3. Studies of Cancer in Experimental Animals

The carcinogenic and toxicological effects of cadmium have been reviewed (Oberdörster, 1986; Peters *et al.*, 1986; Kazantzis, 1987; Oberdörster, 1989; Waalkes & Oberdörster, 1990; Heinrich, 1992; Nordberg *et al.*, 1992; Waalkes *et al.*, 1992a).

3.1 Oral administration

3.1.1 *Mouse*

A group of 48 male and 39 female weanling Swiss mice (Charles River strain) received 5 ppm [5 mg/L] cadmium as **cadmium acetate** in the drinking-water for life. A group of 44 male and 60 female control mice were given 'metal-free' drinking-water. Body weight was generally similar in treated and control animals. Cadmium treatment did not result in an increased incidence of any type of tumour, and the incidence of lung tumours in treated males was reduced compared to controls (0/48 *versus* 8/44; $p < 0.01$, χ^2 test); no such effect was observed in females (5/39 *vs* 9/60) (Schroeder *et al.*, 1964). [The Working Group considered that the single exposure level used was too low for an evaluation of carcinogenicity.]

Groups of 50 eight-week-old male specified pathogen-free (SPF) Swiss mice were administered 0.44, 0.88 or 1.75 mg/kg bw cadmium as **cadmium sulfate** (3 $CdSO_4.8H_2O$) in distilled-water by gavage once a week for 18 months, at which time the experiment was terminated. A control group of 150 male mice received distilled-water alone. No difference in survival or weight gain was observed between cadmium-treated mice and controls. Among the mice surviving after 18 months of treatment, groups of 20 animals were selected at random from the high-dose group and from the control group for histological analysis of selected tissues, including urogenital tract, stomach, lung, liver and kidney, while only macroscopically abnormal tissues were examined from other animals. Tumour incidence was no different in treated and control animals. Special attention was paid to the prostate, but no neoplastic or preneoplastic lesion was observed in this organ (Levy *et al.*, 1975). [The Working Group noted the low dose levels used and the limited histopathological examination in terms of numbers of animals and tissues.]

3.1.2 *Rat*

Groups of 69 male and 58 female weanling Long–Evans rats received 0 or 5 ppm [5 mg/L] cadmium as **cadmium acetate** in the drinking-water until death. Body weights and survival did not differ significantly among treated and control groups; about 50% of test and

control animals survived more than 24 months. Histopathology was performed on gross lesions. Tumour incidences in various organs in the 48 treated male and 36 treated female rats examined were similar to those in the 35 male and 35 female controls examined (Schroeder *et al.*, 1965). [The Working Group noted that the single exposure level used was too low for an evaluation of carcinogenicity.]

A group of 47 weanling Long–Evans rats [sex distribution unspecified] received 5 ppm [mg/L] cadmium as **cadmium acetate** in the drinking-water for life. A control group of 34 rats received drinking-water alone. Growth rate and survival were similar. Only macroscopically visible tumours were sectioned for histological analysis. Tumour incidence (as analysed by χ^2) in the cadmium-treated animals was similar to that in the control group (Schroeder *et al.*, 1963; Kanisawa & Schroeder, 1969). [The Working Group noted the single, low exposure level, the lack of sex-specific data and that histopathology was performed only on macroscopic lesions.]

Three groups of 30 male SPF CB hooded rats, 12 weeks of age, received weekly gastric instillations of 0.09, 0.18 and 0.35 mg/kg bw cadmium as **cadmium sulfate** ($3CdSO_4.8H_2O$) dissolved in sterile distilled-water for 104 weeks, at which time surviving animals were killed. A control group of 90 rats received weekly gastric instillations of sterile distilled-water. Histopathological examination was performed on all animals. There was no difference in body weights or survival. Tumour incidence was similar in cadmium-exposed animals and controls (Levy & Clack, 1975). [The Working Group noted the low doses used and that the spontaneous incidence of interstitial-cell tumours of the testis (seen in 75% of animals) may have obscured any cadmium-induced effects within that tissue.]

Groups of 50 male and 50 female Wistar (W74) rats, four to five weeks of age, were fed diets containing 1, 3, 10 or 50 ppm (mg/kg diet) Cd^{2+} as **cadmium chloride** ($CdCl_2.H_2O$) for a period of 104 weeks. Control groups of 100 rats of each sex were used. Survival was not affected by cadmium treatment, and body weights were significantly reduced only in high-dose males [exact extent or time point not given]. Histological examination was performed on all animals, and tumour incidence data were tested by the Fisher exact test. The incidences of testicular and prostatic tumours and of other tumour types were similar in treated groups and controls (Löser, 1980a).

Groups of 30 male Wistar (TNO/W74) rats, 13–16 weeks old, received a single intragastric dose of 50 mg/kg bw or 10 weekly doses of 5 mg/kg bw cadmium as **cadmium chloride** ($CdCl_2.H_2O$) in distilled-water. Two groups of 10 controls received vehicle only. Five animals in each group of treated animals and two or three animals of each group of control animals were killed at 12 and 18 months, and the remaining animals were killed at 133 weeks. Survival was similar in all groups. Following the single administration of 50 mg/kg bw, growth was slightly retarded [data not shown, extent or time point not given]. Testis, epididymis, seminal vesicles, prostates and gross lesions were examined histologically. Rats treated with cadmium did not have an increased incidence of testicular tumours or of tumours at other sites (Bomhard *et al.*, 1987). [The Working Group noted the short duration of exposure.]

In a study on the effect of chronic dietary deficiency of zinc on the carcinogenicity of orally administered cadmium, groups of 28 male Wistar (WF/NCr) rats, eight weeks of age, were fed diets containing either adequate zinc (60 ppm [60 mg/kg diet]) or a zinc concentration (7 ppm [7 mg/kg diet]) that produced a significant reduction (40%) in serum zinc in

the absence of overt toxicity. Starting two weeks later, these diets were fed together with cadmium at 0, 25, 50, 100 or 200 (maximum-tolerated dose) ppm [mg/kg diet] as **cadmium chloride** hemipentahydrate, for 77 weeks, at which time the study was terminated. Histological examination was performed on all animals and lesions. Zinc deficiency alone did not affect food consumption, weight gain or survival; cadmium did not affect survival or food consumption, and body weight was consistently reduced only at the highest doses (100 and 200 mg/kg diet), by 10% and 12–17%, respectively. At the two highest doses of cadmium, rats fed zinc-deficient diets had a significantly increased food consumption when compared with zinc-deficient controls. The combined incidence of prostatic proliferative lesions (hyperplasia and adenoma), but not those of the lesions separately, was significantly ($p < 0.05$; Fisher exact test) greater in rats given zinc-adequate diets containing 50 ppm cadmium (5/22; 22.7%) than in controls (1/28; 3.6%), and in rats receiving zinc-deficient diets (4/26; 15.4%) than in controls (0/26). At higher doses of cadmium (100 and 200 ppm), an increased incidence of prostatic atrophy was observed in the rats receiving zinc-deficient diets, which may have been responsible for the lower incidence of prostatic lesions seen in rats fed zinc-deficient diets. Cadmium treatment resulted in a dose-related (Cochran–Armitage trend tests) increase in the incidence of leukaemia in rats fed zinc-deficient diets throughout the dose range and in rats fed zinc-adequate diets receiving up to and including 100 ppm. The highest incidence of leukaemia occurred in rats receiving 200 ppm cadmium and the zinc-deficient diet (7/25; 28%), when compared with controls (2.27; 7.4%). Exposure to cadmium at a concentration of 200 ppm in conjunction with the zinc-adequate diet also induced a significant increase in testicular interstitial-cell tumours (6/27, 22.2% compared with controls, 1/28, 3.6%) (Waalkes & Rehm, 1992).

3.2 Inhalation exposure[1]

3.2.1 *Mouse*

Groups of 48 female Han:NMRI mice [age unspecified] were exposed to cadmium at 30 or 90 μg/m^3 as **cadmium chloride**, 30 or 90 μg/m^3 as **cadmium sulfate**, 90, 270 or 1000 μg/m^3 as **cadmium sulfide**, 10, 30, 90 or 270 μg/m^3 as **cadmium oxide dust** or 10, 30 or 90 μg/m^3 as **cadmium oxide fume**. The mass median aerodynamic diameter of all compounds was 0.2–0.6 μm [geometric standard deviation, 1.6]. For each treated group, a control group of 48 animals receiving filtered air was available. Exposure was for 19 or 8 h per day for five days a week, and the exposure time ranged from six to 69 weeks; exposure was terminated in some groups when the mortality rats started to increase. The duration of the study was 71–107 weeks; controls were followed for about 106 weeks. Histological examination was performed on all animals. Survival was reduced in 12 of the 19 experimental groups; survival was similar to that of controls in groups exposed to 90 or 270 μg/m^3 as cadmium sulfide, to 10, 30 or 90 μg/m^3 as cadmium oxide fume and to 10 or 270 μg/m^3 as cadmium oxide dust. The incidence of lung tumours was significantly increased in the groups receiving 30 and 90 μg/m^3

[1]The Working Group was aware of a study by inhalation in progress in rats and mice (Ghess *et al.*, 1992).

as cadmium oxide fumes and 10 μg/m^3 as cadmium oxide dust, but not in the group given 270 μg/m^3 as cadmium oxide (see Table 12). In six other groups receiving cadmium oxide dust at various concentrations, survival was significantly decreased, but the probability of dying with a lung tumour was greater than in the controls (by life-table analysis) (Heinrich *et al.*, 1989; Heinrich, 1992). [The Working Group noted the variable spontaneous lung tumour rate and that the histopathological types of tumours were not reported.]

3.2.2 *Rat*

Four groups of 40 male SPF Wistar (TNO/W75) rats, six weeks old, were exposed to 12.5, 25 or 50 μg/m^3 cadmium as **cadmium chloride** aerosol (mass median aerodynamic diameter, 0.55 μm; geometric standard deviation, 1.8) for 23 h a day on seven days a week for 18 months. Animals were observed for an additional 13 months, at which time the experiment was ended. A group of 41 rats exposed to filtered air served as controls. Histological examination was performed on all animals. Body weights and survival were not affected by cadmium treatment. A dose-related increase in the incidence of malignant pulmonary tumours (mostly adenocarcinomas) was observed in cadmium chloride-treated rats (12.5 μg/m^3, 6/39 [15%]; 25 μg/m^3, 20/38 [53%]; 50 μg/m^3, 25/35 [71%]) compared with controls (0/38). Multiple pulmonary tumours were observed frequently; several tumours showed metastases or were regionally invasive. The incidence of adenomatous hyperplasia was also increased by cadmium treatment (Takenaka *et al.*, 1983).

Groups of 20–40 male and 20 female Wistar (BOR-WISW) [formerly called TNO/W75] SPF rats, nine weeks of age, were exposed to aerosols of **cadmium chloride**, **cadmium sulfate**, **cadmium sulfide**, **cadmium oxide dust** or **cadmium oxide fume** (for all compounds, mass median aerodynamic diameter, 0.2–0.5 μm; geometric standard deviation, 1.6) for up to 18 months and observed for up to an additional 13 months (see Table 13). Two additional groups received both zinc oxide dust and cadmium oxide dust at two different levels. Exposure was generally for 22 h a day for seven days a week, although some groups received continuous exposure for 6 months or discontinuous exposure for 40 h per week for 6 months. Inhalation and observation periods were terminated when mortality reached $\geq$ 25% or $\geq$ 75%, respectively, or at 31 months. Histological examination was performed on all rats. Mortality rates were generally greater in rats treated with the high dose of cadmium [body weights not given]. Generally, all forms of cadmium appeared to increase the incidence of primary pulmonary tumours over that in controls [statistical analysis not performed], to a maximum of 90% (cadmium sulfate in females) compared to 0 in controls; in general, no male:female difference was observed. Except in males exposed to 90 μg/m^3 cadmium oxide and 900 μg/m^3 zinc oxide, zinc oxide reduced the carcinogenicity of cadmium oxide at that dose. The tumours observed were mostly adenomas and adenocarcinomas, but a few rats had bronchioloalveolar adenomas and squamous-cell carcinomas (Glaser *et al.*, 1990). [The Working Group noted that the groups exposed to cadmium oxide fume had significantly lower lung tumour incidences than the groups exposed to the other cadmium compounds ($p < 0.0001$; likelihood ratio by χ^2 test). Animals exposed to cadmium oxide fume, however, had only about half the cadmium content in their lungs as animals exposed to the same concentration of the other cadmium compounds over the same period, which was attributed

Table 12. Percentages of animals bearing lung tumours among mice exposed to cadmium with no reduction in mean survival time

Cadmium compound	Concentration ($\mu g/m^3$ Cd)	Exposure time (weeks)		50% survival (weeks)		Experimental time (weeks)	% Tumour-bearing animals	
		h/day	Weeks	Treated	Controls		Treated	Controls
Cadmium sulfide	90	19	64	76	70	98	21.1	14.6
	270	8	26	78	80	101	25	36.9
Cadmium oxide fume	10	19	55	75	71	98	20.9	20.0
	30	19	50	68	71	93	29.6*	20.0
	90	8	64	74	70	105	34.0*	14.6
Cadmium oxide dust	10	19	64	76	70	105	26.1*	14.6
	270	8	59	66	89	107	25.5	27.7

Modified from Heinrich (1992)
*$p < 0.05$ (χ^2 test)

Table 13. Lung tumour incidence in animals with long-term exposure to cadmium by inhalation

Group	Cadmium aerosol ($\mu g/m^3$)	Duration (months)[a]		Animals bearing primary lung tumours/animals examined
		Exposure	Study	
Males				
Control	0	0	31	0/40
Cadmium chloride	30	18	30	15/20
	90	6	30	11/20
Cadmium sulfate	90	14	31	11/20
Cadmium sulfide	90	18	30	17/20
	270	16	30	14/20
	810	7	30	11/20
	2430	4	30	7/16
	270[b]	6	27	3/20
Cadmium oxide dust	30	18	31	28/39
	90	7	31	12/39
	90[b]	6	31	4/20
	30[c]	18	29	25/38
Cadmium oxide fume	10	18	31	0/40
	30	18	31	8/38
Cadmium oxide/zinc oxide	30/300	18	31	0/20
	90/900	18	31	8/20
Females				
Control	0	0	31	0/20
Cadmium chloride	30	18	31	13/18
	90	6	29	3/18
Cadmium sulfate	90	18	29	18/20
Cadmium sulfide	90	18	31	15/20
	270	16	30	16/19
	810	10	29	13/20
	2430	3	31	6/19
	270[b]	6	29	3/20
Cadmium oxide dust	30	18	31	15/20
	90	11	31	14/19
	90[b]	6	31	3/20
Cadmium oxide/zinc oxide	30/300	18	31	0/20
	90/900	18	31	7/20

Modified from Glaser *et al.* (1990)

[a]Exposure was stopped when 25% mortality had occurred, and the study was terminated when 75% of the animals had died.

[b]Discontinuous exposure for 40 h per week

[c]Rats maintained on a zinc-deficient (24 ppm) diet

to a lower pulmonary deposition (Oberdörster & Cox, 1990) of the chain-like electric arc-generated fume particles. The Working Group was also aware of the fact that the generation of cadmium sulfide aerosols in this study from an aqueous suspension had resulted in the generation of a cadmium sulfate:cadmium sulfide mixture (50:50), due to photo-oxidation of cadmium sulfide, which may have confounded the number of tumours induced by cadmium sulfide (Glaser *et al.*, 1992; König *et al.*, 1992; Oberdörster & Cherian, 1992)].

3.2.3 *Hamster*

Groups of 24 male and 24 female Syrian golden [Hoe:SYHK] hamsters [age unspecified] were exposed to cadmium at 30 or 90 μg/m^3 as **cadmium chloride**, 30 or 90 μg/m^3 as **cadmium sulfate**, 90, 270 or 1000 μg/m^3 as **cadmium sulfide**, 10, 30, 90 or 270 μg/m^3 as **cadmium oxide dust** or 10, 30 or 90 μg/m^3 as **cadmium oxide fume** (mass median aerodynamic diameter, 0.2–0.6 μm [geometric standard deviation, 1.6]) for 19 or 8 h per day on five days a week; the exposure time ranged from 13 to 65 weeks and the total experimental time from 60 to 113 weeks. Control groups received filtered air. Exposure was terminated earlier when mortality started to increase; the experimental time was 61–87 weeks for exposed females and 60–113 weeks for males, as increased mortality occurred earlier in females than in males. Survival was reduced in 12 of the 19 groups of exposed male hamsters, but none showed an increased incidence of lung tumours. Histological examination was performed on all animals. In only six of the exposed groups (males and females combined) was there one or, in one case, two animals with a papilloma or a polypoid adenoma of the trachea; one papilloma was also found in the control group (Heinrich *et al.*, 1989; Heinrich, 1992). [The Working Group noted the limited reporting of the data on tumours and the insensitivity of the hamster to induction of tumours of the lung in studies by long-term inhalation.]

3.3 Intratracheal administration

Rat: Groups of male Fischer 344 rats received either a single intratracheal instillation of 25 μg **cadmium oxide** (median diameter, 0.5 μm) suspended in saline at 70 days of age (48 rats), one instillation at both 70 and 100 days of age (total dose, 50 μg/rat; 46 rats) or one instillation at 70, 100 and 130 days of age (total dose, 75 μg/rat; 50 rats) and were compared with 46 rats receiving intratracheal instillations of saline only. [The dose of 25 μg/rat was approximately 75% of the single intratracheal LD_{50}.] Animals were observed for up to 880 days, and all were examined histologically. Cadmium treatment did not affect survival [body weights not given]. Two pulmonary adenocarcinomas were seen in rats given 50 μg (nonsignificant; χ^2) but none in other groups. Increased incidences of mammary gland fibroadenomas were reported in all groups receiving cadmium oxide: 25 μg/rat, 7/44 (16%); 50 μg/rat, 5/41 (12%); 75 μg/rat, 11/48 (23%); controls, 3/45 (7%). No other significant difference in tumour incidence was seen (Sanders & Mahaffey, 1984). [The Working Group noted that the mammary tumours that occurred in treated groups had to be pooled in order to reach statistical significance.]

Groups of about 40 female Wistar (WU/Kiβlegg) rats, 11 weeks of age, received 20 weekly intratracheal instillations in saline of 1 or 3 μg or 15 weekly instillations of 9 μg

cadmium as **cadmium chloride** hydrate (purity, 99%) or **cadmium oxide** [purity not given] (total doses, 20, 60 or 135 μg/rat for both compounds) or 10 weekly instillations of 63, 250 or 1000 μg cadmium as **cadmium sulfide** (> 99.9% pure; total doses, 630, 2500 or 10 000 μg/rat) and were observed for up to 124 weeks. Concurrent controls received saline only. [Body weights were not given.] Only the lungs and trachea were examined histologically. Cadmium chloride induced moderate, dose-related increases in the incidence of lung tumours: controls, 0/40; 20 μg/rat, 0/38; 60 μg/rat, 3/40 (7.5%); 135 μg/rat, 2/36 (5.6%) [$p < 0.01$; trend test]. Cadmium oxide also induced some lung tumours: 20 μg/rat, 2/37 (5.4%); 60 μg/rat, 2/40 (5.0%); 135 μg/rat, 0/39 (not significant). Cadmium sulfide induced a dose-related increase in the incidence of lung tumours: 2/39 (5.1%) at 630 μg/rat, 8/36 (22.2%) at 2500 μg/rat and 7/36 (19.4%) at 10 000 μg/rat [$p < 0.005$; trend test]. The authors reported that mortality was increased at the highest dose. The lung tumours induced were primarily adenocarcinomas, although a few adenomas and squamous-cell carcinomas were also observed (Pott *et al.*, 1987). [The Working Group noted that the cadmium sulfide particles had been administered in an aqueous suspension, which may have resulted in photo-oxidation of some fraction of the cadmium sulfide to cadmium sulfate; however, even under the assumption of worst-case conditions—24-h exposure of cadmium sulfide suspension to light—the amount of cadmium sulfate should not have exceeded 3% of the total cadmium in the middle dose (Oberdörster & Cherian, 1992). Therefore, photo-decomposition of cadmium sulfide to cadmium sulfate could not have accounted for the carcinogenic response observed.]

3.4 Subcutaneous and/or intramuscular administration

3.4.1 *Mouse*

In a study to examine the effect of zinc on the carcinogenicity of cadmium, a group of 26 male Charles River mice, eight weeks of age, received a single subcutaneous injection of 0.03 mmol/kg bw (5.5 mg/kg bw) **cadmium chloride** [vehicle unspecified] in the interscapular region and were observed for 14 months. Control groups consisted of 25 untreated mice and 25 mice that received a single subcutaneous injection of 0.03 mmol/kg bw cadmium chloride and three concurrent subcutaneous injections of 1.0 mmol/kg zinc acetate (total dose, 3.0 mmol/kg; 550 mg/kg bw) at three different sites over a period of 25 h before and after the cadmium injection. No injection-site tumour was reported. Only testes were examined histologically [weight gains and survival not reported]. Of the mice that received cadmium alone, 77% (20/26) had interstitial-cell tumours of the testis; none occurred in cadmium–zinc treated animals or in untreated controls. Zinc also prevented the induction by cadmium of non-neoplastic lesions of the testes in almost all of the animals (Gunn *et al.*, 1961, 1963).

A group of 20 male CB mice, six to seven weeks of age, received 11 weekly subcutaneous injections of 0.05 mg **cadmium sulfate** tetrahydrate in 0.2 ml sterile distilled-water into the right flank (total dose of cadmium, 0.22 mg). A group of 20 untreated animals served as controls [body weights not reported]. Only gross and testicular lesions were taken for histological analysis. None of the cadmium-treated mice developed tumours at the site of injection, and the incidence of tumours at other sites did not exceed control rates [statistical analysis not given]; however, non-neoplastic changes typical of cadmium treatment, such as

testicular degeneration and interstitial-cell hyperplasia, were observed in 6/16 [17 in table] animals that survived for eight months or more and in none of 15 control mice surviving at least to the same time point. [The Working Group noted the short duration of the study.] In a separate study, 10 six-week-old male CB mice received three weekly subcutaneous injections of 5 mg cadmium sulfate-precipitated rat ferritin into the right flank, followed six weeks later by 12 weekly injections of 0.5 mg of the same preparation (total dose, 0.36 mg cadmium). No tumour was observed at the site of injection during the following 20 months (Haddow *et al.*, 1964; Roe *et al.*, 1964).

3.4.2 *Rat*

The earliest suspicion that cadmium might be carcinogenic came from a brief report by Haddow *et al.* (1961), who detected malignant tumours at the injection site (subcutaneous or intramuscular) of ferritin prepared from rat liver by cadmium precipitation in 8/20 male rats [strain unspecified]. Additionally, 10/20 of these rats had interstitial-cell tumours of the testes.

Groups of 10 female hooded rats, two to three months old, received a single intramuscular injection of 14 or 28 mg **cadmium metal** powder (dimensions: 1.7 μm diameter for spheres, 85 μm × 50 μm for ellipsoids and rods and 220 μm × 50 μm × 50 μm for other shapes) suspended in 0.4 ml fowl serum into the thigh muscle. The total duration of the study was 84 weeks. [Weight gain was not determined and necropsy protocol was not stated.] Two of the rats receiving 28 mg cadmium powder were killed within two week of injection in order to study acute local reactions. Malignant tumours developed at the site of injection in 9/10 rats given 14 mg and in 6/8 rats given 28 mg cadmium powder. Most of the tumours were rhabdomyosarcomas; some fibrosarcomas were seen which metastasized to lymph nodes (Heath *et al.*, 1962; Heath & Daniel, 1964). [The Working Group noted that, while the authors stated that zinc or tungsten metal powders did not induce local sarcomas at the injection site, no details were reported.]

A group of 25 male Wistar rats, 12 weeks of age, received a single subcutaneous injection of 0.03 mmol/kg bw [5.5 mg/kg bw] **cadmium chloride** [vehicle unspecified] in the interscapular region and were observed for 11 months. Control groups consisted of 20 untreated rats and 17 rats that received a single subcutaneous injection of 0.03 mmol/kg bw cadmium chloride and three concurrent subcutaneous injections of 1.0 mmol/kg zinc acetate (total dose, 3.0 mmol/kg; 550 mg/kg bw] in the lumbosacral area over a period of 25 h before and after the cadmium injection. Only testes were examined histologically. [Weight gains and survival were not stated.] Of the rats that received cadmium alone, 17/25 (68%) had interstitial-cell tumours of the testes; 2/17 occurred in cadmium–zinc treated animals, and 0/20 occurred in controls (Gunn *et al.*, 1961, 1963, 1965).

Ten six-month-old female Wistar CB rats received subcutaneous injections of 25 mg **cadmium sulfide** (diameter, 0.5 μm; equivalent to 20 mg cadmium) suspended in 0.25 ml saline into both sides of the dorsal midline. Ten three-month-old rats received subcutaneous injections of 0.25 ml saline and served as controls. [Body weights and necropsy protocol were not stated.] Over the following 12 months, 6/10 treated rats and none of the controls developed fibrosarcomas (Kazantzis, 1963). In a further study, 15 male and 15 female Wistar CB rats, nine months of age, received subcutaneous injections of 25 mg cadmium sulfide

suspended in 0.25 ml saline into both sides of the dorsal midline. Of the 26 rats that survived for more than six months, six developed sarcomas at the site of injection. In a separate experiment, seven male and seven female rats, eight months old, were given a single intramuscular injection of 50 mg cadmium sulfide suspended in 0.5 ml saline into the thigh. [Body weights and necropsy protocol not stated.] Four animals died during the first nine months after injection. Sarcomas at the site of injection developed in 6/14 rats over a 17-month period. In a further study, 10 three-month-old female rats received subcutaneous injections of 25 mg cadmium oxide suspended in 0.25 ml saline into both sides of the dorsal midline; 8/10 developed fibrosarcomas at the site of injection within one year (Kazantzis & Hanbury, 1966).

A group of 22 four-month-old male Wistar rats received single subcutaneous injections of 0.03 mmol/kg bw [5.5 mg/kg bw] **cadmium chloride** (equivalent to 1.35 mg cadmium [vehicle not stated]) into the interscapular region and were observed for 10 months. Only testes and subcutaneous tumours at the site of injection were examined histologically. Sarcomas developed at the site of injection in 9/22 rats, while 21/22 rats developed interstitial-cell tumours of the testis. A group of 17 rats received the same treatment with cadmium chloride and subcutaneous injections of 1 mmol/kg bw (183.5 mg/kg bw) zinc acetate in the lumbosacral area; the zinc acetate treatment resulted in lower incidences of sarcomas at the site of injection (2/17) and of testicular tumours (3/17) than those induced by cadmium. No interstitial-cell tumour of the testis developed in 18 untreated controls (Gunn *et al.*, 1964).

A group of 20 male CB rats, three weeks of age, received an initial subcutaneous injection of 20 mg **cadmium sulfate**-precipitated rat liver ferritin [vehicle unspecified] into the right flank followed by another injection of 20 mg 46 days later and then 2 mg once a week for eight weeks, all in approximately the same area. A group of 16 untreated rats served as controls. Only gross lesions were taken for histological examination. Of the cadmium–ferritin-treated rats, 7/20 (35%) developed injection-site sarcomas and 11/15 (73%) examined developed interstitial-cell tumours of the testis over the total observation period of 28 months. No such lesion was observed in 15 control rats that survived to a similar time (Haddow *et al.*, 1964; Roe *et al.*, 1964). In a subsequent study, no tumour at the site of injection and no testicular tumour was induced in CB Wistar rats by cadmium-free ferritin (Roe *et al.*, 1968). A separate group of 20 male CB rats, six to seven weeks old, received 10 weekly subcutaneous injections into the right flank of 0.5 mg cadmium sulfate tetrahydrate in 0.1 ml sterile distilled-water. By 20 months, 14/20 rats had developed sarcomas at the site of injection (Haddow *et al.*, 1964), while 10/18 examined had developed interstitial-cell tumours of the testis (Roe *et al.*, 1964).

A group of 49 male Wistar rats, four months of age, received a single injection of 1.8 mg cadmium as **cadmium chloride** [vehicle unspecified] either subcutaneously into the interscapular region (23 rats) or intramuscularly into the thigh (26 rats) and were observed for 14 months. No concurrent controls were available [body weights and necropsy protocol not given]. More sarcomas occurred at the site of injection when cadmium was injected subcutaneously (10/23; 43.5%) than intramuscularly (3/26; 11.5%) (Gunn *et al.*, 1967).

Six male Sprague–Dawley rats, three months of age, received a single subcutaneous injection of 10 mg/kg **cadmium chloride** [site and vehicle unspecified] and were observed for

a further 13 months. A group of 16 untreated rats of the same age served as controls. All six cadmium-treated rats developed interstitial-cell tumours of the testis. Baseline urinary testosterone concentrations in cadmium-treated rats bearing tumours were 26–29% those of control animals (Favino *et al.*, 1968). [The Working Group noted the limited reporting of the study.]

Eighty 12-week-old male Wistar rats received a a single subcutaneous injection of 0.03 mmol/kg bw [5.5 mg/kg bw] **cadmium chloride** as the dihydrate (3.4 mg/kg bw cadmium) dissolved in sterile distilled-water into the hip area and were observed for up to two years. Twenty untreated rats served as controls. Animals were examined grossly and histologically [body weights not stated]. Dermal atrophy, ulcerative necrosis, acute and chronic inflammation, fibrosis and mineralization were observed at the site of injection during the two months following administration of cadmium. Of the rats that survived to seven months (the time of appearance of the first sarcoma at the site of injection), 6/45 had local spindle-cell sarcomas after 18 months (Knorre, 1970a,b). In a second experiment with 104 male rats treated by the same schedule, interstitial-cell tumours of the testis were found by 698 days in 10/25 rats still alive at 355 days (when the first tumour of the testis appeared) (Knorre, 1971). One sarcoma at the site of injection metastasized to the peritoneum [possibly invasion], and several others metastasized to regional lymph nodes (Knorre, 1970a). A single cadmium-induced histologically confirmed testicular interstitial-cell tumour metastasized to the colon and liver [a rare event]. No interstitial-cell tumour of the testis was seen in 32 control animals (Knorre, 1971).

A group of 15 male Wistar rats weighing 100–300 g received single subcutaneous injections [site unspecified] of 0.02–0.03 mmol/kg bw [3.7–5.5 mg/kg bw] **cadmium chloride** and were observed for 11 months. Interstitial-cell tumours of the testis developed in 13/13 rats still alive at 11 months, and two rats developed pleomorphic sarcomas at the site of injection (Lucis *et al.*, 1972) .

Three groups of 25 male SPF CB hooded rats, 12 weeks of age, received weekly subcutaneous injections of 0.05, 0.1 or 0.2 mg **cadmium sulfate** (3 $CdSO_4$.8 H_2O; 0.02–0.09 mg Cd) dissolved in sterile distilled-water into alternate flanks over a period of two years. The control group consisted of 75 animals that received weekly injections of distilled-water alone. Extensive macroscopic and microscopic examinations were performed, with special attention to the genital gland complex. Body weights were suppressed ($p < 0.001$) [test unspecified; data not shown; extent not stated] in the high-dose group after two years. Sarcomas at the site of injection were found in 1/25 rats given the low dose, 1/25 given the medium dose and 4/25 given the high dose. No neoplastic change was seen in any other tissue, including the prostate. All groups, including controls, had high incidences of testicular interstitial-cell tumours (67–77%) [which may have obscured any effect of cadmium on that tissue]. The concentration of cadmium in the kidney in the highest dose group was about 500 μg/g tissue [analysed polarographically after dithizone extraction] (Levy *et al.*, 1973).

Twenty male Fischer 344 rats, four to five weeks old, received a single subcutaneous injection of 0.03 mmol/kg bw **cadmium chloride** [5.5 mg/kg bw], and 10 control rats received subcutaneous injections of saline [body weight, survival data and necropsy protocol not given]. Interstitial-cell tumours of the testis (11 bilateral) developed in 16/20 treated rats over the one-year observation period, while none developed in control rats (Reddy *et al.*,

1973). [The Working Group noted that the Fischer 344 strain generally has a > 80% spontaneous incidence of interstitial-cell tumours of the testis by two years of age.]

In a 110-week study to examine the potential effects of calcium and magnesium salts on the carcinogenicity of cadmium, groups of 25 male Wistar rats weighing 120–150 g were kept for two weeks and then given subcutaneous injections of 0.02 or 0.04 mmol/kg bw [3.67 or 7.34 mg/kg bw] **cadmium chloride** hemipentahydrate into the nape of the neck. Rats received either no further treatment or treatment with calcium acetate or magnesium acetate, either in the diet (3%) two weeks prior and two weeks after cadmium chloride treatment or by three separate subcutaneous injections (calcium acetate, 0.16 mmol/kg bw [25.3 mg/kg bw]; magnesium acetate, 4.0 mmol/kg bw [570 mg/kg bw]) in the same area as the cadmium chloride 24 h before, at the same time as and 24 h after cadmium chloride injection. Control rats received saline injections instead of cadmium chloride. All animals were examined histologically. The highest dose of cadmium chloride caused slight weight suppression but only up to 12 weeks after injection. Survival was not affected by any of the treatments. Tumours at the site of injection (predominantly fibrosarcomas) occurred to a similar extent (approximately 33% of rats at risk) in all groups receiving cadmium chloride, regardless of the dose or of other treatments, with the exception of injected magnesium acetate which significantly reduced (to 0; $p < 0.02$; χ^2) the response to cadmium at the injection site. Both levels of cadmium chloride increased the incidence of testicular interstitial-cell tumours (approximately 85%) over that in controls (30%); the increase was generally unchanged by other treatments, with the exception of dietary calcium acetate, which resulted in a lower incidence than in animals receiving cadmium chloride alone, but only at the high dose. When all groups receiving cadmium chloride were considered together, a significantly ($p < 0.02$) higher incidence of pancreatic islet-cell tumours (mainly adenomas) occurred (22/258 rats; 8.5%) when compared with rats not receiving cadmium chloride (3/137; 2.2%) (Poirier *et al.*, 1983).

Groups of 30 male Wistar Crl:(WI)BR rats, six weeks of age, received a single subcutaneous injection of 1.0, 2.5, 5.0, 10.0, 20.0 or 40.0 μmol/kg bw [0.18–7.3 mg/kg bw] **cadmium chloride** dissolved in saline into the dorsal thoracic midline area and were observed for two years. Other groups received either four separate subcutaneous injections of 5 μmol/kg cadmium chloride on days 0, 2, 4 and 7 or a subcutaneous injection of 5 μmol/kg [0.9 mg/kg] cadmium chloride followed two days later by a dose of 10 or 20 [1.8 or 3.6] μmol/kg bw. A group of 45 controls received subcutaneous injections of saline alone. All animals were examined histologically. Cadmium chloride did not modify survival in any group. The highest dose (40 μmol/kg) reduced body weight by about 5–10%. The incidences of sarcomas at the site of injection were found to depend on accumulated dosage at the site and approached 45% incidence at the highest dose of cadmium. The incidences of testicular tumours (mostly interstitial-cell tumours) were correlated with the extent of testicular degeneration induced by cadmium and showed a positive dose-dependence with single doses of cadmium: 83% at 40 μmol/kg and 72% at 20 μmol/kg, as compared with 18% in controls ($p \leq 0.05$; Cochran–Armitage test). The 5-μmol/kg and 20-μmol/kg doses did not increase the incidence of testicular tumours. Prostatic tumour incidence was significantly elevated at the 2.5 μmol/kg dose (8/26; 31%; $p \leq 0.05$, Fisher exact test) compared with controls (5/44; 11%), and a positive dose–effect relationship was seen between 0 and 2.5 μmol/kg in both

tumour incidence and multiplicity ($p \leq 0.05$; Cochran–Armitage test). A reduction to the control level in the tumour response of the prostate to higher doses of cadmium (≥ 5.0 μmol/kg) was attributed by the authors to testicular degeneration and consequent loss of androgenic support. Cadmium chloride suppressed the induction of tumours of the pancreas (both islet-cell and acinar-cell) from 60% in controls to 20% in animals receiving 40 μmol/kg cadmium, with a negative dose dependence ($p \leq 0.05$, Cochran–Armitage test) (Waalkes *et al.*, 1988a).

Groups of 30 male Wistar Crl:(WI)BR rats, six weeks of age, received a single subcutaneous injection of 30 μmol/kg bw (5.5 mg/kg bw) **cadmium chloride** dissolved in saline into the dorsal thoracic midline area and three subcutaneous injections of 0.1, 0.3 or 1.0 mmol/kg [18.4, 55.1 or 183.5 mg/kg bw] zinc acetate in saline into the right, left and midline lumbosacrum 6 h before, at the same time as and 18 h after the cadmium treatment. Animals were observed for up to two years. Two other groups of 30 rats received either 30 μmol/kg cadmium chloride intramuscularly plus 1.0 mmol/kg zinc subcutaneously into the right, left and midline lumbosacrum 4 h before, at the same time as and 18 h after the cadmium treatment or 30 μmol/kg cadmium chloride subcutaneously and, starting two weeks previously, 100 ppm [100 mg/L] zinc as zinc acetate in the drinking-water for the duration of the study. A control group of 84 rats received saline injections and tap water. All animals were examined histologically. The treatments did not modify survival in any group. Injection-site sarcomas occurred in 12/30 rats given only the subcutaneous injection of cadmium, and the incidence was significantly ($p \leq 0.05$; Fisher exact test) reduced by the highest subcutaneous dose of zinc (6/29) and by administration of zinc in the drinking-water (1/30). Intramuscular injection of cadmium produced sarcomas at the site of injection in 3/29 rats given cadmium alone and in 1/29 rats also given zinc subcutaneously. Testicular tumours (mostly interstitial-cell tumours) were observed in 22/30 (73%) rats given only the subcutaneous injection of cadmium chloride and in 3/28 (11%) rats receiving both cadmium chloride subcutaneously and three subcutaneous doses of 1 mmol/kg zinc; 9/83 (11%) were observed in saline control rats. The incidence of testicular tumours overall showed a negative dependence on the subcutaneous dose of zinc ($p \leq 0.05$; Cochran–Armitage test), although zinc in the drinking-water had no effect on induction by subcutaneous cadmium chloride (25/30 rats, 83%). Subcutaneous administration of cadmium caused extensive testicular degeneration, which was prevented in a dose-related fashion by subcutaneous zinc. Intramuscular administration of cadmium did not increase the incidence of tumours of the testis. Prostatic adenoma incidence was elevated in the groups receiving cadmium subcutaneously and the high dose of zinc (8/27; 30%; $p \leq 0.05$, Fisher exact test), intramuscular cadmium (11/26; 42%; $p \leq 0.05$) or intramuscular cadmium and subcutaneous zinc (7/28; 25%; $p \leq 0.05$), compared with controls (8/83; 10%). The tumour response of the prostate to cadmium in animals given the highest dose of zinc was attributed by the authors to prevention of cadmium-induced testicular degeneration and consequent loss of androgenic support (Waalkes *et al.*, 1989).

A group of 70 male Fischer F344/NCr rats, eight weeks old, received a single subcutanous injection of 30.0 μmol/kg bw [5.5 mg/kg bw] **cadmium chloride** hemipentahydrate dissolved in saline into the dorsal thoracic midline area and were observed for 90 weeks. Fifty control animals received a single subcutaneous injection of saline only. In the

33 animals still alive at the time of appearance of the first tumour (32 weeks), cadmium chloride reduced survival but not body weight. Cadmium chloride induced sarcomas (primarily fibrosarcomas) at the site of injection in 21/32 rats (1/50 in controls). The incidence of testicular interstitial-cell tumours was 97% in cadmium chloride-treated rats and 84% in controls. The incidence of large granular lymphocytic leukaemia (2/31) was reduced ($p = 0.028$) by cadmium chloride from that in controls (12/47) (Waalkes *et al.*, 1991a).

Groups of 28 male Wistar Hsd:(WI)BR rats, eight weeks of age, were fed diets either adequate in zinc (60 ppm [60 mg/kg diet]) or marginally zinc-deficient (7 ppm [7 mg/kg diet]), as defined by significant reductions (40%) in serum zinc in the absence of overt weight suppression. The diets were given for two weeks prior to a single subcutaneous injection of 0, 5.0, 10.0 or 30.0 μmol/kg bw (0.92–5.5 mg/kg bw) **cadmium chloride** hemipentahydrate dissolved in saline into the dorsal thoracic midline area. Animals were observed for the next 92 weeks. All animals were examined histologically. Zinc deficiency alone did not affect food consumption, weight gain or survival. Cadmium chloride affected weight gain only at the highest dose (30 μmol/kg), at which body weight was reduced approximately 15%, only for the first 10 weeks after injection; thereafter, weights were not different from those of controls. Survival was reduced in rats fed zinc-adequate diets and given the highest dose of cadmium chloride ($p \leq 0.05$). Injection-site sarcomas occurred in 7/25 rats receiving 30 μmol cadmium chloride and zinc-deficient diets ($p < 0.05$), in 3/24 rats given 30 μmol cadmium chloride and zinc-adequate diets and in 0/49 controls. Dietary zinc level did not affect the incidence of cadmium-induced interstitial-cell tumours of the testis, and a dose–response relationship in tumour incidence occurred with cadmium up to a maximum incidence of approximately 70% (control, < 10%) at both levels of dietary zinc. Rats receiving zinc-deficient diets showed an increased multiplicity of testicular interstitial-cell tumours (Waalkes *et al.*, 1991b).

3.5 Other routes of administration

3.5.1 *Mouse*

In a screening study based on the accelerated induction of lung adenomas in a strain highly susceptible to development of this neoplasm, groups of 20 male and female strain A/Strong mice, six to eight weeks old, received thrice weekly intraperitoneal injections of **cadmium acetate** in saline for a total of 23 injections, while controls received a total of 24 injections of saline alone. The total doses of cadmium acetate were designed to be 7, 14 and 28 mg/kg bw. All mice given 28 mg/kg bw died prior to completion of the study (30 weeks). Lung adenomas occurred in 6/14 (43%) animals given 7 mg/kg bw and in 3/10 (30%) animals given 14 mg/kg bw, compared with 37% of controls. The average number of lung tumours per mouse was unaltered by treatment ($p > 0.05$; Student's *t* test) (Stoner *et al.*, 1976).

3.5.2 *Rat*

A group of 207 male Wistar rats, six weeks of age, received injections of 0.15 ml of a 1-mol [*sic*] solution of **cadmium chloride** [16.86 mg/rat; 241 mg/kg bw] in saline directly into

the prostate. A further group of 50 rats received one to five subcutaneous injections of 0.05 ml of the 1-mol solution of cadmium chloride [5.62–28.1 mg/rat; 80–401 mg/kg bw] in saline [site unspecified]. Concurrent controls were not included [body weights, survival, necropsy protocol and observation time not specified]. Prostatic tumours, generally carcinomas, developed in 17/207 (8.2%) rats given injections directly into the prostate. In the animals given cadmium chloride subcutaneously, a possible early adenocarcinoma of the prostate was observed (Scott & Aughey, 1978). [The Working Group noted that the absence of concurrent controls makes these data difficult to interpret.]

A group of 125 inbred male rats of the Okamoto-toki strain, 12 months of age, were anaesthetized and injected with 0.44 mg cadmium (1.2 mg/kg bw) as **cadmium chloride** in saline into the right lobe of the ventral prostate. Twenty saline-injected rats of the same age served as controls. Animals were observed for 270 days after cadmium chloride injection [body weights and survival not given]. Lesions of the prostate were classified as hyperplasia, atypical hyperplasia, carcinoma *in situ* (Hoffmann *et al.*, 1985a) [modified to atypical hyperplasia with severe dysplasia by Hoffmann *et al.*, 1985b] and invasive carcinoma. The first case of invasive prostatic carcinoma was detected 56 days after treatment, and a total of five cases occurred in 100 rats examined in this group. Other prostatic changes induced by cadmium chloride treatment included 'carcinoma *in situ*' in 11 rats (Hoffmann *et al.*, 1985a), atypical hyperplasia in 29 rats and simple hyperplasia in 38. Of the 20 controls examined, five had simple hyperplasia and one had atypical hyperplasia (Hoffmann *et al.*, 1985a,b). [The Working Group noted the small number of controls and the short obervation period.]

Female Wistar WU/Kiβlegg rats [number not given], 12 weeks of age, each received a single intraperitoneal injection of 50 mg cadmium as **cadmium sulfide** (81 rats examined) or two weekly intraperitoneal injections of 0.125 mg cadmium as **cadmium oxide** (47 rats examined) dissolved in saline. Animals were observed for up to 123 weeks. No concurrent controls were reported. Only gross lesions of the peritoneal cavity were examined histologically. Three of the rats given cadmium oxide and 54/81 given cadmium sulfide had peritoneal cavity tumours, described as sarcomas, mesotheliomas and carcinomas of the abdominal cavity [no further details reported]. In the 204 rats injected with saline alone (combined controls), five intraperitoneal tumours (one carcinoma, one mesothelioma and three sarcomas) were observed (Pott *et al.*, 1987).

Groups of male Okamoto-aoki rats, 12 months of age, were injected twice with 2.25 mg/kg bw (10 rats) or three times with 3.35 mg/kg bw (20 rats) **cadmium chloride** [time course and vehicle unspecified] into the right lobe of the ventral prostate. No concurrent controls were used. Animals were killed after 170 or 240 days, respectively. Prostatic carcinomas occurred in 2/8 rats receiving the lower dose of cadmium and in 9/15 rats given the higher dose (Hoffmann *et al.*, 1988). [The Working Group noted that the absence of a control group makes this study difficult to interpret.]

3.6 Administration with known carcinogens

3.6.1 *Mouse*

Groups of 25 three-week-old female Swiss mice received 5, 10 or 50 ppm [mg/L] cadmium as **cadmium chloride** in deionized water or drinking-water alone (controls) for

15 weeks. After three weeks of exposure, all mice received an intraperitoneal injection of 1.5 mg/g bw [1.5 g/kg bw] urethane in saline. At the end of the 15-week exposure period, all mice were killed. Treatment did not affect average body weight gain or water consumption. Only lungs were examined histologically. Cadmium did not modify the size or number of pulmonary adenomas induced by urethane per animal (Blakley, 1986).

A group of 100 female hybrid CBA × C57Bl/6 mice, weighing 10–12 g, received 0.01 mg/L **cadmium chloride** together with 10 ppm [mg/L] *N*-nitrosodimethylamine (NDMA) in the drinking-water *ad libitum* for nine months, at which time the experiment was terminated. A positive control group of 50 mice received NDMA alone. The total dose of cadmium chloride received from drinking-water was stated to be 0.007 mg. Survival was similar in both groups [body weights not given]. All animals were examined histologically. Treatment with cadmium chloride plus NDMA significantly increased ($p \leq 0.05$, χ^2) the proportion of animals with tumours of any type (95.3%) over that of mice given NDMA alone (80.0%) among animals that survived to the time of appearance of the first tumour. The tumours were primarily pulmonary adenomas, renal adenomas and hepatic haemangiomas and haemangioendotheliomas (Litvinov *et al.*, 1986). [The Working Group noted the high incidence of tumours in the group given NDMA alone and the absence of a concurrent untreated control group and of a group receiving cadmium chloride alone.]

In a study of promotion, groups of 50 male B6C3F1 mice, five weeks of age, were given a single intraperitoneal injection of 90 mg/kg bw *N*-nitrosodiethylamine (NDEA) in tricaprylin or vehicle alone followed two weeks later by administration of 0, 500 or 1000 ppm [mg/L] **cadmium chloride** hemipentahydrate in drinking-water. Groups of 10 mice were killed at 16, 24 and 36 weeks, and the remainder were killed at 52 weeks. Cadmium chloride markedly suppressed body weight gain in the group given 1000 mg/L. All animals were examined histologically. Cadmium chloride was not associated with an increased incidence of tumours, regardless of NDEA treatment, and animals treated with cadmium chloride had a dose-related reduction in NDEA-induced pulmonary adenomas of alveolar-cell origin and liver tumours (typically basophilic adenomas) (Waalkes *et al.*, 1991c).

In a study of initiation, groups of male B6C3F1 mice, five weeks of age, were given a single subcutaneous injection of vehicle (30 mice) or cadmium at 20.0 (30 mice) or 22.5 (60 mice) μmol/kg bw (2.25 or 2.53 mg/kg bw) as **cadmium chloride** hemipentahydrate in saline, followed two weeks later by administration of water or 500 ppm [mg/L] sodium barbital in the drinking-water (540 mice in all). Both doses of cadmium chloride caused focal hepatocellular necrosis; the 20.0 μmol/kg dose caused 8% (5/60) mortality and 22.5 μmol/kg caused 39% (47/120) mortality within the first two days (all treated groups combined). Final body weights were similar in animals that survived the acute toxicity. Groups of 10 mice were killed 40 weeks after cadmium injection, and the remainder were killed at 92 weeks. Histological examination was performed, with special emphasis on liver lesions. Cadmium chloride treatment was not associated with increases in the incidence of any tumours; it reduced ($p \leq 0.05$) liver tumour incidence and the numbers of tumours/liver, but not tumour size (Waalkes *et al.*, 1991c).

3.6.2 *Rat*

Groups of 15 male Fischer 344 rats, seven weeks old, were administered 500 mg/L *N*-nitrosoethyl-*N*-hydroxyethylamine in drinking-water for two weeks followed by 100 mg/L **cadmium chloride** hemipentahydrate for 25 weeks. Cadmium chloride did not affect body weight or survival. Only kidneys and liver were examined histologically. Cadmium chloride did not increase the kidney tumour incidence significantly but it significantly ($p \leq 0.05$; Student's *t* test) increased the mean number of renal dysplastic foci/cm^2 of tissue (0.69 $\pm$ 0.32) in comparison with nitrosamine-treated controls (0.23 $\pm$ 0.28) (Kurokawa *et al.*, 1985, 1989). [The Working Group noted the short duration of the study.]

Groups of 40 male Wistar (Sim:Wistar) rats, nine weeks of age, were treated with estimated daily dietary doses of 50 mg/kg of diet [ppm] cyproterone acetate for three weeks followed by three daily subcutaneous injections of 25 mg testosterone propionate and then a single intravenous injection of 50 mg/kg bw *N*-methyl-*N*-nitrosourea (MNU). One group then received 100 ppm (100 mg/L) **cadmium chloride** in the drinking-water, and another received standard drinking-water and served as controls. Survival and body weight were not affected by cadmium, and the mean survival time was 58 weeks. All animals were examined histologically. One intraductal carcinoma of the prostate occurred in a cadmium-treated rat, but none occurred in controls. The incidences of tumours at other sites were not affected by cadmium (Nakao, 1986). [The Working Group noted the short duration of the study, the minimal effect of MNU alone and the absence of concurrent untreated controls or controls receiving cadmium chloride alone.]

Groups of 20 male Wistar rats [age at onset unspecified] were given 100 ppm [mg/L] *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine in their drinking-water and fed a diet supplemented with 10% sodium chloride for eight weeks to initiate stomach tumour formation. **Cadmium chloride** was then given in the drinking-water at a concentration of 100 ppm [mg/L] for the following 32 weeks. A group of 28 rats received the nitrosamine then distilled drinking-water and served as controls. Cadmium chloride did not modify the incidence of gastroduodenal tumours or preneoplastic lesions (Kurokawa *et al.*, 1989).

Groups of 20 male Fischer 344 rats [age at onset unspecified] were given 50 ppm [mg/L] NDEA in the drinking-water for four weeks followed by 100 ppm [mg/L] **cadmium chloride** in the drinking-water for the following 30 weeks. Controls received NDEA followed by drinking-water. Cadmium chloride significantly ($p < 0.01$) reduced the incidence of hepatocellular carcinomas induced by NDEA (Kurokawa *et al.*, 1989).

Groups of 42–58 female hooded rats, 10–12 weeks of age, were given one treatment of either crocidolite alone (1.82 mg/rat suspended in Tyrode's solution by intratracheal instillation), crocidolite plus cadmium (0.18 mg cadmium/rat as powdered **cadmium metal** suspended in Tyrode's solution by intratracheal instillation), crocidolite plus cadmium plus *N*-nitrosoheptamethyleneimine (NHMI; 1 mg/rat dissolved in saline given subcutaneously into the dorsal thoracic area six weeks after intratracheal instillation of crocidolite and cadmium for 12 weeks) or NHMI alone. Survival was similar in all the cadmium-treated groups. The overall lung tumour incidence in animals receiving crocidolite, cadmium and NHMI (14/45) was significantly ($p \leq 0.05$) higher than that in groups receiving NHMI alone (10/58), crocidolite and cadmium (2/51) or crocidolite and NMHI (7/42). The tumours were

primarily squamous-cell carcinomas (Harrison & Heath, 1986). [The Working Group noted that concurrent untreated controls and groups treated with cadmium metal only were not available.]

Groups of male Wistar Crl:[WI]BR rats, 22 weeks old, were given a single intraperitoneal injection of 18 mg/kg bw NDMA followed 4 h and four days later by intramuscular injections of **cadmium chloride** into the thigh (total doses of cadmium, 1.5 mg/kg bw [20 rats] or 3.0 mg/kg bw [30 rats]) or no further treatment (20 rats). Two other groups of 20 rats were given cadmium alone, and a group of five untreated rats served as controls. The animals were observed for 52 weeks. Cadmium chloride alone was not acutely lethal; NDMA alone caused 5% mortality, low-dose cadmium plus NDMA induced 10% mortality and high-dose cadmium plus NDMA induced 30% mortality. All treatments markedly reduced body weight [extent not stated] within one week of exposure, but by the end of the experiment the weights were similar to those of untreated controls. Only rats surviving to week 30 were included in the tumour analysis. Cadmium chloride increased ($p \leq 0.05$, Fisher exact test) the incidence of renal tumours induced by NDMA (NDMA alone, 2/18 rats examined; NDMA plus low-dose cadmium chloride, 10/18; NDMA plus high-dose cadmium chloride, 11/21) but did not induce significant numbers when given alone (low-dose, 1/20; high-dose, 0/20). Cadmium chloride also increased the incidence of hepatocellular adenoma (NDMA alone, 1/18; NDMA plus pooled cadmium chloride groups, 9/39). In a second experiment, 30 rats (same strain, six weeks old) were given intramuscular injections of cadmium as cadmium chloride at a dose of 1 mg/kg bw into the thigh on days 0, 4, 5 and 6 and of 2 mg/kg bw on day 12; one day later, the animals received an intraperitoneal injection of 18 mg/kg bw NDMA. Further groups received NDMA alone (20 rats), cadmium chloride alone (20 rats) or remained untreated (four rats). Survival and body weights were similar in all groups. Cadmium chloride increased ($p \leq 0.05$, Fisher exact test) the incidence of NDMA-induced renal tumours (NDMA alone, 2/19; NDMA plus cadmium chloride, 15/26) but did not induce any renal tumours when given alone (0/20). The incidences of hepatocellular adenomas were: NDMA alone, 3/19; NDMA plus cadmium chloride, 0/26; cadmium chloride alone, 1/20 (Wade *et al.*, 1987).