but did not chew betel quid. [The Working Group noted that there were too few non smoking betel chewers to calculate a relative risk for them in this study].

2.4.2 Case–control studies

Two case–control studies (Tsai *et al.*, 2001, 2004) and one cross-sectional study (Wu *et al.*, 2009a) from Taiwan, China, and one case–control study from Thailand (Srivatanakul *et al.*, 1991) showed significant associations between chewing betel quid without added tobacco and hepatocellular carcinoma (Table 2.12 available at http://monographs.iarc.fr/ENG/Monographs/vol100E/100E-05-Table2.12.pdf). [It was not specified whether or not tobacco was added to the betel quid in Srivatanakul *et al.*, 1991]. Tsai *et al.* (2001) reported an exposure-response relationship and a synergy with viral infection after adjusting for infection with hepatitis virus (HBV and HCV), tobacco smoking, alcohol consumption and socio-demographic variables. Tsai *et al.* (2004) showed significant associations of hepatocellular carcinoma with betel quid chewing, using two separate control groups (healthy population-based controls and cirrhosis patients). Furthermore, an exposure-response relationship was observed with the duration and quantity of betel quid chewed (P for trend < 0.0001). There was also a positive association between betel quid chewing without tobacco and cirrhosis, a precursor to liver cancer (La Vecchia *et al.*, 1998).

Betel quid appears to act synergistically with viral infections in causing liver cancer. When comparing hepatocellular carcinoma patients to healthy controls, the odds ratio associated with chewing betel quid without tobacco among persons positive for a hepatitis virus (HBV or HCV) was statistically significantly elevated and

orders of magnitude higher than the odds ratio associated with being hepatitis virus positive and a non-chewer or being a chewer and hepatitis virus negative. [No formal test for interaction was presented and the 95% confidence intervals were wide due to the small sample size. It was not possible to determine whether these models were adjusted for alcohol consumption, tobacco smoking or other confounding factors. There was some overlap in cases between Tsai *et al.* (2001) and Tsai *et al.* (2004).]

A population-based study of liver cirrhosis and hepatocellular carcinoma combined was conducted in Keelung, northern Taiwan, China on 60 326 persons aged 30 years and above who were enrolled in a screening programme (Wu *et al.*, 2009a). [Prevalent and incident cases were combined.] There was a statistically significant positive association with chewing betel quid without added tobacco and significant exposure-response relationships for the number of quids chewed daily, number of years of chewing, cumulative exposure (portion-days), and age at initiation (P for trend < 0.01) after adjusting for sex, HBsAg, anti-HCV antibodies, cumulative exposure to alcohol consumption and cigarette smoking. Betel quid chewers who were seronegative for both HBsAg and anti-HCV had a hazard ratio of 5.09 (95%CI: 2.87–9.03); a synergistic association was observed for betel quid chewing and seropositivity for one or both viral markers (hazard ratios ranged from 25–29). [The Working Group noted that the most popular type of betel quid in Keelung includes unripe nuts, betel inflorescence and red lime paste and is swallowed after chewing. It was mentioned that aflatoxin is commonly present in areca nuts, but this was according to a reference from India, where ripe nuts are used for chewing and may be stored for long periods, making them susceptible to mould. Both prevalent and incident cases were included and hepatocellular carcinoma and liver cirrhosis cases were combined, which limits the interpretation of the data for the carcinogenicity of betel quid chewing.]

2.5 Other cancers

2.5.1 Cancer of the stomach

In a case–control study on stomach cancer from Taiwan, China Wu *et al.* (2004b) found a positive association with cumulative chewing of betel quid without added tobacco (betel-years): the odds ratios increased with higher consumption, after adjusting for alcohol consumption, tobacco smoking and *H. pylori* infection (P for trend = 0.03). In a hospital-based case–control study from Chennai, India (Gajalakshmi & Shanta, 1996), elevated odds ratios (not statistically significant) of similar magnitude (range 1.2–1.4) were observed for chewing areca nut only, betel quid only, and betel quid with added tobacco, although the risk disappeared after adjusting for tobacco smoking, alcohol consumption and diet. From a hospital-based case–control study of stomach cancer in Mizoram, India Phukan *et al.* (2005) reported elevated odds ratios for chewing betel quid with or without added tobacco, with significant trends for increasing odds ratios with increasing exposure (according to various exposure metrics) after adjusting for alcohol drinking, smoking, use of *tuibur*, level of education, occupation and income group.

2.5.2 Cancer of the cervix

One study described the association between betel quid chewing (with or without added tobacco) and cervical cancer in which nearly all women were non-smokers and in which all cases, but one, were HPV positive (Rajkumar *et al.*, 2003). There was an association between the use of betel quid without tobacco and cervical cancer; among women who reported using betel quid more than 5 times per day the odds ratio was 4.0

(95%CI: 1.20–13.33) with a significant trend with increasing number of times used per day.

A cross-sectional study derived from a screening programme (Chakrabarti *et al.*, 1990) showed an association between betel quid chewing with and without tobacco and cervical dysplasia. [Women with cytoepidemiological evidence of infection with HPV, HSV, *Trichomonas vaginalis* and *Chlamydia trachomatis* were excluded from the study.]

2.5.3 Cancers of the lung, colon and gallbladder

Several studies have assessed the association between chewing betel quid with or without added tobacco and cancer of the lung (Wen *et al.*, 2005a), colon (Wu *et al.*, 2009b) and gallbladder (Pandey & Shukla, 2003; Shukla *et al.*, 2008).

2.6 Synthesis

2.6.1 Oral cavity

Chewing betel quid, both with and without added tobacco, causes cancer of the oral cavity (IARC, 2004). Recent studies, many of which were adjusted for tobacco smoking, consumption of alcoholic beverages, and/or HPV infection, the major risk factors for oral cancer, confirmed this evaluation. Additionally, positive exposure–response relationships were reported in some studies.

2.6.2 Precancerous lesions of the oral cavity

Many cohort, case–control and cross-sectional studies from a wide range of countries have noted a high prevalence of oral precancerous disorders (leukoplakia, erythroplakia, oral submucous fibrosis) among users of betel quid and areca nut compared to non-users. Among betel quid users with added tobacco in Sri Lanka and India, significant associations were reported in four studies after adjusting for or stratifying by tobacco smoking or consumption of alcoholic beverages. The association between betel quid without added tobacco and precancerous disorders was examined in 6 studies from India, Taiwan, China and Papua New Guinea. A significant positive association was found in all studies and a significant dose–response was observed in 2 of them. Among users of areca nut only, significant associations were reported after adjusting for stratifying by tobacco smoking or consumption of alcoholic beverages. A significant positive association was reported from three studies in Pakistan and India that examined the association between areca nut use and oral submucous fibrosis.

2.6.3 Pharynx

Numerous studies, some of cohort and many of case–control design, have been performed on chewing betel quid, with or without tobacco, and the risk for cancers of the naso-, oro- and hypopharynx, or of the pharynx not otherwise specified. Chewing betel quid with added tobacco is causally associated with cancers of the pharynx and its subsites. Positive exposure–response relationships were noted in some studies, strengthening the credibility of a causal association. In some studies it was possible to demonstrate a synergistic relationship between betel quid chewing, tobacco smoking and consumption of alcoholic beverages on the risk of cancer of the pharynx.

2.6.4 Oesophagus

One cohort and several case–control studies have been performed on chewing betel quid and the risk for cancer of the oesophagus. Chewing betel quid, both with and without added tobacco, causes cancer of the oesophagus. Positive exposure–response relationships were reported in some studies, strengthening the credibility of a causal association. A synergistic relationship

between betel quid chewing, tobacco smoking and consumption of alcoholic beverages on the risk of cancer of the oesophagus was demonstrated in some studies.

2.6.5 Liver

The association between betel quid without added tobacco and cancer of the liver has been evaluated in six studies: 3 cohort studies, 2 case–control and 1 cross-sectional study. Five studies were from Taiwan, China, where betel quid is chewed without added tobacco, and one study was from Thailand, in which the use of betel quid with or without added tobacco was not specified. Significant positive associations were observed in 4 of the 5 Taiwanese studies, although confounding by tobacco smoking, alcohol consumption, hepatitis B or C virus positivity could not be ruled out. Significant positive dose–response relationships with the amount of betel quid chewed were observed in two studies, although confounding could not be ruled out.

2.6.6 Other sites

Several epidemiological studies assessed cancers at other sites but there are not enough data to permit a conclusion.

3. Cancer in Experimental Animals

Several studies investigating the carcinogenicity of betel quid and areca nut in experimental animal have inadequate numbers of animals per group, inadequate frequency and duration of treatment, absence of appropriate controls, ambiguous description of lesions, low survival of animals and inadequate reporting of survival data. Studies that were considered uninformative are not included in the present evaluation.

Representative studies are reported below and are described in Tables 3.1, 3.2, 3.3.

3.1 Mouse

Administration of either areca nut, areca nut and tobacco, arecoline or *pan masala* by skin application did not produce tumours in some studies (Ranadive *et al.*, 1976; Pai *et al.*, 1981; Ramchandani *et al.*, 1998). Topical application of an extract of areca nut extract and tobacco produced epidermoid carcinomas in a small number (2/23) of C17 mice (Ranadive *et al.*, 1976).

A group of 21 male Swiss mice was administered 0.1 mL of an aqueous extract of areca nut (containing 1.5 mg arecoline and 1.9 mg polyphenol) by gavage on five days a week for life. Twelve out of 21 treated mice developed tumours (five hepatocellular carcinomas [$P < 0.05$], two liver haemangiomas, two lung adenocarcinomas, one adenocarcinoma, one squamous cell carcinoma of the stomach, and one leukaemia). No tumour was observed in 20 untreated controls (Bhide *et al.*, 1979).

Administration of 0.1 mL of an aqueous extract of areca nut (containing 1.5 mg arecoline) on five days a week for life by gavage produced lung adenocarcinomas in 47% (9/19, $P < 0.05$) of male Swiss mice. One untreated control mouse of 20 developed a lung adenocarcinoma (Shirname *et al.*, 1983).

Administration by gavage of arecoline hydrochloride, a component of areca nut, induced three squamous cell carcinomas of the stomach, four lung adenocarcinomas and eight liver haemangiomas [not significant] in 43% (15/35) male Swiss mice. One untreated control mouse of 20 developed an unspecified tumour (Bhide *et al.*, 1984).

Dietary feeding of unprocessed areca nut or application of a paste of unprocessed areca nut to the oral cavity of male and female Swiss mice induced squamous cell carcinomas and papillomas in the oesophagus of a small number of

Table 3.1 Carcinogenicity studies of administration of areca nut or betel quid in experimental animals

Species, strain (sex) Duration Reference	Route Dosing regimen Animals/group at start	Incidence and/or multiplicity of tumours	Significance	Comments
Mouse, Swiss (M, F) Lifetime Ranadive *et al.* (1976)	Subcutaneous injection	Fibrosarcomas:		
	Areca nut (hot aqueous extract), 50 mg/mL, 0.2 mL, once/wk for 6 wk; 20/group	14/20	[$P < 0.0001$]	
	Areca nut (cold aqueous extract), 50 mg/mL, 0.2 mL, once/wk for 6 wk; 20/group	10/20	[$P < 0.001$]	
	Distilled water, 0.2 mL, once/wk for 10 wk; 25/group	0/25	-	
Mouse, Swiss (M, F) Lifetime Ranadive *et al.* (1976)	Topical application	Skin tumours:		
	Areca nut/DMSO extract, 30 g areca nut in 20 mL DMSO, 0.1 mL, 3 × /wk; 10M+8F/group	0/18	NS	
	Tobacco/DMSO extract, 5 g tobacco in 20 mL DMSO, 0.1 mL, 3 × /wk; 10M+6F/group	0/16	NS	
	Areca nut + tobacco/DMSO extract, 30 g areca nut + 5 g tobacco in 20 mL DMSO, 0.1 mL, 3 × /wk; 11M+12F/group	1/23 (papillomas), 2/23 (carcinomas)	NS	
	DSMO 0.1 mL, 3 × /wk; 9M+12F/group	0/21	-	
Mouse, Swiss (M) Lifetime Shivapurkar *et al.* (1980)	Subcutaneous injection			Age not specified
	Areca nut/polyphenol fraction, 0.1 mL, once/wk for 13 wk (total dose, 24.7 mg polyphenol)	20/20 (fibrosarcoma, 16/20; hepatoma, 1/20; lung adenocarcinoma, 3/20)	[$P < 0.0001$] (fibrosarcomas)	
	Betel quid aqueous extract, 0.2 mL, once/wk for 13 wk (total dose, 38.4 mg alkaloid + 46.0 mg polyphenol)	7/20 (fibrosarcoma)	[$P < 0.01$]	
	Distilled water, 0.1 mL, once/wk for 13 wk 20/group	0/20	-	
Rat, NIH Black (M, F) 68 wk Kapadia *et al.* (1978)	Subcutaneous injection	Fibrosarcomas:		
	Areca nut/tannin rich areca nut extract, 0.5 mL, once/wk for 56 wk	30/30	Significant	
	Saline, 0.5 mL, once/wk for 56 wk 30/group	0/30	-	

Table 3.1 (continued)

Species, strain (sex) Duration Reference	Route Dosing regimen Animals/group at start	Incidence and/or multiplicity of tumours	Significance	Comments
Hamster, Syrian golden (M) 21 wk Suri *et al.* (1971)	Cheek pouch application	Cheek pouch squamous cell carcinomas:		
	DMSO extract of areca nut, 3 × /wk	8/21	[$P < 0.05$]	
	DMSO extract of areca nut + tobacco, 3 × /wk	16/21	[$P < 0.0001$]	
	DMSO-treated control, 3 × /wk 11–21/group	0/11	-	
Hamster, Syrian golden & white mutant (M, F) 21 mo Ranadive *et al.* (1979)	Cheek pouch application	Forestomach carcinomas–Cheek pouch carcinomas:		Lack of information on sex and strain distribution.
	Areca nut aqueous extract, 3 × /wk	4/21–1/21	[$P < 0.05$]–[NS]	
	Polyphenol fraction of areca nut, 3 × /wk	4/20–1/20	[$P < 0.05$]–[NS]	
	Areca nut pieces + aqueous extract of areca nut, 3 × /wk	6/13–0/13	[$P < 0.001$]–[NS]	
	Betel quid aqueous extract, 3 × /wk	5/20–0/20	[$P < 0.01$]–[NS]	
	Betel quid aqueous extract + tobacco, 3 × /wk	4/13–0/13	[$P < 0.01$]–[NS]	
	Untreated control 13–30/group	0/30–0/30	-	
Hamster, Syrian golden & white mutant (M, F) 21 mo Ranadive *et al.* (1979)	Cheek pouch implantation	Forestomach carcinomas–Cheek pouch carcinomas:		Lack of information on sex and strain distribution.
	Areca nut powder, in capsule, 1 × /2wk	6/19–4/19	[NS]–[NS]	
	Capsule control	0/9–0/9	-	
	Betel quid, 0.8–13 mg of material in wax pellet, once/2wk	8/18–4/18	[$P < 0.001$]–[$P < 0.05$]	
	Betel quid + tobacco, 0.8–13 mg of test material in wax pellet, once/2wk	6/21–3/21	[$P < 0.01$]–[NS]	
	Wax pellet control 9–25/group	0/25–0/25	-	

DMSO, dimethyl sulfoxide; F, female; M, male; mo, month or months; NS, not significant; wk, week or weeks

Table 3.2 Carcinogenicity studies of administration of *pan masala* in mice

Species, strain (sex) Duration Reference	Route Dosing regimen Animals/group at start	Incidence and/or multiplicity of tumours	Significance	Comments
Mouse, Swiss S/RVCri (M, F) Lifetime Bhisey et al. (1999)	Diet		Positive trend for lung adenocarcinoma ($P < 0.004$)	
	Normal diet	0/108		
	Pan masala 2.5% in diet, lifetime	Liver haemangioma, 7/108; lung adenocarcinoma, 3/108; liver adenocarcinoma, 1/108; hepatoma, 1/108; forestomach papilloma, 1/108		
	Pan masala 5% in diet, lifetime 108/group	Liver haemangioma, 1/108; lung adenocarcinoma, 5/108; forestomach carcinoma, 1/108; testicular lymphoma, 1/108		
Mouse, Swiss (M, F) 56 wk Nigam et al. (2001)	Diet		NS	
	Pan masala 2% in diet, 56 wk	Lung tumour, 2/12; haemangioma, 1/12; haemangioendothelioma, 1/12		
	Normal diet, 56 wk 12/group	Lung tumour, 1/12		
Mouse, ICRC (M, F) 6 mo Ramchandani et al. (1998)	Gavage		$P < 0.001$; $P < 0.01$	EPME tested as promoter
	NDEA in drinking-water for 4 d (16 mg/kg bw) followed by EPME by gavage (25 mg/ treatment) 5 × /wk for 6 mo	Forestomach papilloma, 17/26; esophageal papilloma, 11/26		
	NDEA in drinking-water for 4 d (16 mg/kg bw) followed by distilled water by gavage 5 × / wk for 6 mo 30/group	Forestomach papilloma, 5/27; esophageal papilloma, 3/27	-;-	

Table 3.2 (continued)

Species, strain (sex) Duration Reference	Route Dosing regimen Animals/group at start	Incidence and/or multiplicity of tumours	Significance	Comments
Mouse, Swiss Bare (F) 40 wk Ramchandani et al. (1998)	Topical application DMBA (20 nmol in 100 µl acetone) followed by EPME (25 mg in 100 µl acetone) 2x/wk for 40 wk	Skin papilloma: 6.8/mouse	$P < 0.05$	EPME tested as promoter
	DMBA (20 nmol in 100 µl acetone) followed by 100 µl acetone twice/wk for 40 wk 15/group	Skin papilloma: 4.2/mouse	-	

bw, body weight; d, day or days; DMBA, 7,12-dimethylbenz[*a*]anthracene; EPME, Ethanolic *pan masala* extract; F, female; M, male; mo, month or months; NDEA, *N*-nitrosodiethylamine; NS, not significant; wk, week or weeks

Table 3.3 Carcinogenicity studies of administration of areca nut, betel quid or betel leaf with known carcinogens or modifiers of cancer risk in experimental animals

Species, strain (sex) Duration Reference	Route Dosing regimen Animals/group at start	Incidence and/or multiplicity of tumours (%)	Significance	Comments
Mouse, Swiss (M) 180 d Padma *et al.* (1989)	Intragastric instillation		$P < 0.005$, tumour multiplicity inhibition	
	BLE (1 mg/d, 5 × /wk) for 2 wk, followed by B[*a*]P by gavage (1 mg/d, 2 × /wk) for 4 wk, followed by BLE (1 mg/d, twice/wk) for 2 wk	Forestomach papilloma: 0.9/mouse		
	B[*a*]P by gavage (1 mg/d, twice/wk) for 4 wk 20/group	Forestomach papilloma: 4.9/mouse	-	
Mouse, Swiss (M) 180 d Padma *et al.* (1989)	Drinking-water		-	
	NNN, application to tongue (22 mg/mouse)	Total: 13/19 (lung, 4/19; stomach, 5/19; Lung + stomach, 3/19; Liver + stomach, 1/19)		
	NNN, application to tongue (22 mg/mouse); BLE in drinking-water 5 × /wk (2.5 mg/d)	Total: 3/21 (lung)	[$P < 0.005$], inhibition of stomach tumourigenesis	
	NNK, application to tongue (22 mg/mouse)	Total: 10/13 (lung, 8/13; stomach, 1/13;lung + liver, 1/13)	-	
	NNK, application to tongue (22 mg/mouse); BLE in drinking-water 5 × /wk (2.5 mg/d)	Total: 7/15 (lung, 5/15; stomach, 1/15; lung + LIVER, 1/15)	NS	
	Untreated control 20/group	Total: 2/18 (lung)	-	
Hamster, Syrian golden (M) 30 wk Wong *et al.* (1992)	Cheek pouch insertion	Cheek pouch		
	DMBA 0.5%, 3 × /wk for 4 wk	Squamous cell carcinoma: 1/9	-	
	DMBA 0.5%, 3 × /wk for 4 wk, followed by betel quid twice/wk for 24 wk 10/group	Squamous cell carcinoma: 6/9	$P < 0.05$	
Hamster, Syrian golden (M) 18 wk Wong *et al.* (1992)	Cheek pouch insertion	Cheek pouch		
	DMBA 0.5%, 3 × /wk for 6 wk	Squamous cell carcinoma: 1/9	-	
	DMBA 0.5%, 3 × /wk for 6 wk, followed by betel quid twice/wk for 12 wk 10/group	Squamous cell carcinoma: 7/7	$P < 0.01$	

Table 3.3 (continued)

Species, strain (sex) Duration Reference	Route Dosing regimen Animals/group at start	Incidence and/or multiplicity of tumours (%)	Significance	Comments
Hamster, Syrian golden (M) 35 wk Jin *et al.* (1996)	Cheek pouch insertion or painting	Cheek pouch		
	DMBA 0.5%, 3 × /wk for 4 wk	Squamous cell carcinoma: 2/9	-	
	DMBA 0.5%, 3 × /wk for 4 wk, followed by areca nut fibre 3 × / wk for 24 wk	Squamous cell carcinoma: 9/10	$P < 0.01$	
	DMBA 0.5%, 3 × /wk for 4 wk, followed by cold aqueous extract of areca nut 3 × /wk for 24 wk 10/group	Squamous cell carcinoma: 7/10	$P < 0.05$	

BLE, Betel leaf extract; B[*a*]P, benzo[*a*]pyrene; d, day or days; DMBA, 7,12-dimethylbenz[*a*]anthracene; M, male; NNN, *N*′-Nitrosonornicotine; NNK, 4-(*N*-Nitrosomethylamino)-1-(3-pyridyl)-1-butanone; NS, not significant; wk, week or weeks

animals. No oesophageal tumours were observed in control mice (Rao & Das, 1989).

Subcutaneous injections of hot and cold areca nut extracts to Swiss mice increased the incidence of fibrosarcomas at the injection site (Ranadive *et al.*, 1976). Subcutaneous injections of a polyphenol fraction of areca nut to Swiss mice produced fibrosarcomas in 80% (16/20) of the animals (Shivapurkar *et al.*, 1980). In the same study, 35% (7/20) of mice concurrently treated with an aqueous extract of betel quid developed fibrosarcomas.

In a skin tumourigenesis experiment using 7,12-dimethylbenz[*a*]anthracene (DMBA) plus croton oil, feeding of areca nut did not influence the incidence of skin papilloma in Swiss mice (Singh & Rao, 1995).

Betel leaf extract given to mice treated with benzo[*a*]pyrene (B[*a*]P) by gavage reduced the incidence and multiplicity of B[*a*]P-induced forestomach papillomas (Padma *et al.*, 1989; Bhide *et al.*, 1991). It also reduced stomach tumour incidence in mice treated with *N*′-nitrosonornicotine or 4-(*N*-nitrosomethylamino)-1-(3-pyridyl)-1-butanone (Padma *et al.*, 1989).

Lifetime feeding of a diet containing either 2.5% or 5% *pan masala* to Swiss mice induced a variety of benign and malignant tumours in the liver, stomach and lung. No tumours were found in controls. A significant positive trend ($P = 0.004$) with dose was observed in the number of mice with lung adenocarcinoma (Bhisey *et al.*, 1999).

Administration of *pan masala* in the diet produced liver haemangiomas and papillary adenomas of the lung in Swiss mice [not significant]. A few lung adenomas, liver tumours, and benign tumours at some other sites were also observed in mice receiving *pan masala* and tobacco in the diet (Nigam *et al.*, 2001).

Topical application of an extract of *pan masala* to the skin of DMBA-initiated Swiss mice increased significantly the tumour multiplicity of skin papillomas. In the same study, administration of a *pan masala* extract by gavage to ICRC mice given *N*-nitrosodiethylamine (NDEA) in the drinking-water increased the incidence of squamous cell papillomas of the forestomach and oesophagus (Ramchandani *et al.*, 1998).

3.2 Rat

Subcutaneous injection of a tannin rich-extract of areca nut produced fibrosarcomas at the injection site in 30/30 NIH Black rats. No tumours were observed in 30 saline-treated controls (Kapadia *et al.*, 1978).

Dietary administration of areca nut to ACI rats fed vitamin A-sufficient or -deficient diets did not increase tumour incidence (Tanaka *et al.*, 1983).

In ACI rats treated with 4-nitroquinoline-1-oxide in the drinking-water followed by areca nut in the diet, the incidence of squamous cell carcinoma of the tongue was significantly greater (12/17 versus 4/14, $P < 0.0205$) than in animals given 4-nitroquinoline-1-oxide alone (Tanaka *et al.*, 1986).

Oral administration to Holtzman rats of an aqueous extract of betel leaf inhibited DMBA-induced mammary carcinogenesis (6/26 versus 17/27, $P < 0.05$) when given concurrently with DMBA (Rao *et al.*, 1985).

3.3 Hamster

A topical application of either DMSO extracts of areca nut or areca nut with tobacco on the cheek-pouch mucosa increased the incidence of squamous cell carcinoma and leukoplakia in Syrian golden hamster (Suri *et al.*, 1971).

Implantation in the cheek pouch of either (i) areca nut powder or (ii) betel quid with or without tobacco produced cheek-pouch carcinomas and forestomach carcinomas in Syrian golden hamsters and white mutant hamsters. In

the same study, topical application of extracts of betel quid with or without tobacco increased the incidence of forestomach carcinomas. Also, application of either (i) areca nut, (ii) a polyphenol fraction of areca nut, or (iii) areca nut pieces with extract of areca nut increased the incidence of forestomach carcinomas (Ranadive *et al.*, 1979). [The Working Group noted the lack of information on sex and strain distribution.]

Application of an extract of areca nut to the B[*a*]P-initiated cheek pouch of Syrian golden hamsters led to a slight increase in the incidence of squamous cell papillomas and carcinomas compared to B[*a*]P-only-treated animals. In the same study, application of betel leaf extract to B[*a*]P-initiated hamster cheek pouch reduced significantly the incidence of squamous cell papillomas and carcinomas (Rao, 1984).

Administration to the hamsters cheek-pouch of either areca nut fibre or areca nut extract by insertion (Jin *et al.*, 1996) or arecaidine by painting (Lin *et al.*, 1996) increased significantly the incidence of cheek pouch squamous cell carcinomas initiated by application of DMBA.

Concomitant treatment of hamster cheek pouch with DMBA and with an extract of betel quid, by insertion or painting, increased significantly the incidence (Wong *et al.*, 1992) or multiplicity (Lin *et al.*, 1997) of cheek pouch squamous cell carcinomas.

3.4 Baboon

Insertion into a surgically created buccal pouch for 42 months of a betel quid preparation with tobacco in seven baboons or without tobacco in five baboons did not lead to tumour formation (Hamner, 1972).

3.5 Synthesis

In mice, administration by gavage of areca nut extracts containing arecholine increased the incidence of lung adenocarcinoma in one study and of hepatocellular carcinomas in another study. Subcutaneous injections of hot and cold areca nut extracts in one study and of a polyphenol fraction of areca nut in another study increased the incidence of fibrosarcoma. In one study in rats, subcutaneous injection of an areca nut extract also produced fibrosarcomas.

In mice, subcutaneous injection of an extract of betel quid increased the incidence of fibrosarcoma in one study.

In one study in hamsters, topical application of extracts of areca nut or areca nut with tobacco increased the incidence of cheek pouch squamous cell carcinoma. In another similar study, betel quid and betel quid plus tobacco extracts, and areca nut pieces, extracts and polyphenol fractions, increased the incidence of forestomach carcinomas. In a third study, cheek pouch implantation of betel quid increased the incidence of forestomach and cheek pouch carcinomas; implantation of betel quid plus tobacco increased the incidence of forestomach carcinomas. Areca nut or betel quid also promoted DMBA-induced cheek pouch squamous cell carcinomas.

In one study in mice, feeding of a diet containing *pan masala* increased the incidence of lung adenocarcinomas. *Pan masala* also enhanced DMBA-induced skin papillomas and NDEA-induced forestomach and oesophagus papillomas.

Betel leaf extracts reduced the incidence of B[*a*]P-induced squamous cell tumours of the oral cavity in hamsters, of B[*a*]P-induced forestomach papillomas and NNN- and NNK-induced stomach tumours in mice, and of DMBA-induced mammary tumours in rats.

4. Other Relevant Data

For the effects of chewing betel quid and areca nut with tobacco, we refer the reader to Section 4 of the *Monograph* on Tobacco Smoking in this volume.

4.1 Distribution and metabolism of the constituents of betel quid

Metabolism, toxicity, genotoxicity, mutation induction in cancer-related genes, immunomodulatory effects and gene–environment interactions have been investigated for arecoline, the major alkaloid in areca nut, and for other betel-quid ingredients, e.g. catechu, betel leaf and slaked lime. In addition, reactive oxygen species and areca nut-derived nitrosamines are produced in situ in saliva during betel-quid chewing, and their adverse effects have been studied in the oral cavity of betel-quid chewers and in experimental systems.

Areca nut contains several alkaloids and tannins (polyphenols). Arecoline is the most abundant alkaloid, whereas arecaidine, guvacine and guvacoline occur in smaller quantities (Fig. 4.1). In rodents, arecoline is rapidly metabolized in both liver and kidney. In rats, arecoline is de-esterified in the liver to arecaidine, and both arecoline and arecaidine are excreted as the mercapturic acid (Boyland & Nery, 1969). The metabolism of arecoline and arecaidine was investigated in the mouse using a metabolomic approach (Giri *et al.*, 2006). The major metabolite of both alkaloids, *N*-methylnipecotic acid, is a newly discovered metabolite (see Fig. 4.1). A total of 11 metabolites of arecoline were identified. Arecaidine shares six of these with arecoline.

4.1.1 Formation of N-nitroso compounds in the oral cavity

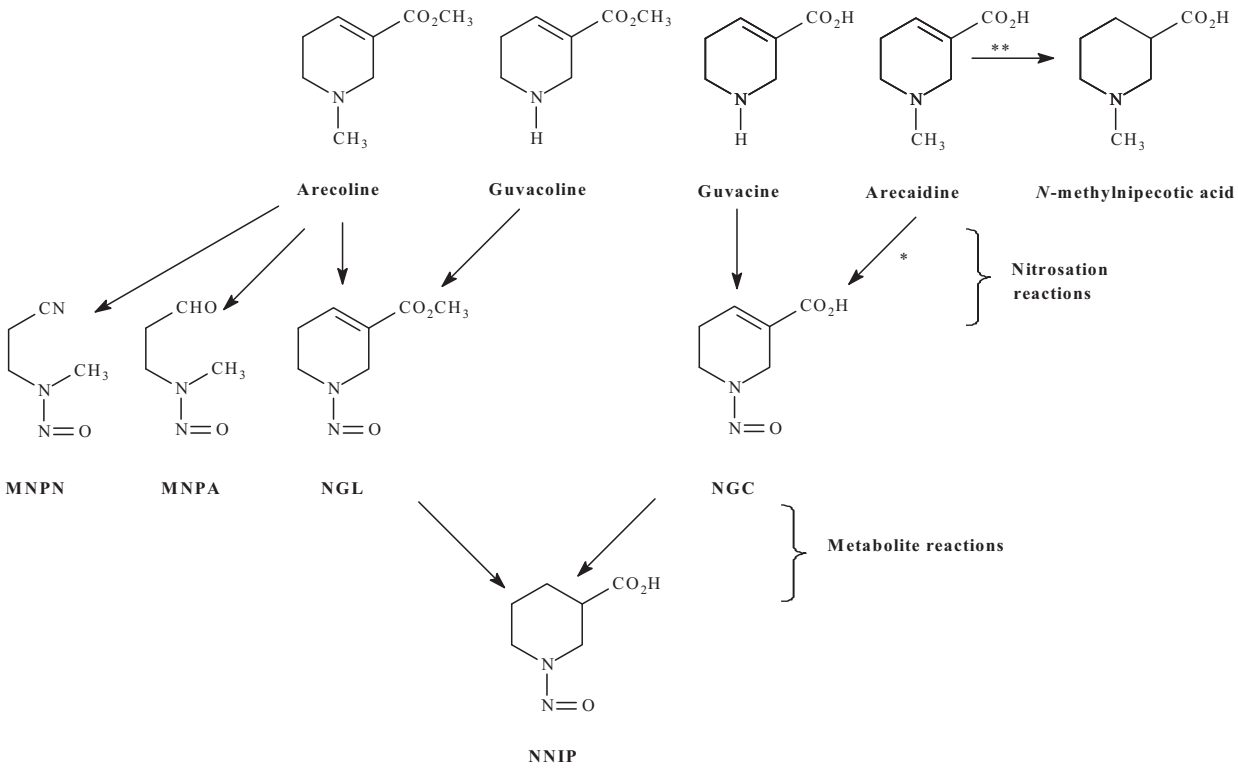
Areca nut contains secondary and tertiary amines that can be nitrosated in saliva during betel-quid chewing by reaction with nitrite in the presence of thiocyanate as a nitrosation catalyst (Fig. 4.1).

Three areca nut-derived nitrosamines, i.e. 3-methylnitrosaminopropionitrile (MNPN; a rodent carcinogen), *N*-nitrosoguvacine (NGC) and *N*-nitrosoguvacoline (NGL) have been detected in the saliva of betel-quid chewers (Nair *et al.*, 1985; Prokopczyk *et al.*, 1987; IARC, 2004). The formation of these nitrosamines can be mimicked *in vitro* by nitrosation with nitrite, thiocyanate and arecoline, which are all present in saliva. Endogenous nitrosation reactions in the oral cavity have been demonstrated in chewers of betel quid mixed with proline (a probe for ingested secondary amines), by measuring increased levels of *N*-nitrosoproline in saliva and urine (Nair *et al.*, 1987a). As chewers often swallow the quid – which contains nitrosamine precursors – the intragastric nitrosation reaction of secondary and tertiary amines may occur at higher rates due to the low pH in the stomach (Nair *et al.*, 1985).

4.1.2 Formation of reactive oxygen species in the oral cavity

Direct evidence that oxidative stress and reactive oxygen species such as the hydroxyl radical ($HO^{\bullet}$) are generated in the oral cavity during betel-quid chewing was provided by measuring the formation of *ortho*- and *meta*-tyrosines from L-phenylalanine in human saliva (Nair *et al.*, 1995). Auto-oxidation of polyphenols in areca nut and catechu generates the superoxide anion ($O_2^{\bullet -}$), especially at the high pH of slaked lime. The superoxide anion is converted to H_2O_2, which reacts in the presence of copper and iron ions (present in µg/gram amounts in

Fig. 4.1 Relationship of areca-nut alkaloids to areca-nut-derived nitrosamines (formed by nitrosation) and a urinary metabolite of *N*-nitrosoguvacoline and *N*-nitrosoguvacine



Adapted from Wenke & Hoffmann (1983), Nair *et al.* (1985), and Ohshima *et al.* (1989)
MNPA, 3-methylnitrosaminopropionaldehyde; MNPN, 3-methylnitrosaminopropionitrile; NGC, *N*-nitrosoguvacine; NGL, *N*-nitrosoguvacoline; NNIP, *N*-nitrosonipecotic acid
* It is likely that nitrosation of arecaidine would produce NGC but this has not been demonstrated.
** *N*-methylnipecotic acid is a recently isolated metabolite of arecoline; nitrosation reactions on this metabolite have not been studied (Giri *et al.*, 2006).

areca nut, catechu and slaked lime) to generate hydroxyl radicals (Nair *et al.*, 1987b). These can induce oxidation of deoxyguanosine to yield 8-hydroxydeoxyguanosine 8-(OH-dG) and DNA strand-breaks (IARC, 2004). Areca-nut extract and arecoline treatment led to depletion of glutathione (GSH) and reduction of glutathione-*S*-transferase (GST) activity in human oral cells and in rodent liver; both processes are known to increase cellular damage and DNA lesions (Chang *et al.*, 2001a, b).

4.2 Genetic and related effects

The genetic and related effects of areca nut and the various constituents of betel quid without tobacco were reviewed in detail by IARC (2004) and are summarized below.

4.2.1 Humans

Elevated formation of micronuclei has been reported in oral exfoliated cells in chewers of betel quid without tobacco. Micronucleus formation has been observed in precancerous lesions in the oral cavity of chewers of betel quid alone (Dave *et al.*, 1991; Kayal *et al.*, 1993), and betel

quid with tobacco (Stich *et al.*, 1989, 1991; Nair *et al.*, 1991).

Elevated sister-chromatid exchange and micronucleus formation have been demonstrated in cultured peripheral lymphocytes collected from chewers of areca nut without tobacco and slaked lime (Dave *et al.*, 1991, 1992; Desai *et al.*, 1996) and with tobacco (Adhvaryu *et al.*, 1986).

In subjects chewing betel quid without tobacco accumulation of p53 protein was observed (Kaur *et al.*, 1994, 1998; Yan *et al.*, 1996; Thongsuksai & Boonyaphiphat, 2001; Chang *et al.*, 2002a). *TP53* *m*RNA was frequently downregulated in betel quid chewing associated oral cancer (Tsai *et al.*, 2008).

Arecoline modulates matrix metalloproteinases (MMPs) and their tissue inhibitors (TIMPs), as well as the activity of lysyl oxidase, which leads to the accumulation of collagen in oral mucosal fibroblasts (Trivedy *et al.*, 1999a, 2001; Chang *et al.*, 2002b). Areca-nut polyphenols inhibit collagenases and increase the cross-linkage of collagen, reducing its degradation (Scutt *et al.*, 1987). These events may underlie the generation of oral submucous fibrosis in betel-quid chewers (Chang *et al.*, 2002b), which could be further enhanced by the release of copper ions, present in areca nut, catechu and slaked lime into the oral cavity of the chewers; inorganic copper salts increased the production of collagen by oral fibroblasts (Trivedy *et al.*, 1999b, 2001).

Cyclooxygenase-2 (COX2) is an inducible enzyme responsible for prostaglandin synthesis in certain inflammatory diseases. Its expression was significantly higher in oral submucous fibrosis specimens than in buccal mucosal fibroblasts (Tsai *et al.*, 2003).

In an oral epithelial cell line, arecoline was found to elevate the expression of the heat-shock protein HSP70 and haem oxygenase HO-1 mRNA in a dose- and time-dependent manner (Lee *et al.*, 2008a, b). Expression of HSP70 and HO-1 was significantly higher in specimens of human oral squamous cell carcinoma associated with areca-quid chewing. Areca-nut extracts increased the expression of inflammatory cytokines, tumour necrosis factor-α, interleukin-1-β, interleukin-6, and interleukin-8, in peripheral blood mononuclear cells (Chang *et al.*, 2009).

Collagen-related genes (*COLA1* and *COLA2*) and collagenase-1 lysil oxidase, transforming growth factor β (TGF-β1) and cystatin C involved in oral submucous fibrosis and DNA-repair genes (X-ray repair cross complementing 1 *XRCC1*) have been investigated in small studies in India, Taiwan, China, in relation to oral and oesophageal cancer and premalignant lesions (Lee *et al.*, 2001; Chiu *et al.*, 2002). No clear gene-environment interactions could be established because of the concurrent confounding by tobacco chewing and smoking or alcohol consumption (IARC, 2004).

4.2.2 Experimental systems

(a) Areca nut extracts

Extracts of betel quid and *pan masala* induced sister chromatid exchange and sperm abnormalities in mice. Betel quid extracts were mutagenic in bacteria and induced chromosomal aberrations, sister chromatid exchange and micronucleus formation in Chinese hamster ovary cells.

Aqueous extracts of areca nut produced gene conversion in yeast, DNA strand-breaks, gene mutation, chromosomal aberrations, sister chromatid exchange and micronucleus formation in rodent cells, both *in vitro* and *in vivo*. It also induced cell transformation in mouse C3H10T1/2 cells and DNA strand-breaks, unscheduled DNA synthesis and DNA–protein crosslinks in cultured human buccal and laryngeal epithelial cells.

(b) Areca nut alkaloids

Arecoline and other areca-nut alkaloids gave positive responses in most bacterial mutagenicity assays, and induced chromosomal aberrations,

micronucleus formation and sister chromatid exchange in mammalian cells, both *in vitro* and *in vivo*. Arecoline inhibited *Tp53* mRNA expression and its transactivating function, repressed DNA repair and triggered DNA damage response in human epithelial cells (Tsai *et al.*, 2008).

(a) Areca-nut-derived nitrosamines

Three areca-nut-derived nitrosamines, i.e. *N*-nitrosoguvacoline (NGL), *N*-nitrosoguvacine (NGC) and 3-methylnitrosaminopropionitrile (MNPN), were detected in the saliva of chewers of betel quid without tobacco. Genotoxic effects of these nitrosamines and of 3-methylnitrosaminopropionaldehyde (MNPA) can be summarized as follows: NGL but not NGC was mutagenic to bacteria. MNPN did not induced DNA single-strand breaks in human buccal epithelial cells (Sundqvist *et al.*, 1989). MNPN formed the DNA adducts 7-methylguanine and O^6-methylguanine (a pro-mutagenic DNA adduct) as well as (2-cyanoethyl)guanines in treated rats (Prokopczyk *et al.*, 1987, 1988). MNPA was not mutagenic in the presence of a metabolic activating system but caused single-strand breaks and DNA crosslinks in human buccal epithelial cells (Sundqvist *et al.*, 1989; Sundqvist & Grafström, 1992).

In Taiwan, China, areca nut is often chewed with fresh betel inflorescence). Betel inflorescence contains safrole and hydroxychavicol at relatively high concentrations (10–15 mg/g fresh nut) (IARC, 2004). Safrole is a possible human carcinogen (IARC, 1987). Taiwanese betel quid-chewers had 3-fold higher urinary excretion of hydroxychavicol, a metabolite of safrole, than non-chewers (Chang *et al.*, 2002c). They also had a high frequency of safrole-like DNA adducts (detected by ^{32}P-postlabelling) in the oral cavity that co-eluted with synthetic safrole-2′deoxyguanosine 3′-monophosphate adducts (Chen *et al.*, 1999). In HBsAg/HCV seronegative hepatocarcinoma, safrole-type DNA adducts were found in hepatic tissues of hepatocarcinoma patients who had chewed betel quid for > 10 years (Chung *et al.*, 2008).

4.3 Mechanistic considerations

Betel quid and areca-nut ingredients and extracts exert a variety of genetic and related effects (Section 4.2.1). Continuous local irritation of buccal epithelial cells caused by betel quid and its ingredients, particularly areca nut and slaked lime, can generate chronic inflammation, oxidative stress and cytokine production. Reactive oxygen species generated during chewing of betel quid and other genotoxic reactants formed from arecoline and areca nut-derived nitrosamines, can lead to DNA- and genetic damage in exposed oral keratinocytes. Persistent oxidative stress can drive affected cells to uncontrolled proliferation and hyperplastic/dysplastic lesions. Chronic occurrence of these toxic insults in the oral cavity of chewers could drive these pre-neoplastic cells towards full malignancy.

4.4 Synthesis

These mechanistic data support the causal associations for carcinogenicity observed in humans at several target sites (indicated in Section 2 of this *Monograph*) for chewers of betel quid without tobacco, and areca nut.

5 Evaluation

There is *sufficient* evidence in humans for the carcinogenicity of betel quid with added tobacco. Betel quid with added tobacco causes cancers of the oral cavity, pharynx and oesophagus.

There is *sufficient* evidence in humans for the carcinogenicity of betel quid without added tobacco. Betel quid without added tobacco causes cancers of the oral cavity and oesophagus. Also, a positive association has been observed between

exposure to betel quid without added tobacco and cancer of the liver.

There is *sufficient* evidence in experimental animals for the carcinogenicity of betel quid with added tobacco.

There is *sufficient* evidence in experimental animals for the carcinogenicity of betel quid without added tobacco.

There is *sufficient* evidence in experimental animals for the carcinogenicity of areca nut.

There is *limited* evidence in experimental animals for the carcinogenicity of *pan masala*.

There is *evidence suggesting lack of carcinogenicity* of betel leaf in experimental animals.

Betel quid with added tobacco is *carcinogenic to humans (Group 1)*.

Betel quid without added tobacco is *carcinogenic to humans (Group 1)*.

Areca nut is *carcinogenic to humans (Group 1)*.

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