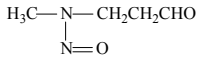
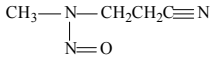
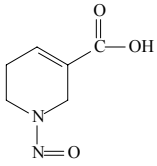
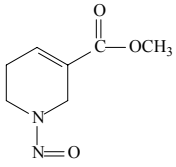
SOME ARECA-NUT-DERIVED *N*-NITROSAMINES

SOME ARECA-NUT-DERIVED *N*-NITROSAMINES

1. Exposure Data

1.1 Chemical and physical data

1.1.1 *Synonyms and structural and molecular formulae*

Chemical name [Chem. Abstr. Services Reg. No.]	Chem. Abstr. Name [Synonym] IUPAC Systematic Name	Structural and molecular formulae and molecular weight		
3-Methylnitrosamino-propionaldehyde [85502-23-4]	Propanal, 3-(methyl-nitrosoamino) [MNPA] 3-(Methylnitrosamino)-propionaldehyde	$C_4H_8N_2O_2$		Mol. wt: 116.1
3-Methylnitrosamino-propionitrile [60153-49-3]	Propanenitrile, 3-(methyl-nitroso-amino) [MNPN] 3-(Methylnitrosamino)-propionitrile	$C_4H_7N_3O$		Mol. wt: 113.1
N-Nitrosoguvacine [55557-01-2]	3-Pyridinecarboxylic acid, 1,2,5,6-tetrahydro-1-nitroso- [NGC; nitrosoguvacine] 1,2,5,6-Tetrahydro-1-nitrosonicotinic acid	$C_6H_8N_2O_3$		Mol. wt: 156.1
N-Nitrosoguvacoline [55557-02-3]	3-Pyridinecarboxylic acid, 1,2,5,6-tetra-hydro-1-nitroso-, methyl ester [NG; NGL; nitrosoguvacoline] Methyl 1,2,5,6-tetra-hydro-1-nitrosonicotinate	$C_7H_{10}N_2O_3$		Mol. wt: 170.2

1.1.2 *Chemical and physical properties*

Chemical/ Physical property	MNPA	MNPN	NGC	NGL
Description	No data	Light-yellow liquid (Chang *et al.* 1976)	Colourless, crystalline solid (Lijinsky & Taylor, 1976)	Yellow oil (Lijinsky & Taylor, 1976)
Melting-point	No data	No data	175.5–177 °C (Lijinsky & Taylor, 1976)	No data
Boiling-point	No data	102–103 °C (0.04 mm Hg) (Chang *et al*., 1976); 97 °C (0.075 mm Hg) (Wenke & Hoffmann, 1983)	No data	137–178 °C (4 mm Hg) (Lijinsky & Taylor, 1976)
Spectroscopy data	NMR, UV and MS data have been reported (Wenke & Hoffmann, 1983; Nishikawa *et al*., 1992)	IR, NMR and MS data have been reported (Chang *et al*., 1976; Wenke & Hoffmann, 1983). MS data reported for MNPN isolated from saliva of betel-quid chewers (Prokopczyk *et al*., 1987)	MS data have been reported (Rainey *et al*., 1978)	MS data have been reported (Rainey *et al*., 1978)
Synthetic compound	Synthetic MNPA is a mixture of E- and Z-isomers in a ratio of 1.4 (Wenke & Hoffmann, 1983)	Synthetic MNPN is a mixture of E- and Z-isomers in a ratio of 1.7 (Wenke & Hoffmann, 1983)		Synthetic NGL is a mixture of E- and Z-isomers in a ratio of 2.5 (Wenke & Hoffmann, 1983)

MNPA, 3-methylnitrosaminopropionaldehyde; MNPN, 3-methylnitrosaminopropionitrile; NGC, *N*-nitrosoguvacine; NGL, *N*-nitrosoguvacoline; NMR, ^{1}H-nuclear magnetic resonance; UV, ultraviolet; MS, mass spectrometry; IR, infrared

1.2 Production

3-Methylnitrosaminopropionaldehyde (MNPA) was prepared by Wenke and Hoffmann (1983) by the reaction of MNPA diethyl acetal with nitrite, followed by hydrolysis. 3-Methylnitrosaminopropionitrile (MNPN) was prepared by Chang *et al.* (1976) by the reaction of sodium nitrite with a solution of MNPN hydrochloride. *N*-Nitrosoguvacine (NGC) was prepared by Lijinsky and Taylor (1976) by nitrosation of guvacine that was synthesized from 3-carbethoxy-4-piperidone by hydrogenation to the piperidonol, followed by dehydration and de-esterification with hydrogen chloride gas at 200 °C, followed by esterification with diazomethane. *N*-Nitrosoguvacoline (NGL) was also prepared by Lijinsky and Taylor (1976) using the same process as that for NGC.

In in-vitro experiments, *N*-nitrosation of arecoline, the major alkaloid of the areca nut, resulted in the formation of NGL, MNPN and MNPA (Wenke & Hoffmann, 1983).

No evidence was found that any of these compounds has ever been produced in commercial quantities or has any use other than as a laboratory chemical.

1.3 Occurrence

NGL, MNPN and MNPA were not detected in three samples of betel quid without tobacco or in three samples of betel quid with tobacco. NGC was detected in one of each of the three samples with and without tobacco (Nair *et al*., 1985).

NGL, NGC and MNPN were found in the saliva of chewers of betel quid with tobacco at nanogram per millilitre levels (Table 1). NGL was also reported in the saliva of three of eight smokers (up to 7.6 ng/mL; Wenke *et al*., 1984a). [The Working Group noted the possibility of analytical problems or the additional use of areca nut by the smokers.] MNPN in saliva was confirmed by mass spectroscopic analysis (Prokopczyk *et al*., 1987); MNPA was not detected in saliva or any other human biological fluid.

Table 1. Levels (range in ng/mL) of areca-nut-derived nitrosamines detected in the saliva[a] of chewers of betel quid with tobacco (BQ + T) and without tobacco (BQ)

Nitrosamine	BQ + T (no.)[b]	BQ (no.)[b]	Reference
NGL	4.3–45 (5)[c]	2.2–9.5 (5)[c]	Wenke *et al*. (1984a)
	0–7.1 (12)	0–5.9 (12)	Nair *et al*. (1985)
	NR	0–142 (9)	Stich *et al*. (1986)
	3.1–23.5 (10)	0.6–8.8 (10)	Nair *et al*. (1987)
NGC	0–30.4 (6)	0–26.6 (6)	Nair *et al*. (1985)
MNPN	NR	0.5–11.4 (10)	Prokopczyk *et al*. (1987)

NGL, *N*-nitrosoguvacoline; NGC, *N*-nitrosoguvacine; MNPN, 3-methylnitrosaminopropionitrile; NR, not reported

[a] Number in parentheses refers to number of samples

[b] The whole saliva or the supernatant was analysed.

[c] In ppb

In-vitro experiments support the hypothesis that, during the chewing of betel quid, nitrosation of arecoline, the major alkaloid of areca nut, produces NGL and MNPN. It was concluded that the conditions prevailing in the oral cavity of betel-quid chewers are likely to favour the formation of these compounds (see Monograph on Betel-quid and Areca-nut Chewing, Section 4.1.1). In-vitro nitrosation of betel quid with and without tobacco under neutral and acidic pH with nitrite in the presence of thiocyanate yielded both NGL and NGC; the formation of NGC was greater than that of NGL when betel quid without tobacco was nitrosated (Nair *et al*., 1985). Small amounts of crude phenolic extracts from fresh green areca fruit (< 60 mg/300 mL) significantly inhibited the formation of NGL from arecoline upon the addition of nitrite, whereas larger amounts (> 250 mg/300 mL) enhanced its formation (Wang & Peng, 1996).

1.4 Analysis

Wenke and Hoffmann (1983) reported a method for analysis of MNPN, MNPA and NGC by gas chromatography with a nitrosamine-selective thermal-energy analyser. Analysis of NGC and NGL has also been reported using similar techniques (Nair *et al*., 1985).

2. Studies of Cancer in Humans

No data were available to the Working Group.

3. Studies of Cancer in Experimental Animals

3.1 3-Methylnitrosaminopropionaldehyde (MNPA)

Subcutaneous administration

Rat: Groups of 15 male and 15 female Fischer 344 rats, 7 weeks of age, received 45 subcutaneous injections of 6.57 mg [0.057 mmol] per animal MNPA (purity, > 99% on the basis of gas chromatography and high-performance liquid chromatography) in 0.3 mL trioctanoin. A group of 12 male and 12 female rats received vehicle only and served as controls. The experiment was terminated 100 weeks after the first injection. Animals were killed at termination or earlier when moribund, and were autopsied and examined histologically. Four of 15 males and 1/14 females developed lung adenoma and one female rat developed lung adenocarcinoma. The total lung tumour incidence was significantly higher in treated groups (6/29) than in controls ($n = 24$), which developed no lung tumours during this period. Other tumours in the treated groups (males and females combined) were nasal papillomas (4/29), liver adenomas (4/29), forestomach papillomas (3/29), nephroblastoma (1/29) and leukaemia (3/29), none of which was found in control animals. A large variety of different tumours occurred in the control group (Nishikawa *et al*., 1992).

3.2 3-Methylnitrosaminopropionitrile (MNPN)

3.2.1 *Oral application*

Rat: MNPN (0.3 mL of a 15 mmol/L solution) was applied by oral swabbing to the oral cavity of 30 male Fischer 344 rats, 10 weeks of age, three times a week for 1 week, once a day for the next 3 weeks and then twice daily until the end of the bioassay (54 weeks). Animals were autopsied and examined histologically. A control group of 30 animals received water alone applied by oral swabbing. In the treated group, 2/30 animals

developed lung adenoma and 2/30 developed lung adenocarcinoma, 10/30 and 14/30 developed nasal cavity adenoma and carcinoma, respectively, 1/30 and 2/30 developed liver adenoma and carcinoma, respectively, 2/30 developed oesophageal papilloma and 1/30 developed oral cavity papilloma. None of 30 control animals developed these lesions (Prokopczyk *et al*., 1991).

3.2.2 *Subcutaneous administration*

Rat: A group of 15 male and 15 female Fischer 344 rats, 7 weeks of age, received thrice-weekly subcutaneous injections of 2.13 mg [0.019 mmol] per animal MNPN (purity, > 99% as determined by high-performance liquid chromatography) in 0.3 mL saline for 20 weeks (total dose, 129 mg per rat or 646 mg/kg bw). A group of 12 males and 12 females served as vehicle controls. The experiment was terminated after 24 weeks because of significant weight loss in the treated animals. Animals were killed at termination or earlier when moribund, and autopsy and histological examination of gross lesions in major organs were carried out. Statistically significant increases in tumour incidence were observed for the following neoplasms: (i) papillomas of the oesophagus in 12/15 treated males ($p < 0.01$) and 14/15 treated females ($p < 0.01$); 3/15 males and 2/15 females treated with MNPN also developed carcinomas of the oesophagus (significant at $p < 0.05$ for both sexes combined); (ii) papillomas of the nasal cavity in 11/15 treated males ($p < 0.01$) and 9/15 treated females ($p < 0.01$); and (iii) papillomas and carcinomas of the tongue in 5/15 treated males ($p < 0.05$) and 6/15 treated females ($p < 0.05$). No tumour was seen in controls (Wenke *et al*., 1984b).

Groups of 21 male and 21 female Fischer 344 rats, 7 weeks of age, received thrice-weekly subcutaneous injections of 0.53 or 2.13 mg/kg bw MNPN in saline for 20 weeks (cumulative dose, 6.4 and 25.7 mg per rat, respectively). A group of 12 male and 12 female rats received saline only and served as controls. Animals were autopsied and gross lesions and major organs were analysed histologically. At the termination of the experiment at 106 weeks, 18/21 male ($p < 0.01$) and 15/21 ($p < 0.01$) female rats had developed nasal carcinomas at the higher dose, whereas only one nasal papilloma had developed with the lower dose. A nasal papilloma was also observed in the control group. The lower dose induced liver tumours [histology not mentioned] in 9/21 male rats, whereas only 1/12 control males developed this tumour. No liver tumours were observed in female rats treated with MNPN, but 3/12 female control rats developed liver tumours. A large variety of other tumours occurred in control and experimental groups (Prokopczyk *et al*., 1987)

3.2.3 *Administration with known carcinogens or modifiers of cancer risk*

Mouse: In a tumour initiation–promotion experiment, a group of 19 female SEN mice, 50–55 days of age, received topical applications of 0.1 mg MNPN in 100 µL acetone every other day for 20 days, amounting to a total dose of 1 mg MNPN. After a 10-day interval, animals were treated with 2 µg 12-*O*-tetradecanoylphorbol-13-acetate in

100 µL acetone twice weekly for 20 weeks. A group of 20 vehicle-treated mice served as controls. Of the MNPN-treated mice, 89% (17/19) developed skin tumours, as did 20% (4/20) of the vehicle-treated controls. Lung adenomas were also found in 89% (17/19) of the MNPN-treated mice but not in the controls. The incidences of both skin tumours and lung adenomas were statistically significant ($p < 0.001$) when compared with the controls (Prokopczyk *et al*., 1991). [The Working Group noted the absence of a group treated with MNPN only and of an adequate histological description of the skin lesions.]

3.3 *N*-Nitrosoguvacoline (NGL)

3.3.1 *Oral administration*

Rat: A group of 15 male and 15 female Sprague-Dawley rats, 8–10 weeks of age, was given drinking-water containing 150 mg/L NGL (no impurity detected by silica-gel thin-layer chromatography) on 5 days per week for 50 weeks (total dose, 750 mg per rat). All animals survived until the end of treatment and were subsequently observed until death or killed at 133 weeks. At 100 weeks, 12 males and nine females were still alive; the four survivors (one male and three females) were killed at 133 weeks. Thirty female and 26 male rats served as untreated [matched or historical, not specified] controls. No statistically significant increase in tumour incidence was found (27 tumours in 30 NGL-treated rats versus 97 tumours in 56 control animals) (Lijinsky & Taylor, 1976). [The Working Group noted that mortality data for the control group were not provided.]

A group of 30 male Fischer 344 rats, 8 weeks of age, received 20 ppm [mg/L] NGL (purity > 99% on the basis of gas chromatography and high-performance liquid chromatography) in the drinking-water for 128 weeks (cumulative dose of NGL, 4.1 mmol/kg). All animals survived until the end of the experiment. Of the treated animals, 4/30 ($p < 0.05$) developed acinar adenoma of pancreas (exocrine pancreas) compared with 1/80 untreated controls (Rivenson *et al*., 1988).

3.3.2 *Administration with known carcinogens or modifiers of cancer risk*

Rat: A group of 30 male Fischer 344 rats, 8 weeks of age, was given 20 ppm [mg/L] NGL concomitantly with 1 ppm 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) in the drinking-water (approximate total doses, 4.1 mmol/kg NGL and 0.17 mmol/kg NNK). Tumour yields observed in these rats were not significantly different from those in rats given NNK only (Rivenson *et al*., 1988).

4. Other Data Relevant to an Evaluation of Carcinogenicity and its Mechanisms

4.1 Absorption, distribution, metabolism and excretion

4.1.1 *Humans*

NGL, NGC and MNPN have been detected in saliva (see Section 1.3).

4.1.2 *Experimental systems*

N-Nitrosonipecotic acid (NNIP) was identified as a major urinary metabolite of NGL in rats. When male BDIV rats were given NGL (50 or 500 μg by stomach tube), urinary NNIP accounted for 66% of the dose in each case. NGC (2.9–4.7% of the dose) was also identified in urine. Only 0.8–1.1% of the dose was excreted in the 24-h faeces (Ohshima *et al*., 1989). In hamsters treated with areca nut and nitrite, NNIP was detected in urine (1.9 ± 0.9 ng/mL; range, 0.57–2.85 ng/mL), indicating endogenous formation of NGL and/or NGC. NNIP was not detected in the urine of hamsters treated with nitrite or areca nut alone (Ernst *et al*., 1987).

NNIP was identified as a major urinary metabolite of NGC in rats. When male BDIV rats were given NGC (50 or 500 μg by stomach tube), urinary NNIP accounted for 82–84% of the dose in the 24-h urine, and 1.6–3.1% of the dose in the 24-h faeces. Unchanged urinary NGC accounted for 2.1–7.6% of the dose. Unchanged NGC (0.5% of the dose) was also observed in the 24-h faeces after administration of the higher dose (Ohshima *et al*., 1989).

4.2 Toxic effects

4.2.1 *Humans*

No data were available to the Working Group.

4.2.2 *Experimental systems*

No toxic effects were reported in rats administered NGL in the drinking-water (0.88 mM [150 mg/L] for 50 weeks; Lijinsky & Taylor, 1976; or 20 ppm [315 mg/rat] for 106 weeks; Rivenson *et al*., 1988). NGL (1.7 mM [289 mg/L]) caused a 50% decrease in the colony-forming efficiency of human buccal epithelial cells (Sundqvist *et al*., 1989).

NGC (up to 5 mM [780 mg/L]) had no significant effect on survival of human buccal epithelial cells (Sundqvist *et al*., 1989).

No toxic effects were reported in male and female Fischer 344 rats treated with 60 subcutaneous injections of MNPN (2.13 mg, 0.019 mmol) thrice weekly over 20 weeks. This dose was highly carcinogenic, however (see Section 3.2) (Wenke *et al*., 1984). No toxic effects were reported in male Fischer 344 rats in which the oral cavity was swabbed with MNPN twice daily up to 5 days per week (0.3 mL of a 15 mmol solution) for 54 weeks. The total dose of MNPN was approximately 259 mg [2.31 mmol] per rat. This dose was carcinogenic (see Section 3.2) (Prokopczyk *et al*., 1991). MNPN (up to 5 mM) had no significant effect on survival of human buccal epithelial cells (Sundqvist *et al*., 1989).

Male and female Fischer 344 rats, 7 weeks of age, received 45 subcutaneous injections of MNPA (6.57 mg, 0.057 mmol, in 0.3 mL trioctanoin) thrice weekly for 15 weeks. The total dose of MNPA per rat was approximately 296 mg (2.6 mmol). Weight gain in treated females was significantly lower than that in controls. Marked liver hydropic degeneration was noted in a female rat that died during week 10. The authors concluded that MNPA was hepatotoxic in females (Nishikawa *et al*., 1992). MNPA (0.15 mM) decreased the colony-forming efficiency of cultured human buccal epithelial cells by 50%; it also (80 μM) decreased low-molecular-weight thiols in these cells by 25% (Sundqvist *et al*., 1989).

4.3 Reproductive and developmental effects

No data were available to the Working Group.

4.4 Genetic and related effects

4.4.1 *Humans*

No data were available to the Working Group.

4.4.2 *Experimental systems*

Results of genotoxicity tests of NGL, NGC, MNPN and MNPA are summarized in Table 2.

In the presence of an exogenous metabolic activation system, NGL was mutagenic to *Salmonella typhimurium* strain TA1535 but only weakly mutagenic to strains TA100 and TA98. Mixed results were obtained in the absence of metabolic activation. It did not cause sex-linked recessive lethal mutations in mature sperm or spermatids of *Drosophila melanogaster*, nor did it induce DNA single-strand breaks in human buccal epithelial cells.

NGC was inactive in *S. typhimurium* TA1535, and did not induce DNA single-strand breaks in human buccal epithelial cells.

MNPN did not induce DNA single-strand breaks in human buccal epithelial cells.

Table 2. Genetic and related effects of areca-nut-derived nitrosamines

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
NGL				
Salmonella typhimurium TA100, reverse mutation	+	(+)	24 µmol [4 mg]/plate	Wang & Peng (1996)
Salmonella typhimurium TA1535, reverse mutation	–	+	200 µg/plate	Rao *et al*. (1977)
Salmonella typhimurium TA98, reverse mutation	(+)[c]	(+)	24 µmol [4 mg]/plate	Wang & Peng (1996)
Drosophila melanogaster, sex-linked recessive lethal mutation	–		20 mM [3400 mg/mL]	Nix *et al*. (1979)
DNA single-strand breaks, human buccal epithelial cells *in vitro*	–	NT	5 mM [850 µg/mL]	Sundqvist *et al*. (1989)
NGC				
Salmonella typhimurium TA1535, reverse mutation	–	–	600 µg/plate	Rao *et al*. (1977)
DNA single-strand breaks, human buccal epithelial cells *in vitro*	–	NT	5 mM [780 µg/mL]	Sundqvist *et al*. (1989)
MNPN				
DNA single-strand breaks, human buccal epithelial cells *in vitro*	+	NT	5 mM [565 µg/mL]	Sundqvist *et al*. (1989)
MNPA				
Salmonella typhimurium TA100, TA1535, TA104, reverse mutation	NT	–	0.4 µmol [46.4 µg]/plate	Chung *et al*. (1994)
DNA single-strand breaks, human buccal epithelial cells *in vitro*	+	NT	0.3 mM [34.8 µg/mL]	Sundqvist *et al*. (1989); Sundqvist & Grafstrom (1992)
DNA–protein cross-links, human buccal epithelial cells *in vitro*	+	NT	0.1 mM [11.6 µg/mL]	Sundqvist & Grafstrom (1992)

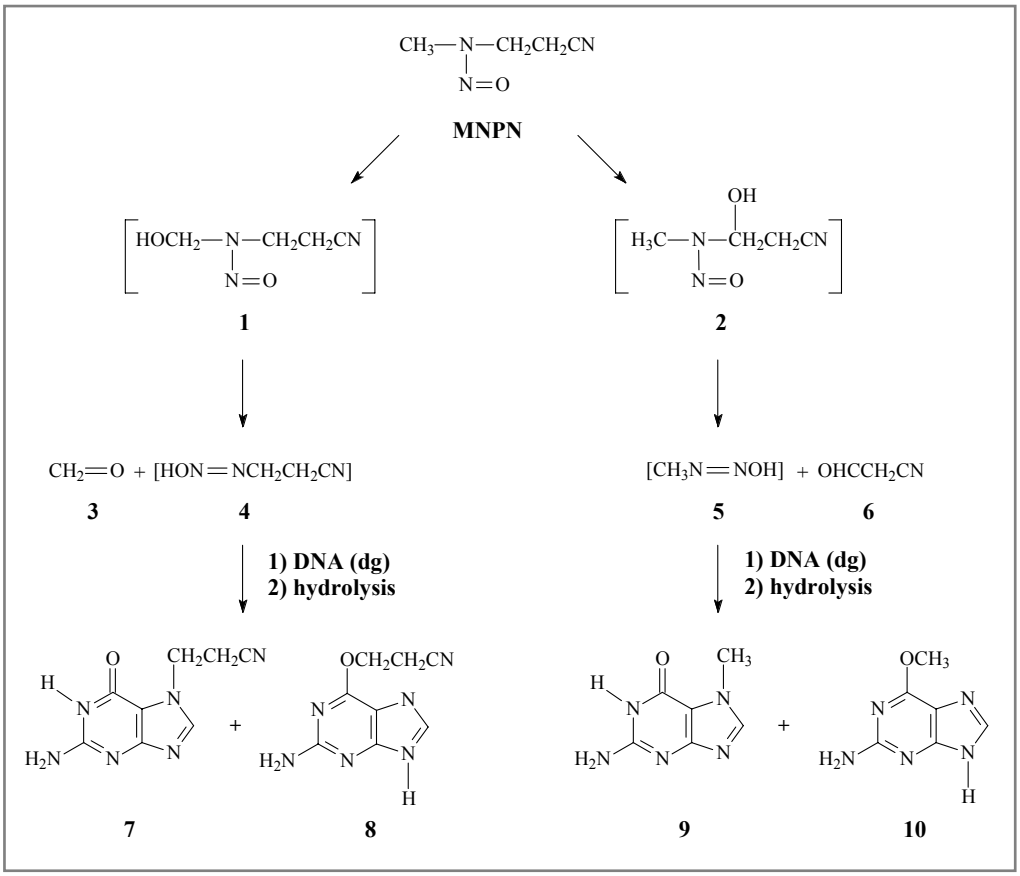
NGL, *N*-nitrosoguvacoline; NGC, *N*-nitrosoguvacine: MNPN, 3-methylnitrosaminopropionitrile; MNPA, 3-methylnitrosaminopropionaldehyde

[a] +, positive; (+), weakly positive; –, negative; NT, not tested

[b] LED, lowest effective dose; HID, highest ineffective dose unless otherwise stated; in-vitro tests, µg/mL

[c] The lowest effective dose tested without an exogenous metabolic system (15 µmol/plate [2.5 mg/plate]) was weakly positive; the dose of 24 µmol was not tested without exogenous system.

Figure 1. Intermediates involved in the α-hydroxylation of 3-methylnitrosamino-propionitrile (MNPN) and their reaction products with deoxyguanosine (dg)



Adapted from Prokopczyk *et al.* (1988)

MNPA was not mutagenic in the presence of rat liver 9000 × g supernatant in *S. typhimurium*. It also induced DNA–protein cross-links at concentrations of 0.1 mM and higher and a significant increase in the levels of DNA single-strand breaks in human buccal epithelial cells in a dose-dependent manner (0.1–1.0 mM). 3-(Carbethoxynitrosamino)-propionaldehyde, a model compound precursor for α-methyl hydroxylation of MNPA, reacted with deoxyguanosine or DNA to form cyclic 1,N^2-propanodeoxyguanosine adducts identical to those derived by the reaction of acrolein with deoxyguanosine and was mutagenic in *S. typhimurium* strains TA100, TA104 and TA1535 without metabolic activation (Chung *et al*., 1994).

Male Fischer 344 rats were given a single intravenous or subcutaneous injection of MNPN (45 mg/kg, 0.4 mmol/kg) in saline or were administered MNPN by swabbing of

the oral cavity (250 mg/kg, 2.21 mmol/kg) and were killed 0.5–36 h later. 7-Methylguanine (**9**) and O^6-methylguanine (**10**) (Figure 1) were detected in DNA of the liver, oesophagus and nasal mucosa. Levels were higher in the liver and nasal mucosa than in the oesophagus. Adducts **9** and **10** were also detected in DNA of the oral cavity after swabbing. These adducts resulted from α-methylene hydroxylation of MNPN via intermediate **2** (Figure 1) (Prokopczyk *et al*., 1987).

Male Fischer 344 rats were given a single subcutaneous injection of MNPN (45 mg/kg, 0.4 mmol/kg) and killed 2–36 h later. 7-(2-Cyanoethyl)guanine (**7**), O^6-(2-cyanoethyl)guanine (**8**), 7-methylguanine (**9**) and O^6-methylguanine (**10**) were detected in the DNA of the liver, nasal mucosa and oesophagus. Adduct ratios (**9**:**7**) ranged from 3.4 to 7.1 in the liver, 1.5 to 2.2 in nasal mucosa and 0.8 to 1.7 in the oesophagus. Levels of adducts **7** and **9** were higher in the DNA of the liver and nasal mucosa than in that of the oesophagus. Adduct ratios (**10**:**8**) ranged from 0.49 to 1.23 in liver and from 0.91 to 3.0 in nasal mucosa. Levels of adducts **8** and **10** were higher in the DNA of the liver and nasal mucosa than in that of the oesophagus; in these latter tissues, the level of adduct **10** was very low, while adduct **8** was not detected. Adducts **7** and **8** result from α-methyl hydroxylation of MNPN (Figure 1; Prokopczyk *et al*., 1988).

5. Summary of Data Reported and Evaluation

5.1 Exposure data

N-Nitrosoguvacoline, *N*-nitrosoguvacine and 3-methylnitrosopropionitrile have been found in the saliva of betel-quid chewers. Thus, there is some evidence that chewers are exposed to these compounds.

5.2 Human carcinogenicity data

No data were available to the Working Group.

5.3 Animal carcinogenicity data

Following subcutaneous administration of 3-methylnitrosaminopropionaldehyde to rats, the incidence of lung adenoma and adenocarcinoma was significantly increased in both males and females. A variety of other benign and malignant tumours was also observed.

Application of 3-methylnitrosopropionitrile to the oral cavity of male rats produced adenomas and adenocarcinomas in lung and nasal cavity, adenomas and carcinomas in the liver and papillomas in the oesophagus and oral cavity.

Subcutaneous administration of 3-methylnitrosaminopropionitrile to rats induced papillomas and carcinomas of the oesophagus and the tongue and papillomas of the nasal

cavity in males and females in a short-term experiment, and an increased incidence of nasal carcinomas in male and female rats and of liver tumours in male rats in a long-term experiment.

In an initiation–promotion study on mouse skin, initiation with 3-methylnitrosopropionitrile led to the development of skin tumours and lung adenomas.

Addition of *N*-nitrosoguvacoline to the drinking-water of rats induced pancreatic adenomas in males in one study, but no increase in tumours in males or females in another.

5.4 Other relevant data

N-Nitrosoguvacoline and *N*-nitrosoguvacine are metabolized in rats to *N*-nitrosonipecotic acid, which is excreted in the urine. *N*-Nitrosonipecotic acid has been detected in the urine of hamsters treated with areca nut plus nitrite, indicating the endogenous formation of *N*-nitrosoguvacoline and *N*-nitrosoguvacine.

3-Methylnitrosopropionitrile induced liver toxicity in female rats.

N-Nitrosoguvacoline but not *N*-nitrosoguvacine was mutagenic to bacteria. 3-Methylnitrosaminopropionitrile caused single-strand breaks and DNA–protein cross-links in human buccal epithelial cells. DNA methylation and cyanoethylation were observed in rats treated with 3-methylnitrosaminopropionitrile. These studies demonstrate that *N*-nitrosoguvacoline, *N*-nitrosoguvacine and 3-methylnitrosaminopropionitrile are genotoxic.

5.5 Evaluation

There is *inadequate evidence* in humans for the carcinogenicity of *N*-nitrosoguvacoline, *N*-nitrosoguvacine and 3-methylnitrosaminopropionitrile.

There is *sufficient evidence* in experimental animals for the carcinogenicity of 3-methylnitrosaminopropionitrile.

There is *limited evidence* in experimental animals for the carcinogenicity of 3-methylnitrosaminopropionaldehyde.

There is *inadequate evidence* in experimental animals for the carcinogenicity of *N*-nitrosoguvacoline and *N*-nitrosoguvacine.

Overall evaluation

N-Nitrosoguvacoline is *not classifiable as to its carcinogenicity to humans (Group 3)*.

N-Nitrosoguvacine is *not classifiable as to its carcinogenicity to humans (Group 3)*.

3-Methylnitrosaminopropionitrile is *possibly carcinogenic to humans (Group 2B)*.

3-Methylnitrosaminopropionaldehyde is *not classifiable as to its carcinogenicity to humans (Group 3)*.

6. References

Chang, S.K., Harrington, G.W., Veale, H.S. & Swern, D. (1976) The unusually mild and facile basic hydrolysis of *N*-nitroso-2-(methylamino)acetonitrile. *J. org. Chem*., **41**, 3752–3755

Chung, F.L., Krzeminski, J., Wang, M., Chen, H.J. & Prokopczyk, B. (1994) Formation of the acrolein-derived 1,N^2-propanodeoxyguanosine adducts in DNA upon reaction with 3-(*N*-carbethoxy-*N*-nitrosamino)propionaldehyde. *Chem. Res. Toxicol.*, **7**, 62–67

Ernst, H., Ohshima, H., Bartsch, H., Mohr, U. & Reichart, P. (1987) Tumorigenicity study in Syrian hamsters fed areca nut together with nitrite. *Carcinogenesis*, **8**, 1843–1845

Lijinsky, W. & Taylor, H.W. (1976) Carcinogenicity test of two unsaturated derivatives of *N*-nitrosopiperidine in Sprague-Dawley rats. *J. natl Cancer Inst.*, **57***,* 1315–1317

Nair, J., Ohshima, H., Friesen, M., Croisy, A., Bhide, S.V. & Bartsch, H. (1985) Tobacco-specific and betel nut-specific *N*-nitroso compounds: Occurrence in saliva and urine of betel quid chewers and formation *in vitro* by nitrosation of betel quid. *Carcinogenesis*, **6**, 295–303

Nair, J., Nair, U.J., Ohshima, H., Bhide, S.V. & Bartsch, H. (1987) Endogenous nitrosation in the oral cavity of chewers while chewing betel quid with or without tobacco. In: Bartsch, H., O'Neill, I.K. & Schulte-Hermann, R., eds, *Relevance of* N-*Nitroso Compounds to Human Cancer: Exposures and Mechanisms* (IARC Scientific Publications No. 84), Lyon, IARC*Press*, pp. 465–469

Nishikawa, A., Prokopczyk, B., Rivenson, A., Zang, E. & Hoffmann, D. (1992) A study of betel quid carcinogenesis. VIII. Carcinogenicity of 3-(methylnitrosamino)propionaldehyde in F344 rats. *Carcinogenesis*, **13**, 369–372

Nix, C.E., Brewen, B., Wilkerson, R., Lijinsky, W. & Epler, J.L. (1979) Effects of *N*-nitrosopiperidine substitutions on mutagenicity in *Drosophila melanogaster*. *Mutat. Res.*, **67**, 27–38

Ohshima, H., Friesen, M. & Bartsch, H. (1989) Identification in rats of *N*-nitrosonipecotic acid as a major urinary metabolite of the areca-nut alkaloid-derived nitrosamines, *N*-nitrosoguvacoline and *N*-nitrosoguvacine. *Cancer Lett.*, **44**, 211–216

Prokopczyk, B., Rivenson, A., Bertinato, P., Brunnemann, K.D. & Hoffmann, D. (1987) 3-(Methylnitrosamino)proprionitrile: Occurrence in saliva of betel quid chewers, carcinogenicity, and DNA methylation in F344 rats. *Cancer Res*., **47**, 467–471

Prokopczyk, B., Bertinato, P. & Hoffmann, D. (1988) Cyanoethylation of DNA *in vivo* by 3-(methylnitrosamino)propionitrile, an *Areca*-derived carcinogen. *Cancer Res.*, **48**, 6780–6784

Prokopczyk, B., Rivenson, A. & Hoffmann, D. (1991) A study of betel quid carcinogenesis. IX. Comparative carcinogenicity of 3-(methylnitrosamino)propionitrile and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone upon local application to mouse skin and rat oral mucosa. *Cancer Lett.*, **60**, 153–157

Rainey, W.T., Christie, W.H. & Lijinsky, W. (1978) Mass spectrometry of *N*-nitrosamines. *Biochem. mass Spectrom.*, **5**, 395–408

Rao, T.K., Hardigree, A.A., Young, J.A., Lijinsky, W. & Epler, J.L. (1977) Mutagenicity of *N*-nitrosopiperidines with *Salmonella typhimurium*/microsomal activation system. *Mutat. Res.*, **56**, 131–145

Rivenson, A., Hoffmann, D., Prokopczyk, B., Amin, S. & Hecht, S.S. (1988) Induction of lung and exocrine pancreas tumors in F344 rats by tobacco-specific and *Areca*-derived *N*-nitrosamines. *Cancer Res.*, **48**, 6912–6917

Stich, H.F., Rosin, M.P. & Brunnemann, K.D. (1986) Oral lesions, genotoxicity and nitrosamines in betel quid chewers with no obvious increase in oral cancer risk. *Cancer Lett.*, **31**, 15–25

Sundqvist, K. & Grafstrom, R.C. (1992) Effects of areca nut on growth, differentiation and formation of DNA damage in cultured human buccal epithelial cells. *Int. J. Cancer*, **52**, 305–310

Sundqvist, K., Liu, Y., Nair, J., Bartsch, H., Arvidson, K. & Grafstrom, R.C. (1989) Cytotoxic and genotoxic effects of areca nut-related compounds in cultured human buccal epithelial cells. *Cancer Res.*, **49**, 5294–5298

Wang, C.-K. & Peng, C.-H. (1996) The mutagenicities of alkaloids and *N*-nitrosoguvacoline from betel quid. *Mutat. Res*, **360**, 165–171

Wenke, G. & Hoffmann, D. (1983) A study of betel quid carcinogenesis. 1. On the in vitro *N*-nitrosation of arecoline. *Carcinogenesis*, **4**, 169–172

Wenke, G., Brunnemann, K.D., Hoffmann, D. & Bhide, S.V. (1984a) A study of betel quid carcinogenesis. IV. Analysis of the saliva of betel chewers. A preliminary report. *J. Cancer Res. clin. Oncol.*, **108**, 110–113

Wenke, G., Rivenson, A. & Hoffmann, D. (1984b) A study of betel quid carcinogenesis. III. 3-(Methylnitrosamino)propionitrile, a powerful carcinogen in F344 rats. *Carcinogenesis*, **5**, 1137–1140