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).