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)

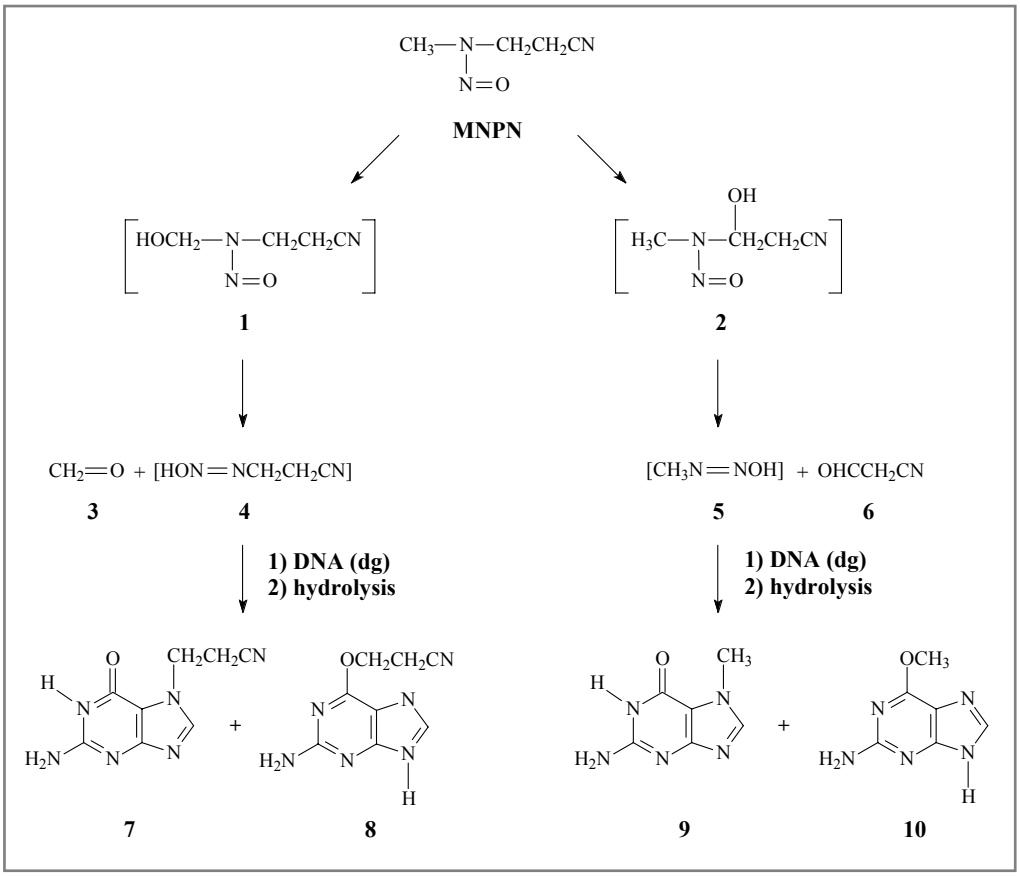
NGL, *N*-nitrosoguvacoline; NGC, *N*-nitrosoguvacine: MNPN, 3-methylnitrosaminopropionitrile; MNPA, 3-methylnitrosaminopropionaldehyde

[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).