3. Studies of Cancer in Experimental Animals

3.1 Oral administration

3.1.1 *Mouse*

Groups of 15–21 male Swiss mice, 8–10 weeks of age, were administered by gavage 0.1 mL of aqueous extracts of areca nut (containing 1.5 mg arecoline and 1.9 mg polyphenol) or betel leaf or a polyphenol fraction of areca nut (containing 1.9 mg tannic acid) on 5 days a week for life. A group of 30 male C17 mice received 0.1 mL of an aqueous extract of areca nut by gavage. Groups of 20 male Swiss and 20 male C17 mice served as untreated controls. Of the animals treated with aqueous areca-nut extract, 12/21 Swiss mice developed tumours (five hepatocellular carcinomas, two haemangiomas of the liver, two adenocarcinomas of the lung, one adenocarcinoma and one squamous-cell carcinoma of the stomach, and one leukaemia) and 8/30 C17 mice developed tumours (three squamous-cell carcinomas and two adenocarcinomas of the stomach, two leukaemias and one adenocarcinoma of the lung). In Swiss mice fed the areca-nut polyphenol fraction, two developed tumours of the salivary gland and one a haemangioma of the liver. No tumour was observed in either of the control groups or in the mice fed aqueous betel-leaf extract (Bhide *et al*., 1979). [The Working Group noted the absence of survival data and indication of duration of the experiment for the treated and control mice.]

A group of 14 male and 18 female C17 mice, 10–12 weeks of age, was fed a diet containing 10% (w/w) areca nut coated with saccharin [concentration not specified] for 40 weeks, and the animals were followed for life. Another group of 12 males and 22 females served as untreated controls. In the group fed areca nut, two males developed squamous-cell carcinomas of the forestomach and three females developed uterine malignancies

(reticular-cell neoplasm type A). Two similar uterine tumours were observed in the untreated controls. No statistically significant increase in tumour incidence was observed in animals treated with areca nut compared with controls (Pai *et al*., 1981).

Groups of 20 male Swiss mice, 8–10 weeks of age, were administered by gavage 0.1 mL of aqueous extracts of betel quid, betel quid and tobacco, areca nut, betel leaf, or areca nut and betel leaf five times per week for life or served as untreated controls. Animals were killed when moribund. All lesions reported were lung adenocarcinomas [histological details not given]; the incidences are given in Table 66 (Shirname *et al*., 1983). [The Working Group noted that the reason for the difference between the initial number of animals and the number of animals assessed for the presence of tumours is unknown.]

Table 66. Design and results of experiments in Swiss mice given aqueous extracts of betel quid and its components by gavage

Group	No. at start	No. of mice alive between 10 and 24 months	No. of mice with lung tumours (%)
Control	20	20	1 (5)
Betel quid	20	15	4 (26)
Betel quid and tobacco	20	18	4 (22)
Areca nut	20	19	9 (47)*
Betel leaf	20	14	1 (7)
Areca nut and betel leaf	20	16	6 (38)*

From Shirname *et al.* (1983)
*Statistically significant compared with controls ($p < 0.05$)

Groups of 8–20 female and 16–35 male Swiss mice, 6 weeks of age, were given 1 mg arecoline hydrochloride (in 0.1 mL distilled water) daily by gavage on 5 days a week, either alone or in combination with potassium nitrate (KNO_3) (1 mg daily), or KNO_3 with slaked lime (1 mg daily); controls were either untreated or received KNO_3 and lime. Treatment was continued for up to 25 months. A total of 15/35 (43%) males given arecoline alone developed tumours (8/18 between 12 and 18 months and 7/17 between 19 and 25 months) compared with 1/20 untreated males. Of the 15 tumours in the arecoline group, eight were liver haemangiomas, four were lung adenocarcinomas and three were squamous-cell carcinomas of the stomach. No tumour was reported in any of the 18 arecoline-treated or 20 control females. The incidence of tumours in male mice given arecoline in combination with either KNO_3 (3/19) or KNO_3 + lime (1/16) did not differ from that in corresponding control males given KNO_3 + lime (2/17). No tumour was found in females treated similarly (Bhide *et al.*, 1984). [The Working Group noted the lack of

information on the time of appearance of specific neoplasms and the inadequate reporting of the pathological findings. The lack of tumours in females was not explained.]

Groups of 18 male and 18 female Swiss mice, 10–12 weeks of age, were administered by gavage a daily ration of 0.1 mL of a fraction of areca nut containing ~1.9 mg tannin for life. Two animals developed tumours of the parotid gland, which were diagnosed as adenocystic carcinomas. One of the tumours was transplantable and was maintained as a model system by serial transplantation in Swiss mice (Gothoskar & Pai, 1986). [This study was only aimed at describing the derivation of a transplantable tumour model and did not yield any data on concentration or constituents of the areca-nut component used. The Working Group deemed it to be inadequate for evaluation.]

A total of 690 inbred Swiss mice, 6–7 weeks of age, divided into groups of 20–25 males and 20–25 females, were fed diets containing the following areca-nut products: (a) ripe, unprocessed, sun-dried areca nut (R-UP-SD), (b) ripe, processed, sun-dried areca nut (R-P-SD), (c) unripe, processed, sun-dried areca nut (UR-P-SD), (d) ripe, unprocessed, sun-dried, water-soaked areca nut (R-UP-SD-WS) or (e) ripe, unprocessed, sun-dried, water-soaked areca nut (R-UP-UD-WS) for 12 months. Diet was prepared using different types of areca nut from the market. The preparations were dehydrated, powdered and added wt/wt to pulverized feed to obtain diets containing 0.25, 0.5 and 1% areca nut. Control mice were maintained on normal diet for 12 months. In a second experiment, areca-nut paste was applied to the oral cavity of groups of 20–25 male and 20–25 female mice using a dispenser at dose levels of 0.25, 0.5 or 1.0 g/kg bw twice a day on 5 days per week for 12 months. The paste was prepared by grinding 10 g powdered areca-nut preparation of each type (as in experiment 1) with 25–30 mL drinking-water to obtain a smooth paste. Drinking-water was not provided to animals for 1 h following administration of the paste to facilitate intimate contact of the paste with the oral, pharyngeal and oesophageal epithelium. Control mice received oral applications of 0.05 mL water. In both experiments, animals were killed at the end of 12 months, internal organs were screened for tumour development and oral, pharyngeal, oesophageal, gastric and liver tissues were processed for histology. Cellular atypia (in three males and two females) and papilloma and carcinoma (in two males and one female) were observed in the oesophagus of animals treated with R-UP-SD at the highest dose (1% in diet). Two of 23 males receiving 1% R-UP-SD-WS developed papillomas in the oesophagus; 2/23 males and 1/21 females receiving 1% R-UP-UD-WS developed oesophageal papillomas and carcinomas. Following oral application of areca-nut paste, animals exposed to 1 g/kg bw R-UP-SD paste developed oesophageal papillomas and carcinomas (2/23 males and 2/24 females). One male and one female mouse receiving 1 g/kg bw R-UP-SD-WS developed papilloma and carcinoma of the oesophagus. In the R-UP-UD-WS-treated group, 2/23 males and 1/21 females developed oesophageal papilloma and carcinoma. In both experiments, oesophageal tumours appeared in different groups that were fed the unprocessed variety of areca-nut preparations (Rao & Das, 1989).

Groups of 15 male and 15 female ICRC/HiCri mice, 6–8 weeks of age, received 25 or 50 mg per animal (quantities denote original dry weight of the product) ethanolic

extract of *pan masala* (EPME; containing areca nut, catechu, lime flavouring agents and unspecified spices), a chewing mixture, by gavage in distilled water or distilled water alone five times per week for 6 months. No forestomach or oesophageal tumour was observed in mice that survived until the end of the experiment or died beforehand (Ramchandani *et al*., 1998).

Groups of 54 male and 54 female Swiss S/RVCri mice, 6–7 weeks of age, were fed diets containing 0, 2.5 or 5% *pan masala* (containing areca nut, catechu, lime, spices and flavouring agents) in the diet for life. Animals were killed when moribund or at the end of 24 months. A significant decrease was observed in the overall survival rate of mice receiving 2.5% and 5% *pan masala* compared with the controls with or without adjustment for sex. Organs were excised and processed for histopathology. A total of 15 benign and 12 malignant tumours were observed in treated mice. The commonest benign tumour was liver haemangioma in 7/108 mice fed 2.5% *pan masala*. In the group fed 5%, more diverse types of benign tumour were found. Malignant tumours were observed in 5/108 and 7/108 mice fed 2.5% and 5% *pan masala*, respectively. Lung and liver adenocarcinomas were observed in 3/108 and 1/108 mice fed 2.5% *pan masala*, as well as one hepatoma. In the 108 mice fed 5%, five adenocarcinomas of the lung, one forestomach carcinoma and one testicular lymphoma were observed. No tumours were found in the 108 controls. A statistically significant positive trend ($p = 0.004$) with dose was observed in the number of mice with lung carcinoma (Bhisey *et al*., 1999).

3.1.2 *Rat*

Groups of eight to nine male and eight female ACI rats, 6 weeks of age, were fed diets containing either 20% Indonesian areca nut or 20% Taiwanese betel-leaf powder for 480 and 300–327 days, respectively. A group of 11 males and eight females received a diet containing 20% Indonesian areca nut and 1% calcium hydroxide for 480 days. A group of nine males and 10 females served as untreated controls. At the end of the treatment periods, the animals were fed a normal diet and observed for life. No statistically significant difference in tumour incidence was observed between the treated and control groups. No tumours were observed in the oral cavity or gastrointestinal tract (Mori *et al*., 1979). [The Working Group noted that addition of 20% (wt/wt) areca-nut powder to the diet results in severe caloric and nutritional imbalances, which may have affected the results. This could not be assessed because of inadequate reporting of the survival data for the various groups.]

The effect of marginal vitamin A deficiency on carcinogenicity was examined in 4–6-week-old male and female ACI rats. Group 1 (32 males and 27 females) was fed a marginally vitamin A-deficient diet (20 IU palmitate/100 g diet) mixed with 20% areca-nut powder and 1% calcium hydroxide; Group 2 (39 males and 28 females) received vitamin A-deficient diet; Group 3 (21 males and 21 females) was fed vitamin A-sufficient diet (1200 IU palmitate/100 g diet) containing 20% areca-nut powder and 1% calcium hydroxide; and Group 4 (20 males and 21 females) received vitamin A-sufficient diet

alone. The experiment lasted for 647 days and 176/209 animals survived beyond 360 days. Two male and two female rats from each group were killed 30 weeks after the start of the experiment to determine vitamin A levels in serum and liver. All other animals were autopsied at natural death, when moribund or at 647 days. Liver tissues were processed for histology. In Group 1 (vitamin A-deficient + areca nut + calcium hydroxide), one papilloma of the tongue and one of the buccal mucosa in males and one papilloma of the tongue and one of the forestomach in females were observed. No upper aerodigestive tract tumour developed in Group 2 or Group 3. Two neoplastic liver nodules were observed in female rats of Groups 1 and 3 and one in Group 4. A solitary hepatocellular carcinoma developed in one female in Group 1. Other tumours that developed were: one spindle-cell carcinoma of the parotid gland in Group 1 males; two transitional-cell carcinomas and two squamous-cell carcinomas of the urinary bladder in Group 2 females; and two endometrial carcinomas, one leiomyosarcoma of the uterus and one mammary adenoma in Group 3 females. Group 4 rats did not develop tumours. The difference in tumour incidence in various groups did not reach statistical significance. Female susceptibility to tumour development could not be interpreted (Tanaka *et al*., 1983).

3.1.3 *Hamster*

Three groups of eight, eight and four Syrian hamsters (males and females approximately equally distributed), 6–7 weeks of age, were given distilled water containing 2% arecoline and 0.5% calcium hydroxide or 2% arecoline alone or were fed a diet that contained 0.1% arecoline with 2.5% calcium hydroxide, respectively, for life. Mean survival was 18 months, animals were killed when moribund or died spontaneously [no specific survival data given for the various experimental groups]. No tumours were observed in any of the treated animals. No tumours were found in four control Syrian hamsters (two males and two females) that ingested distilled water containing 0.5% calcium hydroxide or in four controls that were fed a diet containing 2.5% calcium hydroxide (Dunham *et al.*, 1974). [The Working Group noted the small number of animals.]

Of a group of two males and two females, one male Syrian golden hamster, 25.5 months of age, developed a carcinoid tumour of the glandular stomach after being fed a diet containing 0.1% arecoline and 2.5% calcium hydroxide for 12 months from the age of 1.5 months (Dunham *et al*., 1975). [The Working Group noted the small number of animals used. Because of the lack of available data, a fortuitous finding cannot be excluded.]

In a study aimed at evaluating the effect of concomitant oral administration of areca nut and sodium nitrite, 120 Syrian golden hamsters, 8 weeks of age, were divided into four treatment groups, each consisting of 15 males and 15 females. Group I received a diet containing 0.2% sodium nitrite, group II a diet containing 2% powdered areca nut, group III a diet containing both the sodium nitrite and the areca nut powder added in doses as mentioned above, and group IV received a diet without additives once a day on 5 days a week. The experiment lasted until the animals died spontaneously or were killed when moribund. Controls were killed after the death of the last experimental animal. The group-

based percentage of tumour-bearing animals and the total number of tumours were elevated [not statistically significantly] in animals fed areca nut alone or combined with nitrite as compared with the group fed nitrite only or control. Malignant tumours occurred three times more frequently in the group fed areca powder together with nitrite, but this increase did not reach statistical significance except for an increase in malignant lymphomas in male animals (5/15 versus 1/15; pairwise Fisher's exact probability test, $p < 0.05$). No statistical difference in survival time was seen for the various groups (Ernst *et al*., 1987).

3.2 Skin application

Mouse

A group of 12 Swiss mice [sex and age unspecified] received daily topical applications of an aqueous extract of betel quid (areca nut, stone lime and *gambir*) plus tobacco on the ears for 2 years; two animals developed squamous-cell carcinomas and one a benign squamous papilloma at the site of application (Muir & Kirk, 1960). [The Working Group noted that no controls were reported.]

Groups of 16–23 male and female C17 mice [sexes approximately equally distributed], 2–3 months of age, received thrice-weekly topical applications of 0.1 mL dimethyl sulfoxide (DMSO) extracts of tobacco (5 g ground tobacco in 20 mL DMSO), areca nut (30 g ground areca nut in 20 mL DMSO) or tobacco and areca nut, or applications of 0.1 mL DMSO alone on their backs for life. Animals were allowed to live their normal lifespan or were killed when they showed signs of debility. Skin papillomas were observed in 1/23 animals and epidermoid carcinomas were observed in 2/23 animals that received applications of combined tobacco and areca-nut extracts; no local tumour was observed in the other treatment groups (Ranadive *et al*., 1976).

3.3 Subcutaneous administration

3.3.1 *Mouse*

Groups of 10 male and 10 female Swiss mice, 2–3 months of age, were given subcutaneous injections of 0.2 mL hot or cold filtered aqueous areca-nut extracts (50 mg/mL) once a week for 6 weeks. Animals were allowed to live their normal lifespan or were killed when they showed signs of debility. Fibrosarcomas at the site of injection were observed in 14/20 and 10/20 mice treated with hot and cold areca-nut extracts, respectively; the first tumour appeared after 8 months. No local tumour was seen in a group of 13 male and 12 female controls receiving injections of distilled water for 10 weeks (Ranadive *et al*., 1976). [No further details on follow-up and survival were available.]

Effects on tumour development were investigated in male Swiss mice [age not specified] after subcutaneous injection of distilled water, an aqueous extract of areca nut, a polyphenol fraction of areca nut, a polyphenol-free fraction of areca nut, arecoline, an

aqueous extract of areca nut and betel leaf, an aqueous extract of betel quid or an aqueous extract of betel quid and tobacco. Groups were treated as follows: Group 1 (controls; 20 mice) was injected with 0.1 mL distilled water; group 2 (12 mice) received injections of 0.1 mL of a 1:10 extract of areca nut (prepared by lyophilization of a 1% aqueous extract of areca nut dissolved in 10 mL distilled water); group 3 (20 mice) received injections of 0.1 mL of a polyphenol fraction of areca nut (prepared, concentrated to dryness and dissolved in 10 mL distilled water); group 4 (20 mice) received injections of 0.1 mL of a polyphenol-free fraction of areca nut (1:10 dilution obtained after extraction of ethyl acetate-extracted residue with distilled water, lyophilization and dissolution in 10 mL distilled water); group 5 (10 mice) received injections of 1.5 mg arecoline; group 6 (15 mice) received injections of 0.2 mL of aqueous extract of areca nut and betel leaf; group 7 (20 mice) received injections of 0.2 mL aqueous extract of betel quid; and group 8 (20 mice) received injections of 0.2 mL of aqueous extract of betel quid with tobacco. All groups received weekly injections for 13 weeks. Animals were killed when moribund and abnormal tissues were processed for histopathology. The incidence of tumours was: Group 1, 0/20 tumours; Group 2, two fibrosarcomas, two haemangiomas of the liver (4/12 mice; 33%); Group 3, 16/20 fibrosarcomas, one hepatoma, three lung adenocarcinomas (20/20; 100%); Group 4, 0/20 tumours; Group 5, 0/10 tumours; Group 6, 0/15 tumours; Group 7, 7/20 fibrosarcomas; Group 8, 2/20 tumours at the site of injection [histological identification not mentioned] (Shivapurkar *et al*., 1980).

3.3.2 *Rat*

A group of 15 male and 15 female outbred NIH Black rats, 1–2 months old, received weekly subcutaneous injections of 0.5 mL of a tannin-rich aqueous extract of areca nut for up to 56 weeks and were observed for a further 12 weeks. Injections were discontinued when the first tumour appeared. All treated animals developed fibrosarcomas at the injection site. No local tumour occurred in 15 male and 15 female controls receiving injections of saline (Kapadia *et al*., 1978).

3.4 Intraperitoneal administration

Mouse

Groups of 7–10 male Swiss mice, 6 weeks of age, received weekly intraperitoneal injections of 0.1 mL aqueous extract of areca nut (seven mice), betel leaf (10 mice) or the polyphenol fraction of areca nut (nine mice) [details of doses were insufficiently defined] for 13 weeks. Additional groups were injected with commercial tannin (10 mice), arecoline (1.5 mg; 10 mice) or distilled water (10 mice). All animals were observed for their entire lifespan. No tumour was observed in any of the groups (Shivapurkar *et al*., 1980). [The Working Group noted the small number of animals used, the poor definition of the substances tested and the absence of details on the methods used.]

3.5 Administration to the oral mucosa or cheek pouch

3.5.1 *Rat*

A group of 21 albino (Wistar strain) rats [sex not specified], 3–4 months of age, received applications to the palate and cheek mucosa of a paste (1 g) of *pan masala* [mode of preparation not described; exact site not described; method of application not described] administered every other day for 6 months. A group of 14 untreated animals served as controls. Biopsies were taken after 2, 4 and 6 months of application of *pan masala* and treatment was discontinued for 2 weeks after each biopsy to allow tissue healing. A biopsy was taken in the beginning and at the end of the experiment from control rats. After 6 months of treatment with *pan masala* paste, no tumour was observed; however, a high incidence of dysplastic epithelial changes (loss of nuclear polarity in 65% of animals after 6 months of application) was reported (Khrime *et al*., 1991). [The Working Group noted that the duration of the experiment was only 6 months, and that the incidence of dysplasia is a matter for concern.]

A group of 14 male and 13 female Wistar rats, 2 months of age, received daily applications to the buccal and palatal surfaces of 2% ethanolic extract of arecoline for 2.5 (six animals), 3 (six animals) and 4.5 months (15 animals). Groups of six and 12 animals served as solvent and untreated controls, respectively. Animals were killed at the end of the experiment. Histologically, no tumour was observed (Sirsat & Khanolkar, 1962).

3.5.2 *Hamster*

Groups of 8–50 male and female Syrian golden hamsters, 1–2 months of age, received an implantation in the cheek pouch of a pellet of single beeswax containing 7–50% betel quid or its various components: betel leaf, areca nut, areca nut and betel leaf, or areca nut and tobacco. Animals were allowed to live their normal lifespan or were killed when moribund. Exposure varied from 1 to 35 months. No malignant tumour was observed at the implantation site in any of the groups (Dunham & Herrold, 1962). [The Working Group noted that only a single administration was given.]

A total of 65 male Syrian golden hamsters, 9 weeks of age, was divided into four groups of 11–21. Animals received topical applications on the cheek-pouch mucosa of DMSO extracts of areca nut, tobacco, areca nut plus tobacco or DMSO alone thrice weekly for 21 weeks, at which time all animals were killed. Local squamous-cell carcinomas and leukoplakia were seen in 8/21 and 19/21 of the animals treated with areca-nut extract, and in 16/21 and 18/21 of the groups treated with areca-nut and tobacco extract, respectively; no local tumour was seen in hamsters treated with tobacco extract alone, but 8/12 had leukoplakia. No local tumour was observed in the 11 DMSO-treated controls (Suri *et al*., 1971). [The Working Group noted that development of cheek pouch tumours between 7 and 21 weeks was extremely unusual and would imply an exceedingly powerful carcinogenic effect.]

A group of nine Syrian golden hamsters, 6–7 weeks of age, received applications to the cheek pouch mucosa of 1.5% arecoline in water [quantity not specified] five times per week for life; about 30 mg of 0.5% calcium hydroxide was applied to the cheek pouch mucosa before treatment with arecoline. One papilloma developed in the upper third of the oesophagus in one 15-month-old female (Dunham *et al.*, 1974). [The Working Group noted the small number of animals used.]

Groups of 12–14 male Syrian golden hamsters (total number, 38), 2–3 months of age, received applications to the cheek-pouch mucosa of DMSO extracts of tobacco, areca nut or tobacco plus areca nut thrice weekly for life. A solvent-control group of seven animals received applications of DMSO alone. The combination of tobacco and areca-nut extracts resulted in the development of lesions in the mucous membrane that were diagnosed as early malignant changes in 3/12 animals and in one stomach tumour. One stomach tumour was also found in the 12 animals treated with tobacco extract, but none were found in 14 animals treated with areca-nut extract alone (Ranadive *et al*., 1976). [The Working Group noted the small group size, the ambiguous description of the principal lesions reported and that histology of the stomach tumours was not provided.]

A total of 317 Syrian golden and white mutant hamsters [details on pheno- or genotype of the mutant not provided; sex and distribution of strains in each group unspecified], 2–3 months of age, were administered betel-quid ingredients separately or in various combinations in the cheek pouch, using the following modes: thrice-weekly applications of aqueous extracts of the test materials; deposition of replaceable wax pellets containing the test materials; introduction of gelatin capsules containing the powdered materials; or insertion of the natural components for direct exposure. Animals were killed at 6–12 or 13–14 months. A group of 64 animals served as controls: 25 received a placebo wax pellet, nine received a gelatin capsule and 30 were untreated. The incidences of cheek-pouch and forestomach carcinomas in animals receiving topical applications of the aqueous extracts of test materials are given in Tables 67 and 68. Of the group receiving implants of wax pellets containing betel quid, 4/18 and 8/18 developed cancers of the cheek pouch and forestomach, respectively. Cheek-pouch carcinomas [histology not specified] and forestomach squamous-cell carcinomas were observed in 3/21 and 8/21 (two of these eight carcinoms were located in the oesophageal region) animals receiving implants of wax pellets containing betel quid plus tobacco. The incidence of tumours in hamsters given betel-quid ingredients in their natural form was not markedly different from that seen after other modes of administration. Cheek-pouch carcinomas and forestomach carcinomas developed in 5/16 hamsters given cheek-pouch implantations of capsules containing areca-nut powder, tobacco and lime. Cheek-pouch carcinomas (4/19) and forestomach carcinomas (6/19) occurred in hamsters given capsules containing areca-nut powder (Ranadive *et al.*, 1979). [The Working Group noted the lack of information on sex and strain distribution, the lack of data on the number of tumours per animal and the lack of details on the quantitative composition of the mixtures tested.]

A total of 243 male Syrian golden hamsters, 6–7 weeks of age, were divided into eight groups. Group 1 served as controls. In the other groups, the following test substances were

Table 67. Design and results of experiments in Syrian golden hamsters given topical applications of aqueous extracts of betel-quid ingredients on the cheek pouch

Group	No. of hamsters at start	Age (months)	Cheek-pouch carcinoma		Forestomach carcinoma	
			No.	%[a]	No.	%[a]
Control	19	6–12	–		–	
	11	13–21	–		–	
Areca-nut extract[a]	6	6–12	–		1	16.6
	15	13–21	1	6.6	3	20.0
Polyphenol fraction of areca nut[b]	4	6–12	–		–	
	16	13–21	1	6.2	4	25.0
Areca nut and tobacco extract[b]	6	6–12	–	16.6	–	
	12	13–21	2		3	25.0
Betel-quid extract[c]	16	6–12	–		4	25.0
	4	13–21	–		1	25.0
Betel quid with tobacco extract[d]	7	6–12	–		1	14.2
	6	13–21	–		3	50.0
Areca-nut pieces with extract of areca nut[b]	–	6–12	–		–	
	13	13–21	–		6	46.1
Areca-nut pieces with extracts of areca nut and tobacco[b]	4	6–12	–		2	50.0
	10	13–21	–		1	10.0

From Ranadive *et al*. (1979)

[a] Both hot and cold extracts of areca nut; filtered from an initial mixture of 0.5 g/mL

[b] Details of preparation not mentioned

[c] Filtered from a mixture containing betel leaf (0.5 mg/mL), areca-nut powder (0.2 mg/mL), slaked lime (0.01 g/mL) and catechu (0.01 g/mL)

[d] Filtered from a mixture containing betel leaf (0.5 mg/mL), areca-nut powder (0.2 mg/mL), slaked lime (0.01 g/mL), catechu (0.01 g/mL) and 0.04 g/mL tobacco

applied: liquid paraffin, liquid paraffin with 0.5% 7,12-dimethylbenz[*a*]anthracene (DMBA), DMSO, extract of cured tobacco, extract of Thai areca nut, extract of cured tobacco with Thai areca nut and extract of Indian areca nut. The mode of application was according to Salley (1954) and the extracts were prepared by the methods of Suri *et al*. (1971), with a remark that an additional 10 mL DMSO had to be added to the areca nut before the extract could be obtained by squeezing. Groups 1, 2 and 3 (six animals per group) were killed at week 16. Other groups (45 animals per group) were killed serially at 2-week intervals, from weeks 2 to 30. Squamous-cell carcinoma was seen in the

Table 68. Design and results of experiments in hamsters given implantations of wax pellets and capsules containing betel-quid ingredients[a] on the hamster cheek pouch

Group	No. of hamsters at start	Age (months)	Cheek-pouch carcinoma		Forestomach carcinoma	
			No.	%	No.	%
Wax pellet control	20	6–12	–		–	
	5	13–21	–		–	
Betel quid	8	6–12	1	12.5	3	37.5
	10	13–21	3	30	5	50
Betel quid + tobacco	9	6–12	–		1	11
	12	13–21	3	25	7[b]	42
DMBA (standard carcinogen control)	15	6–12	12	80	7[b]	33
	0	13–21	–		–	
Capsule control	5	6–12	–		–	
	4	13–21	–		–	
Areca-nut powder	10	6–12	2	20	2	20
	9	13–21	2	22	4	44
Areca-nut powder + tobacco powder + lime	4	6–12	1	25	1	25
	12	13–21	4	33	4	33

From Ranadive *et al*. (1979)
DMBA, 7,12-dimethylbenz[*a*]anthracene
[a] Each pellet weighed 2–3 g and contained 0.82–1.3 g of test substance [proportion of the various components not mentioned].
[b] Two of the tumours were oesophageal carcinomas, and were not included in the percentages.

DMBA-treated group [number of tumours not stated]. No tumour was seen in any of the other groups (Weerapradist & Boonpuknavig, 1983).

In a study in which the tumour-promoting activity of arecaidine was tested in DMBA-treated hamster buccal pouches, 112 male adult Syrian hamsters, 10–12 weeks of age, were divided into 16 groups, each containing seven animals. Three of these groups received applications of 1000, 2000 or 3000 μg/mL arecaidine solutions six times per week for 12 weeks without DMBA pretreatment, and one group was untreated. [Arecaidine was obtained from Sigma but the chemical form was not provided.] At the end of 12 weeks, all animals were killed. In all four groups, no tumour was observed (Lin *et al*., 1996).

In an initiation–promotion study, 24 male Syrian golden hamsters, 8 weeks of age, received applications to the cheek pouch of 25 μg/mL arecaidine in distilled water thrice weekly for 12 weeks. Ten weeks later, 1% croton oil in acetone was applied thrice weekly

to the cheek pouches of the 13 remaining animals for another 3 weeks, after which the animals were killed and the last three animals were killed 34 weeks after the start of the experiment. No tumours were found in any of the animals (MacDonald, 1986).

In a study of the tumour-promoting activity of betel quid on DMBA-treated hamster cheek pouches, two groups of male Syrian golden hamsters, about 6–8 weeks of age at the start of the experiment, received insertions of betel quid alone into the cheek pouch. Treatment with betel quid alone for 36 weeks (10 males) or 52 weeks (nine males) did not lead to tumour formation (Wong *et al*., 1992).

The tumour-promoting activity of betel quid on DMBA-treated hamster cheek pouch was tested in groups of 42 non-inbred male adult Syrian hamsters, 8–10 weeks of age, one group was tested with Taiwanese betel-quid extract alone, which was painted six times weekly. The quid extract consisted of the filtrate of a mixture of areca nut (450 g), 'unripe betel fruit' (120 g) [assumed to be inflorescence of *Piper betle* L.] and slaked lime (50 g) to which 300 mL DMSO were added as solvent. At the end of 2 weeks, six animals were taken from each group and killed; this was repeated after periods of 2 weeks each, thus enabling evaluation of the effect of treatment in relation to its duration. After 14 weeks, all animals had been killed. No tumours were seen in the group treated with betel quid alone (Lin *et al*., 1997).

Groups of 20 female Syrian golden hamsters, 6–7 weeks of age, received topical applications to the cheek pouch mucosa of aqueous extracts of tobacco (1 mg per pouch), areca nut (1 mg per pouch) or betel leaf (5 mg per pouch) twice a day for either 10 days or 6 months and were killed 6 months after the last treatment. In the short-term study, none of the animals developed any lesion. In the long-term study, one local squamous-cell papilloma and two local squamous-cell carcinomas of the cheek pouch developed in 3/20 animals in the tobacco-treated group and one local papilloma and one local squamous-cell carcinoma were found in 2/20 animals in the areca nut-treated group. No cheek-pouch tumour was observed in 20 animals treated with betel leaf or in 10 untreated or 10 vehicle controls (Rao, 1984).

Epithelial atypia only was observed in the cheek pouches of 3/6 male and female Syrian golden hamsters after repeated application of 250 mg calcium hydroxide once a day five times a week for 2 weeks and thereafter three times each week between the 2nd and 40th weeks of treatment. Treatment started when the hamsters were 3.5–4.5 weeks old and they reached an average age of 81 weeks. They were either killed when moribund or found dead (Dunham *et al*., 1966). [The Working Group noted the large amount of slaked lime.]

Eight Syrian golden hamsters (males and females), 6–7 weeks of age, received 0.5% calcium hydroxide in DMSO painted on the cheek pouch epithelium, without yielding any lesions [no data on frequency and duration of treatment mentioned] (Dunham *et al*., 1974).

Slaked lime either alone (38 animals) or with tobacco (24 animals) [concentrations not specified] was painted onto the cheek-pouch epithelium of 62 Syrian golden hamsters [sex unspecified] (initial weight, 40–50 g) thrice weekly until they were killed after 2, 4,

8, 16, 24 and 52 weeks of exposure. No tumours were observed (Kandarkar & Sirsat, 1977).

3.5.3 *Baboon*

Twelve young adult baboons (one male and 11 females) were divided into two groups and fed a diet intended to simulate protein deficiency. With this diet, the normal average serum protein level dropped from 7.4 to 6.4% and was maintained for over 3 years. Five animals received a basic betel quid (a freshly prepared ground mixture of betel leaves, areca nut and calcium hydroxide) and seven baboons received the basic quid with added Maharashtran tobacco. Thrice weekly, 3.5 g of the test substance were administered into a surgically created buccal mucosal pouch for 42 months. Biopsies were taken after 1, 6, 9, 12, 16, 23, 29, 34 and 42 months. Severe epithelial atypia was seen microscopically in 1/7 animals treated with betel quid with tobacco after 34 months of quid insertion and in 3/7 of the same group after 42 months. After 42 months of treatment with betel quid without tobacco, 5/5 baboons showed epithelial atypia but not to the degree observed in the group treated with betel quid and tobacco (Hamner, 1972).

3.6 Intravaginal instillation

Mouse

A group of 60 virgin female Swiss mice, about 40 days old, received daily instillation into the vagina of a betel-quid mixture (shell lime and areca nut) with tobacco for up to 380 days, at which time 13 animals were still alive. A group of 10 females received instillations of isotonic saline and served as controls. Of the 50 animals that survived for periods ranging from 324 to 380 days, seven developed carcinomatous changes in the vagina; no tumour was found in controls (Reddy & Anguli, 1967). [The Working Group noted the ambiguous description of the lesions and considered the study to be inadequate for evaluation.]

3.7 Administration with known carcinogens or modifiers of cancer risk

3.7.1 *Mouse*

The anticarcinogenic effect of betel-leaf extract on the development of benzo[*a*]pyrene-induced forestomach papilloma and oral neoplasia induced by the tobacco-specific carcinogenic nitrosamines *N′*-nitrosonornicotine (NNN)- and 4-(methylnitrosoamino)-1-(3-pyridyl)-1-butanone (NNK) was investigated. Groups of 20 inbred male Swiss mice, 8 weeks of age, were administered betel-leaf extract (1 mg/day; 5 days per week) by intragastric instillation for 2 weeks. Thereafter, animals received eight twice-weekly doses of 1 mg benzo[*a*]pyrene in sesame oil by gavage for 4 weeks. The mice again received betel-leaf extract for 2 weeks following cessation of carcinogen treatment. In a second set of experiments,

betel-leaf extract was given in drinking-water on 5 days per week (2.5 mg per day per mouse) during administration of NNN or NNK on the tongue of animals. Treatment with betel-leaf extract in drinking-water reduced the incidence and the yield (number per mouse) of benzo[*a*]pyrene-induced forestomach papilloma (4.9 ± 0.28 versus 0.9 ± 0.22; $p < 0.0005$). The decrease was statistically significant. Betel-leaf extract reduced the number of tumour-bearing mice in NNN- and NNK-treated animals from 49/82 to 23/80; the effect was pronounced in animals receiving low doses of NNN or NNK (Padma *et al*., 1989).

In order to test the chemopreventive efficacy of an extract of betel leaves on benzo[*a*]pyrene-induced forestomach tumours, groups of 20 male Swiss mice, 6–8 weeks of age, were administered an extract of betel leaf or its constituents including eugenol, hydroxychavicol, β-carotene and α-tocopherol in drinking-water (2.5 mg per animal in 6 mL) for 2 weeks. From the 3rd week, the mice were administered eight doses of 1 mg benzo[*a*]pyrene in 0.1 mL peanut oil by gavage twice a week for 4 weeks. Betel-leaf extract or one of its constituents was continued for a further 2 weeks after the cessation of carcinogen treatment. Other groups were treated with betel-leaf extract or each of its constituents only and one group of animals served as peanut oil-vehicle controls. After completion of the treatment, animals were observed and killed at the age of 180 days. In studies on benzo[*a*]pyrene-induced forestomach neoplasia, treatment with betel-leaf extract or its constituents did not influence the number of animals with tumours. However, the average number of papillomas per mouse was significantly lower in mice treated with betel-leaf extract or its constituents. Maximal inhibition of papilloma development was observed in mice receiving hydroxychavicol (Bhide *et al*., 1991).

Effects of topically applied betel-leaf extract and its constituents, β-carotene, α-tocopherol, eugenol and hydroxychavicol, on DMBA-induced skin tumours were evaluated in two strains of mice. Betel-leaf extract, β-carotene and α-tocopherol significantly inhibited papilloma formation by 83, 86 and 86% in Swiss mice and by 92, 94 and 89% in male Swiss bare mice, respectively. Hydroxychavicol showed 90% inhibition in Swiss bare mice at 23 weeks of treatment. Eugenol showed minimal protection in both strains of mice. The mean latency period and survival in betel-leaf extract-, β-carotene-, α-tocopherol- and hydroxychavicol-treated groups were remarkably long compared with the group treated with DMBA alone (Azuine *et al*., 1991). [The results may have been confounded by low survival of the animals.]

A group of 12 male and 12 female C17 mice, 10–12 weeks of age, was fed a diet containing 10% (w/w) areca nut coated with saccharin [concentration not specified] for 40 weeks and also received 0.2 mL of a 0.1% solution of 1,4-dinitrosopiperazine per day by gavage. A group of 15 males and 14 females received 1,4-dinitrosopiperazine only. The animals were followed for life. In the group treated with 1,4-dinitrosopiperazine only, seven male and four female mice developed squamous-cell carcinomas of the forestomach. Five females also developed uterine malignancies. In the group treated with 1,4-dinitrosopiperazine and fed areca nut, six male and three female mice developed squamous-cell carcinomas of the forestomach and five females developed uterine malig-

nancies. The authors concluded that treatment with areca nut did not potentiate the carcinogenicity of 1,4-dinitropiperazine (Pai *et al*., 1981).

Groups of 20 male and female random-bred Swiss mice, 4 weeks of age, were initiated with topical applications of 50 μg DMBA in 0.1 mL acetone, treated 2 weeks later with twice-weekly topical applications of 0 or 1% croton oil for 18 weeks and fed diets containing 0 or 1% ripe, unprocessed, sun-dried areca nuts ground into a fine powder. No influence of areca nut on the incidence of skin papillomas was observed (Singh & Rao, 1995).

Groups of 15 male and 15 female ICRC-strain mice, 6–8 weeks of age, were administered a cumulative dose of 16 g/kg bw *N*-nitrosodiethylamine (NDEA) in the drinking-water for 4 days. One week later, mice were fed by gavage 12.5, 25 or 50 mg ethanolic *pan masala* extract, 5 nmol 12-*O*-tetradecanoylphorbol-13-acetate or distilled water on 5 days a week for 3 or 6 months to assess promoting–progression activity on the incidence of oesophageal and stomach tumours. The cumulative rate and yield of forestomach and oesophageal tumours (squamous-cell papillomas) increased significantly in animals treated with 25 mg ethanolic *pan masala* extract (Ramchandani *et al*., 1998).

3.7.2 *Rat*

The effect of areca nut on chemical carcinogenesis in the upper digestive tract and liver was examined in two different experimental models using ACI rats. The incidences of neoplasms and preneoplastic lesions of the tongue in animals given [5 mg/L] 4-nitroquinoline-1-oxide (4NQO) in the drinking-water for 16 weeks followed by 20% areca nut in the diet for 40 weeks were significantly higher than those in the 14 animals given 4NQO alone. No enhancing effect from areca nut on the incidences of neoplastic and preneoplastic lesions in the upper digestive tract was found in animals administered 4NQO for 12 weeks (Tanaka *et al*., 1986).

When an aqeous extract of the leaves of *Piper betle* was given orally at different dose levels during the initiation phase of DMBA-induced mammary carcinogenesis in rats, higher doses of the extract inhibited the emergence of tumours. However, when the extract was fed to the rats bearing DMBA-induced mammary tumours for 8 weeks, no appreciable degree of inhibition of tumour growth was noted. Betel-leaf extract at the dose levels used did not affect the body-weight gain of rats (Rao *et al*., 1985).

3.7.3 *Hamster*

Groups of female Syrian golden hamsters, 6–7 weeks old, were treated with topical exposures to graded doses of benzo[*a*]pyrene (25 μg, 50 μg and 100 μg per pouch) thrice weekly either alone (20 animals per group) or combined with areca nut (1 mg per pouch; 25 animals per group) or betel leaf (5 mg per pouch; 25 animals per group) twice a day for 6 months and killed 6 months later. The incidence of squamous-cell papilloma or carcinoma in the group treated with the three different doses of benzo[*a*]pyrene alone was

20, 35 and 61%. In the animals treated with the three different doses of benzo[*a*]pyrene together with areca nut, tumour incidence was 26.1, 52.4 and 77.3%, respectively. In the animals treated with the three different doses of benzo[*a*]pyrene together with betel leaf, the tumour incidence was 12.5, 18.2 and 27.3% for the three groups (Rao, 1984).

In a short-term study, groups of 25 female Syrian golden hamsters, 6–7 weeks old, were treated with topical exposures to graded doses of benzo[*a*]pyrene (25 µg, 50 µg and 100 µg per pouch) daily either alone or combined with areca nut (1 mg per pouch) or betel leaf (5 mg per pouch) twice a day for 10 days and killed 6 months later. For the three different doses of benzo[*a*]pyrene, an incidence of squamous-cell papilloma or carcinoma of 4, 8.7 and 16.7%, respectively, was found. Addition of areca nut yielded tumour incidences of 0, 4.2 and 8.3%, respectively. In the animals that were treated with benzo[*a*]-pyrene and betel leaf, tumour incidence was 0, 0 and 4.0% for the three groups, respectively (Rao, 1984).

The inhibitory effect of oral administration of betel-leaf extract and two of its constituents, β-carotene and α-tocopherol, as single agents or in combination with dietary turmeric, on methyl(acetoxymethyl)nitrosamine (DMN-OAC)-induced oral carcinogenesis was studied in 226 Syrian hamsters. DMN-OAC was administered twice monthly for 6 months. The chemopreventive effect of betel-leaf extract or its constituents with turmeric was determined by comparing tumour incidence observed in treated groups with that seen in control animals. The apparent site-specific chemopreventive effect of betel-leaf extract or its constituents was demonstrated by inhibition of tumour incidence, reduction of tumour burden, extension of the tumour latency period and regression of established, obvious tumours. The inhibitory effect of betel-leaf extract or its constituents combined with turmeric was higher than that of the individual constituents (see Table 69; Azuine & Bhide, 1992). [Data regarding survival were incomprehensible.]

The tumour-promoting activity of arecaidine on DMBA-treated hamster buccal pouches was tested in 112 male adult Syrian golden hamsters, 10–12 weeks of age. The animals were divided into 16 groups, each containing seven animals. The buccal pouch of each hamster was painted three times weekly for 12 consecutive weeks with a heavy mineral oil containing 0.5% DMBA in group 1, for 8 consecutive weeks followed by 4 weeks without any additional treatment in group 2, and for 8 consecutive weeks followed by application of 200, 300, 400 and 500 µg/mL arecaidine in polyethylene glycol, respectively, six times per week for 4 weeks in groups 3–6. In six additional groups, DMBA was painted thrice weekly for 4 weeks, without additional treatment in group 7 and with arecaidine solutions incremented from 600 to 1000 µg/mL applied six times per week for a further 8 weeks in groups 8–12. In groups 13–15, arecaidine in solutions of 1000, 2000 or 3000 µg/mL was applied six times a week for 12 weeks. Group 16 was untreated. At the end of 12 weeks, all animals were killed. In groups 2–4, 5/7 hamsters per group had tumours, with a total of seven, nine and eight tumours, respectively, whereas in groups 5 and 6, all hamsters (7/7 per group) had tumours (13 tumours in each of the groups). The increase in the number of tumours in groups 5 and 6 was statistically significant ($p < 0.05$, *t*-test). In groups 7–12, the number of hamsters with tumours

Table 69. Effect of betel-leaf extract and its constituents on DMN-OAC-induced oral tumours in Syrian golden hamsters at 13 months

DMN-OAC[a]	Test compound[b]	Weight gain (g)[c]	Latency period (months)	Tumour incidence				Tumour burden (mm^3)[c]
				Overall		At death		
				5–13 months	%	5–13 months	%	
+	–	52 ± 2	5–10	14/15	93	14/15*	93	600 ± 72
+	BLE	28 ± 2	6–11	4/15*	27	4/15*	27	1.4 ± 0.9*
+	β-Carotene	35 ± 4	8–12	4/15*	27	1/15*	7	0.5 ± 0.0*
+	α-Tocopherol	48 ± 3	9	1/15*	7	1/5	7	4.2 ± 0.0*
–	–	58 ± 2	–	0/15	0	0/15	0	–

From Azuine & Bhide (1992)

[a] DMN-OAC (methyl(acetoxymethyl)nitrosamine) at 2 mg/kg bw twice a month for 6 months

[b] BLE (betel-leaf extract; 2.5 mg), β-carotene (3.1 mg, 5.8 μM) and α-tochopherol (2.5 mg, 5.8 μM) administered daily in drinking-water 2 weeks prior to and simultaneously with DMN-OAC treatment and continued until the end of the experiment

[c] Results are mean ± SE.

* $p < 0.01$ as compared with DMN-OAC alone (χ^2 for tumour incidence data and Student's *t* test for the difference in total tumour burden between the control and treated groups)

in the various groups was 0/7, 5/7, 5/7, 4/7, 7/7 and 7/7, respectively, whereas the total number of tumours was zero, seven, eight, seven, 13 and 15, respectively ($p < 0.05$, *t*-test, for the number of tumours in the groups treated with 900 and 1000 μg/mL arecaidine). In groups 13–15, no tumour was observed (Lin *et al*., 1996).

Six groups of 10 male Syrian golden hamsters, 6–8 weeks of age, received topical applications of 0.5% DMBA three times per week for 2, 4 or 6 weeks, followed by insertion of Taiwanese betel quid, consisting of fresh unripe areca nut, betel stem, slaked lime and catechu, for 12 or 24 weeks. The betel quid was renewed twice a week for the duration of the experiment. The incidence of tumours in cheek pouches was recorded 6 weeks after removal of the insert. Six groups similarly treated with DMBA only served as concurrent control groups. The incidences of tumours (carcinomas) were significantly higher in the group treated with DMBA for 4 weeks followed by betel quid for 24 weeks and in the group treated with DMBA for 6 weeks followed by betel quid for 12 weeks than in their concurrent control groups (6/9 versus 1/9, $p < 0.05$ and 7/7 versus 1/9, $p < 0.01$, respectively) (Wong *et al*., 1992).

Groups of 10 male Syrian hamsters, 2 months of age, were used to test the promoting activity of the various components of Taiwanese betel quid. Following an initial application of 0.5% DMBA three times per week for 4 weeks, the animals remained untreated for 1 week. Twelve of the 13 groups served as the experimental groups and their buccal pouches were filled or painted [for further technical details on application, the authors

refer to another publication (Wong *et al*., 1992)] with components of betel quid thrice weekly for 24 weeks. Thereafter, animals were untreated for a further 6 weeks before being killed. The 13th group that served as a control was left untreated after the initial 4-week application of DMBA and killed at the same time as the other groups. The following substances were applied: group 1, slaked lime; group 2, areca-nut fibre; group 3, *Piper betle*; group 4, *Piper betle* and slaked lime; group 5, hot aqueous extract of areca nut; group 6, hot aqueous extract of areca nut and slaked lime; group 7, cold aqueous extract of areca nut; group 8, cold aqueous extract of areca nut and slaked lime; group 9, hot aqueous extract of areca nut and *Piper betle*; group 10, cold aqueous extract of areca nut with *Piper betle*; group 11, hot aqueous extract of areca nut with *Piper betle* and slaked lime; and group 12, cold aqueous extract of areca nut with *Piper betle* and slaked lime. The incidence of tumours was significantly higher in groups exposed to dry areca-nut fibre (9/10; $p < 0.01$, two-tailed Student's *t*-test) and cold aqueous areca-nut extract only (7/10; $p < 0.05$, two-tailed Student's *t*-test) versus the control in which the tumour incidence was 2/9 (Jin *et al.*, 1996).

Groups of 42 non-inbred male adult Syrian golden hamsters, 8–10 weeks old, received applications to the buccal pouch of 0.5% DMBA thrice weekly concurrently with betel-quid extract six times a week, DMBA alone or betel-quid extract alone. The betel-quid extract consisted of the filtrate of a mixture of areca nut (450 g), unripe betel fruit (120 g) and slaked lime (50 g) to which 300 mL DMSO was added as solvent. At the end of 2 weeks, six animals were taken from each group and killed; this was repeated after periods of 2 weeks each, thus enabling evaluation of the effect of treatment in relation to its duration. After 14 weeks, all animals had been killed. In three other groups, the treatment regimen was as follows: DMSO six times a week, mineral oil six times a week and no treatment at all. After 8 weeks of treatment, tumours occurred in the groups treated with DMBA alone and in the group treated with DMBA and concomitant betel quid. Both the number of tumours and their size were greater in the group with the combined treatment than in the group treated with DMBA alone ($p < 0.05$, two-tailed Student's *t*-test). No tumours were seen in any of the other groups (Lin *et al*., 1997).

DMBA-impregnated sutures (300–400 µg DMBA/cm suture) approximately 1.5 cm in length were placed in the buccal pouch of 165 adult Syrian golden hamsters [age and sex not specified]. The placement of the suture was confirmed every 2 weeks and replaced if lost. After 12 weeks, the DMBA-coated sutures were removed. The cheek pouches were painted with a solution of arecaidine (0.5 mg/mL mineral oil) thrice weekly for an additional 4 weeks or until tumours reached a size of 100 mm^2. With this protocol, all of 133 hamsters that were still alive after 16 weeks of initiation–promotion treatment developed squamous-cell carcinomas (Wani *et al*., 2001). [The Working Group noted that no animals treated with DMBA only were included.]

In a large experiment investigating 130 Syrian golden hamsters, aged 2 months, four males and four females received applications to the buccal pouch of 0.5% DMBA solution thrice weekly for 4 consecutive weeks and were then left untreated for 1 week. After this period, the buccal pouches were treated with slaked lime for 24 weeks and animals were

left untreated for a further 6 weeks. No differences were seen between the group treated with slaked lime and the control that received DMBA only (Jin *et al.*, 1996).