3. Studies of Cancer in Experimental Animals

3.1 Ethanol and alcoholic beverages

Previous studies

Ethanol was evaluated by an IARC Working Group in 1988 (IARC, 1988). At the time, some early studies were available in which ethanol was administered to mice (Krebs, 1928: Ketcham *et al.*, 1963, Horie *et al.*, 1965) and hamsters (Elzay, 1966; Henefer, 1966; Elzay, 1969; Freedman & Shklar, 1978) by use of various protocols, but these studies were found to be inadequate for evaluation.

The 1988 Working Group evaluated studies published between 1965 and 1987, most of which were criticized for various reasons, including small numbers of experimental animals, absence of histopathological examination, absence of an untreated control group, limited dose of ethanol administered, short duration of the study and unpaired feeding regimen. Thus, the conclusion was that ethanol *per se* could not be considered to be carcinogenic in animal experiments.

Studies on the administration of ethanol and the development of cancer in experimental animals that have been published since that time are reviewed below.

3.1.1 *Oral administration*

(a) Mouse

As part of a study to investigate the effects of ethanol on the carcinogenicity of NDMA, three groups of 50 male strain A (A/JNCr) mice (a strain that is prone to develop spontaneous lung tumours), 4 weeks of age, received 10% ethanol in the drinking-water. One group received ethanol from week 1 to week 16, the second group received ethanol from week 4 to week 16 and the third group received ethanol from week 5 to week 16. [Ethanol intake calculated from the average water consumption was between 0.4 and 0.48 g per day per animal.] The lung-tumour incidence was between

12 and 14%, which was not significantly different from that in two control groups that did not receive ethanol. The spontaneous lung-tumour occurrence was 10% (Anderson, 1988).

As part of a study to investigate the effect of ethanol on the carcinogenicity of ethyl carbamate, 15 female strain A/Ph mice, 6.5 weeks of age, received 5, 10 or 20% ethanol in the drinking-water for 12 weeks; 15 animals that did not receive ethanol served as controls. Body weight (bw) was reduced with 20% ethanol. The percentages of mice with lung tumours were 67, 47 and 67%, respectively, compared with 40% in the control group, a difference that was not statistically significant. The tumour multiplicity also did not differ (Kristiansen *et al.*, 1990). [The Working Group noted the small number of animals, and that ethanol blood concentrations and intake data were not specified.]

As part of another study to investigate the effect of ethanol on the carcinogenesis of ethyl carbamate, 25 female NMRI mice, 10 weeks of age, were treated daily for 3 days with 10% ethanol by gavage (0.3 mL/25 g bw) and then with 20% ethanol for a total of 8 weeks. Eight weeks after the last dose, the animals were killed; 9–24% of mice in the ethanol-treated group developed lung adenomas compared with 17–21% in the control group, a difference that was not significant (Altmann *et al.*, 1991). [The Working Group noted the short duration of exposure to ethanol.]

Groups of 30 male and 30 female inbred Swiss mice, 8 weeks of age, received either 10% Indian country liquor or 1% ethanol in the drinking-water or pure water only from the age of 2 months until 18 months. The experiment was terminated at 26 months of age. The total tumour incidence in untreated male and female mice was 3% (1/29; one lung and forestomach) and 4% (1/27; one forestomach), respectively, compared with 5% (1/22; one lung) and 11% (2/19; two forestomach), respectively, in animals that received 1% ethanol in the drinking-water. Indian country liquor at 10% induced a tumour incidence of 28% (7/25; one liver, one lung, four forestomach, one lung and forestomach) [$P = 0.0186$] in male mice and 7% (2/29; one kidney and one forestomach) in female mice (Zariwala *et al.*, 1991). [The Working Group noted that Indian country liquor may contain a wide variety of congeners that may be responsible for the results obtained. No significantly different effect was observed between controls and animals treated with 1% ethanol. One per cent ethanol is a rather low dose and may not be sufficient to induce tumours. The Working Group also noted that very few animals survived to the end of the study.]

Groups of 30 male BALB/c mice, 8 weeks of age, received 10% Indian country liquor or 1% ethanol in the drinking-water or pure water from the age of 2 months until 18 months. The experiment was terminated when the mice were 26 months of age. Untreated controls had a 4% tumour incidence (1/24; one forestomach); 10% liquor and 1% ethanol resulted in a tumour incidence of 22% (5/23; three lung, two forestomach) and 0% (0/28), respectively (Zariwala *et al.*, 1991). [The Working Group noted that Indian country liquor may contain a wide variety of congeners that may be responsible for the results obtained. No difference in effect was observed between untreated

controls and animals that received 1% ethanol in the drinking-water. One per cent ethanol in the drinking-water is a rather low dose and may not be sufficient to induce tumours. The Working Group noted also that very few animals survived to the end of the study.]

To investigate the effect of ethanol on the carcinogenesis of *N*-nitrosodimethylamine (NDMA), a group of 25 male A/JNCr mice, 4–6 weeks of age, received a 10% solution of ethanol in the drinking-water for 4 weeks and was then kept for another 12 weeks. [Intake of ethanol could be calculated from the amount of water consumed and was approximately 0.34 g per mouse per day.] The experiment was terminated at 16 weeks. In the ethanol-treated group, 16% (4/25) developed lung tumours compared with 8% (2/25) in the control group, a difference that was not statistically significant. In another experiment, 48 animals received 10% ethanol in the drinking-water for 69 ± 6 weeks and another 48 animals served as a control group for 70 ± 5 weeks without ethanol. The lung tumour rate was 69% in the ethanol-treated group and 83% in the control group (difference not significant). In a third experiment, groups of 30 animals each received 0 (controls), 5, 10 or 20% ethanol in the drinking-water for 16 weeks. The experiment was terminated at 16 weeks. The numbers of animals with lung tumours were 3.3, 20, 23.3 and 13.3%, respectively. These values were not statistically different (Anderson *et al.*, 1992). [The Working Group noted that no blood ethanol measurements were taken.]

Two groups of 15 female C3H/Ou mice, 6 weeks of age, received 12% ethanol in the drinking-water or water alone for 65 weeks. In the ethanol-treated group, development of mammary tumours was delayed ($P = 0.03$). The median incidence was reached 17 weeks later than in the controls. Ethanol consumption was approximately 15 g/kg bw per day. Ethanol-treated animals gained less weight and consumed fewer calories (controls consumed 13% more calories) and drank 40% less fluid (Hackney *et al.*, 1992). [The Working Group noted that the number of animals was small, that variables such as calories and drinking-water were not controlled for and that no ethanol blood concentrations were given.]

Ten female C3H/Ou mice, 6 weeks of age, received 4 g/kg bw ethanol per day by gavage five times per week for 65 weeks, while 16 animals received a control gavage with Sustacal. The animals received the same calories per day in an isocaloric pair-feeding model provided by semipurified solid diets. Diet restriction was necessary for controls but water was given *ad libitum*. Both groups developed similar numbers of mammary tumours at a similar rate. The highest ethanol blood level achieved was 0.25% (250 mg/100 mL) (Hackney *et al.*, 1992). [The Working Group noted the small number of animals, the adequate design with pair feeding and the adequate blood ethanol concentrations.]

Two groups of 20 and 14 female C3H/Ou mice, 6 weeks of age, received Lieber-DeCarli diets with 29% ethanol as total calories (20 g/kg per day) and control diet for 65 weeks, respectively. No difference in weight gain and no difference in mammary tumour development were observed (Hackney *et al.*, 1992). [The Working Group noted the small number of animals and the adequate design with pair feeding.]

As part of a study to investigate the effect of ethanol on the carcinogenesis of *N*-nitrosomethylbenzylamine (NMB_zA), groups of 13 and 12 female C57BL/6 mice, 4–6 weeks of age, received ethanol [purity not specified] as 30% of total calories (Lieber-DeCarli diets) for 22 weeks or control diet, respectively. The experiment was terminated at 22 weeks. No difference in tumour incidence was observed between the ethanol-treated and control groups (one tumour in each group) (Eskelson *et al.*, 1993). [The Working Group noted the small number of animals. One control mouse developed an oesophageal tumour without carcinogen treatment, which is difficult to explain.]

As part of a study that investigated the effect of ethanol on the carcinogenicity of nitrosamines, 25 male strain A/JNCr mice, 4 weeks of age, received 10% ethanol in the drinking-water for 4 weeks. The experiment was terminated 32 weeks later. The incidence of lung tumours in the ethanol-treated group was 60% [15/25], which was slightly, but not significantly, greater than that in the untreated control group (38% [9/24]). In a second experiment, 49 female Swiss NIH:Cr(S) mice, 4 weeks of age, received 15% ethanol for 12 weeks [presumably in the drinking-water] and were killed when ill or at 18 months of age; 48 animals served as a saline control group. No difference in body weight or survival was observed. No significant difference in tumour yield was reported. In the ethanol-treated group, besides lung tumours, five lymphomas, one thymic tumour, four uterine tumours and two sarcomas were also reported. In the control group, six lymphomas, one thymic tumour, one uterine tumour and one sarcoma were noted (Anderson *et al.*, 1993). [The Working Group noted that blood ethanol concentrations were not determined.]

A group of 20 female ICR mice, 40 days of age, was administered 10% ethanol (v/v) [purity not specified] in the drinking-water for 2 months and then 15% ethanol (v/v) in the drinking-water for 23 months. An additional group of 20 females was given tap-water as their drinking fluid. The experiment was terminated after 25 months. Mammary tumours were assessed macroscopically and microscopically. Body weights did not differ between the two groups. Mice that received drinking-water that contained ethanol consumed 4.7 ± 0.60 mL/day (13.2 ± 2.66 g/kg bw ethanol per day), which did not differ from that consumed by control mice (5.3 ± 0.64 mL/day). Beginning 8 months after treatment, mammary gland tumours (papillary or medullary adenocarcinoma) were detected in 45% (9/20) mice given ethanol in the drinking-water compared with 0/20 control mice [$P = 0.0012$; two-tailed Fisher's exact test] (Watabiki *et al.*, 2000).

As part of a study that investigated the effect of ethanol on the carcinogenicity of ethyl carbamate, three groups of 48 male and 48 female $B6C3F_1$ mice, 28 days of age, received either 0, 2.5 or 5.0% ethanol orally in the drinking-water for 104 weeks. No impurities except water were detected. The average daily consumption of ethanol was 100 and 180 mg in male mice that received 2.5 and 5% ethanol, respectively. The comparable values for females were 80 and 155 mg. [This is equivalent to approximately 2.2 and 4.2 g/kg bw per day for both sexes.] No serum ethanol concentrations could be measured with the doses of ethanol administered (< 8 mg/100 mL). Increasing ethanol

content in the drinking-water had no effect on cell-cycle distribution in the liver or on cell proliferation in the lungs. Increasing ethanol content in the drinking-water increased cytochrome P-450 2E1 (CYP2E1) in the livers of female but not of male animals. Ethanol had no effect on body weight. Male mice showed a dose-related increase in survival as a function of increasing ethanol concentrations ($P = 0.053$), while female mice did not. Complete histopathology was performed. In female mice, ethanol had no effect on tumour incidence. In male mice, a dose-related trend ($P < 0.05$; Poly-3 test) was found for the incidence of hepatocellular adenoma (control, 15% (7/46); 2.5% ethanol, 25% (12/47); 5% ethanol, 39% (19/48) and for that of hepatocellular adenoma or carcinoma (control, 26% (12/46); 2.5%, 34% (16/47); 5%, 52% (25/48)). The increase in the incidence of hepatocellular adenoma was significant in the 0.5% ethanol-treated group (National Toxicology Program, 2004; Beland *et al.*, 2005). [The Working Group noted that the ethanol serum concentrations were too low to measure and that the lack of induction of hepatic CYP2E1 in the liver of male animals could be due to low ethanol levels. Despite the low amount of ethanol given, it is remarkable that the incidence of hepatocellular tumours was increased in male animals. The Working Group also noted that the maximum tolerated dose may have not been used in this study.]

(b) Rat

As part of a study to investigate the effect of ethanol on the carcinogenicity of synthetic estrogens and progestins, one group of female and one group of male Wistar JCL rats, 4 weeks of age, received 10% ethanol in the drinking-water on 5 days a week *ad libitum*. On the remaining 2 days of each week, the animals received pure water. In addition, 0.5 mL olive oil per day was given through a stomach tube. The treatment lasted 12 months and rats were killed at 2, 4, 6, 8 (five females and four males for each time point) and 12 months (10 females and eight males). Control rats that did not receive ethanol were also available (five female and four males for each time point). No hepatocellular carcinoma or hyperplastic nodules were found in any of the animals during the experimental period (Yamagiwa *et al.*, 1994). [The Working Group noted the small number of animals, the non-pair-feeding regime and the lack of measurements of ethanol blood levels.]

Eight groups of 50 male and 50 female Sprague Dawley rats, 6–7 weeks of age, received a semi-synthetic liquid diet either with low (1%) or high (3%) ethanol content or low glucose or high glucose content (20.2 or 62.0 g/L of diet glucose to serve as equicaloric controls). Males were given 70 mL/day and females were given 60 mL/day [which corresponded to an alcohol (and glucose) intake of 0.56 g/day (11.1 g glucose/day) and 1.68 g/day (14 g glucose/day) in males and 0.48 g/day (9.5 g glucose/day) and 1.44 g/day (12 g glucose/day) in females]. Liquid diet was given to the animals until death, but no glucose or ethanol was added after 104 weeks. Animals were killed when moribund or when the study was terminated, after 120 weeks. Treatment with 3% ethanol led to lower body weight in males after 13 weeks and in females after 69

weeks. Statistical analysis of survival showed that females treated with 3% ethanol survived longer than the controls ($P = 0.002$). Those treated with 1% ethanol also had a longer survival, which was not statistically significant. No statistical difference in organ weights was noted. For males, no effect of ethanol was observed on the occurrence of overall neoplasms (benign or malignant). In females, there was a statistically significant decrease in the incidence of all tumours among ethanol-exposed animals ($P < 0.01$). Pituitary tumours [not specified] were more common among high-dose ethanol-treated females (80%) than among high-dose glucose-treated animals (58%) ($P < 0.05$). Among low-dose ethanol-treated females, there was a statistically significant increase in the incidence of benign tumours in all organs as well as in mammary gland fibroma, fibroadenoma or adenoma [no incidence provided] (Holmberg & Ekström, 1995). [The Working Group noted that the ethanol intake was low relative to the high rate of ethanol metabolism in these rats and the low dose used, and that ethanol blood concentrations were not measured.]

As part of a study to investigate the influence of various chemicals on the carcinogenesis of *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine (MNNG), 16 male Fischer 344 rats, 5 weeks of age, received 10% ethanol in the drinking-water for 51 weeks starting at 7 weeks of age; 15 untreated male Fischer 344 rats served as a control. No forestomach tumours or glandular stomach neoplasms were observed in any of the groups (Wada *et al.*, 1998). [The Working Group noted the poor reporting of the study, the small number of animals, that the rats were not pair fed and the absence of ethanol blood measurements.]

Groups of 110 male and 110 female Sprague-Dawley rats and their offspring (30 males and 39 females) received 10% ethanol (purity > 99.8%) or no ethanol (49 male and 55 female offspring) in the drinking-water *ad libitum* starting at 39 weeks of age (breeders), 7 days before mating or from embryo life (offspring) until spontaneous death (last death at 179 weeks for offspring). Control animals received tapwater. The intake of fluid was lower in the treated compared with the control group, but no difference in body weight was noted. No significant differences in survival occurred with the exception of lower survival of female offspring treated with ethanol from 104 to 152 weeks. Full necropsies and histopathology were performed. An increase in the incidence of total malignant tumours was noted in female breeders (72% (79/110) versus 43% (48/110); $P < 0.0001$) and male offspring (76% (23/30) versus 47% (23/49); $P < 0.02$). This was due to an increase in the incidence of head and neck carcinoma (oral cavity, lips, tongue) in male breeders (13% (15/110) versus 2.7% (3/110); [$P = 0.0054$] 33% (10/30) versus 4% (2/49); [$P = 0.0014$]) and female offspring (41% (16/39) versus 5% (3/55); [$P = 0.0001$]) and that of carcinoma of the forestomach in male (7% (8/110) versus 0/110; [$P = 0.0012$]) and female (2.7% (3/110) versus 0/110 [not significant]) breeders. Increases in the incidence of interstitial-cell adenomas of the testis (21% (23/110) versus 8% (9/110); [$P = 0.013$]) and osteosarcoma of the head and other sites were also observed in male breeders (11% (12/110) versus 0.9% (1/110); [$P = 0.0042$]) (Soffritti *et al.*, 2002a). [The Working Group noted that this was not a

pair-feeding experiment, that the number of animals per litter was not reported, that ethanol intake may have been low and that no ethanol blood concentrations were measured. However, even under these experimental conditions, administration of ethanol caused an increase in tumour development, which is important to note. The Working Group also noted that some statements reporting increased incidences were not supported by statistical analyses performed by the Working Group.]

(c) Hamster

A total of 90 male and 90 female Syrian golden hamsters, 8 weeks of age, were divided into six groups and received 10% Indian country liquor or 1% ethanol in the drinking-water or pure drinking-water from the age of 2 months until 18 months. No tumours were observed after treatment with liquor in either sex. A 3% (1/29) incidence of forestomach papillomas was seen in untreated control male hamsters (Zariwala *et al.*, 1991).

3.1.2 *Dermal application*

Mouse

As part of a study on modifying effects, 24 female C3H/HeNCr(MTV-) mice, 9–10 weeks of age, were treated locally with a 25% ethanol solution on the dorsal skin, ear and tail three times a week for 30 weeks. None of the animals developed skin tumours (melanoma, squamous-cell carcinoma or fibrosarcoma) (Strickland *et al.*, 2000). [The Working Group noted the small number of animals and the absence of untreated controls.]

3.1.3 *Transplacental and neonatal administration*

(a) Mouse

A group of 27 female Swiss mice, 8 weeks of age, received 10% Indian country liquor in the drinking-water from day 12 of gestation until weaning of the progeny (total, 38 days). Weaned offspring were kept under observation until death with no further treatment. No significant changes in tumour incidence [tumour type not specified] were observed in either sex of offspring of mothers treated with liquor (3% (2/62) of males, 4% (2/53) of females) compared with untreated controls (6% (2/34) of males, 2% (1/45) of females). Breeders treated with liquor had 1/18 (5%) lung adenoma compared with none in controls (Zariwala *et al.*, 1991). [The Working Group found that the data reported were insufficient to evaluate.]

(b) Hamster

A group of four female Syrian hamsters received 10% ethanol in the drinking-water on days 5–16 of pregnancy. A control group received water only. No difference

in tumour incidence in the offspring was observed between the ethanol-treated and control groups (Schüller *et al.*, 1993).

3.1.4 *Genetically modified animals*

Mouse

Twenty-four male C57/B6 APC MIN mice, 7–8 weeks of age, received alternately 15 and 20% ethanol [purity not specified] in the drinking-water every other day for 10 weeks. The experiment was terminated after 10 weeks and histopathology was performed. Ethanol supplementation resulted in a 35% increase in intestinal tumour multiplicity (26.8 ± 8.9 versus 36.9 ± 10.1; $P < 0.05$). The increase in tumour incidence was most pronounced (67%) [multiplicity not given] in the distal small bowel ($P < 0.05$) (Roy *et al.*, 2002). [The Working Group noted that the effect of ethanol was investigated in a genetically susceptible mouse model of intestinal cancer.]

3.2 Modifying effects of ethanol on the activity of known carcinogens

Previous studies

More than 30 studies were included in this section of the previous Monograph (IARC, 1988). Long-term experiments were performed in mice, rats and hamsters, with different known carcinogens, mostly *N*-nitrosamines (see Table 3.1 for details and reference).

In experiments in which various carcinogens were administered orally with ethanol as a vehicle, ethanol enhanced the incidence of tumours of the nasal cavity induced in mice by NDMA and that of oesophageal/forestomach tumours and lung tumours induced in mice by *N*-nitrosodiethylamine (NDEA) or *N*-nitrosodi-*n*-propylamine.

In further studies, various carcinogens were administered by different routes simultaneously with ethanol in water as the drinking fluid or in liquid diets. Ethanol enhanced the incidence of benign tumours of the nasal cavity induced in rats by *N'*-nitrosonornicotine (NNN) given in a liquid diet and the incidence of nasal cavity and tracheal tumours and of neoplastic nodules of the liver induced in hamsters by *N*-nitrosopyrrolidine (NPYR) given by intraperitoneal injection. Administration of ethanol in the drinking-water enhanced the incidence of hepatocellular carcinomas and liver angiosarcomas induced in rats by inhalation of vinyl chloride.

In several other experiments, ethanol had no modifying effect on the overall incidence of tumours in mice, rats or hamsters given *N*-nitrosomethylbenzylamine (NMB_zA), *N*-nitrosobis(2-oxopropyl)amine, *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG), 7,12-dimethylbenz[*a*]anthracene (DMBA) or 1,2-dimethylhydrazine (DMH) by various routes of administration.

An increase in tumour morbidity (mostly in target organs characteristic of the carcinogens used) was observed in all experiments in which ethanol was used as a vehicle

Table 3.1 Modifying effects of ethanol on the activity of various carcinogens in experimental animals (studies published before 1987 in their order of citation in IARC Monograph Volume 44, 1988)

Species, strain	No., sex, age or weight	Carcinogen: doses, route of administration	Ethanol: doses, route of administration	Control	Duration of experiment	Length of observation	Results	Reference
Mice, C57BL	Groups of 29–37 males and females; 8 weeks	NDMA 0.03 mg × 2/week ig; total dose, 3 mg	40%; 0.2 mL as vehicle; total dose 20 mL	NDMA in water	50 weeks	72 weeks	Increase; olfactory tumours infiltrating brain in 12/36 (33%) males, 12/30 (40%) females; 0 in controls	Griciute *et al*. (1981)
Mice, hybrid CBA × C57BL/6	50 or 100 females/ group; weighing 10–12 g	NDMA 10 mg/L as drinking fluid	6000 mg/L as drinking fluid with NDMA	NDMA in drinking-water	9 months	9 months	No effect	Litvinov *et al*. (1986a)
Rats, Sprague-Dawley	17 females/ group; weighing 130 g	NDMA 1.5 mg ip, 5 days/week × 4 weeks; total dose, 30 mg	In liquid diet (36% of total calories) 3 weeks before carcinogen; no ethanol 1 week during and 1 week after carcinogen; 5-week cycles repeated 4 times	NDMA in isocaloric liquid diet	20 weeks	For life	No effect	Teschke *et al*. (1983)

Table 3.1 (continued)

Species, strain	No., sex, age or weight	Carcinogen: doses, route of administration	Ethanol: doses, route of administration	Control	Duration of experiment	Length of observation	Results	Reference
Mice, hybrid CBA × C57BL/6	100 females/ group; weighing 10–12 g	NDEA 10 mg/L as drinking-water	6000 mg/L as drinking-water simultaneously with NDEA	NDEA in drinking-water	12 months	12 months	Increase in pulmonary tumours, mainly adenomas; 49/86 (57%) ethanol-treated, 22/79 (27.8%) controls	Litvinov *et al*. (1986b)
Mice, C57BL	32 or 38 females/ group; 8 weeks	NDPA 0.03 mg ig, 2 × week; total dose, 3 mg	40% (w/v) 0.2 mL; total dose, 20 mL (6.4 g 100% ethanol) as vehicle	NDPA in water	50 weeks	72 weeks	Increase in spinocellular carcinoma, oesophagus/ forestomach carcinoma; 36/70 (51%) ethanol-treated, 7/70 (10%) controls; $p<0.00005$	Griciute *et al*. (1982)
Rats, albino (similar to BDII)	28 or 20 animals/ group, sex distribution unspecified; 10–12 weeks	NDEA 3 mg/kg bw in drinking-water daily; total dose, 700 ± 71 mg/kg bw; 730 ± 67 mg/kg bw in brandy-treated group	40 mL commercial brandy (38% alcohol) as drinking fluid simultaneously; total dose, 8100 mL/kg bw	NDEA in drinking-water	For life	For life	Reduction in hepatocellular carcinoma; 16/20 (80%) brandy-treated, 28/28 (100%) controls [no weight gain and high mortality in brandy-treated group]	Schmähl *et al*. (1965)

Table 3.1 (continued)

Species, strain	No., sex, age or weight	Carcinogen: doses, route of administration	Ethanol: doses, route of administration	Control	Duration of experiment	Length of observation	Results	Reference
Rats, Sprague-Dawley	13–27 males and females/ group; 3 months	NDEA 2.5 or 10 mg/kg bw daily ig; total dose, 607 or 1867 mg/kg bw; 529 or 1806 mg/kg bw in ethanol-treated group	0.5 mL 30% (w/v) as vehicle; total dose 106 or 90 mL/kg bw	NDEA in water	For life	For life	Increase in benign and malignant oesophago-forestomach tumours	Gibel (1967)
Rats, Sprague-Dawley	90 males/ group; 14 weeks	NDEA 0.1 mg/ kg bw day in drinking-water; 5 days/week	5 mL 25% in water as drinking fluid; 5 days/week	NDEA in water	For life	For life	Decrease in oesophago-forestomach and liver tumours	Habs & Schmähl (1981)
Rats, Sprague-Dawley	72 females; weighing 100 g	NDEA 100 mg/ kg bw ip 1 day prior to the start of ethanol and 2 months later; 1 group choline-supplemented, another choline-deficient diet	32–25% w/v as drinking fluid	NDEA without ethanol (2 groups); choline-deficient diet only (neither NDEA nor ethanol; 1 group)	10 months	10 months	No effect; several lung and kidney tumours in rats fed choline-deficient diet only	Porta *et al.* (1985)

Table 3.1 (continued)

Species, strain	No., sex, age or weight	Carcinogen: doses, route of administration	Ethanol: doses, route of administration	Control	Duration of experiment	Length of observation	Results	Reference
Rats, Wistar	Males [number not indicated]; weighing 120 g	NDEA 30 mg/kg bw ip × 1	5% in water as drinking fluid 1 week after carcinogen	NDEA in tap-water	18 months	18 months	Carcinoma formation with a high incidence of clear-cell foci or basophilic foci and hyperplastic nodules	Driver & McLean (1986)
Wistar rats	10 or 5 males/group; weighing 180–200 g	NDEA 10 mg/kg bw; 24 h after partial hepatectomy	20% ethanol + 10% sucrose as drinking fluid; 110 mL/kg bw (15.4 g/kg bw daily) 8 weeks after	NDEA in tap-water	40 weeks	40 weeks	Increase in hepatocellular nodules in ethanol-treated group $p<0.05$	Takada *et al*. (1986)

Table 3.1 (continued)

Species, strain	No., sex, age or weight	Carcinogen: doses, route of administration	Ethanol: doses, route of administration	Control	Duration of experiment	Length of observation	Results	Reference
Mice, C57BL	38 males and 32 females/ group; 8 weeks	NDEA 0.03 mg ig, 2 ×/week; total dose, 3 mg	40% 0.2 mL ethanol:water solution; total dose, 20 mL (6.4 g 100% ethanol) as vehicle	NDEA in tap-water	50 weeks	78 weeks	Increase in spinocellular oesophageal/ forestomach cancer in ethanol-treated group: 13/38 (34%) males, 19/31 (61%) females versus 4/38 (10%) male, 3/32 (9%) female controls; decrease in lymphomas in ethanol-treated group: 21/69 (30%) versus 45/70 (64%) controls	Griciute *et al*. (1984)

Table 3.1 (continued)

Species, strain	No., sex, age or weight	Carcinogen: doses, route of administration	Ethanol: doses, route of administration	Control	Duration of experiment	Length of observation	Results	Reference
Mice, C57BL	70 animals/ group; [age and weight unspecified]	Mixture of 0.01 mg NDMA, 0.01 mg NDEA, 0.01 mg NDPA; ig 2 ×/week; total doses: NDMA, 1.0 mg; NDEA, 1.0 mg; NDPA, 1.0 mg	40% as vehicle	NDMA in water	50 weeks	79 weeks	Increase in forestomach/ oesophageal carcinoma: 35/70 (50%) versus 8/70 (11%) controls; pulmonary adenoma, 55/70 (78%) versus 34/70 (48%) controls; olfactory tumours: 2/70 (3%) versus 0/70 controls	Griciute *et al*. (1987)
Rats, Sprague-Dawley	40 males/ group, weanling	NMB_zA 2 mg/ kg bw ig 2 × week, 4 weeks; zinc-deficient diet	4% in deionized water as drinking fluid, 4 weeks before carcinogen	NMB_zA in deionized water without ethanol	29 weeks	29 weeks	No effect	Gabrial *et al*. (1982)

Table 3.1 (continued)

Species, strain	No., sex, age or weight	Carcinogen: doses, route of administration	Ethanol: doses, route of administration	Control	Duration of experiment	Length of observation	Results	Reference
Rats, Sprague-Dawley	48 animals/ group; 13 weeks	NMPhA 2.0 or 10.0 mg/kg bw sc weekly for 39 or 24 weeks; or 0.3 or 1.5 [presumably mg/kg bw] in drinking-water for 29 or 22 weeks	25% (about 30 mL/kg bw) in water 5 ×/week	NMPhA without ethanol in drinking-water or sc	22–39 weeks	For life	No effect	Schmähl (1976)
Rats, Fischer 344	28 males/ group; weighing 160 g	NPIP 0.06% in basal diet; 8 weeks	10% in drinking-water for 12 weeks; 1 mL 50% into pharynx 2 ×/week for 8 weeks with or without 10% in drinking-water for 12 weeks	NPIP without ethanol	20 weeks	20 weeks	No effect	Konishi *et al*. (1986)

Table 3.1 (continued)

Species, strain	No., sex, age or weight	Carcinogen: doses, route of administration	Ethanol: doses, route of administration	Control	Duration of experiment	Length of observation	Results	Reference
Rats, Sprague-Dawley	20 animals/group [sex distribution unspecified]; 3 months	DNPIP 5 mg/kg bw ig/day; total dose, 2605 mg; 2250 mg in ethanol-treated group	0.5 mL 30% (v/v) as vehicle ig for life	DNPIP	For life	For life	No differences in number of tumours; appearance of the first tumour at day 450 in ethanol-treated groups and day 521 in control group	Gibel (1967)
Rats, Fischer 344	26–30 males/group; 9 weeks	NNN at 13 weeks of age; groups 1, 2: 10 mg/kg bw sc; 3 alternate days/week (56–66 injections); total dose, 177 mg/rat; groups 3, 4: 17.5 mg/L NNN in liquid diet for 27 weeks; total dose, 177 mg/rat	Groups 2 and 4 6.6% w/v (35% of calories) in liquid diet simultaneously	Control liquid diet (groups 1 and 3)	22–27 weeks	To 98 weeks of age	Groups 1 and 2, no effect; groups 3–6 increase in nasal cavity tumours ($p<0.05$)	Castonguay *et al*. (1984)

Table 3.1 (continued)

Species, strain	No., sex, age or weight	Carcinogen: doses, route of administration	Ethanol: doses, route of administration	Control	Duration of experiment	Length of observation	Results	Reference
Rats, BD	50 animals/ group; young adult	NNN 0.3, 1.0 or 3.0 mg/rat ig 2 ×/week; total dose, 46.8, 156 or 468.0 mg/rat	40% aqueous solution as vehicle	NNN in water	78 weeks	Until 120 weeks of age	Morbidity from olfactory tumours slightly elevated in ethanol-treated groups; time of appearance of the first tumour shorter in all ethanol-treated groups	Griciute *et al*. (1986)
Hamsters, Syrian golden	21 males/ group; 9 weeks	NNN at 13 weeks of age; 0.5 mL ip of 2.37 or 4.74 mg/animal 3 ×/week, 25 weeks; total dose, 177 or 354 mg	6% w/v; 35% caloric intake in liquid diet before and during administration of NNN	NNN and liquid diet without ethanol	29 weeks	4 weeks and 18 months	No effect	McCoy *et al*. (1981)
Hamsters, Syrian golden	21 males/ group; 9 weeks	NPYR at 13 weeks; 0.5 mL ip of 1.33 or 2.67 mg/animal 3 ×/week, 25 weeks; total dose, 100 or 200 mg	6% w/v; 35% in isocaloric diet before and during administration of NPYR	NPYR in liquid diet without ethanol	29 weeks	4 weeks and 18 months	Higher morbidity from nasal cavity and tracheal tumours; $p<0.05$	McCoy *et al*. (1981)

Table 3.1 (continued)

Species, strain	No., sex, age or weight	Carcinogen: doses, route of administration	Ethanol: doses, route of administration	Control	Duration of experiment	Length of observation	Results	Reference
Hamsters, Syrian golden	27 males/ group; 9 weeks	NPYR 1.33 mg/ animal ip 3 ×/week, 25 weeks; total dose, 100 mg/ animal	7.4% or 18.5% in water as drinking fluid for 4 weeks before and during NPYR administration	NPYR and tap-water without ethanol	29 weeks	4 weeks and 17 months	Increase in hepatic neoplastic nodules; $p<0.01$	McCoy *et al*. (1981)
Hamsters, Syrian	15 males/ group; 6 weeks; weighing 80–100 g	NDOPA 20 mg/ kg bw sc × 1, 2 weeks after the start of ethanol treatment	25% in water w/v as drinking fluid	Water	24 weeks	24 weeks	Reduction in pancreatic tumours: 0/13 ethanol-treated, 11/14 (78%) non-ethanol-treated	Tweedie *et al*. (1981)
Hamsters, Syrian golden	20 or 40 animals/ group; 8 weeks	NDOPA 20 mg/ kg bw sc before or 4 weeks after beginning of ethanol treatment	5% (w/v) in water as drinking fluid	NDOPA single injection, no ethanol	46 weeks	46 weeks	No significant difference in pancreatic tumours	Pour *et al*. (1983)
Rats, Wistar	21 or 30 males/group; 7 weeks	MNNG 100 mg/L in drinking-water simultaneously with a 10% saline-supple-mented diet for 8 weeks	10% in drinking-water after MNNG administration	MNNG for 8 weeks in drinking-water	40 weeks	40 weeks	No increase in adenocarcinomas in glandular stomach	Takahashi *et al*. (1986)

Table 3.1 (continued)

Species, strain	No., sex, age or weight	Carcinogen: doses, route of administration	Ethanol: doses, route of administration	Control	Duration of experiment	Length of observation	Results	Reference
Rats, inbred Fischer	4–12 animals/ group; 4–6 weeks	OH-AAF 160 mg/kg in semisynthetic diet	10 or 20% by vol. in drinking-water simultaneously with or after treatment with OH-AAF	Drinking-water, without ethanol	12–20 weeks	40 weeks	No effect	Yamamoto *et al*. (1967)
Rats, NIH random-bred black	20 animals/ group; weanling	OH-AAF 80 mg/kg in the diet	10% in drinking-water	Water alone	64 weeks	64 weeks	No significant increase in hepatomas	Yamamoto *et al*. (1967)
Rats, Fischer 344	26 males/ group; 10 weeks; weighing 170–210 g	Azoxymethane 7 mg/kg bw sc in sterile water 1 ×/week, 10 weeks, 3 weeks after start of experiment	Isocaloric liquid diet containing 12 or 23% of calories as beer, 9 or 18% as ethanol (before and during carcinogen administration)	Liquid diet without ethanol	26 weeks	26 weeks	Decrease in colon cancers in high-dose group (18 versus 45 controls); no effect with low dose (37 versus 45 controls)	Hamilton *et al*. (1987a)

Table 3.1 (continued)

Species, strain	No., sex, age or weight	Carcinogen: doses, route of administration	Ethanol: doses, route of administration	Control	Duration of experiment	Length of observation	Results	Reference
Rats, Fischer 344	35 males/ group; 10 weeks; weighing 210–260 g	Azoxymethane 7 mg/kg bw sc 1 ×/week, 10 weeks	11, 22, 33% of calories from ethanol in liquid diets either 3 weeks before and during or for 16 weeks after carcinogen treatment	Liquid diet without ethanol	29 weeks	29 weeks	No effect when liquid ethanol diet given after carcinogen; decrease in colon cancer when higher doses given before and during carcinogen treatment	Hamilton *et al*. (1987b)
Mice, NMRI	30 or 20 females/ group [age unspecified]	DMBA 0.02 mL of a 1% solution v/v skin applications 3 ×/week	Vehicle (purity 99.5%)	Acetone as solvent	20 weeks	Unknown	Increase in skin tumours: 11/20 (55%) ethanol-treated, latency 6 weeks; 4/30 (13%) acetone-treated, latency 9 weeks; p=0.002	Stenbäck (1969)
Mice, CF1	72 and 70 males; 2 months	DMBA 0.02 mL in 1.5% acetone skin application × 1	50% aqueous solution; 0.04 mL applications in same region 1 month after DMBA; 2 ×/ week, 40 weeks	No further treatment after DMBA	Ethanol: 1 month and 40 weeks	20 weeks	No effect	Kuratsune *et al*. (1971)

Table 3.1 (continued)

Species, strain	No., sex, age or weight	Carcinogen: doses, route of administration	Ethanol: doses, route of administration	Control	Duration of experiment	Length of observation	Results	Reference
Mice, CF1	46–55 males/ group; 1 month	DMBA 0.025 mL in 1.5% acetone skin application × 1	0, 12, 43% applications in same region 1 month after DMBA; 2 ×/ week, 40 weeks	No applications of ethanol	Ethanol: 1 month and 40 weeks	20 weeks	No effect at the end of treatment period	Kuratsune *et al*. (1971)
Rats, Sprague-Dawley	16 males/ group; 60 days	DMH 30 mg/ kg bw sc 1 ×/ week, 4 weeks, 4 weeks after beginning ethanol	36% of total calories (6.6 v/v) in liquid diet for 4 weeks; 3 weeks standard diet during DMH; ethanol again for 4 weeks; 4 cycles	Isocaloric diet without ethanol	28 weeks	32 weeks	Number of rectal tumours significantly higher in group given ethanol (17 versus 6)	Seitz *et al*. (1984)
Rats, D/A	20 or 40 males/group; 4–6 weeks; weighing 150–250 g	DMH 20 mg/kg bw sc 1 ×/week, 20 weeks; high- or low-fat diet	Beer or 4.8% ethanol as drinking fluid	No applications of beer or ethanol	28 weeks	28 weeks	No effect	Howarth & Pihl (1984)

Table 3.1 (continued)

Species, strain	No., sex, age or weight	Carcinogen: doses, route of administration	Ethanol: doses, route of administration	Control	Duration of experiment	Length of observation	Results	Reference
Rats, Sprague-Dawley	22 males/ group; 5 weeks	DMH 15 mg/kg bw sc 1 ×/week, 16 weeks	5% (95% laboratory grade) v/v as drinking fluid from 3 weeks before carcinogen	Water as drinking fluid	19 weeks	25 weeks	No difference in number of colonic cancers	Nelson & Samelson (1985)
Rats, Sprague-Dawley	12 males/ group; 5 weeks	DMH 20 mg/kg bw sc 1 ×/week, 10 weeks	Beer as drinking fluid from 3 weeks before carcinogen	Water as drinking fluid	13 weeks	27 weeks	Decrease in gastrointestinal tumours in beer-treated (8/12 (66%) versus 12/12 (100%) DMH alone)	Nelson & Samelson (1985)
Rats, Sprague-Dawley	80 males/ group [age unspecified]	VC 600 ppm (1560 mg/m^3) inhalation 4 h/ day, 5 days/ week	5% in water as drinking fluid for life from 4 weeks before carcinogen	Water without ethanol as drinking fluid	1 year	10 months	Increases in hepatocellular carcinomas (35/80 (43%) VC, 48/80 (60%) VC + ethanol) and liver angiosarcomas (18/80 (22%) VC, 40/80 (50%) VC + ethanol); p=0.002	Radike *et al*. (1981)

Table 3.1 (continued)

Species, strain	No., sex, age or weight	Carcinogen: doses, route of administration	Ethanol: doses, route of administration	Control	Duration of experiment	Length of observation	Results	Reference
Mice, C3H	30 males/ group; 8 weeks	Ethyl carbamate 2 mg/animal ig 2 ×/week; total dose, 10 mg	40% 0.2 mL as vehicle simultaneously or 24 h after ethyl carbamate	Ethyl carbamate in water	5 weeks	6 months	Increase in pulmonary adenomas with ethanol as vehicle; no effect with ethanol given 24 h after ethyl carbamate	Barauskaite (1985)
Mice, white outbred [strain unspecified]	12 males and 14 females/ group; 8 weeks	Ethyl carbamate 10 mg in 0.2 mL saline ip 2 ×/ week; total dose, 100 mg	40% 0.2 mL as vehicle	Ethyl carbamate in saline	5 weeks	12 weeks	Increase in average no. of lung adenomas per animal: 30 ethanol-treated, 13 saline-treated; p=0.002	Griciute (1981)

From IARC (1988) DMBA, 7,12-dimethylbenz[*a*]anthracene; DMH, 1,2-dimethylhydrazine; DNPIP, *N*,*N*′-dinitrosopiperazine; ig, intragastric intubation; ip, intraperitoneal injection; NDEA, *N*-nitrosodiethylamine; NDMA, *N*-nitrosodimethylamine; NDOPA, *N*-nitrosobis(2-oxopropyl)amine; NDPA, *N*-nitrosodi-*n*-propylamine; NMBzA, *N*-nitrosomethylbenzylamine; NMPhA, *N*-nitrosomethylphenylamine; MNNG, *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine; NNN, *N*′-nitrosonornicotine; NPIP, *N*-nitrosopiperidine; NPYR, *N*-nitrosopyrrolidine; OH-AAF, *N*-hydroxy-2-acetylaminofluorene; sc, subcutaneous injection; VC, vinyl chloride

for *N*-nitrosamines and other carcinogens (DMBA). Similar results were obtained in some but not all experiments when the animals received ethanol just before the administration of the carcinogen or separately but at the same time as the carcinogen. There was no effect on carcinogenesis in most experiments when ethanol was given separately and after administration of the carcinogen, or when the concentration of ethanol in the fluid used was low (5%). This suggests that ethanol may influence the initiation of carcinogenesis in some manner, but it is also possible that the process is enhanced due to some mechanistic events: the facilitation of entry into the target cell by ethanol, a change in intracellular metabolism or suppression of DNA repair. The hypothesis of competitive inhibition of hepatic metabolism of the carcinogen, which allows it to reach the target organs, has also been proposed. A change in the target organ specificity of NDMA by ethanol was observed: when NDMA was given in combination with ethanol, rats and mice developed tumours in the nasal cavity, which is not a target site for this nitrosamine.

Studies published after 1987 are reviewed below and summarized in Table 3.2.

3.2.1 *Aflatoxin B1*

Rat

A group of 29 male inbred ACI/N rats [age unspecified] received twice-weekly intraperitoneal injections of 1.5 mg/kg bw aflatoxin B_1 [purity not specified] in 200 µL dimethyl sulfoxide (DMSO) for 10 weeks (total dose, 30 mg/kg bw). One week after the last injection, 15 of the aflatoxin B_1-injected rats were given drinking-water that contained 10% ethanol [purity not specified] for 56 weeks, while the remaining 14 rats continued to receive control drinking-water. Additional rats received injections of DMSO without aflatoxin B_1 and received drinking-water that contained ethanol (15 rats) or control drinking-water (10 rats) for 56 weeks. The experiment was terminated after a total of 67 weeks, at which time the extent of liver neoplasia was assessed macroscopically and microscopically. The body weights in all groups were similar. The tumour incidence in rats treated with aflatoxin B_1 and ethanol was 13% (2/15) neoplastic nodules and 7% (1/15) hepatocellular carcinoma. Neither neoplastic nodules nor hepatocellular carcinoma were detected in any of the other groups (Tanaka *et al.*, 1989).

3.2.2 *Acetoxymethylnitrosamine*

Rat

Two groups of 20 male Sprague-Dawley rats [age unspecified], weighing 215–220 g, were fed liquid diets that contained 36% of total calories as ethanol or for which 36% was isocalorically replaced by carbohydrates for 2 weeks, after which time 2 mg/kg bw acetoxymethylnitrosamine were applied locally to the rectal mucosa once every 2 weeks. At weeks 15 and 18, the animals underwent colonoscopy and were then killed

Table 3.2 Modifying effects of ethanol on the activity of various carcinogens in experimental animals (studies published after 1987)

Species, strain	No., sex, age or weight	Carcinogen: doses, route of administration	Ethanol: doses, route of administration	Control	Duration of experiment	Length of observation	Results	Reference
Rats, inbred ACI/N	10–15 males/ group [age unspecified]	AFB_1 1.5 mg/ kg bw in 200 µL DMSO ip; 2 ×/week; total dose, 3 mg/ kg bw	10% in drinking-water, 1 week after last injection	DMSO without AFB_1 + ethanol or + drinking-water	10 weeks	67 weeks	AFB_1 + ethanol: 2/15 (13%) neoplastic nodules; 1/15 (6%) hepatocellular carcinoma; none in other groups	Tanaka *et al*. (1989)
Rats, Sprague-Dawley	20 males/ group; weighing 215–220 g	AMMN 2 mg/kg bw on rectal mucosa 1 ×/2 weeks; colonoscopy	36% of total calories 2 weeks before and during AMMN	Isocaloric diet	21 weeks	21 weeks	Incidence of tumours significantly increased in ethanol-treated at week 15 (p<0.05) but not at weeks 18 or 21	Seitz *et al*. (1990)
Rats, Sprague-Dawley	20 males/ group; weighing 215–220 g	AMMN 2mg/ kg bw on rectal mucosa 1 ×/2 weeks	2.5 mL (4.8 g/kg bw) by gavage 2 ×/day, 10 weeks before AMMN	Saline by gavage before AMMN	21 weeks	21 weeks	No effect on incidence; time to tumour occurrence significantly decreased (p=0.0295)	Seitz *et al*. (1990)

Table 3.2 (continued)

Species, strain	No., sex, age or weight	Carcinogen: doses, route of administration	Ethanol: doses, route of administration	Control	Duration of experiment	Length of observation	Results	Reference
Rats, Fischer 344/ DuCrj	5–40 males/ group; 21 days	MeIQx 200 ppm in diet	0.1, 0.3, 1, 3. 10 or 20% (purity, 99.%) in drinking-water 8 weeks after start of MeQIx	Drinking-water only	24 weeks	24 weeks	Dose-dependent increase in incidence (p<0.001) and multiplicity (p<0.01) of liver tumours with 10 and 20%, and 20% ethanol, respectively	Kushida *et al*. (2005)
Rats, SPF albino Wistar	20 males/ group [age unspecified]	Azaserine 30 mg/kg bw ip × 1 at 19 days of age; high-fat diet	5% for first 2 weeks increased to 10% by 6 weeks in high-fat diet	No ethanol	447–448 days	447–448 days	No effect on pancreatic tumours	Woutersen *et al*. (1989)
Rats, Fischer 344	20 and 23 males/group; 10 weeks; weighing 210–260 g	Azoxymethane 14 mg/kg bw sc 1 ×/week, 10 weeks	33% of total calories in diet 3 weeks before and during azoxymethane	Isocaloric diet	13 weeks	29 weeks	Decrease in incidence and multiplicity of all tumours and colonic and small intestine tumours	Hamilton *et al*. (1988)

Table 3.2 (continued)

Species, strain	No., sex, age or weight	Carcinogen: doses, route of administration	Ethanol: doses, route of administration	Control	Duration of experiment	Length of observation	Results	Reference
Rats, Sprague-Dawley	11–18 males/group; [age unspecified] weighing 340 g	Azoxymethane 15 mg/kg bw ig 1 ×/week, 2 weeks	8 g/kg bw/day ig in diet increased to 13 g/kg bw/day at day 10; 35 days later, reduced to no ethanol on day 39, at 9 h before and during azoxymethane; resumed 6 h later; 1-week cycle repeated once then stopped	Diet with no ethanol ig or water ig and standard diet	49 days + 2 weeks	49 days + 30 weeks	Azoxymethane and ethanol: 2/18 (11%) mucinous duodenal adenocarcinomas and 1/18 (5%) duodenal focal adenomatous changes; none in other groups	Hakkak *et al.* (1996)

Table 3.2 (continued)

Species, strain	No., sex, age or weight	Carcinogen: doses, route of administration	Ethanol: doses, route of administration	Control	Duration of experiment	Length of observation	Results	Reference
Rats, Fischer 344	53 or 40 males/group; 4.5 weeks	Azoxymethane 15 mg/kg bw in saline sc 1 ×/ week, 2 weeks	Beer as drinking-water 1 week before azoxymethane	No beer and no beer and saline sc only drinking-water	42 weeks	42 weeks	Azoxymethane and beer: decreased incidence and multiplicity of colonic adenomas (46% versus 82% [p<0.01] and 0.55±0.67/rat versus 1.41±1.10/ rat [p<0.005]) and adenocarcinomas (5% versus 64% [p<0.01] and 0.09±0.43/rat versus 1.00±0.98 [p<0.05]) compared with azoxymethane and control drinking-water	Nozawa *et al*. (2004)
Mice, BALB/c	111 animals [sex unspecified]; 8 weeks	Benzo[*a*]pyrene 2 mg in 200 µL olive oil sc 1 ×	10% in drinking-water after benzo[*a*] pyrene	No ethanol	58 weeks	58 weeks	Ethanol reduced incidence of subcutaneous fibrosarcomas from 84.0% to 65.4%	Uleckiene & Domkiene (2003)

Table 3.2 (continued)

Species, strain	No., sex, age or weight	Carcinogen: doses, route of administration	Ethanol: doses, route of administration	Control	Duration of experiment	Length of observation	Results	Reference
Rats, Sprague-Dawley	50 females/ group; 21 days; weighing 40–55 g	DMBA 20 mg/ kg bw in 0.1–0.2 mL sesame oil by gavage at 55 days of age	20% of calories × 3 days; 10% of calories × 4 days then 20% of calories in liquid diet	Pair fed no ethanol	34 days + 20–25 weeks	25–30 weeks	No statistically significant effect	Rogers & Conner (1990)
Rats, Sprague-Dawley	32 or 20 females/ group; 21 days; weighing 40–55 g	DMBA 30 mg/ kg bw in 0.1–0.2 mL sesame oil by gavage at 55 days of age	10% of calories × 4 weeks; 3.5 g/kg bw ethanol by gavage; control diet 1 day before and 1 day after DMBA; 10% of calories × 1 week then 25% of calories	10% fat × 1 week; no ethanol	34 days + 12–13 weeks	17–18 weeks	No effect on mammary tumorigenesis	Rogers & Conner (1990)
Rats, Sprague-Dawley	15 or 17 females; 30 days; weighing 72.6±1.0 (SE) g	DMBA 5 mg/ rat in 0.5 mL corn oil ig at 58 days of age	20% of calories in diet 4 weeks before and 1 week after DMBA	No ethanol	5 weeks	25 weeks	Incidence of mammary tumours: 82% versus 47–48% in controls ($p<0.05$)	Singletary *et al*. (1991)

Table 3.2 (continued)

Species, strain	No., sex, age or weight	Carcinogen: doses, route of administration	Ethanol: doses, route of administration	Control	Duration of experiment	Length of observation	Results	Reference
Rats, Sprague-Dawley	24–33 females/ group; 25 days; weighing 49.0 ± 0.5 (SE) g	DMBA 5 mg/ rat in 0.5 mL corn oil ig at 53 days of age	10 or 20% of calories in diet 4 weeks before and 1 week after DMBA	No ethanol	5 weeks	31 weeks	Incidence of mammary tumours (mainly adenocarcinoma): 74% in 20% ethanol-treated (p<0.05) versus 47–48% in controls; no increase with 10% ethanol	Singletary *et al*. (1991)
Rats, Sprague-Dawley	92 females; 42 days; weighing 177.4±2.3 (SE) g	DMBA 5 mg/ rat in 0.5 mL corn oil ig at 56 days of age	15 or 30% of calories from 63 days of age	No ethanol	21 weeks	21 weeks	T_{50}: 150, 84 and 105 days for 0%, 15% and 30% ethanol; 0% versus 15% (p<0.05)	Singletary *et al*. (1991)
Rats, Sprague-Dawley	20 females/ group; 40 days	DMBA 15 mg in 1 mL sesame oil ig at 50 days of age	5% v/v in drinking-water	No ethanol	130 days	130 days	Tumour incidence: 100% in controls versus 40% in ethanol-treated (p<0.001)	McDermott *et al*. (1992)
Rats, Sprague-Dawley	15 pregnant females/ group; [age not specified]; 23–25 female offspring/ group	DMBA 10 mg in 1 mL peanut oil on postnatal day 47	16 or 25 g/kg diet (7 and 15% of total energy) on days 7–18 of gestation	No ethanol		17 weeks	Total number of palpable tumours/ rat significantly increased with 16 g/kg diet ethanol (p<0.006)	Hilakivi-Clarke *et al*., 2004)

Table 3.2 (continued)

Species, strain	No., sex, age or weight	Carcinogen: doses, route of administration	Ethanol: doses, route of administration	Control	Duration of experiment	Length of observation	Results	Reference
Hamster, Syrian golden	36 males; 4–6 weeks	DMBA 1% solution in heavy mineral oil on right buccal pouch × 3	5% ethanol (v/v) in liquid diet 1 week after DMBA	No ethanol (pair-fed isocaloric diet)	33 weeks	35 weeks	Tumour multiplicity significantly greater with ethanol (3.29±1.02 versus 1±0.0 in controls)	Nachiappan *et al*. (1993)
Rats, Sprague-Dawley	16 males/ group; weighing 250–300 g	DMH 30 mg/ kg bw sc × 1/ week, 4 weeks; 4 cycles	36% of total calories, 4 weeks; control diet 4 weeks during DMH	No ethanol; isocaloric carbohydrates	32 weeks	32 weeks	No change in number, size or distribution of largee bowel tumours	McGarrity *et al*. (1988)
Rats, Sprague-Dawley	20–30 males and females/ group; 10 weeks	DMH 21 mg/ kg bw in water + EDTA sc 1 ×/ week	1.23 g/kg bw ethanol in drinking-water	No ethanol	18 weeks	25–27 weeks	No significant difference in tumour incidence or multiplicity	Pérez-Holanda *et al*. (2005)
Mice, A/ Ph	15 females/ group; 6.5 weeks	Ethyl carbamate 200, 500 or 1000 ppm in drinking-water	5, 10 or 20% as drinking fluid	No ethanol; no ethyl carbamate	12 weeks	12 weeks	Ethanol decreased ethyl carbamate-induced tumour multiplicity (p<0.001 with 10% and 20% ethanol)	Kristiansen *et al*. (1990)
Mice, Han/ NMRI	25 females/ group; approximately 10 weeks	0.3 mL/25 g bw of 1.5, 3.0, 7.5 or 15 g/L ethyl carbamate in tap-water by gavage daily	10% for first 3 days then 20% by gavage daily	No ethanol	8 weeks	16 weeks	No effect on ethyl carbamate-induced lung adenomas	Altmann *et al*. (1991)

Table 3.2 (continued)

Species, strain	No., sex, age or weight	Carcinogen: doses, route of administration	Ethanol: doses, route of administration	Control	Duration of experiment	Length of observation	Results	Reference
Mice, C3H/HeJ	18–21 males/ group; weanling	Ethyl carbamate 10 or 20 mg/kg bw per day	12% as drinking-water or Concord red, Concord white or Johannesburg Riesling as drinking-water	No ethanol; no ethyl carbamate only water	41 weeks	41 weeks	Ethanol and wine decreased frequency of ethyl carbamate-induced tumours	Stoewsand *et al*. (1991)
Mice, BALB/c	20 males and 20 females/ group; 8 weeks	Ethyl carbamate 10 mg ip; 2 ×/ week; total dose, 100 mg	10% in drinking-water [duration not specified]	No ethanol	5 weeks	4 months	No significant differences in tumour mulciplicity	Uleckiene & Domkiene (2003)
Mice, $B6C3F_1$	48 males and 48 females/ group; 28 days	Ethyl carbamate 10, 30 or 90 ppm in drinking-water	2.5 or 5% ethanol in the drinking-water	No ethanol; no ethyl carbamate	104 weeks	104 weeks	Ethanol increased tumour incidence in females and decreased tumour incidence in males	National Toxicology Program (2004); Beland *et al*. (2005)
Rat, Wistar JCL	Females [initial number unspecified]; 4 weeks	Ethinylestradiol (0.075 mg) and norethindrone acetate (6.0 mg) in 0.5 mL olive oil ig daily	10% w/v in the drinking-water, 2–5 days/week	No ethanol; no hormones	12 montths	12 months	Ethanol increased incidence of hepatocellular carcinomas from 1/12 (8%) to 8/21(38%) ($p<0.05$)	Yamagiwa *et al*. (1991)

Table 3.2 (continued)

Species, strain	No., sex, age or weight	Carcinogen: doses, route of administration	Ethanol: doses, route of administration	Control	Duration of experiment	Length of observation	Results	Reference
Rats, ACI/N	20 or 19 males/group; 6 weeks	MAMA 25 mg/kg bw in saline ip 1 ×/week, 2 weeks	10% in drinking-water	No ethanol	414 days	414 days	Ethanol increased incidence of large intestinal adenocarcinomas (15/17 (94%) versus 9/16 (56%) controls; p=0.040) and rectal neoplasms (10/17 (59%) versus 3/16 (19%); p=0.019)	Niwa *et al.* (1991)
Rats, ACI/N	15 females/group; 6 weeks	MAMA 25 mg/kg bw in saline ip 1 ×/week, 2 weeks	Saké (ethanol content, 15–16%), 50% saké (ethanol content, 7.5%), 15% ethanol, 7.5% ethanol	No ethanol; no MAMA	280 days	294 days	Ethanol increased non-significantly incidences of rectosigmoidal colonic neoplasms	Niwa *et al.* (1991)
Rats, Wistar	80 males/group; 55 weeks	MeDAB 0.06% in diet, 4 weeks	5, 10 or 15% in drinking-water 2 weeks after MeDAB	No ethanol; no MeDAB	47 weeks	53 weeks	No significant effect	Yanagi *et al.* (1989)

Table 3.2 (continued)

Species, strain	No., sex, age or weight	Carcinogen: doses, route of administration	Ethanol: doses, route of administration	Control	Duration of experiment	Length of observation	Results	Reference
Rats, Fischer 344	Males [initial number unspecified]; 4–6 weeks	NNK 20 mmol/kg gavage 3 ×/week, 4 weeks	36% of total calories in liquid diet	No ethanol	55 weeks	55 weeks	Ethanol increased incidences of tumours of oesophagus, oral cavity, lungs and liver (p<0.05); increase in mean frequency and size of tumours (p<0.001)	Nachiappan *et al*. (1994)
Hamster, Syrian	4 pregnant females/group; [age unspecified]	NNK 50 mg/kg bw on day 15	10% in drinking-water on gestation days 5–16	No ethanol	2 weeks	45 weeks	Ethanol increased incidence of tumours in male and female offspring (p<0.01)	Schüller *et al*. (1993)
House musk shrews, Jic:SUN	4, 25 or 30 females/group; 5 weeks	MNNG 50 ppm in tap-water	2, 5 or 10% in drinking-water	Tap-water	30 weeks	45 weeks	No significant effect	Shikata *et al*. (1996)
Rats, Wistar	15 males/group; 6 weeks	MNNG 50 µg/mL in drinking-water, 20 weeks	2.5 mL/kg 20% in saline ip, every other day from week 21 to week 52	No ethanol	52 weeks	52 weeks	Ethanol increased tumour incidence (p<0.02) and multiplicity (p<0.01)	Iishi *et al*. (1989)
Rats, ACI	30 and 25 males; 4 weeks; weighing 58 g	MNNG 0.25 mL/10 g bw of 5 g/L solution ig × 1	10% in drinking-water	No ethanol	1 year	1 year	No effect	Watanabe *et al*. (1992)

Table 3.2 (continued)

Species, strain	No., sex, age or weight	Carcinogen: doses, route of administration	Ethanol: doses, route of administration	Control	Duration of experiment	Length of observation	Results	Reference
Rats, Wistar	20 males/ group; weighing 150–200 g	MNNG 100 µg/mL in drinking-water	MNNG in 11% ethanol or wine	No ethanol	6 months	13 months	Ethanol significantly reduced the development of gastroduodenal tumours	Cerar & Pokorn (1996)
Rats, Fischer	15 males/ group; 6 weeks	MNNG 150 mg/kg bw ig × 1	10% in drinking-water 1 week after MNNG, 51 weeks	No ethanol	51 weeks	52 weeks	Ethanol significantly reduced incidence of stomach and oesophageal papillomas and carcinomas	Wada *et al*. (1998)
Mice, Swiss (NIH: Cr(S))	Females [initial number unspecified]; 4 weeks	MNA 60 or 180 mg/kg bw ig 3 ×/week, 12 weeks	15% in drinking-water	No ethanol	12 months	18 months	Ethanol significantly increased incidence of thymic lymphomas (from 21/49 (43%) to 32/50 (64%); $p<0.05$)	Anderson *et al*. (1993)

Table 3.2 (continued)

Species, strain	No., sex, age or weight	Carcinogen: doses, route of administration	Ethanol: doses, route of administration	Control	Duration of experiment	Length of observation	Results	Reference
Rats, Sprague-Dawley	32 females/ group; 23 days	30 mg/kg bw MNU ip × 1 at 50 days of age	15, 20 and 30% of calories in diet 22 days before MNU and 26 days after	No ethanol	4 weeks	8 weeks	15% ethanol significantly increased incidence of mammary adenocarcinomas/ rat (2.2±0.3 versus 1.4±0.2); no effect with other doses. No significant difference was observed for 20% and 30% ethanol-treated groups.	Singletary *et al*. (1995)

Table 3.2 (continued)

Species, strain	No., sex, age or weight	Carcinogen: doses, route of administration	Ethanol: doses, route of administration	Control	Duration of experiment	Length of observation	Results	Reference
Rats, Sprague-Dawley	30–32 females/ group; 38 days	30 mg/kg bw MNU ip × 1 at 51 days of age	15, 20 and 30% of calories in diet 1 week after MNU	No ethanol	4 weeks	7 weeks	15% ethanol significantly increased incidence of palpable mammary tumours/rat (3.2±0.4 versus 2.0±0.3) and mammary adenocarcinomas/ rat (4.4±0.5 versus 2.3±0.4); adenocarcinomas also increased with 20% ethanol compared with calorically restricted controls (3.0±0.5 versus 1.8±0.3)	Singletary *et al*. (1995)
Hamsters, Syrian golden	40 males; weanling [age unspecified]	BOP 20 mg/ kg bw sc × 1 at 6 weeks of age and × 1 at 7 weeks of age	5–10% in high-fat diet	No ethanol	372–373 days after BOP	372–373 days after BOP	No effect	Woutersen *et al*. (1989)

Table 3.2 (continued)

Species, strain	No., sex, age or weight	Carcinogen: doses, route of administration	Ethanol: doses, route of administration	Control	Duration of experiment	Length of observation	Results	Reference
Mice, A/JNCr	Males [initial number unspecified]; 4 weeks	NDEA 6.8 ppm in drinking-water	10% in drinking-water	No ethanol	4 weeks	36 weeks	Ethanol increased incidence (from 42/50 (84%) to 50/50 (100%)) and multiplicity (from 1.5±1.2 to 5.8±2.2; $p<0.01$) of lung tumours and forestomach tumours (from 1/50 (2%) to 16/50 (32%))	Anderson *et al*. (1993)
Rats, Fischer 344	30 or 28 males/group; 6 weeks	NDEA 50 ppm in drinking-water	10% in drinking-water	No ethanol	8 weeks	104 weeks	Ethanol increased incidence of oesophageal papillomas and carcinomas (from 2/28 (7%) and 1/28 (3%) to 10/26 (38%) and 8/26 (30%), respectively; $p<0.01$)	Aze *et al*. (1993)

Table 3.2 (continued)

Species, strain	No., sex, age or weight	Carcinogen: doses, route of administration	Ethanol: doses, route of administration	Control	Duration of experiment	Length of observation	Results	Reference
Mice, A/JNCr	50 males/group; 4 weeks	NDMA 0.5, 1 or 5 ppm in drinking-water	10 or 20% in drinking-water	No ethanol	16 weeks	16 weeks	10% ethanol increased incidence of lung tumours; 20% ethanol increased average number of lung tumours with high-dose but not low-dose NDMA	Anderson (1988)
Mice, A/JNCr	25–50 males/group; 4–6 weeks	NDMA 5 ppm in drinking-water, 4 weeks; 1 ppm in drinking-water, 16, 32, 48 or 72 weeks; 1 or 5 mg/kg bw ig × 1; 1 mg/kg bw ig, ip, sc or iv 5 ×/week, 4 weeks	5, 10 or 20% in drinking-water	No ethanol	4 weeks; 16, 32, 48 or 72 weeks; 16 weeks; 36 weeks	16 weeks; 16, 32, 48 or 72 weeks; 16 weeks; 36 weeks	Ethanol at all doses increased the incidence and multiplicity of tumours in mice treated with NDMA in drinking-water or 5 mg/kg bw ig; no effect with other routes of administration	Anderson *et al*. (1992)
Rats, MRC Wistar	25 or 40 males/group; 6 weeks	NMAA 25 mg/kg bw in 5 mL water ip × 1/week, 3 weeks, at 7, 8 and 9 weeks of age	20% (21% of 95%) in water, 2 weeks; then 10%	No ethanol	For life	For life	No significant difference in tumour incidence	Mirvish *et al*. (1994)

Table 3.2 (continued)

Species, strain	No., sex, age or weight	Carcinogen: doses, route of administration	Ethanol: doses, route of administration	Control	Duration of experiment	Length of observation	Results	Reference
Mice, C57BL/6	15 or 17 females/ group; 4–6 weeks of age	NMB_zA 0.2 mg/kg bw orally in corn oil; 3 ×/week, 3 weeks (total dose, 1.8 mg/ kg bw)	30% total calories, 3 weeks	No ethanol	25 weeks	25 weeks	Ethanol increased incidence of oesophageal tumours (from 6/15 (40%) to 10/17 (59%)) and multiplicity (from 8.2±2.5 to 14.3±2.8; $p<0.001$)	Eskelson *et al*. (1993)
Rats, Sprague-Dawley	Males [initial number unspecified]; weanling; weighing 70–120 g	NMB_zA 2.5 mg/kg bw ip 3 ×/week, 3 weeks	7% in diet 1 week after NMB_zA or 9 weeks before and during NMB_zA	No ethanol	17 months or 13 weeks	20 months of age	Ethanol after NMB_zA decreased frequency and size but increased incidence of oseophageal tumours; ethanol before NMB_zA significantly decreased incidence of oesophageal tumours (from 10/26 (38%) to 3/13 (23%) ; $p<0.01$)	Mufti *et al.* (1989)

Table 3.2 (continued)

Species, strain	No., sex, age or weight	Carcinogen: doses, route of administration	Ethanol: doses, route of administration	Control	Duration of experiment	Length of observation	Results	Reference
Rats, Sprague-Dawley	39 or 35 males; [age unspecified]	NMB_zA 2.5 mg/kg bw in diet × 2/week, 3 weeks	10% in drinking-water 2 weeks before NMB_zA	No ethanol	5 weeks	~20 weeks	No difference in tumour incidence	Newberne *et al*. (1997)
Rats, Fischer 344/DuCrj	15 males/group; 6 weeks	NMB_zA 500 µg/kg bw in DMSO sc 3 ×/week, 5 weeks	3.3 and 10% in drinking-water after end of NMB_zA, 15 weeks	No ethanol; no NMB_zA	20 weeks	20 weeks	No difference in incidence or multiplicity of oesophageal tumours	Morimura *et al*. (2001)
Rats, Fischer 344/DuCrj	15 males/group; 6 weeks	NMB_zA 100 or 500 µg/kg bw in DMSO sc 3 ×/week, 5 weeks	10% in drinking-water, 5 or 24 weeks	No ethanol	24 weeks	29 weeks	No difference in incidence or multiplicity of oesophageal tumours	Kaneko *et al*. (2002)
Rats, albino Wistar	10 males/group; weighing 156±15 g	NMB_zA 100 µg/kg bw ip 2 ×/week, 10 weeks	5% (36% of total calories) in liquid diet 8 weeks before and after NMB-zA	No ethanol	30 weeks	30 weeks	Ethanol increased the incidence, mean size and mean number per rat of oesophageal tumours	Tsutsumi *et al*. (2006)
Rats, Fischer 344	Males [initial number unspecified]; 4–6 weeks	NNN 40 mmol/kg by gavage 3 ×/week, 4 weeks	7% (36% of total calories) in diet 1 week after end of NNN	No ethanol	60 weeks	60 weeks	Ethanol increased incidence ($p<0.05$), mean frequency and size ($p<0.001$) of tumours of oesophagus, oral cavity and lung	Nachiappan *et al*. (1994)

Table 3.2 (continued)

Species, strain	No., sex, age or weight	Carcinogen: doses, route of administration	Ethanol: doses, route of administration	Control	Duration of experiment	Length of observation	Results	Reference
Mice, *Mus musculus*	16–48 females/ group; 3 months	NDEA/NNN: 0.04 mL/L NDEA on days 4–7; 30 mg/L NNN on days 1–3 then NDEA on days 4–7 in drinking-water	6% in drinking-water	No ethanol	28 weeks	28 weeks	No difference in incidence of invasive oesophageal carcinoma	Gurski *et al*. (1999)
Mice, A/ JNCr	Males [initial number unspecified]; 4 weeks	NPYR 6.8 or 40 ppm in drinking-water, 4 weeks	10% in drinking-water	No ethanol	4 weeks	36 weeks	Ethanol increased incidence and multiplicity of lung tumours	Anderson *et al*. (1993)
Rats, white [not further specified]	140 males; [age unspecified]	NSEE 50 mg/ kg bw io 5 ×/ week, 4 months	0.5 mL 40% io 3 ×/week, 8 months	No ethanol	8 months	8 months	No effect on incidence or multiplicity of tumours	Alexandrov *et al*. (1989)

AFB_1, aflatoxin B_1; AMMN, acetoxymethylnitrosamine; BOP, *N*-nitrosobis(2-oxopropyl)amine; DMBA, 7,12-dimethylbenz[*a*]anthracene; DMH, dimethylhydrazine; DMSO, dimethylsulfoxide; EDTA, ethylene diamine tetraacetic acid; ig, intragastric administration; io, intraoesophageal administration; ip, intraperitoneal injection; iv, intravenous injection; MAMA, methylazoxymethanol acetate; MeDAB, 3′-methyl-4-dimethylaminobenzene; MeIQx, 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline; MNA, N^6-(methylnitroso)adenosine; MNNG, *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine; MNU, *N*-methyl-*N*-nitrosourea; NDEA, *N*-nitrosodiethylamine; NDMA, *N*-nitrosodimethylamine; NMAA, *N*-nitrosomethylamylamine; NMB_zA, *N*-nitrosomethylbenzylamine; NNK, 4-(methylnitrosamino)-1-(3-pyridyl)butanone; NNN, *N*′-nitrosonornicotine; NPYR, *N*-nitrosopyrrolidine; NSEE, *N*-nitrososarcosin ethyl ester; sc, subcutaneous injection; SE, standard error; T_{50}, number of days required for 50% of rats to develop palpable tumours

after 21 weeks. The tumour incidence was significantly increased in ethanol-treated rats compared with controls at week 15 ($P < 0.05$), but not at weeks 18 or 21. The time-to-tumour occurrence was significantly decreased in ethanol-treated rats compared with controls ($P = 0.0245$, two-sided). In a second experiment, 40 male Sprague-Dawley rats [age unspecified], weighing 280–290 g, received either 2.5 mL ethanol (4.8 g/kg bw) or saline by gavage twice daily for 10 weeks, followed by local application of 2 mg/kg bw acetoxymethylnitrosamine to the rectal mucosa once every 2 weeks. No significant difference in tumour incidence was seen between ethanol-treated and control rats at weeks 15, 18 or 21; the time-to-tumour occurrence was significantly decreased in ethanol-treated rats compared with controls ($P = 0.0295$, two-sided) (Seitz *et al.*, 1990).

3.2.3 *2-Amino-3,8-dimethylimidazo[4,5-f]quinoxaline (MeIQx)*

Rat

A total of 210 male Fischer 344/DuCrj rats, 21 days of age, were fed 200 ppm 2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline (MeIQx) [purity not specified]. Water was provided *ad libitum* for the first 8 weeks. After 8 weeks and during 16 weeks, the rats continued to receive MeIQx in the diet but were subdivided such that 40 rats received control drinking-water, 30 rats each received 0.1%, 0.3%, 1%, 3% or 10% ethanol (purity, 99.5%) in the drinking-water and 20 rats received 20% ethanol in the drinking-water. An additional 10 rats were fed control diet for the first 8 weeks. Five of these rats were then given 20% ethanol in the drinking-water, while the other five continued to receive control drinking-water. The experiment was terminated after 24 weeks and livers were examined histologically. Rats administered 20% ethanol had significantly decreased body weights. Liver neoplasms were present only in groups administered MeIQx. [The Working Group noted the small number of rats that were not exposed to MeIQx.] In rats that were given MeIQx in the diet, the incidence of hepatocellular adenoma, hepatocellular carcinoma and hepatocellular adenoma plus hepatocellular carcinoma was increased by consumption of ethanol in a dose-dependent manner ($P < 0.001$). The incidence of hepatocellular adenoma and hepatocellular adenoma plus hepatocellular carcinoma was significantly and dose-dependently increased in groups administered MeIQx and 10% or 20% ethanol compared with the group that received MeIQx alone ($P < 0.01$); the incidence of hepatocellular carcinoma was increased significantly in rats that received MeIQx and 20% ethanol ($P < 0.01$). The multiplicity of hepatocellular adenoma and hepatocellular adenoma plus hepatocellular carcinoma was significantly and dose-dependently increased in the groups administered MeIQx and 20% ethanol compared with the group that received MeIQx alone ($P < 0.01$) (Kushida *et al.*, 2005).

3.2.4 *Azaserine*

Rat

A group of 40 male weanling SPF albino Wistar rats [age not specified] received either a high-fat diet (25% corn oil) or a high-fat diet plus ethanol. Ethanol was dissolved in tap-water and the concentration was gradually increased starting at day 25 from 5% during the first 2 weeks to a final concentration of 10% which was reached within 6 weeks. The animals received a single intraperitoneal injection of 30 mg/kg bw azaserine at 19 days of age and were killed on days 447 and 448 thereafter. No effect of ethanol on pancreatic adenomas or carcinomas was noted (Woutersen *et al*., 1989).

3.2.5 *Azoxymethane*

Rat

Groups of 20 and 23 male Fischer 344 rats, 10 weeks of age and weighing 210–260 g, were fed diets that contained 33% of total calories as ethanol or for which 33% was isocalorically replaced by carbohydrates for 3 weeks before and during subcutaneous administration of 14 mg/kg bw azoxymethane per week for 10 weeks. The ethanol-fed group was then given the ethanol-free diet until they were killed, 16 weeks after the last injection. The prevalence and multiplicity of all tumours observed as well as those of colonic and small intestinal tumours separately were found to be decreased by ethanol (Hamilton *et al*., 1988).

Male Sprague-Dawley rats [age not specified], weighing 340 g, were implanted with a single gastric cannula; 14 days later, rats were randomly assigned to three different groups. One group of 18 rats was infused with a liquid diet that contained ethanol, a second group of 11 rats was infused with the same diet without ethanol and a third group of 13 rats was infused with a volume of water equal to that of the liquid diet given to the other two groups. The liquid diets were infused at a rate of 160 kcal/kg$^{0.75}$/day over 23 hours. Ethanol was initially provided at a dose of 8 g/kg bw per day and this was gradually increased to 13 g/kg bw ethanol per day by day 10. All rats had ad-libitum access to drinking-water. Rats in the third group were given ad-libitum access to standard rat chow. Thirty-five days after the start of gastric infusion, the amount of ethanol was gradually decreased over a period of 4 days, for rats on the ethanol diet, at which time the dietary infusions were stopped. Nine hours later, 15 mg/kg bw azoxymethane [purity not specified] in sterile water were infused and dietary infusion was resumed 6 hours later. This sequence was repeated 1 week later. After the second azoxymethane infusion, all rats were maintained on standard rat chow until the end of the experiment at 30 weeks, at which time the extent of gastrointestinal neoplasia was determined histologically. Two of 18 rats that received azoxymethane and ethanol developed well-differentiated mucinous adenocarcinomas in the duodenum and another rat in the same group had focal adenomatous changes in the duodenum. No

neoplastic or preneoplastic changes were observed in the gastrointestinal tract in any of the other groups (Hakkak *et al.*, 1996).

A group of 93 male Fischer 344 rats, 4.5 weeks of age, were administered either control drinking-water (53 rats) or drinking-water consisting of beer (brewed from Munich malt, Pilsner malt and hops; 40 rats). One week later, 40 of the rats that received the control drinking-water and all of the rats that received beer were given two subcutaneous injections of 15 mg/kg bw azoxymethane [purity not stated] in saline [volume not stated] at 1-week intervals. The remaining 13 rats that received control drinking-water were given two subcutaneous injections of saline. The experiment lasted 42 weeks. Body weights of the rats injected with azoxymethane were significantly lower than those of rats injected with saline ($P < 0.05$). All of the saline-treated rats survived to the end of the experiment; 45% of the rats from each of the azoxymethane-treated groups died. Colonic tumours were assessed histologically: none were observed in rats treated with saline. In rats administered azoxymethane and control drinking-water, the incidence and multiplicity (± SD) of colonic adenomas were 46% and 0.55 ± 0.67 tumours/rat and those of colonic adenocarcinomas were 82% and 1.41 ± 1.10 tumours/rat, respectively. The incidence ($P < 0.01$) and multiplicity ($P < 0.005$) of adenomas was significantly decreased in rats that were injected with azoxymethane and received beer compared with rats that were injected with azoxymethane and received control drinking-water. In rats administered azoxymethane and beer, the incidence and multiplicity of adenomas were 5% and 0.09 ± 0.43 tumours/rat and those of adenocarcinomas were 64% and 1.00 ± 0.98 tumours/rat, respectively. The multiplicity ($P < 0.05$) of adenocarcinomas was significantly decreased in rats that were injected with azoxymethane and received beer compared with rats that were injected with azoxymethane and received control drinking-water (Nozawa *et al.*, 2004).

3.2.6 *Benzo[a]pyrene*

Mouse

Male and female BALB/c mice [number and sex distribution per group not specified], 8 weeks of age, were given a single subcutaneous injection of 2 mg benzo[*a*]pyrene in 200 µL olive oil and were then administered 0 or 10% ethanol in the drinking-water *ad libitum* [duration of ethanol administration not specified]. All mice survived until 58 weeks after the start of the experiment, at which point it was terminated. At 10 weeks, 20% of the mice in the benzo[*a*]pyrene-treated group and 3.8% of the mice in the benzo[*a*]pyrene plus ethanol-treated group had developed tumours. At 18 weeks, the tumour incidence was 60 and 46.1% in the benzo[*a*]pyrene- and benzo[*a*]pyrene plus ethanol-treated groups, respectively. At the end of the experiment, the tumour incidences were 84.0 and 65.4%, respectively. All tumours were subcutaneous fibrosarcomas (Uleckiene & Domkiene, 2003).

3.2.7 *7,12-Dimethylbenz[a]anthracene (DMBA)*

(a) Rat

Two experiments were performed to investigate the effect of ethanol on DMBA-induced mammary gland carcinogenesis. Three groups of 50 female Sprague-Dawley rats, 21 days of age and weighing 40–55 g, were fed a liquid diet that supplied 20% of calories as fat for 3 days. One group was then continued on the same diet (*ad libitum*), one group was fed 10% of calories as ethanol for 4 days and then 20% of calories as ethanol for the remainder of the experiment (*ad libitum*) and the third group was fed control diet pair-fed by calories (20% of calories as fat) each day to an individually matched ethanol-treated rat (experiment 1). Rats had free access to distilled water at all times. At 55 days of age, the animals were given a single dose of 20 mg/kg bw DMBA in 0.1–0.2 mL sesame oil by gastric gavage. All animals were necropsied 20–25 weeks after exposure to DMBA. No statistically significant effect of ethanol ingestion on mammary gland tumorigenesis was observed between the ethanol-treated and pair-fed control groups or between the control group and either of the other groups (64–70% mammary tumour incidence). [Blood ethanol concentrations were measured.] In the second experiment, female Sprague-Dawley rats, 21 days of age, were fed a liquid diet that provided 10% of calories as fat for 1 week and were then kept on the same diet (20 rats), or paired by weight into ethanol-treated (32 rats) and pair-fed control (32 rats) groups. Ethanol-treated rats were fed 10% of calories as ethanol for 4 weeks; at the beginning of the 4th week, all ethanol-treated rats were given a single dose of 50% ethanol (3.5 g/kg bw by gavage); their pair-fed partners were given the equivalent calories as sucrose. One week later, at 55 days of age, all rats were given 30 mg/kg bw DMBA in 0.1–0.2 mL sesame oil by gavage; ethanol-treated rats were fed control diet for 1 day before and 1 day after DMBA administration, returned to 10% of calories as ethanol for 1 week and then fed 25% of calories as ethanol for the remainder of the experiment. For one 24-hour period at 10, 13, 14, 15 and 18 weeks of age, dietary ethanol was raised to 35% of calories. The experiment was terminated and rats were necropsied 12–13 weeks after exposure to DMBA. Histological diagnoses were made of mammary tumours, liver and other organs when abnormal. No detectable effect of ethanol ingestion on mammary tumorigenesis (80–94% mammary tumour incidence) was observed (Rogers & Conner, 1990). [The Working Group noted the very high tumour response in all groups.]

The influence of chronic ethanol intake on the initiation and promotion stages of mammary tumour development was evaluated in three separate studies. Experiments 1 and 2 were designed to evaluate the influence of ethanol intake on the initiation stage of DMBA-induced mammary tumorigenesis. Female Sprague-Dawley rats, 21–22 days of age, were fed a liquid control diet. At 30 days of age, rats in experiment 1, weighing 72.6 ± 1.0 (SE) g, were fed diets that contained ethanol at 0 (15 rats) and 20% (17 rats) of calories. At 25 days of age, rats in experiment 2, weighing 49.0 ± 0.5 (SE) g, were fed ethanol at 0 (33 rats), 10 (24 rats) and 20% (31 rats) of calories. Rats were

pair-fed on a daily basis. Serum ethanol concentration was measured after 4 and 12 hours in subgroups of animals fed the diet that contained ethanol. Diets were removed 18–24 hours before intragastric administration of 5 mg/rat DMBA in 0.5 mL corn oil at 58 (experiment 1) or 53 (experiment 2) days of age. The rats were returned to the diets that contained ethanol until 1 week after DMBA treatment, after which time all rats were fed a powdered control diet. Experiments 1 and 2 were terminated at 20 and 26 weeks after administration of DMBA, respectively. Experiment 3 was designed to evaluate the effect of ethanol intake on the promotion or post-initiation stage: 92 female Sprague-Dawley rats, 42 days of age, were fed the powdered control diet for 2 weeks. At 56 days of age, all rats were administered 5 mg/rat DMBA in 0.5 mL corn oil intragastrically. At 63 days of age, the animals, weighing 177.4 ± 2.3 (SE) g, were separated into three treatment groups that were pair-fed diets that contained ethanol at 0 (31 rats), 15 (30 rats) or 30% (31 rats) of calories for the remainder of the study. The experiment was terminated 21 weeks after administration of DMBA. At necropsy, tumours were removed and examined histologically. For statistical analysis, the χ^2 test, median test and the Student's *t* test were applied. Rats that consumed ethanol at 20% of total calories before administration of DMBA had a mammary tumour incidence of 82 (experiment 1; $P < 0.05$) and 74% (mainly adenocarcinomas; experiment 2; $P < 0.05$) compared with an incidence of 47–48% in rats fed the control diet. No increased tumour incidence was found in rats fed the 10% ethanol diet in experiment 2. Classification of tumours from experiment 1 was not performed. No differences in multiplicity or latency of tumours were observed in experiments 1 and 2. In experiment 3, the final tumour incidence in rats that consumed ethanol at 15% of calories was significantly increased compared with rats fed the control diet. In rats fed ethanol at 30% of calories, the tumour incidence did not differ from that of rats fed the control diet. The number of days required for 50% of rats to develop palpable tumours (T_{50}) was 150, 84 and 105 for rats fed the diets containing ethanol at 0, 15 and 30% of calories, respectively (0% versus 15%, $P < 0.05$). The tumours were mainly adenocarcinomas (Singletary *et al.*, 1991).

Two groups of 20 female Sprague-Dawley rats, 40 days of age, were given 95% laboratory-grade ethanol diluted in tap-water to 5% by volume as their sole water source or tap-water alone. At 50 days of age, under general anaesthesia, all animals were given 15 mg DMBA in 1 mL sesame oil by intragastric instillation. The animals were killed at 120 days after administration of DMBA or when a tumour bulk was apparent. Tumours were counted and measured by calipers. Two animals in the control group died within 24 hours after administration of DMBA and were excluded from further analysis. No animal in the ethanol-treated group died before the end of the study. All 18 surviving animals in the control group had developed tumours by 116 days after administration of DMBA in contrast with a tumour incidence of 40% ($P < 0.001$) in the 20 ethanol-treated rats. The mean time to first tumour appearance following administration of DMBA was 67.3 ± 19 days for the control group and 63 ± 16.3 days for the ethanol-treated group. The mean number of tumours per tumour-bearing animal in

control and ethanol-treated groups was 2.9 ± 2.7 and 3.2 ± 2.2, respectively. The mean tumour growth rate was 25.5 ± 11.8 mm^3 per day in the control group versus 30.7 ± 17.7 mm^3 per day in the ethanol-treated group. The histology of the tumours was similar in both groups (McDermott *et al.*, 1992). [The Working Group noted that the intake of ethanol was rather low considering the high rate of metabolism of these animals. Blood levels of ethanol were not measured.]

Groups of 15 pregnant Sprague-Dawley rats [age not specified] were pair-fed isocaloric liquid diets that contained either 0, 16 (7% ethanol of total energy) or 25 g/kg diet (15% ethanol of total energy) ethanol [purity not stated] on days 7–18 of gestation. Blood levels of ethanol were not measured but, based upon previous experiments, were estimated to be 61.3 ± 5.0 mg/dL and 95.8 ± 6.1 mg/dL for the 16-g and 25-g groups, respectively. On postnatal day 47, 23–25 female offspring per group were administered 10 mg (~50 mg/kg bw) DMBA [purity not stated] in 1 mL peanut oil, after which mammary gland tumour development was monitored for 17 weeks. The total number of palpable tumours per rat was significantly higher ($P < 0.006$) in rats exposed *in utero* to diets that contained ethanol than in those exposed to the control diet. Post-hoc analysis indicated that the increase in the incidence of mammary gland tumours was significant in rats exposed *in utero* to 16 g/kg diet ethanol compared with those not exposed to ethanol in the diet. The mean tumour latency did not differ among the groups (Hilakivi-Clarke *et al.*, 2004).

(b) Hamster

The right buccal pouch of 36 male Syrian golden hamsters, 4–6 weeks of age, was painted three times on alternate days for 1 week with a 1% solution of DMBA [purity not specified] in heavy mineral oil. The left buccal pouch remained unpainted to serve as a control. One week later, 16 of the hamsters were placed on a liquid diet that contained 5% ethanol (v/v) and the remaining 20 hamsters were pair-fed an isocaloric control diet. Periodic sampling indicated blood–ethanol levels at a concentration range of 80–180 mg/dL (mean, 95 mg/dL) in ethanol-fed hamsters. At 22 weeks after the start of the experiment, seven control and six ethanol-treated hamsters were killed; the remaining seven controls and 10 ethanol-treated hamsters were killed at 35 weeks. At the end of the experiment, the ethanol-treated hamsters weighed significantly less than the pair-fed controls ($P < 0.005$). Buccal pouch tumours were assessed macroscopically and representative tumours were examined histologically. The incidence and multiplicity of tumours (epidermoid carcinomas) in the right buccal pouch of hamsters treated with DMBA and the control diet was 38% (5/13) and 1 ± 0.0 tumours/tumour-bearing hamster. The incidence and multiplicity of tumours in the right buccal pouch of hamsters treated with DMBA and fed the ethanol diet was 70% (7/10) and 3.29 ± 1.02 tumours/tumour-bearing hamster. Tumour multiplicity in the ethanol-treated hamsters was significant greater than that in pair-fed controls. No tumours were observed in the left buccal pouches of any of the hamsters, which served as an internal control (Nachiappan *et al.*, 1993).

3.2.8 *Dimethylhydrazine (DMH)*

Rat

The effect of chronic administration of ethanol on DMH-induced colorectal carcinogenesis was evaluated in two groups of 16 adult male Sprague-Dawley rats [age unspecified], initially weighing 250–300 g, that were pair-fed nutritionally complete liquid diets that contained 36% of total calories as ethanol or isocaloric carbohydrates, respectively, for 4 weeks. Thereafter, the animals were given the first of four weekly subcutaneous injections of 30 mg/kg bw DMH, during which time standard laboratory chow replaced the liquid diet to avoid competitive inhibition of pro-carcinogen activation by ethanol. This 8-week cycle was completed four times during a total of 32 weeks. At the end of each 8-week cycle, two to five rats from each group were killed. All surviving rats were killed at the end of 32 weeks. The incidence, size and distribution of colon tumours was recorded. Sample specimens of normally appearing proximal and distal colon and rectum and gross tumours were studied microscopically. At the end of the first 4 weeks of ethanol consumption, blood ethanol levels were measured in five randomly chosen rats. Chronic ethanol consumption did not alter the number, size or distribution of large-bowel tumours in DMH-treated animals (McGarrity *et al*., 1988).

Groups of 20–30 male and 20–30 female Sprague-Dawley rats, 10 weeks of age, were given 18 weekly subcutaneous injections of 21 mg/kg bw DMH [purity not specified] in distilled water [concentration not specified] (pH 6.5) that contained ethylene diamine tetraacetic acid (EDTA) as a stabilizing agent (37 mg EDTA:400 mg DMH) and 0 or 1.23 g/kg bw ethanol [purity not specified] daily in the drinking-water for the duration of the study. Daily food consumption and ethanol intake were controlled throughout the experiment. All surviving animals were killed between weeks 25 and 27. At the end of the study, 28% (2/14) male and 78% (11/14) female rats in the DMH-treated group were tumour-free compared with 14% (1/7) and 55% (5/9), respectively, in the group that received DMH and ethanol. The mean numbers of tumours (adenocarcinomas and mucinous carcinomas) per rat (± SD) in the DMH-treated group were 1.83 ± 1.34 and 1.00 ± 0.00 for male and female rats, respectively. The corresponding numbers in the DMH/ethanol-treated group were 2.00 ± 0.89 and 1.00 ± 0.00, respectively. No significant differences in tumour incidence or multiplicity were found between the two groups (Pérez-Holanda *et al*., 2005).

3.2.9 *Ethyl carbamate (urethane)*

Mouse

Groups of 15 female specific pathogen-free strain A/Ph mice, 6.5 weeks of age, were administered 0, 200, 500 or 1000 ppm ethyl carbamate (purity, < 99%) dissolved in tap-water and 0, 5, 10 or 20% ethanol solutions as drinking fluid for 12 weeks, after which time the mice were killed. Survival was > 90%. Lung tumours were counted.

Table 3.3 Pulmonary tumours in female strain A/Ph mice treated for 12 weeks with combinations of ethanol and ethyl carbamate in the drinking-water

Concentration of ethyl carbamate (ppm)	Concentration of ethanol (%)	No. of tumours/mouse (mean±SD)
0	0	0.4±0.7
0	5	1.1±1.5
0	10	1.0±1.7
0	20	1.0±1.0
200	0	11.8±3.8
200	5	9.9±4.7
200	10	4.7±2.7*
200	20	3.8±3.2*
500	0	45.4±12.0
500	5	46.0±9.4
500	10	22.1±6.5*
500	20	9.6±4.9*
1000	0	70.9±15.5
1000	5	61.3±12.4
1000	10	39.3±9.9*
1000	20	21.6±6.9*

From Kristiansen *et al.* (1990) SD, standard deviation * $p<0.001$ in comparison with the respective control group representing 0% ethanol and equivalent concentration of ethyl carbamate (Wilcoxon rank test)

Random samples of nodules were taken from the lungs for histopathological evaluation and confirmation of adenoma. The numbers of nodules were analysed by the Spearman rank correlation and Wilcoxon rank test (see Table 3.3). Ethyl carbamate induced lung tumour multiplicity in a dose-dependent manner both alone and in combination with all three concentrations of ethanol. Ethanol inhibited ethyl carbamate-induced lung tumour multiplicity in a dose-dependent manner. The inhibition was not statistically significant with 5% ethanol but was highly significant with 10 and 20% ethanol (Kristiansen *et al.*, 1990).

In two series of experiments, 12 groups of 25 female Han/NMRI mice, approximately 10 weeks of age, received 0.3 mL/25 g bw of one of the following solutions: 1.5, 3.0, 7.5 or 15 g/L ethyl carbamate [purity unspecified] in tap-water or in 20% ethanol [during the first 3 days of the experiment, 10% ethanol rather than 20% ethanol was administered] by gavage daily during the first 8 weeks of the study. After a further 8 weeks without treatment, the animals were weighed and killed. The fixed lungs were inspected for the presence of lung adenomas using a binocular magnifying glass, then confirmed histologically. The rank sum test was used for statistical significance. Simultaneous application of 20% ethanol [approximately 2.3 g/kg bw per day] had no effect on the number of ethyl carbamate-induced lung adenomas (Altmann *et al.*, 1991).

Groups of 18–21 male weanling C3H/HeJ mice [age unspecified] were given control drinking-water, 12% ethanol [purity not stated] as the drinking-fluid or Concord white, Concord red or Johannesburg Riesling wine as the drinking-fluid simultaneously with 0, 10 or 20 mg/kg bw ethyl carbamate [purity not specified] per day. The ethanol content of the wines had been adjusted to 12%. The experiment lasted 41 weeks. Survival was > 80%, except for the group given 20 mg/kg bw ethyl carbamate and control drinking-water in which survival was 57%. Livers and lungs were examined histologically. Hepatocellular adenoma was detected in all treatment groups (5.6–57.1% incidence) except in those treated with Concord red wine in the absence of ethyl carbamate. Compared with the respective control groups that received only 0, 10 or 20 mg/kg bw ethyl carbamate, the frequency of hepatocellular adenoma/tumour-bearing mouse was decreased significantly in all groups except in mice administered 20 mg/kg bw ethyl carbamate plus 12% ethanol or Concord red wine, respectively. Liver haemangiosarcomas were detected in mice given 10 mg/kg bw ethyl carbamate without ethanol or wine (4.8% incidence) and in all groups given 20 mg/kg bw ethyl carbamate (4.8–23.8% incidence) except for those that also received 12% ethanol. Compared with the control group that was given only 20 mg/kg bw ethyl carbamate, the frequency of haemangiosarcoma/tumour-bearing mouse was decreased significantly in all groups given 20 mg/kg bw ethyl carbamate plus 12% ethanol or wine. Lung Clara-cell adenomas were detected in all treatment groups given 10 or 20 mg/kg bw ethyl carbamate (4.8–57.1% incidence). Compared with the control group that was given only 10 mg/kg bw ethyl carbamate, the frequency of Clara-cell adenoma/tumour-bearing mouse was decreased significantly in all groups given 20 mg/kg bw ethyl carbamate plus wine. Lung alveolar adenomas were detected in all treatment groups given 10 or 20 mg/kg bw ethyl carbamate (4.8–47.6% incidence), except for mice given 10 mg/kg bw ethyl carbamate plus 12% ethanol. Compared with the control group that was given only 20 mg/kg bw ethyl carbamate, the frequency of alveolar adenoma/tumour-bearing mouse was decreased significantly in all groups administered 20 mg/kg bw ethyl carbamate plus ethanol or wine (Stoewsand *et al.*, 1991).

Groups of 20 male and 20 female BALB/c mice, 8 weeks of age, received twice-weekly intraperitoneal injections of 10 mg ethyl carbamate ('pure'; total dose, 100 mg). Two groups also received 10% ethanol [purity not specified] in the drinking-water *ad libitum* [duration of ethanol administration not specified]. All surviving mice were killed after 4 months. The lungs were examined macroscopically and microscopically. The tumour incidence (lung adenomas) was 100% in all groups. Seventeen males and 20 females in the ethyl carbamate-treated group and 20 males and 19 females in the ethyl carbamate plus ethanol-treated group survived until the end of the experiment. Tumour multiplicities (± SD; males and females combined) were 9.9 ± 3.2/mouse in the ethyl carbamate-treated group and 8.1 ± 2.5/mouse in the ethyl carbamate plus ethanol-treated group. No significant differences between sexes or between dose groups were observed (Uleckiene & Domkiene, 2003).

Groups of 48 male and 48 female B6C3F$_1$ mice, 28 days of age, were exposed to 0, 10, 30 or 90 ppm ethyl carbamate in the presence of 0, 2.5 or 5% ethanol in the drinking-water *ad libitum* for 104 weeks. Complete histopathology was performed. Serum levels of ethyl carbamate and ethanol were assessed. The results are summarized in Table 3.4. In female mice administered 10 and 90 ppm ethyl carbamate, ethanol caused dose-related increases in the incidence of alveolar/bronchiolar adenoma or carcinoma and haemangiosarcoma of the heart, respectively. In male mice, a different relationship was observed: ethanol caused a dose-related decrease in the incidence of alveolar/bronchiolar adenoma or carcinoma and of Harderian gland adenoma or carcinoma after exposure to 30 ppm ethyl carbamate. The decrease in the incidence of alveolar/bronchiolar adenoma or carcinoma was significant at 5% ethanol (National Toxicology Program, 2004; Beland *et al.*, 2005).

3.2.10 *Hormones*

Rat

Four groups of female Wistar JCL rats, 4 weeks of age, received 0.075 mg ethinylestradiol and 6.0 mg norethindrone acetate in 0.5 mL olive oil by stomach tube daily for 12 months; the same doses administered by the same method and 10% ethanol w/v in the drinking-water on 2–5 consecutive days a week and pure water for the 2 remaining days each week; 0.5 mL olive oil alone and 10% ethanol and water as in the previous group; or 0.5 mL olive oil only daily throughout the experiment, which lasted 12 months. Daily ethanol intake in the group administered ethinylestradiol and norethindrone acetate was 9.6 ± 2.6 g/kg bw at the beginning of experiment and 11.3 ± 3.7 g/kg bw at 12 months. In the ethanol-treated group, the corresponding intakes were 9.9 ± 2.5 g/kg bw at the beginning and 11.7 ± 4.1 g/kg bw at 12 months. Animals were killed at 2, 4, 6, 8 and 12 months. Histological analysis of liver tissue was performed. Statistical analysis was carried out using the paired Student's *t* and χ^2 tests. Liver tumours observed were well differentiated hepatocellular carcinoma. There was an increased incidence of hepatocellular carcinoma in the group treated with ethinylestradiol and norethindrone acetate plus ethanol (38%; 8/21) compared with the group treated with ethinylestradiol and norethindrone acetate alone (8% (1/12); $P < 0.05$) (Yamagiwa *et al.*, 1991).

3.2.11 *Methylazoxymethanol acetate*

Rat

Two experiments were performed to evaluate the effect of ethanol or saké on methylazoxymethanol acetate-induced large bowel cancer. In the first experiment, 39 male ACI/N rats, 6 weeks of age, were divided into two groups. All animals were given two weekly intraperitoneal injections of 25 mg/kg bw methylazoxymethanol acetate

Table 3.4 Incidence of neoplasms in B6C3F$_1$ mice administered 0, 10, 30 or 90 ppm ethyl carbamate with 0, 2.5 or 5% ethanol in the drinking-water for two years[a]

Neoplasm	Ethanol (%)	Ethyl carbamate (ppm)		
		10	30	90
Females				
Alveolar/bronchiolar adenoma or carcinoma	0	8/48 (16.7%)[&]	28/48 (58.3%)[*]	39/47 (83.0%)[*]
	2.5	11/47 (23.4%)	21/48 (43.8%)[*]	38/48 (79.2%)[*]
	5	17/48 (35.4%)[*,‡]	24/48 (50.0%)[*]	37/48 (77.1%)[*]
Heart haemangiosarcoma	0	0/48 (0.0%)	1/48 (2.1%)	0/48 (0.0%)[&]
	2.5	0/47 (0.0%)	0/48 (0.0%)	3/48 (6.3%)
	5	0/48 (0.0%)	0/48 (0.0%)	6/47 (12.8%)[*,‡]
Males				
Alveolar/bronchiolar adenoma or carcinoma	0	18/48 (37.5%)[*]	29/47 (61.7%)[*,&]	37/48 (77.1%)[*]
	2.5	19/48 (39.6%)	24/47 (51.1%)[*]	43/48 (89.6%)[*]
	5	11/48 (22.9%)	14/48 (29.2%)[‡]	40/48 (83.3%)[*]
Harderian gland adenoma or carcinoma	0	12/47 (25.5%)[*]	30/47 (63.8%)[*,&]	38/47 (80.9%)[*]
	2.5	14/48 (29.2%)[*]	21/47 (44.7%)[*]	38/48 (79.2%)[*]
	5	14/48 (29.2%)[*]	17/48 (35.4%)[*,‡]	35/45 (77.8%)[*]

From National Toxicology Program (2004); Beland *et al*. (2005) [a] The data are reported as the number of animals with a neoplasm per number of animals examined microscopically and (in parentheses) the percentage incidence. An ampersand (&) associated with a 0% ethanol incidence indicates a significant ($p<0.05$) dose-related trend with respect to ethanol. An asterisk (*) associated with a specific treatment indicates a significant ($p<0.05$) difference compared with the 0 ppm urethane incidence. (A double dagger (‡) associated with a specific treatment indicates a significant ($p<0.05$) difference compared with the 0% ethanol incidence. *p* Values for the effects of ethanol are two-sided.

[purity unspecified] dissolved in normal saline. One week after the termination of the injections, one group of 20 rats was given 10% ethanol as drinking-water and a second group of 19 rats received distilled water alone. The experiment was terminated 414 days after the study began. Most tumours in the large intestine were macroscopically sessile or pedunculated polyps and, histologically, were diagnosed as adenomas or adenocarcinomas. In ethanol-treated rats, 16/17 effective animals developed large bowel neoplasms (94%); among these, adenomas were seen in seven rats (41%) and adenocarcinomas in 15 animals (88%). In control rats, 11/16 effective animals had large bowel neoplasms (69%); four animals developed adenomas (25%) and nine had adenocarcinomas (56%). The incidence of large intestinal adenocarcinomas in the ethanol-treated group (88%, 15/17) was significantly higher than that in controls (56% (9/16); $P = 0.040$). No significant differences were noted for the incidence of adenomas between the two groups. The incidence of rectal neoplasms in ethanol-treated rats (59%, 10/17) was significantly higher than that in controls (19% (3/16); $P = 0.019$). In

the second experiment, six groups of 15 female ACI/N rats, 6 weeks of age, were given two weekly intraperitoneal injections of 25 mg/kg bw methylazoxymethanol acetate. A group of seven rats received two injections of saline alone. After a 1-week interval, rats in all treated groups were given isocaloric drinks (105–110 cal/100 mL) as follows: one group was given commercially available saké (approximately 110 cal/100 mL; ethanol content, 15–16%); one group was given 50% saké (approximately 110 cal/100 mL; ethanol content, 7.5%); two groups were given 15% ethanol (approximately 105 cal/100 mL); one group was given 7.5% ethanol (approximately 105 cal/100 mL); and one group was given water without ethanol supplement (approximately 105 cal/100 mL). Glucose (4 cal/g) was added to the 50% saké, 7.5% ethanol and water to make isocaloric drinks. The volume of all drinks was adjusted to 15 mL/rat/12 hour, because the mean fluid intake was found to differ among the groups in a preliminary experiment. The experiment was terminated 280 days after the first administration of methylazoxymethanol acetate. All surviving animals were killed and autopsied. All major organs, especially the intestines, were carefully inspected grossly, and suspected lesions were taken for histological examination. To determine tumour distribution, the large bowel was divided into three parts, and the distal 5 cm from the anus was treated as the rectosigmoidal colon. The first intestinal tumour was observed in an animal that died on the 189th day. [The group was not indicated.] No significant differences among the groups were noted. The incidence of rectosigmoidal colonic neoplasms in the groups given saké (53%, 8/15 effective animals), 50% saké (46%, 6/13) and 15% ethanol (50%, 5/10) was non-significantly higher than that in the group given water (38%, 5/13). The numbers of rectosigmoidal colonic neoplasms per total large intestinal neoplasms in the groups given saké (68%, 11/16) and 50% saké (67%, 8/12) were also non-significantly higher those than in the group given water (45%, 5/11). The incidence of colonic tumours in the second experiment was lower than that in the first, which may have been due to the shorter duration of the former (Niwa *et al.*, 1991).

3.2.12 *3′-Methyl-4-dimethylaminobenzene (MeDAB)*

Rat

Groups of 80 male Wistar rats, 5 weeks of age, were fed powdered diets containing 0 or 0.06% 3′-methyl-4-dimethylaminoazobenzene (MeDAB) [purity not specified] for an initiation period of 4 weeks. Another group of 80 rats was fed the same diets without carcinogen. After a 2-week recovery period on a pelleted diet, each of the two groups was divided in four identical subgroups that were given distilled drinking-water that contained 0, 5, 10 or 15% ethanol (‘of the highest grade’). The rats were fed a pelleted diet and the drinking solutions *ad libitum*. Rats not treated with MeDAB were killed 45 weeks after the start of ethanol administration at week 51. The rats fed MeDAB were killed at the end of week 53 after initiation. In the groups that were not initiated with MeDAB, no macroscopic tumours were observed in the liver or other organs. In contrast, macroscopical liver changes, including variable tumour size and irregularity

of the surface, were observed in rats initiated with MeDAB. The incidence of hepatocellular carcinomas in the initiated groups was 37% (7/19), 37% (7/19), 16% (3/19) and 42% (8/19) in the rats administered 0, 5, 10 and 15% ethanol, respectively (Yanagi *et al.*, 1989).

3.2.13 *4-(Methylnitrosamino)-1-(3-pyridyl)butanone (NNK)*

(a) Rat

Male Fischer 344 rats [initial number unspecified], 4–6 weeks of age, were treated by gavage with a total dose of 20 mmol/kg 4-(methylnitrosamino)-1-(3-pyridyl) butanone (NNK) three times a week for 4 weeks. One week after initiation, the animals received liquid diets that contained 36% of total calories as ethanol or an isocaloric equivalent of carbohydrates for 55 weeks. Ethanol increased the incidence of tumours of the oesophagus, oral cavity, lungs and liver initiated by NNK ($P < 0.05$) and caused an increase in the mean frequency and size of the tumours ($P < 0.001$) (Nachiappan *et al.*, 1994).

(b) Hamster

Two groups of four pregnant female Syrian hamsters [age not specified] received 10% ethanol in the drinking-water on days 5–16 of pregnancy and two groups of four hamsters served as untreated controls. On day 15, 50 mg/kg bw NNK were intratracheally instilled into animals that did or did not receive the ethanol. The control group received identical intratracheal instillation with distilled water only. The offspring were weaned at 4 weeks of age and were observed until weight loss or symptoms occurred and were then killed. Treatment with ethanol and NNK resulted in a significant increase in the incidence of tumours in male and female offspring compared with those treated with NNK alone ($P < 0.01$). This was also found for tumours of the nasal cavity in females, of the pancreas in males and females and of pheochromocytoma in both sexes (Schüller *et al.*, 1993).

3.2.14 N-*Methyl*-N′-*nitro*-N-*nitrosoguanidine (MNNG)*

(a) Shrew

Groups of female Jic:SUN house musk shrews, 5 weeks of age, were administered tap-water (four animals), 2% ethanol (purity, > 99.5%) in tap-water (four animals), 50 ppm MNNG [purity not specified] in tap-water (25 animals), or 50 ppm MNNG in tap-water that contained 2% (25 animals), 5% (30 animals) or 10% (25 animals) ethanol. The treatment lasted for 30 weeks, after which the animals were returned to tap-water. Average water consumption (approximately 10 mL/day) was not affected by the presence of MNNG and/or ethanol. All animals were autopsied. No significant differences in body weight or organ weights were observed among groups. All MNNG-treated

animals that survived to 20 weeks of age were included in the analysis. Randomly selected animals were killed sequentially at 20, 30, 35, 40 and 45 weeks of age. The animals in the 2% ethanol-treated control group were killed at 35 weeks of age. Organs and tissues were examined grossly and microscopically after routine histological procedures and haematoxylin/eosin staining. At the highest doses (5 and 10%), co-administration of ethanol with 50 ppm MNNG produced an acute toxic response: 20% (6/30) of the animals in the 5% ethanol-treated group died within 7 days and 40% (10/25) of the animals in the 10% ethanol-treated group died within 4 days after the start of the treatment. Acute toxicity was not observed in any of the other groups. Thus, the MNNG- and MNNG plus 2% ethanol-treated groups were selected for the long-term (30-week) study. Five animals were selected from each of these two groups for analysis at 20, 30, 35, 40 and 45 weeks of age. Oesophageal papillomas or squamous-cell carcinomas were not observed in either of the two groups at 20 weeks of age. At 30 weeks of age, two papillomas in the MNNG-treated group and one papilloma in the MNNG plus ethanol-treated group were observed. At later time-points, the incidence of papillomas and squamous-cell carcinomas, respectively, was: five and four in the MNNG-treated group compared with three and three in the MNNG plus ethanol-treated group at 35 weeks of age; five and five in the MNNG-treated group compared with five and five in the MNNG plus ethanol-treated group at 40 weeks of age; and five and five in the MNNG-treated group compared with five and five the MNNG plus ethanol-treated group at 45 weeks of age. Oesophageal tumours were not found in the water-treated or ethanol-treated control groups (Shikata *et al*., 1996).

(b) Rat

Two group of 15 male Wistar rats, 6 weeks of age, received 50 μg/mL MNNG in the drinking-water for 20 weeks. The average dose of MNNG consumed by each rat was 120 mg. From week 21, the rats received tap-water *ad libitum*. The rats also received intraperitoneal injections of either 2.5 mL/kg 0.9% saline solution or 2.5 mL/kg 20% ethanol in 0.9% saline solution per day every other day until week 52, at which time the animals were killed. Animals that survived 50 weeks were included. Ethanol treatment increased tumour incidence ($P < 0.02$) and multiplicity ($P < 0.01$) (Iishi *et al*., 1989).

Groups of 30 and 25 male ACI rats, 4 weeks of age and weighing 58 g, received 0.25 mL/10 g bw of a stock solution of 5 g/L MNNG by gastric intubation. Thereafter, the animals received either tap-water or 10% ethanol in the drinking-water for 1 year. Additional groups of rats that received water or ethanol only served as controls. Ethanol had no effect on the incidence of squamous-cell carcinoma of the forestomach or adenocarcinoma of the glandular stomach induced by MNNG. Ethanol alone had no effect on tumour yield compared with rats that received water (Watanabe *et al*., 1992).

Three groups of 20 male Wistar rats [age unspecified], weighing 150–200 g, were given 100 μg/mL MNNG in tap-water (control group), 100 μg/mL MNNG in 11% ethanol or 100 μg/mL MNNG in wine for 6 months, and the experiment was terminated

at 13 months. In the glandular stomach, six carcinomas, one carcinoma and one carcinoma plus one sarcoma were observed in the control, ethanol- and wine-treated groups, respectively. In the forestomach, one carcinoma, two carcinomas plus one papilloma and one carcinoma were found in the same groups, respectively. In the duodenum, four carcinomas were found in the control group (Cerar & Pokorn, 1996). [The Working Group noted that the application of MNNG solutions in the experimental groups was prolonged for 10 days to equalize the MNNG consumption per group, which confounds the interpretation of the study.]

Two groups of 15 male Fischer 344 rats, 6 weeks of age, received a single intragastric administration of 150 mg/kg bw MNNG [solvent not specified.]. One week later, one group was administered 10% ethanol in the drinking-water for 51 weeks. Animals were killed at 52 weeks and histopathological examination of the stomach and oesophagus was performed. In the MNNG plus ethanol-treated group, the incidence of papilloma and carcinoma was 2/15 (18%) (significantly reduced; $P < 0.01$) and 6% (1/15) versus 66% (10/15) and 6% (1/15), respectively, in the MNNG-treated group (Wada *et al.*, 1998).

3.2.15 N^6-*(Methylnitroso)adenosine*

Mouse

Groups of female Swiss (NIH:Cr(S)) mice [initial number unspecified], 4 weeks of age, received three intragastric doses of 60 or 180 mg/kg bw N^6-(methylnitroso) adenosine per week with or without 15% ethanol for 12 weeks. Thereafter, the mice were killed when ill or at 18 months of age. A complete necropsy was performed and tumours were examined histologically. Ethanol statistically significantly increased the incidence of thymic lymphomas induced by N^6-(methylnitroso)adenosine (at the 180-mg/kg bw dose): the incidence increased from 43% (21/49) in the N^6-(methylnitroso) adenosine-treated group to 64% (32/50) in the N^6-(methylnitroso)adenosine plus 15% ethanol-treated group ($P < 0.05$) (Anderson *et al.*, 1993).

3.2.16 N-*Methyl*-N-*nitrosourea (MNU)*

Rat

A study was conducted to evaluate the influence of low and high ethanol intake (15, 20 or 30% of calories) as part of a liquid diet on both the initiation and promotion stages of *N*-methyl-*N*-nitrosourea (MNU)-induced rat mammary tumorigenesis. In the first experiment (an initiation study), groups of 32 female Sprague-Dawley rats, 23 days of age, were fed a powdered control diet until 28 days of age, after which time the animals were randomly assigned to groups and fed *ad libitum* diets that contained ethanol at 0, 15, 20 and 30% of calories. At 50 days of age, 30 mg/kg bw MNU were administered intraperitoneally; all animals received the control diet between 18

and 24 hours before treatment. Four hours following the injections, the animals were returned to the previous control diets or diets that contained ethanol until 57 days of age. At this time, all animals were fed the powdered control diet for the remainder of the study. Two additional control groups were added in case the diet intake for rats fed the 20% and 30% ethanol diets decreased significantly compared with controls fed *ad libitum*. Beginning 4 weeks after treatment with MNU, animals were palpated weekly for the appearance of mammary tumours. Analysis of the incidence of cumulative, palpable mammary tumours indicated a significant difference in trends between the 15% ethanol-treated and control groups. A significant 64% increase in the number of adenocarcinomas per rat was observed for animals in the 15% ethanol-treated group (2.2 ± 0.3) compared with the control group (1.4 ± 0.2). No significant differences in the numbers of tumours were observed for the 20 and 30% ethanol-treated groups compared with their respective controls. In the second experiment (influence of ethanol intake on promotion), female Sprague-Dawley rats were fed a powdered control diet from 38 to 51 days of age, at which time MNU was administered intraperitoneally at a dose of 30 mg/kg bw. At 58 days of age, the animals were randomized into four groups to be fed *ad libitum* diets that contained ethanol at 0% (32 rats), 15% (30 rats), 20% (30 rats) or 30% (30 rats) of calories. A fifth group of 32 rats was pair-fed the 0% ethanol diet according to the average daily intakes of the rats fed the diet that contained 30% ethanol. At necropsy, tumours were removed and examined histopathologically. No significant difference in trends was observed for the incidence of cumulative, palpable mammary tumours between the 0 and 15% ethanol-treated groups nor between the group that underwent caloric restriction and the 20 or 30% ethanol-treated groups. The average number of palpable tumours and adenocarcinomas per rat increased significantly in animals in the 15% ethanol-treated group compared with those in the 0% ethanol-treated group (3.2 ± 0.4 versus 2.0 ± 0.3 palpable tumours/rat; 4.4 ± 0.5 versus 2.3 ± 0.4 adenocarcinomas/rat). The number of adenocarcinomas per rat was also significantly increased in animals fed the 20% ethanol diet compared with the calorie-restricted controls (3.0 ± 0.5 versus 1.8 ± 0.3). No significant difference between the calorie-restricted and 30% ethanol-treated groups was observed with regard to palpable tumours and adenocarcinomas (Singletary *et al.*, 1995).

3.2.17 N'-*Nitrosobis(2-oxopropyl)amine*

A group of 40 male weanling Syrian golden hamsters [age not specified] received either a high-fat diet (25% corn oil) or a high-fat diet plus ethanol, the concentration of which was gradually increased starting at day 25 from 5% during the first 2 weeks to a final concentration of 10% within 6 weeks. The hamsters received two subcutaneous injections of 20 mg/kg bw *N*-nitrosobis(2-oxopropyl)amine at 6 and 7 weeks of age and were killed 372 and 373 days after the second injection. Ethanol had no effect on the incidence of pancreatic adenomas or carcinomas (Woutersen *et al.*, 1989)

3.2.18 N-*Nitrosodiethylamine (NDEA)*

(a) Mouse

Groups of male strain A/JNCr mice [initial number unspecified], 4 weeks of age, were administered 6.8 ppm NDEA in sterilized distilled drinking-water with or without 10% ethanol for 4 weeks and were held without further treatment for 32 weeks. Complete necropsy was performed and tumours were examined histologically. Treatment with 6.8 ppm NDEA resulted in an 84% (42/50) incidence of lung tumours. When 10% ethanol was included with the NDEA, 100% (50/50) of the mice developed tumours and the multiplicity of lung tumours was increased (5.8 ± 2.2 versus 1.5 ± 1.2; $P < 0.01$). Ethanol also strongly potentiated the tumorigenic effect of NDEA in the forestomach from 2% (1/50) in NDEA-treated animals (one carcinoma) to 32% (16/50) in NDEA plus ethanol-treated animals (16 forestomach tumours including 14 carcinomas) (Anderson *et al.*, 1993).

(b) Rat

The enhancing effect of ethanol on oesophageal tumour development in rats following initiation with NDEA was evaluated. Groups of 30 and 28 male Fischer 344 rats, 6 weeks of age, were administered 50 ppm NDEA (purity, > 99%) dissolved in 10% ethanol (purity, > 99%) solution and 50 ppm NDEA solution in distilled water, respectively, for 8 weeks and were maintained thereafter on tap-water and basal diet for 96 weeks, at which time all rats were killed. The total intake of NDEA in the group given NDEA plus water was 134% that of the group given NDEA dissolved in water that contained ethanol. The numbers of nodules and masses in the oesophagus were counted, and all gross lesions were examined histopathologically. The effective numbers of rats were 26 and 28, respectively, and the number of survivors after 104 weeks was four and 10, respectively. The first animal with an oesophageal tumour died in the group administered 50 ppm NDEA in water that contained ethanol at week 43. The incidence of papillomas and carcinomas in the group given NDEA in water that contained ethanol were 38% (10/26) and 30% (8/26), respectively, compared with 7% (2/28) and 3% (1/28), respectively, in the group that received NDEA alone ($P < 0.01$) (Aze *et al.*, 1993).

3.2.19 N-*Nitrosodimethylamine (NDMA)*

Mouse

Groups of 50 male A/JNCr mice, 4 weeks of age, received 0.5, 1 or 5 ppm NDMA in sterile distilled drinking-water with or without 10 or 20% ethanol for 16 weeks. When the animals were killed, the lungs were removed and examined for primary lung tumours. Questionable lesions were subjected to histopathology (see Table 3.5). Mice treated with 0.5, 1 or 5 ppm NDMA and 10% ethanol had an increased incidence

of lung tumours and/or average number of lung tumours per mouse compared with those that received only 0.5, 1 or 5 ppm NDMA. Mice treated with 5 ppm NDMA and 20% ethanol had an increased average number of lung tumours per mouse compared with those that received 5 ppm NDMA only; this increase was not observed in mice treated with 0.5 ppm NDMA and 20% ethanol compared with mice that received only 0.5 ppm NDMA. In an additional experiment, mice were treated with 5 ppm NDMA with or without 10% ethanol for 4 weeks and then kept for an additional 12 weeks. Another group received 5 ppm NDMA for 4 weeks and then 10% ethanol for 12 weeks. Mice treated simultaneously with 5 ppm NDMA and 10% ethanol for 4 weeks had an increased incidence of lung tumours and average number of lung tumours per mouse compared with mice that received 5 ppm NDMA only. Treatment with 10% ethanol after administration of the 5 ppm NDMA did not affect the tumour incidence or multiplicity (Anderson, 1988).

Groups of 25 and 50 male Strain A/JNCr mice, 4–6 weeks of age, received 0 and 5 ppm NDMA [purity unspecified] in sterilized distilled drinking-water, respectively, with or without 10% reagent-grade ethanol for 4 weeks and were then held for an additional 12 weeks before being killed (experiment 1). Further groups of 50 males received 0 or 1 ppm NDMA with or without 10% ethanol in the drinking-water for 16, 32, 48 or 72 weeks after which time they were killed (experiment 2). Groups of 30 males received a single intragastric dose of 1 or 5 mg/kg bw NDMA and 0, 5, 10 or 20% ethanol in the drinking-water and were killed after 16 weeks (experiment 3); and groups of 25 males received doses of 1 mg/kg bw NDMA five times a week for 4 weeks by intragastric, intraperitoneal, subcutaneous or intravenous administration, with or without 0 or 10% ethanol in the drinking-water, and were killed 32 weeks after the last treatment (experiment 4). Complete necropsies were performed on all animals. In experiment 1, in mice exposed to 5 ppm NDMA in the drinking-water, inclusion of 10% ethanol almost doubled the incidence of tumour-bearing mice and increased average multiplicity fourfold. A similar enhancement was obtained with 1 and 5% ethanol, with no significant difference in numbers of tumours among the NDMA–ethanol-treated groups (Table 3.6). In experiment 2, in mice exposed to 1 ppm NDMA for up to 72 weeks, the inclusion of 10% ethanol increased the incidence of lung tumours after 48 weeks of exposure and increased lung tumour multiplicity at 72 weeks of exposure (Table 3.7). The incidence of kidney tumours was increased after 72 weeks of exposure. In experiment 3, a single intragastric dose of 5 mg/kg NDMA co-administered with 5, 10 or 20% ethanol resulted in a significant increase in tumour incidence and multiplicity compared with administration of NDMA without ethanol. This was not observed with doses of 1 mg/kg NDMA (Table 3.8). In experiment 4, when 10% ethanol was included in the drinking-water, no effect on the incidence or multiplicity of lung tumours was observed, regardless of the route of administration (Anderson *et al.*, 1992).

Table 3.5 Lung tumour incidence in male A/JNCr mice treated with *N*-nitrosodimethylamine (NDMA) with or without ethanol

NDMA (ppm)	Ethanol (%)	Treatment period (weeks)	Lung tumour incidence	Tumours/mouse (SD)
0.5	0	1–16	3/50 (6%)	0.06 (0.24)
0.5	10	1–16	9/50 (18%)	0.22 (0.51)*
0.5	0	1–16	4/50 (8%)	0.08 (0.27)
0.5	20	1–16	8/50 (16%)	0.16 (0.37)
1	0	1–16	9/50 (18%)	0.18 (0.39)
1	10	1–16	14/50 (28%)	0.44 (0.90)*
5	0	1–16	32/39 (82%)	2.1 (1.0)
5	10	1–16	21/22 (95%)*	4.2 (2.9)*
5	0	1–16	31/48 (65%)	1.5 (1.7)
5	10	1–16	50/50 (100%)*	5.4 (3.4)*
5	20	1–16	43/45 (86%)	3.2 (3.6)*
5	0	1–4 (NDMA) 5–16 (nothing)	19/50 (38%)	0.6 (0.9)
5	10	1–4 (NDMA + ethanol) 5–16 (nothing)	47/50 (94%)*	3.6 (2.5)*
5	10	1–4 (NDMA) 5–16 (ethanol)	26/50 (52%)	0.8 (0.9)

From Anderson (1988) SD, standard deviation *Significantly different ($p<0.05$) from groups that did not receive ethanol.

3.2.20 N-*Nitrosomethylamylamine*

Rat

To evaluate the effect of ethanol on *N*-nitrosomethylamylamine-induced oesophageal carcinogenesis, groups of 25 and 40 male MRC Wistar rats were given intraperitoneal injections of 25 mg/kg bw *N*-nitrosomethylamylamine in 5 mL distilled water once a week at 7, 8 and 9 weeks of age and received either drinking-water (controls) or 20% ethanol (21% of 95% ethanol) in distilled water containing 2 g/L catechol from 6 weeks of age continuously for 2 weeks. The ethanol content was then reduced to 10% because liquid consumption had decreased by about 25%. All rats were maintained on these treatments until they died or appeared ill. Full necropsies were performed and all oesophagi (which were slit) and tissues with apparent tumours were sectioned and examined histologically. In the oesophagus, *N*-nitrosomethylamylamine alone induced

Table 3.6 Enhancement of lung tumorigenesis by 5 ppm *N*-nitrosodimethylamine (NDMA) at different concentrations of ethanol in the drinking-water

Ethanol concentration in water	No. with tumour/total (%)	Average no. of tumours per mouse at risk±SD
0	27/50 (54%)	1.0±1.4
1%	47/49 (96%)[a]	4.3[a]±3.2
5%	46/48 (96%)[a]	5.4[a]±4.0
10%	49/50 (98%)[a]	4.1[a]±2.8
No NDMA		
0	2/25 (8%)	0.1±0.3
10%	4/25 (16%)	0.2±0.4

From Anderson *et al*. (1992) SD, standard deviation Water consumption values are the average for the last week of the 4-week treatment period. [a] Difference statistically significant compared with controls, $p < 0.05$

papillomas in 69% (27/39) of the rats and squamous-cell carcinomas in 18% (7/39) of the rats. In rats administered ethanol, the incidence of oesophageal papilloma and carcinoma was 75% (18/24) and 29% (7/24), respectively. The tumour incidences were not significantly different (Mirvish *et al.*, 1994).

3.2.21 N-*Nitrosomethylbenzylamine (NMBzA)*

(a) Mouse

Groups of 15 or 17 female C57BL/6 mice, 4–6 weeks of age, were fed a control diet or a diet that contained ethanol and were administered 0.2 mg/kg bw NMB_zA orally in a corn oil vehicle three times a week for 3 weeks (total dose, 1.8 mg/kg bw). Following oesophageal tumour induction by NMB_zA, the ethanol-fed mice received a diet in which ethanol was isocalorically substituted for maltose dextrin to provide 30% of the total dietary calories. The experiment was terminated 22 weeks after the end of the NMB_zA treatment. The incidence of oesophageal tumours was 6/15 (40%) in the NMB_zA-treated group compared with 59% (10/17) in the NMB_zA plus ethanol-treated group. The mean multiplicity was 8.2 [± 2.5, estimated from a figure] compared with 14.3 [± 2.8, estimated from a figure]. [The Working Group found that this increase in multiplicity was statistically significant, Student's-*t*-test; $P < 0.001$] (Eskelson *et al.*, 1993).

(b) Rat

The effect of chronic dietary ethanol consumption on the initiation and promotion of chemically induced carcinogenesis was evaluated in male Sprague-Dawley weanling rats [initial number and age unspecified], weighing 70–120 g, that received thrice-weekly intraperitoneal injections of 2.5 mg/kg bw NMB_zA for 3 weeks. To study the effect of ethanol on tumour promotion, an ethanol (7% content) or carbohydrate control

Table 3.7 Tumorigenesis by 1 ppm *N*-nitrosodimethylamine (NDMA) in drinking-water with or without 10% ethanol at increasing time intervals

Exposure time and treatment	Lung tumour-bearing mice (no./total; average no.±SD)	Kidney tumours	Other tumours	Average terminal body weight (g±SD)
16 weeks				
NDMA	14/50 (28%); 0.3±0.6	0	0	35.9±4.6
NDMA + ethanol	22/50 (44%); 0.5±0.5	0	0	34.3±5.0
32 weeks				
NDMA	24/50 (48%); 0.7±0.9	0	0	37.8±6.9
NDMA + ethanol	30/50 (60%); 1.0±1.1	0	0	38.0±6.9
48 weeks				
NDMA	32/48 (67%)[a]; 1.6±1.7	0	0	35.2±6.6
NDMA + ethanol	45/49 (92%)[a]; 2.2±1.5	0	1 lymphocytic lymphoma	42.2±5.9
72 weeks				
NDMA (69±8 weeks)	42/48 (88%); 2.4[a]±1.9	1[b]	1 mammary CA, 1 FCC lymphoma	37.6±5.6
NDMA + ethanol (70±6 weeks)	48/49 (98%); 3.4[a]±1.8	7[b]	4 haemangiomas, 1 haemangiosarcoma (liver), 2 lymphomas (1 FCC, 1 myelogenous), 1 adrenal pheochromocytoma, 1 hepatocellular CA, 1 sarcoma (bladder)	35.3±8.3

From Anderson *et al*. (1992) CA, carcinoma; FCC, follicular centre cell; SD, standard deviation Average water consumption did not vary between groups or over time and averaged 4.1 (± 0.7) mL/mouse/day. [a] $p<0.05$ or better [b] $p=0.032$, one-tailed Fisher exact test

diet was administered 1 week following the NMB_zA treatment and continued until termination of the experiment at 20 months of age, by which time the animals had received ethanol for a total of 17 months. To study the effect of ethanol on initiation, the rats were given ethanol or control diet for 12 weeks, and the NMB_zA treatment was given during the last 3 weeks. The ethanol content of the diet was then gradually reduced over 1 week, and the animals were fed regular chow diet thereafter until termination of the experiment at 20 months of age. These rats had received ethanol before and during initiation; their oesophagi were excised and examined for the incidence of nodules. Lesions that exhibited a three-dimensional structure with a height of at least 1 mm were designated as tumours. When ethanol was administered after treatment with NMB_zA, the mean frequency and size of oesophageal tumours decreased; however, the

Table 3.8 Effects of co-administration of ethanol on lung tumorigenesis induced by a single intragastric dose of *N*-nitrosodimethylamine (NDMA)

Treatment	No. of mice with tumour/ total	Average no. of tumours per mouse at risk±SD
NDMA, 1 mg/kg		
No ethanol	7/30 (23.3%)	0.30±0.59
+ 5% ethanol	6/30 (20%)	0.20±0.40
+ 10% ethanol	6/30 (20%)	0.30±0.69
+ 20% ethanol	9/29 (31%)	0.37±0.66
NDMA, 5 mg/kg		
No ethanol	15/30 (50%)[a]	0.93[a]±1.40
+ 5% ethanol	27/30 (90%)[a]	1.80[a]±1.40
+ 10% ethanol	30/30 (100%)[a]	4.27[a]±2.00
+ 20% ethanol	30/30 (100%)[a]	7.10[a]±4.10

From Anderson *et al*. (1992) SD, standard deviation [a] Values statistically different, $p<0.05$ or better

incidence increased. There was only one small tumour among 32 of the control animals; 18.7% (14/75) of animals that received ethanol had tumours ($P < 0.05$) and two of these animals had multiple (two and four) tumours. Treatment with ethanol before and during initiation significantly reduced the incidence of oesophageal tumours: 38% (10/26) of control rats but only 23% (3/13) of ethanol-treated rats had such tumours ($P < 0.01$; reduction). [The Working Group did not confirm the significance of this reduction.] The oesophageal tumours were predominantly papillomas (Mufti *et al*., 1989). [The Working Group noted that, in the experiment on initiation, ethanol was given for 12 weeks and, in the experiment on promotion, it was given for 17 months.]

As part of a study to investigate the effect of zinc deficiency on oesophageal carcinogenesis, groups of 39 and 35 male Sprague-Dawley rats [age not specified] were given control drinking-water and drinking-water that contained 10% ethanol [purity not specified], respectively, for 2 weeks and were then dosed with 2.5 mg/kg bw NMB_zA [purity not specified] twice a week for 3 weeks [vehicle and route of administration not specified]. After 14 weeks, the weight of rats given control-drinking-water was 378 ± 16 g compared with 268 ± 28 g for rats given the drinking-water that contained 10% ethanol. The animals were observed for 20 or more weeks [exact time not specified], at which time the extent of oesophageal tumorigenesis was assessed macroscopically and microscopically. The incidence oesophageal tumours was 37% (13/35) in rats administered control drinking-water compared with 33% (13/39) in rats given 10% ethanol in the drinking-water, a difference that was not statistically significant (Newberne *et al*., 1997).

Three groups of 15 male Fischer 344/DuCrj rats, 6 weeks of age, received thrice-weekly subcutaneous injections of 500 μg/kg bw NMB_zA (purity, > 99%) in 20% DMSO [volume not specified] for 5 weeks. Two additional groups of 10 rats each were

similarly injected with 20% DMSO. After receiving the last injection of NMB_zA, two of the groups were given 3.3 and 10% ethanol (purity, > 98%) in the drinking-water; the other group continued to receive control drinking-water. After the last injection of 20% DMSO, one of the groups was given 10% ethanol in the drinking-water, while the other group continued to receive control drinking-water. The experiment was terminated 15 weeks after the rats were placed on drinking-water solutions that contained ethanol. Oesophageal tumours were examined macroscopically and microscopically, and were only present in rats administered NMB_zA. In rats that received NMB_zA only, the incidence and multiplicity (± SD tumours/rat) of oesophageal tumours were 47% (7/15) and 0.8 ± 1.1. The corresponding values for rats that received NMB_zA and 3.3% ethanol were 33% (4/12) and 0.9 ± 1.6 and those for rats that received NMB_zA and 10% ethanol were 46% (6/13) and 0.8 ± 1.0. All of the tumours were characterized as squamous-cell papillomas, with the exception of a single squamous-cell carcinoma that was detected in the NMB_zA and 10% ethanol-treated group. Neither the incidence nor the multiplicity of oesophageal tumours differed among any of the groups that had been treated with NMB_zA (Morimura *et al.*, 2001).

Groups of 15 male Fischer 344/DuCrj rats, 6 weeks of age, received thrice-weekly subcutaneous injections of 100 or 500 μg/kg bw NMB_zA (purity, > 98%) [injection volume and solvent not specified] for 5 weeks and were also given control drinking-water for 24 weeks, 10% ethanol (purity, > 99%) in the drinking-water for 5 weeks and then control drinking-water for 19 weeks or 10% ethanol in the drinking-water for 24 weeks. The experiment was terminated 24 weeks after the first injection of NMB_zA, at which time the extent of papillary oesophageal tumorigenesis was assessed macroscopically and microscopically. Rats that received 10% ethanol in the drinking-water for 24 weeks weighed significantly less than those that received control drinking-water or 10% ethanol in the drinking-water for 5 weeks. No oesophageal tumours were observed in rats treated with 100 μg/kg bw NMB_zA and either control drinking-water or drinking-water that contained ethanol. In rats that received 500 μg/kg bw NMB_zA, the incidence and multiplicity (± SD tumours/rat) of oesophageal tumours, respectively, were 13% (2/15) and 0.1 ± 0.4 in those given control drinking-water, 33% (5/15) and 0.4 ± 0.6 in those given 10% ethanol in the drinking-water for 5 weeks and 46% (7/15) and 0.6 ± 0.6 in those given 10% ethanol in the drinking-water for 24 weeks. Neither the tumour incidence nor tumour multiplicity differed significantly among these groups (Kaneko *et al.*, 2002).

Two groups of 10 male albino Wistar rats [age not specified], weighing 156 ± 15 g, were either fed a liquid diet that contained ethanol (5% ethanol (v/v) high-grade absolute, 36% of total calories) or pair-fed a diet in which the ethanol was replaced isocalorically with glucose. Eight weeks after being placed on the diets, each of the rats received twice-weekly intraperitoneal injections of 100 μg/kg bw NMB_zA [purity not specified] for 10 consecutive weeks. The liquid diets were removed 1 h before the injections, and blood was collected for analysis of ethanol; none was detected [limit of detection not specified]. The liquid diets were replaced 5 hours after the injections. The experiment

was terminated after 30 weeks and oesophageal tumours were assessed macroscopically and microscopically. The average intake for both groups was 80 mL/day (4.0 mL ethanol/day for the ethanol group). Body weights did not differ significantly between the groups. In NMB_zA-treated rats administered the ethanol diet, the oesophageal tumour incidence was 100% (10/10), the mean size of oesophageal tumours was 7.3 ± 3.6 mm, the mean number of oesophageal tumours per rat was 6.1 ± 1.0 and the incidence of squamous-cell carcinoma of the oesophagus was 50% (5/10). In NMB_zA-treated rats administered the pair-fed control diet, the oesophageal tumour incidence was 5/10 (50%), the mean size of oesophageal tumours was 5.0 ± 0.7 mm, the mean number of oesophageal tumours per rat was 0.5 ± 0.5 and the incidence of squamous-cell carcinoma of the oesophagus was 0/10. Each of these parameters was significantly increased in the ethanol-fed group compared with the pair-fed control rats (Tsutsumi *et al.*, 2006).

3.2.22 N-*Nitrosonornicotine (NNN)*

Rat

Male Fischer 344 rats [initial number unspecified], 4–6 weeks of age, were treated by gavage with NNN at a total dose of 40 mmol/kg three times a week for 4 weeks. One week after initiation, the animals received liquid diets that contained 36% of total calories either as ethanol or isocalorically as carbohydrates for 55 weeks. Ethanol increased the incidence of tumours initiated by NNN in the oesophagus (79%, 40/52), oral cavity (29%, 15/52) and lungs (15%, 8/52) ($P < 0.05$) compared with the control-fed rats (35%, 14/40), (17%, 7/40), (5%, 2/40) respectively) and caused an increase in the mean frequency and size of the tumours ($P < 0.001$) (Nachiappan *et al.*, 1994).

3.2.23 *NNN in combination with* N-*nitrosodiethylamine (NDEA)*

Mouse

Four groups of 48 female mice (*Mus musculus*), 3 months of age, received either water on days 1–3 and then 0.04 ml/L NDEA in the drinking-water on days 4–7, 30 mg/L NNN on days 1–3 followed by NDEA on days 4–7, 6% ethanol followed by NDEA or 6% ethanol plus NNN followed by NDEA. A control group of 16 mice received water only for 7 days. The experiment was terminated after 180 days. The incidence of invasive carcinoma of the oesophagus was 0% (control), 64%, 58%, 69% and 65% in the different groups, respectively, which was not significant (Gurski *et al.*, 1999).

3.2.24 N-*Nitrosopyrrolidine (NPYR)*

Mouse

Groups of male strain A/JNCr mice [initial number unspecified], 4 weeks of age, were administered 6.8 or 40 ppm NPYR in sterilized distilled drinking-water with or without 10% ethanol for 4 weeks. The mice were held without further treatment for 32 weeks. Complete necropsy was performed and tumours were examined histologically. NPYR alone did not cause a significant number of tumours at either dose. The inclusion of 10% ethanol with the 6.8 ppm dose increased the incidence of lung tumours from 41 (20/49) to 67% (33/49) and average multiplicity from 0.5 ± 0.8 to 1.2 ± 1.2 tumours/mouse (the differences were statistically significant). With the 40-ppm NPYR dose, inclusion of ethanol resulted in 98% (47/48) of the mice with lung tumours and a 5.5-fold increase in multiplicity (3.3 ± 1.7) compared with NPYR alone (0.6 ± 0.8; $P < 0.01$) (Anderson *et al.*, 1993).

3.2.25 N-*Nitrososarcosin ethyl ester*

One hundred and forty male white rats [age unspecified], average weight of 100 g, were divided into eight groups. Rats received an intraoesophageal dose of 50 mg/kg bw *N*-nitrososarcosin ethyl ester five times a week for 4 months. Some groups received in addition 0.5 mL 40% ethanol intraoesophageally three times a week for 8 months. Ethanol was given 5–10 minute after the carcinogen. Ethanol had no effect on the incidence or multiplicity of tumours in the oesophagus or forestomach (Alexandrov *et al.*, 1989).

3.3 Acetaldehyde

Previous studies

Acetaldehyde was considered by two previous Working Groups in June 1984 (IARC, 1985) and February 1998 (IARC, 1999).

The 1984 Working Group evaluated bioassays in which rats and hamsters had been exposed to acetaldehyde by inhalation and intratracheal instillation. Rats exposed by inhalation showed an increased incidence of adenocarcinomas and squamous-cell carcinomas of the nasal mucosa. Hamsters exposed by inhalation had an increased incidence of laryngeal carcinomas; however, in another inhalation study in hamsters with a lower level of acetaldehyde, an increase in tumours was not observed. Exposure of hamsters to acetaldehyde by inhalation enhanced the incidence of respiratory tract tumours induced by intratracheal instillation of benzo[*a*]pyrene. Intratracheal instillation of acetaldehyde into hamsters did not result in an increased tumour incidence. A study that involved subcutaneous administration of acetaldehyde to rats was judged to be inadequate for evaluation. From these data, the Working Group concluded that

there was *sufficient evidence* for the carcinogenicity of acetaldehyde to experimental animals (see IARC 1985 for details and references).

The 1998 Working Group evaluated one bioassay in which rats were exposed to acetaldehyde by inhalation. A preliminary report of this bioassay had been considered by the 1984 Working Group. Exposure to acetaldehyde vapour increased the incidence of respiratory tract tumours, particularly nasal adenocarcinomas and squamous-cell carcinomas. From these data and those considered by the previous Working Group, the 1998 Working Group concluded that there was *sufficient evidence* for the carcinogenicity of acetaldehyde to experimental animals (see IARC 1999 for details and references).

3.3.1 *Oral administration*

Rat

Groups of 50 male and 50 female Sprague-Dawley rats, 6 weeks of age, were exposed to 0, 50, 250, 500, 1500 or 2500 mg/L acetaldehyde (purity, > 99.0%) in the drinking-water for 104 weeks. The experiment was terminated when the last animal died at 161 weeks of age. The administration of acetaldehyde in the drinking-water did not affect water or food consumption, body weight or survival. Complete histopathology was performed on all animals. The incidence of malignant mammary tumours (adenocarcinomas) was 6% (3/50), 18% (9/50), 6% (3/50), 20% (10/50) [$P = 0.0357$ compared with controls; one-tailed Fisher's exact test], 16% (8/50) and 12% (6/50) in female rats administered 0, 50, 250, 500, 1500 and 2500 mg/L acetaldehyde, respectively. Slight treatment-related increases were observed in the incidence of Zymbal gland carcinomas, ear duct carcinomas and oral cavity carcinomas in both sexes [not statistically significant]. Nasal cavity carcinomas (4%, 2/50) were only observed in male rats administered 2500 mg/L acetaldehyde. Sporadic incidences of lung adenomas and adenocarcinomas, forestomach acanthomas and squamous-cell carcinomas and intestinal fibromas and adenocarcinomas were observed in male and/or female rats administered acetaldehyde [no statistically significant difference]. Testicular interstitial-cell tumours were observed in all groups [not statistically significant]. The incidence of uterine adenocarcinomas was increased in rats administered 250 mg/L acetaldehyde (10% (5/50) versus 0/50 controls) [$P = 0.0281$; one-tailed Fisher's exact test]. The incidence of cranial osteosarcomas was increased in male rats administered 50 mg/L (10% (5/50) versus 0/50 controls) [$P = 0.0281$; one-tailed Fisher's exact test] and 2500 mg/L acetaldehyde (14% (7/50) versus 0/50 controls) [$P = 0.0062$; one-tailed Fisher's exact test]. Lymphomas and leukaemias combined were observed in all groups; compared with the controls (12% (6/50) males and 4% (2/50) females), the incidences were increased in male rats administered 50 mg/L (28%, 14/50) [$P = 0.0392$; one-tailed Fisher's exact test] and 1500 mg/L acetaldehyde (30%, 15/50) [$P = 0.0239$; one-tailed Fisher's exact test] and in female rats administered 500 mg/L acetaldehyde (8/50) [$P = 0.0458$; one-tailed Fisher's exact test] (Soffritti *et al.*, 2002b). [The Working Group noted that a variety of tumours were observed in male and female rats administered acetaldehyde in the

drinking-water. In some instances, the incidence in the treated groups was significantly greater than that in the respective control groups; nevertheless, these increases may be due to chance because no obvious dose–response relationship was observed in any of the tissues. The Working Group expressed concerns whether the doses were accurate due to the volatility of acetaldehyde.]

3.3.2 *Administration with a known carcinogen*

Rat

Groups of 18–20 male Fischer 344 rats, 6 weeks of age, were given a single intraperitoneal injection of 200 mg/kg bw NDEA [purity not specified] dissolved in 0.9% saline [volume not specified]. Two weeks later, the rats were administered 0, 2.5 or 5% acetaldehyde [purity not specified] in the drinking-water for 6 weeks. One week after being transferred to drinking-water that contained acetaldehyde, all rats were subjected to a two-thirds partial hepatectomy. One additional group was injected intraperitoneally with 0.9% saline instead of NDEA in 0.9% saline. Two weeks after the injection of saline, this group was placed on 5% acetaldehyde in the drinking-water; the group was also subjected to a partial hepatectomy. The experiment was terminated 8 weeks after the initial intraperitoneal injection and liver sections were prepared for immunohistochemical examination of glutathione *S*-transferase (GST) (placental type)-positive foci, a short-term marker for liver carcinogenesis. Rats injected with NDEA and exposed to 5% acetaldehyde consumed more drinking-water than those exposed to 2.5% acetaldehyde [$P < 0.001$; Student's *t*-test]. The administration of NDEA did not affect water consumption in rats given 5% acetaldehyde. Body weights, absolute liver weights and relative liver weights were significantly decreased ($P < 0.05$; Student's *t*-test) in rats given NDEA and 2.5 or 5% acetaldehyde compared with those given NDEA only; the effect was greater with 5% acetaldehyde. Body weights and absolute liver weights were significantly decreased [$P \leq 0.007$; Student's *t*-test] in rats given NDEA in 0.9% saline and 5% acetaldehyde compared with those given 0.9% saline and 5% acetaldehyde. GST (placental type)-positive foci were not detected in rats injected with 0.9% saline and given 5% acetaldehyde in the drinking-water but were observed in rats injected with NDEA; however, the number/cm^2, total area and mean diameter of the foci were not affected by the administration of either 2.5 or 5% acetaldehyde (Ikawa *et al.*, 1986) (Table 3.9).

A total of 250 Sprague-Dawley rats, 1 day of age, were given a single intraperitoneal injection of 15 mg/kg bw NDEA [purity not specified] in 100 µL normal saline. At 3 weeks of age, a subgroup of the rats (females only [number not specified]) was given 5% acetaldehyde [purity not specified] in the drinking-water for 9 weeks, an additional subgroup (females only [number not specified]) was given twice weekly injections of a 250-µL solution of 33% carbon tetrachloride [purity not specified] in mineral oil and 5% acetaldehyde in the drinking-water; and a further subgroup (females only [number not specified]) was given twice weekly injections of a 250-µL solution of 33% carbon

Table 3.9 Quantitative values of glutathione *S*-transferase (GST) (placental type)-positive foci in liver of male Fischer 344 rats treated with combinations of *N*-nitrosodiethylamine (NDEA) and acetaldehyde

NDEA (mg/kg bw)	Acetaldehyde (%)	GST-positive focal lesion		
		No./ cm^2	Total area (mm^2/ cm^2)	Mean diameter of focus (mm)
200	5	9.6±2.9	0.45±0.22	0.24±0.03
200	2.5	10.9±3.0	0.55±0.18	0.25±0.02
0	5	0	0	0

From Ikawa *et al*. (1986)

tetrachloride in mineral oil and control drinking-water. An additional group of 10 rats received a single intraperitoneal injection of 100 μL normal saline at 1 day of age. This group and a subgroup [number not specified] of the NDEA-treated animals were given control drinking-water only. The experiment was terminated when the rats were 12 weeks of age. Liver sections were prepared for examination by haematoxylin/eosin staining and by immunohistochemistry for the presence of GST (placental type)-positive foci. Of the rats administered carbon tetrachloride and acetaldehyde, 27% died during the experiment. Rats that received NDEA and acetaldehyde or NDEA, acetaldehyde and carbon tetrachloride weighed significantly less than those that received NDEA and carbon tetrachloride, NDEA alone or the normal saline ($P < 0.001$; Student's *t*-test). Liver foci or nodules were not present in normal saline-treated rats. Liver foci were present in rats treated with NDEA (100%, 10/10) or with NDEA and acetaldehyde (90%, 18/20); the incidence did not differ between these groups [two-tailed Fisher's exact test]. Liver nodules were present in rats treated with NDEA and carbon tetrachloride (65%, 13/20) or with NDEA, carbon tetrachloride, and acetaldehyde (100%, 10/10); the incidence was significantly greater in the group treated with NDEA, carbon tetrachloride and acetaldehyde ($P < 0.05$; χ^2 test). [The Working Group felt it was inappropriate to use a χ^2 test in this situation; a two-tailed Fisher's exact test indicated $P = 0.064$]. The extent of GST (placental type)-positive foci and/or nodules, as measured by number/cm^2 or area/cm^2, did not differ between rats treated with NDEA or with NDEA and acetaldehyde or between rats treated with NDEA and carbon tetrachloride or with NDEA, carbon tetrachloride and acetaldehyde. These data indicate that acetaldehyde does not potentiate the hepatocarcinogenic response induced by NDEA or by NDEA and carbon tetrachloride (Cho & Jang, 1993; Table 3.10).

Table 3.10 Glutathione *S*-transferase (GST) (placental type)-positive foci and/or nodules in liver of female Sprague-Dawley rats treated with combinations of *N*-nitrosodiethylamine (NDEA), acetaldehyde and carbon tetrachloride

Treatment group (no. of animals)	Foci (%)	Nodules (%)
Untreated (10)	0 (0)	0 (0)
NDEA (10)	10 (100)	0 (0)
NDEA/acetaldehyde (20)	18 (90)	0 (0)
NDEA/acetaldehyde/carbon tetrachloride (10)	3 (30)	10 (100)

From Cho & Jang (1993)

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