3. Studies of Cancer in Experimental Animals

3.1 Metallic mercury

Intraperitoneal administration

Rat: A group of 39 male and female BDIII and BDIV rats, three months old, received two intraperitoneal injections of 0.05 ml **metallic mercury** [purity unspecified] over 14 days (total dose, 0.1 ml); mean survival was 580 days in treated rats and 780 days in controls. Only gross lesions were investigated histopathologically. At 22 months, when 12/39 animals were still alive, one female rat had a spindle-cell sarcoma in the abdominal cavity. Two females and two males of the 11 remaining rats developed similar tumours (Druckrey *et al.*, 1957). [The Working Group noted the incomplete reporting of the study and the possibility that the lesions seen were the result of a solid-state effect.]

3.2 Mercuric chloride

3.2.1 *Oral administration*

(*a*) *Mouse*

A group of 54 male and 54 female Swiss mice (Charles River CD strain), 20 days old, were given drinking-water containing **mercuric chloride** (5 ppm [mg/L] mercury) [purity unspecified] for life. A control group of 54 male and 54 female mice was given the drinking-water alone. Of the controls, 50% of the males were still alive at 602 days and 10% at 789 days, and 50% of females were still alive at 539 days and 10% at 691 days. Of the treated mice, 50% of males were still alive at 540 days and 10% at 697 days, and 50% of females at 575 days and 10% at 736 days. The numbers of mice autopsied were 38 control males and 47 control females and 48 male and 41 female treated mice. The authors reported that 11/41 treated female mice and 3/47 control females developed lymphoma or leukaemia [$p = 0.09$, Fisher exact test] (Schroeder & Mitchener, 1975). [The Working Group noted the incomplete reporting of the study and that only some of the animals were autopsied.]

Groups of 60 male and 60 female B6C3F1 mice, six weeks old, received 0, 5 or 10 mg/kg bw **mercuric chloride** (purity > 99%) in deionized water by gavage (10 ml/kg bw) on five days a week for 103–104 weeks. Ten animals from each group were killed at 15 months for evaluation. Survival at the end of the two-year study was 36/50, 36/50 and 31/50 in the control, low-dose and high-dose male groups and 41/50, 35/50 and 31/50 in the corresponding female groups. Body weights of both female and male treated mice were similar to those of controls throughout. Of the high-dose male mice, 2/49 developed renal tubular adenomas and 1/49 a renal tubular adenocarcinoma. No such tumour was seen in either the control or low-dose groups. No increase in the incidence of tumours was seen in the treated female mice (US National Toxicology Program, 1993).

(*b*) *Rat*

Groups of 60 male and 60 female Fischer 344/N rats, six weeks old, received 0, 2.5 or 5 mg/kg bw **mercuric chloride** (purity, > 99%) in deionized water by gavage (5 ml/kg bw) on five days a week for 103–104 weeks. Ten animals from each group were killed at 15 months for evaluation. Body weights of low- and high-dose males and high-dose females were lower than those of controls. Survival at two years was 26/50 male controls, 10/50 low-dose and 5/50 high-dose rats and 35/50, 28/49 and 30/50 in the females. The decrease in survival in male rats was due, in part, to an increased incidence of treatment-related renal disease. High-dose males had a greater incidence of renal tubular hyperplasia than control males (12/50 *versus* 3/50; $p = 0.005$), but the incidence of renal tubular adenomas was similar (control, 4/50; high-dose, 5/50). In female rats, renal tubular hyperplasia occurred in 5/50 high-dose rats and 2/50 controls; two high-dose female rats had renal tubular adenomas, but none was seen in controls. Treated male rats had a dose-related increase in the incidence of forestomach hyperplasia compared to controls (control, 3/49; low-dose, 16/50; high-dose, 35/50), as did high-dose female rats (control, 5/50; low-dose, 5/49; high-dose, 20/50). In addition, there was a dose-related increase in the incidence of squamous-cell papilloma of the forestomach in treated males (control, 0/50; low-dose, 3/50; high-dose, 12/50); such tumours also occurred in 2/50 high-dose female rats. High-dose males also had an increased

incidence of thyroid follicular-cell carcinoma (control, 1/50; low-dose, 2/50; high-dose, 6/50), but not of hyperplasia (control, 2/50; low-dose, 4/50; high dose, 2/50) or adenoma (control, 1/50; low-dose, 4/50; high-dose, 0/50) (US National Toxicology Program, 1993). [The Working Group noted the low survival rate of male animals.]

3.2.2 *Administration with known carcinogens*

As the purpose of the investigations described below was to study interactions with known carcinogens, the studies were limited to specific target sites, were often of short duration and were not intended to address the carcinogenicity of mercury *per se*.

(*a*) *Mouse*

Twenty female Sencar mice [age unspecified] received single topical applications of 0.2 ml of 10 nmol [2.6 μg] 7,12-dimethylbenz[*a*]anthracene (DMBA) followed by twice weekly topical applications of 200 μg **mercuric chloride** in 0.2 ml of a 90% acetone solution for 26 weeks. A positive control group of 20 mice, initiated with DMBA, received promotion with 12-*O*-tetradecanoylphorbol 13-acetate [dose and dosing regime unspecified]. All mice in the positive control group developed skin papillomas, and two developed carcinomas. No skin tumour occurred in the mercuric chloride-treated mice (Kurokawa *et al.*, 1989). [The Working Group noted the incomplete reporting of the study.]

(*b*) *Rat*

A group of 15 male Fischer 344 rats, seven weeks old, was administered *N*-nitroso-*N*-hydroxydiethylamine (NHDEA) at 500 ppm [mg/L] in the drinking-water for two weeks followed by drinking-water containing 40 ppm [mg/L] **mercuric chloride** (99.5% pure) for 25 weeks. A further group of 15 rats received only mercuric chloride at 40 ppm for 25 weeks; a control group of 15 rats received drinking-water for the 27-week experimental period; and a further group of 15 rats was given NHDEA for two weeks followed by 25 weeks of drinking-water alone. There was no significant difference in the number of renal-cell tumours in rats receiving NHDEA and mercuric chloride (5/15) and those receiving NHDEA alone (2/15), but there was a significant ($p < 0.01$, Student's t test) increase in the mean number of dysplastic foci/cm^2 in the NHDEA- plus mercuric chloride-treated group (1.09) over that in the group treated with NHDEA alone (0.23). No renal-cell tumour or dysplastic focus was reported in the group receiving mercuric chloride alone (Kurokawa *et al.*, 1985).

Groups of 20 male Fischer 344 rats [age unspecified] were given drinking-water containing 50 ppm [mg/L] *N*-nitrosodiethylamine (NDEA) for four weeks to initiate liver carcinogenesis, followed by 30 weeks of treatment with drinking-water containing 40 ppm [mg/L] **mercuric chloride** [purity unspecified], water alone or 1000 ppm [1 g/L] phenobarbital (positive control). All animals were killed at week 34. Mercuric chloride treatment did not increase the number of hepatocellular carcinomas, adenomas or hyperplastic nodules over that in rats treated with NDEA alone (Kurokawa *et al.*, 1989). [The Working Group noted the incomplete reporting of the study.]

Groups of 20 male Wistar rats [age unspecified] were given *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine in the drinking-water at 100 ppm [mg/L], together with a diet supplemeted with 10% sodium chloride for eight weeks to initiate gastroduodenal carcinogenesis,

followed by 32 weeks of treatment with drinking-water containing 40 ppm [mg/L] **mercuric chloride** and basal diet without the 10% sodium chloride; controls were given the nitrosamine and basal diet containing sodium chloride for eight weeks then basal diet and standard drinking-water. The incidences of carcinoma and hyperplasia of the fundic and pyloric regions of the glandular stomach and of carcinoma of the duodenum were not increased by treatment with mercuric chloride over those caused by the nitrosamine alone (Kurokawa *et al.*, 1989). [The Working Group noted the incomplete reporting of the study.]

(*c*) *Hamster*

A group of 20 female Syrian golden hamsters [age unspecified] received three weekly injections [site unspecified] of *N*-nitrosobis(2-oxopropyl)amine (NBOPA) at a dose of 10 mg/kg bw to initiate pancreatic carcinogenesis, followed by treatment with drinking-water containing 40 ppm [mg/L] **mercuric chloride** for a further period [presumed to be 30 weeks]. A further group of 32 hamsters received NBOPA followed by drinking-water alone for 30 weeks. At the end of the study [duration unspecified], there was no difference in the multiplicity of either pancreatic adenocarcinomas or dysplastic lesions between the two groups (Kurokawa *et al.*, 1989). [The Working Group noted the incomplete reporting of the study.]

3.3 Methylmercury chloride

3.3.1 *Oral administration*

(*a*) *Mouse*

Groups of 60 male and 60 female ICR mice, five weeks of age, were fed a diet containing 0, 15 or 30 ppm [mg/kg] **methylmercury chloride** (99.3% purity) for 78 weeks. All animals were examined macroscopically, but histopathological examination was carried out only on kidneys of animals that died after week 53 and on lungs of mice with renal masses. The first renal tumour was detected in a male treated with 15 ppm and necropsied at week 58. Most mice given 30 ppm had severe neurotoxic effects and died or became moribund by week 26; similar, but less marked toxic effects occurred in the group treated with 15 ppm. At 78 weeks, survival among male mice was 24/60 given 0 ppm, 8/60 given 15 ppm and 0/60 given 30 ppm; survival among female mice was 33/60 given 0 ppm, 18/60 given 15 ppm and 0/60 given 30 ppm. The numbers of male mice that died after 53 weeks with renal tumours were: 1/37 (an adenoma) in the group given 0 ppm, 13/16 (total numbers of tumours: 11 adenocarcinomas [$p < 0.001$] and five adenomas [$p < 0.01$]) in the group given 15 ppm and none in the one surviving animal treated with 30 ppm. No renal tumour was reported in the female mice (Mitsumori *et al.*, 1981). [The Working Group noted the poor survival in the groups exposed to high doses of methylmercury chloride and the limited number of tissues subjected to histopathological evaluation.]

Groups of 60 male and 60 female ICR mice, five weeks of age, were administered diets containing 0, 0.4, 2 or 10 ppm (mg/kg) **methylmercury chloride** (99.3% pure) for 104 weeks. Six males and six females from each group were killed at 26-week intervals and subjected to histological examination, as were all other animals. No neurotoxic effect was observed in the

treated animals, and, although all male mice given 10 ppm were dead by week 98, there was no difference in survival rates between the control and treated groups. The first renal tumour occurred in a male treated with 10 ppm at 58 weeks. Epithelial degeneration of the renal proximal tubules was seen in both males (40/59) and females (19/60) given 10 ppm, and similar but milder degeneration was seen in males given 2 ppm (12/58). The incidence of renal tumours in male mice was 1/58 (an adenoma) at 0 ppm, 0/59 at 0.4 ppm, 0/58 at 2.0 ppm and 13/59 (10 adenocarcinomas and three adenomas) at 10 ppm. [The effective numbers of animals at risk for renal tumours could not be determined.] No such tumour was seen in treated female mice (Hirano *et al.*, 1986).

Groups of 60 male and 60 female specific-pathogen-free (SPF) B6C3F1 mice, five weeks of age, were fed diets containing 0, 0.4, 2.0 or 10 ppm [mg/kg] **methylmercury chloride** (99.3% pure) for 104 weeks. All animals were subjected to histopathological examination. In the group treated with 10 ppm, neurotoxicity was recorded in male mice at week 59 and in females at week 80; at termination, neurological signs were seen in 33/60 males and 3/60 females. Survival was similar to that of controls (48%) in all groups except males treated with 10 ppm, which had 17% survival. The incidence of chronic nephropathy was increased in male mice treated with 2 ppm (27/60) or 10 ppm (59/60) and in females given 10 ppm (56/60). The first renal tumour was seen in a male given 10 ppm and killed at week 70. Renal epithelial tumours occurred in 0/60 control males, 0/60 given 0.4 ppm, 1/60 (an adenoma) given 2 ppm and 16/60 (13 adenocarcinomas and five adenomas) given 10 ppm; among female mice, a single adenoma (1/60) was found in those given 10 ppm (Mitsumori *et al.*, 1990). [The Working Group noted the lower survival of high-dose males after 60 weeks.]

(*b*) *Rat*

Groups of 25 male and 25 female weanling SPF Wistar rats were administered diets containing 0, 0.1, 0.5 or 2.5 ppm [mg/kg] **methylmercury chloride** (100% pure) for two years. Apart from a slight reduction in growth of females treated with 2.5 ppm, there was no effect of treatment on growth. No clinical or neurological sign of methylmercury chloride toxicity was reported during the study; mortality at 104 weeks was: 6/25 female and 7/25 male controls, 10/25 females and 8/25 males at 0.1 ppm, 9/25 females and 13/25 males at 0.5 ppm and 11/25 females and 13/25 males at 2.5 ppm. Histopathological examination was carried out on the control and 2.5 ppm-treated animals and on all animals that died. The authors reported no difference in tumour incidence or latency among the groups [no further detail reported] (Verschuuren *et al.*, 1976a,b). [The Working Group noted the limited nature of the study.]

Groups of 56 male and 56 female SPF Sprague–Dawley rats, five weeks of age, were administered diets containing 0, 0.4, 2 or 10 ppm [mg/kg] **methylmercury chloride** (99.3% purity) for 130 weeks. Ten animals of either sex were killed at 13 and 26 weeks and 10 at 52 and 78 weeks. Neurological signs of methylmercury chloride toxicity were apparent in the 10 ppm-treated group from week 22 in males and from week 46 in females. All animals were subjected to necropsy and histopathological examination. Survival in the groups given 10 ppm was lower than in controls or in the other two treated groups; the cause of death was related to nephrotoxicity. The incidence of tumours did not differ significantly among the

treated and control groups. A single renal adenoma was found in a high-dose female (Mitsumori *et al.*, 1983, 1984).

3.3.2 *Administration with known carcinogens*

As the purpose of the investigations described below was to study interactions with known carcinogens, the studies were limited to specific target sites, were often of short duration and were not intended to address the carcinogenicity of mercury *per se*.

Mouse: Groups of 16–20 female Swiss-cross mice, 21–24 days old, were given 0, 0.2, 0.5, or 2.0 μg/ml (mg/L) **methylmercury chloride** [purity unspecified] in deionized drinking-water for 15 weeks and then killed. After the first three weeks of the exposure, mice received intraperitoneal injections of 1.5 mg/g [g/kg bw] urethane in normal saline [volume unspecified] or saline alone. The lung tumour incidence in the mice injected with saline was reported to be less than one tumour per mouse in all test groups [no further detail reported]. The number of pulmonary adenomas induced by urethane alone (21.5 ± 3.0) was exceeded only in the group that received the high dose of methylmercury chloride (33.1 ± 3.8) (Blakley, 1984).

Groups of 20 female W rats were maintained either on basal diet or on basal diet containing 10 ppm [mg/kg] **methylmercury chloride** [purity unspecified] dissolved in corn oil, from weaning until they delivered pups. They were also given either 0.159, 0.318 or 0.636% ethylurea in the diet from day 14 of the breeding period to parturition or 50 or 100 mg/kg bw by gavage on days 17, 18 and 19 of gestation; at the same time, they received 0.5, 1.0 or 2.0 g/L sodium nitrite in drinking-water or 25 or 50 mg/kg bw by gavage. Control groups received either the basal diet alone or the methylmercury chloride diet alone. All dams were returned to the basal diet at parturition, and progeny (generally about 25: 13 males and 12 females) were maintained on the basal diet for their lifespan. Survival was poor in some treatment groups. The incidence of neurogenic tumours was nearly 100% in some ethylurea/sodium nitrite-treated groups; there were 0/25 neurogenic tumours in the methylmercury chloride control group. Methylmercury chloride did not increase the incidence of neurogenic tumours in the groups receiving ethylurea/sodium nitrite, but schwannomas of the central nervous system tended to appear earlier than in the group given ethylurea/sodium nitrite alone (Nixon *et al.*, 1979). [The Working Group noted the reduced sensitivity of the study, due to the very high incidence of neurogenic tumours in ethylurea/sodium nitrite-treated groups, and the poor survival.]

3.3.3 *Hormonal influences*

Mouse: Groups of 50 intact male and 50 intact female SPF ICR mice, seven weeks of age, were fed basal diet or basal diet containing 10 ppm [mg/kg] **methylmercury chloride** (purity, 99.3%) for 80 weeks. Groups of 50 orchiectomized male and 50 ovarectomized female mice, operated at five weeks of age, were fed basal diet containing 10 ppm methylmercury chloride only or also received weekly subcutaneous injections of 0.2 mg/mouse testosterone propionate in a 0.2% suspension (w/v) of sesame oil for 80 weeks. All groups receiving methylmercury chloride had nephrotoxic changes and caecal ulceration. No renal tumour was seen in intact males receiving basal diet alone, but one renal adenoma was seen in an

intact female mouse; renal adenocarcinomas (14/50) and an adenoma (1/50) were seen in intact male mice given the basal diet with methylmercury chloride but not in intact female mice. In addition, 6/50 intact male mice given methylmercury chloride in the diet had tubular-cell hyperplasia, a lesion that the authors considered to be preneoplastic. No renal tumour was seen in orchiectomized or ovarectomized mice receiving methylmercury chloride only, but two adenocarcinomas occurred in males and three in females that received methylmercury chloride together with testosterone propionate (Hirano *et al.*, 1988).