4. BIOLOGICAL DATA RELEVANT TO THE EVALUATION OF CARCINOGENIC RISK TO HUMANS

Imbalances in caloric and micronutrient intake are known to influence the incidence of spontaneous and experimentally induced tumours and most aspects of reproduction. In experiments evaluated in this section on the carcinogenicity and reproductive effects of ethanol in animals, including the modifying effects of ethanol, the controls and treated groups were not always maintained on isocaloric diets and no experiment involved iso-nutrient diets.

Some of the minor components of alcoholic beverages have carcinogenic, mutagenic and teratogenic activity; however, only studies referring to ethanol and alcoholic beverages are covered in this monograph.

Unless otherwise mentioned, the purity of the ethanol used in the carcinogenicity experiments described below was not specified. Similarly, when ethanol was given as a percentage in drinking-water, unless mentioned, it was not specified whether the percentage was calculated on a weight or volume basis.

4.1 Carcinogenicity studies in animals

(*a*) *Ethanol and alcoholic beverages*

The Working Group was aware of some early studies in which ethanol was administered to mice (Krebs, 1928; Ketcham *et al.*, 1963; Horie *et al.*, 1965) and to hamsters (Elzay, 1966; Henefer, 1966; Elzay, 1969; Freedman & Schklar, 1978) using various protocols, but these were found to be inadequate for evaluation.

(i) *Oral administration*

Mouse: A group of 108 male and 42 female CF1 mice, 75-120 days old, was given 43% ethanol in water as the drinking fluid intermittently (five days a week; Horie *et al.*, 1965) for periods of up to 1020 days. A group of 44 male CF1 mice, 65 days old, was given 14% ethanol similarly for up to 735 days. Two mice given 43% ethanol developed papillomas of the forestomach; a few other tumours, malignant lymphomas (four) and lung adenomas (three) were also found in the high-dose group. A further group of 100 male ddN mice, 130 days old, was given 19.5% as the drinking fluid intermittently for a maximum of 664 days; one mouse

developed a papilloma of the forestomach. Groups of 42-69 male or female CF1 mice, 65-120 days old, were given Japanese whisky, Scotch whisky or saké as the drinking fluid intermittently for periods of up to 978 days. Three mice treated with Japanese whisky developed malignant lymphomas; one mouse treated with Scotch whisky developed a forestomach papilloma; and three malignant lymphomas and one forestomach papilloma were observed in the group treated with saké. A further group of 100 male ddN mice, 130 days old, was given sherry as the drinking fluid for periods of up to 536 days; papillomas of the forestomach were found in three mice (Kuratsune *et al.*, 1971). [The Working Group noted the absence of untreated control groups and the inadequate reporting of the data, such as survival rates.]

A group of 15 female C3H/St mice, 20-35 days old, received 12% ethanol (v/v) in water as the drinking fluid for 80 weeks. A control group of 30 females was maintained on deionized water. Mammary tumours developed between six and 11 months of age in 8/11 (73%) mice in the ethanol-treated group and between 12 and 16 months of age in 22/27 (82%) control mice. The tumour incidence in ethanol-treated mice was not statistically different from that in controls, but the shorter median time to tumour appearance in the ethanol-treated group (eight months *versus* 14.2 months of age) was significant ($p < 0.001$; Schrauzer *et al.*, 1979). [The Working Group noted the small number of animals used and the absence of histopathological examination of the tumours.]

A group of 16 female C3H/St weanling mice received decarbonated light beer (6% ethanol v/v) as the drinking fluid for life after weaning. A control group of 16 mice was maintained on deionized water. The incidence of 'mammary adenocarcinomas' (58.3% and 64%) and tumour latency (16.8 and 14.5 months of age) were similar in the two groups. Both groups reached 50% survival at about 15.5 months (Schrauzer *et al.*, 1982). [The Working Group noted the small number of animals and absence of histopathological examination of the tumours.]

As part of a study on modifying effects (see pp. 105-106, 108), 36 male and 32 female C57Bl mice, eight weeks of age, were administered 0.2 ml 40% ethanol by intragastric intubation twice a week for 50 weeks (total dose, 20 ml). All 68 surviving mice were killed at 80 weeks of age, at which time no treatment-related tumour was observed (Griciute *et al.*, 1981, 1982, 1984). [The Working Group noted the absence of an untreated control group and the limited dose of ethanol administered.]

Three groups of 100 male C57Bl/10J mice, 14 weeks old, were housed individually and given 3.5-15% ethanol solution for five weeks and subsequently 3.5, 7.5 or 12% v/v ethanol in distilled water as the drinking fluid *ad libitum* for their lifespan. A control group of 100 male mice received distilled water *ad libitum*. All groups were treated for up to 160 weeks. No difference in survival was noted between the group fed 3.5% ethanol and the controls (mean survival, 742 days); the longest survival time was in the group given 7.5% ethanol (792 days), and the group receiving 12% ethanol had a mean survival time of 760 days. Increased incidences of liver sarcomas [probably lymphomas], but not of hepatocellular carcinomas, were observed in mice given 7.5 and 12% ethanol (13/87 and 10/72) compared with 4/79 in the controls and 6/77 in the group given 3.5% ethanol, but not all mice were necropsied (Schmidt *et al.*, 1987). [The Working Group noted that many mice in each group were

autolysed and could therefore not be used for pathological examination, and the inadequate reporting of the histopathological findings.]

Rat: In a study on modifying effects (see pp. 106-107), 40 albino (similar to BDII) rats [sex distribution unspecified], 10-12 weeks of age, were given a commercial brandy (38% ethanol) *ad libitum* as the drinking fluid. The rats failed to gain weight during the experiment and more than half had died by week 32. No liver tumour was reported in animals that had died by 56 weeks or in 20 controls given tap-water (Schmähl *et al.*, 1965). [The Working Group noted the short duration of the study and the short survival.]

As part of a study on modifying effects (see pp. 107, 109), groups of 40 Sprague-Dawley rats [sex distribution unspecified], three months old, were given 0.5 ml of 30% or 50% ethanol (v/v) daily by gavage for life. The average lifespan of rats treated with 30% ethanol was 500 days, and that of the group given 50% ethanol was 396 days. A group of ten rats given 1 ml saline orally served as controls. No oesophageal, stomach or hepatic tumour was found in any of these groups (Gibel, 1967). [The Working Group noted the limited extent of pathological examination.]

As part of a study on modifying effects (see p. 109), 48 Sprague-Dawley rats [sex distribution unspecified], three months old, were given 25% ethanol as the drinking fluid five times a week until their natural death. Another group of 48 rats was untreated and served as controls. Mean survival was 780 and 730 days in the two groups, respectively. No statistically significant increase in tumour incidence was observed (Schmähl, 1976). [The Working Group noted the inadequate reporting of the data.]

A group of 15 male Holtzman rats, weighing 80-100 g, was fed a synthetic diet containing ethanol (contributing 35% of total calories; mean ethanol consumption, 5 g/kg bw per day) for up to 370 days. A group of 15 males served as pair-fed controls. When animals were killed at 14 months, no liver tumour was found in either the ethanol-treated or control group (Mendenhall & Chedid, 1980). [The Working Group noted the short duration of the study, the small group size and that pathological examination was limited to the liver.]

Groups of 20-25 male and 20-25 female Wistar rats, six weeks of age, were given 15 or 55% ethanol, 15 or 55% farm apple brandy or 15 or 40% industrial apple brandy as the drinking fluid for up to 23 months. The higher concentrations were given on alternate days. Groups of 20 male and 20 female rats given water alone served as controls. After 23 months, survivors (80-93% of animals) were killed. No excess of tumours of any kind was found in any of the groups (Mandard *et al.*, 1981). [The Working Group noted that the daily intake of ethanol was lower at the higher concentrations.]

As part of a study on modifying effects (see p. 114), 80 male Sprague-Dawley rats [age unspecified] were given 5% ethanol (v/v) in water as the drinking fluid for up to 30 months. A group of 80 male rats given water alone served as controls. About 70% of animals in both groups were still alive at 18 months. Hepatocellular carcinomas were found in 8/79 ethanol-treated and in 1/80 control animals [$p = 0.016$]. Hyperplastic nodules [inadequately described] occurred in the livers of ten controls and 29 ethanol-treated animals. Endocrine tumours (benign and malignant) developed in 57/79 ethanol-treated animals (26 of the pituitary [$p = 0.0004$], 14 of the adrenal, 14 of the pancreas and three of the testis) and in

8/80 control animals (in the pituitary) (Radike *et al.*, 1981). [The Working Group noted that isocaloric and isonutrient intakes were not controlled in either treated or control groups.]

As part of a study on modifying effects (see pp. 109-110), groups of 26 male Fischer 344 rats, nine weeks old, were fed a liquid diet containing 6% ethanol (w/v; corresponding to 35% of the total caloric content) or control liquid diet for 26 weeks, after which they were returned to normal laboratory diet. All surviving rats were killed at 98 weeks of age. No statistically significant difference in tumour incidence was found between the treated group and controls (Castonguay *et al.*, 1984). [The Working Group noted the short period of exposure to ethanol.]

As part of a study on modifying effects (see p. 110), 25 male and 25 female young adult BDVI rats were given 40% ethanol [amount unspecified] by intragastric instillation twice a week for 78 weeks. A group of 25 males and 25 females served as untreated controls. Average lifespan was 98 weeks in male and 105 weeks in female treated animals and 107 and 113 weeks in controls; all surviving animals were killed at 120 weeks of age. No tumour that could be related to ethanol treatment was observed in these animals (Griciute *et al.*, 1986). [The Working Group noted that the dose of ethanol administered was not specified.]

As part of a study on modifying effects (see p. 112), groups of ten male Wistar rats [age unspecified] were given either 10% ethanol in water as the drinking fluid or water alone for up to 40 weeks, at which time they were killed. No tumour was found in either group (Takahashi *et al.*, 1986). [The Working Group noted the small sizes of the groups and the short duration of the experiment.]

Hamster: Groups of 58 male and six female or seven male and three female golden hamsters, 75 days old, were given sherry or 19.5% ethanol in water intermittently (on five days per week; Horie *et al.*, 1965) as the drinking fluid for up to 807 days. One hamster given sherry developed a papilloma of the forestomach (Kuratsune *et al.*, 1971). [The Working Group noted the absence of untreated control groups, the unusual group sizes and the inadequate reporting of the experiment.]

As part of a study on modifying effects (see pp. 110-111), 19 male outbred Syrian golden hamsters, nine weeks of age, were maintained on a liquid diet containing 6% (w/v) ethanol (35% of total caloric content) for a total of 29 weeks, with a four-week interruption after the first 20 weeks of the experiment. A group of 21 male hamsters served as controls. The animals were followed for up to a total of 19 months. No statistically significant difference in tumour incidence was found (McCoy *et al.*, 1981). [The Working Group noted the limited number of animals and the short duration of ethanol treatment.]

As part of a study on modifying effects (see p. 111), 20 male and 20 female outbred Syrian golden hamsters, eight weeks old, were given 5% (w/v) ethanol in water as the drinking fluid for up to 46 weeks, at which time all surviving animals were killed. No pancreatic tumour was observed (Pour *et al.*, 1983). [The Working Group noted the short duration of the experiment and that histological examination was limited to the pancreas, common duct and gall-bladder.]

As part of a study on modifying effects (see p. 111), groups of 27 male outbred Syrian golden hamsters, nine weeks old, were given 7.4% or 18.5% ethanol in water as the drinking

fluid for 29 weeks, after which they were given tap-water only. A group of 27 male hamsters given tap-water served as controls. All surviving animals were killed 18 months after the beginning of the experiment. No statistically significant difference in tumour incidence was found (McCoy *et al.*, 1986). [The Working Group noted the short duration of ethanol treatment.]

(ii) *Skin application*

Mouse: A group of 29 female and 36 male C57Bl mice, 95-125 days old, received skin applications of a distillation residue of a saké (33% residue in 50% ethanol) three times a week for up to 829 days. A control group of 31 females and 33 males was painted on the skin with 50% ethanol and observed for up to 830 days. A skin papilloma developed in one mouse in the control group, but in none of the saké-treated group. A further group of 58 male CF1 mice, about 100 days old, received skin applications of a distillation residue of a Japanese whisky (33% residue in 50% ethanol) three times a week for up to 814 days. A control group of 57 males received applications of 50% ethanol for up to 802 days. No skin tumour was found in either group (Kuratsune *et al.*, 1971). [The Working Group noted the limited reporting of the experiment, such as on survival rates.]

(iii) *Transplacental and neonatal administration*

Mouse: Two groups of female C3H mice [age not specified] were given 0.5 or 5% ethanol (v/v) as the drinking fluid during pregnancy and their male offspring were observed for 15 months. Two additional groups were given water during pregnancy and 0.5 or 5% ethanol for one week beginning when their pups were one week old; male offspring were followed for 15 months after weaning. In offspring exposed to ethanol during embryogenesis, liver tumours (diagnosed grossly and described as hepatomas) developed in 3/25 exposed to 0.5% and in 1/10 exposed to 5% ethanol. In those exposed to ethanol *via* the milk for one week, liver tumours developed in 5/31 exposed to 0.5% and 5/45 exposed to 5%. The incidence of hepatomas in pooled control males was 27/62. The lower incidence of hepatomas in male mice exposed to ethanol during embryogenesis or for one week during suckling was statistically significant ($p < 0.005$ for combined experiments; Kahn, 1968). [The Working Group noted the short duration of treatment, the absence of information on initial group sizes and the lack of histopathological examination of the liver tumours.]

(*b*) *Modifying effects of ethanol on the activity of known carcinogens*

(i) N-*Nitrosodimethylamine* (NDMA)

Groups of 31-32 female and 37-38 male C57Bl *mice*, eight weeks of age, received gastric intubations of 0.03 mg NDMA in 0.2 ml water (total dose, 3 mg) or 0.03 mg NDMA in 0.2 ml 40% ethanol (total doses, 3 mg NDMA and 20 ml 40% ethanol [6.4 g 100% ethanol]) twice a week for 50 weeks. The experiment was terminated 72 weeks after the start of treatment, at which time all survivors were sacrificed. At that time, 25/68 mice given NDMA and 3/70 given NDMA plus ethanol were still alive. In animals given NDMA plus ethanol, 12/36 males and 12/30 females developed aesthesioneuroepitheliomas (olfactory tumours that infiltrate the frontal lobe of the brain); no such tumour was observed in animals given

NDMA. No significant difference in the incidence of other tumours was observed (Griciute *et al.*, 1981).

A group of 50 hybrid CBA × C57Bl/6 female *mice*, weighing 10-12 g, received NDMA (10 mg/l) in the drinking-water; another group of 100 mice received NDMA (10 mg/l) in combination with ethanol (6000 mg/l) in water as the drinking fluid. At nine months, all survivors were killed. There was no significant difference in the incidences of liver, lung or kidney tumours between the two groups (Litvinov *et al.*, 1986a). [The Working Group noted the short duration of the study.]

Two groups of 17 female Sprague-Dawley *rats*, weighing 130 g, were pair-fed for three weeks on a nutritionally adequate liquid diet containing either ethanol (36% of total calories) or isocalorically substituted carbohydrates (control diet) and were then maintained on laboratory chow and tap-water *ad libitum* for two weeks, during the first week of which they each received five daily intraperitoneal injections of 1.5 mg NDMA. This five-week cycle was repeated four times (total dose of NDMA, 30 mg/animal), after which time the animals were fed laboratory chow and observed for life. Survival in the group receiving NDMA plus ethanol was significantly longer than in the group receiving NDMA alone. The total number of tumours, the histological type and the target organs (liver, kidney, lung and thyroid gland; subcutaneous sarcomas) were similar in the two groups (Teschke *et al.*, 1983). [The Working Group noted the limited number of animals used and the short duration of exposure to ethanol.]

(ii) N-*Nitrosodiethylamine* (NDEA)

Groups of 38 male and 32 female C57Bl *mice*, eight weeks of age, received gastric intubations of 0.03 mg/animal NDEA in 0.2 ml tap-water (total dose, 3 mg/animal) or 0.03 mg/animal NDEA in 0.2 ml 40% aqueous ethanol (total doses, 3 mg/animal NDEA and 20 ml 40% ethanol [6.4 g 100% ethanol]) twice a week for 50 weeks. Animals were held for a further 28 weeks, at which time all survivors were sacrificed. A higher incidence of lymphomas was observed in the group given NDEA (45/70) than in mice given NDEA plus ethanol (21/69). The incidence of malignant oesophageal/forestomach tumours (mainly spinocellular [squamous-cell] carcinomas) was higher in the NDEA plus ethanol group (13/38 males, 19/31 females) than in the NDEA group (4/38 males, 3/32 females; Griciute *et al.*, 1984).

Groups of 100 female hybrid CBA × C57Bl/6 *mice*, weighing 10-12 g, received NDEA (10 mg/l) or NDEA in combination with ethanol (6000 g/l) in the drinking-water for 12 months, at which time all survivors were killed. The incidence of pulmonary tumours (mainly adenomas) was 49/86 in the group that received NDEA plus ethanol as compared to 22/79 in mice treated with NDEA only [$p = 0.0002$]. No difference in the incidences of other tumours was observed (Litvinov *et al.*, 1986b).

Groups of albino (similar to BDII) *rats* [sex distribution unspecified], 10-12 weeks of age, received 3 mg/kg bw NDEA in the drinking-water daily (28 rats; total dose, 700 ± 71 mg/kg bw), or 3 mg/kg bw NDEA as well as 40 ml of a commercial brandy (38% alcohol) as the drinking fluid (20 rats; total dose of brandy, 8100 ml/kg bw) [exact length of survival and of the experiment unspecified]. Hepatocellular carcinomas developed in 28/28 animals

given NDEA and in 16/20 animals given NDEA plus brandy (Schmähl *et al.*, 1965). [The Working Group noted the inadequate reporting of the experiment.]

Groups of 13-27 male and female Sprague-Dawley *rats* [sex distribution unspecified], three months of age, received daily intragastric intubations of 2.5 mg/kg bw NDEA (total dose, 607 mg/kg bw), 2.5 mg/kg bw NDEA in 0.5 ml 30% (v/v) ethanol (total doses, 529 mg/kg bw NDEA and 106 ml/kg bw ethanol), 10 mg/kg bw NDEA (total dose, 1867 mg/kg bw) or 10 mg/kg bw NDEA in 0.5 ml 30% ethanol (total doses, 1806 mg/kg bw NDEA and 90 ml/kg bw ethanol) for life [duration of the study unspecified]. Mean times to tumour appearance in these groups were 242, 211, 186 and 180 days, respectively. The combination of NDEA plus ethanol increased the incidence of papillomas in the oesophagus and/or forestomach [sites not clearly specified]: 2.5 mg/kg bw NDEA, 5/23; 2.5 mg/kg bw NDEA plus ethanol, 17/27; 10 mg/kg bw NDEA, 10/23; 10 mg/kg bw NDEA plus ethanol, 13/13. In contrast, the incidence of epidermoid carcinomas of the oesophagus and/or forestomach [sites not clearly specified] was increased only in the high-dose group: 10 mg/kg bw NDEA, 3/23; 10 mg/kg bw NDEA plus ethanol, 7/13 [$p = 0.013$] (Gibel, 1967). [The Working Group noted inconsistencies in the reporting.]

Two groups of 90 male Sprague-Dawley *rats*, 14 weeks of age, received daily administrations of 0.1 mg/kg bw NDEA in the drinking-water on five days per week for life or the same NDEA treatment and subsequent daily administration of 5 ml 25% ethanol in water as the drinking fluid on five days per week for life. Average survival times were 116 weeks in the group receiving NDEA alone and 104 weeks in the group receiving NDEA plus ethanol; in the latter group, 31 animals died prior to the appearance of the first tumours. The incidence of liver tumours (described as malignant hepatomas) was lower in the group receiving both NDEA and ethanol (4/59; 7%) than in animals treated only with NDEA (36/80; 45%; $p < 0.01$). There was also a decrease in the incidence of squamous-cell carcinomas and/or papillomas of the oesophagus in animals treated with both NDEA and ethanol (18/59; 31%) as compared to the NDEA-treated group (33/80; 41%; Habs & Schmähl, 1981). [The Working Group noted the high mortality of animals in the group treated with both agents which precludes a meaningful evaluation of the study.]

Two groups of 19 female Sprague-Dawley *rats* weighing 100 g were fed semisynthetic choline-deficient or choline-supplemented diets and 25% sucrose plus 32% (w/v) ethanol (decreased to 25% during the first five days) as the drinking fluid for up to ten months. One day prior to the start of the experiment and two months later, the rats were given an intraperitoneal injection of 100 mg/kg bw NDEA. Two additional groups of 12 female rats were fed the same isocaloric diets without ethanol and received the two doses of NDEA. A further group of ten rats were pair-fed a choline-deficient diet but not treated with NDEA, and served as controls. Seven months after the initiation of the experiment, 12, 11, six, six and four rats in the different groups, respectively, were killed; the remaining animals were killed at the end of the tenth month. No tumour of the liver or kidney was found in any of the rats killed at seven months. At termination of the experiment at ten months, hepatocellular carcinomas and renal 'adenomas' were found in 3/6 and 2/6 rats given NDEA and fed the choline-deficient diet, but in none of the other groups (Porta *et al.*, 1985). [The Working

Group noted the short duration of the study and the small number of animals left after the interim kill.]

Male Wistar *rats* weighing 80-120 g received a single intraperitoneal injection of 30 mg/kg bw NDEA, and, one week later, received either tap-water or 5% ethanol in water as the drinking fluid, and were observed for 18 months. Hepatocellular carcinomas developed in 2/8 animals given NDEA plus ethanol and in 0/18 controls (Driver & McLean, 1986). [The Working Group noted the small number of animals used.]

Either tap-water or 20% ethanol plus 10% sucrose in water was given as the drinking fluid to male Wistar *rats* that had been subjected to a 70% partial hepatectomy, followed by an intraperitoneal injection of 10 mg/kg bw NDEA 24 h after surgery. The group that received ethanol plus sucrose as the drinking fluid was placed on this regimen eight weeks after surgery and consumed an average of 110 ml/kg bw (15.4 g/kg bw ethanol) daily. All rats were killed 40 weeks after NDEA treatment. In five rats given NDEA alone, two hepatocellular nodules [adenomas] less than 2 mm in diameter were found; in ten rats given NDEA followed by ethanol, 15 nodules ranging in diameter from less than 2 mm (11 nodules) to 6 mm were found and confirmed histologically ($p < 0.05$; Takada *et al.*, 1986). [The Working Group noted the small number of animals used and the short duration of the study.]

(iii) N-*Nitrosodi*-n-*propylamine* (NDPA)

Groups of 38 male and 32 female C57Bl *mice*, eight weeks of age, received intragastric intubations of 0.03 mg NDPA in 0.2 ml water (total dose, 3 mg) or 0.03 mg NDPA in 0.2 ml 40% ethanol (v/v) in water (total doses, 3 mg NDPA; 20 ml 40% ethanol [6.4 g 100% ethanol]) twice a week for 50 weeks. At 72 weeks after the beginning of the experiment, all survivors were killed. A statistically significant increase in the incidence of spinocellular [squamous-cell] carcinoma of the oesophagus/forestomach was observed in the group give NDPA plus ethanol (36/70; $p < 0.00005$) as compared to the group given NDPA alone (7/70) (Griciute *et al.*, 1982).

Groups of 70 C57Bl *mice* [sex distribution and age unspecified] received intragastric instillations of a mixture of 0.01 mg NDMA plus 0.01 mg NDEA plus 0.01 mg NDPA in 40% ethanol [volume unspecified] or in water twice a week for 50 weeks. All surviving animals were killed after 79 weeks. Tumour incidences in the two groups were: malignant forestomach/oesophageal tumours, 35/70 and 8/70; pulmonary adenomas, 55/70 and 34/70; and aesthesioneuroepitheliomas infiltrating the brain, 2/70 and 0/70, respectively (Griciute *et al.*, 1987).

(iv) N-*Nitrosomethylbenzylamine* (NMBzA)

Two groups of 40 male weanling Sprague-Dawley *rats* were fed a zinc-deficient diet containing 7 mg/kg zinc and were given either deionized water or 4% ethanol in deionized water as the drinking fluid. After four weeks, NMBzA was administered intragastrically to both groups at a dose of 2 mg/kg bw twice weekly for four weeks and the dietary regimes were maintained for a further 29 weeks. Oesophageal tumours were observed in 25/33 rats

fed the diet without ethanol as drinking fluid and in 29/34 rats fed the diet with 4% ethanol in water as the drinking fluid (Gabrial *et al.*, 1982).

(v) N-*Nitrosomethylphenylamine* (NMPhA)

Groups of 48 Sprague-Dawley *rats* [sex distribution unspecified], 13 weeks of age, received either weekly subcutaneous injections of 10.0 mg/kg bw NMPhA for 24 weeks (group 1); weekly subcutaneous injections of 2.0 mg/kg bw NMPhA for 39 weeks (group 2); daily administration of 1.5 [presumably mg/kg bw] NMPhA in the drinking-water for 22 weeks (group 3); or daily administration of 0.3 [presumably mg/kg bw] NMPhA in the drinking-water for 29 weeks (group 4). Four other groups received the same treatments in combination with administration of 25% ethanol (about 30 ml/kg bw) in water five times per week. The animals were observed until natural death; the mean life expectancy was 780 ± 120 days for all treated groups. The incidences of oesophageal tumours (squamous-cell carcinomas, transition from papilloma to carcinoma often seen) were group 1, 41/48 (84%); group 2, 22/48 (46%); group 3, 42/48 (87%); and group 4, 39/48 (80%); administration of ethanol did not alter these incidences (Schmähl, 1976). [The Working Group noted limitations in the experimental design and reporting.]

(vi) N-*Nitrosopiperidine* (NPIP)

Groups of about 28 male Fischer 344 *rats* weighing 160 g received either 0.06% NPIP in the basal diet for eight weeks; 0.06% NPIP in the basal diet for eight weeks followed by 10% ethanol in water as the drinking fluid for 12 weeks; 0.06% NPIP in the basal diet plus administration of 1 ml 50% ethanol through a tube inserted into the pharynx once every two days for eight weeks followed by no further treatment or followed by 10% ethanol as the drinking fluid for 12 weeks. The study was terminated 20 weeks after the start of the experiment. No significant difference in the incidence of oesophageal carcinomas was found (Konishi *et al.*, 1986). [The Working Group noted the relatively short duration of the experiment.]

(vii) N,N'-*Dinitrosopiperazine* (DNPIP)

Groups of 20 Sprague-Dawley *rats* [sex distribution unspecified], three months of age, received daily administration of 5 mg/kg bw DNPIP (total dose, 2605 mg) or 5 mg/kg bw DNPIP plus 0.5 ml 30% (v/v) ethanol (total doses: DNPIP, 2250 mg; ethanol, unspecified) by gastric intubation for life [duration unspecified]. The numbers of oesophageal and/or forestomach tumours [sites not clearly specified] induced by DNPIP were 11 papillomas and one carcinoma in DNPIP-treated animals and 16 papillomas and one carcinoma in those given DNPIP plus ethanol. Time to appearance of tumours was 521 days in the groups given DNPIP compared with 450 days in the group given DNPIP plus ethanol (Gibel, 1967). [The Working Group noted inconsistencies in the reporting.]

(viii) N'-*Nitrosonornicotine* (NNN)

Groups of 26 or 30 male Fischer 344 *rats*, nine weeks of age, were maintained on either a control liquid diet (groups 1 and 3) or a liquid diet containing 6.6% w/v ethanol (35% of calories; groups 2 and 4). At 13 weeks of age, rats in groups 1 and 2 were given a

subcutaneous injection of 10 mg/kg bw NNN in saline on three alternate days per week (56-66 injections; total dose, 177 mg/rat); the liquid diets were replaced with basal diet 24 h after the last injection. At 13 weeks of age, rats from groups 3 and 4 received addition of 17.5 mg/l NNN to their respective liquid diets for 27 weeks (total dose, 177 mg/rat), after which time they were placed on basal diets during the observation period. The study was terminated when rats were 98 weeks of age. No significant difference was observed in the incidence of nasal cavity tumours between animals receiving subcutaneous injections of NNN (group 1, 24/30; group 2, 22/30); however, in rats administered NNN in the liquid diet, nasal cavity tumours developed in 18/30 rats in group 3 (NNN; 11 benign and seven malignant) and in 26/30 rats in group 4 (NNN plus ethanol; 20 benign and six malignant; $p < 0.05$). The incidence of oesophageal tumours was 25/30 in rats in group 3 (16 benign and nine malignant) and 20/30 in group 4 (13 benign and seven malignant; Castonguay *et al.*, 1984).

Groups of 25 male and 25 female young adult BDVI *rats* received gastric instillations of 0.3, 1.0 or 3.0 mg/rat NNN in water, or 0.3, 1.0 or 3.0 mg/rat NNN in a 40% aqueous ethanol solution [volume unspecified] twice a week for 78 weeks (total doses of NNN, 46.8, 156 and 468 mg/rat). Animals were held for observation until 120 weeks of age, at which time survivors were killed. Only seven rats receiving 3.0 mg NNN in water and none receiving 3.0 mg NNN in ethanol survived for more than 78 weeks. The time to appearance of the first tumour of the nasal cavity was shorter in all rats given NNN in ethanol solution. The incidences of malignant nasal cavity tumours (mainly aesthesioneuroepitheliomas [neuroblastomas]) were slightly elevated in ethanol-treated rats: males — 0/25, 2/25 and 20/25 given NNN in water; 0/25, 5/25 and 24/25 given NNN in ethanol; and females — 1/25, 3/25 and 22/25 given NNN in water; 0/25, 2/25 and 25/25 given NNN in ethanol. No difference in the incidence of tumours at other sites was noted (Griciute *et al.*, 1986). [The Working Group noted that animals were given the same amount of NNN irrespective of body weight, that few animals received the total dose of NNN, and that the volume of ethanol given was not stated.]

Groups of 21 male outbred Syrian golden *hamsters*, nine weeks of age, were fed control liquid diets or liquid diets containing 6% (w/v) ethanol (35% of caloric intake). At 13 weeks of age, the animals received 0.5-ml intraperitoneal injections of 2.37 or 4.74 mg/animal NNN three times a week for 25 weeks (total dose, 177 or 354 mg) and were maintained on their respective diets. Treatment was suspended during weeks 17-21 because of weight loss. At the end of the treatment, animals in both groups were returned to a diet of laboratory chow and water. Animals were sacrificed when moribund, and survivors were sacrificed 18 months after the beginning of carcinogen administration. There was no significant difference in the incidence of nasal cavity or tracheal tumours in the two treated groups (McCoy *et al.*, 1981). [The Working Group noted the short duration of exposure to ethanol and that animals were given the same amount of NNN, irrespective of body weight.]

(ix) N-*Nitrosopyrrolidine* (NPYR)

Groups of 21 male outbred Syrian golden *hamsters*, nine weeks of age, were fed control liquid diets or liquid diets containing 6% (w/v) ethanol (35% of caloric intake). At 13 weeks

of age, the animals received 0.5-ml intraperitoneal injections of 1.33 or 2.67 mg/animal NPYR three times a week for 25 weeks (total dose, 100 or 200 mg) and were maintained on their respective diets. Treatment was suspended during weeks 17-21 because of weight loss. At the end of the treatment, animals in both groups were returned to a diet of laboratory chow and water. All survivors were killed 15 months after the beginning of carcinogen administration. The incidences of nasal cavity tumours were: low-dose NPYR, 1/20; low-dose NPYR plus ethanol, 8/18 ($p < 0.05$); high-dose NPYR, 14/21; and high-dose NPYR plus ethanol, 16/17 ($p < 0.05$). The incidences of tracheal tumours were: low-dose NPYR, 4/20; low-dose NPYR plus ethanol, 9/18 ($p < 0.05$); high-dose NPYR, 8/21; and high-dose NPYR plus ethanol, 11/17 (McCoy *et al.*, 1981). [The Working Group noted that animals were given the same amount of NPYR, irrespective of body weight.]

In a subsequent experiment, the effect of changes in the amount of ethanol consumed on the carcinogenicity of NPYR was determined. Groups of 27 male outbred Syrian golden *hamsters*, nine weeks of age, were administered tap-water, 7.4% ethanol or 18.5% ethanol in water as the drinking fluid for four weeks, followed by 0.5-ml intraperitoneal injections of 1.33 mg/animal NPYR three times a week for 25 weeks (total dose, 100 mg/animal), after which ethanol-treated animals were administered tap-water only. Animals were killed 17 months after the beginning of carcinogen administration. The incidences of tracheal papillomas were: tap-water plus NPYR, 3/26; low-dose ethanol plus NPYR, 6/26; and high-dose ethanol plus NPYR, 4/26. The incidences of hepatic neoplastic nodules were 6/26, 17/26 ($p < 0.01$) and 17/26 ($p < 0.01$), respectively (McCoy *et al.*, 1986). [The Working Group noted that animals were given the same amount of NPYR, irrespective of body weight.]

(x) N-*Nitrosobis(2-oxopropyl)amine* (NDOPA)

Two groups of 15 male Syrian *hamsters*, six weeks old, received either a single subcutaneous injection of 20 mg/kg bw NDOPA alone or 25% ethanol in water (w/v) as the drinking fluid; two weeks after the start of ethanol treatment, animals were given a single subcutaneous injection of 20 mg/kg bw NDOPA. Treatment continued for 24 weeks, at which time the hamsters were killed. Histopathological examination of the exocrine pancreas showed fewer neoplastic lesions [details not given] in animals treated with NDOPA plus ethanol (0/13) than in those treated with NDOPA alone (11/14; Tweedie *et al.*, 1981). [The Working Group noted the small number of animals used, the short duration of the study and the inadequate reporting.]

Groups of 20 male and 20 female outbred Syrian golden *hamsters*, eight weeks old, received 5% (w/v) ethanol in water as the drinking fluid for 46 weeks. Then, one group received a single subcutaneous injection of 20 mg/kg bw NDOPA prior to ethanol treatment, and the other received a subcutaneous injection of 20 mg/kg NDOPA four weeks after the beginning of ethanol treatment. A further group of 20 males and 20 females received the injection of NDOPA and were maintained on tap-water. All survivors were killed at the end of 46 weeks. No significant different in the incidence of pancreatic tumours was noted (Pour *et al.*, 1983).

(xi) N-*Methyl*-N'-*nitro*-N-*nitrosoguanidine* (MNNG)

Two groups of male Wistar *rats*, seven weeks of age, received 100 mg/l MNNG continuously in their drinking-water simultaneously with a 10% sodium chloride supplemented diet for eight weeks, after which time they were returned to nonsupplemented diets and either tap-water (30 rats) or 10% ethanol in water as the drinking fluid (21 rats). At the end of 40 weeks, all survivors were killed. Adenocarcinomas of the glandular stomach or duodenum occurred in 4/30 and 2/21 rats given tap-water and 10% ethanol, respectively (Takahashi *et al.*, 1986).

(xii) N-*Hydroxy-2-acetylaminofluorene* (OH-AAF)

Groups of four to ten male and six to 12 female inbred Fischer *rats*, four to 16 weeks of age, were fed a semisynthetic diet with or without 160 mg/kg OH-AAF for 12-20 weeks, after which they were maintained on basal diet for up to 20 weeks. Groups also received 10 or 20% (by volume) ethanol in water as the drinking fluid or water alone, simultaneously with the OH-AAF diet. Other groups were given the OH-AAF treatment and water in the first experimental period followed by control diet and ethanol in the second period. In a second series, groups of 20 male and 20 female weanling random-bred NIH black rats were fed a diet containing 80 mg/kg OH-AAF for 64 weeks and received either water alone or 10% ethanol in water as the drinking fluid. No significant treatment-related increase in the incidence of hepatocellular adenomas was observed in any of the groups of either strain (Yamamoto *et al.*, 1967). [The Working Group noted the small numbers of animals per group and the large variations in age at the start of treatment.]

(xiii) *Azoxymethane*

Groups of 26 male Fischer 344 *rats*, ten weeks of age, were pair-fed isocaloric liquid diets containing 12 or 23% of calories as beer or 9 or 18% as ethanol. Three weeks after the start of treatment, the rats received ten weekly subcutaneous injections of 7 mg/kg bw azoxymethane in sterile water and were maintained on the same diets until sacrifice at week 26. The high-dose ethanol group showed a trend towards reduced number of tumours in the right colon but not in the left. The number of right colonic tumours was inversely correlated with ethanol consumption in all animals (Spearman's rank correlation coefficient: r, −0.350; $p < 0.001$), but there was no correlation with left colonic tumours. The total number of colonic tumours in the high-dose ethanol group was markedly reduced in comparison with controls (18 tumours *versus* 45) but not in the low-dose ethanol group (37 tumours *versus* 45). Similar effects were found in the group given 23% calories as beer (Hamilton *et al.*, 1987a). [The Working Group noted that the exact incidences and the histopathological nature of the tumours were not reported.]

In a further study, groups of 35 male ten-week-old Fischer 344 *rats* were given liquid diets providing 11, 22 or 33% of calories from ethanol. All rats were given ten weekly subcutaneous injections of 7 mg/kg bw azoxymethane, and the liquid-ethanol diets were given either three weeks prior to and during treatment or for 16 weeks after treatment with azoxymethane, at which time all animals were killed. Administration of the liquid-ethanol diet after injection of the carcinogen had no effect on the incidence of tumours of the right and transverse colon or of the left colon. The incidence of tumours of the left colon was

significantly reduced by the 22 and 33% liquid-ethanol diets, as was that of tumours of the right and transverse colon by the 33% liquid-ethanol diet given prior to and during administration of the carcinogen (Hamilton *et al.*, 1987b). [The Working Group noted the absence of data on the histopathological nature of the tumours.]

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

Two groups of 30 or 20 female NMRI *mice* [age unspecified] received skin applications of 0.02 ml of a 1% solution (v/w) of DMBA in acetone or ethanol (purity, 99.5%) three times a week for 20 weeks. Skin tumours, including some squamous-cell carcinomas, occurred in 4/30 mice treated with DMBA in acetone (11 tumours; latency, nine weeks) compared with 11/20 [$p = 0.002$] mice treated with DMBA in ethanol (48 tumours; latency, six weeks; Stenbäck, 1969).

Two groups of 72 or 70 male CF1 *mice*, two months of age, received a single skin application of 0.02 ml of a 1.5% DMBA solution in acetone. One group received no further treatment, but, one month later, the second group received applications of 0.04 ml of a 50% aqueous ethanol solution on the same region twice a week for 40 weeks. At the end of the treatment period, the number of tumour-bearing animals in the ethanol-treated group (25/59; papillomas and one squamous-cell carcinoma) was not significantly different from that in controls (15/46; all papillomas). In a second experiment, groups of mice received a single application of 0.025 ml DMBA (1.5%) in acetone. One month later, animals received applications to the skin of 0, 12 or 43% ethanol twice a week for 40 weeks. There was no statistically significant difference in tumour yield between mice treated with 12% ethanol (6/55; one squamous-cell carcinoma) or with 43% ethanol (9/56; all papillomas) or controls (5/46; Kuratsune *et al.*, 1971).

(xv) *1,2-Dimethylhydrazine* (DMH)

Two groups of 16 male Sprague-Dawley *rats*, 60 days of age, were fed a liquid diet containing 36% of total calories as ethanol (concentration, 6.6% v/v) or as isocaloric carbohydrates. After four weeks of treatment, the animals were fed a standard laboratory diet for three weeks and received four weekly subcutaneous injections of 30 mg/kg bw DMH. This feeding schedule was repeated for a total of four cycles. After 32 weeks, the 28 survivors were killed; all had intestinal tumours. A higher number of rectal tumours (17 *versus* 6) was seen in rats treated with DMH and ethanol than in rats treated with DMH and an isocaloric diet, but no difference was seen in tumour incidences at other sites in the intestine (Seitz *et al.*, 1984, 1985).

Four groups of 20 male inbred D/A *rats*, four to six weeks of age, were fed a low-fat diet (5%, w/w; standard diet), and four other groups were fed a high-fat diet (33.5%, w/w). Both diets had a ratio of polyunsaturated:saturated fat of 3:2. One group on each diet received commercially available beer, and another received 4.8% ethanol (v/v) in water as the drinking fluid. All of these groups received 20 weekly subcutaneous injections of 20 mg/kg bw DMH. The other four groups served as low- or high-fat untreated or DMH-treated controls and received tap-water. Animals were killed 28 weeks after the first injection. The number of tumours per animal in both the small and large intestines was significantly greater in DMH-treated rats receiving the high-fat as compared to those receiving the

low-fat diet. However, no significant difference in the incidence of intestinal cancer was noted in the groups given ethanol or beer as compared to those given water (Howarth & Pihl, 1985).

Two groups of 22 male Sprague-Dawley *rats*, five weeks of age, received either tap-water alone or 95% laboratory-grade ethanol diluted to 5% (v/v) in tap-water as the drinking fluid. After three weeks, both groups also received 16 weekly subcutaneous injections of 15 mg/kg bw DMH. A third control group of 22 rats received neither ethanol nor DMH. Animals were killed 22 weeks after the first injection of DMH. All animals given DMH had colonic cancers; ethanol did not affect the number of tumours per rat (3.9 ± 0.45 without DMH *versus* 3.5 ± 0.45 with DMH) (Nelson & Samelson, 1985).

In the same study, two groups of 12 male Sprague-Dawley *rats*, five weeks of age, received either tap-water alone or a commercially available beer as the drinking fluid. After three weeks, both groups also received ten weekly [presumably subcutaneous] injections of 20 mg/kg bw DMH. Animals were killed 14 weeks after the last injection of DMH. All 12 rats given DMH alone developed gastrointestinal tumours, compared to 8/12 rats given DMH plus beer, and there were fewer of these tumours per rat compared with rats treated with DMH alone (1.33 ± 0.43 *versus* 2.91 ± 0.52; $p = 0.043$; Nelson & Samelson, 1985). [The Working Group noted the small number of animals used and the absence of histological diagnosis of the tumours.]

(xvi) *Vinyl chloride* (VC)

Groups of 80 male Sprague-Dawley *rats* [age unspecified] received 5% ethanol in water or water alone as the drinking fluid for life. Four weeks after the start of treatment, one ethanol-treated and one untreated group were exposed by inhalation to 600 ppm (1560 mg/m^3) VC for 4 h per day on five days per week for one year. At 18 months, survival was 40% in rats exposed to VC and 18% in animals exposed to VC plus ethanol. All survivors were killed 30 months after the first exposure to VC. Of the rats exposed to VC and to VC plus ethanol, 35/80 and 48/80 [$p = 0.028$] had hepatocellular carcinomas and 18/80 and 40/80 [$p = 0.002$] had liver angiosarcomas, respectively (Radike *et al.*, 1981).

(xvii) *Urethane*

In a study reported as an abstract, three groups of 30 male C3H *mice*, eight weeks of age, received gastric intubations of 2 mg/animal urethane in 0.2 ml water, 2 mg/animal urethane in 0.2 ml 40% ethanol or 2 mg/animal urethane in 0.2 ml water followed 24 h later by 0.2 ml 40% ethanol twice a week for five treatments. They were then given basal diet and tap-water for six months, at which time all survivors were killed. The presence of ethanol as a solvent enhanced pulmonary adenoma development; subsequent administration of ethanol had no influence on the carcinogenicity of urethane (Barauskaite, 1985).

In a study reported as an abstract, groups of 12-14 white outbred male and female *mice* [strain unspecified], eight weeks of age, received intraperitoneal injections of 10 mg urethane in 0.2 ml saline or in 0.2 ml 40% ethanol solution twice weekly for five weeks. Mice were held for 12 weeks, at which time all survivors were killed. All animals given urethane

developed pulmonary tumours; the average number of pulmonary adenomas per animal was 30 in the group given urethane in ethanol and 13 in the group given urethane in saline ($p = 0.002$; Griciute, 1981).

(*c*) *Carcinogenicity of metabolites*

Acetaldehyde, the major intermediary metabolite of ethanol, was tested for carcinogenicity in rats by inhalation exposure and in hamsters by inhalation and by intratracheal instillation. It produced tumours of the respiratory tract following its inhalation, particularly adenocarcinomas and squamous-cell carcinomas of the nasal mucosa in rats and laryngeal carcinomas in hamsters. In hamsters, it did not result in an increased incidence of tumours following intratracheal instillation. Inhalation of acetaldehyde enhanced the incidence of respiratory-tract tumours induced by intratracheal instillation of benzo[*a*]pyrene in hamsters. Previous IARC working groups have concluded that there is *sufficient evidence* for the carcinogenicity of acetaldehyde in experimental animals (IARC, 1985, 1987a).

4.2 Other relevant data from experimental systems

(*a*) *Absorption, distribution and excretion*

The absorption, distribution, excretion and metabolism of ethanol have been reviewed extensively (Wallgren & Barry, 1970; Kalant, 1971; Hawkins & Kalant, 1972; Khanna & Israel, 1980; Lieber, 1982, 1983, 1984a, 1985a,b).

Ethanol is absorbed from the gastrointestinal tract by simple diffusion. The rate of absorption is decreased by delayed gastric emptying and by the intestinal contents. It has been demonstrated in several animal species that food delays absorption, producing a slower rise and lower peak value of the blood ethanol in fed than in fasting animals. The absorption of ethanol was slower from beer and wine than from distilled beverages. High ethanol concentrations may delay gastric emptying (Rasmussen, 1940; Wallgren & Barry, 1970) as does intraperitoneal administration of glucose (Wallgren & Hillbom, 1969). In contrast, carbohydrates (Broitman *et al.*, 1976), amino acids and dipeptides (Hajjar *et al.*, 1981) have been shown to enhance ethanol absorption in perfused rat jejunum *in vivo*.

The diffusion of ethanol through cell boundaries is slow and is affected by blood flow. Ethanol in the blood passes almost immediately into brain tissue (Harger *et al.*, 1937; Hulpieu & Cole, 1946; Crone, 1965). In contrast, the distribution of ethanol to resting skeletal muscle is particularly slow (Harger & Hulpieu, 1956).

After oral dosage, ethanol disappeared from the blood of dogs linearly in the post-absorption phase, irrespective of the concentration of ethanol in the body; zero-order kinetics was also found in dogs, cats, rabbits, pigeons and chicken after intravenous administration (Newman & Lehman, 1937).

(b) Metabolism

Ethanol is eliminated from the body mainly by metabolism in the liver and only minimally by urinary excretion and pulmonary exhalation (Wallgren & Barry, 1970). Other tissues such as kidney (Leloir & Muñoz, 1938), stomach and intestine oxidize ethanol to a small extent (Carter & Isselbacher, 1971; Lamboeuf *et al.*, 1981).

The hepatic metabolism of ethanol proceeds in three basic steps. First, ethanol is oxidized within the cytosol of hepatocytes to acetaldehyde; second, acetaldehyde is converted to acetate, mainly in the mitochondria; and third, acetate produced in the liver is released into the blood and is oxidized by peripheral tissues to carbon dioxide, fatty acids and water. The main pathway for ethanol metabolism proceeds *via* alcohol dehydrogenase (ADH). However, alternative pathways for ethanol oxidation have been described, which are situated in other subcellular compartments.

(i) *Alcohol dehydrogenase (ADH)*

ADH occurs in the soluble fraction of the liver and is also found in other tissues in the body, such as gastrointestinal mucosa, kidney, lung and possibly brain (Mistilis & Garske, 1969; Hawkins & Kalant, 1972). It catalyses the oxidation of alcohols, including ethanol, to their corresponding aldehydes in the presence of NAD, according to the following scheme:

$$CH_3CH_2OH + NAD^+ \dashrightarrow CH_3CHO + NADH + H^+$$

(Hawkins & Kalant, 1972). The optimal pH for ADH-mediated oxidation of ethanol is 10-11 (von Wartburg *et al.*, 1965; Lieber, 1970). Several factors affect the pathway, including the activity of ADH, the intracellular acetaldehyde concentration, the activity of the shuttle mechanisms that transport reducing equivalents into the mitochondria and the rate of oxidation of reducing equivalents *via* the mitochondrial respiratory chain. The rate-limiting step in the ADH pathway can therefore vary depending on the experimental conditions (Thurman, 1977).

The rate of elimination of ethanol *in vivo* correlates with the basal metabolic rate, indicating that the rate of mitochondrial NADH oxidation is a major rate-limiting step in the ADH pathway. Horse liver, for example, contains large amounts of ADH, yet the rate of ethanol metabolism is relatively low, reflecting the animal's basal metabolic rate (Lester & Keokosky, 1967). In smaller animals, such as rats, although a greater proportion of total ADH activity (50-85%) may be used for ethanol oxidation, the theoretical capacity of ADH to metabolize ethanol still exceeds the actual rate *in vivo* (Crow *et al.*, 1977). This conclusion is supported by studies demonstrating an increase in ethanol elimination following administration of fructose (Thieden & Lundquist, 1967). The effect of fructose in both fasting and fed animals is due to the fact that ATP is consumed during its phosphorylation, thus increasing the capacity of the respiratory chain to oxidize NADH derived from the oxidation of ethanol and acetaldehyde (Thieden *et al.*, 1972; Scholz & Nohl, 1976).

ADH activity is under hormonal control. Thus, castration of rats increases ADH activity in liver (Mezey *et al.*, 1980; Lumeng & Crabb, 1984); and chronic administration of testosterone to male rats castrated before puberty and to female rats decreases both the

metabolic rate of ethanol and ADH activity to values similar to those found in mature males (Rachamin *et al.*, 1980).

Decreased activity of hepatic ADH, leading to a corresponding reduction in the rate of ethanol elimination, is observed in rats with advancing age (Hahn & Burch, 1983), with protein deficiency (Horn & Manthei, 1965; Bode *et al.*, 1970; Wilson, J.S. *et al.*, 1986) and in spontaneously hypertensive animals (Rachamin *et al.*, 1980). Numerous studies support the conclusion that, while increased ADH activity is not associated with increased rates of ethanol oxidation, reduction in ADH activity does lead to a decrease in ethanol elimination (Lieber, 1983).

(ii) *Cytochrome P450*

In 1965, a NADPH-dependent ethanol oxidase was found in microsomes from pig's liver which catalyses the oxidation of methanol to formaldehyde and that of ethanol to acetaldehyde (Orme-Johnson & Ziegler, 1965). This system, which was subsequently designated the microsomal ethanol-oxidizing system (MEOS; Lieber & DeCarli, 1968), carries out the following reaction:

$$CH_3CH_2OH + NADPH + O_2 + H^+ \longrightarrow CH_3CHO + NADP^+ + 2H_2O$$

(Lieber, 1970).

Ethanol binds to hepatic cytochrome P450 and gives a modified type-II binding spectrum (Rubin *et al.*, 1971). The K_m of cytochrome P450 for ethanol (about 8 mM) is greater than that for ADH (Lieber & DeCarli, 1970; Lindros *et al.*, 1974). An isozyme of cytochrome P450 that is induced by ethanol, IIE1 (previously described as $P450_{3a}$, $P450_{ALC}$ or P450j; Nebert *et al.*, 1987) has been purified from the liver of rabbits (Koop *et al.*, 1982), rats (Peng *et al.*, 1982), hamsters, deermice (*Peromyscus maniculatus*) and baboons (Lasker *et al.*, 1986a); and its presence has been demonstrated in rabbit kidney and nasal mucosa (Ding *et al.*, 1986; Ueng *et al.*, 1987) and rat kidney (Thomas *et al.*, 1987), but not in microsomes from a variety of other tissues (Ding *et al.*, 1986). Cytochrome P450-dependent ethanol oxidizing capacity has also been demonstrated in mucosal cells from the upper gastrointestinal tract and colon of rats (Seitz *et al.*, 1979, 1982) and in macrophages from a variety of tissues in mice (Wickramasinghe *et al.*, 1987).

(iii) *Catalase*

Catalase is a haemoprotein located in the peroxisomes of many tissues (de Duve & Baudhuin, 1966). An early suggestion that catalase might play a role in the metabolism of ethanol (Keilin & Hartree, 1936) was later confirmed by Laser (1955), who showed that ethanol could be oxidized effectively in the presence of hydrogen peroxide and catalase. The scheme of the reaction is as follows:

$$\text{Catalase} + H_2O_2 \longrightarrow \text{Cat-}H_2O_2$$

$$\text{Cat-}H_2O_2 + CH_3CH_2OH \longrightarrow CH_3CHO + 2H_2O + \text{catalase}$$

(Thurman *et al.*, 1988).

Catalase can oxidize ethanol *in vitro* only in the presence of a hydrogen peroxide generating system (Keilin & Hartree, 1945); however, the reaction is limited by the rate of

hydrogen peroxide generation rather than by the amount of catalase haem (Oshino *et al.*, 1973). It had been thought previously that the rate of hydrogen peroxide production represented only a small fraction of the rate of ethanol oxidation *in vivo* (Boveris *et al.*, 1972; Sies, 1974), but more recent experiments with physiological concentrations of fatty acid bound to albumin indicate that the role of catalase may be more important (Handler & Thurman, 1987).

(iv) *Aldehyde dehydrogenases* (ALDH)

A NAD-dependent ALDH with a broad substrate specificity for aldehydes was described in 1949 (Racker, 1949), and reviews on ALDH are available (e.g., Lindros, 1978). The enzyme has very low K_m values and a high reaction rate (Grunnet, 1973); therefore, under normal circumstances, only low concentrations of acetaldehyde are found outside the liver (Jacobsen, 1952; Kiessling, 1962). The metabolism of acetaldehyde has been reviewed (IARC, 1985). At low concentrations of acetaldehyde (<50 μM) during ethanol oxidation, acetaldehyde oxidation is predominantly a mitochondrial process (Grunnet, 1973; Marjanen, 1973; Lindros *et al.*, 1974; Parrilla *et al.*, 1974). Several isozymes of ALDH have been identified (for reviews, see Lindros, 1978; Salaspuro & Lindros, 1985). Tissues other than the liver may also produce acetaldehyde after ethanol administration (Baraona *et al.*, 1985), and intestinal bacteria have been shown to produce small amounts (Baraona *et al.*, 1986).

(v) *Ethanol acyltransferase*

Ethanol acyltransferase activity has been described in rat liver microsomes (Grigor & Bell, 1973; Bakken *et al.*, 1979) and in the pancreas (Estival *et al.*, 1981). The enzyme is responsible for esterification of fatty acids with ethanol.

(vi) *Modifying effects of repeated ethanol consumption on the metabolism of ethanol and acetaldehyde*

Most studies show that repeated or long-term consumption of ethanol enhances its clearance (e.g., Lieber & DeCarli, 1970; Misra *et al.*, 1971; Feinman *et al.*, 1978; Yuki & Thurman, 1980; for review, see Eriksson & Deitrich, 1983).

Feeding of ethanol (36% of total calories for 24 days) to rats enhanced its metabolism in microsomes (Lieber & DeCarli, 1968; Rubin & Lieber, 1968) and in isolated hepatocytes (Cederbaum *et al.*, 1978) and increased the rate of its disappearance from the blood (Tobon & Mezey, 1971; Lieber & DeCarli, 1972; Bleyman & Thurman, 1979; Wendell & Thurman, 1979). Enhanced clearance of ethanol from the blood has also been demonstrated *in vivo* in deermice (*Peromyscus maniculatus*; Shigeta *et al.*, 1984), in chimpanzees, in rhesus monkeys (Pieper & Skeen, 1973) and in baboons (Pikkarainen & Lieber, 1980; Nomura *et al.*, 1983). Further examples are listed in Table 41. The biochemical background for this phenomenon has been attributed to a hypermetabolic state in the liver (Israel *et al.*, 1975) or to increased ethanol oxidation *via* cytochrome P450 (Lieber & DeCarli, 1970).

Blood concentrations of acetaldehyde in baboons were increased following chronic ethanol consumption; these correlated positively with rates of ethanol elimination and negatively with liver mitochondrial ALDH activity (Pikkarainen *et al.*, 1981).

Table 41. Enhanced xenobiotic metabolism in the liver due to prior ethanol treatment

Substrate tested	Species examined	Reference
Alcohols and ketones		
Ethanol	Rat	Lieber & DeCarli (1968)
	Rabbit	Morgan et al. (1981)
	Hamster	McCoy (1980)
	Deermouse (Peromyscus maniculatus)	Burnett & Felder (1980)
	Rabbit	Koop & Casazza (1985)
Acetone	Rat	Casazza & Veech (1985)
2-Butanol	Rat	Krikun & Cederbaum (1984)
Halogenated alkanes, alkenes and ethers		
Carbon tetrachloride	Rat	Johansson & Ingelman-Sundberg (1985)
Chloroform	Rat	Sato, A. et al. (1981); Sato & Nakajima (1985)
Trichloroethylene	Rat	Sato, A. et al. (1981); Sato & Nakajima (1985)
1,1-Dichloroethylene	Rat	Sato, A. et al. (1981); Sato & Nakajima (1985)
Enflurane	Rat	Pantuck et al. (1985)
Aromatic compounds		
Benzene	Rat	Sato, A. et al. (1981); Sato & Nakajima (1985)
Toluene	Rat	Sato, A. et al. (1981); Sato & Nakajima (1985)
para-Nitrophenol	Rat	Reinke & Moyer (1985)
Acetaminophen	Rat	Sato, C. et al. (1981)
	Mouse	Walker et al. (1983)
	Hamster	Elliott et al. (1985)
Benzo[a]pyrene	Rat	Seitz et al. (1978)
	Hamster	Murphy & Hecht (1986)
	Rabbit	Koop & Casazza (1985)
Aflatoxin B_1	Rat	Toskulkao & Glinsukon (1986)
Aromatic amines		
Benzidine	Rat	Neis et al. (1985)
para-Aminobenzoic acid	Rat	Neis et al. (1985)
2-Acetylaminofluorene	Rat	Smith & Gutmann (1984)
Nitrosamines		
N-Nitrosodimethylamine	Rat	Garro et al. (1981)
	Rabbit	Yang et al. (1985b)
N-Nitrosopyrrolidine	Rat	Farinati et al. (1985)
	Hamster	McCoy et al. (1979)
N'-Nitrosonornicotine	Rat	Castonguay et al. (1984)
Azoxymethane	Rat	Sohn et al. (1987)
Testosterone	Rat	Rubin et al. (1976)
Vitamin D	Rat	Gascon-Barré & Joly (1981); Gascon-Barré (1982)

Following repeated alcohol consumption, ALDH activity has been reported to decrease (Koivula & Lindros, 1975; Greenfield *et al.*, 1976; Lebsack *et al.*, 1981), to remain the same (Redmond & Cohen, 1971; Tottmar *et al.*, 1974) and to increase (Horton, 1971; Greenfield *et al.*, 1976; Väänänen *et al.*, 1984), depending mainly on the acetaldehyde concentrations used.

Chronic alcohol consumption has been shown to increase ADH activity in the distal colon of rats (Seitz, 1985).

(*c*) *Modifying effects of ethanol on the metabolism of xenobiotics*

Pretreatment of animals with ethanol enhances the hepatic metabolism of a variety of agents, such as volatile hydrocarbons, alcohols and ketones, aromatic amines and nitrosamines (for examples, see Table 41).

Long-term ethanol consumption results in proliferation of smooth hepatic endoplasmic reticulum and an increase in the level of cytochrome P450 (Iseri *et al.*, 1966; Rubin *et al.*, 1968; Joly *et al.*, 1973). The induction of cytochrome P450 by ethanol is associated with an increase in metabolism (see Table 41) and, in some instances, in the toxicity of many compounds, including *N*-nitrosodimethylamine (Garro *et al.*, 1981; Olson *et al.*, 1984), acetaminophen (paracetamol; Strubelt *et al.*, 1978; Sato, C. *et al.*, 1981; Walker *et al.*, 1983), carbon tetrachloride (Traiger & Plaa, 1971; Hasumura *et al.*, 1974), aflatoxin B_1 (Glinsukon *et al.*, 1978) and benzene (Nakajima *et al.*, 1985). It increases the hydroxylation of aniline (Morgan *et al.*, 1981; Villeneuve *et al.*, 1976). The unique cytochrome P450 isozyme (IIE1) induced by ethanol catalyses the oxidation of some other alcohols, aniline (Morgan *et al.*, 1982) and *N*-nitrosodimethylamine (Yang *et al.*, 1985a,b).

In contrast, ethanol inhibited the metabolism of xenobiotics *in vitro* (for examples, see Table 42). Acute administration of ethanol also inhibited the metabolism of some drugs *in vivo* (see Rubin *et al.*, 1970; Mezey, 1976; Sato *et al.*, 1985). There are two possible explanations for this phenomenon: direct competition for cytochrome P450 (Sato, A. *et al.*, 1981) or a decrease in the supply of a cofactor for the monooxygenase system (Reinke *et al.*, 1980).

The hepatic enzymes that catalyse conjugation of xenobiotics, such as UDP-glucuronyltransferase (Dioguardi *et al.*, 1970; Idéo *et al.*, 1971; Yost & Finley, 1983; Reinke *et al.*, 1986; Sweeny & Reinke, 1987) and glutathione-*S*-transferases (Younes *et al.*, 1980; Hétu *et al.*, 1982; David & Nerland, 1983; Schnellmann *et al.*, 1984), are similarly induced by feeding ethanol.

Conjugation reactions have also been found to be inhibited by concomitant ethanol administration; for example, glucuronidation was inhibited in isolated hepatocytes (Moldéus *et al.*, 1978; Sundheimer & Brendel, 1984) and in rat liver microsomes (Marniemi *et al.*, 1975), and glucuronidation and sulphation were inhibited in perfused rat liver (Reinke *et al.*, 1986).

Pretreatment of rats with ethanol increases the metabolism of *N*-nitrosamines in the lung, oesophagus (Farinati *et al.*, 1985) and nasal mucosa (Castonguay *et al.*, 1984). When ethanol was given concomitantly with NDMA, concentrations of the nitrosamine in brain,

Table 42. Inhibition of cytochrome P450-dependent metabolism by ethanol in the liver *in vitro*

Substrate tested	Species examined	Reference
Halogenated alkanes and alkenes		
Chloroform	Rat	Sato, A. *et al.* (1981)
1,2-Dichloroethane	Rat	Sato, A. *et al.* (1981)
Trichloroethylene	Rat	Sato, A. *et al.* (1981)
Aromatic compounds		
Aniline	Rat	Rubin & Lieber (1968)
Benzene	Rat	Sato, A. *et al.* (1981)
Styrene	Rat	Sato, A. *et al.* (1981)
Toluene	Rat	Sato, A. *et al.* (1981)
Polycyclic aromatic compound		
Benzo[*a*]pyrene	Rat	Rubin & Leiber (1968)
Nitrosamines		
N-Nitrosodimethylamine	Rat	Swann *et al.* (1984); Tomera *et al.* (1984)
N-Nitrosoethylmethylamine	Rat	Peng *et al.* (1982)
Drugs		
Meprobamate	Rat	Rubin *et al.* (1970)
Pentobarbital	Rat	Rubin *et al.* (1968)

lung, liver, kidney and blood were increased markedly (Anderson *et al.*, 1986). First-pass clearance of NDMA by the liver in rats was prevented when it was administered in ethanol (1 ml, 5% v/v) instead of in water. The prevention of first-pass clearance has a dramatic effect on alkylation of kidney and oesophageal DNA: at dose levels of 30 μg/kg bw NDMA, methylation of kidney DNA of ethanol-treated rats was five times that in controls; lower doses of NDMA induced methylation in the kidneys of ethanol-treated rats but not in controls (Swann, 1984). Similarly, administration of *N*-nitrosodiethylamine (NDEA; 20 μg/kg bw) in ethanol (1 ml; 5% v/v) led to a five-fold increase in *N*7 ethylation of guanine in oesophageal DNA over that seen with a similar dose of NDEA alone (Swann *et al.*, 1984).

(*d*) *Modifying effects of ethanol on intermediary metabolism*

As described above, when ethanol is oxidized to acetaldehyde *via* ADH, NAD is required as a cofactor and is reduced to NADH during the reaction. Under normal conditions, the rate of NADH production exceeds its rate of reoxidation, resulting in an increase in the ratio of NADH:NAD in the liver. Oxidation of ethanol *via* acetaldehyde to acetate also results in the reduction of both cytosolic and mitochondrial redox states

(Lieber, 1983, 1984a), reflected in increases in the ratios of lactate to pyruvate and β-hydroxybutyrate to acetoacetate (Forsander, 1970). These changes are used widely as markers of cytosolic and mitochondrial redox states (Bücher & Klingenberg, 1958; Hohorst *et al.*, 1959) and of ethanol-induced changes in the redox balance of the liver (Forsander *et al.*, 1965; Salaspuro & Mäenpää, 1966). Most of the acute metabolic effects of ethanol, such as inhibition of hepatic gluconeogenesis (Krebs *et al.*, 1969), decreases in citric acid cycle activity (Thurman, 1977) and impairment of fatty acid oxidation (Lieber & Schmid, 1961), are due to this major effect on the intermediary metabolism of the liver (see Table 43).

Table 43. Effects of ethanol on intermediary metabolism in the liver

Metabolic effect	Reference
Redox change in liver	Lieber (1983, 1984a)
NADH:NAD ratio increases	Smith & Newman (1959); Räihä & Oura (1962)
Lactic:pyruvic acid ratio increases	Forsander et al. (1965)
β-Hydroxybutyrate:acetoacetate ratio increases	Forsander et al. (1965)
Inhibition of citric acid cycle in liver	Thurman (1977)
Inhibition of glycolysis in liver	Thurman (1977)
Inhibition of gluconeogenesis in liver	Krebs et al. (1969)
Inhibition of fatty acid oxidation and accumulation of triglycerides in liver	Lieber & Davidson (1962); Rebouças & Isselbacher (1961); Mallov & Bloch (1956)
Inhibition of protein synthesis and secretion in liver	Perin et al. (1974); Baraona et al. (1980)
Accumulation of protein in liver	Baraona et al. (1977)
Inhibition of glycoprotein secretion in liver	Tuma et al. (1980)
Potentiation of hepatic lipid peroxidation	Shaw et al. (1981)
Inhibition of intestinal amino acid transport	Mezey (1985)
Elevation of blood lipoproteins	Baraona (1985)
Increase in peripheral fat mobilization	Mallov & Bloch (1956); Lieber (1985b)
Increased formation and decreased urinary excretion of urates	Lieber (1985b)

In rats, administration of ethanol lowers the levels of vitamin A in the liver (Sato & Lieber, 1981), an effect which is potentiated by, e.g., phenobarbital and butylated hydroxytoluene (Leo *et al.*, 1987).

(e) *Major toxic effects*

The single-dose acute oral toxicity (LD_{50}) of ethanol has been reported to be 6.2-17.8 g/kg bw in rats (Smyth *et al.*, 1941; Kimura *et al.*, 1971; Bartsch *et al.*, 1976), 8.3-9.5 g/kg bw in mice (Spector, 1956; Bartsch *et al.*, 1976), 5.6 g/kg bw in guinea-pigs (Smyth *et al.*, 1941), 6.3-9.5 g/kg bw in rabbits and 5.5-6.5 g/kg bw in dogs (Spector, 1956). The LD_{50} after a single intraperitoneal administration of ethanol was 4.3 g/kg bw in bats (Greenwald *et al.*, 1968) and 8.6 g/kg bw in mice (Forney *et al.*, 1963); 29-38% of mice given a single

intraperitoneal injection of 6.5 g/kg ethanol survived over 24 h (Macdonald *et al.*, 1977). An intraperitoneal LD_{50} of 5.0 g/kg was reported in rats (Barlow *et al.*, 1936; Bartsch *et al.*, 1976); however, it was reported that LD_{50} values decrease in male Wistar rats with age: the oral LD_{50} was 10.6 g/kg bw in three- to four-month-old rats and 7.06 g/kg bw in 10- to 12-month-old animals; the intraperitoneal LD_{50} was 6.71 g/kg bw and 5.10 g/kg bw at the two ages, respectively (Wiberg *et al.*, 1970).

(i) *Alterations in the liver*

Liver injury due to ethanol ingestion was initially attributed to malnutrition rather than to toxicity (Best *et al.*, 1949). Later, many experimental studies showed that ethanol can cause morphological alterations and effects on liver metabolism in well-fed animals (Lieber & DeCarli, 1974; Lieber *et al.*, 1975). [The Working Group noted that since ethanol interferes with the uptake of some vitamins from the gut in humans (see section 4.3(*e*)), it is unlikely that nutritional effects can be distinguished from the effects of ethanol *per se*.]

The degree and type of liver injury depend on a number of factors, including ethanol concentration, nutritional state, time of administration and type of animal. Liver changes may occur in several steps, from hepatomegaly, fatty liver, necrosis and fibrosis to cirrhosis.

Hepatomegaly and the accumulation of lipids in hepatocytes (steatosis) are the earliest manifestations of acute administration of ethanol. A significant increase in the lipid content of the liver of rats may be produced by a single dose of ethanol (Mallov & Bloch, 1956). The fatty acid composition of the lipids which accumulate resembles that of adipose tissue (Brodie *et al.*, 1961), which indicates that they are derived from extrahepatic tissue. Other possible mechanisms behind the acute fatty infiltration of the liver are related to the ethanol-induced increase in the ratio of NADH:NAD in the liver, which inhibits fatty acid oxidation (Lieber & Schmid, 1961; see Baraona, 1985) and may enhance the synthesis of glycerolipids (Nikkila & Ojala, 1963).

Subchronic and chronic administration of ethanol to rats also causes fatty liver (Lieber *et al.*, 1963), hepatocellular enlargement and a decrease in total liver tubulin (Baraona *et al.*, 1975, 1977, 1984). The latter result has been shown to be associated with the inhibition of tubulin polymerization and decreased formation of microtubules (Baraona *et al.*, 1977, 1981a; Matsuda *et al.*, 1979; Okanoue *et al.*, 1984), a phenomenon which may be mediated *via* acetaldehyde (Matsuda *et al.*, 1979). This conclusion is supported by the demonstration that disulfiram, which inhibits acetaldehyde oxidation, produces a further decrease in microtubules (Baraona *et al.*, 1981a).

Subchronic administration of ethanol to rats in a liquid diet was reported in one study to cause dose-related acidophilic degeneration, necrosis, formation of Mallory bodies and centrolobular steatosis (Holmberg *et al.*, 1986). The production of necrosis, inflammation and fibrosis is enhanced in rats when the ethanol-containing liquid diet is supplemented with either vitamin A (Leo & Lieber, 1983) or 4-methylpyrazole (Lindros *et al.*, 1983).

When rats were given ethanol by an intragastric intubation at levels rising from 8 to 15 g/kg bw per day (32-47% of total calories) over four months, together with a diet containing 25% of total calories as fat, focal necrosis, inflammation and fibrosis were observed in the liver. Serum levels of transaminases were also elevated. Alcoholic hepatitis and Mallory

bodies were not detected (Tsukamoto *et al.*, 1986). When ethanol was given to rats continuously by intragastric intubation at doses which resulted in blood concentrations of 2.16 ± 1.2 g/l, together with a low-fat diet (4.9% of total calories), progressive fatty infiltration of the liver was detected; after 30 days, one-third of the animals also showed focal necrosis with infiltration of macrophages in the liver (Tsukamoto *et al.*, 1985).

It has not been possible to reproduce hepatitis or cirrhosis by feeding ethanol to rats (Salaspuro & Lieber, 1980). A high-fat diet together with alcohol has been shown to produce fatty liver in dogs, and four of 16 animals developed cirrhosis (Connor & Chaikoff, 1938).

Advanced alcoholic liver injury has also been produced in primates. All of nine baboons fed ethanol at 50% of total calories developed fatty liver, and four animals developed hepatitis within nine to 12 months (Lieber *et al.*, 1975). Of 18 baboons fed ethanol, 15 developed focal fibrous septa, five with diffuse septal fibrosis, of which four proceeded to septal cirrhosis and one each to micronodular and to mixed micro-macronodular cirrhosis; cirrhosis can occur in baboons without the polymorphonuclear inflammation characteristic of human alcoholic hepatitis (Popper & Lieber, 1980). The production of liver fibrosis in baboons fed ethanol has been confirmed, even in studies in which choline was given in excess (Nakano & Lieber, 1982; Lieber *et al.*, 1985; Miyakawa *et al.*, 1985); however, long-term feeding of ethanol produced fatty infiltration but not liver fibrosis in monkeys (Mezey *et al.*, 1983).

In rats, large single or multiple doses of ethanol (5-6 g/kg bw) produced lipid peroxidation in the liver, as measured by malonaldehyde production (Di Luzio & Hartman, 1967; Macdonald, 1973; Valenzuela *et al.*, 1980; Videla *et al.*, 1980), whereas smaller single doses (≤3 g/kg) had no effect (Hashimoto & Recknagel, 1968). However, after long-term administration of ethanol (36% of total calories for five to six weeks) to rats, a single 3-g/kg bw dose of ethanol caused increased hepatic diene conjugation; diene conjugation could be prevented, in part, by the administration of methionine (Shaw *et al.*, 1981).

(ii) *Alterations at other sites*

Ethanol can, depending on dose, irritate the oral cavity, oesophagus and gastric mucosa in experimental animals. Tissue damage has been observed in the gastric epithelium of mice (Eastwood & Kirchner, 1974) and in the small intestinal mucosa of rats (Baraona *et al.*, 1974). An increase in cell proliferation in rat oesophageal epithelium was found after ethanol had been fed in a liquid diet (Mak *et al.*, 1987). Cellular proliferation has also been observed in the gastrointestinal tract of rats and dogs after repeated administration of ethanol (Willems *et al.*, 1971; Baraona *et al.*, 1974).

A significant enhancement of rectal-cell production and an extension of the proliferative compartment in rectal crypts following chronic ethanol ingestion has been demonstrated in rats (Simanowski *et al.*, 1986).

(iii) *Effect of vitamins*

Consumption of ethanol under conditions of vitamin A deficiency increased the incidence of squamous metaplasia in rat trachea. Abnormal cilia and increased numbers of

lysosomes in ciliated cells were also observed in rats depleted of vitamin A and administered ethanol (Mak *et al.*, 1984).

(iv) *Effect on hormones*

In male rats, plasma testosterone and luteinizing hormone levels fell following ethanol administration (Cicero *et al.*, 1977, 1979). Chronic ethanol ingestion produced testicular atrophy in rats (see Gavaler & Van Thiel, 1987); in addition, ethanol reduced the levels of luteinizing hormone as well as the receptor for the hormone on the Leydig cells of rat testis (Bhalla *et al.*, 1979).

In female mice, rats, rabbits and monkeys, subchronic and chronic administration of ethanol results in significant disturbances of the oestrous cycle, ovulatory function and fertility (see Gavaler & Van Thiel, 1987 and below).

(v) *Hepatic and pancreatic foci*

Effects on ATPase-deficient foci were investigated in six-week-old female Wistar rats administered 10% ethanol in water as the drinking fluid (daily intake, 8 g/kg bw) either continuously during intragastric administration of NDEA (3 mg/kg bw) or *N*-nitrosomorpholine (NMOR; 40 mg/l in the drinking-water; daily intake, 5 mg/kg bw) on four days per week, or alternately with the carcinogens (on the remaining three days per week), for 11 or 15 weeks, respectively. Another group of 12-week-old rats received 3 mg/kg bw NDEA in a 3-ml aqueous solution by stomach tube three times a week for nine weeks, followed subsequently by 10% ethanol in water as the drinking fluid continuously or intermittently (at weekly intervals) for 16 weeks. Fourteen days after the termination of treatment, both the size and number of ATPase-deficient foci in the liver were increased in animals that had received ethanol continuously or intermittently during NDEA or NMOR administration, but no such enhancement was obtained in animals that had received ethanol after the cessation of carcinogen treatment (Schwarz *et al.*, 1983, 1984). [The Working Group noted that a single marker was used for preneoplastic foci.]

In male Sprague-Dawley rats that received ten intragastric intubations of 0.38 mg/kg bw aflatoxin B_1, followed five days later by a low-fat (11% of calories as corn oil) or high-fat (35% of calories as corn oil) diet containing ethanol (35% of calories) for 12 weeks, no difference in the incidence of γ-glutamyl transferase-positive foci was observed in rats in either group (Misslbeck *et al.*, 1984). [The Working Group noted that a single marker was used for preneoplastic foci.]

A significant increase in the number of acidophilic and basophilic acinar-cell foci was observed in the exocrine pancreas of male weanling SPF Wistar rats that received 15% ethanol (v/v) in the drinking-water for 17 weeks, as compared to controls not receiving ethanol, after a single intraperitoneal injection of 30 mg/kg bw azaserine followed by a low-fat (5% corn oil) or high-fat (25% corn oil) diet. There was a positive interaction between dietary fat and ethanol in terms of the size of basophilic foci (Woutersen *et al.*, 1986).

No modulating effect of ethanol was observed after 17 weeks on the number or size of putative preneoplastic cystic or ductular exocrine pancreatic lesions in male, weanling

Syrian golden hamsters that received subcutaneous injections of 20 mg/kg bw *N*-nitrosobis(2-oxopropyl)amine at six and seven weeks of age and were fed low-fat (5% corn oil) or high-fat (25% corn oil) diets with or without 15% ethanol (v/v) in water as the drinking fluid (Woutersen *et al.*, 1986).

(*f*) *Effects on reproduction and prenatal toxicity*

The effects of acetaldehyde, a metabolite of ethanol, on reproduction and prenatal toxicity have been reviewed. Fetal malformations and resorptions were found in rats and fetal malformations were found in mice treated with acetaldehyde (IARC, 1985).

(i) *Reproductive effects*

The effects of ethanol on reproduction have been reviewed recently (Gavaler & Van Thiel, 1987). Studies in mice and rats have shown effects on the testis and on other reproductive tissues but generally have not shown an effect on reproductive performance.

When female C57Bl/Crgl *mice* were given 10% ethanol (v/v) in water as the drinking fluid before mating, throughout gestation and lactation, no significant effect on reproductive capacity or pup development or behaviour was seen (Thiessen *et al.*, 1966).

When female Wistar *rats* were given 20-25% of the calories consumed as 12% ethanol in a sucrose solution as the drinking fluid before mating and throughout gestation and lactation, there was no effect on reproductive performance or on development of offspring (Oisund *et al.*, 1978). Offspring of female Sprague-Dawley rats that were administered ethanol in water as the drinking fluid at a concentration of 20% v/v for four weeks before mating and at a concentration of 30% v/v during gestation were physically and developmentally retarded and failed to catch up with control offspring during the first four weeks postpartum (Leichter & Lee, 1979).

In a study in which male C57Bl/6J *mice* were given 5 or 6% (v/v) ethanol in a liquid diet for 70 days or 35 days, respectively, there was a significant decrease in testicular weight and in seminal vesicle/prostate weight, an increase in the frequency of germ-cell desquamation, inactive seminiferous tubules, inhibition of in-vitro fertilization of mouse oocytes by epididymal spermatozoa, as well as a significant decrease in the total number of motile sperm. During a ten-week recovery period, improvement was greater in the group given 5% than in those given 6% ethanol (Anderson *et al.*, 1985). Preparation of sperm from the cauda epididymis five weeks after oral administration of ethanol (1, 2 or 4.0 ml/kg bw) to male (CBA × Balb/c)-F_1 mice five times daily did not show sperm anomalies (Topham, 1980, 1983). Addition of ethanol to ram spermatozoa (0.62 M; 15 μl in 0.4 ml semen samples containing 2-dioxy-D-glucose) inhibited sperm motility (Mayevsky *et al.*, 1983).

There is evidence *in vitro* and *in vivo* that ethanol is toxic to animal and human Leydig cells and seminiferous tubules (Gavaler & Van Thiel, 1987; Van Thiel *et al.*, 1983). Male Sprague-Dawley *rats* maintained on a liquid diet containing 6% ethanol (95% v/v) for one week followed by four weeks on a 10% ethanol liquid diet showed adverse effects on sex organs (testes, seminal vesicles, ductules) as well as a significant decrease in serum testosterone levels (Klassen & Persaud, 1978). Male Sprague-Dawley rats that received an

intraperitoneal injection of 2.5 g/kg bw ethanol showed a significant decrease in the levels of luteinizing hormone and testosterone and marked attenuation of testicular steroidogenesis (Cicero *et al.*, 1979).

Exposure of male *rats* [strain unspecified] to ethanol *in utero* or as neonates by administration of a liquid diet containing ethanol (36% of total calories) resulted in adverse effects on gonadal growth and development and disturbances in their sexual behaviour and performance when adult (Parker *et al.*, 1984). Subcutaneous administration of 7.9 g/kg bw ethanol to female CD rats inhibited ovulation, primarily by blocking ovulatory surges of luteinizing hormone (Kieffer & Ketchel, 1970). Blood levels of luteinizing hormone varied with dose and timing of treatment, but ethanol administered by intraperitoneal injection increased the secretion of prolactin by female Wistar rats (Alfonso *et al.*, 1985). Exposure of Sprague-Dawley rats to ethanol (average, 11.6 g/kg bw) *in utero* altered the adult patterns of luteinizing hormone secretion in male and female offspring, indicating an effect on the central mechanisms that control secretion of pituitary luteinizing hormone (Handa *et al.*, 1985). Administration of 5% ethanol (36% of total calories) in a liquid diet to female Wistar rats for 49 days decreased ovarian weight by 60% and significantly decreased plasma oestradiol-17β levels and the development of oestrogen target organs (Van Thiel *et al.*, 1978). Ovarian function in female Holtzman rats, 20 days of age, was suppressed by feeding of liquid diets containing 5% ethanol (36% of total caloric intake) for up to 55 days, in which blood ethanol concentrations averaged 2.5 g/l, but not by 2.5% ethanol (Bo *et al.*, 1982).

Vaginal opening was delayed in female Holtzman rats fed a liquid diet containing 5% ethanol for eight or 16 weeks. Among rats treated for 16 weeks, irregular oestrous cycles and cycles longer than those in controls were observed. Mating of these females with untreated males resulted in no adverse effect on fertility, litter size or neonatal body weight (Krueger *et al.*, 1982).

In female macaque *monkeys* that administered ethanol to themselves intravenously on a schedule of reinforcement used for food acquisition, providing 2.9-4.4 g/kg bw ethanol per day for three to 6.5 months, amenorrhoea, atrophy of the uterus, decreased ovarian mass and significant decreases in luteinizing hormone levels were observed (Mello *et al.*, 1983). In female rhesus monkeys infused intravenously with 2-4 g/kg bw ethanol after spontaneous onset of labour or following the induction of labour by infusion of oxytocin, partial suppression of labour was observed only in preterm animals with irregular uterine contractions (Horiguchi *et al.*, 1971).

(ii) *Male-mediated developmental effects*

Male C3H *mice* were fed ethanol (20 or 30% of total calories) in a liquid diet and, after four weeks of treatment, were mated to untreated females. The resulting litters showed no change in the number of implants, prenatal mortality, fetal weight, sex ratio or soft-tissue malformations (Randall *et al.*, 1982).

Male Sprague-Dawley *rats* maintained for six weeks on a liquid diet containing 10% ethanol were paired with untreated females. There was body weight loss and central nervous system impairment, and only half of the treated animals had successful matings, compared to all of the controls. There was a decrease in litter size and an increase in prenatal mortality

among the litters (Klassen & Persaud, 1976). When 20% ethanol in water (v/v) was given as the drinking fluid for 60 days to male Long-Evans rats, which were mated with untreated females one to three weeks after cessation of treatment, the incidence of congenital malformations in the offspring was increased (Mankes *et al.*, 1982).

(iii) *Placental transfer*

CD-1 *mice* received intraperitoneal injections of 4, 6 or 7 g/kg bw ethanol on gestation day 10. Ethanol crossed the placenta rapidly and was found in the embryo 5 min after dosage. The blood ethanol concentrations in maternal blood and liver were dose dependent; acetaldehyde was detectable in maternal blood at all dose levels and in maternal liver and embryos after administration of 7 g/kg. Within 5 min after intraperitoneal injection of pregnant dams with 200 mg/kg bw acetaldehyde on gestation day 10, acetaldehyde was detectable in the embryos. Thus, both ethanol and acetaldehyde were found to be accessible to the embryo during a sensitive period of organogenesis (Blakley & Scott, 1984a).

Sprague-Dawley *rats* received an intraperitoneal dose of 2 g/kg bw ethanol four to five days before term. Maternal and fetal ethanol levels were similar; the concentration of acetaldehyde in maternal blood was four times that in the placenta, and none was found in fetal tissues (Kesäniemi & Sippel, 1975). With similar analytical techniques, acetaldehyde was found in fetal blood 150 min after intravenous or oral administration of 2.5 g/kg bw ethanol to pregnant Wistar rats on gestation day 21. Ethanol concentrations in the fetal compartment were found to be similar to those in the mother's blood 150 min after dosage. The authors concluded that, since ADH activity is very low in the fetus, the acetaldehyde was probably transported across the placenta (Espinet & Argilés, 1984). In a subsequent study with improved methods of analysis, the acetaldehyde levels were much higher (~five fold) in maternal blood than in either umbilical or fetal blood of rats (Gordon *et al.*, 1985).

Intraperitoneal injection of golden *hamsters* with 1.5 g/kg bw ^{14}C-labelled ethanol on gestation days 6 or 15 or intravenous administration of 0.5 g/kg bw ^{14}C-labelled ethanol to cynomolgus *monkeys* during the last 30 days of pregnancy resulted in transfer of ^{14}C-labelled ethanol to the fetus in both species (Ho *et al.*, 1972).

Dorset *ewes* were infused intravenously with a total dose of 15 ml/kg bw of 9.75% ethanol over a period of 1-2 h on gestation days 121-138. Diffusion across the placenta was rapid, and blood ethanol concentrations in the maternal and fetal compartments reached about 2.3 g/l (Mann *et al.*, 1975).

Ewes were given six doses of 0.8 ml/kg bw ethanol intravenously between days 125 and 135 of gestation. At dose levels of ethanol sufficient to cause about 2 g/l maternal and fetal blood ethanol, there was rapid depression of fetal myocardial contractility, which was maintained for several hours after cessation of ethanol administration (Lafond *et al.*, 1985).

Cynomolgus and rhesus *monkeys* were administered 0.8-1.5 g/kg bw ethanol intravenously over a period of 30 min on days 106-160 of gestation. The rate of clearance from the fetus paralleled that of the mother and was determined by the rate of elimination from the mother (Hill *et al.*, 1983). Similar findings were reported by Horiguchi *et al.* (1971).

(iv) *Preimplantation effects*

In a study on the effects of preimplantation exposure, RAP strain *mice* were given ethanol intravenously on days 3 and 4 of pregnancy and offspring were examined on day 19 of pregnancy. Mean fetal and placental weights were significantly lowered, but there was no effect on skeletal development (Checiu & Sandor, 1986).

Treatment of $(C_3H \times C57Bl)F_1$ female *mice* with a single dose of 1 ml of 12.5% ethanol by gavage 2 h after a 30-min mating period produced an increase in late (after day 11) fetal deaths. The same treatment given 1 h after mating did not produce this effect. The authors proposed that the effect was due to a specific action on the fertilized ovum at the time of second meiotic division, causing aneuploidy (see also pp. 138-139), but the numbers of embryos available for examination in this study were inadequate to confirm this hypothesis (Washington *et al.*, 1985).

Preimplantation effects were studied by the examination of uterine contents of albino *rats* following consumption of plum brandy (reported as 24% ethanol) or cognac (reported as 20% ethanol) for 40-45 days before mating and during pregnancy until the rats were killed on day 5. Development was retarded, and there was an increased number of pathological morulae and blastocysts (Fazakas-Todea *et al.*, 1985).

(v) *Fetotoxicity and structural teratology*

Adverse development and teratogenic effects caused by ethanol have been reviewed (Chernoff, 1979; Abel, 1980; Streissguth *et al.*, 1980; Abel, 1985a).

Groups of C3H *mice* were given a liquid diet or a fortified liquid diet, each either alone or with 4.1% w/v ethanol, from days 0-17 of pregnancy; a further group was given an amount of liquid diet equal to that consumed by the group given liquid diet plus ethanol. Ethanol consumption inhibited fetal growth and development but did not affect litter size, irrespective of the diet used (Goad *et al.*, 1984). Retardation of muscle growth was seen in offspring killed at 12 weeks of age of inbred mice [strain unspecified] given 10-20% ethanol in the drinking-water for 11 weeks before mating and 30% ethanol after breeding until delivery. Prenatally, there was suppression of hyperplasia of muscle fibres during myogenesis; postnatally, there was suppression of normal hypertrophy of individual muscle fibres (Ihemelandu, 1984).

Some studies in several *mouse* strains have shown no teratogenic effect, even at dose levels providing blood ethanol concentrations of 2 g/l or higher. CF-1 (Schwetz *et al.*, 1978), CD-1 (Hood *et al.*, 1979) and C3H (Lochry *et al.*, 1982) mice given ethanol orally or in the drinking fluid had pups with minor skeletal variants or decreased fetal body weight, but there was no increase in resorptions or malformations. Other studies in *mice* showed teratogenic effects and resorptions, typically at blood ethanol concentrations in excess of 2 g/l. The effects, such as fetal resorptions, intrauterine growth retardation, cleft palate, altered craniofacial development and exencephaly, limb defects and heart defects, varied with the strain of mice, mode of administration and stage of gestation at which ethanol was administered (Kronick, 1976; Chernoff, 1977; Randall *et al.*, 1977; Boggan *et al.*, 1979; Randall & Taylor, 1979; Chernoff, 1980; Giknis *et al.*, 1980; Rasmussen & Christensen, 1980; Webster *et al.*, 1980; Sulik *et al.*, 1981; Bannigan & Burke, 1982; Checiu & Sandor,

1982; Sulik & Johnston, 1983; Padmanabhan *et al.*, 1984; Stuckey & Berry, 1984; Webster *et al.*, 1984; Martinez *et al.*, 1985; Padmanabhan & Muawad, 1985; Daft *et al.*, 1986).

In a study to evaluate the role of zinc deficiency in the developmental toxicity of ethanol, CBA/J *mice* were given a liquid diet, either fortified with zinc or deficient in zinc, and ethanol (15 or 20% of total calories). Zinc deficiency potentiated the ethanol-induced increase in resorptions and external malformations and the decrease in fetal weight (Keppen *et al.*, 1985). Similarly there was an increase in the incidence of both external and internal malformations in C57Bl/6 mice given a marginally zinc-deficient diet and ethanol during gestation, in comparison with mice given a control diet and with mice treated with ethanol alone (Miller, S.I. *et al.*, 1983).

Long-Evans *rats* administered 6 g/kg bw ethanol orally on gestation days 5-19 had blood ethanol concentrations of over 2.6 g/l. Fetuses had decreased body weight, increased body water and sodium content and decreased lipid-free solid content (Abel & Greizerstein, 1979).

Sprague-Dawley *rats* were given 15 or 25% ethanol-derived calories in liquid diets 20 days before mating, throughout mating and until gestation day 19; additional groups were pair-fed an isocaloric diet. There was decreased caloric intake in the group given 25% ethanol-derived calories and in the pair-fed controls, and in both of these groups there were associated decreases in fetal body weight, organ weights and DNA and protein contents compared to the pair-fed controls of the group given 15% ethanol-derived calories. The effects of 15% ethanol-derived calories were attributed to ethanol, while the effects of 25% ethanol-derived calories were attributed partly to decreased caloric intake (Sorette *et al.*, 1980).

Sprague-Dawley *rats* were provided with 18, 25 and 32% protein-derived calories and 36% ethanol-derived calories in a liquid diet on gestation days 1-21. The maternal ethanol blood levels were 1.5-2 g/l. Ethanol caused a significant decrease in fetal body weight and brain weight but an increase in relative brain weight, irrespective of the protein content of the diet (Weinberg, 1985).

Sprague-Dawley *rats* received 15% ethanol in drinking-water on days 6-15 of gestation. Decreased maternal weight gain and fetal growth retardation but no teratogenic effect were observed. The maximal blood ethanol concentration was about 400 mg/l (Schwetz *et al.*, 1978). The offspring of Sprague-Dawley rats given 20% ethanol in the drinking-water four weeks before mating and 30% ethanol in drinking-water until gestation day 20 had retarded skeletal development and decreased body weight but no gross malformation (Lee & Leichter, 1983). Following oral administration of 4 g/kg bw ethanol to Sprague-Dawley rats twice daily for three-day periods between days 7 and 15 of gestation, an increased incidence of resorptions and a marginal effect on fetal body weight but no teratogenic effect were observed (Fernandez *et al.*, 1983). Long-Evans rats were administered 5% ethanol in drinking-water for 90 days prior to mating and during gestation or during gestation only. The effects were similar. No gross malformation was observed, but there was a significant decrease in fetal body weight (Samson, 1981).

In contrast to studies in which gross malformations were not observed, polydactyly and polysyndactyly were reported in the offspring of Sprague-Dawley *rats* given 5 g/kg bw (but not in those given 6 g/kg bw) per day ethanol orally on gestation days 1-15 or 1-20. Maximal blood ethanol concentrations of 2.5-3.25 g/l were reported with the two doses (West *et al.*, 1981a). In Long-Evans rats given 4 ml/kg bw ethanol as a single oral dose between days 6 and 15 of gestation, a variety of gross malformations was reported in 72-100% offspring compared to 12% of controls (Mankes *et al.*, 1983).Prenatal treatment of Long-Evans *rats* with 35% ethanol-derived calories in a liquid diet shortened the umbilical cord (Barron *et al.*, 1986).

When Sprague-Dawley *rats* were given ethanol as 35% of total calories in a liquid diet on gestation days 1-21, offspring had abnormal distribution of nerve fibres in the temporal regions of the hippocampus, which persisted to maturity (West *et al.*, 1981b). Sprague-Dawley rats were exposed *in utero* and/or postnatally to ethanol as 36% of total calories in a liquid diet from gestation day 16 until postnatal day 14 or from birth until postnatal day 14. The sexually dimorphic nucleus in the preoptic area of the brain of adult male offspring was significantly decreased in volume (Rudeen *et al.*, 1986). In hooded rats given a liquid diet containing 37% ethanol-derived calories from day 6 of gestation to time of birth (gestation day 23 for ethanol-exposed rats; day 22 for controls), delayed and extended period of cortical neuron generation, reduced number of neurons and altered distribution of neurons were seen (Miller, 1986).

Sprague-Dawley *rats* were exposed by inhalation to concentrations of up to 20 000 ppm (37 800 mg/m^3) ethanol for 7 h per day on gestation days 1-19; blood levels as high as 1.5-2 g/l were reported. At 20 000 ppm, the dams showed signs of narcosis and had decreased food consumption; the incidence of malformations in the offspring was of borderline significance. At 16 000 and 10 000 ppm (30 240 and 19 000 mg/m^3), corresponding to blood levels of 40-80 mg/100 ml and 3 mg/100 ml, respectively, there was no increase in malformations (Nelson *et al.*, 1985a).

The fetuses of New Zealand white *rabbits* given 15% ethanol in the drinking-water on gestation days 6-18 showed no adverse effect. The maximal blood ethanol concentration in the mothers was 250 mg/l (Schwetz *et al.*, 1978).

When *ferrets* were administered 1.5 g/kg bw ethanol daily as a 25% solution orally on days 15-35 of gestation, there was a significant increase in the number of fetuses and litters with malformations but no effect on fetal weight or resorptions. The peak blood ethanol concentration was 2 g/l (McLain & Roe, 1984).

Dogs were administered 1.8 g/kg bw ethanol as a 25% solution by gavage twice daily and were given either a normal-protein or low-protein diet throughout gestation. Ethanol consumption and low dietary protein intake, independently of each other, significantly decreased maternal weight gain as well as the weight of the neonates (Switzer *et al.*, 1986).

Oral administration of 3 or 3.6 g/kg bw ethanol to *dogs* by gavage throughout gestation resulted in no gross or histological abnormality, a slight decrease in the number of offsprings per litter and in pup weight, and an increase in the number of still births. Blood ethanol concentrations were 1.3-1.75 g/l (Ellis *et al.*, 1977).

In miniature *swine* given 20% ethanol in drinking-water (>3 g/kg bw/day) as gilts (18 months old) or sows (three years old), there was a significant decrease in mean litter size and in the birth weight of piglets and a significant increase in the incidence of multiple malformations (Dexter *et al.*, 1980).

Cynomolgus *monkeys* administered up to 5 g/kg bw ethanol daily on gestation days 20-150 revealed an increase in pregnancy wastage (abortions and still births) but no structural malformation or facial change (Scott & Fradkin, 1984). Pregnant pigtailed macaque monkeys were administered 0.3-4.1 g/kg bw ethanol by gavage once a week throughout gestation starting either before day 10 or on day 40. Spontaneous abortion frequency increased at peak plasma ethanol concentrations above 2 g/l. Developmental alterations were observed consistently in offspring of monkeys with blood levels greater than 1.5 g/l when treatment was initiated at the start of gestation; infants exposed only after gestation day 40 were less consistently abnormal despite higher blood ethanol levels (5.5 g/l; Clarren *et al.*, 1987a,b). In rhesus and cynomolgus monkeys given 3 g/kg bw ethanol intravenously over 1-2 min on gestation days 120-147, transient but marked collapse of umbilical vasculature was observed within 15 min. This resulted in severe hypoxia and acidosis in the fetus, but recovery occurred during the succeeding hour (Mukherjee & Hodgen, 1982).

When ethanol is given in combination with other chemicals which tend to increase the blood level of ethanol by reducing its metabolism, e.g., 4-methylpyrazole (Blakley & Scott, 1984b) and pyrazole (Varma & Persaud, 1979), the teratogenic and fetotoxic effects are increased. Administration of ethanol with chemicals that tend to increase the acetaldehyde level, however, e.g., disulfuram (Webster, W.S. *et al.*, 1983), does not increase the teratogenicity of ethanol.

Combined administration of ethanol and metronidazole to Swiss-Webster *mice* increased the number of resorptions, decreased fetal body weight and had a marginal effect on the incidence of malformations (Giknis & Damjanov, 1983).

Administration of ethanol in combination with an unspecified extract of marijuana containing Δ^9-tetrahydrocanabinol to Swiss-Webster *mice* by subcutaneous injection on days 1-15 of gestation and to Long-Evans *rats* intragastrically on days 7-15 of gestation produced a significant decrease in maternal weight gain and an increased incidence of resorptions. In both species, the incidence of resorptions was increased with marijuana alone, but the increase was more than additive with the combination of marijuana and ethanol (Abel, 1985b).

Ethanol increased the incidence of cleft palate in Swiss *mice* administered methylmercuric chloride and retinyl acetate (Lee, 1985). Ethanol in combination with lithium carbonate had a synergistic effect on the induction of fetal abnormalities in albino rats (Sharma & Rawat, 1986).

(vi) *Behavioural and functional teratology*

Male and female Sprague-Dawley *rats* were exposed by inhalation to concentrations of 10 000 or 16 000 ppm (19 000 or 30 240 mg/m^3) ethanol for 7 h per day; males were exposed for six weeks before mating and females throughout gestation. With 16 000 ppm, the blood

ethanol concentration was about 500 mg/l. There was no physical or behavioural difference among offspring of treated and control animals. Behavioural testing included rotorod, open-field and wheel activity and avoidance conditioning tests (Nelson *et al.*, 1985b).

In a study of Long-Evans hooded *rats* gives daily doses of 1 or 2 g/kg bw ethanol orally throughout gestation, there were decreases in litter size, litter weight and mean pup weight, but no gross malformation or evidence of behavioural teratogenic effects (Abel, 1978).

In other studies, the most frequently reported behavioural teratogenic effect was alteration in motor activity. When Long-Evans hooded *rats* were administered 4 or 6 g/kg bw ethanol daily througout gestation, there was decreased litter weight but not litter size at birth and increased postnatal mortality. Motor activity of neonates raised by surrogate mothers was impaired at 16 and 20 days of age (Abel & Dintcheff, 1978). Increased motor activity was also reported in offspring of Long-Evans hooded rats given liquid diets containing ethanol (35% of total calories) on gestation days 6-20 (Zimmerberg *et al.*, 1986). A significant increase in motor activity was reported at 16 days of age among offspring of Wistar rats given liquid diets containing 5% ethanol on days 6-19 of gestation. There was no significant effect on length of gestation, litter size at birth or pup weight from birth up to 28 days of age (Bond, 1986).

Offspring of pregnant Long-Evans *rats* administered liquid diets containing ethanol (35% of total calories) during days 6-20 of gestation exerted a lower maximal suckling pressure, spent less time suckling during test sessions and displayed an altered suckling pattern (Rockwood & Riley, 1986).

Confirming evidence of behavioural teratogenic effects has been reported in rats (Viirre *et al.*, 1986; Vorhees & Fernandez, 1986) and mice (Yanai & Ginsburg, 1976; Randall *et al.*, 1986).

Open-field activity was significantly increased in the offspring of ethanol-treated miniature *swine* treated as described on p. 132 (Dexter *et al.*, 1980).

(vii) *In-vitro studies*

In studies with explanted cultured embryos from rats and mice, demonstrating the effect of ethanol on embryonal development independent of maternal metabolism, growth retardation and malformations were seen following exposure to ethanol in the culture medium. Adverse effects were generally found at concentrations of 1.5 g ethanol/l medium or greater (Brown *et al.*, 1979; Sandor *et al.*, 1980; Priscott, 1982; Wynter *et al.*, 1983). Acetaldehyde has been shown to have similar effects (IARC, 1985).

(viii) *Other effects*

In Sprague-Dawley *rats* given 30% ethanol-derived calories in a liquid diet throughout gestation, reduced placental transfer of the glucose analogue 2-deoxyglucose was observed (Snyder *et al.*, 1986). There is also evidence that administration of ethanol to rats during gestation decreases placental transport of amino acids (Henderson *et al.*, 1981; Snyder *et al.*, 1986) and sodium-potassium ATPase activity (Fisher *et al.*, 1986).

In Sprague-Dawley *rats* exposed to increasing concentrations of ethanol in a liquid diet (up to 36% of ethanol-derived calories) before and during gestation, there was a significant

increase in placental weight and a decrease in membrane-associated placental folate receptor activity. The authors considered this to be evidence for the role of placental toxicity in ethanol-associated intrauterine growth retardation (Fisher *et al.*, 1985).

(ix) *Effects on sexual differentiation*

When Long-Evans *rats* were given 10% ethanol in drinking-water from gestation day 7 to delivery, gestation was prolonged, and offspring of each sex showed decreased anogenital distances at birth. Pups nursed by ethanol-drinking mothers had a significantly earlier preputial separation, but there was no effect on adult masculine sex behaviour, plasma testosterone or weights of accessory sex glands (Chen & Smith, 1979).

When Sprague-Dawley *rats* were given liquid diets containing ethanol (35% of total calories) on day 7 of gestation through parturition, absence of sexual dimorphism (saccharin preference and maze learning) was seen among offspring, suggesting disrupted perinatal androgen status (McGivern *et al.*, 1984). In the offspring of Long-Evans hooded rats given a liquid diet containing 35% ethanol-derived calories on gestation days 6-20, males showed feminized behaviour and females showed masculinized behaviour, suggesting disruption of the hormonal environment prenatally (Meyer & Riley, 1986). No evidence of altered sexual dimorphism in saccharin preference was found among offspring of Long-Evans hooded rats administered 3.5 g/kg bw ethanol twice daily intragastrically on gestation days 11-21 (Abel & Dintcheff, 1986).

Among offspring of Long-Evans *rats* fed liquid diets containing 35% ethanol-derived calories during gestation days 6-20, there was evidence of behavioural deficits, which persisted until adulthood. Female offspring showed a variety of deficits in maternal behaviour when adult, which may have been related to prenatal hormonal alterations (Barron & Riley, 1985).

In male progeny of Wistar *rats* given ethanol in a liquid diet (36% of total calories) from gestation day 12 to ten days postpartum, there was decreased anogenital distance; the weights of the testes and seminal vesicles/prostate were decreased 55 and 110 days postpartum; serum testosterone and luteinizing hormone levels were decreased on day 55 but not on day 110; and sexual motivation and performance were reduced. The authors concluded that there was less phenotypic masculinization at birth in the treated offspring (Udani *et al.*, 1985).

(x) *Lactational and postnatal effects*

Ethanol and acetaldehyde were measured in milk and peripheral blood of Wistar *rats* given ethanol as 36% of total calories in a liquid diet. Ethanol levels in the blood increased slightly (26-29 mmol/l; 1.2-1.3 g/l) from day 5 to day 15 of lactation, and levels of acetaldehyde increased from 41 to 53 μM/l (1.9-2.4 mg/l). The concentration of acetaldehyde in milk was 50% of that in the blood, while the concentration of ethanol was 44-80% of the blood level. Blood ethanol concentrations in suckling pups (around 0.3 μM/l, 14 mg/l) were much lower than those in maternal blood but increased after day 15; by the end of lactation, some pups had started to consume the liquid diet (Guerri & Sanchis, 1986). Increases in pH, protein and lipids and a decrease in lactose were seen in milk from

ethanol-treated Wistar rats (Sanchis & Guerri, 1986). Sprague-Dawley rats receiving 5% ethanol in drinking-water during the second half of pregnancy showed decreased mammary gland development; when they were treated after parturition, no effect was observed (Jones & Stewart, 1984).

Pups of Sprague-Dawley *rats* were given feed containing ethanol (6.6-9.8 g/kg bw per day) by gavage on postnatal days 4-10. An oral dose of 7.4 g/kg bw gave a blood ethanol concentration of 1.6 g/l. At dose levels of 7.4 g/kg bw and above, microencephaly was observed on day 10 (Pierce & West, 1986). In Sprague-Dawley rats administered 6 and 10% ethanol in the drinking-water during lactation, blood ethanol concentrations were about 190 and 410 mg/l, respectively. A significant decrease in neonatal body weight gain was observed. However, when neonates were given ethanol directly by exposure to vapour at concentrations of 3.5-4.2% v/v, providing blood ethanol concentrations of more than 2.5 g/l, there was only minimal growth retardation (Swiatek *et al.*, 1986).

Sprague-Dawley *rats* administered 4 g/kg bw ethanol per day orally on days 6-16 of age or 6 g/kg bw ethanol on day 6 showed significant decreases in whole brain weight, in cerebellar weight and in the number of cerebellar cells, and altered balancing ability on days 17 and 70 postnatally (Burns *et al.*, 1986). Exposure of Sprague-Dawley rats to high levels of ethanol (7.2-12.0 g/kg bw per day) during days 4-8 of age decreased the weights of the brain, cerebral cortex and cerebellum, increased receptor affinity and decreased the number of neurotransmitter receptors (Serbus *et al.*, 1986).

(g) *Genetic and related effects*

Ethanol

The genetic effects of ethanol were reviewed recently (Obe & Anderson, 1987).

(i) *Prokaryotes*

In the *rec*-type repair test with *Proteus mirabilis*, ethanol (0.1 ml per plate) was inactive in the absence of an exogenous metabolic system (Braun *et al.*, 1982). Rapid lysis mutants in bacteriophage T4D were not induced by ethanol (up to 130.4 μl/ml) in the absence of an exogenous metabolic system, but the yield of phages was reduced by concentrations of ethanol greater than 69.7 μl/ml (Kvelland, 1983). In DNA repair tests with different strains of *Escherichia coli* WP2, ethanol gave very weak positive results at 5 mg/well (De Flora *et al.*, 1984a,b). It was not mutagenic in the presence or absence of an exogenous metabolic system in *Salmonella typhimurium* strains TA97, TA98, TA100, TA1535, TA1537 or TA1538 (McCann *et al.*, 1975; Cotruvo *et al.*, 1977; Arimoto *et al.*, 1982; Blevins & Taylor, 1982; Blevins & Shelton, 1983; De Flora *et al.*, 1984a,b).

In the presence of an exogenous metabolic system, De Flora *et al.* (1984a,b) consistently found a small (approximately two-fold) increase in the number of revertants in *S. typhimurium* strain TA102 in the presence of 200 and 300 μl ethanol (160 and 240 mg/plate). [The Working Group noted that *S. typhimurium* strain TA102 is considered to respond to the presence of oxygen radicals.]

(ii) *Plants*

Ethanol (up to 0.5 M) produced chromosomal aberrations in root-tip meristems of *Vicia faba* (Michaelis *et al.*, 1959; Rieger & Michaelis, 1960, 1961, 1970, 1972; Schubert *et al.*, 1979; Rieger *et al.*, 1982, 1985); exclusively chromatid-type aberrations were induced dose-dependently. Treatment of roots with ethanol (0.2 M) led to a significant elevation of sister chromatid exchange frequencies (Schubert *et al.*, 1979). Anomalies of anaphases and micronuclei were not observed when *Allium cepa* root-tip cells were exposed to up to 0.17 M ethanol, but sister chromatid exchanges were induced dose-dependently following exposure of roots (Cortés *et al.*, 1986). Exposure of cuttings of *Tradescantia paludosa* to ethanol (5-12.5%) for 6 h in a nutrient solution led to micronuclei in tetrads 24 h after exposure (Ma *et al.*, 1984).

(iii) *Fungi*

In *Saccharomyces cerevisiae*, ethanol produced respiration-deficient (or petite) mutants (Bandas & Zakharov, 1980; Bandas, 1982; Cabeça-Silva *et al.*, 1982; Hamada *et al.*, 1985). When cells were treated and held at 4°C in water instead of at 30°C in complete medium, ethanol was no longer mutagenic, showing that metabolic processes are necessary for its activity in this system (Bandas, 1982). Ethanol (5%) had no effect on the frequencies of gene conversions in *S. cerevisiae* in the absence of an exogenous metabolic system (Barale *et al.*, 1983).

In *Aspergillus nidulans*, ethanol (approximately 5%) led to nondisjunction (Käfer, 1984) and to nondisjunction and mitotic crossing-over (Harsanyi *et al.*, 1977). Treatment of germinating but not of quiescent conidia of *A. nidulans* with ethanol (6 and 7%) led to induction of nondisjunction; point mutations were not induced in this test (Morpurgo *et al.*, 1979; Gualandi & Bellincampi, 1981).

(iv) *Insects*

When ethanol (10%) was fed to *Drosophila melanogaster* larvae for 2-96 h, neither somatic mutation nor recombination was induced (Graf *et al.*, 1984).

Feeding of ethanol to male *Drosophila* larvae (9-11 days, 4% ethanol in the food; Vogel *et al.*, 1983) or adults (three days, 5% ethanol in fluid; Vogel, 1972; Vogel & Chandler, 1974) did not lead to sex-linked recessive lethal mutation. When *Drosophila* eggs were seeded in medium containing 1 ml ethanol in 25 ml food and allowed to grow until hatching (Creus *et al.*, 1983), or when adult flies were fed with 5% aqueous sucrose containing up to 30% ethanol (Woodruff *et al.*, 1984), no sex-linked recessive lethal mutation was induced.

(v) *Mammalian cells* in vitro

Treatment of primary cultures of rat hepatocytes for 3 h with 1% ethanol did not induce DNA damage as measured by the alkaline elution technique (Sina *et al.*, 1983). Ethanol (up to 0.78 M) did not induce mutations in mouse lymphoma L5178Y $TK^{+/-}$ cells (Amacher *et al.*, 1980).

In the absence of an exogenous metabolic system, ethanol (0.1% for 44 h) did not induce sister chromatid exchanges in mouse kidney fibroblasts (Garcia Heras *et al.*, 1982). In the absence of an exogenous metabolic system, ethanol (50 μl/ml for 1 h) did not induce

micronuclei in Chinese hamster V79 cells (Lasne *et al.*, 1984). In Chinese hamster ovary cells, it did not induce sister chromatid exchanges (Obe & Ristow, 1977 — 0.1% one application per day for 8 days; Schwartz *et al.*, 1982 — 1% for 28 h; Darroudi & Natarajan, 1987 — 0.16 M for 30 min) or chromosomal aberrations (Darroudi & Natarajan, 1987 — 0.16 M for 30 min) in the absence of an exogenous metabolic system, although a slight increase in sister chromatid exchange frequencies was reported in one study (de Raat *et al.*, 1983 — 31.6 g/l, 1 h treatment). The frequencies were higher in the presence of an exogenous metabolic system (de Raat *et al.*, 1983). In another study, also in the presence of an exogenous metabolic system, up to 0.1 M for 3 h induced exchanges (Takehisa & Kanaya, 1983). Sister chromatid exchanges (Takehisa & Kanaya, 1983; Darroudi & Natarajan, 1987) and chromosomal aberrations (Darroudi & Natarajan, 1987) were also induced in the presence of extracts from plants. These are unusual exogenous metabolic systems.

Treatment of primary cultures of Syrian golden hamster embryo cells (ELa/ENG strain) with 0.5% ethanol did not lead to transformed cell colonies (Bokkenheuser *et al.*, 1983). As reported in an abstract, treatment of mouse C3H/10T1/2 cells with 4-32 mg/ml ethanol for 24 h led to a small fraction (1.2-2.6%) of dishes with transformed foci (Abernethy *et al.*, 1982). Ethanol inhibited intercellular communication in a dose-dependent manner (up to 200 µl/5 ml [32 mg/ml]), as measured by metabolic cooperation between cultured $6TG^S$ and $6TG^r$ Chinese hamster V79 cells (Chen *et al.*, 1984).

(vi) *Human cells* in vitro

Treatment of HeLa cells with 0.1% ethanol (one application per day for nine days) did not lead to an elevation of exchange-type aberrations or of micronuclei in the absence of an exogenous metabolic system (Obe & Ristow, 1979). Ethanol (0.1%) did not produce sister chromatid exchanges in human lymphoid cells in the presence (1-h treatment) or in the absence (48-h treatment) of an exogenous metabolic system (Sobti *et al.*, 1982, 1983).

In human lymphocyte cultures, both chromosome- and chromatid-type aberrations were found with different doses of ethanol (up to 3.5 mg/ml for 50 h) in the absence of an exogenous metabolic system (Badr *et al.*, 1977). Treatment of human lymphocytes in culture with ethanol (up to 0.5% for 72 h) led to a dose-related elevation of sister chromatid exchange frequencies in the absence of an exogenous metabolic system (Alvarez *et al.*, 1980a). In other analyses in lymphocytes, ethanol in the absence of an exogenous metabolic system did not induce chromosomal aberrations (Cadotte *et al.*, 1973 — up to 500 mg/100 ml [0.6%] for 72 h; Obe *et al.*, 1977 — 0.5% for 24 h; Königstein *et al.*, 1984 — up to 1% for 24 h; Banduhn & Obe, 1985 — 1% for 24 or 48 h; Kuwano & Kajii, 1987 — up to 1% for 26 h) or sister chromatid exchanges (Obe *et al.*, 1977 — 0.5% for 72 h; Véghelyi & Osztovics, 1978 — 0.5% for 72 h; Athanasiou & Bartsocas, 1980 — 0.2% for 72 h; Jansson, 1982 — up to 2% for up to 68 h; Hill & Wolff, 1983 — 20 µl/5 ml [0.4%] for 70 h; Königstein *et al.*, 1984 — up to 1% for up to 48 h).

In human lymphocytes, ethanol (1%) alone had no effect on the frequencies of sister chromatid exchanges. When alcohol dehydrogenase (ADH) was added together with ethanol, the sister chromatid exchange frequencies were elevated; exposure of cells to

ethanol, ADH and aldehyde dehydrogenase led to fewer sister chromatid exchanges than in cells treated with ethanol and ADH alone (Obe *et al.*, 1986).

(vii) *Mammals* in vivo

Oral or subcutaneous administration of 0.05 ml of 95% ethanol to male ddY mice or up to 40% ethanol given in water as the drinking fluid to male Swiss mice for 26 days did not increase the frequency of micronuclei in the polychromatic erythrocytes of the bone marrow (Chaubey *et al.*, 1977; Watanabe *et al.*, 1982). Giving male CBA mice 10 and 20% ethanol as their only liquid supply for up to 16 weeks led to an elevation in the frequencies of sister chromatid exchanges in the bone-marrow cells (Obe *et al.*, 1979), but oral administration of 10% ethanol to male Swiss Webster mice once a day for four days did not (Nayak & Buttar, 1986).

Feeding male CD rats for six weeks with a liquid diet containing 36% of the calories as ethanol led to an elevation in the frequencies of micronuclei in the erythrocytes of the bone marrow (Baraona *et al.*, 1981b), but male Wistar rats receiving 10 and 20% ethanol as the only liquid supply for three or six weeks did not show an increase in the frequency of micronuclei in the erythrocytes of the bone marrow or in hepatocytes. Nor did the treatment lead to chromosomal aberrations in bone-marrow cells or in lymphocytes; the frequencies of sister chromatid exchanges were elevated in the peripheral lymphocytes but not in bone-marrow cells (Tates *et al.*, 1980).

Giving Chinese hamsters 10% ethanol as their only liquid supply for nine weeks did not lead to an elevation in the frequencies of chromosomal aberrations in the bone marrow (Korte *et al.*, 1979); the same treatment for 46 weeks did not lead to an increase in the frequency of sister chromatid exchanges in bone-marrow cells or chromosomal aberrations in lymphocytes (Korte & Obe, 1981). Treatment of Chinese hamsters with ethanol *via* the drinking-water (10% in week 1, 15% in weeks 2 and 3, 20% in weeks 4-12) did not lead to an elevation in the frequencies of chromosomal aberrations or sister chromatid exchanges in bone-marrow cells (Korte *et al.*, 1981).

Intubation of pregnant ICR mice with ethanol in such a way that the total dose was 4-8 g/kg bw led to a dose-dependent elevation of the frequencies of sister chromatid exchanges in embryonal liver cells (Alvarez *et al.*, 1980b). A single intraperitoneal injection of 4 g/kg bw 10% ethanol to ICR mice on day 10 of pregnancy induced sister chromatid exchanges in embryonal cells (Czajka *et al.*, 1980).

Intragastric administration to Wistar rats of 2 ml 40% ethanol daily on days 10-25 of pregnancy led to an elevation in the number of anaphases with bridges and fragments in embryonic liver cells (Kozachuk & Barilyak, 1982).

Intra-amniotic injection of 0.02 ml of a 40% ethanol solution to 13-day-old embryos of Wistar rats did not lead to chromosomal aberrations or aneuploidy (Barilyak & Kozachuk, 1983).

No elevation in the frequency of chromosomal aberrations was seen in spermatogonia of male Wistar rats given 10% ethanol *via* the drinking-water (Kohila *et al.*, 1976) or of male Sprague-Dawley rats given ethanol as their only liquid supply (7% in weeks 1-2, 10% in weeks 3-4, 15% in weeks 5-6, 20% in weeks 7-36; Halkka & Eriksson, 1977). Oral

administration of 0.8 ml of 12.5 or 15% ethanol to CBA/CA or MF1 mice with analysis of second meiotic metaphases in the testes 2-6 h later, significantly increased the frequencies of hypo- and hyperploidies (2.99 and 4.2%, respectively, compared to a control frequency of 0.5%; Hunt, 1987). Intubation of ethanol (1.5 ml of 12.5%) in male Chinese hamsters was reported not to induce aneuploidies in spermatogonia or in spermatocytes I and II (Daniel & Roane, 1987).

A high incidence of aneuploid female pronuclei was found in fertilized eggs of female (C57Bl × CBA) F1 mice given ethanol orally (1 ml of 10-15%, 1.5-2.5 h after the predicted time of ovulation; Kaufman, 1983). Intragastric administration of ethanol to female CFLP mice (1.5 ml of a 12.5% solution) 1.5 h before, or 1.75 h, 4 h, 13.5 h or 17 h after treatment with human chorionic gonadotrophin (given to induce superovulation) led to high frequencies of aneuploid (hypo- and hyperploid) first-cleavage mitoses (15.3-25.0%) of the female pronuclear set in fertilized eggs, which was independent of the treatment schedule. Essentially the same result was found when female MF1 mice were treated with ethanol (15.7-24.0% aneuploid first-cleavage mitoses; Kaufman & Bain, 1984a). In another analysis, female CFLP mice were intubated with 1.5 ml of a 12.5% ethanol solution in distilled water 4 h, 13.5 h or 17 h after injection of human chorionic gonadotrophin. The animals were sacrificed 20-21 h after the injection (one-cell-stage embryos), three days later (morula stage) or 10-11 days later. The first-cleavage metaphases showed a high incidence of aneuploidy (15.3-22.8%), and aneuploidy was also found in the morula stages (11.1-15.4%). The time of administration of ethanol did not influence the frequencies of aneuploidies considerably. At the 10th or 11th day following ethanol administration, morphologically abnormal conceptuses were seen, and triploid and trisomic embryos were found (Kaufman & Bain, 1984b). [The Working Group noted that the effects of ethanol and acetaldehyde on the formation of microtubules (see p. 123) could explain the ability of ethanol to induce aneuploidy in various test systems.]

Genetic effects of ethanol in the male germ line have been reviewed (Anderson, 1982).

Administration of up to 30% ethanol as the only liquid supply for 35 days to male Wistar rats did not lead to dominant lethal mutations (Chauhan *et al.*, 1980). Feeding male Sprague-Dawley rats a liquid diet containing 6% ethanol for one week, followed by 10% ethanol for four weeks (Klassen & Persaud, 1976) and administration of 20% ethanol in water as the drinking fluid to male Long-Evans rats for 60 days led to the induction of dominant lethal mutations (Mankes *et al.*, 1982). Dominant lethal mutations have been reported in male CBA mice after intubation on three consecutive days with 0.1 ml 40-60% ethanol (Badr & Badr, 1975). Conflicting results were obtained in CFLP mice after oral administration of 2 ml/kg bw 40% ethanol on five consecutive days (James & Smith, 1982; Smith & James, 1984).

[The Working Group noted that chromosomal aberrations, sister chromatid exchanges and point mutations are induced by ethanol in test systems in which ethanol can be metabolized. This may indicate that acetaldehyde rather than ethanol is the mutagen in these systems.]

The activity profile for ethanol in short-term tests is shown in Appendix 2.

Alcoholic beverages

The mutagenicity of several components of alcoholic beverages has been discussed in various volumes of *IARC Monographs*: formaldehyde (IARC, 1987b), acetaldehyde (IARC, 1987b), acrolein (IARC, 1987b), *N*-nitrosamines (IARC, 1978), benzene (IARC, 1987b), styrene (IARC, 1987b), benzo[*a*]pyrene (IARC, 1983c) and tannins (IARC, 1976b).

Different alcoholic beverages (30 home-made apple brandies, 18 commercial apple brandies and 28 other commercial alcoholic beverages: eight whiskies, eight rums, eight cognacs, four armagnacs) were not mutagenic to *S. typhimurium* TA98 or TA100 either in the presence or absence of an exogenous metabolic system when 200-μl samples were tested. Alcoholic fractions of some of these beverages were mutagenic in the presence or absence of an exogenous metabolic system in TA98 and TA100 (Loquet *et al.*, 1981).

Alcohol-free extracts prepared from alcoholic beverages have generally been found to be mutagenic in the presence and absence of an exogenous metabolic system (Table 44). Extracted residues of pooled and concentrated Canadian beers were not mutagenic to *S. typhimurium* TA98, TA100 or TA102 in the presence or absence of an exogenous metabolic system (Brusick *et al.*, 1988).

Treatment of Chinese hamster ovary cells with geneva (15.8 and 32.6 g/l), rum (15.8 and 32.6 g/l) or port (15.8 g/l) led to the induction of sister chromatid exchanges in the presence but not in the absence of an exogenous metabolic system. Port (32.6 g/l) and sherry (15.8 g/l) also induced sister chromatid exchanges in the absence of an exogenous metabolic system (de Raat *et al.*, 1983).

Treatment of human lymphocytes *in vitro* with different types of alcoholic beverages for 24 h, such that the final ethanol concentration was always 0.5%, did not lead to the induction of chromosomal aberrations (Obe *et al.*, 1977). Alcohol-free extracts of whisky, rum, brandy (Hoeft & Obe, 1983) and red wine (Rueff *et al.*, 1986) induced sister chromatid exchanges in human lymphocytes *in vitro* in the absence of an exogenous metabolic system.

When tested with *S. typhimurium* strains, microsomes from liver, lung, intestine and oesophagus of ethanol-treated animals more effectively transformed indirect mutagens to mutagenic compounds than did microsomes from control animals. These effects were specific to the organs from which the microsomes were prepared and to the mutagens tested (Seitz *et al.*, 1978; McCoy *et al.*, 1979; Garro *et al.*, 1981; Seitz *et al.*, 1981a,b; Lieber, 1982; Olson *et al.*, 1984; Smith & Gutmann, 1984; Farinati *et al.*, 1985; Neis *et al.*, 1985; Sato *et al.*, 1986; Steele & Ioannides, 1986; Lieber *et al.*, 1987).

Genetic and related effects of metabolites

Acetaldehyde increased the incidence of sister chromatid exchanges in bone-marrow cells of mice and hamsters treated *in vivo* and induced chromosomal aberrations in rat embryos exposed *in vivo*. It induced DNA cross-links, chromosomal aberrations and sister chromatid exchanges in human cells *in vitro* and chromosomal aberrations, micronuclei and sister chromatid exchanges in cultured rodent cells. It induced chromosomal aberrations, micronuclei and sister chromatid exchanges in plants and DNA damage and mutation in bacteria. Acetaldehyde induced cross-links in isolated DNA (IARC, 1987b). The activity profile for acetaldehyde is given in Appendix 2.

Table 44. Mutagenicity of alcohol-free extracts of alcoholic beverages

Beverage	Test system[a]	Comment	Reference
17 Chinese spirits	TA98, TA100, TA1535; +/-	- in some cases only	Lee & Fong (1979)
Chinese wine treated with nitrite	TA98, TA100, TA1535, TA1538; +/-	No effect when not treated with nitrite	Lin & Tai (1980)
Home-made and commercial apple brandies, whiskies, cognacs, armagnacs, rums	TA98, TA100; +/-	Not always congruent	Loquet et al. (1980, 1981)
Red wine	TA98; +/-		Tamura et al. (1980)
White wine	TA98; +/-	Slight effect	Tamura et al. (1980)
Apple cider-based drinks, commercially available beverages	TA98, TA100; +/-		Tuyns et al. (1980)
Whiskies, brandies	TA100; -		Nagao et al. (1981)
Red wine	TA98, TA100; +/-	+ more effective	Stoltz et al. (1982a)
Red wine, white wine, light beer	TA98, TA100; +	Dark beer, gin, liqueur no effect	Stoltz et al. (1982b)
Red wine	TA98; +	White wine, one rosé wine no effect	Stavric et al. (1983)
Red wine	TA98, TA100; +		Subden et al. (1984)
Red wine	TA98; +	Beer, saké, whisky no effect	Kikugawa et al. (1985)
Red wine	TA98, TA100; +/-	+ more effective	Sousa et al. (1985)
Red wine	TA98; +		Ong et al. (1986)
Red wine	TA98; +; SOS chromotest with E. coli PQ37		Rueff et al. (1986)
Red wine	TA98, TA100; +		Yu et al. (1986)
Chilean commercial and home-made red and white wines, European red wines	TA98, TA100; +/-		Bull et al. (1987)

[a] +, with exogenous metabolic system; -, without

4.3 Other relevant data in humans

The term 'alcoholics' is used in this monograph in a broad sense to indicate persons who have a high alcohol consumption or who are considered to be alcohol-dependent. Information that would allow a more precise categorization was usually not given in the reports reviewed.

(*a*) *Absorption, distribution and excretion*

Ethanol occurs at concentrations similar to those of alcoholic beverages in the stomach and upper jejunum after ingestion (Halsted *et al.*, 1973). Absorption of ethanol from the gastrointestinal tract occurs by simple diffusion (Wallgren & Barry, 1970). Most ingested ethanol is absorbed within the first hour from the stomach and upper intestine, resulting in ethanol concentrations in the ileum and colon similar to that of the vascular space (Halsted *et al.*, 1973).

(*b*) *Metabolism*

(i) *Ethanol metabolism*

The rate of ethanol metabolism varies among individuals, and studies of twins indicate that interindividual variability in the rate of ethanol metabolism is under genetic control (Vesell *et al.*, 1971; Kopun & Propping, 1977).

The disappearance of ethanol from the blood follows zero-order kinetics; the elimination rate is approximately 0.1 g/kg bw per h (Newman & Lehman, 1937).

It is generally accepted that the main pathway for ethanol oxidation in man is *via* the ADH pathway. Human ADH is coded by three structural gene loci, the corresponding products of which, α, β- and γ-polypeptides, combine to form active dimeric isozymes. The ADH molecule may appear in at least nine electrophoretically different isozyme forms (Smith *et al.*, 1971, 1973).

An 'atypical' ADH has been described which differs from the usual enzyme in its catalytic activity, pH optimum, kinetic parameters and molecular structure (von Wartburg *et al.*, 1965; von Wartburg & Schürch, 1968; Yoshida *et al.*, 1981). In European countries, the incidence of atypical ADH ranges from 4 to 20% (von Wartburg & Schürch, 1968). In Japan, however, 85-98% of the population carries the atypical ADH (Fukui & Wakasugi, 1972; Agarwal *et al.*, 1981; Agarwal & Goedde, 1986). In spite of the presence of highly active atypical ADH, however, the rate of ethanol metabolism in normal and atypical ADH phenotype carriers is not significantly different (see p. 145; Edwards & Price Evans, 1967). Other isozyme forms found in human liver include π-ADH (Li & Magnes, 1975; Li *et al.*, 1977) and ADH-Indianapolis (Bosron *et al.*, 1980).

Human hepatic ADH catalyses the oxidation of not only alcohols but also endogenous and exogenous sterols (Frey & Vallee, 1980).

A cytochrome P450 isozyme immunologically identical to the ethanol-inducible forms from rats and rabbits, P450 IIE1, has also been isolated from human liver (Lasker *et al.*, 1986b; Song *et al.*, 1986; Lasker *et al.*, 1987).

A nonoxidative pathway of ethanol metabolism — esterification of ethanol with fatty acids — has been described in many human organs (Lange, 1982; Laposata & Lange, 1986); its importance remains to be determined.

(ii) *Acetaldehyde metabolism*

Acetaldehyde is oxidized further to acetate by aldehyde dehydrogenase (ALDH), which also occurs in several isoenzyme forms (Agarwal *et al.*, 1981). Acetaldehyde concentrations during ethanol oxidation in the blood following ingestion of 0.8 g/kg bw were low in healthy male Caucasians: 2-20 μM (0.1-1 mg/l) in hepatic venous blood; less than 2 μM (0.1 mg/l) in peripheral venous blood (Nuutinen *et al.*, 1984). In contrast, alcohol ingestion by Orientals resulted in marked elevations of blood acetaldehyde levels concentrations ranging from 0.4 to 3 mg/l (Ijiri, 1974); and individuals developed facial flushing and tachycardia as a direct consequence of elevated blood acetaldehyde levels (Ijiri, 1974; Mizoi *et al.*, 1979; Inoue *et al.*, 1980). Acetaldehyde-mediated facial flushing occurs in individuals in whom one of the ALDH isoenzymes, ALDH2, occurs in low concentrations or is absent (Agarwal *et al.*, 1981; Ikawa *et al.*, 1983).

In one study of volunteers, it was found that infusion of fructose caused a marked elevation of acetaldehyde in the blood of four nonalcoholic control subjects but not in four alcoholics. The oxidation of acetaldehyde in blood *in vivo* and *in vitro* was similar in alcoholics and nonalcoholics (Nuutinen *et al.*, 1984).

(iii) *Modifying effects of chronic ethanol consumption on the metabolism of ethanol*

A 30-80% increase in the metabolism of ethanol in alcoholics who consume 100 g or more ethanol per day has been described in several studies (Bernhard & Goldberg, 1935; Kater *et al.*, 1969; Ugarte *et al.*, 1972; Salaspuro & Lieber, 1979; Keiding *et al.*, 1983; Nuutinen *et al.*, 1983, 1984). Consumption of smaller amounts of ethanol (45 g per day) for three weeks did not affect the rate of its disappearance in volunteers of either sex (Holtzman *et al.*, 1985).

Cirrhotic patients with jaundice who had not taken alcohol for more than four weeks showed decreased rates of ethanol elimination due to liver injury (Lieberman, 1963). In contrast, ethanol elimination rates were elevated even in the presence of relatively severe liver damage when measurements were taken during the first month of abstinence (Ugarte *et al.*, 1977).

(iv) *Modifying effects of chronic ethanol consumption on the metabolism of acetaldehyde*

Early studies of the concentration of acetaldehyde in the blood of alcoholics may have been limited by methodological difficulties (Majchrowicz & Mendelson, 1970; Truitt, 1971; Magrinat *et al.*, 1973), as pointed out by Stowell *et al.* (1977). Following intravenous alcohol administration, blood acetaldehyde concentrations were demonstrated to be higher in

alcoholic than in nonalcoholic subjects (Korsten *et al.*, 1975). More recent studies with better methods have confirmed that blood acetaldehyde concentrations increase following chronic alcohol consumption (Lindros *et al.*, 1980; Palmer & Jenkins, 1982). In alcoholic patients, peak blood acetaldehyde concentrations were higher at high than at low ethanol blood levels (Nuutinen *et al.*, 1983). Blood acetaldehyde values correlated positively with rates of ethanol elimination (Lindros *et al.*, 1980) and negatively with liver ALDH activity (Jenkins & Peters, 1980; Nuutinen *et al.*, 1983; Jenkins *et al.*, 1984; Matthewson *et al.*, 1986).

Several factors, such as hepatomegaly (Pelkonen & Sotaniemi, 1982), increased reoxidation of NADH (Thurman *et al.*, 1988) and increased cytochrome P450 levels (Pelkonen & Sotaniemi, 1982), may be responsible for the enhanced elimination rates of ethanol by alcoholics.

Sera of alcoholic patients were found to contain antibodies to acetaldehyde-protein adducts. Anti-acetaldehyde adduct immunoglobulin titres in 21 healthy nondrinking individuals ranged from 10 to 80, whereas 25 of 34 alcoholics had titres of 160 or above ($p < 0.001$). These results suggest that acetaldehyde-induced immunogenic determinants can initiate an immune response which may be used to differentiate alcoholics from nonalcoholics (Hoerner *et al.*, 1986).

(*c*) *Modifying effects of ethanol on the metabolism of xenobiotics*

In both alcoholics and nonalcoholics, acute drinking of alcohol results in inhibition of xenobiotic metabolism as observed in experimental systems (see Table 42). For example, the metabolism of meprobamate and pentobarbital is inhibited by acute administration of ethanol (Rubin *et al.*, 1970). After ethanol intake, blood xylene levels in volunteers exposed to xylene by inhalation were increased 1.5-2.0 fold, while urinary excretion of methylhippuric acid, a xylene metabolite, declined by about 50% (Riihimäki *et al.*, 1982). During exposure to toluene by inhalation (3.2 mmol/m^3) for 4.5 h, moderate doses of ethanol (15 mmol/kg bw) given orally to volunteers almost doubled the maximum toluene concentration in blood and decreased the blood clearance of toluene by approximately 44% (Wallén *et al.*, 1984). Trichloroethylene concentrations in plasma increased two fold, and decreased urinary excretion of a major metabolite of trichloroethylene — trichloroethanol — was observed when ethanol was ingested immediately prior to exposure to trichloroethylene by inhalation (Müller *et al.*, 1975). Drinking of alcoholic beverages inhibits liver metabolism of nitrosamines, such as *N*-nitrosodimethylamine and *N*-nitrosodiethylamine. As a result, nitrosamines are excreted in urine of beer drinkers and volunteers given amines and ethanol (Eisenbrand *et al.*, 1981; Spiegelhalder *et al.*, 1982; Spiegelhalder & Preussmann, 1985).

In chronic alcoholics with normal liver function, xenobiotic metabolism is enhanced in the absence of alcohol. Consumption of 46% of total calories as ethanol for one month by volunteers resulted in a striking increase in the rate of clearance from the blood of meprobamate and pentobarbital (Misra *et al.*, 1971). Similarly, increases in the metabolism of antipyrine (Cushman *et al.*, 1982), tolbutamide (Carulli *et al.*, 1971), warfarin and phenytoin, but not aminopyrine (Iber, 1977), have been described.

When volunteers were given ethanol in the diet in increasing amounts up to 46% of total calories for 16-18 days, electron microscopy of biopsy specimens of the liver revealed a marked increase in the smooth endoplasmic reticulum (Lane & Lieber, 1966). No such change was detected in 17 alcoholics with hepatic steatosis (but not fibrosis; Hakim *et al.*, 1972). These results are probably due to induction of cytochrome P450; for example, hepatic pentobarbital hydroxylase activity, measured in biopsy specimens, was doubled in three nonalcoholic volunteers after 12 days of feeding 42% of total calories as ethanol. No change was detected in the activity of benzo[*a*]pyrene hydroxylase (Rubin & Lieber, 1968).

Alcoholics with normal liver histology had elevated levels of hepatic cytochrome P450 and *in-vitro* activities of monooxygenase, as well as increased clearance of antipyrine *in vivo* (Pelkonen & Sotaniemi, 1982); in contrast, alcoholics with hepatitis or cirrhosis had lower than normal values in all these analyses (Pelkonen & Sotaniemi, 1982; Woodhouse *et al.*, 1983).

It is well accepted that chronic consumption of ethanol enhances the metabolism of many drugs and halogenated hydrocarbons to reactive intermediates, resulting in increased toxicity (Lieber, 1982; Zimmerman, 1986), due probably to induction of P450 IIE1 by ethanol (Coon & Koop, 1987). For example, severe hepatic failure has been reported to develop in chronic alcoholics after ingestion of normally nontoxic doses of acetaminophen (Emby & Fraser, 1977; McClain *et al.*, 1980; Seeff *et al.*, 1986).

(*d*) *Modifying effects of ethanol on intermediary metabolism*

Most of the acute metabolic effects of ethanol that have been observed in experimental animals have also been detected in humans, as reviewed by Lieber (1982). These include elevation of the NADH:NAD redox state (i.e., increases in the lactate:pyruvate and β-hydroxybutyrate:acetoacetate ratios), inhibition of carbohydrate and lipid metabolism and accumulation of hepatic triglycerides (Lieber, 1984a).

It is generally accepted that ethanol metabolism in humans is regulated by the ADH system. This conclusion is supported by the numerous studies in humans which have demonstrated that administration of fructose, which increases rates of NADH reoxidation, elevates rates of ethanol metabolism from 30 to 80%. The absence of elevated alcohol elimination rates in individuals with atypical ADH, who have increased enzyme activity (von Wartburg *et al.*, 1965), further supports the conclusion that the ADH system is regulated predominantly by the NADH:NAD redox state (Thurman *et al.*, 1988).

(*e*) *Major toxic effects*

The clinical features of ethanol intoxication are related to blood ethanol levels: mild intoxication (500-1500 mg/l) is manifested in emotional lability and slight impairment of visual acuity, muscular coordination and reaction time; moderate intoxication (1500-3000 mg/l) results in visual impairment, sensory loss, muscular incoordination, slowed reaction time and slurred speech; severe intoxication (3000-5000 mg/l) is characterized by marked muscular incoordination, blurred or double vision, sometimes stupor and hypothermia, and

occasionally hypoglycaemia and convulsions; in coma (>5000 mg/l), there are depressed reflexes, respiratory depression, hypotension and hypothermia. Death may occur from respiratory or circulatory failure or as the result of aspiration of stomach contents in the absence of the gag reflex (Weatherall *et al.*, 1983).

Long-term, high-level alcohol consumption caused toxicity in almost all organ systems of the body (Lieber, 1982).

(i) *Gastrointestinal tract*

Alcoholism commonly affects the mouth, with enlargement of the parotid gland and an increase in salivary secretion in patients with alcoholic liver injury (Dürr *et al.*, 1975; Bode & Menge, 1978). Glossitis and stomatitis are common in alcoholics.

Oesophageal complications that are often diagnosed in alcoholics include oesophagitis, columnar metaplasia (Wienbeck & Berges, 1985) and functional alterations in peristaltic contractions, especially in patients with peripheral neuropathy (Winship *et al.*, 1968).

Ethanol also has acute and chronic effects on gastric secretion and the gastric mucosa. Increased gastric acid secretion occurs as an acute effect (Cooke, 1972). Long-term consumption of alcohol decreases basal and maximal acid output (Chey *et al.*, 1968) and can cause chronic antral gastritis (Parl *et al.*, 1979). Acute mucosal lesions ('haemorrhagic gastritis') are a significant cause of upper gastrointestinal blood loss in alcoholics (Katz *et al.*, 1976).

A number of syndromes or pathological effects of alcoholic beverages in the upper intestine have been attributed to the high alcohol concentrations attained in the upper small intestine, including alterations in intestinal motility (decreased impeding waves in the jejunum; Robles *et al.*, 1974) and impaired transport of, for example, glucose (Thomson & Majumdar, 1981), amino acids (Israel *et al.*, 1969), electrolytes (Mekhjian & May, 1977), thiamine (Wilson & Hoyumpa, 1979), vitamin B12 (Lindenbaum & Lieber, 1969) and folic acid (Halsted *et al.*, 1967).

Since malnutrition impairs the nutrient absorptive function of the gastrointestinal tract, the malnutrition that often accompanies alcoholism may itself contribute to, or exacerbate, the malabsorption seen in alcoholics (Mezey, 1975; Lieber, 1982).

Pyridoxine deficiency occurs in alcoholics, and acetaldehyde has been incriminated in the accelerated destruction of vitamin B6 (Lumeng & Li, 1974). Abnormally low blood levels of vitamin E have been reported in alcoholics (Losowsky & Leonard, 1967). Deficiencies in other vitamins and trace elements have been reviewed (Thomson & Majumdar, 1981).

(ii) *Liver*

Alcoholic liver disease ranges from fatty liver, alcoholic hepatitis and fibrosis to irreversible cirrhosis (for review, see Lieber, 1984b).

Alcoholic hepatitis is characterized by an inflammatory reaction of the liver with necrosis of hepatocytes and may be associated with the occurrence of hyalin bodies (Mallory, 1911; Denk *et al.*, 1975; French & Burbige, 1979; Phillips, 1982; Denk, 1985).

Most studies suggest that intake of more than 120-180 g ethanol per day for more than 15 years is the critical dose-duration factor for the development of cirrhosis (Lelbach, 1975). Some other reports (Péquignot *et al.*, 1978; Tuyns & Péquignot, 1984; Norton *et al.*, 1987) have shown an elevated risk for cirrhosis following even lower daily consumption of ethanol. Risk factors for the development of alcoholic cirrhosis include poor nutrition, genetic susceptibility and female gender (Burnett & Sorrell, 1981; Tuyns & Péquinot, 1984; Norton *et al.*, 1987).

It has been suggested that viral hepatitis B is more common among alcoholics than among corresponding nonalcoholic populations, and an increased prevalence of serological markers of viral hepatitis has been reported in alcoholics (Mills *et al.*, 1979; Pimstone & French, 1984). The extent to which joint exposure to hepatitis B virus and alcohol leads to mutual modification of effects has not been clearly established.

(iii) *Pancreas*

Alcoholic pancreatitis is generally described as both acute and chronic (Sarles & Laugier, 1981). The acute form is associated with considerable mortality (Geokas, 1984). Chronic alcoholic pancreatitis generally develops after eight to ten years of heavy drinking (Strum & Spiro, 1971). Drinking binges (heavy drinking during weekends) often precipitate relapses of pancreatitis in alcoholics; similar excesses by nonalcoholics rarely provoke the disorder (Sarles & Laugier, 1981).

(iv) *Endocrine organs*

Alcohol interacts with the endocrine system, including the hypothalamus, pituitary and gonads. In addition, liver injury may disturb peripheral metabolism of hormones by affecting hepatic blood flow, protein binding, enzymes, cofactors or receptors.

Chronic ethanol abuse increases plasma cortisol levels (Mendelson & Stein, 1966; Mendelson *et al.*, 1971). Ethanol stimulates adrenal medullary secretion of catecholamines; in addition, the peripheral metabolism of the released catecholamines is altered by ethanol (Davis *et al.*, 1967). Alcohol and alcoholic liver injury affect thyroid function; acute administration of ethanol increases the liver:plasma ratio of thyroid hormones (Szilagyi, 1987), a finding that may explain some of the metabolic effects of ethanol.

Blood testosterone concentrations fall reversibly in normal male volunteers within hours of their ingesting sufficient amounts of alcohol to produce hangover (Van Thiel *et al.*, 1983). Chronic intake of alcohol further decreases serum testosterone levels which may lead to testicular atrophy and impotence (Mendelson & Mello, 1974; Gordon *et al.*, 1976; Van Thiel & Lester, 1977; Välimäki *et al.*, 1982; Van Thiel & Gavaler, 1985; Gavaler & Van Thiel, 1987). The finding of simultaneously elevated luteinizing hormone levels, especially in cirrhotics (Välimäki *et al.*, 1982), suggests a primary testicular effect of ethanol.

It is generally accepted that acute ethanol administration has little or no effect on human female hypothalamic-pituitary-gonadal function. On the contrary, chronic ethanol abuse leads to early menopause, lower postmenopausal gonadotropin levels and increased plasma levels of the classic female sex hormones, despite the presence of amenorrhoea (Gavaler

& Van Thiel, 1987). Elevated basal prolactin levels and exaggerated prolactin responses to thyrotropin-releasing hormone have been described in alcoholics (Ylikahri *et al.*, 1980).

(v) *Immune system*

The effects of alcohol on the immune system have been reviewed (Kanagasundaram & Leevy, 1981; Lieber, 1982; MacSween & Anthony, 1985). Studies in patients with alcoholic liver disease have shown decreased immune responsiveness (Berenyi *et al.*, 1974).

(vi) *Heart*

Acute and prolonged ingestion of alcohol has a deleterious effect on left ventricular function (Gould *et al.*, 1971; Regan *et al.*, 1975). The association between heavy drinking and cardiomyopathy is widely recognized (New York Heart Association Criteria Committee, 1964; Wendt *et al.*, 1966; Friedberg, 1971; McDonald *et al.*, 1971; Perloff, 1971; Demakis *et al.*, 1974; Regan *et al.*, 1975).

Protective effects of moderate alcohol consumption with regard to cardiovascular disease are discussed on p. 37.

(f) Effects on reproduction and prenatal toxicity

Numerous reviews of the reproductive effects and prenatal toxicity of alcohol are available (Jones & Smith, 1975; Warner & Rosett, 1975; Majewski, 1978; Neugut, 1981; Colangelo & Jones, 1982; Streissguth, 1983; Barrison *et al.*, 1985; Gavaler & Van Thiel, 1987). The adverse effects of alcohol in pregnancy have been known since biblical times, and occasional reports were published in the eighteenth and nineteenth centuries about the effects of excessive drinking on pregnancy. Following publication of the term 'fetal alcohol syndrome' by Jones and Smith (1973), contemporary interest in the effects of alcohol in pregnancy increased extensively, and, within eight years, Abel (1981) had published a comprehensive bibliography on the subject containing more than one thousand references.

The 'fetal alcohol syndrome' is characterized by both physical and mental effects. The major physical features are pre- and postnatal growth deficiency with regard to both weight and length, microcephaly, and characteristic facial features, including short palpebral fissures, short upturned nose with hypoplastic philtrum, thinned upper vermilion and retrognathia. The major neurological features are mild to moderate mental retardation, poor coordination, hypotonia, irritability in infancy and hyperactivity in childhood (Clarren & Smith, 1978). The two sexes seem to be equally at risk (Abel, 1979). Not all affected children have all of the features of the syndrome, and difficulty in recognizing the facial features seems to present the major problem in diagnosis. The syndrome was first described in the children of chronic alcoholic women, and much of the subsequent research has been devoted to finding whether the effects are dose-related and whether a threshold exists below which no adverse effect is detectable.

Hanson *et al.* (1978), as part of a large study on drinking, smoking, diet and use of of medicines in 1529 women in Seattle, WA, USA, separated a subgroup of infants born to 70 mothers who drank at least 1 oz (29.6 ml) ethanol per day on average, and these were compared with 93 infants born to mothers drinking <1 oz ethanol per day (five or more

drinks per occasion). These 163 infants underwent special examination for features of fetal alcohol syndrome without prior knowledge of the mothers' drinking habits. Fetal alcohol syndrome-type features were diagnosed in 9/70 infants from mothers who consumed 1 oz [≃ 23 g] ethanol per day or more, compared with 2/93 from mothers who consumed less than 1 oz/day ($p = 0.023$). Significantly lower body weight, body length and reduced mental and motor development were found among infants of the mothers who drank more heavily on follow-up of infants of the original cohort at eight months of age (Streissguth *et al.*, 1981).

A prospective study by Olegård *et al.* (1979) in Göteborg, Sweden, in 1977/1978 identified 28 pregnancies in 25 alcoholic women. During the study period, there were about 7600 deliveries in the whole of Göteborg, and the study included antenatal clinics covering about one-third of the population. Five of the pregnancies ended in induced abortions, and two babies died during birth. Of the remaining 21 pregnancy outcomes, seven infants had full fetal alcohol syndrome and seven others partial features of the syndrome.

In a number of other studies, evidence was found for adverse effects of alcohol consumption during pregnancy, but not necessarily typical features of the fetal alcohol syndrome. A prospective study in Boston, MA, USA, by Ouellette *et al.* (1977) on 633 women who had registered for prenatal care at the Boston City Hospital, addressed alcohol intake, smoking, drug use and diet. At publication, 322 babies had been delivered. The abnormality rate, defined as infants with congenital anomalies, growth abnormalities or neurological abnormalities, was 29/42 (71%) in heavy drinkers, 45/128 (36%) in moderate drinkers and 52/150 (35%) in rare drinkers or abstainers ($p < 0.001$). The authors defined heavy drinkers as having an average daily intake of >45 ml [~ 36 g] ethanol per day or more, and abstinent/rare drinkers as less than one drink per month; the remainder were classified as moderate drinkers. There was a marked excess of infants of heavy drinkers who were small for their gestational age or had congenital anomalies or microcephaly. There was no specific pattern of anomalies, such as the fetal alcohol syndrome. [The Working Group noted that this is a high-risk population, among which 35% of all newborns are admitted to intensive care. Also, there was an association between heavy alcohol intake and both smoking and previous use of psychotropic drugs, but these were not corrected for in the analysis.]

A large prospective study in France (Kaminski *et al.*, 1976) was carried out on 9236 women delivered in Paris between 1963 and 1969. Using as the criterion for heavy drinking consumption of 400 ml wine containing 11% or more alcohol [~ 35 g ethanol] (or equivalent in other alcoholic beverages) per day, an excess of stillbirths was reported (2.6 *versus* 1.0%), as well as an excess of babies small for their gestational age (4.8 *versus* 2.5%) and reduced placental weight. The effects of alcohol were still significant ($p \leqslant 0.01$) after adjusting for maternal age, marital state, smoking and previous pregnancy outcomes. Two further studies (Kaminski *et al.*, 1981), one retrospective and the other prospective, did not confirm the increase in stillbirths found in the first study, and none of the three studies showed an increase in congenital malformations associated with alcohol intake.

Sokol *et al.* (1980) reported the results of a study of women from one obstetric hospital in Cleveland, OH, USA, who were classified as alcohol abusers (204), compared with 11 923 in a 'no alcohol group'. Alcohol abuse was associated with reduced birth weight (average

reduction, 190 g), a significant increase in congenital anomalies and problems during delivery. Five cases of fetal alcohol syndrome were diagnosed. [The Working Group noted that a number of confounding factors, such as drug abuse and gravidity, were identified but were not controlled for.]

In a later study, the same group of workers (Ernhart *et al.*, 1987) analysed data from 359 mother-infant pairs classified according to alcohol intake, almost all of whom were from lower social classes. The prevalence of the cranio-facial anomalies associated with the fetal alcohol syndrome was related to the amount of maternal drinking during the first trimester of pregnancy. The relationship was most marked at higher levels of consumption (above 3 oz [88.8 ml] ethanol).

A prospective study on 32 019 women attending 13 clinics in California, USA, in 1974-77 was reported by Harlap and Shiono (1980), who studied the relationship between alcohol consumption, smoking and first- and second-trimester abortions. They found a significant increase in the incidence of second-trimester abortions with alcohol intake, giving age-adjusted relative risks of 1.03 (not significant), 1.98 ($p < 0.01$) and 3.53 ($p < 0.01$) for women taking less than one, one to two and three or more drinks [not defined] per day. There was no relationship with the incidence of first-trimester abortions; the effects observed were not explained by age, parity, race, marital status, smoking or the number of previous abortions.

Kline *et al.* (1980) conducted a case-control study on the relationship between alcohol intake and spontaneous abortion. Cases were a consecutive series of spontaneous abortions in three hospitals in Manhattan (NY, USA) between 1974 and 1978. Controls, matched for age and hospital and who had been delivered after 28 weeks' gestation, were selected concurrently, and 657 case-control pairs were included in the analysis. The authors found that 17.0% of cases and only 8.1% of controls reported drinking twice per week or more. The odds ratio, adjusted for three variables (age, interval between last menstrual period and interview and drinking before pregnancy) was 2.6 (95% confidence interval, 1.6-4.2). No significant interaction was found between alcohol intake and several other variables, such as smoking, previous spontaneous abortion, nausea/vomiting, weight, age, race, marijuana use or caffeine use. The effect was dose-related and, for those who drank twice a week or more, each type of beverage was significantly associated with increased risk of abortion. The association for wine and spirits (average intake per occasion, >1 oz [~ 23 g] ethanol) was slightly greater than that for beer (average intake, <1 oz ethanol, except for daily drinkers). The authors concluded that 1 oz ethanol twice per week is about the threshold amount that may produce an abortion.

Marbury *et al.* (1983) reported on a large study on alcohol consumption in 12 440 women from two hospitals in Boston, USA, interviewed at the time of delivery. After controlling for confounding by demographic factors, smoking, parity and obstetric history, alcohol consumption of 14 drinks per week or more was associated only with an increase in abruptio placenta. There was no adverse effect below 14 drinks per week, and no increase in malformations at any intake level.

Full fetal alcohol syndrome is seen only in the children of very heavy drinkers, usually chronic alcoholics. Some of the features of the syndrome are seen, however, at lower doses, and one of the most sensitive effects seems to be reduced birth weight. Little (1977), using

multiple linear regression, showed in a study on 263 women that an average intake of 1 oz [~ 23 g] ethanol daily before pregnancy was associated with a decrease in birth weight of 91 g, and the same amount in late pregnancy with a decrease of 160 g. Wright *et al.* (1984) published a review of previous studies, including a detailed analysis of their own study on 1122 pregnancies in the UK, in which they used both the Mantel-Haenszel method and stepwise logistic regression. They found that intake of more than 10 g ethanol per day before or in very early pregnancy doubled the risk of a light (<10th centile) baby. Both of these studies showed an association between alcohol intake and smoking and showed that the effect of smoking on birth weight is independent of, and additive with, the effect of alcohol. Smith *et al.* (1986) showed, in a group of moderate drinkers, that women who stop drinking by mid-pregnancy have a lower risk in terms of both growth and behavioural outcomes than those who continue throughout pregnancy.

Follow-up of children with fetal alcohol syndrome shows that the effects remain severe and permanent, with little or no evidence of catching up either physically or mentally. This observation is independent of whether the child is brought up in his own home or in a foster home, and suggests that the majority of the defects are the result of prenatal exposure to alcohol (Kyllerman *et al.*, 1985; Streissguth *et al.*, 1985).

As well as the facial dysmorphology, growth and mental retardation, a number of other malformations have been observed in association with fetal alcohol syndrome. The most common, occurring in up to 50% of cases, are cardiac malformations, especially atrial and ventricular septal defects (Löser & Majewski, 1977; Dupuis *et al.*, 1978; Sandor *et al.*, 1981), renal defects (DeBeukelaer *et al.*, 1977; Quazi *et al.*, 1979; Havers *et al.*, 1980) and a variety of skeletal defects (Spiegel *et al.*, 1979; Herrmann *et al.*, 1980; Halmesmäki *et al.*, 1985; Pauli & Feldman, 1986). Although mental retardation is a major feature of fetal alcohol syndrome, there have been few reports of brain dysmorphology; however, severe disorders in brain development have been reported (Clarren *et al.*, 1978; Goldstein & Arulanantham, 1978; Peiffer *et al.*, 1979; Wisniewski *et al.*, 1983), suggesting an action at different stages. Other less frequent effects include alteration in palmar creases (Tillner & Majewski, 1978), liver abnormalities (Habbick *et al.*, 1979; Møller *et al.*, 1979) and eye defects (Strömland, 1981).

Studies of hypothalamic-pituitary hormonal function in children with fetal alcohol syndrome have shown no abnormality, indicating that the deficit in height and weight is not due to lack of growth hormone (Root *et al.*, 1975; Tze *et al.*, 1976; Castells *et al.*, 1981).

(g) *Mutagenicity and chromosomal effects*

Chromosomal aberrations and aneuploid metaphases were found in the peripheral lymphocytes of alcoholics. In 200 alcoholics, de Torok (1972) found a high frequency of metaphases with nonmodal chromosome numbers, particularly in those with alcoholism-related organic brain syndrome, in whom 43.7% of the cells had 45 chromosomes and only 4.4% of the cells had the normal chromosome number of 46. In 100 alcoholics without organic brain syndrome, 23.6% of the cells had 46 chromosomes. In 20 ex-alcoholics who no longer drank, 47.2% of the cells had 46 chromosomes, and, in 60 nonalcoholics, there were

91.6% metaphases with 46 chromosomes. The author stated that a high incidence of structural changes was also observed.

Mitelman and Wadstein (1978), using 72-h cultures, found a significant elevation of the frequencies of hyperploid and hypoploid metaphases and of chromosomal aberrations in cells from ten alcoholics. Significant increases in the incidences of hyperploid and hypoploid metaphases and of metaphases with aberrations were observed using 72-h cultures of cells from 77 alcoholics (Kucheria *et al.*, 1986).

[The Working Group noted that a culture time of three days was used in these studies, which is not ideal since second- and third-division metaphases are present in such cultures. In addition, aneuploidies, especially metaphases with 45 chromosomes, could arise during preparation of the cells.]

In a series of studies with two-day cultures, Obe and his coworkers analysed chromosomal aberrations in lymphocytes of alcoholics (Obe *et al.*, 1977; Obe & Ristow, 1979; Obe *et al.*, 1979, 1980; Obe & Salloch-Vogel, 1985; Obe *et al.*, 1985; Obe, 1986) and found that they had higher frequencies of chromosomal aberrations than nonalcoholics. A comparison of the numbers of exchange-type aberrations per 10^4 metaphases in 379 alcoholics (number of metaphases analysed, 65 952) with historical control values gave the following results: chromatid translocations, 15.62 *versus* 5.13; dicentrics, 19.56 *versus* 8.00; rings, including minutes, 7.88 *versus* 1.77. These results indicate that there are nearly three times more exchange-type aberrations in alcoholics as in nonalcoholics (Obe & Anderson, 1987). In a study of 200 alcoholics, the frequencies of exchange-type aberrations were not correlated with age or sex but were correlated positively with the duration of alcohol dependence; smoking alcoholics had a higher frequency of exchange-type aberrations than nonsmoking alcoholics (Obe *et al.*, 1980). Alcoholics who were not currently drinking alcohol had fewer aberrations than those drinking currently (Obe *et al.*, 1980, 1985).

In a study of peripheral blood lymphocytes from alcohol drinkers and 20 controls, cultured for 48 h, there were more chromosome-type aberrations in those with a daily ethanol consumption of more than 80 ml (Horvat *et al.*, 1983).

Several studies have suggested that alcoholics have higher sister chromatid exchange frequencies in their lymphocytes than nonalcoholics (Butler *et al.*, 1981; Seshadri *et al.*, 1982; Horvat *et al.*, 1983; Hedner *et al.*, 1984; Kucheria *et al.*, 1986). Butler *et al.* (1981) showed that in nine alcoholics who were not currently drinking alcohol there was no elevation in such frequencies. Seshadri *et al.* (1982) reported that sister chromatid exchange frequencies in babies of ten alcoholic mothers who had elevated sister chromatid exchange frequencies were similar to those in babies from nonalcoholic mothers.

[The Working Group noted that in these studies smoking and other confounding factors were frequently not controlled for, which may have influenced the results.]

Activity profiles appear in Appendix 2.