

3. Studies of Cancer in Experimental Animals

3.1 Lead acetate

3.1.1 *Mouse*

(a) *Oral administration*

In a study by Waszynski (1977), a total of 137 male and female F1(RIII × C57Bl) mice, 6–8 weeks of age, were divided into four groups and fed diets containing lead acetate (analytical grade) (daily intake, 6 mg/mouse), sulfathiazole (daily intake, 0.3 mg/mouse), lead acetate plus sulfathiazole, or unaltered diet (control) for 18 months and were observed for an additional 7 months. Some animals died during the observation period. Histological analysis revealed that none of the treatments produced renal tumours. [The Working Group noted that the group sizes were not specified.]

Blakley (1987) exposed groups of 42–46 female albino Swiss mice, 8 weeks of age, to lead acetate [purity unspecified] in drinking-water at concentrations of 0 (control), 50 or 1000 $\mu\text{g}/\text{mL}$ for up to 280 days. This mouse strain has a high spontaneous incidence of lymphocytic leukaemia. Mice that died during exposure or were killed at the end of the study were examined grossly for evidence of lymphocytic leukaemia of thymic origin (enlarged thymus). Survival was significantly reduced ($p = 0.007$, Lee-Desu survival statistic) in the lead-treated mice, suggesting that lead enhanced death due to leukaemia. [The Working Group noted the absence of histological analysis of the tumours.]

(b) *Pre- and perinatal administration*

In a study by Waalkes *et al.* (1995), groups of 10–15 primigravid C57Bl/6NCr mice, previously bred with C3H/HeNCr males to produce B6C3F₁ offspring, were given *ad libitum* drinking-water containing lead acetate [purity unspecified] at doses that provided lead concentrations of 0 (control), 500, 750 and 1000 ppm, from gestation day 12 through to birth and until 4 weeks postpartum when offspring were weaned. Exposure to lead acetate did not alter litter size. The offspring were placed into same-sex groups of 23–25 based on maternal exposure level to lead acetate. They were given water without added lead and with or without 500 ppm sodium barbital as a renal tumour promoter and observed for a total of 112 weeks postpartum. Exposure to lead acetate did not alter body weight of the offspring nor the survival of any group during the observation period. A complete necropsy was performed on each animal. In male offspring, histological examination of the kidneys revealed that exposure to lead acetate was associated with a dose-related increase ($p = 0.0006$, Cochran-Armitage test for trend) in the incidence of proliferative lesions (including atypical tubular-cell hyperplasia) and a significant increase in the incidence of renal adenomas ($p < 0.05$, two-tailed Fisher's exact test) and occasional renal carcinomas. In female offspring, exposure to lead acetate was associated with a significant dose-related increase ($p = 0.017$; Cochran-Armitage test for trend) in the incidence of renal proliferative lesions, including hyperplasia, adenoma and carcinoma. Proliferative lesions were exclusively of renal tubular cell origin. Sodium barbital did not increase the incidence of renal proliferative lesions in any group. The authors noted that the transplacental/translactational lead acetate-induced tumours arose in the absence of extensive chronic nephropathy typically seen in lead carcinogenesis in rodents chronically exposed as adults. Exposure to lead acetate was not associated with tumours at extra-renal sites in any of the experimental groups (Waalkes *et al.*, 1995).

(c) *Administration with known carcinogens or modifiers of carcinogenesis*

Bull *et al.* (1986) tested various chemicals with potential to initiate tumours, including lead acetate (purity, > 99%), in groups of 30 female SENCAR mice, 6–8 weeks of age. The mice received a single oral dose of 600 mg/kg bw [presumably by gastric intubation; vehicle either emulphor, saline or water] or a single dermal application of 600 mg/kg bw to the shaved back [vehicle either acetone or ethanol]. Two weeks later, mice received

dermal applications of 1.0 μg of the skin tumour promoter, 12-*O*-tetradecanoyl phorbol-13-acetate (TPA), in 0.2 mL acetone three times per week for up to 52 weeks. Skin tumours were assessed histologically; other tumours were not reported. Oral and topical applications of lead acetate did not affect the incidence of skin tumours. [The Working Group noted that the study was limited by the use of a single dose and the inadequate reporting of experimental details.]

Blakley (1987) also studied the effects of oral administration of lead acetate on the incidence of urethane-induced lung adenoma in female Swiss mice. Groups of 19–25 mice, 3 weeks of age, were exposed to lead acetate [purity unspecified] in the drinking-water at concentrations of 0 (control), 50, 200 or 1000 $\mu\text{g}/\text{mL}$ for 15 weeks. A single intraperitoneal injection of 1.5 mg/kg bw urethane was administered 3 weeks after the start of lead treatment. The lead acetate and urethane treatments did not alter water consumption or body weight gain. The animals were killed at the end of the lead exposure period (15 weeks). Lungs were inflated, fixed and inspected visually for the number and size of adenomas. Treatment with lead acetate did not alter average lung tumour size or multiplicity (tumours/animal). [The Working Group noted that the absence of a group treated with lead acetate alone limited the utility of this study.]

3.1.2 *Rat*

(a) *Oral administration*

Boyland *et al.* (1962) fed a group of 20 male Wistar rats, 10 weeks of age, a diet containing 1% lead acetate [purity unspecified] for 1 year and observed them for up to 629 days. Histological evaluation of rats that died revealed the first renal tumour after 331 days. Subsequently, 14 more rats died with renal tumours. Among the total of 15 renal tumours, 14 were carcinomas. [The Working Group noted the absence of a control group but the remarkable incidence of renal carcinomas in lead acetate-exposed animals.]

In a lifetime study testing metals for nutritional essentiality, groups of 50 male and 50 female Long Evans rats [age unspecified] were exposed to 5 ppm lead as lead acetate [purity not specified] in drinking-water from weaning to natural death and compared with control animals given water without added lead. Lead acetate significantly increased mortality in both sexes ($p < 0.05$, Student's *t*-test). Not all animals underwent necropsy and only grossly visible tumours were evaluated microscopically [tumour location not specified]. The authors indicated no significant differences in total tumour incidence between control and lead acetate-treated rats (Chi-squared test) (Schroeder *et al.*, 1965; Kanisawa & Schroeder, 1969). [The Working Group noted the low dose used, the limited pathological evaluation, that animals would have been deficient in other metals, and that the data were inconsistent between the two reports.]

Zawirska and Medras (1968) gave groups of 94 male and 32 female Wistar rats [age not specified] a diet supplemented with lead acetate [purity unspecified] to achieve a dose of lead of 3 mg/rat per day for 2 months followed by a dose of 4 mg/rat per day for 16 months. The groups were compared with 19 male and 13 female control rats fed unaltered

diets. After 18 months, 40 of the lead acetate-treated rats [sex unspecified] were killed while the rest were allowed to live to a natural death. Weight loss was evident in the lead acetate-treated rats [actual body weight data not given; survival data not given]. Extensive histological examination was performed on all animals. The authors stated that no tumours were observed in control rats except for one adenoma and one carcinoma of the mammary gland. This included an absence of spontaneous tumours of the kidney and endocrine organs in control animals. The 94 lead acetate-treated male rats had 58 renal tumours (43 adenomas, 15 carcinomas), 23 adrenal gland tumours (22 adenomas, one carcinoma), 23 interstitial-cell tumours of the testes, 22 prostatic tumours (21 adenomas, one carcinoma), 10 lung tumours (eight adenomas, two carcinomas), four pituitary adenomas, three liver tumours, three brain gliomas, three thyroid adenomas, two spermatic duct carcinomas, one leukaemia and one sarcoma [given a tumour incidence of 0/19 in control males, incidences of renal, adrenal, testes and prostatic tumours were significantly increased in lead acetate-treated male rats ($p < 0.05$, two-tailed Fisher's exact test)]. The 32 lead acetate-treated female rats had 14 renal tumours (12 adenomas, two carcinomas), nine adrenal gland adenomas, five lung tumours (four adenomas, one carcinoma), three mammary gland tumours, two liver tumours, two thyroid tumours, one pituitary adenoma, one oesophageal carcinoma, one leukaemia and two sarcomas [given a tumour incidence of 0/13 in control females, incidences of renal and adrenal tumours were significantly increased in lead-treated female rats ($p < 0.05$, two-tailed Fisher's exact test)]. [The Working Group noted that the spontaneous incidence of gliomas in rats is a very rare event.]

In further studies (Zawirska & Medras, 1972; Zawirska, 1981), groups of 47 male and 47 female Wistar rats, 31 weeks of age, were fed lead acetate [purity unspecified] in the diet to achieve a dose of lead of 3 mg per day [based on 20 g food/rat given to 10 rats/cage] for periods ranging between 60 and 504 days and were observed for times ranging from 60 days to the point of natural death (maximum 572 days). Control animals [stated variously as 31 males and 31 females or 47 males and 47 females] were fed unaltered diet and were observed for up to 800 days [survival was imprecisely defined]. All rats were examined histologically. No tumours were reported in the control group. In the 94 lead acetate-treated rats, examination revealed 102 tumours including 12 rats with kidney adenomas, 15 with lung adenomas, 17 with pituitary adenomas, 10 with brain gliomas, 11 with thyroid adenomas, five with parathyroid adenomas, 11 with prostate adenomas, eight with mammary adenomas and 13 with adrenal cortical adenomas [all incidences were significantly increased, except parathyroid adenoma incidence, versus 0/62 control animals ($p < 0.05$, two-tailed Fisher's exact test)]. The authors stated that renal tumour incidence appeared to be related to length of treatment with lead acetate. [The Working Group noted some inconsistencies between the two reports. The Working Group also noted that the spontaneous incidence of kidney adenomas and brain gliomas is a very rare event.]

Azar *et al.* (1973) fed groups of 50 male and 50 female rats [strain and age unspecified] diets containing concentrations of lead acetate [purity unspecified] to give 10, 50, 100 or 500 ppm lead for 2 years. A control group of 100 males and 100 females remained untreated.

In a second study, started shortly after the first, groups of 20 male and 20 female rats were fed 0, 1000 and 2000 ppm lead [presumably as lead acetate] for 2 years. Weight gain was depressed in animals receiving 1000 and 2000 ppm lead. Data on survival rates at 2 years indicated increased mortality in males fed 500 and 2000 ppm lead [test not specified]. Complete necropsy with histological examination was carried out on all animals. No pathological lesions were reported in rats fed up to 100 ppm lead. No renal tumours occurred in either male or female control rats. In male rats treated with lead, the incidence of renal tumours was 5/50, 10/20 and 16/20 in groups fed 500 ppm, 1000 ppm and 2000 ppm, respectively [all three incidences were statistically significant, two-tailed Fisher's exact test; a χ^2 test for trend proved significant ($p < 0.001$)]. In female rats treated with lead, the incidence of renal tumours was 0/50, 0/20 and 7/20 in the groups fed 500 ppm, 1000 ppm and 2000 ppm, respectively [the incidence in the last group was significantly increased; two-tail Fisher's exact test]. Most renal tumours were adenomas derived from the tubular epithelium. The doses of lead acetate used resulted in the following blood lead concentrations: no treatment, 12.7 $\mu\text{g/dL}$; 10 ppm lead acetate, 11.0 $\mu\text{g/dL}$; 50 ppm, 18.5 $\mu\text{g/dL}$; 100 ppm, 35.2 $\mu\text{g/dL}$; 500 ppm, 77.8 $\mu\text{g/dL}$.

Waszynski (1977) fed groups of 15–20 male and 19–26 female [individual group size not specified] Wistar rats, aged 2–2.5 months, diets containing either lead acetate (analytical grade) alone, sulfathiazole alone, lead plus sulfathiazole or unaltered diet (control) for 18 months and observed them for an additional 7 months. Diets were prepared to give a dose of 3 mg/rat per day lead acetate and 54 mg/rat per day sulfathiazole. Some animals died during the observation period. Histological examination of the 42 male and female rats that survived until the end of the observation period showed that lead acetate treatment alone induced 14 renal tumours including five carcinomas in males and one carcinoma in a female. In 43 male and female rats that survived until the end of the observation period, lead plus sulfathiazole treatment induced 17 renal tumours including one renal carcinoma in a female. Controls and rats fed sulfathiazole alone did not develop renal tumours. [The Working Group noted some deficiencies in reporting. The Working Group also noted that the spontaneous occurrence of renal carcinomas in rats is a very rare event.]

Nogueira (1987) fed groups of 10–12 male Wistar rats, 6 weeks of age, diets containing 0 (control), 0.5 or 1.0% lead acetate [purity unspecified] for up to 24 weeks. Survival and body weight were unaltered by lead acetate treatment. At necropsy, kidneys were assessed histologically in a median transverse section and tumours were categorized as basophilic or chromophobic, and the incidence was reported separately. Renal tumours were not reported in the 10 control rats [the Working Group noted the absence of other data on tumours in controls]. Renal tumours did not occur in the 12 rats fed 0.5% lead acetate, but of the 10 rats fed 1.0% lead acetate, two developed basophilic tumours and seven developed chromophobic tumours [it is unclear if any rats had both types of tumours].

In a study by Fears *et al.* (1989) on carcinogenic mixtures, groups of 24 male and 24 female Fischer 344 rats [age unspecified] were fed 500, 2000 or 8000 ppm lead as lead acetate [purity unspecified] in the diet for up to 725 days. Control groups of 213 male and 214 female rats received unaltered diet. Other groups of male and female rats received

lead together with various other carcinogens (*N*-butyl-*N*-(4-hydroxybutyl)nitrosamine [NBBN], aflatoxin B₁ or thiouracil) in the diet to evaluate carcinogenic synergy. Survival of lead-treated rats did not differ from that of controls. All animals underwent complete necropsy. Tissues were examined histologically, and only malignant tumours were tabulated [pathological type not specified]. No malignant renal tumours were found in the 213 male and 214 female control rats. In male rats treated with lead acetate alone, the incidence of malignant renal tumours was 0/24, 11/24 [statistically significant, two-tailed Fisher's exact test, $p < 0.05$], and 19/24 [statistically significant; two-tailed Fisher's exact test, $p < 0.05$; χ^2 test for trend, $p < 0.0001$] in animals that received 500 ppm, 2000 ppm and 8000 ppm lead, respectively. In female rats treated with lead acetate alone, the incidence of malignant renal tumours was 0/24, 1/24, and 4/24 [statistically significant, two-tailed Fisher's exact test] in animals that received 500 ppm, 2000 ppm and 8000 ppm lead, respectively. No carcinogenic interactions were observed between lead and NBBN, aflatoxin B₁ or thiouracil.

(b) *Subcutaneous administration*

In a study undertaken by Teraki and Uchiumi (1990) to analyse the metal content in metal-induced injection-site tumours, a group of 13 male Fischer 344/NS1e rats, 5 weeks of age, received subcutaneous dorsal injections of 60 mg/kg bw lead acetate [purity unspecified] in distilled water weekly for 5 weeks. Another group of 13 rats was injected with saline and served as controls. Rats were observed for 80 weeks following the start of the injections [survival data not given]. [Although not explicitly stated, histological analysis appears to have been performed.] Of the rats injected with lead acetate and available for review, 42% [probably five rats with tumours/12 rats injected] developed injection-site sarcomas [No data were given for the incidence of injection-site tumours in controls and the Working Group noted the incomplete reporting of experimental details.]

(c) *Administration of lead with known carcinogens or modifiers of carcinogenesis*

Hinton *et al.* (1979) fed groups of 150 male Fischer 344 rats, weighing 125–175 g [age unspecified], unaltered diet (control) or diets containing 1% lead as lead acetate [purity unspecified], 0.04% *N*-(4'-fluoro-4-biphenyl) acetamide (FBPA) or 1% lead acetate plus FBPA. Ten to 20 rats from each group were killed after 3, 7 and 14 days and 4, 8, 16, 24, 36 and 52 weeks of exposure [survival unspecified] and a gross examination of the kidneys and livers for the appearance of tumours was made. This revealed one mass in the kidney of a rat fed lead acetate alone for 52 weeks. Lead acetate treatment also appeared to increase FBPA-induced gross renal tumour multiplicity [not statistically evaluated]. Upon histological examination, these lesions were confirmed as renal adenocarcinomas. [The Working Group noted that the experimental design and reporting was insufficient to evaluate the role of lead acetate on FBPA-induced carcinogenesis.]

Tanner and Lipsky (1984) fed groups of male Fischer 344 rats [initial group size of 50 but substantially reduced due to lead-induced mortality], weighing 125–175 g, diets con-

taining either 10 000 ppm lead as lead acetate [purity unspecified], 400 ppm FBPA, FBPA plus lead, or unaltered diet (control). Five to 10 animals per group were killed after 16, 24, 36 and 52 weeks of feeding and kidneys were examined microscopically. No details were given on survival. The number of control animals killed at each time point was 5–10 [exact number unspecified]. No renal lesions were seen in the controls at any time. The incidence of renal hyperplasia, adenoma and adenocarcinoma was reported irrespective of concurrent lesions in the same animal. Of the 26 rats fed FBPA alone, 19 had hyperplasia, eight had adenomas and eight had carcinomas. Of the 29 rats fed lead alone, 21 had hyperplasia, two had adenomas and one had an adenocarcinoma. Of the 27 rats fed FBPA and lead, 27 had hyperplasia, 18 had adenomas and 10 had carcinomas. Statistical evaluation was not carried out. [The Working Group noted the incomplete reporting and the high early mortality of the treated rats.]

Koller *et al.* (1985) gave groups of 7–16 male weanling Sprague-Dawley rats 0, 26 or 2600 ppm lead as lead acetate [purity unspecified] in drinking-water continuously for a total of 76 weeks. Twenty-eight weeks after start of lead acetate exposure, each group was simultaneously exposed to diets containing sodium nitrite (6.36 g/kg diet) and ethyl urea (2.0 g/kg diet) for 20 weeks and thereafter to unaltered diet until the end of the study (76 weeks). A control group received unaltered water and feed, and a group received 2600 ppm lead acetate alone for 76 weeks. Of the lead-exposed animals, 3/36 were lost to observation due to early death. All rats were subjected to histological examination. Renal tumours occurred with the following incidence (tumour bearing rats/number of rats examined): control, 0/7; ethyl urea and sodium nitrite only, 0/8; 26 ppm lead acetate plus ethyl urea and sodium nitrite, 0/7; 2600 ppm lead acetate plus ethyl urea and sodium nitrite, 6/10 [statistically significant versus controls, two-tailed Fisher's exact test]; 2600 ppm lead acetate only, 13/16 [statistically significant versus controls, two-tailed Fisher's exact test]. All kidney tumours were classified as renal tubule carcinomas with the exception of a clear cell adenoma in the group of rats treated with 2600 ppm lead plus ethyl urea and sodium nitrite.

Nogueira (1987) fed groups of 10–12 male Wistar rats, 6 weeks of age, diets containing 0 (control), 0.5 or 1.0% lead acetate [purity unspecified] for up to 24 weeks. Separate groups were given 0.01 or 0.025% *N*-nitrosodiethylamine (NDEA) in water at a dose of 5 mg/kg per day [presumably 5 mg of solution/kg per day by intubation] with or without 0.5 or 1.0% lead acetate. Lead acetate did not appear to affect NDEA-induced carcinogenesis.

In a study of the effects of calcium and lead on blood pressure, Bogden *et al.* (1991) fed groups of 4–8 male weanling Wistar rats diets containing either 0.2% or 4.0% calcium. The animals were given drinking-water containing 0, 1 or 100 µg/mL lead as lead acetate [purity unspecified]. After 31 weeks, rats were killed and one kidney from each rat was prepared for histological examination. Proliferative lesions of the kidney were observed only in rats fed the 4.0% calcium diet and given 100 µg/mL lead in drinking-water; among five rats there were three with transition cell hyperplasia and two with invasive carcinoma. [The Working Group noted the small group sizes.]

3.1.3 *Dog*

Azar *et al.* (1973) fed groups of four male and four female beagle dogs [age unspecified] diets containing 0 (control), 10, 50, 100 and 500 ppm lead acetate [purity unspecified] for 2 years, at which time the experiment was terminated. These doses of lead did not affect weight gain or mortality. A complete necropsy with histological examination was carried out on all animals. There were no pathological effects of dietary lead in any organ system in the females. Two male dogs fed 500 ppm lead showed a slight degree of cytomegaly in the proximal convoluted tubule of the kidneys. No tumours of any type were reported. [The Working Group noted the small group sizes and the short duration of treatment.]

3.1.4 *Monkey*

A case of a rhesus macaque (*Macaca mulatta*) that developed chronic myelocytic leukaemia after having been exposed to lead acetate has been reported by Krugner-Higby *et al.* (2001). The malignancy occurred in a female monkey that had received daily oral exposures to lead for a total of 2 years in order to achieve a target blood lead concentration of 35 µg/dL. Beginning at day 8 postpartum and continuing for the next 6 months, lead was given to the monkey mixed in a commercial milk formula [dose unspecified]. After weaning at 6 months, lead was administered in a fruit-flavoured diet for an additional 18 months [dose unspecified]. Regular blood samples were drawn to test for blood lead concentrations. The mean concentration of lead in blood over the lifetime of the leukaemic macaque was 37.6 µg/dL. The first symptoms of haematopoietic abnormality developed when the monkey was 25 months of age and, after an attempt at chemotherapeutic intervention, the animal was sacrificed 4 months later. The author noted that this was the first report of chronic myelocytic leukaemia in this species and that it is a rare malignancy in non-human primates. The animal was seronegative for several retroviruses that have been associated with lymphoid neoplasia in non-human primates.

3.2 **Lead subacetate**

3.2.1 *Mouse*

(a) *Oral administration*

Van Esch and Kroes (1969) fed groups of 25 male and 25 female Swiss mice, 5 weeks of age, diets containing either 0 (control), 0.1% or 1.0% lead subacetate [purity unspecified] for up to 2 years. The higher dose caused an early decrease in survival. Although the dose was reduced to 0.5% at 92 days of treatment for male mice and 114 days for female mice, few animals survived beyond 1 year. Survival was similar in controls and mice fed 0.1% lead subacetate. In the latter group, histological examination showed renal tumours in six males (two adenomas, four carcinomas) and one female (adenoma) while none occurred in controls. One carcinoma occurred in a female fed 1.0/0.5% lead

subacetate. [The Working Group noted that the number of mice subjected to pathological analysis was not specified].

Stoner *et al.* (1986) gave groups of 16 male and 16 female strain A/J mice, 6–8 weeks of age, three oral doses of lead subacetate [purity unspecified] per week for up to 24 weeks (total dose, 190 mg/kg bw) and compared them with untreated controls. Of the lead-treated mice, 81% of the females and 100% of the males survived to the end of the study. At the end of the experiment, lungs were removed, fixed, and gross lesions representing lung tumours were counted. A few lesions were subjected to histological examination to confirm typical histopathology of pulmonary adenoma. Lead subacetate-treated mice did not show a significant lung tumour response.

(b) *Intraperitoneal administration*

In an earlier study, Stoner *et al.* (1976) gave groups of 20 male and female strain A/Strong mice, 6–8 weeks of age, intraperitoneal injections of lead subacetate (> 97% pure) dissolved in tricapylin, three times per week for 5 weeks (total doses, 30, 75 or 150 mg/kg bw) and observed them for up to 30 weeks after the first injection. The high dose was considered to be a maximum tolerated dose. Of the lead-treated mice, 60–75% survived to the end of the study compared with 90% of the controls. At the end of the experiment, lungs were removed, fixed, and gross lesions representing lung adenomas were counted. A few lesions were subjected to histological examination and confirmed the typical histopathology of pulmonary adenoma. Lung tumour multiplicity (average number of tumours/mouse) was increased approximately threefold in mice given the highest dose of lead subacetate compared with controls given vehicle alone (1.47 ± 0.38 versus 0.50 ± 0.12 ; $p < 0.05$, Student's *t*-test) (Stoner *et al.*, 1976; Shimkin *et al.*, 1977).

Poirier *et al.* (1984) gave groups of 30 (equal numbers of male and female) strain A/Strong mice, 6–8 weeks of age, intraperitoneal injections of lead subacetate (reagent grade) dissolved in tricapylin (0.04 mmol/kg per injection) three times per week (total of 20 injections; total dose, 0.8 mmol/kg) and observed them for up to 30 weeks after the first injection. This dose schedule was said to constitute a maximum tolerated dose of lead in this strain of mouse. To define potential antagonism, separate groups received intraperitoneal injections of calcium acetate or magnesium acetate at 1:1, 3:1 and 10:1 molar ratios with lead subacetate. Calcium or magnesium were admixed with the lead prior to injection and given with the same dosage schedule as lead subacetate. Survival of mice to the end of the study was 70–87% in controls, 67% in mice treated with lead alone and 53–77% in mice treated with combined calcium and lead. Survival ranged from 43–60% in mice given magnesium at 3:1 or 10:1 molar ratios with lead. When magnesium was given as an equimolar mixture with lead, only 1/30 mice survived, preventing further analysis of this group. At the end of the experiment, surviving animals were killed, lungs were removed and fixed, and gross lesions representing lung adenomas were counted. A few lesions were subjected to histological examination and confirmed the typical histopathology of pulmonary adenoma. Lead alone caused a significant increase ($p < 0.05$, Student's *t*-test) in lung tumour multiplicity compared with controls (0.86 ± 0.20 versus

0.32 ± 0.12). At all doses used, calcium significantly reduced lead-induced increases in lung tumour multiplicity ($p < 0.05$). In the study with magnesium, lead alone caused a significant increase in lung tumour multiplicity ($p < 0.05$) compared with mice given the vehicle while magnesium given at molar ratios of 3:1 or 10:1 with lead significantly reduced lead-induced increases in lung tumour multiplicity ($p < 0.05$).

Stoner *et al.* (1986) gave groups of 16 male and 16 female strain A/J mice, 6–8 weeks of age, intraperitoneal injections of lead subacetate [purity unspecified] dissolved in water three times per week for up to 24 weeks (total doses, 38, 95 or 190 mg/kg bw) and compared them with water-treated controls. Of the lead-treated mice, 81–100% survived except in the group of males that received the high dose, of which only 3/16 (19%) survived to the end of the study. At the end of the experiment, surviving animals were killed, lungs were removed and fixed, and gross lesions representing lung tumours were counted. A few lesions were subjected to histological examination and confirmed the typical histopathology of pulmonary adenoma. Lung tumour multiplicity was significantly increased in the males at the low dose (38 mg/kg bw; 0.5 ± 0.18) and at the high dose (190 mg/kg bw; 0.67 ± 0.33), compared with controls (0.07 ± 0.07) ($p < 0.05$; Wilcoxon nonparametric rank test). The four other lead-treated groups did not show a significant lung tumour response. [The Working Group noted the mortality in the high dose-treated male group.]

(c) *Administration of lead with known carcinogens or modifiers of carcinogenesis*

Sakai *et al.* (1990) gave groups of 14–18 of male ddy mice [age unspecified], weighing 22–24 g, weekly intraperitoneal injections of 10 mg/kg bw lead subacetate [purity, > 99.99%] suspended in 50% glycerine solution for 18 weeks with or without either two or three intraperitoneal injections of 10 mg/kg bw *N*-nitrosodimethylamine (NDMA) in saline per week. Controls received injections of vehicles alone (saline and 50% glycerine solution). The treatments did not affect survival [test unspecified]. Lung tumours were evaluated histologically [stage was not specified, but included adenomas and adenocarcinomas]. Control mice and mice given lead alone did not develop lung tumours. Although lead did not alter NDMA-induced lung tumour incidence, lung tumour multiplicity was significantly increased in groups given lead compared with those that received NDMA alone. Animals given two injections of NDMA per week developed an average of 0.33 tumours/lung compared with 0.78 tumours/lung in mice also receiving lead ($p < 0.05$, Student's *t*-test) [descriptive statistics not given]. Mice injected three times a week with NDMA developed an average of 3.4 tumours/lung compared with 5.7 tumours/lung in mice also receiving lead subacetate ($p < 0.05$, Student's *t*-test) [standard errors not given].

3.2.2 Rat

(a) Oral administration

Van Esch *et al.* (1962) fed groups of 11–16 male and 11–16 female Wistar rats [age incompletely specified] diets containing 0.1% lead subacetate [purity unspecified] or unaltered diet (control) for 29 months or 1.0% lead subacetate or unaltered diet (control) for 24 months. Both concentrations of lead reduced body weight ($p < 0.005$, Wilcoxon's test) and 1.0% dietary lead subacetate reduced survival [test unspecified]. The incidence of renal tumours in rats fed 0.1% lead subacetate was 5/16 in males and 6/16 in females compared with 0/14 in control males and 0/15 in control females [incidences in both males and females were significantly elevated, Fisher's exact test]. The incidence of renal tumours in rats fed 1.0% lead subacetate was 6/13 in males and 7/11 in females compared with 0/13 in control males and 0/13 in control females [incidences in both males and females were significantly elevated, Fisher's exact test; χ^2 test for trend for both male and female, and $p < 0.006$ and $p < 0.0004$ respectively]. Three carcinomas occurred in rats fed 0.1% lead subacetate and six occurred in rats fed 1.0% lead subacetate; the remainder were adenomas. In the group fed 1.0% lead subacetate, one animal had a carcinoma with multiple metastases.

Mao and Molnar (1967) fed a group of 40 male Wistar rats (weighing ~200 g) [age unspecified] a diet containing 1% lead subacetate [purity unspecified] and a group of 20 rats an unaltered diet. Rats were killed or died from 238 to 690 days (controls) or from 213 to 677 days (lead-treated) [average survival unspecified]. Necropsies were performed on all animals and kidneys were examined histologically. Evaluation [presumably of the kidney only] revealed a single renal sarcoma among the 20 control rats while 31/40 lead-treated rats developed renal tumours [significantly different from controls, Fisher's exact test], including adenomas and carcinomas. One lead-treated rat with a renal tumour showed a pulmonary metastasis.

In a study by Oyasu *et al.* (1970) in which the effects of 2-acetylaminofluorene (2-AAF) in combination with lead subacetate was studied, two groups of male CD Sprague-Dawley rats, 5–8 weeks of age, were fed diets containing 1.0% lead subacetate [purity unspecified] or 1.0% lead subacetate and 1.6% indole and were observed for 12–17 months. Average survival in these groups was 53–69 weeks. A pool of various control groups (age range, 58–67 weeks) was used, including a mixed group of 130 male and female CD Sprague-Dawley ex-breeders over 60 weeks of age, a mixed group of 155 male and female CD Sprague-Dawley rats fed unaltered diets, 23 Wistar rats [sex unspecified] fed 3.2% indole in the diet and 17 CD Sprague-Dawley rats [sex unspecified] fed unaltered diets but whose cerebrum was damaged by focal freezing. Necropsy and histological examination was performed on all animals. The authors pooled the groups of rats fed lead subacetate alone with those fed lead acetate plus indole for statistical analysis. The reported incidence (tumour bearing rats/rats examined) of cerebral gliomas was: 1/325 (0.3%) in control rats; and 5/58 (8.6%) ($p < 0.05$, test unspecified) in rats fed either lead subacetate alone or lead subacetate plus indole. Two of 17 rats fed lead sub-

acetate alone developed gliomas and 3/41 rats fed lead subacetate + indole developed gliomas. [The Working Group considered there were only 285 untreated controls, in which one glioma occurred.] The incidence of renal cortical tumours [pathological stage undefined] was: 13/17 (76%) in rats fed lead subacetate alone and 25/41 (61%) in rats fed lead subacetate plus indole. The incidence of renal tumours in controls was not reported. [The Working Group noted the unusual design of analysis and incomplete reporting of this investigation.]

In a study of the histopathology of chemically-induced renal tumours carried out by Ito *et al.* (1971), a group of 10 male Wistar rats, 6–8 weeks of age, was fed 1.5% lead subacetate [purity unspecified] in the diet for 48 weeks and renal tumours were assessed histologically. All 10 rats developed either renal adenomas (60%) or renal carcinomas (40%). [The Working Group noted the absence of a control group.]

In another study by Ito (1973) focusing on the histopathology of tumours of the urinary system of rats, groups of 11–13 male Wistar rats, 6–8 weeks of age, some of which were also subjected to unilateral nephrectomy, were fed 1.5% lead subacetate [purity unspecified] in the diet for 23 weeks (intact or nephrectomized) or 48 weeks (intact). Tumours of the urinary system were assessed histologically. In the 13 intact rats fed the lead subacetate-containing diet for 23 weeks, no renal tumours were observed while 2/11 lead subacetate-treated unilaterally-nephrectomized rats developed renal tumours. After 48 weeks of exposure, renal tumours developed in 9/11 lead subacetate-treated intact rats. Lead subacetate-induced tumours were either renal-cell adenomas (64%) or carcinomas (36%). [The Working Group noted the absence of control groups.]

In a study performed by Kasprzak *et al.* (1985) of the effects of dietary calcium on the carcinogenicity of lead subacetate in the kidney, groups of 28–30 male Sprague-Dawley rats were fed 1% lead subacetate (AR grade) admixed with 0, 0.3, 1, 3 or 6% calcium acetate in the diet and observed for 79 weeks. Controls received unaltered basal diet and a separate group was fed 3% calcium acetate alone. All additions to the basal diet caused significant suppression of weight gain ranging from 7 to 46% ($p < 0.05$, Student's *t*-test). No significant differences in survival were observed (two-tailed Fisher's exact test). At the time of the detection of the first renal tumour (58 weeks), surviving rats were killed, tissues were examined microscopically and renal tumour incidence was determined. Renal tumours did not occur in control rats or rats fed calcium alone. In rats fed lead subacetate alone, 13/29 (45%) developed renal tumours [statistically significant; Fisher's exact test, $p < 0.05$] including 11 adenomas and two adenocarcinomas. Addition of 0.3, 1, 3 or 6% calcium (calcium reduced renal lead content by up to 72%) to the diet significantly increased ($p = 0.035$ – 0.014 , two-tailed Fisher's exact test) the incidence of renal tumours in lead-treated rats to 62–79%. The number of rats with bilateral renal tumours was also significantly increased ($p < 0.05$, two-tailed Fisher's exact test) in comparison with rats treated with lead subacetate alone.

(b) *Administration of lead with known carcinogens or modifiers of carcinogenesis*

The effects of lead subacetate in combination with 2-AAF, indole or boiled linseed oil containing 'lead drier' [lead drier is a compound made of lead, cobalt and manganese naphthenates that was added to improve the drying qualities of oil-based paints made with boiled linseed oil] on renal carcinogenesis were studied by Hass *et al.* (1967) in male CD rats, 6–8 weeks of age. For up to 74 weeks the animals were fed diets supplemented with two or more of the following components: 0.06% 2-AAF, 1.6% indole, 1.0% lead subacetate [specified as 'chemically pure'] and 10.0 g/100 mL [presumably mL of diet] commercial boiled linseed oil containing 'lead drier'. Some animals in each group were killed at 52 weeks. The concentrations of naphthenates in the linseed oil were given as 0.20% lead, 0.35% manganese and 0.30% cobalt. In total, 64 rats received 2-AAF plus indole, 24 rats received lead subacetate plus indole, 50 rats received lead subacetate, indole and linseed oil, and 74 rats received lead subacetate, 2-AAF, indole and linseed oil. A few rats died during exposure [precise survival and body weight data not given]. Complete autopsies were done and tissues from all tumours were examined microscopically. None of the animals fed diets containing 2-AAF and indole developed renal tumours. All of the groups fed diets containing lead subacetate developed renal cortical tumours. Of the 24 rats fed lead subacetate plus indole, 22 had 'cystomas' [presumably cystic adenomas], 19 had adenomas [presumably solid adenomas] and 11 (46%) had adenocarcinomas. Of the 50 rats fed lead acetate, indole and linseed oil, 30 had cystomas, 20 had adenomas and 14 (28%) had adenocarcinomas. Of the 74 rats fed lead subacetate, indole, linseed oil and 2-AAF, 37 had cystomas, 29 had adenomas and 25 (34%) had adenocarcinomas. [The Working Group noted the absence of a control group or a group receiving lead subacetate alone].

Oyasu *et al.* (1970) fed three groups of male CD Sprague-Dawley rats, 5–8 weeks of age, diets containing 1.0 % lead subacetate [purity unspecified], or 1.0% lead subacetate and 1.6% indole, or 1.0% lead subacetate, 1.6% indole (added to prolong the lifespan of 2-AAF-treated rats) and 2-AAF. The animals were observed for 12–17 months. Average survival was 53–69 weeks. Histological examination was performed on all animals that died or were killed at the end of the experiment. For statistical analysis the authors pooled the groups of rats fed lead subacetate alone with those fed lead acetate plus indole. The reported incidence (tumour bearing rats/rats examined) of cerebral gliomas was 5/58 (8.6%) in rats fed either lead subacetate alone or lead subacetate plus indole (2/17 rats fed lead subacetate alone developed gliomas and 3/41 rats fed lead subacetate + indole developed gliomas), and 6/72 (8.3%) in rats fed lead subacetate, indole and 2-AAF. The incidence of renal cortical tumours [pathological stage undefined] was 13/17 (76%) in rats fed lead subacetate alone; 25/41 (61%) in rats fed lead subacetate plus indole, and 36/72 (50%) in rats fed lead subacetate, indole and 2-AAF. [The Working Group noted the unusual design of analysis and incomplete reporting of this investigation.]

Hiasa *et al.* (1983) studied the development of renal tumours in groups of 17–24 male Wistar rats, 6 weeks of age, fed diets containing 500 or 1000 ppm *N*-ethyl-*N*-hydroxyethyl-

nitrosamine (EHEN) for 2 weeks followed by 1000 ppm lead subacetate (purity, 99.5%) for 20 weeks with an additional observation period of 10 weeks (total duration, 32 weeks). Two groups were fed EHEN alone (1000 ppm) and lead subacetate alone, respectively. Controls received unaltered diets for 32 weeks. Rats that died before the end of the study were excluded from evaluation; these included two rats given EHEN alone and four rats given EHEN plus lead subacetate. All groups fed lead subacetate and the group fed 1000 ppm EHEN alone showed significant reduction ($p < 0.05$, test unspecified) of final body weight (maximum, 14%). Histological examination revealed the following renal tumour incidence (tumour-bearing rats/rats examined): 500 ppm EHEN, 0/24; 1000 ppm EHEN, 9/18 ($p < 0.05$ versus control, χ^2 test); 500 ppm EHEN plus lead subacetate, 10/22 ($p < 0.05$ versus 500 ppm EHEN alone); 1000 ppm EHEN plus lead subacetate, 17/17 ($p < 0.05$ versus 1000 ppm EHEN alone); lead subacetate alone, 0/24; control 0/24. No adenocarcinomas occurred in rats fed EHEN alone, one renal adenocarcinoma occurred in a rat fed EHEN 500 ppm plus lead subacetate and 10 adenocarcinomas in rats fed 1000 ppm EHEN and lead subacetate. [The Working Group noted the short duration of the study for the assessment of lead-induced tumours.]

The effects of various nephrotoxic chemicals, including lead subacetate, in promoting EHEN-induced renal carcinogenesis were studied by Shirai *et al.* (1984) in groups of 23–25 male Fischer 344 rats [age unspecified; weighing ~130 g] that were given 0.1% EHEN in the drinking-water for 1 week followed by 0.1% lead subacetate [purity unspecified] in the diet for 35 weeks. A separate group received lead subacetate alone from week 2 to week 36. All rats were subjected to unilateral nephrectomy during the third week of the experiment. All rats were killed after 36 weeks and five transverse kidney sections from each animal were taken for histological evaluation. Lead subacetate alone did not induce renal tumours. EHEN alone induced renal-cell tumours [size and histology unspecified] in 5/23 rats (22%); exposure to lead subacetate after EHEN increased the incidence of renal-cell tumours to 13/25 ($p < 0.05$ versus EHEN alone, test unspecified). [The Working Group noted that no untreated control group was included and noted the short duration of the study for the assessment of lead subacetate-induced tumours.]

Groups of 15 male Wistar rats, 6 weeks of age, were fed diets containing 1000 ppm EHEN for 2 weeks. Unilateral nephrectomies were then performed on all rats and they were provided with unaltered diets or diets containing 1000 ppm lead subacetate [purity unspecified] for up to 18 additional weeks. Five animals from each group were killed at weeks 8, 12 and 20 of the experiment. In rats given EHEN alone, 2/15 had simple renal hyperplasia (hyperplasia with a tubular pattern), 0/15 had renal 'adenomatous hyperplasia' (hyperplasia with loss of tubular pattern) and 0/15 had renal tumours. In rats given EHEN plus lead subacetate, 11/15 had simple hyperplasia, 8/15 had adenomatous hyperplasia and 1/15 had a renal-cell tumour. Incidence and multiplicity (lesions/rat) of simple hyperplasia was increased in the rats fed EHEN plus lead and killed at week 20 versus rats fed EHEN alone ($p < 0.05$, tests unspecified) (Hiasa *et al.*, 1991; Nishii 1993). [The Working Group noted that no untreated control group was included and noted the short duration of the study for the assessment of lead subacetate-induced tumours.]

3.2.3 *Hamster*

Van Esch and Kroes (1969) fed groups of 22 male and 23–24 female Syrian golden hamsters, 3–4 weeks of age, either 0 (control), 0.1 or 0.5% lead subacetate [purity unspecified] in the diet for up to 2 years. The higher dose in females and both doses in males appeared to reduce survival, mostly during the first year [not statistically evaluated]. At the end of the experiment all animals were killed and underwent histological examination. No renal tumours or hyperplasia occurred in any group, although pleomorphic cells with hypertrophic nuclei were commonly observed in the kidneys of lead-treated hamsters.

3.2.4 *Rabbit*

Hass *et al.* (1967) fed a total of 85 male rabbits (primarily New Zealand albino with a few German Checker and Belgian Hare), 3 months of age, diets containing 0.5–1.0% lead subacetate (specified as chemically pure) for 3–78 weeks. Twenty-one animals received lead alone while the others were given various other compounds (linseed oil containing lead drier, 2-AAF, cholesterol, chloroform, carbon tetrachloride and vitamin D) in the diet or by injection. Precise survival and body weight data were not given. None of the lead-treated rabbits developed renal tumours, although chronic lead nephropathy was common. [The Working Group noted the absence of a control group, the use of a variety of strains of rabbits and the incomplete reporting of the study.]

3.3 **Lead carbonate**

3.3.1 *Rat*

Oral administration

Fairhall and Miller (1941) fed male albino rats, 70–90 g, [strain and age unspecified] diets that contained 0.1% lead arsenate (49 rats) (see section 3.10 for assessment of the lead arsenate experiment) or an equivalent amount of lead as lead carbonate (55 rats) for up to 2 years. A control group of 24 rats of similar age and weight was fed the same diet without the test substances. At the end of the first year, approximately half of the surviving rats in each group were killed, and tissues were taken for histological examination. The remaining rats in each group were maintained on the same treatments and were killed at the end of the second year; their tissues were examined similarly. Histological examinations performed at 1 and 2 years showed moderate to marked kidney changes (enlargement of cells in the convoluted tubules, intranuclear inclusions and accumulation of brown pigment in cells of the convoluted, proximal and distal tubules), but no tumours in the rats fed lead carbonate. No pathological changes of note were observed in other tissues and organs in the lead carbonate-treated rats compared with controls. No tumours were observed at any site in any of the groups. [The Working Group noted the incomplete reporting of the study.]

3.4 Lead nitrate

3.4.1 Mouse

Administration of lead with known carcinogens or modifiers of carcinogenesis

Litvinov *et al.* (1984) gave groups of 50 female CBA × C57/BL6 mice [age unspecified] 10 mg/L NDMA or 0.3 mg/L lead nitrate or both compounds together in drinking-water for either 26 or 39 weeks. None of the mice receiving lead nitrate alone for 39 weeks developed tumours. Treatment with lead nitrate did not appear to impact NDMA-induced renal tumours. [The Working Group noted the short duration of the study for assessment of lead-induced tumours.]

3.4.2 Rat

(a) *Oral administration*

Schroeder *et al.* (1970) gave groups of male Long-Evans rats [age and initial number unspecified] 25 ppm lead nitrate (25 mg/L lead) in the drinking-water from weaning until death. An epidemic of pneumonia of 3 weeks duration killed 22 lead-treated rats and 19 controls. Sufficient rats survived in each group (52 lead-treated rats and 52 controls) to continue the experiment. After corrections for the early mortality, the survival of lead-treated rats was lower ($p < 0.05$) than that of the controls. Grossly visible tumours [type and location unspecified] were found in 7/43 lead-treated rats at necropsy; the tumour incidence (10/50) in controls was not significantly different. [The Working Group noted the low dose of lead nitrate that was used and the incomplete reporting of this experiment.]

(b) *Administration of lead with known carcinogens or modifiers of carcinogenesis*

Litvinov *et al.* (1982) gave groups of 50 albino outbred male rats, weighing 150–200 g, NDMA in the drinking-water (effective dose, 0.5 mg/kg bw per day) alone or in the presence of lead nitrate (0.5 mg/L drinking-water). A group of 50 control rats were given drinking-water only; no group treated with lead nitrate alone was available. The experiment lasted 19 months. A complete necropsy was performed on all animals and tissues were examined histologically. No renal or liver tumours occurred in control animals. Renal tumours occurred in 8/42 rats treated with NDMA alone and in 19/41 rats treated with NDMA and lead nitrate. Liver tumours occurred in 15/42 rats receiving NDMA alone compared with 5/41 rats receiving NDMA and lead nitrate combined. The authors concluded that lead nitrate increased the incidence of kidney tumours and decreased the incidence of liver tumours.

3.5 Lead powder

3.5.1 Rat

(a) Oral administration

Furst *et al.* (1976) gave groups of 25 male and 25 female young Fischer 344 rats [age unspecified] weighing ≤ 119 g, lead powder (99.9% pure; 10 mg lead; particle size unspecified) in corn oil by stomach tube twice a month for 12 months and observed them for 24 months. Groups of 20 male and 20 female control rats were given 0.5 mL corn oil by stomach tube according to the same schedule. A complete necropsy was performed on all of the animals that were killed and included histological examination of suspicious tissues. Survival data were not reported. One lymphoma and four leukaemias were found at necropsy in 47 lead powder-treated rats; this did not differ significantly from the incidence of three lymphomas found at necropsy of 29 controls. No other neoplasms were reported in the lead powder-treated or control rats.

(b) Intramuscular administration

In a similar experiment, Furst *et al.* (1976) gave groups of 25 male and 25 female young Fischer 344 rats [age unspecified], weighing ≤ 111 g, monthly intramuscular injections of 10 mg lead powder (99.9% pure; particle size unspecified) in trioctanoin for 9 months and then monthly injections of 5 mg lead powder for 3 months and observed the animals until the end of the experiment (24 months). Equal numbers of rats were given the trioctanoin vehicle alone and served as controls. A complete necropsy was performed on all animals that were killed and included histological examination of suspicious tissues. Fibrosarcomas developed at the injection site in one of the lead powder-treated rats and in one of the control rats. The incidences of lymphoma were 6/50 in the lead powder-treated rats and 3/50 in the trioctanoin-treated controls. The authors noted the following tumours in the lead powder-treated rats that were not seen in the controls: a metastasis of an osteogenic sarcoma in the lungs without primary site identification, a mesothelioma of the urogenital tract and two tumours of the pancreas (a fibrolipoma and a villous adenoma). Statistical analyses were not reported, but the authors concluded that 'lead powder did not seem to produce any appreciable number of tumours'.

(c) Intrarenal administration

Jasmin and Riopelle (1976) gave a group of 20 young female Sprague-Dawley rats, weighing 120–140 g, injections of 5 mg lead powder [purity and particle size not specified] suspended in 50 μ L glycerine into the cortical sections of both poles of the right kidney (total dose, 10 mg lead powder/rat). A negative control group of 16 rats received similar intrarenal injections of 50 μ L glycerine alone and a positive control group of 16 rats received intrarenal injections of 5 mg nickel subsulfide powder (total dose, 10 mg/rat) in 50 μ L glycerine. The experiment lasted 12 months, during which time the animals were examined at regular intervals for development of erythrocytosis and renal tumours. All of

the kidneys were removed at necropsy for histological examination. No erythrocytosis or renal tumours developed in the lead powder-treated rats or in the negative controls, whereas marked erythrocytosis ($p < 0.05$ versus controls) developed in all of the 16 positive control rats, and renal carcinomas were found in 7/16 of these near the nickel subsulfide injection sites [p -value not reported].

3.6 Lead oxide

3.6.1 Rat

Inhalation exposure

Monchaux *et al.* (1997) exposed a group of 50 male Sprague-Dawley rats, 12 weeks of age, to lead oxide particles [purity unspecified] (mean particle size, $0.69 \mu\text{m}$; mass median aerodynamic diameter (MMAD), $5.1 \mu\text{m}$) in a whole-body chamber. The mean airborne concentration of lead oxide measured within the chamber was $5.3 \pm 1.7 \text{ mg/m}^3$, and rats were exposed for 6 h per day on 5 days per week for 1 year. The authors estimated that this exposure was approximately equivalent to the dose of oral lead acetate that would give rise to a 10% incidence of kidney cancers in rats, according to the experiments of Azar *et al.* (1973). A group of 785 untreated rats served as the control. In addition to the rats treated with lead oxide alone, a group of 63 rats was initially exposed to 0.6 Gy fission neutrons by placing the animals at a distance of 4 m from a Silene experimental reactor core (time unspecified); another group of 50 rats was exposed to fission neutrons and 2 months later to lead oxide by inhalation. A fifth group of 25 rats was exposed to lead oxide by inhalation and then received six intramuscular injections of 25 mg/kg bw 5–6 benzo[*b*]fluoranthene (βNF) [schedule unspecified] for lung tumour promotion starting 1 month after lead exposure ended. All animals were kept until moribund [precise survival time unspecified] except those exposed to lead oxide and βNF , which were killed 100 days after the last injection of βNF . A complete necropsy was performed on each animal. Lungs were fixed by intratracheal instillation of fixative. Lead oxide exposure did not reduce survival compared with controls. No lung tumours occurred. One renal tumour occurred among the group of animals receiving lead oxide alone. The addition of βNF did not alter lead oxide-induced tumours. Lead oxide did not alter cancer rates induced by neutron exposure.

3.6.2 Hamster

Intratracheal administration

Kobayashi and Okamoto (1974) gave groups of 15 male and 15 female Syrian golden hamsters, 6 weeks of age, intratracheal instillations weekly for 10 weeks of either 1 mg lead oxide powder (99.8% purity; particle diameter $< 20 \mu\text{m}$), or a mixture of 1 mg benzo[*a*]pyrene and lead oxide, or 1 mg benzo[*a*]pyrene alone. Each treatment sample was suspended in 0.2 mL isotonic saline plus 0.5% carboxymethylcellulose solution. One group received vehicle alone. All suspensions were ultrasonicated and homogenized, so

that the final size of most of the particles (95%) within the mixture was $< 10 \mu\text{m}$. Fifteen males and 15 females were kept as untreated controls. The hamsters were killed when moribund or at 60 weeks after the initial intratracheal instillation. The hamsters treated with lead oxide alone, benzo[*a*]pyrene alone or the combination showed lower survival rates than those that received the vehicle alone or untreated controls. At necropsy, in addition to examination of any visible tumour foci, the five pulmonary lobes of each hamster were sectioned for histological examination. Atypical epithelial proliferations, adenomas (11 in males and females) and an adenocarcinoma (in a female) were observed in the lungs of hamsters given benzo[*a*]pyrene mixed with lead oxide. The neoplastic changes originated mostly in the bronchiolo-alveolar area. No neoplastic changes were found in the other groups. Lead oxide alone induced hyperplastic and squamous metaplastic foci of the alveolar area, while benzo[*a*]pyrene alone affected the lung only slightly. Although statistical confirmation was not provided, the authors concluded that lead oxide showed a cocarcinogenic effect with benzo[*a*]pyrene in the bronchiolo-alveolar area of hamster lungs. [The Working Group noted that lead could have acted as a carrier for benzo[*a*]pyrene as in other particle studies, but this does not exclude other lead-related mechanisms of carcinogenesis.]

3.7 Lead naphthenate

3.7.1 Mouse

Skin application

Baldwin *et al.* (1964) painted a 20% solution of lead naphthenate [purity unspecified] in benzene on shaved dorsal skin of a group of 59 adult male Schofield albino mice. The total dose of 6 mL was administered over 12 months as weekly or twice-weekly applications [dosing schedule unclear]. Kidney damage [no further details reported] was observed in treated animals and, after 648 days, less than 50% of the mice had survived. Of the 59 treated animals, two developed skin papillomas, one developed renal carcinoma and four showed tubular adenomas of the kidney. [The Working Group noted that no vehicle-control group was included and that a known carcinogen was used as the vehicle.]

3.8 Lead chromate

Chromium[VI] was previously evaluated as *carcinogenic to humans* (Group 1) in the Monograph on Chromium and Chromium Compounds (IARC, 1990).

3.8.1 Mouse

Intramuscular administration

Furst *et al.* (1976) gave a group of 25 female weanling NIH-Swiss mice intramuscular injections of 3 mg/mouse lead chromate (98% pure) in trioctanoin (tricaprylin; total dose,

12 mg/mouse) monthly for 4 months. The authors noted that a higher dose (8 mg/mouse) of lead chromate was not tolerated. Two control groups of mice were included; one received the vehicle alone and the other served as uninjected controls. Necropsies were performed at termination of the study at around 25 months, and showed that 2/17 mice in the lead chromate-treated group had developed lymphoma and 3/17 had alveogenic carcinomas [further details not reported]. Necropsies of 22/25 mice in the vehicle-control group revealed two animals with lymphocytic leukaemia and one with an alveogenic carcinoma. In the uninjected controls, necropsies of 15/25 mice showed one lymphoma, five lymphocytic leukaemias and one alveogenic carcinoma. [The Working Group noted the incomplete reporting of the study.]

3.8.2 *Rat*

(a) *Subcutaneous injection*

Groups of 40 male and 40 female Sprague-Dawley rats, 13 weeks of age, were given a single subcutaneous injection of 30 mg/rat lead chromate (chromium yellow) or basic lead chromate (chromium orange) [purity unspecified] suspended in saline. Within 150 weeks, 26/40 animals injected with lead chromate and 27/40 injected with basic lead chromate had developed sarcomas (rhabdomyosarcomas and fibrosarcomas). No sarcomas developed in 60 control animals (Maltoni, 1976; Maltoni *et al.*, 1982).

(b) *Intramuscular administration*

Hueper (1961) gave a group of 33 rats [strain, age and sex unspecified] intramuscular implantations of lead chromate [amount and purity unspecified] in sheep fat. One tumour was reported at the implantation site [no further details stated]. None of the rats in two vehicle-control groups developed tumours. [The Working Group noted the incomplete reporting of the study.]

Furst *et al.* (1976) gave groups of 25 male and 25 female weanling Fischer 344 rats intramuscular injections of 8 mg lead chromate (98% pure) in trioctanoin (tricaprylin) once a month for 9 months. At the termination of the study at around 25 months, two lymphomas, 11 injection-site fibrosarcomas, 10 injection-site rhabdomyosarcomas and one osteogenic sarcoma had developed in 24 lead-chromate-treated female rats. Among 23 lead chromate-treated male rats, three injection-site fibrosarcomas, seven rhabdomyosarcomas and three renal carcinomas had developed. In the vehicle-control group, necropsy revealed two lymphomas among 16 females and one lymphocytic leukaemia and one injection-site fibrosarcoma among 12 males. [The Working Group noted the development of tumours of the kidney distant to the site of administration in male rats.]

(c) *Intrapleural administration*

Hueper (1961) also gave a group of 33 rats [strain, age and sex unspecified] intrapleural implantations of lead chromate [amount and purity unspecified] in sheep fat. Three

tumours were reported at the implantation site [no further details stated]. None of the rats in two vehicle-control groups developed tumours. [The Working Group noted the incomplete reporting of the study.]

(d) *Intrabronchial administration*

In a study to investigate the potential carcinogenicity of a range of chromium-containing materials, groups of 50 male and 50 female Porton-Wistar rats, 8–10 weeks of age, received intrabronchial implantations of stainless-steel mesh pellets (5×1 mm) loaded with about 2 mg of a series of seven commercial types of lead chromate test materials (lead chromate (99.8% pure), primrose chrome yellow, Supra LD chrome yellow, molybdate chrome orange, light chrome yellow, medium chrome yellow and silica-encapsulated medium chrome yellow). The lead chromates contained between 60–64% lead with the exception of the silica-encapsulated medium chrome yellow (40% lead). Groups of 50 male and 50 female rats receiving pellets loaded with cholesterol alone acted as negative controls and similar groups receiving pellets loaded with 2 mg calcium chromate (96.7% purity) suspended in 50:50 cholesterol acted as positive controls. Animals were maintained for 24 months at which time surviving animals were killed and full necropsies were performed. All lungs and any suspected lesions were examined histologically. Survival was 95.7% at 400 days and overall, 53.9% at 700 days. No bronchial carcinomas (0/100) were seen in the cholesterol-alone control group whilst 25/100 bronchial carcinomas (24 squamous-cell carcinomas and one adenocarcinoma) were found in the calcium chromate positive control group. Among the seven lead chromates tested, one squamous-cell tumour of the lung was found in one male in each of the four groups which received lead chromate (99.8% pure), primrose chrome yellow, Supra LD chrome yellow or medium chrome yellow, respectively (in each of the four groups, incidence was 1/100, males and females combined; $p = 0.37$; χ^2 test) (Levy & Venitt, 1986; Levy *et al.*, 1986).

3.8.3 *Guinea-pig*

Intratracheal administration

Steffee and Baetjer (1965) gave a group of 13 guinea-pigs [strain and sex unspecified], 3 months of age, 0.3-mL intratracheal instillations of 1% lead chromate [purity and particle size unspecified] in saline at 3-monthly intervals for 18 months with no further exposure until death or termination of the experiment. After an experimental period of 40–50 months, none of the lead chromate-exposed animals and none of the vehicle-control animals (18 guinea-pigs) had developed pulmonary tumours. [The Working Group noted the small numbers of animals and the insufficient experimental details provided.]

3.8.4 *Rabbit*

Intratracheal administration

Steffe and Baetjer (1965) gave a group of seven rabbits [strain and sex unspecified], 4 months of age, 1-mL intratracheal instillations of 1% lead chromate [purity and particle size unspecified] in saline at 3-monthly intervals for 9–15 months with no further exposure until death or termination of the experiment. After an experimental period of 40–50 months, none of the lead chromate-exposed animals and none of the vehicle-control animals (five rabbits) had developed pulmonary tumours. [The Working Group noted the small numbers of animals and the insufficient experimental details provided.]

3.9 Lead phosphate

3.9.1 *Rat*

(a) *Subcutaneous injection*

Zollinger (1953) gave groups of 10 albino randomly-bred rats [strain, age and sex unspecified] weekly subcutaneous injections of 20 mg lead phosphate [purity unspecified] as a 2% suspension in 1 mL [vehicle unspecified] for up to 16 months (total doses, 40–760 mg/rat). No kidney epithelial tumours were reported for the 40 rats in an untreated control group. Among treated rats, many animals died during the experiment and the incidence of renal tumours (adenomas, cystadenomas, papillomas and cortical carcinomas) was 19/29 for rats surviving ≥ 10 months from the start of treatment. Each of the 19 had received a total dose of 120–680 mg lead phosphate. Histological analysis revealed that 21/112 treated rats had renal tumours (the earliest developing 4 months after the start of treatment), and in 11, the renal tumours were bilateral. The author reported that tumour incidence increased with time. [The Working Group noted the renal tumour response with a water-insoluble lead compound.]

Tönz (1957) gave groups of 36, 33, 14 and 29 albino rats [strain, age and sex unspecified] subcutaneous injections of 20 mg lead phosphate [purity unspecified] suspended in pectin weekly for up to 9.5 months and observed them for a total experimental period of up to 16.5 months after the start of the injections. Total doses were: 340 mg over 4 months; 440 mg over 4–9.5 months; 250 mg over a total experimental period of 10 months or less; and 360 mg from a total experimental period of 10 to 16.5 months; for the above four groups, respectively. Kidney weight increased in all animals given lead phosphate. All 36 rats that received a total dose of 340 mg and all 33 rats that received a total dose of 440 mg lead phosphate died during or shortly after the treatment. The incidences of renal adenomas and carcinomas were 19/29 and 3/29 respectively in the group that received an average total dose of 360 mg lead phosphate. Spontaneous epithelial kidney tumours were not observed in more than 2000 historical control animals in the author's laboratory. [The Working Group noted that no concurrent control group was included but noted the renal tumour response with a water-insoluble lead compound.]

Baló *et al.* (1965) gave a group of 80 albino rats [age and sex unspecified] subcutaneous injections of lead phosphate [purity and vehicle unspecified] at weekly or fortnightly intervals for 18 months. Rats surviving to the end of treatment had received a total dose of 1.3 g lead phosphate. Renal adenomas developed in 29 lead phosphate-treated rats and in none of 20 control animals. [The Working Group noted the renal tumour response with a water-insoluble lead compound.]

(b) *Subcutaneous and intraperitoneal administration combined*

Roe *et al.* (1965) gave three groups of 24 male Chester Beatty Wistar rats [age unspecified] combined subcutaneous and intraperitoneal injections of a technical grade of lead phosphate in distilled water at repeated intervals for 34 weeks (total doses, 29, 145 and 450 mg lead phosphate for the three groups, respectively). No carcinoma developed in the 23 rats that survived to 200 days in the lowest-dose group. Twenty-one animals in the highest-dose group died before 200 days and two of the three remaining animals developed renal adenomas. In the mid-dose group, 14/23 rats surviving to 200 days developed renal tumours (13 adenomas, seven adenocarcinomas and one undifferentiated malignant renal tumour) [statistically significant, Fisher's exact test; $p < 0.05$]. Two of the 24 control rats developed renal tumours (one undifferentiated malignant tumour and one transitional-cell carcinoma in the renal pelvis).

3.10 Lead arsenate

Arsenic compounds were previously evaluated as *carcinogenic to humans* (Group 1) in Supplement 7 of the *IARC Monographs* (IARC, 1987) and in the Monograph on Arsenic in Drinking-Water (IARC, 2004b).

3.10.1 Rat

(a) *Oral administration*

Fairhall and Miller (1941) fed a group of 49 white male rats [strain unspecified], weighing 70–90 g, a diet containing 10 mg/animal lead arsenate [purity unspecified] daily for 2 years (total approximate dose, 7.2 g). Of the animals that were given lead arsenate 45% had died after 1 year and 61% at 2 years [cause of death unspecified]. No tumours were reported from necropsies of the experimental animals nor from the 24 animals in the control group. [The Working Group noted the high early mortality.]

Kroes *et al.* (1974) fed groups of 40 or 29 male and 40 or 19 female weanling Wistar rats a diet containing 463 or 1850 ppm, respectively, of technical-grade lead arsenate (60% lead; 20.9% arsenic) for up to 120 weeks. At necropsies of 17 surviving males that had received the higher dose, one bile duct adenocarcinoma, one renal cortical adenoma and one lymphangioma were observed. No tumours were seen at necropsies of 11 surviving females that received the higher dose. In 38 males in the low-dose group, one renal hamartoma and two pituitary adenomas were observed. Among 40 females in the low-dose

group, eight developed pituitary adenomas. In the control groups, necropsy of 39 males revealed one nephroblastoma, one pituitary adenoma, one lymphatic leukaemia and one lymphoblastosarcoma, and among 59 females, one thoracic sarcoma and 13 pituitary adenomas were observed. The authors stated that no definite conclusions could be drawn from the study.

(b) *Administration of lead with known carcinogens or modifiers*

Kroes *et al.* (1974) also fed two groups of 40 male and 40 female weanling SPF-derived Wistar rats a diet containing 463 ppm technical-grade lead arsenate (60% lead; 20.9% arsenic) and 0.3 mL water by intubation (five times/week for the duration of the study) for up to 120 weeks. One of the groups also received 5 µg/rat NDEA in water by intubation five times/week for the duration of the study. Control groups comprised 50 male and 60 female rats receiving 0.3 mL water by intubation and 50 male and 60 female rats receiving 5 µg/day NDEA as well as 0.3 mL water by intubation (five times/week for the duration of the study). Necropsies were performed on all the animals at the end of the study. In the group that received lead arsenate plus NDEA, one heart endothelial sarcoma and one pituitary adenoma were seen among 34 male rats; in the corresponding 40 females, seven pituitary adenomas were observed. In the water-only control group, one nephroblastoma, one pituitary adenoma, one lymphatic leukaemia and one lymphoblastosarcoma were observed among 39 males. In the corresponding group of 59 females, one thoracic sarcoma and 13 pituitary adenomas were observed. In the water plus NDEA control groups, one splenic lymphosarcoma, three pituitary adenomas, one lymphosarcoma and one lymphatic leukaemia were observed among 40 males. In the corresponding female group of 58 rats, one renal hamartoma, 14 pituitary adenomas and one lymphosarcoma were observed. [The Working Group noted the absence of an effect of lead arsenate on NDEA-induced tumours.]

3.11 Tetraethyl lead

3.11.1 *Mouse*

Subcutaneous administration

Epstein and Mantel (1968) gave groups of 109, 79 and 69 male and female randomly bred neonatal (0–21 days old) Swiss mice subcutaneous injections of total doses of 0.6, 1.2 and 2.0 mg/mouse, respectively, of tetraethyl lead [purity unspecified] in tricaprylin into the nape of the neck. The low-dose group received 0.1 mg on days 1 and 7 and 0.2 mg on days 14 and 21; the mid-dose group received 0.2 mg on days 1 and 7 and 0.4 mg on days 14 and 21; and the high-dose group received a single injection of 2 mg on day 1. All 69 mice injected with 2 mg, 92% of the 79 mice injected with 1.2 mg and 20% of the 109 mice injected with 0.6 mg tetraethyl lead died prior to weaning. At 36 weeks, the incidence of lymphomas in the mice that survived treatment with 0.6 mg tetraethyl lead (low dose) was 1/26 males and 5/41 females. The incidence of lymphomas in vehicle-control animals was 1/39 males and 0/48 females. Thus the incidence of lymphomas in treated

female mice was significantly elevated ($p < 0.05$, χ^2 test) compared with female control mice but not in treated male mice compared with male control mice. [The Working Group felt that this study was limited by high mortality and the lack of concordance in tumour response between lead-treated male and female mice.]