3. Biological Data Relevant to the Evaluation of Carcinogenic Risk to Humans

3.1 Carcinogenicity studies in animals[1]

The carcinogenicity of chromium and chromium-containing compounds in experimental animals has been reviewed recently (Yassi & Nieboer, 1988; Fairhurst & Minty, 1990).

The description and evaluation of the available carcinogenicity studies in experimental animals have been subdivided into four subsections, mainly according to the chemical and physical properties of different chromium-containing materials: (*a*) Metallic chromium; (*b*) chromium [III] compounds; (*c*) chromium [VI] compounds; and (*d*) other chromium compounds. Chromium-containing alloys used in implants will be considered in a subsequent volume of *IARC Monographs* (Volume 52), in a monograph on cobalt and cobalt compounds.

(*a*) *Metallic chromium*

(i) *Intrapleural administration*

Mouse: No tumour was observed after 14 months in a group of 50 male C57Bl mice, approximately six weeks of age, that received six intrapleural injections of 10 μg chromium powder in 0.2 ml of a 2.5% gelatin-saline solution every other week. A total of 32 mice lived for up to 14 months (Hueper, 1955).

Rat: Groups of 17 female and eight male Osborne-Mendel rats, approximately four months old, were given six monthly intrapleural injections of 16.8 mg chromium powder in 50 μl lanolin; and 25 male Wistar rats, of approximately the same age, received six weekly intrapleural injections of 0.5 mg chromium powder suspended in 0.1 ml of a 2.5% gelatin-saline solution. Six Osborne-Mendel rats survived up to 19-24 months and 12 Wistar rats up to 25-30 months. Three female Osborne-Mendel rats developed adenofibromas of the thoracic wall; in addition, one rat also had a retroperitoneal haemangioma. Two other rats [group unspecified] had a haemangioma and an angiosarcoma, and another rat [group unspecified] had an intra-abdominal round-cell sarcoma. Of 12 male Wistar rats receiving gelatin alone, three developed intra-abdominal round-cell sarcomas (Hueper, 1955).

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[1]The Working Group was aware of carcinogenicity studies in progress with sodium chromate by intraperitoneal administration in mice and rats, with calcium chromate and chromite ore residue by inhalation in rats and with chromium by intratracheal administration in hamsters (IARC, 1988).

(ii) *Intramuscular administration*

Rat: A group of 24 male Fischer rats, eight weeks of age, received a single intramuscular injection of 2 mg chromium dust (elemental Cr, 65%, chromium oxides as Cr_2O_3, 35%; Ni, Al, Cu, Mn and Co, < 0.1%; mean particle diameter, 1.6 μm) suspended in 0.5 ml penicillin G procaine. No local tumour was reported in the 22 survivors at 24 months (Sunderman *et al.*, 1974). [The Working Group noted that only a single low dose was given.]

Two groups of 18 and 20 male Fischer-344 rats, aged eight weeks, received a single intramuscular injection of 4.4 mg chromium dust (Cr, 76%; O_2, 24%; Mn, 0.2%; median particle diameter, 1.4 μm) suspended in 0.2 ml penicillin G procaine. The study was terminated at two years, when 13/18 and 0/20 were still alive in the two groups, respectively; the low survival in the second group was due to an epidemic of pulmonary pneumonia. No local tumour developed in either group (Sunderman *et al.*, 1980). [The Working Group noted that only a single dose was given.]

A group of 25 male and 25 female weanling Fischer-344 rats received monthly intramuscular injections of 100 mg chromium powder (99.9% pure) in 0.2 ml tricaprylin. Treatment was continued until definite nodules appeared at the injection site in more than one animal [time unspecified]. The study was terminated at 644 days [survival figures not given]. A single injection-site fibrosarcoma was reported in a male rat. No local tumour was seen in 50 vehicle-control rats (Furst, 1971).

(iii) *Intraperitoneal administration*

Mouse: A group of 50 male C57Bl mice, approximately six weeks old, was given weekly intraperitoneal injections for four consecutive weeks of 10 μg chromium powder (diameter, > 100 μm to colloidal particle size) suspended in 0.2 ml of a 2.5% gelatin-saline solution. Forty mice survived up to 21 months, at which time the experiment was terminated. One mouse developed a myeloid leukaemia; no other tumour was noted (Hueper, 1955). [The Working Group noted the low dose given.]

Rat: A group of 25 male Wistar rats, three to four months old, was given weekly intraperitoneal injections for six consecutive weeks of 50 μg chromium powder in 0.1 ml of a 2.5% gelatin-saline solution. One rat developed a scirrhous carcinoma of the caecal submucosa, two rats developed intra-abdominal round-cell sarcomas, one rat had both a sarcoma of the leg of cartilaginous osteoid origin and an insulinoma of the pancreas, and one rat had an insulinoma (Hueper, 1955). [The Working Group noted that no vehicle control group was reported and that the authors stated that, although round-cell sarcomas also occurred in controls, insulinomas were found only in treated rats.]

(iv) *Intravenous administration*

Mouse: A group of 25 C57Bl mice [sex unspecified], about eight weeks of age, received six weekly injections into the tail vein of 2.5 μg chromium powder (particle size, ≤4 μm) in 0.05 ml of a gelatin-saline solution. Six animals lived up to 12 months, but none to 18 months. No tumour was observed (Hueper, 1955).

Rat: A group of 25 male Wistar rats, approximately seven months of age, was given six weekly injections of 90 μg chromium powder in 0.18 ml of a 2.5% gelatin-saline solution into the left vena saphena. Fifteen were still alive at one year and 13 at two years, at which time the study was terminated. Round-cell sarcomas were observed in four rats - three in the ileocaecal region and one in the intrathoracic region. One rat had a haemangioma of the renal medulla, and two rats had papillary adenomas of the lungs, one of which showed extensive squamous-cell carcinomatous changes. Use of vehicle-treated controls was not reported. The author stated that, although round-cell sarcomas also occurred in groups of control rats in this series of studies, lung adenomas were found only in treated rats (Hueper, 1955).

Rabbit: Eight albino rabbits [sex unspecified], approximately six months of age, received six weekly intravenous injections of 25 mg/kg bw chromium powder in 0.5 ml of a 2.5% gelatin-saline solution into the ear vein; the same course of treatment was given four months later; and, three years after the first injection, a third series of injections was given to the three surviving rabbits. Four rabbits given intravenous injections of the vehicle alone served as controls. One of three rabbits that survived six months after the last injection developed a tumour of uncertain origin (apparently an immature carcinoma) involving various lymph nodes, but no tumour occurred in controls (Hueper, 1955).

(v) *Intrafemoral administration*

Rat: A group of 25 male Wistar rats, approximately five months old, received an injection into the femur of 0.2 ml of a 50% (by weight) suspension of chromium powder (approximately 45 mg) in 20% gelatin-saline and was observed for 24 months; 19 survived over one year. No tumour developed at the injection site. Similarly, a group 25 male Osborne-Mendel rats, approximately five months of age, was injected in the femur with a similar dose of chromium powder in 0.2 ml lanolin and observed for 24 months; 14 survived for one year, and one rat developed a fibroma at the injection site (Hueper, 1955).

[The Working Group noted that many of the above studies suffered from various limitations, including the use of low doses, low effective numbers of animals and inadequate reporting.]

(vi) *Administration with known carcinogens*

Rat: Groups of 35-62 female Wistar rats, four to six weeks old, were given one intratracheal instillation of 10 mg powdered chromium (purity, 99.4%; diameter, 1-3 μm) in combination with 1 or 5 mg 20-methylcholanthrene (MC) or MC alone in saline and were killed at various intervals up to 12 weeks. Squamous-cell carcinomas of the lung developed 12 weeks after treatment in 7/12 (58%) rats given Cr + 5 mg MC, in 3/12 (25%) given Cr + 1 mg MC, in 3/7 (43%) given 5 mg MC alone, in 1/8 (12.5%) given 1 mg MC alone and in 0/12 given Cr alone (Mukubo, 1978.) [The Working Group noted the short duration of the study.]

(*b*) *Chromium[III] compounds*

(i) *Intratracheal instillation*

Rat: Random-bred and Wistar rats [age, sex and distribution unspecified] were given single intratracheal instillations of 50 and 20 mg chromic oxide, respectively. Malignant lung tumours developed in 7/34 and 6/18 animals; and four and five of these, respectively, were lung sarcomas, which appeared between 11 and 22 months after treatment (Dvizhkov & Fedorova, 1967). [The Working Group noted that use of controls was not reported and other details were not given.]

(ii) *Intrabronchial administration*

Rat: A group of 98 rats [strain, age and sex unspecified] received implants of intrabronchial stainless-steel mesh pellets (5 × 1 mm) loaded with 3-5 mg of a 50:50 mixture of chromic oxide with a cholesterol binder. Animals were observed up to 136 weeks. No lung tumour was found in treated or in 24 cholesterol binder-treated controls (Laskin *et al*., 1970).

Groups of 100 male and female Porton-Wistar rats received intrabronchial pellets loaded with 2 mg chromite ore [purity not given], 2 mg chromic oxide (metallurgical-grade, 99-100% pure), 2 mg chromic chloride hexahydrate (95% pure) or 2 mg chrome tan ($Cr_2(OH)_2(SO_4)_2Na_2SO_4{\cdot}xH_2O$ [purity not given]) suspended 50:50 in cholesterol. The incidence of squamous metaplasia in the left bronchus of treated animals was similar to that in controls. An increased incidence was seen with all five Cr[VI] compounds examined in the same study (see p. 125; Levy & Venitt, 1986).

In a second study using the same technique, no lung tumour was seen in a group of 101 rats treated with high silica chrome ore (TSS 695, containing 46.1% chromic oxide) (Levy *et al*., 1986).

(iii) *Oral administration*

Mouse: Groups of 54 male and 54 female weanling Swiss mice received 5 mg/l chromic acetate in drinking-water for life. No difference was found in the survival

of treated females compared with controls, but treated males died earlier than control males (mean survival, 831 *versus* 957 days); only 60% of males survived 18 months. The incidence of tumours in treated animals was no greater than that in controls (Schroeder *et al.*, 1964).

Rat: Chromic acetate was given in drinking-water at a level of 5 mg/l to 46 male and 50 female weanling Long Evans rats for life. At least 70% of the animals survived for up to two years; treated females lived as long as control females, but treated males lived up to 100 days longer than control males. The incidences of tumours at various sites in rats of either sex were not significantly different from those in controls. The total numbers of autopsied animals with tumours were: 16/39 treated males, 18/35 treated females, 9/35 male controls and 15/35 female controls (Schroeder *et al.*, 1965).

Chromic oxide (green) obtained by the reduction of chromate at 600°C was baked in bread with other nutrients at levels of 1, 2 and 5%, and the bread was fed to groups of 60 male and female inbred BD rats, 100 days of age, on five days per week for two years. At the high-dose level, the total dose consumed was about 1800 g/kg bw. Average survival times were 860-880 days. Mammary fibroadenomas were found in three rats given 1%, in one given 2% and in three given 5%. One mammary carcinoma and two fibroadenomas were detected in controls (Ivankovic & Preussmann, 1975).

(iv) *Intrapleural administration*

Mouse: Only granulomas were produced when 10 mg chromite ore dust [$FeO(CrAl)_2O_3$] particles (average diameter, 1 μm; range, 0.1-5 μm) were injected intrapleurally in 0.5 ml distilled water into 25 Balb/c mice [sex and age unspecified]. Animals were killed at intervals from two weeks to 18 months after the injection (Davis, 1972). [The Working Group noted the lack of detailed reporting.]

Rat: A group of 14 male and 11 female Osborne-Mendel rats, four months of age, received six monthly intrapleural injections of 37 mg chromite ore suspended in 0.05 ml lanolin. Thirteen survived one year and all animals were dead at 24 months. One thoracic tumour (fibrosarcoma) was found in a treated animal but none in 25 controls (Hueper, 1955).

A group of 34 rats [strain, sex and age unspecified] received intrapleural implantations of chromic acetate [dose unspecified] in sheep fat. Eighteen rats were still alive at 15 months and 15 at 21 months. One implantation-site tumour [type unspecified] was seen. Of 34 control rats administered sheep fat alone, 30 were alive at one year and 11 at 21 months; none developed a tumour (Hueper, 1961).

A group of 42 Bethesda Black [NIH Black] rats [sex unspecified], approximately three months of age, received eight intrapleural implantations over 13 months of 25 mg chromic acetate in gelatin capsules. No implantation-site tumour

was seen after two years (Hueper & Payne, 1962). [The Working Group noted the lack of controls.]

(v) *Intramuscular administration*

Rat: A group of 34 rats [sex, age and strain unspecified] received intramuscular implantations of chromic acetate [dose unspecified]. Thirty were still alive at one year and 17 at 21 months. One animal developed an injection-site tumour [type unspecified]. Of 32 controls given implants of sheep fat alone 30 were alive at one year and ten at 21 months. None developed a local tumour (Hueper, 1961).

A group of 35 Bethesda Black [NIH Black] rats [sex unspecified], approximately three months of age, received an intramuscular implantation of 25 mg chromic acetate in a gelatin capsule; a further seven intramuscular implantions were made over a period of 24 months, at which time the rats were sacrificed. One spindle-cell sarcoma was observed at the site of implantation (Hueper & Payne, 1962). [The Working Group noted that no control group was reported.]

(vi) *Intraperitoneal administration*

Mouse: In a strain A mouse assay for lung adenomas, three groups of ten male and ten female strain A/Strong mice, six to ten weeks of age, were given intraperitoneal injections of chromic sulfate suspended in tricaprylin three times a week for eight weeks (total doses, 480, 1200 and 2400 mg/kg bw). Animals were killed 30 weeks after the first injection. No significant increase in the incidences of pulmonary adenomas over those in 20 vehicle-treated or 20 untreated control mice of each sex was observed (Stoner *et al.*, 1976; Shimkin *et al.*, 1977). [The Working Group noted the small number of animals used.]

Rat: In experiments with Wistar and random-bred rats [sex, age and distribution unspecified], 4/20 animals developed lung sarcomas 16-19 months after a single intraperitoneal injection of 20 mg chromic oxide (Dvizhkov & Fedorova, 1967). [The Working Group noted that no control group was reported.]

(vii) *Intravenous administration*

Mouse: Strain A mice in a group of 25 males and 25 females [age unspecified] were each given an intravenous injection into the tail vein of 5 mg chromite ore (39-60% chromic oxide; particle size, 1.6 μm) suspended in saline. The animals were killed at three, 4.5 and six months. There was no difference in the incidence of pulmonary adenomas between treated mice and 75 untreated controls (Shimkin & Leiter, 1940; Shimkin *et al.*, 1977).

Rabbit: A group of six female albino rabbits, six months of age, received six weekly intravenous injections of 25 mg chromite ore suspended in 5 ml of a 2.5% gelatin-saline solution; treatment was repeated in four of the six rabbits nine months later. The six rabbits died or were killed at 13, 20, 22, 22, 48 and 48 months.

No tumour was observed during these periods (Hueper, 1955). [The Working Group noted the small number of animals used and the lack of controls.]

(viii) *Intrafemoral administration*

Rat: A group of 15 male and 10 female Osborne-Mendel rats, five months of age, each received an injection into the femur of 0.05 ml of a 50% (by volume) suspension containing about 58 mg chromite ore (44% chromic oxide) in lanolin; 13 survived one year. No tumour developed at the injection site (Hueper, 1955).

(ix) *Administration with known carcinogens*

Rat: Groups of 15 male Fischer 344 rats, seven weeks of age, received drinking-water (distilled) containing 0 or 500 mg/l *N*-nitrosoethylhydroxyethylamine (NEHEA) for two weeks. Thereafter, rats received drinking-water alone or drinking-water containing 600 mg/l chromic chloride hexahydrate (98% pure) for 25 weeks, when the study was terminated. There was no significant increase in the incidence of renal-cell tumours in the group receiving NEHEA and chromic chloride (6/15) over that in the group given NEHEA alone (2/15). No renal tumour was reported in the group receiving chromic chloride alone (Kurokawa *et al.*, 1985). [The Working Group noted that the experiment was not intended as a test for the overall carcinogenicity of chromic chloride.]

(c) Chromium[VI] compounds

(i) *Inhalation*

Mouse: Groups of 136 C57Bl/6 mice of each sex, eight weeks old, were exposed by inhalation to calcium chromate dust (reagent grade; particle size, 99.9% < 1.0 μm) at 13 mg/m^3 for 5 h per day on five days per week over their lifespan. The median survival time was 93 weeks for treated and 80 weeks for control mice. Six lung adenomas appeared in treated males and eight in females, compared with three and two in the 136 respective controls [p = 0.04 for males and females combined]. No carcinoma was seen; no information was given on the occurrence of tumours at other sites (Nettesheim *et al.*, 1971).

A group of 50 female ICR/JcI mice [age unspecified] was exposed by inhalation to chromic acid (chromium trioxide) mist (particle size, 84.5% > 5 μm) generated by a miniaturized electroplating system at a chromium concentration of 3.63 mg/m^3 for 30 min per day on two days per week for up to 12 months. Mice surviving at that time were maintained for a further six months; two groups of ten mice killed at 12 and 18 months served as controls. A single lung adenoma was reported in 1/15 mice that died or were killed between six and nine months; lung adenomas occurred in 3/14 mice that died between ten and 14 months; and 1/19 adenoma and 2/19 adenocarcinomas in mice that died at 15-18 months. In the control groups, no lung

tumour was reported in ten mice killed at 12 months, but 2/10 adenomas occurred in those killed at 18 months. The authors observed nasal perforations in six mice exposed for more than ten months and time-related inflammatory changes, including squamous metaplasia, in the trachea and bronchus of exposed mice (Adachi *et al.*, 1986). [The Working Group noted the incomplete reporting of lesions.]

A group of 43 female C57Bl mice [age unspecified] was exposed by inhalation to chromic acid (chromium trioxide) mist (85% of particles > 5 μm) generated by a miniaturized electroplating system at a chromium concentration of 1.81 mg/m³ for 120 min twice a week for 12 months, at which time 23 mice were killed. The remaining 20 were killed six months after the last exposure. Nasal perforation was seen in 3/23 and 3/20 mice killed at 12 and 18 months, respectively; 0/23 and 6/20 nasal papillomas occurred in these groups. A single lung adenoma was reported in the group killed at 18 months. No nasal inflammatory change or lung tumour was seen in a group of 20 untreated control mice (Adachi, 1987). [The Working Group noted the inadequate reporting of lesions.]

Rat: Groups of 20 male TNO-W74 Wistar rats, six weeks of age, were exposed by inhalation to sodium dichromate at 25, 50 or 100 μg/m³ Cr (average mass median diameter, 0.36 μm), produced from an aqueous sodium dichromate solution, for 22-23 h per day on seven days per week for 18 months. The rats were then held for a further 12 months, at which time the study was terminated. A control group consisted of 40 untreated male rats. Survival was about 90% at 24 months; at termination at 30 months, survival was 65, 55, 75 and 57.5% in the 25, 50, 100 μg/m³ and control groups respectively. In rats that survived 24 or more months, lung tumours occurred in 0/37, 0/18, 0/18 and 3/19 in the control, 25, 50 and 100 μg/m³ groups, respectively. The three lung tumours were two adenomas and an adenocarcinoma; a squamous carcinoma of the pharynx was also reported in this group. The incidence of treatment-related tumours was not increased at other sites (Glaser *et al.*, 1986). [The Working Group noted the small number of animals used.]

(ii) *Intratracheal instillation*

Mouse: A group of 62 strain A mice [sex unspecified], ten to 11 weeks of age, received six intratracheal injections of 0.03 ml of a 0.2% saline suspension of zinc chromate [basic potassium zinc chromate, $K_2O.4ZnO.4CrO_3.3H_2O$ (Baetjer *et al.*, 1959b)] at six-week intervals and were observed until death. No pulmonary carcinoma was found; pulmonary adenomas occurred in 31/62 exposed, in 7/18 untreated control and 3/12 zinc carbonate-treated control animals (Steffee & Baetjer, 1965).

Groups of 40 male and 40 female Sprague-Dawley rats, ten weeks of age, received intratracheal instillations of 1 ml/kg bw sodium dichromate (99.95% pure) or calcium chromate (chemically pure) in 0.9% sodium chloride solution once a week or five times a week. Equal numbers of male and female rats were used as vehicle

and untreated controls. Administered doses and schedules are given in Table 20. Treatment and study of all groups was continued for 30 months; median survival was approximately 800 days in the sodium dichromate-treated groups. [The Working Group noted that survival was not reported for the calcium chromate-treated groups.] No lung tumour was reported in the groups treated five times weekly with sodium dichromate. Among animals treated weekly with sodium dichromate, 14/80, 1/80 and 0/80 animals developed lung tumours in the groups receiving 1.25, 0.25 and 0.05 mg/kg bw, respectively, with 0/80 in controls [$p < 0.001$, Cochran-Armitage test for trend]. Of the 14 animals that developed lung tumours after receiving 1.25 mg/kg bw sodium dichromate weekly 12 had adenomas and eight had malignant lung tumours, described as two adenocarcinomas (bronchioalveolar) and six squamous-cell carcinomas. The authors noted that two of the tumours were questionable and that the majority of the observed lung tumours were small, non-metastasizing, non-fatal and co-existed with scarring and other treatment-related inflammatory changes not seen in animals treated five times a week with lower doses. In the groups receiving calcium chromate, similar findings were made, with a total of six lung tumour-bearing rats (five adenomas ($p < 0.01$) and one squamous-cell carcinoma) in the group receiving 0.25 mg/kg bw five times a week, and 13 lung-tumour-bearing rats (11 adenomas ($p < 0.01$) and three with two squamous-cell carcinomas and one adenocarcinoma ($p < 0.01$)) in the group receiving 1.25 mg/kg bw once a week. [The Working Group assumed that one rat had both an adenoma and a squamous-cell carcinoma.] The authors noted that one of the squamous-cell carcinomas may have been a metastasis from a primary tumour of the jaw (Steinhoff *et al.*, 1986).

Hamster: Groups of 35 male Syrian golden hamsters, about six weeks old, received weekly intratracheal instillations of 0.1 mg calcium chromate in 0.2 ml saline for 56 weeks and were maintained for a further 44 weeks. No lung tumour was reported (Reuzel *et al.*, 1986).

Guinea-pig: Groups of 21 or 13 guinea-pigs [sex and strain unspecified], three months of age, received six intratracheal instillations of 0.3 ml of a 1% suspension in saline of 3 mg zinc chromate as basic potassium zinc chromate (Baetjer *et al.*, 1959b) or 3 mg lead chromate, at three-monthly intervals. The animals were observed until death. A single pulmonary adenoma was seen in the group given zinc chromate, but no pulmonary carcinoma. No pulmonary adenoma was seen in the lead chromate group or in 18 vehicle controls (Steffee & Baetjer, 1965). [The Working Group noted the limited reporting of the study.]

Rabbit: Groups of seven rabbits [sex and strain unspecified], four months of age, received three to five intratracheal instillations of 1 ml of a suspension in saline of 1% (10 mg) zinc chromate (basic potassium zinc chromate, Baetjer *et al.*, 1959b) or lead chromate at three-monthly intervals. No lung tumour was reported in

Table 20. Protocol and results of test by intratracheal instillation of various chromium[VI] compounds to rats[a]

Compound	No. of animals		Dose (mg/kg bw)	Schedule	No. of lung tumours		Total no. of tumour-bearing animals
	Males	Females[b]			Benign	Malignant	
Sodium dichromate	40	50	0.25	5 × weekly	–	–	–
Sodium dichromate	40	45	0.05	5 × weekly	–	–	–
Sodium dichromate	40	45	0.01	5 × weekly	–	–	–
Sodium dichromate	40	40	1.25	1 × weekly	12*	8*	14
Sodium dichromate	40	40	0.25	1 × weekly	–	1	1
Sodium dichromate	40	40	0.05	1 × weekly	–	–	–
Calcium chromate	40	50	0.25	5 × weekly	5*	1	6
Calcium chromate	40	40	1.25	1 × weekly	11*	3*	13
Benzo[*a*]pyrene	0	10	5.0	1 × weekly			
Dimethyl carbamoyl chloride	10	10	1.0	1 × weekly			
Sodium chloride (0.9%)	40	50	1 ml/kg	5 × weekly	–	–	–
Sodium chloride (0.9%)	40	40	1 ml/kg	1 × weekly	–	–	–
Untreated	40	50	–	–	–	–	–

[a]From Steinhoff *et al.* (1986)

[b]Only 40 females used for carcinogenicity tests (five to ten extra in test groups)

*Significant at $p < 0.01$

treated animals or in five saline-treated controls (Steffee & Baetjer, 1965). [The Working Group noted the limited reporting of the study.]

(iii) *Intrabronchial administration*

Rat: A group of 100 rats [strain, sex and age unspecified] received intrabronchial implantations of stainless-steel mesh pellets (5 × 1 mm) loaded with 3-5 mg of a 50:50 mixture of calcium chromate with a cholesterol binder. Six squamous-cell carcinomas and two adenocarcinomas of the lung were found in animals observed up to 136 weeks. The median time to appearance of tumours was 540 days. A group of 100 rats similarly treated with chromium trioxide and observed up to 136 weeks had no such tumour, nor did 24 controls treated with cholesterol binder (Laskin *et al*., 1970). [Although the incidence of lung tumours was not statistically significant, the Working Group noted the probable biological significance of these tumours.]

Groups of approximately 50 male and 50 female Porton-Wistar rats, six to eight weeks old, received intrabronchial implantations into the left lung of stainless-steel mesh pellets (5 × 1 mm) loaded with about 2 mg of a series of chromium-containing test materials suspended 50:50 in cholesterol. Groups of approximately 75 male and 75 female rats receiving blank pellets or pellets loaded with cholesterol alone acted as negative controls. Animals were maintained for 24 months, at which time the study was terminated and all lungs and abnormal tissues examined. [The Working Group noted that survival was not reported]. No lung tumour was seen in either control group or among rats receiving pellets loaded with sodium dichromate (99-100% pure) or sodium chromate (98-99% pure); a single squamous-cell carcinoma of the left lung was seen in the group treated with chromic acid (chromium trioxide; 99-100% pure) and eight squamous-cell carcinomas ($p < 0.05$) of the left lung in the group treated with calcium chromate (95% pure). There was a significant increase in the incidence of bronchial squamous metaplasia of the left lung in rats without lung tumours in all treatment groups when compared to the groups receiving cholesterol or a blank pellet. Of animals that received intrabronchial pellets loaded with zinc potassium chromate ($K_2CrO_4.3ZnCrO_4.Zn(OH)_2$, 99-100% pure) suspended in cholesterol, 3/61 developed squamous-cell carcinomas of the left lung (Levy & Venitt, 1986).

In a second study using the techniques and protocol described above, the incidences of squamous-cell carcinomas of the left lung in groups of animals given a range of lead chromates, zinc chromates and strontium chromates were as shown in Table 21. Significance was calculated by comparing the incidence of bronchial carcinomas in each test group with that in a reference group comprising the two negative control groups and all groups treated with chromium-containing materials. Survival was 96% at 400 days and 54% at 700 days. Calcium chromate (96.7% pure), included as a positive control, induced 25/100 left-lung bronchial carcinomas

(24 squamous-cell carcinomas and one adenocarcinoma); chromium trioxide (99.9% pure) induced 2/100 left-lung bronchial carcinomas (one squamous-cell carcinoma and one anaplastic carcinoma); sodium dichromate dihydrate (TSS 612; 99.7% pure) gave 1/100 left-lung squamous-cell carcinoma; and a residue material (vanadium solids) from the bichromate production process, containing 5.3% calcium chromate and 17.2% sodium dichromate, induced 1/100 squamous-cell carcinoma of the left lung. No bronchial carcinoma was seen in the 100 rats given cholesterol alone; and 22/48 bronchial carcinomas (21 squamous-cell carcinomas and one anaplastic carcinoma) were seen in a positive control group receiving 20-methylcholanthrene (Levy *et al.*, 1986).

Table 21. Incidence of bronchial carcinomas in rats administered various chromium[VI] compounds by intrabronchial implantation into the left lung[a]

Compound	Composition	Incidence of bronchial carcinomas
Lead chromates		
Lead chromate (99.8% pure)	Pb, 64%; CrO_4, 35.8%	1/98
Primrose chrome yellow	Pb, 62.1%; Cr, 12.6%	1/100
Molybdate chrome orange[b]		0/100
Light chrome yellow	Pb, 62.1%; Cr, 12.5%	0/100
Supra (70 FS) LD chrome yellow	PbO, 61.5%; CrO_3, 26.9%	1/100
Medium chrome yellow	Pb, 60.2%; Cr, 16.3%	1/100
Silica encapsulated medium chrome yellow	Pb, 40.4%; Cr, 10.5%	0/100
Barium chromate (98% pure)	Ba, 54.1%; CrO_4, 42.1%	0/101
Zinc chromate IW (low water solubility)	ZnO, 39.4%; CrO_3, 40.8%	5/100[c] [$p = 0.004$]
Zinc chromate (Norge composition)	ZnO, 39.2%; CrO_3, 43.5%	3/100[c] [$p = 0.068$]
Zinc tetroxychromate	Zn, 56.6%; Cr, 8.8%	1/100
Strontium chromate	Sr, 42.2%; CrO_4, 54.1%	43/99
Strontium chromate	Sr, 43.0%; Cr, 24.3%	62/99
Cholesterol control		0/100
20-Methylcholanthrene control		22/48

[a]From Levy *et al.* (1986)

[b]Composition incompletely described by Levy *et al.* (1986); it is a mixture of lead chromate, lead sulfate and lead molybdate (Pb, 62.9%; Cr, 12.9%; Mo, 4.2%).

[c]Significance for each treatment group based on a reference group composed of a combination of the negative control group and all groups treated with chromate-containing materials, except those treated with calcium or strontium chromate

(iv) *Intrapleural administration*

Rat: A number of chromium[VI] compounds [doses unspecified] administered to rats [sex, age and strain unspecified] by intrapleural implantation in experiments lasting 27 months gave the following numbers of implantation-site tumours [type unspecified]: strontium chromate, 17/28 (nine alive at one year); barium chromate, 1/31 (30 alive at one year); lead chromate, 3/34 (32 alive at one year); zinc yellow [unspecified composition], 22/33 (11 alive at one year); calcium chromate, 20/32 (none alive at one year); sintered calcium chromate, 17/33 (nine alive at one year, one alive at 21 months); and sodium dichromate, 0/26 (20 alive at one year, none alive at 18 months). None of 34 control rats had tumours (30 alive at one year, five alive at two years) (Hueper, 1961).

Groups of 20 male and 19 female Bethesda Black [NIH Black] rats, three months of age, received 16 monthly intrapleural injections of 2 mg sodium dichromate in gelatin and were observed for up to two years. One adenocarcinoma of the lung was observed; no tumour at the injection site was observed in 60 control rats treated with gelatin solution. After intrapleural implantation of 12.5 mg calcium chromate in a gelatin capsule to 14 rats, eight developed malignant tumours [type unspecified] at the site of implantation after two years compared with none in 35 controls (Hueper & Payne, 1962).

(v) *Subcutaneous administration*

Mouse: A group of 26 female and 26 male C57Bl mice received calcium chromate or sintered calcium chromate (prepared by heating calcium chromate with less than 1% impurities to about 1100°C for about 1 h) by subcutaneous injection of 10 mg chromium compound in tricaprylin, and the animals were observed for 18-26 months. One sarcoma was observed in 13 mice treated with calcium chromate that lived longer than six months, but none was seen at the injection site in the other treated groups or in vehicle controls. Histologically, the injection-site tumours were spindle-cell sarcomas or fibrosarcomas (Payne, 1960a).

Rat: Groups of 40 male and female Sprague-Dawley rats, 13 weeks of age, received a single subcutaneous injection of 30 mg lead chromate (chromium yellow) or basic lead chromate (chromium orange) in water. Sarcomas (rhabdomyosarcomas and fibrosarcomas) developed at the injection site in 26/40 and 27/40 animals, respectively, within 117-150 weeks. No local tumour occurred in 60 vehicle-control rats, and a single local sarcoma occurred in 80 control rats that received comparable subcutaneous injections of iron yellow or iron red (Maltoni, 1974, 1976; Maltoni *et al.*, 1982).

Groups of 20 male and 20 female Sprague-Dawley rats, 13 weeks of age, received single subcutaneous injections of 30 mg zinc yellow (basic zinc chromate) at 20% or 40% CrO_3 in 1 ml saline. Local sarcomas (rhabdomyosarcomas and fibrosarcomas) were seen in 6/40 and 7/40 rats given 20% and 40% CrO_3 at 110 and 137 weeks, respectively. No local tumour had occurred in the 40 control animals by 136 weeks (Maltoni *et al.*, 1982).

A further group of 20 male and 20 female Sprague-Dawley rats received single subcutaneous injections of 30 mg molybdenum orange (described as a mixture of lead chromate, sulfate and molybdate) in 1 ml saline. At termination of the study at 117 weeks, 36/40 rats had injection-site sarcomas; no local tumour occurred in 45 male and 15 female untreated controls (Maltoni, 1974; Maltoni *et al.*, 1982).

(vi) *Intramuscular administration*

Mouse: Groups of 26 female and 26 male C57Bl mice [age unspecified] received calcium chromate or sintered calcium chromate (prepared by heating calcium chromate with less than 1% impurities to about 1100 °C for about 1 h) by intramuscular implantation of 10 mg of the chromium compound mixed with 20 mg sheep fat. Animals were observed for a total of 14 months. Nine implantation-site sarcomas were observed among 46 mice given sintered calcium chromate that lived longer than six months; one sarcoma was observed in 50 mice given non-sintered calcium chromate; and no sarcoma was found among 50 control mice that lived six months or more (Payne, 1960a).

A group of 25 female NIH-Swiss weanling mice was given intramuscular injections of 3 mg lead chromate in trioctanoin every four months. Two lymphomas were seen within 16 months and three lung adenocarcinomas within 24 months among 17 mice that were necropsied. The incidences of these tumours were 1/15 and 1/15 in untreated control mice and 2/22 and 1/22 among vehicle-injected control mice (Furst *et al.*, 1976).

Rat: Groups of 35 Bethesda Black [NIH Black] male and female rats, approximately three months of age, received an intramuscular implantation of pellets containing 25 mg calcium chromate (99% pure), 25 mg sintered calcium chromate or 25 mg sintered chromium trioxide, all in 50 mg sheep fat. Sarcomas (spindle-cell sarcomas and fibrosarcomas) at the implantation site were seen after 12-14 months in 8/35 rats given calcium chromate, 8/35 given sintered calcium chromate and 15/35 given sintered chromium trioxide. No local tumour was seen in 35 controls or in groups of 20 males and 15 females given implants of 25 mg barium chromate (99% pure) in 50 mg sheep fat (Hueper & Payne, 1959). [The Working Group noted that sintered chromium trioxide at 1100 °C would contain appreciable amounts of chromic chromate; it also noted the short duration of the experiment.]

In groups of 32-34 rats [sex, strain and age unspecified] that received intramuscular implantation of various chromium compounds in sheep fat [doses unspecified], the following incidences of implantation-site tumours [type unspecified] were recorded after 27 months: calcium chromate, 9/32 (22 alive at one year, seven alive at two years); sodium dichromate, 0/33 (25 alive at one year, 16 alive at 18 months); sintered calcium chromate, 12/34 (22 alive at one year, none alive at two years); strontium chromate, 15/33 (20 alive at one year); barium chromate, 0/34 (30 alive at one year); lead chromate, 1/33 (28 alive at one year); and zinc yellow [composition unspecified], 16/34 (22 alive at one year). None of 32 control rats given implants of sheep fat alone developed local tumours (30 alive at one year, six alive at two years) (Hueper, 1961).

A group of 20 male and 19 female Bethesda Black [NIH Black] rats, three months of age, was given 16 intramuscular injections of 2 mg sodium dichromate in gelatin at monthly intervals and observed for two years (17 alive at 16 months). No tumour appeared at the injection site. After intramuscular implantation of 12.5 mg calcium chromate in a gelatin capsule to eight rats, four malignant tumours developed at the implantation site in animals observed for two years; compared with none in 35 controls (Hueper & Payne, 1962).

Each of a group of 24 male CB stock rats, five to six weeks of age, was given an intramuscular injection of calcium chromate in arachis oil (total dose, 19 mg) once a week for 20 weeks. Eighteen developed spindle-cell or pleomorphic-cell sarcomas at the injection site, none of which metastasized [$p < 0.01$]. The mean time to tumour appearance was 323 days (duration of experiment, 440 days). No tumour developed in 15 control rats given arachis oil only that were alive at 150 days (Roe & Carter, 1969).

Groups of 25 male and 25 female weanling Fischer-344 rats received intramuscular injections of 8 mg lead chromate suspended in trioctanoin once a month for nine months or 4 mg calcium chromate in the same vehicle once a month for 12 months. Lead chromate induced 14 fibrosarcomas and 17 rhabdomyosarcomas at the site of injection in 31/47 rats. In addition, renal carcinomas were observed in 3/23 male rats at 24 months. Calcium chromate induced tumours (three fibrosarcomas, two rhabdomyosarcomas) in 5/45 animals. No such tumour appeared in a group of 22 controls injected with the vehicle (Furst *et al.*, 1976). [The Working Group noted that the renal tumours might be attributable to the lead content of the compound (IARC, 1980b)].

(*d*) *Other chromium compounds*

(i) *Inhalation*

Mouse: Groups of mice, eight to ten weeks of age, were exposed in dust chambers for 4 h per day on five days per week to a mixed chromium dust[1] containing 1-2 mg/m³ soluble chromium (as chromium trioxide) until they died or were killed (total dose of chromium trioxide inhaled, 272-1330 mg-h): 127 Swiss females were exposed for up to 58 weeks, ten Swiss males and 11 Swiss females for up to 39 weeks, 34 strain A females for 16 weeks, 45 strain A females for 24 weeks, 110 strain A females for 38 weeks, 52 strain A males for 46 weeks, 50 C57Bl males for 42 weeks and 61 C57Bl females for 41 weeks. No lung carcinoma was observed, and the incidence of lung adenomas did not significantly exceed that in control mice in any strain. The experiment lasted for up to 101 weeks (Baetjer *et al.*, 1959b). [The Working Group noted the low doses and the small numbers of animals used.]

Rat: A group of 78 Wistar rats [sex unspecified], six to eight weeks of age, was exposed by inhalation to a mixed chromium dust[1] for 4-5 h per day on four days a week for life, to give an average chromium trioxide concentration of 3-4 mg/m³. No significant difference in tumour incidence was observed between treated and control groups. A group of 38 Sherman rats [sex unspecified], six to eight weeks of age, received 16 monthly intratracheal injections of 0.1 ml of a suspension consisting of 0.5% mixed chromium dust plus 0.6% potassium dichromate, equivalent to 0.07 mg chromium/dose. No lung tumour occurred (Steffee & Baetjer, 1965).

A group of 20 male TNO-W74 Wistar rats, six weeks of age, was exposed by inhalation to 100 μg/m³ pyrolysed Cr[VI]/Cr[III] oxides (3:2; average mass median diameter, 0.39 μm) for 22-23 h per day on seven days a week for 18 months. The rats were then held for a further 12 months. A control group consisted of 40 untreated male rats. Survival was over 90% at 24 months and at 30 months was 50% and 42% in treated and controls, respectively. A single lung adenoma was found in the treated group and none in the controls (Glaser *et al.*, 1986). [The Working Group noted the small number of animals used].

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[1]The Working Group noted that, in the publications of Baetjer *et al.*, the mixed chromium dust used was prepared by grinding to a fine powder the roast that is produced when chromite ore is heated at a high temperature with sodium carbonate and calcium hydroxide. The mixture contained approximately 12% chromium, consisting of water-soluble sodium chromate (chromium[VI]), water-insoluble but acid-soluble chromium[VI] and [III] chemicals and some unchanged chromite ore; to this mixture was added 1% potassium bichhromate. The final analysis of the dust gave 13.7% chromium trioxide and 6.9% chromic oxide. The Working Group commented that this roasted mixture, known as 'frit', is the product of the first stage of the bichromate production process prior to leaching. This first-stage process may or may not involve the addition of calcium hydroxide or limestone.

Groups of 120 male and 120 female Sprague-Dawley rats, six to seven weeks of age, were exposed by inhalation to 0.5 mg/m^3 'unstabilized', 0.5 or 25 mg/m^3 'stabilized' chromium[IV] dioxide particles (mass median aerodynamic diameter, 2.6-2.8 μm) for 6 h a day on five days per week for two years. Ten rats from each group were killed at 12 months for interim observation. Between 101 and 108 lungs were examined for each group exposed up to 24 months. [The Working Group noted that no survival data were reported.] In the 25 mg/m^3 stabilized group, there was treatment-related alveolar bronchiolization. In addition, lung adenomas occurred in 1/106 males and 1/108 females in this group, and keratin cysts and 'cystic keratinized squamous-cell carcinomas' in 108 females. The authors considered that the cystic keratinized squamous-cell carcinomas were related to a dust reaction alone and were not true malignant tumours (Lee *et al.*, 1988). [The Working Group noted that similar lesions of the lung were described in a previous *IARC Monograph* on titanium dioxide (IARC, 1989).]

Guinea-pig: Three-month-old guinea-pigs [sex unspecified] were exposed by inhalation to a combination of mixed chromium dust[1] for 4-5 h per day on four days per week for lifespan (average dose, 3-4 mg/m^3 chromic trioxide); 3/50 developed pulmonary adenomas. No pulmonary adenoma occurred in 44 controls (Steffee & Baetjer, 1965).

Rabbit: Eight rabbits [sex and strain unspecified], four months of age, were exposed by inhalation for 4-5 h per day on four days per week for up to 50 months to mixed chromium dust[1] according to a complex dosage schedule (average dose, 3-4 mg/m^3 chromium trioxide). No pulmonary tumour was seen (Steffee & Baetjer, 1965).

(ii) *Intratracheal instillation*

Mouse: Five to six intratracheal instillations of a mixed chromium dust[1], equivalent to 0.04 mg chromium trioxide per instillation, were given either to 14 and 20 Swiss males, which were then observed for 26 and 32 weeks, respectively; to 45 and 110 Swiss females, observed for up to 32 and 48 weeks, respectively; to 28, 52, 77 and 48 strain A females observed for up to 31, 37, 43 and 52 weeks respectively; to 17 strain A males observed for up to 52 weeks; or to 48 C57Bl males and 47 C57Bl females observed for up to 32 weeks. Treated animals developed no more lung tumours than did untreated control animals (Baetjer *et al.*, 1959b).

Guinea-pig: Groups of 19 guinea-pigs [sex unspecified], three months old, were given six intratracheal instillations of 0.3 ml of a 1% suspension in saline of a mixed chromium dust[1] or a pulverized residue dust (roast material from which solu-

[1]See footnote on p. 130

ble chromates had been leached) at intervals of three months. The animals were observed until they died. No pulmonary carcinoma developed in any experimental group or in 18 vehicle controls (Steffee & Baetjer, 1965).

Rabbit: Groups of rabbits received three to five intratracheal injections of 1 ml of a 1% suspension in saline of mixed chromium dust (ten rabbits) or 'pulverized residue dust' (roast material from which soluble chromates had been leached) (seven rabbits) at intervals of three months. No pulmonary tumour was seen in either group (Steffee & Baetjer, 1965).

(iii) *Intrabronchial administration*

Rat: A group of 100 rats [strain, sex and age unspecified] received intrabronchial implants of stainless-steel mesh pellets (5 × 1 mm) loaded with 3-5 mg of a 50:50 mixture of a chromate process residue (an intermediate process residue from the bichromate-producing industry, which may have contained up to 3% calcium) with a cholesterol binder. Animals were observed up to 136 weeks. One squamous-cell carcinoma was observed after 594 days in 1/93 rats that lived more than 150 days. No lung tumour was seen in 24 cholesterol binder-treated controls (Laskin *et al.*, 1970).

In a study using intrabronchial implantation, described previously (p. 125), no bronchial tumour was seen in five groups of 100 Porton-Wistar rats that received pellets loaded with five residues from bichromate production: Bolton high lime residue, residue after alumina precipitation, residue from slurry tank (free of soluble chromium), residue from vanadium filter and residue from slurry disposal tank. All five materials contained less than 5% hexavalent chromium (Levy & Venitt, 1986; Levy *et al.*, 1986).

In a second study using the technique described above, but examining bichromate production residues containing lime, the following incidences of squamous-cell carcinomas of the left lung were seen: high lime residue from old tip (TSS 643D; Cr_2O_3, 2.4%; $CaCrO_4$, 2.7%; Na_2CrO_4, 1.0%), 1/99; kiln frit (TSS 643B, with 2% limestone added to feedmix; Cr_2O_3, 13.0%; Na_2CrO_4, 29.0%), 2/100; and recycled residue (TSS 643C, with 2% limestone added to feedmix; Cr_2O_3, 20.4%; Na_2CrO_4, 2.2%), 0/100 (Levy *et al.*, 1986).

(iv) *Intrapleural administration*

Mouse: Groups of 30 male and 25 female strain A mice, eight to ten weeks of age, were given four intrapleural injections of 0.05 ml of a 2 or 4% suspension of mixed chromium dust[1] in olive oil at intervals of four to six weeks. The incidence of

[1]See footnote on p. 130.

lung tumours during an observation period of 38 weeks was similar to that in a control group of 23 males and 18 females (Baetjer *et al.*, 1959b).

Rat: A group of 25 male Bethesda Black [NIH Black] rats, three months of age, received sheep-fat cubes containign 25 mg roasted chromite ore implanted into the pleural cavity. Squamous-cell carcinomas of the lungs were observed in 2/24 rats that survived 19-24 months. One lung adenoma occurred in the 4/15 female controls given an implant of sheep fat that survived this period (Hueper, 1958). [The Working Group noted that the roasted chromite ore tested in this study was a process-derived material that contained unspeciated chromium compounds formed during oxidative heating of a chromium ore that had been subjected to alkaline leaching. Hueper sometimes referred to this material as 'chromate' and sometimes as 'chromite'.]

Of a group of 32 rats [age, sex and strain unspecified] that received intrapleural implantations of chromite roast residue [amount unspecified], 5/32 (28 alive at one year, ten alive at 24 months) developed malignant tumours at the implantation site. In a group of 34 rats given chromic chromate [precise chemical nature unspecified], 25 tumours developed at the implantation site. None of 34 control rats had a tumour (30 alive at one year, five alive at 24 months) (Hueper, 1961).

When 25 mg roasted chromite ore in 50 mg sheep fat (equivalent to 2 mg Cr) were implanted intrapleurally into 15 male and 20 female Bethesda Black rats [age unspecified], implantation-site sarcomas occurred in three rats over 17 months. No tumour was seen in 35 rats injected intrapleurally with the sheep-fat vehicle only (Payne, 1960b)

(v) *Intramuscular administration*

Mouse: A group of 26 male and 26 female C57Bl mice [age unspecified] was given intramuscular implantations of 10 mg roasted chromite ore (equivalent to 0.79 mg chromium) in sheep fat. None developed tumours at the implantation site within 22 months. No local tumour developed in 52 controls treated with sheep fat alone (Payne, 1960b). [The Working Group noted that no data on survival were reported.]

Rat: A group of 31 female Bethesda Black [NIH Black] rats, approximately three months old, was given intramuscular implants of small cubes composed of 25 mg roasted chromite ore suspended in 75 mg sheep fat. Three rats developed fibrosarcomas at the site of implantation within 24 months. No implantation-site tumour occurred in 15 vehicle-treated controls (Hueper, 1958).

In a group of 34 rats [age, sex and strain unspecified] that received intramuscular implantations of chromite roast residue, 1/34 (32 alive at one year, two alive at 27 months) developed a malignant tumour at the injection site [type unspecified]. In a further group of 22 rats given intramuscular implantations of chromic chromate [precise chemical nature unspecified], 24 local tumours were observed after 24

months. None of 32 controls given implants of sheep fat alone developed local tumours (30 alive at one year, six alive at 24 months) (Hueper, 1961).

(vi) *Injections into subcutaneously implanted tracheal grafts*

Rat: Seventy-two tracheal rings excised from female Wistar-Lewis rats were implanted subcutaneously into the backs of 13 rats of the same strain (weighing 100-150 g at the start of the experiment). Two weeks later, the grafts were filled by injection with 0.05 ml of an agar suspension of 2.5 mg chromium carbonyl with or without 2.5 mg benzo[*a*]pyrene. Biopsies were performed at intervals. Ten squamous-cell carcinomas developed in 24 tracheas that received the mixture, and two carcinomas developed in 22 tracheas treated with chromium carbonyl alone. Three of the tracheal carcinomas produced by the mixture metastasized within nine months. The time to appearance of the tumours was four to 14 months. No tumour occurred in the four trachea that received the vehicle only (Lane & Mass, 1977).

The experiments described in section 3.1 are summarized in Table 22, by compound.

Table 22. Summary of studies used to evaluate the carcinogenicity to experimental animals of metallic chromium and chromium compounds

Compound	Route	Species (No. at start)	Tumour incidence[a]	Reference
Metallic chromium				
Chromium	Intratracheal	Rat (53)	0/12	Mukubo (1978)
Chromium	Intrapleural	Mouse (50)	0/50	Hueper (1955)
Chromium	Intrapleural	Rat/2 groups (25; 25)	A few tumours, also in controls	Hueper (1955)
Chromium	Intramuscular	Rat (24)	0/22 local tumour	Sunderman *et al.* (1974)
Chromium	Intramuscular	Rat (38)	0/38 local tumour	Sunderman *et al.* (1980)
Chromium	Intramuscular	Rat (50)	1/50 *vs* 0/50	Furst (1971)
Chromium	Intraperitoneal	Mouse (50)	0/50 local tumour	Hueper (1955)
Chromium	Intraperitoneal	Rat (25)	5/25 (mixed)	Hueper (1955)
Chromium	Intravenous	Mouse (25)	0/25	Hueper (1955)
Chromium	Intravenous	Rat (25)	6/25 (mixed)	Hueper (1955)
Chromium	Intravenous	Rabbit (8)	1/3 *vs* 0/4	Hueper (1955)
Chromium	Intrafemoral	Rat/2 groups (25; 25)	0/25; 1/25 local tumour	Hueper (1955)

Table 22 (contd)

Compound	Route	Species (No. at start)	Tumour incidence[a]	Reference
Chromium[III] compounds				
Chromic acetate	Drinking-water	Mouse (108)	M 6/39 *vs* 11/44 F 9/29 *vs* 22/60	Schroeder *et al.* (1964)
Chromic acetate	Drinking-water	Rat (96)	M 16/39 *vs* 9/35 F 18/35 *vs* 15/35	Schroeder *et al.* (1965)
Chromic acetate	Intrapleural	Rat (34)	1/34 *vs* 0/34	Hueper (1961)
Chromic acetate	Intrapleural	Rat	0/42 local tumour	Hueper & Payne (1962)
Chromic acetate	Intramuscular	Rat (34)	1/34 *vs* 0/32 local tumour	Hueper (1961)
Chromic acetate	Intramuscular	Rat (35)	1/35 local tumour	Hueper & Payne (1962)
Chromic oxide	Oral (in bread)	Rat (3 groups of 60)	As controls	Ivankovic & Preussman (1975)
Chromic oxide	Intratracheal	Rat (?)	Malignant lung tumours 7/34 (50 mg) and 6/18 (20 mg)	Dvizhkov & Fedorova (1967)
Chromic oxide	Intrabronchial	Rat (98)	0/98 *vs* 0/24	Laskin *et al.* (1970)
Chromic oxide	Intrabronchial	Rat (100)	0/100 *vs* reference[b]	Levy & Venitt (1986)
Chromic oxide	Intraperitoneal	Rat (?)	Lung sarcomas 4/20	Dvizhkov & Fedorova (1967)
Chromic chloride hexahydrate	Intrabronchial	Rat (100)	0/100 *vs* reference[b]	Levy & Venitt (1986)
Chromic chloride	Drinking-water	Rat (15)	0/15	Kurokawa *et al.* (1985)
Chrome tan	Intrabronchial	Rat (100)	0/100 *vs* reference[b]	Levy & Venitt (1986)
Chromic sulfate	Intraperitoneal	Mouse (10 per group; 3 dose levels)	As controls	Stoner *et al.* (1976)
Chromite	Intrabronchial	Rat (100)	0/100 *vs* reference[b]	Levy & Venitt (1986)

Table 22 (contd)

Compound	Route	Species (No. at start)	Tumour incidence[a]	Reference
Chromite (high silica chrome ore TSS 645)	Intrabronchial	Rat	0/99 *vs* reference[b]	Levy *et al.* (1986)
Chromite	Intrapleural	Mouse (25)	0/25	Davis (1972)
Chromite	Intrapleural	Rat (25)	1/25 *vs* 0/25	Hueper (1955)
Chromite	Intravenous	Mouse (50)	As controls	Shimkin & Leiter (1940)
Chromite	Intravenous	Rabbit (6)	0/6	Hueper (1955)
Chromite	Intrafemoral	Rat (25)	0/25 local tumour	Hueper (1955)
Chromium[VI] compounds				
Calcium chromate	Inhalation	Mouse (136)	Lung adenomas M 6/136 *vs* 3/136 F 8/136 *vs* 2/136	Nettesheim *et al.* (1971)
Calcium chromate	Intrabronchial	Rat (100)	Bronchial carcinomas 8/100 *vs* 0/24 (NS)	Laskin *et al.* (1970)
Calcium chromate	Intrabronchial	Rat (100)	Squamous-cell carcinomas 8/84 *vs* reference[b] $p < 0.05$	Levy & Venitt (1986)
Calcium chromate	Intrabronchial	Rat (100)	Bronchial carcinomas 25/100 ($p < 0.01$) positive control	Levy *et al.* (1986)
Calcium chromate	Intratracheal	Rat (80)	Lung: 5 x weekly; 0.25 mg/kg 6/80 *vs* 0/80 ($p < 0.01$) Lung: 1 x weekly; 1.25 mg/kg 13/80 *vs* 0/80[c] ($p < 0.01$)	Steinhoff *et al.* (1986)
Calcium chromate	Intratracheal	Hamster (35)	No lung tumour	Reuzel *et al.* (1986)
Calcium chromate	Intramuscular	Mouse (52)	1/50 *vs* 0/50 local tumour (NS)	Payne (1960a)
Calcium chromate sintered	Intramuscular	Mouse (52)	9/46 *vs* 0/50 local tumours [$p < 0.01$)]	Payne (1960a)
Calcium chromate	Intramuscular	Rat	9/32 *vs* 0.32 local tumours [$p < 0.01$]	Hueper (1961)
Calcium chromate sintered	Intramuscular	Rat	12/34 *vs* 0/32 local tumours [$p < 0.01$]	Hueper (1961)

Table 22 (contd)

Compound	Route	Species (No. at start)	Tumour incidence[a]	Reference
Calcium chromate	Intramuscular	Rat (50)	5/45 *vs* 0/22 local tumours (NS)	Furst *et al.* (1976)
Calcium chromate	Intramuscular	Rat (8)	4/8 *vs* 0/35 local tumours	Hueper & Payne (1962)
Calcium chromate	Intramuscular	Rat (24)	18/24 *vs* 0/15 local tumours [$p < 0.01$]	Roe & Carter (1969)
Calcium chromate	Intramuscular	Rat (35)	8/35 *vs* 0/35 [$p < 0.01$]	Hueper & Payne (1959)
Calcium chromate sintered	Intramuscular	Rat (35)	8/35 *vs* 0/35 [$p < 0.01$]	Hueper & Payne (1959)
Calcium chromate	Intraperitoneal	Rat (14)	8/14 *vs* 0/35 local tumours	Hueper & Payne (1962)
Calcium chromate	Intraperitoneal	Rat (?)	20/32 *vs* 0/34 [$p < 0.01$]	Hueper (1961)
Calcium chromate sintered	Intraperitoneal	Rat (?)	17/33 *vs* 0/34 [$p < 0.01$]	Hueper (1961)
Calcium chromate	Subcutanous	Mouse (52)	1/13 *vs* 0/52 (NS)	Payne (1960a)
Calcium chromate sintered	Subcutaneous	Mouse (52)	0/31 *vs* 0/52	Payne (1960a)
Chromic acid (chromium trioxide)	Inhalation	Mouse (50)	Lung adenomas, 10-14 months: 3/14 *vs* 0/10 (NS) Adenomas, 15-18 months: 1/19 *vs* 2/10 Adenocarcinoma: 2/19 *vs* 0/10 (NS)	Adachi *et al.* (1986)
Chromic acid (chromium trioxide)	Inhalation	Mouse (43)	Nasal papilloma, 18 months: 6/20 *vs* 0/20 ($p < 0.05$); 1/20 adenoma of lung	Adachi (1987)
Chromic acid (chromium trioxide)	Intrabronchial	Rat (100)	Squamous-cell carcinoma: *vs* reference[b] (NS)	Levy & Venitt (1986)
Chromic acid (chromium trioxide)	Intrabronchial	Rat (100)	Bronchial carcinoma: 2/100 *vs* 0/100 (NS)	Levy *et al.* (1986)
Chromic acid (chromium trioxide)	Intrabronchial	Rat (100)	Lung: 0/100 *vs* 0/24	Laskin *et al.* (1970)
Chromic oxide sintered	Intramuscular	Rat (35)	15/35 local tumours	Hueper & Payne (1959)

Table 22 (contd)

Compound	Route	Species (No. at start)	Tumour incidence[a]	Reference
Sodium dichromate	Inhalation	Rat (20 per group)	Lung tumours: controls, 0/37 25 μg, 0/18 50 μg, 0/18 100 μg, 3/19 (2 adenomas) 1 adenocarcinoma + 1 squamous-cell carcinoma of pharynx	Glaser *et al.* (1986)
Sodium dichromate	Intrabronchial	Rat (100)	0/89 *vs* reference[b]	Levy & Venitt (1986)
Sodium dichromate	Intrabronchial	Rat (100)	Bronchial carcinoma: 1/100 *vs* 0/100 (NS)	Levy *et al.* (1986)
Sodium dichromate	Intratracheal	Rat (80)	5 × weekly: 0/80 in all groups 1 × weekly: control, 0/80; 0.05 mg/kg, 0/80; 0.25 mg/kg, 1/80; 1.25 mg/kg, 14/80[c] ($p < 0.01$)	Steinhoff *et al.* (1986)
Sodium dichromate	Intrapleural	Rat (39)	Lung adenocarcinoma: 1/34	Hueper & Payne (1962)
Sodium dichromate	Intrapleural	Rat (?)	0/26 *vs* 0/34 local tumour	Hueper (1961)
Sodium dichromate	Intramuscular	Rat (39)	0/39 local tumour	Hueper & Payne (1962)
Sodium dichromate	Intramuscular	Rat	0/33 *vs* 0/32 local tumour	Hueper (1961)
Sodium chromate	Intrabronchial	Rat (100)	Lung: 0/89 *vs* reference[b]	Levy & Venitt (1986)
Bichromate residue (vanadium solids)	Intrabronchial	Rat (100)	Bronchial carcinoma: 1/100 *vs* reference[b]	Levy *et al.* (1986)
Zinc chromates				
Basic potassium zinc chromate	Intratracheal	Mouse (62)	Pulmonary adenomas: 31/62 *vs* 7/18	Steffee & Baetjer (1965)
Basic potassium zinc chromate	Intratracheal	Guinea-pig (21)	Pulmonary adenomas: 1/21 *vs* 0/18	Steffee & Baetjer (1965)

Table 22 (contd)

Compound	Route	Species (No. at start)	Tumour incidence[a]	Reference
Basic potassium zinc chromate	Intratracheal	Rabbit (7)	0/7 *vs* 0/5	Steffee & Baetjer (1965)
Zinc potassium chromate	Intrabronchial	Rat (100)	Squamous-cell carcinoma: 3/61 *vs* reference ($p < 0.05$)	Levy & Venitt (1986)
Zinc chromate (IW)	Intrabronchial	Rat (100)	Lung: 5/100 *vs* reference[b] [$p = 0.004$]	Levy *et al.* (1986)
Zinc chromate (Norge)	Intrabronchial	Rat (100)	3/100 *vs* reference[b] [p = 0.068] NS according to authors	Levy *et al.* (1986)
Zinc tetroxychromate	Intrabronchial	Rat (100)	1/100 *vs* reference[b] (NS)	Levy *et al.* (1986)
Zinc yellow	Intrapleural	Rat	22/33 *vs* 0/34	Hueper (1961)
Zinc yellow	Subcutaneous	Rat (40)	Local tumours: control, 0/40 20% CrO_3, 6/40 40% CrO_3, 7/40	Maltoni *et al.* (1982)
Zinc yellow	Intramuscular	Rat	16/34 *vs* 0/32	Hueper (1961)
Lead chromates				
Lead chromate	Intrabronchial	Rat (100)	Bronchial carcinoma: 1/98 *vs* 0/100 (NS)	Levy *et al.* (1986)
Primrose chrome yellow	Intrabronchial	Rat (100)	1/100 *vs* reference[b] (NS)	Levy *et al.* (1986)
Molybdate chrome orange	Intrabronchial	Rat (100)	0/100	Levy *et al.* (1986)
Molybdenum orange	Subcutaneous	Rat (40)	36/40 *vs* 0/60	Maltoni (1974); Maltoni *et al.* (1982)
Light chrome yellow	Intrabronchial	Rat (100)	0/100	Levy *et al.* (1986)
Supra LC chrome yellow	Intrabronchial	Rat (100)	1/100 *vs* reference[b] (NS)	Levy *et al.* (1986)
Medium chrome yellow	Intrabronchial	Rat (100)	1/100 *vs* reference[b]	Levy *et al.* (1986)
Silica encapsulated	Intrabronchial	Rat (100)	0/100 (NS)	Levy *et al.* (1986)
Lead chromate	Intratracheal	Guinea-pig (13)	0/13	Steffee & Baetjer (1965)

Table 22 (contd)

Compound	Route	Species (No. at start)	Tumour incidence[a]	Reference
Lead chromate	Intrapleural	Rat	3/34 *vs* 0/34	Hueper (1961)
Lead chromate	Intramuscular	Mouse (25)	Lymphoma, lung adenocarcinoma: not different from controls	Furst *et al.* (1976)
Lead chromate	Subcutaneous	Rat (40)	26/40 *vs* 0/60 and 1/80 local tumours	Maltoni (1974, 1976); Maltoni *et al.* (1982)
Basic lead chromate	Subcutaneous	Rat (40)	27/40 *vs* 0/60 and 1/80 local tumours	Maltoni (1974, 1976); Maltoni *et al.* (1982)
Lead chromate	Intramuscular	Rat (50)	31/47 *vs* 0/22 local tumours and 3/23 M *vs* 0/22 renal carcinomas	Furst *et al.* (1976)
Lead chromate	Intramuscular	Rat	1/33 *vs* 0/32 local tumour	Hueper (1961)
Barium chromate	Intrabronchial	Rat (101)	0/101	Levy *et al.* (1986)
Barium chromate	Intrapleural	Rat (?)	1/31 *vs* 0/34	Hueper (1961)
Barium chromate	Intramuscular	Rat (?)	0/34 *vs* 0/32 local tumour	Hueper (1961)
Barium chromate	Intramuscular	Rat (35)	0/35	Hueper & Payne (1959)
Strontium chromate	Intrabronchial	Rat (100)	Bronchial carcinoma: 43/99 *vs* reference[b]	Levy *et al.* (1986)
Strontium chromate	Intrabronchial	Rat (100)	Bronchial carcinoma: 64/99 *vs* reference[b]	Levy *et al.* (1986)
Strontium chromate	Intrapleural	Rat (?)	17/28 *vs* 0/34	Hueper (1961)
Strontium chromate	Intramuscular	Rat	15/33 *vs* 0/32 local tumour	Hueper (1961)
Other chromium compounds and chromium-containing mixtures				
Mixed chromium dust	Inhalation	Mouse (500)	As controls	Baetjer *et al.* (1959b)
Mixed chromium dust	Inhalation	Rat (78)	As controls	Steffee & Baetjer (1965)

Table 22 (contd)

Compound	Route	Species (No. at start)	Tumour incidence[a]	Reference
Mixed chromium dust	Inhalation	Guinea-pig (50)	Pulmonary adenomas: 3/50 *vs* 0/44	Steffee & Baetjer (1965)
Mixed chromium dust	Inhalation	Rabbit (8)	0/8 local tumour	Steffee & Baetjer (1965)
Mixed chromium dust	Intratracheal	Mouse (506)	As controls	Baetjer *et al.* (1959b)
Mixed chromium dust	Intratracheal	Guinea-pig (19)	0/19 *vs* 0/18	Steffee & Baetjer (1965)
Mixed chromium dust plus $K_2Cr_2O_4$	Intratracheal	Rat (38)	0/38 local tumour	Steffee & Baetjer (1965)
Mixed chromium dust	Intratracheal	Rabbit (10)	0/10 local tumour	Steffee & Baetjer (1965)
Mixed chromium dust	Intrapleural	Mouse (55)	As controls	Baetjer *et al.* (1959b)
Residue dust	Intratracheal	Guinea-pig (19)	0/19 *vs* 0/18	Steffee & Baetjer (1965)
Residue dust	Intratracheal	Rabbit (7)	0/7 local tumour	Steffee & Baetjer (1965)
Roasted chromite ore	Intrapleural	Rat (25)	Bronchial carcinoma: 2/24	Hueper (1958)
Roasted chromite ore	Intrapleural	Rat (35)	3/35 *vs* 0/35	Payne (1960b)
Roasted chromite ore	Intramuscular	Mouse (52)	0/52 local tumour	Payne (1960b)
Roasted chromite ore	Intramuscular	Rat (31)	3/31 *vs* 0/15	Hueper (1958)
Roasted chromite residue	Intrapleural	Rat (32)	5/32 *vs* 0/34	Hueper (1961)
Roasted chromite residue	Intramuscular	Rat (34)	1/34 vs 0/32	Hueper (1961)
Chromate process residue	Intrabronchial	Rat (100)	1/93 *vs* 0/24	Laskin *et al.* (1970)

Table 22 (contd)

Compound	Route	Species (No. at start)	Tumour incidence[a]	Reference
Bichromate production residues (all with < 5% Cr[VI])	Intrabronchial			Levy & Venitt (1986); Levy *et al.* (1986)
Bolton high lime residue		Rat (100)	0/100	
Alumina precipitation residue		Rat (100)	0/100	
Slurry tank residue		Rat (100)	0/100	
Vanadium filter residue		Rat (100)	0/100	
Slurry disposal residue tank		Rat (100)	0/100	
Bichromate production residues with lime				
High lime residue (TSS 643D)	Intrabronchial	Rat (99)	Bronchial carcinoma: 1/99 (NS)	Levy *et al.* (1986)
Kiln frit (CTSS 643B) + 2% limestone	Intrabronchial	Rat (100)	2/100 (NS)	Levy *et al.* (1986)
Recycled residue (CTSS 643C) + 2% limestone	Intrabronchial	Rat (100)	0/100	Levy *et al.* (1986)
Pyrolysed Cr[VI]/Cr[III] 3:2 oxide	Inhalation	Rat (20)	Lung adenoma: 1/20 *vs* 0/40	Glaser *et al.* (1986)
Chromium[IV] dioxide	Inhalation	Rat		
Unstabilized (0.5 mg/m^3)		(240)	0/240 *vs* 0/240	Lee *et al.* (1988)
Stabilized (0.5 and 25 mg/m^3)		(480)	2/210 adenomas 6/108 keratin cysts 2/108 cystic keratin squamous lesions	

[a]NS, not significant

[b]*p*-Value calculated by comparing the incidence of bronchial carcinomas in each test group with that in a reference group comprising the two negative control groups and all the groups receiving chromium-containing materials

[c]No. of tumour-bearing animals

3.2 Other relevant data in experimental systems

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

The metabolism of chromium has been reviewed (Aitio *et al.*, 1988; Nieboer & Jusys, 1988; World Health Organization, 1988). De Flora and Wetterhahn (1990) have specifically reviewed the redox chemistry of chromium[VI] with respect to cellular metabolism; a metabolic model has been suggested by Elinder *et al.* (1988).

(i) *Metallic chromium and chromium alloys*

Chromium-cobalt alloys appear to release chromium[VI] after intramuscular implantations in rats (Wapner *et al.*, 1986). Chromium metal powder released chromium[VI] when incubated in aerated phosphate buffer, Ringer's solution, phosphate buffer with added bicarbonate and Locke's physiological buffer (Grogan, 1957).

(ii) *Chromium[III] compounds*

In contrast to chromium[VI] compounds, less than 1% of chromium[III] is absorbed from the gastrointestinal tract of animals (Mertz, 1969).

Four hours after intratracheal instillation of chromic chloride in rabbits, 85% of the chromium remained in the lungs and 8% was found in the urine; after uptake, chromium was confined mainly to plasma, and the peak concentration was reached after 20 min (Wiegand *et al.*, 1984a).

After exposure of rats by inhalation to chromic chloride particles (mass median aerodynamic diameter (MMAD), 1.8 and 1.5 μm; 19 and 27% less than 1 μm; 8-10.7 mg chromium/m^3), only one clearance phase was demonstrated, with a half-time of about 160 h (Suzuki *et al.*, 1984). As with chromium[VI] compounds, the highest organ concentrations in both rats and rabbits were found in the kidney and liver after exposure to chromic chloride by the pulmonary route, although the concentrations found were lower than those after a corresponding exposure to chromium[VI] (Suzuki *et al.*, 1984; Wiegand *et al.*, 1984a).

Chromium (especially trivalent chromium) strongly accumulated in the interstitial tissues of the gonads of male mice, but not in seminiferous epithelium (Danielsson *et al.*, 1984).

Little chromium[III] is taken up by cells (Aaseth *et al.*, 1982; Nieboer & Jusys, 1988), but more of some organic chromium[III] complexes may be taken up (Yamamoto *et al.*, 1981; Norseth *et al.*, 1982).

After parenteral administration of chromium[III] to rats (as with chromium[VI]), chromium is excreted predominantly in the urine (National Research Council, 1974; Langård, 1980, 1982). Less than 2% of an intravenous dose of chromic chloride was found in the faeces of rats 8 h after injection (Hopkins, 1965). In a

subsequent study, seven days after intraperitoneal injection of chromic chloride to mice, the cumulated amounts excreted in faeces and urine were about equal (Bryson & Goodall, 1983).

Studies on the mechanism of excretion of chromium[III] by the kidneys indicate that glomerular filtration is the major mechanism (Donaldson *et al.*, 1986).

As with chromium[VI], biliary excretion of chromic chloride has been demonstrated in rats (Cikrt & Bencko, 1979; Norseth *et al.*, 1982); less than 1% of an intravenously injected dose of chromic chloride was excreted in 5 h (Norseth *et al.*, 1982).

The elimination curve for chromium, as measured by whole-body determination, has an exponential form. In rats, three different components of the curve have been identified, with half-times of 0.5, 5.9 and 83.4 days after intravenous injection of chromic chloride at 1 μg/kg bw Cr (Mertz *et al.*, 1965).

In contrast to results with hexavalent chromium, a single intraperitoneal injection of chromic chloride to mice resulted in 45% retention of chromium three weeks after the injection (Bryson & Goodall, 1983).

In mice administered sodium dichromate, chromium was shown to cross the placenta throughout gestation; transfer was more effective than with chromic chloride, which was not detectably transferred during early gestation, although placental transfer of chromium[III] did occur during late gestation (Danielsson *et al.*, 1982).

A total of 25-30% of chromium administered as chromic chloride to pregnant rats on days 17-20 of gestation was transferred to the placental-fetal unit (Wallach & Verch, 1984). Groups of ICR mice were given a single intraperitoneal injection of [^{51}Cr]chromic chloride on day 8 of gestation and were sacrificed 4, 8 and 12 h after injection. The radioactivity in the fetus increased with time since injection, whereas maternal blood levels decreased (Iijima *et al.*, 1983a).

(iii) *Chromium[VI] compounds*

Gastrointestinal absorption of chromates has been reported. In a review, 3-6% of an administered dose was reported to appear in the urine of rats; this may be an underestimate of the absorption from the gastrointestinal tract, which also takes part in chromium excretion (Mertz, 1969). The absorption of chromates depends on the degree of reduction of chromium[VI] to chromium[III], which is poorly absorbed from the gastrointestinal tract (Donaldson & Barreras, 1966; De Flora *et al.*, 1987a).

Following intratracheal administration of sodium chromate solution to rabbits, about 45% (as Cr) remained in the lungs 4 h after instillation; 15% was excreted in urine. The highest concentration of chromium[VI] was reached in red cells after about 3 h, and the corresponding plasma concentration at that time was about one-third of that in red cells (Wiegand *et al.*, 1984a). Absorption from the lungs may be decreased by extracellular reduction of the hexavalent form (Suzuki, 1988).

Zinc chromate was absorbed in rats exposed to known atmospheric concentrations (6.3-10.7 mg/m³, equivalent to 1.3-2.2 mg/m³ Cr) in an inhalation chamber: a five-fold increase in the blood chromium level was observed after 100 min of exposure by inhalation, and this level increased at a similar rate during the next 150 min (Langård *et al.*, 1978).

Suzuki *et al.* (1984) exposed rats by inhalation to potassium dichromate particles (MMAD, 1.6-2.0 μm: 12-25% of particles < 1 μm; determined by multistage impactor (Andersen Sampler) and controlled by electron microscopy). A two-phase clearance pattern for chromium was demonstrated, the smaller particles having half-times of 30 h and 700 h; for larger particles, a single phase with a half-time of 160 h was demonstrated. [The Working Group noted that no statistical evaluation of the differences is given in the paper.] The authors stated that there might also be an undetected rapid component for the larger particles and noted that reduction of the hexavalent form may explain the two-phase clearance from the respiratory tract after exposure to chromium[VI]. This reduction was demonstrated by Suzuki (1988).

Sodium chromate (69 μg Cr), zinc chromate (66 μg Cr) and lead chromate (38 μg Cr), all at 20 μl, were injected intratracheally into Wistar rats; 30 min later, 36, 25 and 81% of the doses, respectively, were still present in the lungs. From 30 min and up to six days, lung clearance followed first-order kinetics, with half-times of 2.4 days for sodium chromate, 1.9 days for zinc chromate and 1.8 days for lead chromate. Limited amounts of chromium were found in blood and organs after exposure to lead chromate; the concentrations found were similar with sodium and zinc chromates. At ten days, 20% of the dose of sodium and zinc chromates had been excreted in the urine; negligible amounts of lead chromate were found. After exposure to lead chromate, about 80% of the chromium was excreted in faeces during the same interval (Bragt & van Dura, 1983).

Percutaneous absorption of labelled sodium chromate occurred in guinea-pigs (Wahlberg & Skog, 1963): a maximum of 4% of the dose applied on the skin disappeared within 5 h, and labelled chromium was detected in a number of organs.

Following administration of chromium[VI], most of the chromium found in the blood is bound to red blood cells (Mutti *et al.*, 1979; Suzuki *et al.*, 1984; Wiegand *et al.*, 1984a). After exposure of rats by inhalation to potassium dichromate or of rabbits by inhalation to sodium dichromate, the highest concentrations were found in the kidney and liver (Suzuki *et al.*, 1984; Wiegand *et al.*, 1984a). The spleen also contained high concentrations of chromium after subcutaneous administration of potassium dichromate to rats (Mutti *et al.*, 1979). The organ concentrations after exposure to chromium[VI] were always much higher than after a corresponding exposure to chromium[III] (Suzuki *et al.*, 1984; Wiegand *et al.*, 1984a).

After parenteral administration of chromium[VI] to rats, chromium was excreted predominantly in the urine (National Research Council, 1974; Langård, 1980, 1982). Seven days after intraperitoneal injection of potassium chromate to mice, urinary excretion was twice as high as faecal excretion; following administration of chromium[III], faecal excretion was three times as high as urinary excretion (Bryson & Goodall, 1983).

Subcutaneous injections of 3 mg/kg bw potassium dichromate were given to rats every other day for eight weeks. Urinary elimination of chromium increased steadily during the experiment and was correlated with the concentration of chromium in the renal cortex (Franchini *et al.*, 1978).

Elimination of chromium from the blood of rats exposed by inhalation to zinc chromate was slow: the blood chromium level fell by less than 50% during the first three days after exposure; and after 18 and 37 days 20% and 9% of the initial concentration, respectively, remained. Excretion occurred mainly *via* the urine (Langård *et al.*, 1978).

Biliary excretion of chromium following administration of sodium dichromate has been demonstrated in rats (Cikrt & Bencko, 1979; Norseth *et al.*, 1982); 6-8% of an intravenous dose of sodium dichromate was excreted in 5 h (Norseth *et al.*, 1982).

Three weeks after a single intraperitoneal injection of potassium dichromate to mice, 7.5% chromium was retained. After repeated weekly intraperitoneal injections of potassium dichromate, about 3% of chromium was retained eight weeks after the first injection. In both cases, this level is about one-sixth of that observed after administration of chromium[III] (Bryson & Goodall, 1983).

Chromium[VI] (tested as sodium dichromate and as an unspecified chromate *in vitro*) was transported effectively through mammalian cell membranes by the carboxylate, sulfate and phosphate carrier systems; the kinetics of uptake also involve intracellular reduction to the trivalent form (Sanderson, 1976; Wetterhahn-Jennette, 1981; Aaseth *et al.*, 1982; Alexander *et al.*, 1982). Chromium[VI] (tested as sodium dichromate) was rapidly reduced to chromium[III] after cellular uptake, but such reduction may also take place outside the cell, with decreased uptake as a result (De Flora *et al.*, 1987a; Suzuki, 1988). Glutathione seems to be the most important factor for intracellular reduction of chromium[VI], but ascorbic acid, microsomes in the presence of NAD/NADH microsomal cytochrome P450, mitochondria and proteins such as haemoglobin and glutathione reductase in red blood cells may also be active in the reduction process (Connett & Wetterhahn, 1983; Ryberg & Alexander, 1984; Wiegand *et al.*, 1984b; Connett & Wetterhahn, 1985; De Flora & Wetterhahn, 1990). Once absorbed and retained in biological tissue, chromium compounds occur in the trivalent form (Mertz, 1969). Initial binding may involve the pentavalent form (Rossi & Wetterhahn, 1989). When the reducing

capacity of liver cells is decreased, the hexavalent form may be found in bile (Norseth *et al.*, 1982).

After treatment of rats with sodium dichromate at 20 mg/kg bw intraperitoneally (134 μmol/kg bw Cr), more of the chromium associated with chromatin was bound to DNA than was the case after chromic chloride treatment (Cupo & Wetterhahn, 1985a).

The intracellular reduction of hexavalent chromium implies the generation of short-lived species of pentavalent and tetravalent chromium with affinities that differ from that of the trivalent form (Connett & Wetterhahn, 1983). The pentavalent form is stabilized by increased amounts of glutathione (Kitagawa *et al.*, 1988). The reduction process thus serves as a detoxification process even intracellularly, when it takes place at a distance from the target site for toxic or genotoxic effect; it serves to activate if it takes place near the cell nucleus, presumably of target organs (De Flora & Wetterhahn, 1990). It has been suggested that phagocytosis may be important for the uptake of hexavalent compounds — in particular soluble forms — as it would allow the slow intracellular release of chromate ions over a long time (Norseth, 1986).

(*b*) *Toxic effects*

As a general rule, chromium[VI] is much more toxic than chromium[III] when administered to animals, and very marked differences in the cytotoxicity of compounds of the two oxidation states have been observed *in vitro*. Effects on the kidney and the respiratory organs are the most important (for reviews, see Nieboer & Jusys, 1988; World Health Organization, 1988).

The mean intravenous lethal dose in mice is 85 mg/kg bw chromic sulfate, 400-800 mg/kg bw chromic chloride and 2290 mg/kg bw chromic acetate (National Research Council, 1974). The LD_{50} in rats for potassium dichromate administered by stomach tube was reported to be 177 mg/kg for males and 149 mg/kg for females (World Health Organization, 1988).

(i) *Chromium[III] compounds*

Morphological changes in rabbit alveolar macrophages occurred after exposure by inhalation to chromic nitrate (0.6 mg/m^3 Cr) for four to six weeks. Fewer macrophages were obtained by lavage than with chromium[VI], but only chromium[III] caused functional changes in macrophages, measured by increased metabolic activity and reduced phagocytotic activity. The authors speculated that these effects may be due to the release of chromium[III] ions from phagocytized particles, with subsequent binding to macromolecules in the cell. Such particles were not seen after exposure to chromium[VI] (Johansson *et al.*, 1986).

Chromium[III] has been found in ribonucleic acids from all sources examined. It is possible that chromium helps stabilize the structure of RNA (Wacker & Vallee,

1959). Chromium bound to only a limited extent to chromatin and DNA from the liver and kidney of rats treated intraperitoneally with chromic chloride at 80 mg/kg bw (290 μmol/kg bw Cr), as indicated in a study by Tsapakos *et al.* (1983a); DNA damage, as measured by alkaline elution, was not demonstrable in kidney after injection of chromium[III]. The binding of chromic nitrate to denatured or native DNA was limited and relatively unaffected by the presence of microsomes and NADPH (Tsapakos & Wetterhahn, 1983).

Chromic chloride was 100 times less effective than chromium[VI] in inhibiting DNA synthesis (Levis *et al.*, 1978a). A large difference in cytotoxic activity between chromium[VI] and chromium[III] was also noted when the effects of 11 water-soluble chromium compounds on BHK cells were compared: of the chromium[III] compounds, only chromic nitrate appeared to be cytotoxic, but it was contaminated with chromium[VI] at about 0.2% (Levis & Majone, 1979).

Chromic chloride inhibited the uptake of ribo- and deoxyribonucleosides by BHK cells (Levis *et al.*, 1978a). In contrast to chromium[VI], chromic chloride inhibited the plasma membrane Mg^{2+}-ATPase activity of BHK cells only when it was present in the incubation medium and not when cells were pretreated with it (Luciani *et al.*, 1979).

Chromic oxide particles are taken up by cells by phagocytosis; chromium[III] may thus reach its target sites even if it is not derived from intracellular chromate ion. An inhibitory effect on cell cycle progression in Chinese hamster cells was demonstrated after exposure to crystalline chromic oxide (particle size, 91% <1 μm; purity, 99.8%) at concentrations ranging from 50 to 200 μg/ml (Elias *et al.*, 1986). Chromic chloride does, however, stimulate RNA synthesis both *in vitro* and *in vivo* in mouse liver and in regenerating rat liver (Okada *et al.*, 1981, 1983, 1984).

(ii) *Chromium[VI] compounds*

Renal lesions in animals are confined to the proximal convoluted tubules (for review, see National Research Council, 1974; Aitio *et al.*, 1988; World Health Organization, 1988). In rats exposed to a single subcutaneous dose of 15 mg/kg bw potassium dichromate, increases in urinary β-glucuronidase, lysozyme, glucose and protein as well as morphological changes in renal tubules were observed, although the glomerular filtration rate was unchanged (Franchini *et al.*, 1978).

Ngaha (1981) demonstrated that urinary volume was increased with increased amounts of acid and alkaline phosphatases in the urine in rats after subcutaneous injection of potassium dichromate at 25 mg/kg. The concentrations of the phosphatases and of lactate dehydrogenase in kidney tissue decreased. No significant change in the levels of these enzymes in liver tissue was demonstrated.

Morphological changes occurred in rabbit alveolar macrophages after exposure by inhalation to sodium chromate (0.9 mg/m^3 Cr) for four to six weeks. Significantly more macrophages were present in the lavage fluid from the chromium[VI]-exposed animals than in those exposed to chromium[III], but functional changes in macrophages were observed after exposure to chromium[III] and not after exposure to chromium[VI] (Johansson *et al.*, 1986). Activation of phagocytosis was demonstrated in rat alveolar macrophages after exposure to 25-50 μg/m^3 sodium dichromate by inhalation for 28 days; exposure to 200 μg/m^3 for the same interval inhibited phagocytotic function. Lung clearance of inhaled [^{59}Fe] iron oxide was significantly decreased after exposure to the high dose of sodium dichromate. The antibody response to sheep red blood cells and the mitogen-stimulated T-lymphocyte response were stimulated at the low doses but inhibited at the high dose (Glaser *et al.*, 1985).

Exposure of cats by inhalation to 11-23 mg/m^3 chromium[VI] as dichromate for 2-3 h/day during five days caused bronchitis and pneumonia. In rabbits exposed similarly, no effect was observed. Mixed dusts containing chromates (7 mg/m^3 as chromium trioxide) were fatal to mice when inhaled for 37 h over ten days; whereas no marked effect was noted in rabbits or guinea-pigs that inhaled 5 mg/m^3 (as chromium trioxide) for 4 h/day on five days/week for one year (National Research Council, 1974). Increased subepithelial connective tissue and flattened epithelium in the large bronchi were observed in mice exposed to chromate (Nettesheim *et al.*, 1971).

In chronically treated cell cultures, chromium[VI] was much more active than chromium[III] in reducing cell growth and survival, independently of the particular compound used (Bianchi *et al.*, 1980). Chromium bound to chromatin and DNA from liver and kidney of rats treated intraperitoneally with sodium dichromate (140 μmol [7.2 mg]/kg as Cr) (Tsapakos *et al.*, 1983a) Binding of chromium to nucleic acids *in vitro* depends on the reduction of the chromium[VI] to chromium[III]. In contrast to chromium[III], binding to denaturated or native DNA was demonstrated with potassium dichromate only in the presence of the complete microsomal reducing system (Tsapakos & Wetterhahn, 1983).

Potassium dichromate induced a rapid blockage of DNA replication in Syrian hamster fibroblasts (BHK line), whereas RNA and protein synthesis were inhibited secondarily (Levis *et al.*, 1978b). It also reduced the colony-forming ability of BHK cells at 10^{-7}-10^{-4}M. It facilitated the uptake of ribo- and deoxyribonucleosides in BHK cells (Levis *et al.*, 1978a). The effect of potassium dichromate on the nucleoside pool in BHK cells could not be explained solely by changes in transport (Bianchi *et al.*, 1979). Plasma membrane Mg^{2+}-ATPase activity of BHK cells was inhibited when the cells were pretreated with potassium dichromate, even when chromate was absent from the assay medium (Luciani *et al.*, 1979). Mitochondrial

respiration was inhibited by about 50% by the addition of 25 μM [1.3 mg] sodium chromate in rat liver (Ryberg & Alexander, 1984).

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

(i) *Chromium[III] compounds*

Treatment of sea-urchin sperm with potassium chromic sulfate or chromic nitrate (5×10^{-5}-5×10^{-4} M [2.6-26 mg]) before fertilization failed to induce larval malformation (Pagano *et al.*, 1983).

In cultured mouse embryos, chromium nitrate (0.02-2 μg/ml Cr) caused less impairment of blastocyst formation and inhibition of hatching from the zona pellucida to the formation of the inner cell mass than the chromium[VI] salts tested (Jacquet & Draye, 1982).

In contrast to chromium[VI], chromic chloride showed no overt cytotoxicity in chick limb bud mesenchymal cells *in vitro* (Danielsson *et al.*, 1982).

Groups of 30 pregnant ICR mice were given a single intraperitoneal injection of [^{51}Cr]chromic chloride (19.5 mg/kg bw Cr) on day 8 of gestation and were sacrificed at intervals of 4-192 h after injection. More pyknotic cells were observed in the neural plate of experimental embryos than controls [percentages not given], especially by 8 h after injection (Iijima *et al.*, 1983a).

A dose-dependent increase in the frequency of rib fusion in fetuses (6-16%, depending on dose) and exencephaly and anencephaly were seen occasionally at higher dose levels following intraperitoneal injection of 9.8-24.4 mg/kg bw chromic chloride to mice on day 8 of gestation. Maternal effects were not described (Matsumoto *et al.*, 1976).

(ii) *Chromium[VI] compounds*

Treatment of sea-urchin sperm with sodium chromate before fertilization resulted in a number of abnormal larvae, depending on length of exposure and concentration. Sea-urchin embryos reared in the presence of chromate at 5×10^{-5}-5×10^{-4} M had retarded differentiation of the gut and skeleton (Pagano *et al.*, 1983).

Cultured mouse embryos at the two-cell stage were incubated in Brinster's medium with potassium chromate or calcium chromate (0.02-2 μg/ml Cr). Blastocyst formation was damaged, and hatching of the blastocyst from the zona pellucida to the formation of the inner cell mass was inhibited (Jacquet & Draye, 1982).

As reported in an abstract, male mice were administered 3×10^{-3} M [882 mg] potassium bichromate by either intratesticular or intraperitoneal injection, then mated weekly. A decrease in the number of sperm was seen after three weeks of treatment, and abnormalities in shape reached about 50% of the total sperm after four weeks of treatment. Decreases in the number of implantation sites, in litter size and in fetal body weight were observed. No conspicuous malformation of fetuses

was detected. None of the females became pregnant after three weeks of treatment of males (Yasuda, 1980).

Sodium chromate inhibited chondrogenesis in chick limb bud mesenchymal cells *in vitro* at concentrations of about 0.1 μg/ml Cr (Danielsson *et al.*, 1982).

Chromium trioxide dissolved in saline was injected into the air sacs of embryonated chicken eggs at doses of 0.002-0.05 mg/egg on days 0-4 of incubation. Control eggs were injected with a comparable volume of saline. All embryos were examined on day 8, and malformations, such as short and twisted limbs, microphthalmia, exencephaly, short and twisted neck, everted viscera, oedema and reduced body size, were observed in treated eggs. Most embryos showed unilateral or bilateral limb defects (Gilani & Marano, 1979).

Chromium trioxide was administered intravenously at doses of 5, 7.5, 10 or 15 mg/kg bw to groups of ten pregnant golden hamsters early on day 8 of gestation. Fetuses were collected on gestation days 12, 14 and 15 and were examined for frequency and types of malformations. In the different dose groups, 6-40% of the fetuses were resorbed and 1-100% of fetuses had growth retardation; 2% of control fetuses were resorbed. Maternal toxicity (mortality, decreased weight gain, kidney tubular necrosis) was seen in treated animals. Cleft palate occurred in 34-85% of exposed fetuses (2% in controls) and defects in skeletal ossification in up to 96% (Gale, 1978). On comparing five strains of hamsters (ten animals per group, exposure to 8 mg/kg bw on day 8), different susceptibilities were observed: three strains were very susceptible to the embryotoxic effects, while the others were more resistant. In the more susceptible strains, the percentage of resorption sites was 13-28%, whereas in the less susceptible strains it was 7-11%. External abnormalities observed were cleft palate (0-30%) and hydrocephalus. Maternal toxicity (decreased weight gain) was seen in all groups. The time of administration of the chromium trioxide was important: cleft palate was induced only when chromium was administered on day 7, 8 or 9 of gestation and not when it was given on day 10 or 11 (Gale & Bunch, 1979; Gale, 1982).

(*d*) *Genetic and related effects*

The activity of chromium and chromium compounds in tests for genetic and related effects was evaluated in previous *IARC Monographs* (1980a, 1982, 1987a,b). Moreover, a number of reviews on this subject are available in the literature (e.g., Heck & Costa, 1982a,b; Léonard & Lauwerys, 1980; Petrilli & De Flora, 1980; Paschin & Kozachenko, 1981; Levis & Bianchi, 1982; Petrilli & De Flora, 1982; Baker, 1984; Bianchi & Levis, 1984; Hansen & Stern, 1984; Bianchi & Levis, 1985; Petrilli *et al.*, 1986a,b; Sunderman, 1986; Venitt, 1986; Bianchi & Levis, 1987, 1988; Nieboer & Shaw, 1988; World Health Organization, 1988; De Flora *et al.*, 1990).

Over 600 reports have been published on 32 chromium compounds of various oxidation states and solubilities, and the data base covers 125 experimental systems with different endpoints and/or targets. The studies described below are summarized in Appendix 1 to this volume.

(i) *Metallic chromium*

Metallic chromium was assayed for the ability to induce cell transformation (anchorage-independent growth) in Syrian hamster BHK fibroblasts. Although chromium particles were phagocytized by cells, no significant increase in the number of cell foci growing in soft agar was observed (Hansen & Stern, 1985). [See General Remarks, p. 44, for concerns about this assay.]

As reported in an abstract, male Sprague-Dawley rats were exposed to chromium fumes generated from powders of chromium metal by a plasma flame sprayer at concentrations of 1.84 ± 0.55 mg/m^3 or 0.55 ± 0.07 mg/m^3 fume for 5 h/day on five days a week for one week or two months. Significant increases in the frequencies of sister chromatid exchange and of chromosomal aberrations were observed in peripheral blood lymphocytes, whereas chromosomal aberration frequencies in bone-marrow cells were unchanged (Koshi *et al.*, 1987). [The Working Group noted that some oxidation of metallic chromium may have occurred during generation of the fumes.]

(ii) *Chromium[III] compounds*

Twelve chromium[III] compounds of various water solubilities were assayed in a number of short-term tests, often at the same time as chromium compounds of other oxidation states. They included: (a) highly soluble compounds, such as chromic chloride, chromic acetate, chromic nitrate, chromic sulfate and chromic potassium sulfate; (b) sparingly soluble products, such as basic chromic sulfate or neochromium, chromium alum and chromic phosphate; and (c) almost insoluble compounds, such as chromic hydroxide, chromic oxide, chromite ore and cupric chromite. In addition, several reports dealt with the activity of chromium[III] tannins and of chromium[III] compounds bound to organic ligands, as described below. In evaluating the results, summarized in Appendix 1, it should be noted that some of the positive results, obtained with both pure laboratory compounds and industrial products, might be due to contamination by traces of chromium[VI] (indicated as [+] in Appendix 1); therefore, reported positive results with chromium[III] compounds should be interpreted with caution, particularly for those studies in which the purity of test compounds was not checked.

In several studies, the activity of chromic chloride in acellular (i.e., purified nucleic acids) or subcellular (i.e., cell nuclei) systems was investigated. Depurination of calf thymus DNA did not occur, as shown by the unchanged release of adenine detectable by thin-layer chromatography. In addition, it did not induce

mutation of single-stranded ϕ X 174 *am*3 DNA, transfected into *Escherichia coli* spheroplasts and then tested for reversion in a progeny phage assay (Schaaper *et al.*, 1987). As reported previously, chromium[VI] trioxide was also inactive in this system; chromic chloride, however, induced *lacZ*α forward mutation in double-stranded M13mp2 DNA, transfected into JM101 *E. coli* (Snow & Xu, 1989). Moreover, chromic chloride suppressed the infectivity of tobacco mosaic virus RNA, probably by nonenzymic cleavage of internucleotide phosphodiester bonds (Huff *et al.*, 1964). Assessments of viscosity, ultraviolet absorption spectra and thermal denaturation of purified DNA and RNA showed that, at variance with chromium[VI] which (as an oxidizing agent) breaks the polynucleotide chain (see potassium dichromate), chromium[III] is responsible for physicochemical alterations of nucleic acids by interacting with the phosphate groups and nitrogen bases (Tamino & Peretta, 1980; Tamino *et al.*, 1981). As evaluated by nucleotide incorporation into calf thymus DNA in the presence of *E. coli* DNA polymerase, chromic chloride inhibited DNA synthesis more potently than chromium[VI] (potassium dichromate); however, at levels below the inhibitory concentration, it enhanced nucleotide incorporation (Nishio & Uyeki, 1985). Like chromium[VI] (potassium dichromate), chromic chloride increased misincorporation of nucleotide bases into daughter DNA strands synthesized from a synthetic polynucleotide template, poly[d(A-T)], in the presence of avian myeloblastosis virus or *E. coli* DNA polymerases (Sirover & Loeb, 1976; Tkeshelashvili *et al.*, 1980). The misincorporated bases were present as single-base substitutions (Tkeshelashvili *et al.*, 1980). In contrast to chromium[VI] salts (potassium chromate and potassium dichromate), chromic chloride favoured cross-links between *E. coli* DNA and bovine serum albumin, as assessed by checking ^{3}H-DNA-bovine serum albumin binding in a filtration assay (Fornace *et al.*, 1981). The same chromium[III] compound produced DNA fragmentation (alkaline elution technique), as determined by single-strand breaks and cross-links in isolated calf thymus nuclei (Beyersmann & Köster, 1987) and in purified DNA from V79 cells (Bianchi *et al.*, 1983). DNA-protein cross-links were also detected by exposing nuclei of mouse leukaemia L1210 cells to chromic chloride; chromium[VI] (potassium chromate) was inactive (Fornace *et al.*, 1981).

Most studies in which the activity of chromium[III] compounds was evaluated in prokaryotes yielded negative results. Chromic chloride did not induce λ prophage in *E. coli* WP2$_s$(λ) (Rossman *et al.*, 1984) after overnight incubation at concentrations near the growth inhibitory concentration of the compound. No SOS response was induced in *E. coli* GC2375, UA4202 or PQ30 by chromic chloride, chromic nitrate or chromic acetate (Llagostera *et al.*, 1986), or in strain PQ37 by chromic potassium sulfate (De Flora *et al.*, 1985a) or chromic chloride (Olivier & Marzin, 1987). Chromic nitrate, chromic chloride and chromic potassium sulfate

were confirmed to be inactive in strain PQ37, whereas chromic acetate produced a low but significant increase in SOS-inducing activity (Venier *et al.*, 1989).

In differential killing assays with *E. coli*, chromic chloride was equally toxic in the wild strain AB1157 and in the repair-deficient strains AB1886 (*uvrA6*⁻), GW801 (*recA56*⁻), GW802 (*recA56*⁻*uvrA6*⁻), PAM AA34 (*recA56*⁻*lexA2*⁻) and PAM5717 (*lexA2*⁻) (Warren *et al.*, 1981).

Chromic chloride, chromic phosphate and chromic oxide (spotted in powder form) were equally toxic in *E. coli* WP2 (wild strain) and in the repair-deficient strains WP2 *uvrA* (*uvrA*⁻), CM571 (*rec A*⁻) and WP100 (*uvrA*⁻ *rec A*⁻), as assessed by the streak method on agar and, in the case of chromic phosphate, by means of a test-tube assay (Yagi & Nishioka, 1977).

Chromic chloride and chromic acetate were equally toxic to WP2 and to the repair-deficient strains WP67 (*uvrA*⁻*polA*⁻) and CM871 (*uvrA*⁻*recA*⁻*lexA*⁻) when assayed in the treat-and-plate test, but these compounds, chromic nitrate and chromic potassium sulfate were more toxic in the repair-deficient strains when assayed by a liquid micromethod (De Flora *et al.*, 1984a). Four conditions were required to elicit this unusual positivity of chromium[III] in this system: (a) performance of the test in a liquid medium, (b) long contact between chromium[III] and bacteria (at least 6-8 h), (c) a physiological pH (7.0-7.4) and (d) the presence of high, subtoxic concentrations (0.2-0.3 M) of phosphate (De Flora *et al.*, 1990). Chromic chloride, chromic sulfate and chromic potassium sulfate did not induce differential killing of *S. typhimurium* TA1978 (wild strain) or TA1538 (*rec*⁻) (Gentile *et al.*, 1981). In the *rec* assay in *Bacillus subtilis* H17 (wild strain) and M45 (*rec*⁻), negative results were obtained with chromic chloride (Nishioka, 1975; Nakamuro *et al.*, 1978; Matsui, 1980; Gentile *et al.*, 1981), chromic sulfate and chromic potassium sulfate (Kada *et al.*, 1980; Kanematsu *et al.*, 1980; Gentile *et al.*, 1981). Positive results were obtained in this system, using the spot test procedure, with chromic acetate and chromic nitrate. Chromic acetate also gave positive results in the $arg^- \rightarrow arg^+$ reversion test in *E. coli* Hs30R (Nakamuro *et al.*, 1978).

No reversion of $trp^- \rightarrow trp^+$ was produced in *E. coli* by chromic chloride or chromic acetate (strains WP2 and WP2*uvrA*) (Petrilli & De Flora, 1982) or by chromic sulfate (strain DG1153) (Arlauskas *et al.*, 1985). In a $lacI^+/lacI^-$ forward mutation assay in *E. coli* KMBL3835, chromic chloride yielded unclear results (Zakour & Glickman, 1984).

In more than 30 reports (see Appendix 2), highly soluble or sparingly soluble chromium[III] compounds were inactive in the $his^- \rightarrow his^+$ reversion test in several strains of *Salmonella typhimurium* (TA1535, TA1537, TA1538, TA92, TA94, TA97, TA98, TA100 and TA102) in the absence of exogenous metabolic systems (Tamaro *et al.*, 1975; Petrilli & De Flora, 1977, 1978a; De Flora, 1981a; Tso & Fung, 1981; Venier *et al.*, 1982; Bennicelli *et al.*, 1983; Bianchi *et al.*, 1983; De Flora *et al.*, 1984a,b;

Arlauskas *et al*., 1985; Langerwerf *et al*., 1985; Loprieno *et al*., 1985; Marzin & Phi, 1985; Petrilli *et al*., 1985). Chromic chloride, chromic nitrate, chromic potassium sulfate, chromium alum and neochromium were still not mutagenic in the presence of metabolic systems, including post-mitochondrial supernatants of rat liver, lung or muscle, rat muscle mitochondria (with or without ATP), human erythrocyte lysates and oxidized glutathione. Mutagenic effects were produced by all these compounds only in the presence of a strong oxidizing agent, such as potassium permanganate (Petrilli & De Flora, 1978a). The inactivity of chromic sulfate was unaffected by the presence of nitrilotriacetic acid (NTA) (Loprieno *et al*., 1985). Surprisingly, high amounts of chromic chloride [unspecified source and purity] were reported to be weakly mutagenic to strains TA98 and TA94 in one study (Langerwerf *et al*., 1985). Other positive results can be ascribed to contamination with traces of chromium[VI], in a chromic nitrate sample (Venier *et al*., 1982; Bianchi *et al*., 1983) and in an industrial chromite sample (Petrilli & De Flora, 1978a; De Flora, 1981a; Venier *et al*., 1982; Bianchi *et al*., 1983).

Chromic chloride was reported to induce mitotic gene conversion at the *trp5* locus and point reverse mutation at the *ilv* locus in strain D7 of *Saccharomyces cerevisiae*. Such activity, usually detected when the yeast was in the logarithmic growth phase, was very weak, was obtained only with high doses of chromium[III] and occurred only in the presence of 0.1 M phosphate; no activity was seen when 0.05 M Tris-hydrochloric acid was used as the buffer (Galli *et al*., 1985; Bronzetti *et al*., 1986).

Chromic potassium sulfate gave weakly positive results in an unscheduled DNA synthesis assay in mature pollen of *Petunia hybrida* (W166K) (Jackson & Linskens, 1982). [The Working Group noted that the effect was very weak and that the existence of a dose-response relationship was not investigated.] Chromic nitrate induced chromosomal aberrations in the root tips of *Vicia faba* (Gläss, 1955).

A study of the cytotoxic effects produced in Syrian hamster BHK monolayers and of mitotic cycle alterations in human epithelial-like heteroploid HEp2 cells produced by chromic chloride, chromic sulfate, chromium alum, neochromium and chromium[VI]-contaminated chromite provided evidence that chromium[III] is far less active than chromium[VI] (Levis & Majone, 1981). Chromic chloride did not induce unscheduled DNA synthesis in mouse kidney A18BcR cells (Raffetto *et al*., 1977) or human heteroploid EUE cells (Bianchi *et al*., 1983). It did not inhibit DNA synthesis in Syrian hamster BHK fibroblasts, even when the cells were reversibly permeabilized in hypertonic medium (Bianchi *et al*., 1984), or in mouse L cells unless they were permeabilized with detergents (Nishio & Uyeki, 1985).

Chromic chloride did not induce DNA fragmentation in mouse leukaemia 1210 cells (Fornace *et al*., 1981), in Chinese hamster V79 cells (Bianchi *et al*., 1983) or in human embryo lung IMR-90 fibroblasts (Fornace *et al*., 1981), as assessed by al-

kaline elution, or in human white blood cells, as assessed by the alkaline unwinding technique (McLean *et al.*, 1982). Similarly, chromic nitrate, even at concentrations up to 25 times those of chromium[VI] compounds needed to damage DNA, did not produce DNA fragmentation (alkaline elution) in chicken embryo hepatocytes (Tsapakos *et al.*, 1983b). In contrast, a sample of cupric chromite induced DNA-protein cross-links in Novikoff ascites hepatoma cells, as evaluated by high-speed centrifugation of detergent-treated cells followed by polyacrylamide gel electrophoresis; chromous chloride was inactive in this test (Wedrychowski *et al.*, 1986a).

Using the *hprt* forward mutation assay, negative results were obtained with chromic sulfate in mouse mammary carcinoma Fm3A cells (8-azaguanine resistance) (Nishimura & Umeda, 1978 [Abstract]), with chromic acetate in Chinese hamster V79/4 cells (8-azaguanine resistance) (Newbold *et al.*, 1979) and in CHO cells (6-thioguanine resistance) (Bianchi *et al.*, 1983). In V79 cells, a sample of chromic oxide, uncontaminated with chromium[VI] was phagocytized, as shown by electron microscopic detection of intracytoplasmic vacuoles containing crystalline chromic oxide particles, after an 18-h exposure of cells; it also induced 6-thioguanine resistance (Elias *et al.*, 1986).

Mostly negative results have been reported with regard to the induction of sister chromatid exchange by chromium[III] compounds in various types of cultured mammalian cells (Table 23). Both positive and negative results have been reported in the literature concerning the ability of these compounds to induce chromosomal aberrations in mammalian cells (Table 24). In parallel assays, aberrations were induced more frequently than sister chromatid exchange by chromium[III] compounds, but much higher concentrations of chromium[III] than chromium[VI] were generally needed to induce chromosomal aberrations, due perhaps to an indirect effect of high doses, such as the release of lysosomal nucleases (Levis & Majone, 1981). The decreased frequency of chromium[III]-induced chromosomal aberrations in the presence of superoxide dismutase, copper (salicylate), copper (tyrosine), catalase and mannitol suggests the involvement of active oxygen species (Friedman *et al.*, 1987). The occasional finding of both sister chromatid exchange and chromosomal aberrations in CHO cells was ascribed by the authors to contamination of their chromium[III] samples with traces of chromium[VI] (Levis & Majone, 1979; Bianchi *et al.*, 1980; Venier *et al.*, 1982).

Chromic chloride inhibited spindle formation in human skin fibroblasts, but only at the highest concentration tested (100 μM), which was several orders of magnitude higher than the concentration required for chromium[VI] compounds (sodium chromate and calcium chromate) to produce the same effect (Nijs & Kirsch-Volders, 1986).

Table 23. Induction of sister chromatid exchange by chromium[III] compounds in cultured mammalian cells

Chromium compound	Cell line	Result and comments	Reference
Chromic acetate	Chinese hamster CHO	-	Levis & Majone (1979)
		-	Bianchi *et al.* (1980)
	Mouse macrophage P388D₁	+	Andersen (1983)
	Human peripheral lymphocytes	+	Andersen (1983)
Chromic chloride	Mouse primary lymphocytes (BALB/c)	-	Venier *et al.* (1982)
		-	Majone *et al.* (1983)
		-	Bianchi *et al.* (1983)
	Mouse primary lymphocytes (BALB/Mo)	-	Venier *et al.* (1982)
		-	Majone *et al.* (1983)
		-	Bianchi *et al.* (1983)
	Mouse LSTRA lymphocytes	-	Bianchi *et al.* (1983)
	Syrian hamster embryo primary	-	Tsuda & Kato (1977)
	Chinese hamster CHO	-	Macrae *et al.* (1979)
		-	Levis & Majone (1979)
		-	Levis & Majone (1981)
		-	Majone & Rensi (1979)
		-	Bianchi *et al.* (1980)
		-	Bianchi *et al.* (1983)
		-	Venier *et al.* (1982)
		-	Uyeki & Nishio (1983)
		+ (48-h incubation)	Venier *et al.* (1985a)
	Chinese hamster lung Don	-	Koshi (1979)
		+	Ohno *et al.* (1982)
	BHK fibroblasts	-	Bianchi *et al.* (1984)
	BHK fibroblasts (permeabilized)	-	Bianchi *et al.* (1984)
	Human peripheral lymphocytes	-	Ogawa *et al.* (1978)
		-	Stella *et al.* (1982)
	Chinese hamster V79	+ (300 × dose needed for Cr[VI])	Elias *et al.* (1983)
Chromic nitrate	Chinese hamster CHO	-	Levis & Majone (1979)
		-	Bianchi *et al.* (1980)
		-	Venier *et al.* (1982)

Table 23 (contd)

Chromium compound	Cell line	Result and comments	Reference
Chromic potassium sulfate	Chinese hamster CHO	-	Levis & Majone (1979)
		-	Bianchi *et al.* (1980)
Chromic sulfate	Chinese hamster CHO	-	Levis & Majone (1981)
		-	Loprieno *et al.* (1985)
	Chinese hamster lung Don	-	Ohno *et al.* (1982)
Chromium alum	Chinese hamster CHO	-	Levis & Majone (1981)
		-	Venier *et al.* (1982)
Neochromium	Chinese hamster CHO	-	Levis & Majone (1981)
Chromic oxide	Mouse macrophage $P388D_1$	- (taken up by cells after 48 h)	Andersen (1983)
	Chinese hamster V79	+ (1000 × dose needed for Cr[VI])	Elias *et al.* (1983)

Chromic chloride (Bianchi *et al.*, 1983; Hansen & Stern, 1985) and chromic oxide (Hansen & Stern, 1985) did not induce anchorage-independent growth in Syrian hamster BHK fibroblasts (see General Remarks, p. 44, for concern about this assay). Chromic chloride was reported to produce morphological transformation of mouse fetal cells (Raffetto *et al.*, 1977), but it did not transform Syrian hamster embryo primary cells nor, in contrast to chromium[VI] (potassium chromate), did it affect the transforming capacity of benzo[*a*]pyrene (Rivedal & Sanner, 1981).

In vivo, intraperitoneal injection of chromic chloride did not induce DNA fragmentation in rat liver or kidney cells (Tsapakos *et al.*, 1983b; Cupo & Wetterhahn, 1985a), as assessed by the alkaline elution technique in the same laboratory from which positive results were reported with chromium[VI] (see sodium dichromate). The number of micronucleated erythrocytes in bone-marrow cells of BALB/c mice was not increased after intraperitoneal injection of 250-500 mg/kg bw chromic nitrate, which contrasts with the positive result recorded with a soluble chromium[VI] compound (potassium dichromate) at ten-fold lower doses (Fabry, 1980).

Table 24. Induction of chromosomal aberrations by chromium[III] compounds in cultured mammalian cells

Chromium compound	Cell line	Result	Reference
Chromic acetate	Chinese hamster CHO	+	Levis & Majone (1979)
		+	Bianchi *et al.* (1980)
	Human peripheral lymphocytes	+	Nakamuro *et al.* (1978)
Chromic chloride	Mouse fetal	+	Raffetto *et al.* (1977)
	Syrian hamster embryo	-	Tsuda & Kato (1977)
	Chinese hamster CHO	+	Levis & Majone (1979)
		+	Levis & Majone (1981)
		+	Majone & Rensi (1979)
		+	Bianchi *et al.* (1980)
		+	Venier *et al.* (1982)
	Human peripheral lymphocytes	-	Nakamuro *et al.* (1978)
		-	Sarto *et al.* (1980)
		+	Kaneko (1976)
		+	Stella *et al.* (1982)
Chromic nitrate	Chinese hamster CHO	-	Levis & Majone (1979)
		-	Bianchi *et al.* (1980)
		-	Venier *et al.* (1982)
	Human peripheral lymphocytes	-	Nakamuro *et al.* (1978)
Chromic potassium sulfate	Chinese hamster CHO	+	Levis & Majone (1979)
		+	Bianchi *et al.* (1980)
Chromic sulfate	Mouse mammary carcinoma Fm3A	-	Umeda & Nishimura (1979)
	Syrian hamster embryo primary	-	Tsuda & Kato (1977)
	Chinese hamster CHO	+	Levis & Majone (1981)
	Chinese hamster 237-2a	+	Rössner *et al.* (1981)
Chromium alum	Chinese hamster CHO	+	Levis & Majone (1981)
Neochromium	Chinese hamster CHO	+	Levis & Majone (1981)
Chromic oxide	Chinese hamster CHO	[+]	Levis & Majone (1981)
		[+]	Venier *et al.* (1982)

The results of these studies with pure and industrial chromium[III] products are summarized in Appendix 1. Data on the genotoxicity of chromium tanning liquors used in the hide and leather industry, most of which are composed of almost insoluble sulfates, are also available. None of 17 tanning liquors, dissolved in water, acids or alkali, reverted *his*$^-$ *S. typhimurium* strains; however, the frequency of sister chromatid exchange was increased by eight (including chromium alum) of 13

tannins tested in Chinese hamster CHO cells. Contamination with chromium[VI] was detected in four of the active compounds (Venier *et al.*, 1982, 1985a).

The marked differences in potency seen in parallel assays with chromium[III] and chromium[VI] compounds have already been commented upon. The positive results sometimes obtained with chromium[III] compounds (46 positive and 141 negative results) can be ascribed to a variety of factors, which emerged from analyses of the literature (Levis *et al.*, 1978b; Levis & Bianchi, 1982). These include unquantified contamination with trace amounts of chromium[VI], nonspecific effects of very high doses, and penetration of chromium[III] by endocytosis following long exposure *in vitro* or under special treatment conditions, such as exposure to detergents or to subtoxic concentrations of phosphate. In addition, a technical artefact may result from interaction of chromium[III] with DNA released from disrupted cells during extraction procedures.

Chromium[III] complexes

Unlike chromium[VI] (potassium chromate), a chromic glycine complex did not produce unscheduled DNA synthesis in cultured human skin fibroblasts (Whiting *et al.*, 1979). In a differential killing assay with *E. coli* AB1157 (wild strain) and various repair-deficient strains and in the *S. typhimurium his*⁻ reversion test, four of 17 hexacoordinate chromium[III] compounds gave positive results only in the DNA repair test and four in both tests (Warren *et al.*, 1981). The most active complexes were those containing aromatic amine ligands, like 2,2′-bipyridine and 1,10-phenanthroline. [The Working Group noted that the genotoxicity of these ligands was not checked.] Complexation with salicylate and citrate (but not with NTA, ethylenediaminetetraacetic acid, Tiron, glucose, glycine, pyrophosphate or acetate) rendered chromic chloride weakly active in the *rec* assay with *B. subtilis* (Gentile *et al.*, 1981). The mutagenicity of $[Cr(bipy)_2Cl_2]Cl$ and $[Cr(phen)_2Cl_2]Cl$ was confirmed in *S. typhimurium* TA100 (Beyersmann *et al.*, 1984). Water-soluble complexes of chromium[III] (chromic sulfate and chromic chloride) with five amino acids (arginine, aspartic acid, glycine, hydroxyproline and lysine) or with salicylic acid or ascorbic acid did not revert various *his*⁻ *S. typhimurium* strains (Langerwerf *et al.*, 1985). Initial observations were reported in an abstract concerning the ability of $[Cr(bipy)_2Cl_2]Cl$ to induce predominantly extragenic suppressors in TA103 (Vieux *et al.*, 1986). This complex was shown in the same laboratory to revert *his*⁻ *S. typhimurium* TA92, TA100 and TA98 (Warren *et al.*, 1981). In a further abstract (Rogers *et al.*, 1987), the same compound was reported to revert TA102 and TA104, with an appreciable reduction of activity under anaerobiosis and in the presence of the hydroxyl ion scavenger mannitol. Exposure of calf thymus nuclei to $Cr(glycine)_3$ produced DNA-protein cross-links as well as DNA strand-breaks, i.e., the same type of lesions caused by hexahydrated chromic chloride (Beyersmann & Köster,

1987). In the same study, $[Cr(phen)_2Cl_2]Cl$ also produced DNA fragmentation, but with a lower fraction of cross-links, when applied to intact Chinese hamster V79 cells; this phenanthroline complex, in contrast to $Cr(glycine)_3$, also induced 6-thioguanine resistance. No mutagenic effect was detected in *his*⁻ *S. typhimurium* with a commercial preparation of glucose tolerance factor, a yeast-extracted natural complex of chromium[III] with nicotinic acid, glycine, glutamic acid and cysteine, which is prescribed as a dietary supplement in cases of deficient chromium[III] intake with food and impaired glucose tolerance (De Flora *et al.*, 1989a). $Cr(maltolate)_3$, a chromium[III] complex with a low lipophilic ligand, did not induce gene mutations in *S. typhimurium* TA92, TA98, TA100 or TA104 nor sister chromatid exchange in mammalian cell culture (CHO line), whereas a complex with a high lipophilic ligand, $Cr(acetyl\ acetonate)_3$, although inactive to TA92, TA98 and TA100 strains, was clearly mutagenic to strain TA104 and increased the frequency of sister chromatid exchange (Gava *et al.*, 1989a).

(iii) *Chromium[VI] compounds*

Potassium dichromate, sodium dichromate, ammonium dichromate, potassium chromate, sodium chromate, ammonium chromate and chromium trioxide

Highly soluble chromium[VI] compounds were assayed in several acellular systems. Potassium dichromate did not induce cross-links of *E. coli* [^{3}H]DNA to bovine serum albumin (Fornace *et al.*, 1981). It inhibited DNA synthesis by decreasing nucleotide incorporation into calf thymus DNA in the presence of *E. coli* DNA polymerase (Nishio & Uyeki, 1985). Potassium dichromate (Sponza & Levis, 1980 [Abstract]; Bianchi *et al.*, 1983) and chromium trioxide (Sirover & Loeb, 1976; Tkeshelashvili *et al.*, 1980) decreased the fidelity of DNA synthesis by altering the ratio of incorporation of radiolabelled complementary to noncomplementary nucleotides. In these studies, the synthetic polynucleotide poly[d(A-T)] was used as a template in the presence of viral (avian myeloblastosis virus), bacterial (*E. coli*) and mammalian (calf thymus) DNA polymerase (Miyaki *et al.*, 1977). Chromium trioxide induced depurination in calf thymus DNA by enhancing the release of guanine, whereas no effect was produced on the release of adenine (Schaaper *et al.*, 1987). Potassium dichromate induced breakage in the polynucleotide chain of purified DNA and RNA, as inferred from studies of viscosity, ultraviolet absorption spectra and thermal denaturation patterns (Tamino & Peretta, 1980; Tamino *et al.*, 1981). Sodium chromate induced single-strand breaks in supercoiled circular DNA of the bacterial phage PM2, but only when combined with glutathione. Of two purified reaction products, the chromium[V] complex $Na_4(glutathione)_4CrV.8H_2O$ cleared supercoiled PM2 DNA, whereas the final product, the chromium[III]-glutathione complex was inactive (Kortenkamp *et al.*, 1989). A positive (Tkeshelashvili *et al.*, 1980) and a negative result (Schaaper *et al.*, 1987) were reported for the

recovery of viral infectivity of single-stranded ϕX174 DNA which, following treatment with chromium trioxide, was transfected into *E. coli* spheroplasts (*am3* reversion). Potassium chromate induced *lacZ*α forward mutation in double-stranded M13mp2 DNA transfected into JM101 *E. coli* (Snow & Xu, 1989). Gel electrophoresis analysis demonstrated production of oligonucleotides from [^{32}P-5′]-end-labelled DNA fragments treated with sodium chromate only in the presence of hydrogen peroxide (Kawanishi *et al.*, 1986). Several of these studies showed that chromium[VI] compounds are less active than chromium[III] compounds in simplified systems (See General Remarks, p. 43, for a discussion of the biological activity of chromium[VI] and chromium[III] compounds.)

DNA-damaging effects were observed by treating bacteria with highly soluble chromium[VI] compounds. Thus, potassium dichromate produced DNA fragmentation in strain WP2 of *E. coli*; this effect, detected by alkaline sucrose gradient sedimentation, was attenuated by α-tocopherol (Kalinina & Minseitova, 1983a,b). λ Prophage was induced by potassium chromate in *E. coli* WP2_S (Rossman *et al.*, 1984). An SOS response, inferred from induction of an SOS operator gene coupled with a gene coding for β-galactosidase in *E. coli*, was elicited by sodium dichromate in strain PQ37 (De Flora *et al.*, 1985a), by potassium dichromate, potassium chromate and chromium trioxide in strains GC2375, UA4202 and PQ30 (Llagostera *et al.*, 1986), and by potassium dichromate and potassium chromate in strain PQ37 (Venier *et al.*, 1989) and in strains PQ35 and PQ37 (Olivier & Marzin, 1987). Potassium dichromate also showed SOS-inducing activity in strain TA1535/pSK1002 of *S. typhimurium* (Nakamura *et al.*, 1987).

As shown by means of various techniques (spot test, streak method, treat-and-plate test, liquid test), soluble chromium[VI] compounds induce nonreparable DNA damage in repair-deficient bacteria. In particular, potassium dichromate, sodium dichromate, ammonium dichromate, potassium chromate, sodium chromate, ammonium chromate and chromium trioxide were active in the *rec* assay in *B. subtilis* in strains H17 (wild-type) and M45 (*rec*$^-$) (Nishioka, 1975; Nakamuro *et al.*, 1978; Kada *et al.*, 1980; Kanematsu *et al.*, 1980; Matsui, 1980; Gentile *et al.*, 1981). Potassium dichromate, sodium dichromate, ammonium dichromate, sodium chromate and chromium trioxide were more toxic in strain TA1538 (*rec*$^-$) than in the parental strain (TA1978) of *S. typhimurium* (Gentile *et al.*, 1981). Potassium dichromate, ammonium dichromate and potassium chromate were equally toxic in wild strain WP2 and in WP2*uvrA* (*uvrA*-) but more toxic in CM571 (*recA*$^-$) and in WP100 (*uvrA*$^-$*recA*$^-$) than in WP2 (Yagi & Nishioka, 1977). Sodium dichromate was more toxic in TM1080 (*polA*$^-$ *lexA*$^-$ R factor) and CM871 (*uvrA*$^-$*recA*$^-$*lexA*$^-$) than in the *E. coli* wild strain (WP2). No lethality was observed in WP2*uvrA* (*uvrA*$^-$) or WP67 (*uvrA*$^-$*polA*$^-$) (Petrilli & De Flora, 1982). The lethality of sodium dichromate,

potassium chromate, ammonium chromate and chromium trioxide to the triple mutant CM871 (*uvrA⁻recA⁻lexA⁻*) was greater than to WP2; this was not the case for WP67 (*uvrA⁻polA⁻*) (De Flora *et al.*, 1984a). [The Working Group noted that these results indicate the importance of the *rec* and *lex* SOS functions, rather than of the polymerase mechanism and *uvr* excision repair system, in repairing DNA damage produced by chromium[VI] in bacteria.]

Reversion to luminescence (bioluminescence test) was induced by potassium dichromate in strain Pf-13 of *Photobacterium fischeri* (Ulitzur & Barak, 1988). Reversion to *arg* autotrophy was induced by potassium dichromate and potassium chromate in *E. coli* strain Hs30R (Nakamuro *et al.*, 1978), and by sodium chromate in K12-343-113(λ) (Mohn & Ellenberger, 1977). The ability of soluble chromium[VI] compounds to revert *E. coli* to *trp* auxotrophy was reported by Venitt and Levy (1974) for sodium chromate (strains WP2 and WP2*uvrA*) and potassium chromate (WP2, WP2*uvrA* and WP2*exrA*), by Nishioka (1975) for potassium dichromate (WP2 and WP2*uvrA*, CM871 being insensitive), by Green *et al.* (1976) for potassium chromate (WP2), by Nestmann *et al.* (1979) for chromium trioxide (WP2*uvrA*, but only in a fluctuation test), by Petrilli and De Flora (1982) for sodium dichromate (WP2 and WP2*uvrA*), by Venitt and Bosworth (1983) for potassium dichromate (WP2*uvrA*, further increased under anaerobic growth conditions) and by Venier *et al.* (1987) for potassium dichromate (WP2*uvrA*, further increased by NTA). The only negative result was reported by Kanematsu *et al.* (1980), who identified potassium dichromate as a mutagen (in WP2*hcr⁻* only, WP2 *try⁻* being insensitive) but failed to detect the mutagenicity of chromium trioxide (in either WP2 or WP2*hcr⁻*). Potassium chromate had no effect on ultraviolet-induced mutagenesis in WP2 (Rossman & Molina, 1986).

The mutagenicity of soluble chromium[VI] compounds in *his- S. typhimurium* and its modulation were investigated in a large number of laboratories, using various techniques (spot test, spiral test, plate test and preincubation test). A number of studies yielded positive results (Tamaro *et al.*, 1975; Petrilli & De Flora, 1977; De Flora, 1978; Petrilli & De Flora, 1978b; Nestmann *et al.*, 1979; De Flora, 1981a,b; Tso & Fung, 1981; Petrilli & De Flora, 1982; Venier *et al.*, 1982; Bennicelli *et al.*, 1983; Bianchi *et al.*, 1983; Beyersmann *et al.*, 1984; De Flora *et al.*, 1984a,b; Arlauskas *et al.*, 1985; Langerwerf *et al.*, 1985; Loprieno *et al.*, 1985; Marzin & Phi, 1985; LaVelle, 1986a,b; Vieux *et al.*, 1986 [Abstract]; Farrell *et al.*, 1989). Negative results were reported in all the strains tested in one study only (Kanematsu *et al.*, 1980) [doses were not reported]. Using the replicate plate technique, Pedersen *et al.* (1983) claimed that a high proportion of *S. typhimurium his*$^+$ revertant colonies were false, but this conclusion was criticized by Baker *et al.* (1984), using the same technique. In general, with the exception of TA1535, all *his S. typhimurium* strains tested were reverted by chromium[VI]. The following ranking of sensitivity was reported:

TA102 > TA100 > TA97 > TA92 > TA1978 > TA98 > TA1538 > TA1537 (Bennicelli *et al.*, 1983). Other sensitive strains included TA103 (Gava *et al.*, 1989b), TA104 (De Flora *et al.*, 1988; Gava *et al.*, 1989b), TA94 (Langerwerf *et al.*, 1985), TS26 (LaVelle, 1986a) and GV19 (LaVelle, 1986b). The nitroreductase-deficient derivative strain TA100NR was even more sensitive than TA100, which suggests a diminution of the mutagenicity of chromium[VI] by bacterial nitroreductases (De Flora *et al.*, 1989b). Reversion patterns indicate that chromium[VI] induces frameshift errors in bacterial DNA and, to a greater extent, base-pair substitution at both GC base-pairs (TA100) and AT base-pairs (TA102, TA104). The latter two strains are known to be sensitive to oxidative mutagens. In any case, the presence of plasmid pKM101 in the most sensitive strains indicates that the mutagenicity of chromium[VI] is amplified through error-prone DNA repair pathways, which is consistent with the results of the DNA-repair tests reported above. The potency of chromium[VI] compounds correlated with their chromium[VI] content, being in the range of a few revertants per nanomole chromium[VI] in TA100 and TA102 (De-Flora, 1981a; De Flora *et al.*, 1984a,b). Since the potency of mutagens of various chemical classes tested in the same laboratory varied between 2×10^{-6} and 1.4×10^{4} revertants/nmol (6.8×10^{9}-fold range), chromium[VI] compounds can be classified as mutagens of medium potency in this test system (De Flora *et al.*, 1984b).

The bacterial mutagenicity of potassium dichromate was also confirmed in forward mutation assay in *E. coli*, testing acquired resistance to rifampicin in AB1157 and derived *recA*$^-$, *recB*$^-$, *recC*$^-$, *recF*$^-$ and *sbc*$^-$ strains (Kalinina & Minseitowa, 1983b,c), *lacI*$^+$/*lacI*$^-$ mutation in strain KMBL3835 (Zakour & Glickman, 1984), and replication of integrated λ genes in strain CHY832 (RK test) (Hayes *et al.*, 1984). Chromium trioxide induced Ara^r forward mutation in strains BA9 and BA13 of *S. typhimurium* (Ruiz-Rubio *et al.*, 1985). Potassium chromate did not induce forward or back mutations in a fluctuation test with K-12-derived *E. coli* strains but enhanced the frameshift mutagenicity of 9-aminoacridine (LaVelle, 1986a).

Reducing chemicals (ascorbic acid and sodium sulfite) and glutathione, NADH and NADPH decreased the bacterial mutagenicity of various chromium[VI] compounds (Petrilli & De Flora, 1978b; De Flora *et al.*, 1985b; Petrilli *et al.*, 1986a). A similar reducing effect was induced by other sulfur compounds, such as *N*-acetylcysteine (De Flora *et al.*, 1984c), cysteine (Petrilli *et al.*, 1986a) and dithiothreitol (Rogers *et al.*, 1987 [Abstract]). The mutagenicity of potassium dichromate was also considerably decreased by anaerobic growth conditions, but not by addition of the hydroxyl ion scavenger mannitol (Rogers *et al.*, 1987 [Abstract]). The mutagenicity of soluble chromium[VI] compounds was not affected or was poorly affected by addition of soda ash, diethyl ether, prostaglandin or ethylenediaminetetraacetic acid (Petrilli & De Flora, 1982; Petrilli *et al.*, 1986a) or complex mixtures

(crude oil, oil dispersants) (Petrilli *et al.*, 1980; De Flora *et al.*, 1985a), whereas it was inhibited by other metals (Sokolowska & Jongen, 1984 [Abstract]). Sodium dichromate and unfractionated cigarette smoke condensate had antagonistic mutagenic effects (Petrilli & De Flora, 1982). The mutagenicity of potassium dichromate was increased by nitrilotriacetic acid (Gava *et al.*, 1989a). Potassium chromate decreased the mutagenicity of ethyl methanesulfonate and increased that of sodium azide (LaVelle & Witmer, 1984) and of its metabolite azidoalanine (LaVelle, 1986b); more than additive effects were observed with 9-aminoacridine (LaVelle, 1986a).

The mutagenicity of soluble chromium[VI] compounds in *S. typhimurium* was consistently decreased by rat liver post-mitochondrial supernatant in all studies in which this aspect was evaluated (De Flora, 1978; Löfroth, 1978; Petrilli & De Flora, 1978b; Nestmann *et al.*, 1979; Petrilli & De Flora, 1980; De Flora, 1981a; Petrilli & De Flora, 1982; Venier *et al.*, 1982; Bianchi *et al.*, 1983; De Flora *et al.*, 1984a; Loprieno *et al.*, 1985; Petrilli *et al.*, 1986b). The polychlorinated biphenyl Aroclor 1254 was the most efficient inducer of this effect, followed in activity by phenobarbital and 3-methylcholanthrene (Petrilli *et al.*, 1985). Pretreatment of rats with *N*-acetylcysteine also stimulated reduction of chromium[VI] by liver and lung post-mitochondrial supernatant (De Flora *et al.*, 1985c). The effect of the rat liver fraction on the mutagenicity of various chromium[VI] compounds was inhibited by dicoumarol, a specific inhibitor of the cytosolic enzyme DT diaphorase (De Flora *et al.*, 1987b); purified DT diaphorase itself decreased the mutagenicity of sodium dichromate (De Flora *et al.*, 1988). The mutagenicity of sodium dichromate was also decreased by liver preparations from other animal species, including fish (*Salmo gairdneri*) (De Flora *et al.*, 1982), chicken, hamster (De Flora *et al.*, 1985d), Pekin duck (De Flora *et al.*, 1989a), mouse (De Flora, 1982), woodchuck (De Flora *et al.*, 1987c, 1989c) and humans (De Flora, 1982). Moreover, mutagenicity was decreased by thermostable components of human gastric juice (De Flora & Boido, 1980; De Flora *et al.*, 1987a), with peaks of reducing activity during post-meal periods following stimulation of gastric secretion (De Flora *et al.*, 1987a). Human erythrocyte lysates decreased the mutagenicity of chromium[VI] (Petrilli & De Flora, 1978b); human and rat pulmonary alveolar macrophages were particularly efficient (Petrilli *et al.*, 1986c). Human peripheral lung parenchyma decreased the mutagenicity of chromium[VI] more efficiently than a post-mitochondrial supernatant of bronchial tree (Petruzzelli *et al.*, 1989); as assessed from 71 surgical specimens from cancer and noncancer patients, the ability of lung parenchyma to decrease the mutagenicity of chromium[VI] was significantly enhanced in cigarette smokers (De Flora *et al.*, 1987d). In rats, the inhibitory effect of lung was autoinduced by repeated intratracheal instillations of sodium dichromate (Petrilli *et al.*, 1985). Comparative assays provided evidence that the reducing capacity of rat tissue post-mitochondrial supernatant ranked as follows: liver > adrenal > kidney >

testis > stomach and lung; preparations of skeletal muscle, spleen and intestine had no effect on the mutagenicity of chromium[VI] (Petrilli & De Flora, 1978b, 1980, 1982). Further assays confirmed the negligible effect of rat muscle (which is a typical target of the carcinogenicity of chromium[VI] in bioassays) in decreasing the mutagenicity of chromium[VI], as compared to liver and, to a lesser extent, to cutis and subcutis (De Flora *et al.*, 1989a). The selective loss of chromium[VI] mutagenicity was accompanied by the disappearance of measurable chromium[VI] in the presence of various body fluids and cell and tissue preparations. [The Working Group interpreted these findings as indicating mechanisms that limit the activity of chromium[VI] compounds *in vivo*.]

Forward mutation and mitotic gene conversion were induced by potassium dichromate in the yeast *Schizosaccharomyces pombe* (Bonatti *et al.*, 1976). The same compound induced reversion ($ilv^- \rightarrow ilv^+$) and mitotic gene conversion in strain D7 of *Saccharomyces cerevisiae* (Singh, 1983; Galli *et al.*, 1985; Kharab & Singh, 1985). Conversely, chromium trioxide elicited gene conversion and mitotic crossing-over but failed to revert the same yeast strain (Fukunaga *et al.*, 1982). Potassium dichromate slightly enhanced recombination frequency in strain D1513 of *Saccharomyces cerevisiae* and produced disomic and diploid gametes (Sora *et al.*, 1986), but it did not induce mitochondrial 'petite' mutants in strain D7 (Kharab & Singh, 1987).

Neither potassium chromate nor chromium trioxide induced micronuclei in pollen mother cells of *Tradescantia paludosa* (Ma *et al.*, 1984). In contrast, further studies indicated a dose-dependent increase in the induction of micronuclei by chromium trioxide, which was significantly inhibited by cysteine (Zhang *et al.*, 1984).

In *Drosophila melanogaster*, sodium dichromate gave positive results in a somatic eye-colour test (*zeste* mutation) (Rasmuson, 1985). Both potassium dichromate and chromium trioxide induced sex-linked recessive lethal mutations, but only potassium dichromate induced non-disjunction and X-Y chromosome loss at a dose corresponding to the LD_{50} (Rodriguez-Arnaiz & Molina Martinez, 1986). The induction of sex-linked recessive lethal mutations by potassium dichromate was enhanced by NTA (Gava *et al.*, 1989b).

A variety of genetic and related endpoints were explored in cultured mammalian cells. Potassium dichromate produced alterations of the mitotic index and of mitotic phases in human epithelial-like heteroploid HEp-2 cells (Majone, 1977; Levis & Majone, 1981) and NHIK 3025 cervix tissue cells (Bakke *et al.*, 1984), and imbalance of the endogenous adenylate pool in Syrian hamster BHK fibroblasts (Levis *et al.*, 1978b; Bianchi *et al.*, 1982a). It inhibited DNA synthesis, as evaluated by ^{3}H-thymidine incorporation, in mouse L cells (Nishio & Uyeki, 1985), in BHK fibroblasts and in HEp-2 cells (Levis *et al.*, 1977, 1978a,b), in which a secondary inhibition of RNA and protein syntheses was also observed (Levis *et al.*, 1978b).

Inhibition of DNA synthesis was further enhanced following reversible permeabilization of cells in hypertonic medium (Bianchi *et al.*, 1984). Potassium dichromate also reduced the colony-forming ability of BHK cells by a multi-hit mechanism of cell inactivation (Levis *et al.*, 1978a). Unscheduled DNA synthesis was induced by potassium dichromate in mouse kidney A18BcR cells (Raffetto *et al.*, 1977) but not in human EUE heteroploid cells (Bianchi *et al.*, 1982b, 1983); potassium chromate induced unscheduled DNA synthesis in human skin fibroblasts (Whiting *et al.*, 1979). Chromium trioxide inhibited repair of γ-ray-induced chromosome breaks in human peripheral blood lymphocytes (Morimoto & Koizumi, 1981).

DNA fragmentation and cross-links were produced by soluble chromium[VI] compounds in a number of cultured mammalian cell lines, as assessed by various techniques, including alkaline elution, alkaline sucrose gradient, nucleoid sedimentation, alkaline unwinding, the nick translation assay, and polyacrylamide gel electrophoresis (Table 25). An exception was a study by alkaline elution in Chinese hamster V79 cells with potassium dichromate (Bianchi *et al.*, 1983).

In the *hprt* forward mutation assay, potassium chromate did not induce 8-azaguanidine–resistant mutants in mouse mammary carcinoma FM3A cells, in contrast to the activity of potassium dichromate and chromium trioxide in the same system (Nishimura & Umeda, 1978 [Abstract]). An unspecified chromate induced 6-thioguanine resistance in Chinese hamster V79 cells (Beyersmann & Köster, 1987). Potassium dichromate induced 8-azaguanidine resistance and 6-thioguanidine resistance in Chinese hamster V79 cells (Newbold *et al.*, 1979; Rainaldi *et al.*, 1982; Paschin *et al.*, 1981; Bianchi *et al.*, 1983); its mutagenic activity was decreased by thiotepa (Paschin & Kozachenko, 1982), unaffected by nitrilotriacetic acid (Celotti *et al.*, 1987) and enhanced by nickel[II] (Hartwig & Beyersmann, 1987). In comparative assays, Chinese hamster V79 cells were found to be more sensitive to chromium[VI] than Chinese hamster CHO cells (Paschin *et al.*, 1983). The combined use of selective 8-azaguanidine-resistant and ouabain-resistant systems showed that potassium dichromate can also induce base-pair substitutions in the DNA of V79 cells (Rainaldi *et al.*, 1982). Potassium dichromate and potassium chromate induced forward mutation at the thymidine kinase locus in mouse lymphoma L5178Y cells (Oberly *et al.*, 1982). As assessed in an assay for the synthesis of P-100$^{gag\text{-}mos}$ viral proteins, sodium chromate induced expression of the *v-mos* gene in MuSVts110-infected rat kidney 6m2 cells (Biggart & Murphy, 1988).

Soluble chromium[VI] compounds consistently increased the frequency of sister chromatid exchange (Table 26). The highest frequency of induction was observed in the early S-phase of the human lymphocyte cycle (Stella *et al.*, 1982).

Table 25. Studies in which DNA fragmentation and DNA–DNA and DNA–protein cross-linking were induced in cultured mammalian cells by soluble chromium[VI] compounds

Chromium compound	Cell line	Comment	Reference
Potassium dichromate	Mouse L1210 leukaemia		Fornace *et al.* (1981)
	Novikoff ascitic hepatoma		Wedrychowski *et al.* (1986a)
	Chinese hamster CHO		Brambilla *et al.* (1980) [Abstract]; Hamilton-Koch *et al.* (1986)
	Human foreskin HSBP fibroblasts	Detected by alkaline sucrose gradient but not nick translation, nucleoid sedimentation or alkaline unwinding Enhanced by glutathione, unaffected by hydroxyl radical scavengers (mannitol, iodine), diminished by superoxide dismutase and catalase	Hamilton-Koch *et al.* (1986); Snyder (1988)
	Human white blood		McLean *et al.* (1982)
Sodium dichromate	Rat primary hepatocytes		Sina *et al.* (1983)
Potassium chromate	Mouse L1210 leukaemia		Fornace *et al.* (1981)
	Chinese hamster CHO fibroblasts	DNA–protein cross-linkage, probably due to chromium[III]	Miller & Costa (1988, 1989)
	Human embryo lung IMR-90 fibroblasts		Fornace *et al.* (1981)
	Human skin fibroblasts	At 7th–8th passage	Whiting *et al.* (1979)
	Human CRL1223 fibroblasts		Fornace (1982)
	Human AG1522 fibroblasts		Fornace (1982)
	Human XP12BE fibroblasts	Similar effect in normal xeroderma pigmentosum cells	Fornace (1982)
	Novikoff ascitic hepatoma		Wedrychowski *et al.* (1986b)
	Human bronchial epithelium		Fornace *et al.* (1981)

Table 25 (contd)

Chromium compound	Cell line	Comment	Reference
Sodium chromate	Chick embryo hepatocytes	Related to glutathione and cytochrome P450 metabolism	Tsapakos *et al.* (1983b); Cupo & Wetterhahn (1984, 1985b)
	Chinese hamster V79	Decreased by α-tocopherol; increased by riboflavin and sodium sulfite; DNA-protein breaks recognized by poly(ADT-ribose)polymerase	Sugiyama *et al.* (1987, 1988)
Chromium trioxide	Novikoff ascitic hepatoma		Wedrychowski *et al.* (1986a)

Table 26. Studies in which sister chromatid exchange was induced in cultured mammalian cells by chromium[VI] compounds

Chromium compound	Cell line	Comment[a]	Reference
Potassium dichromate	Mouse lymphocytes LSTRA		Bianchi *et al.* (1983)
	BALB mouse primary lymphocytes	BALB cells carrying endogenized Moloney leukaemia virus more sensitive than uninfected cells	Bianchi *et al.* (1983); Majone *et al.* (1983)
	Mouse macrophage $P388D_1$		Andersen (1983)
	Mouse embryo blastocytes		Iijima *et al.* (1983b)
	Chinese hamster CHO		Levis & Majone (1979); Majone & Levis (1979); Bianchi *et al.* (1980); Levis & Majone (1981); Majone *et al.* (1982); Venier *et al.* (1982); Bianchi *et al.* (1983); Uyeki & Nishio (1983); Loprieno *et al.* (1985); Montaldi *et al.* (1987b)
	Chinese hamster V79		Rainaldi *et al.* (1982)
	Chinese hamster lung Don		Ohno *et al.* (1982)
	Syrian hamster BHK fibroblasts	Increased in permeabilized cells	Bianchi *et al.* (1984)
	Human peripheral blood lymphocytes		Ogawa *et al.* (1978); Gómez-Arroyo *et al.* (1981); Imreh & Radulescu (1982 [Abstract]); Stella *et al.* (1982); Andersen (1983)
	Human skin fibroblasts		Macrae *et al.* (1979)
Sodium dichromate	Chinese hamster CHO		Levis & Majone (1979); Majone & Levis (1979); Bianchi *et al.* (1980)
	Chinese hamster V79		Elias *et al.* (1983)
Potassium chromate	Chinese hamster CHO		Levis & Majone (1979); Macrae *et al.* (1979); Majone & Rensi (1979); Bianchi *et al.* (1980); Majone *et al.* (1982)
	Chinese hamster V79		Price-Jones *et al.* (1980); Elias *et al.* (1983)

Table 26 (contd)

Chromium compound	Cell line	Comment[a]	Reference
Potassium chromate (contd)	Chinese hamster lung Don		Ohno *et al.* (1982)
	Human skin fibroblasts		Macrae *et al.* (1979)
	Human peripheral blood lymphocytes		Douglas *et al.* (1980)
Sodium chromate	Chinese hamster CHO		Levis & Majone (1979); Bianchi *et al.* (1980)
	Chinese hamster V79		Elias *et al.* (1983)
Chromium trioxide	Chinese hamster CHO		Levis & Majone (1979); Bianchi *et al.* (1980)
	Chinese hamster lung Don		Koshi (1979); Ohno *et al.* (1982)
	Human peripheral blood lymphocytes		Gómez-Arroyo *et al.* (1981)
Calcium chromate	Chinese hamster CHO	Increased in presence of NTA	Venier *et al.* (1985b); Sen & Costa (1986)
Lead chromate	Human peripheral blood lymphocytes	Dissolved in NaOH	Douglas *et al.* (1980)
Strontium chromate	Chinese hamster CHO	Increased in presence of NTA	Venier *et al.* (1985b)
Zinc chromates	Chinese hamster CHO	Increased in presence of NaOH or NTA	Levis & Majone (1981); Venier *et al.* (1985b)
	Chinese hamster V79		Elias *et al.* (1983)
Basic zinc chromates	Chinese hamster CHO	Increased in presence of NaOH or NTA	Levis & Majone (1981); Venier *et al.* (1985b)
Zinc chromate	Chinese hamster CHO	Increased in presence of NTA	Venier *et al.* (1985b)
Barium chromate	Chinese hamster CHO	Increased in presence of NTA	Venier *et al.* (1985b)

Table 26 (contd)

Chromium compound	Cell line	Comment[a]	Reference
Lead chromate	Chinese hamster CHO	Increased in presence of NTA	Montaldi *et al.* (1987a,b)
	Chinese hamster CHO	Increased in presence of NTA	Loprieno *et al.* (1985)
	Human peripheral blood lymphocytes	Dissolved in NaOH	Douglas *et al.* (1980)
Chromium yellow	Chinese hamster CHO	Increased in presence of NTA	Venier *et al.* (1985b)
	Chinese hamster CHO	Increased in presence of NaOH	Levis & Majone (1981)
Chromium orange	Chinese hamster CHO	Increased in presence of NaOH	Levis & Majone (1981)
	Chinese hamster CHO		Loprieno *et al.* (1985)
Molybdenum orange	Chinese hamster CHO	Increased in presence of NTA	Venier *et al.* (1985b)
	Chinese hamster CHO	Increased in presence of NaOH	Levis & Majone (1981)

[a]NTA, nitrilotriacetic acid

As reported in an abstract, potassium dichromate also increased the frequency of micronucleated cells in human lymphocytes cultured *in vitro* (Imreh & Radulescu, 1982).

In many studies, the induction of chromosomal aberrations was investigated, often in parallel with assessments of the frequency of sister chromatid exchange; all of them gave positive results (Table 27). Chromatid-type aberrations, mainly gaps, breaks and chromatid exchanges, were the most frequently reported aberrations (Levis & Bianchi, 1982).

Two studies dealt with the induction of aneuploidy by soluble chromium[VI] salts in cultured mammalian cells: no increase in the number of aneuploids or polyploids was detected following treatment of Chinese hamster V79 cells with potassium chromate (Price-Jones *et al.*, 1980). Sodium chromate, however, exhibited spindle-modifying properties in human skin fibroblasts, as assessed by means of a differential staining technique for chromosomes and spindles, alterations in which may represent one of the major causes of aneuploidy (Nijs & Kirsch-Volders, 1986).

The majority of studies provided evidence that soluble chromium[VI] salts can induce cell transformation in different experimental systems. In particular, as evaluated by means of the soft agar assay, potassium dichromate produced anchorage-independent growth of Syrian hamster BHK fibroblasts (Bianchi *et al.*, 1983; Hansen & Stern, 1985), which was further enhanced in the presence of NTA (Lanfranchi *et al.*, 1988). [See General Remarks, p. 44, for concern about this assay.] The same compound induced morphological transformation of Syrian hamster embryo (SHE) primary cells (Tsuda & Kato, 1977; Hansen & Stern, 1985) and of mouse fetal cells at the third passage (Raffetto *et al.*, 1977), whereas a negative result was reported in mouse embryo C3H10T1/2 cells (Patierno *et al.*, 1988). [See General Remarks, p. 44, for concerns about this assay.] Sodium chromate also induced morphological transformation of SHE primary cells (DiPaolo & Casto, 1979), but potassium chromate did not, although it potentiated the transforming capacity of benzo[*a*]pyrene (Rivedal & Sanner, 1981); it also enhanced the morphological transformation induced by the simian adenovirus SA7 in SHE primary cells (Casto *et al.*, 1979).

Several studies were also carried out with soluble chromium[VI] compounds *in vivo*. Following intraperitoneal injection to Sprague-Dawley rats, sodium dichromate induced a selective DNA fragmentation in different tissues, as assessed by means of the alkaline elution technique. In particular, liver nuclei contained protein-associated DNA single-strand breaks as well as DNA-protein cross-links, whereas kidney nuclei contained mainly DNA-protein cross-links (Tsapakos *et al.*, 1981) and lung nuclei contained both DNA interstrand and DNA-protein cross-links (Tsapakos *et al.*, 1983a). These lesions were repaired most rapidly in the liver, which may provide a partial explanation of the differential toxicity of

Table 27. Studies in which chromosomal aberrations were induced in cultured mammalian cells by chromium[VI] compounds

Chromium compound	Cell line	Comment[a]	Reference
Potassium dichromate	Mouse tertiary fetal		Raffetto *et al.* (1977)
	Mouse mammary carcinoma Fm3A		Umeda & Nishimura (1979)
	Rat peripheral blood lymphocytes		Newton & Lilly (1986)
	Rat embryo fibroblasts		Bigaliev *et al.* (1977a)
	Syrian hamster embryo primary	Inhibited by sodium sulfite	Tsuda & Kato (1977)
	Chinese hamster CHO		Levis & Majone (1979); Majone & Levis (1979); Bianchi *et al.* (1980); Levis & Majone (1981); Venier *et al.* (1982)
	Chinese hamster V79		Newbold *et al.* (1979)
	Human peripheral blood lymphocytes		Nakamuro *et al.* (1978); Imreh & Radulescu (1982 [Abstract]); Stella *et al.* (1982)
Sodium dichromate	Chinese hamster CHO		Levis & Majone (1979); Majone & Levis (1979); Bianchi *et al.* (1980)
	Human peripheral blood lymphocytes		Sarto *et al.* (1980)
Potassium chromate	Mouse mammary carcinoma Fm3A		Umeda & Nishimura (1979)
	Chinese hamster CHO		Levis & Majone (1979); Majone & Rensi (1979); Bianchi *et al.* (1980)
	Chinese hamster lung Don		Koshi & Iwasaki (1983)
	Human skin fibroblasts		Macrae *et al.* (1979)
	Human peripheral blood lymphocytes		Nakamuro *et al.* (1978); Douglas *et al.* (1980)
Sodium chromate	Chinese hamster CHO		Levis & Majone (1979); Bianchi *et al.* (1980)

Table 27 (contd)

Chromium compound	Cell line	Comment[a]	Reference
Chromium trioxide	Mouse mammary carcinoma Fm3A		Umeda & Nishimura (1979)
	Syrian hamster embryo primary		Tsuda & Kato (1977)
	Chinese hamster CHO		Levis & Majone (1979); Bianchi *et al.* (1980)
	Chinese hamster lung Don		Koshi (1979)
	Human peripheral blood lymphocytes		Kaneko (1976)
Calcium chromate	Mouse embryo C3H10T1/2	Random damage	Sen *et al.* (1987)
	Chinese hamster CHO	Random damage	Levis & Majone (1979); Sen *et al.* (1987)
	Chinese hamster lung Don		Koshi & Iwasaki (1983)
Basic zinc chromates	Chinese hamster CHO	Increased in presence of NaOH	Levis & Majone (1981)
Zinc chromate	Chinese hamster lung Don		Koshi & Iwasaki (1983)
Lead chromate	Chinese hamster CHO	Increased in presence of NaOH or NTA	Levis & Majone (1981); Montaldi *et al.* (1987b)
	Chinese hamster lung Don		Koshi & Iwasaki (1983)
	Human peripheral blood lymphocytes	Increased in presence of NaOH	Douglas *et al.* (1980)

[a]NTA, nitrilotriacetic acid

chromium[VI] in these organs (Tsapakos *et al.*, 1983a). Following its injection onto the inner shell membrane of eggs, sodium dichromate produced single-strand breaks in blood cells and DNA cross-links in liver cells of chicken embryos (Hamilton & Wetterhahn, 1986). Intraperitoneally injected potassium dichromate inhibited DNA repair synthesis in rat lymphocytes (Rudnykh & Saichenko, 1985); it was active in a mammalian spot test in C57Bl/6J/BOM mice, but only when administered at 10 mg/kg and not at 20 mg/kg (Knudsen, 1980).

Intraperitoneal injection of potassium dichromate or potassium chromate to Chinese hamsters induced sister chromatid exchange in bone-marrow cells and an increased frequency of micronucleated polychromatic erythrocytes (Kaths, 1981). Micronucleated polychromatic erythrocytes were also enhanced by potassium dichromate in BALB/c mice (Fabry, 1980) and in CBA × C57Bl/6J mice (Paschin & Toropsev, 1982, 1983) and by potassium chromate in NMRI mice (Wild, 1978), ms and ddY mice, the former strain being more sensitive (Hayashi *et al.*, 1982). Comparative trials in various mouse strains showed no important sex-related variation in induction of micronucleated cells by chromium[VI] and confirmed the different susceptibilities of different strains (rank of sensitivity: ms > BDF1 > CD-1 > ddY) (Collaborative Study Group for the Micronucleus Test, 1986, 1988).

Chromosomal aberrations were induced in gill tissue cells of *Boleophthalmus dussumieri* fish by intramuscular injection or addition to water of sodium dichromate (Krishnaja & Rege, 1982). Potassium dichromate induced chromosomal rearrangements and aneuploidy in rat bone-marrow cells when given orally or intratracheally (Bigaliev *et al.*, 1977b). Following intraperitoneal or intravenous injection, it produced chromosomal aberrations in lymphocytes and bone-marrow cells (Newton & Lilly, 1986). Intraperitoneal injection of potassium dichromate was also clastogenic to bone-marrow cells of BALB/c mice (Léonard & Deknudt, 1981 [Abstract]), but no increase in chromosomal aberrations was observed in CBA × C57Bl/6J hybrid mice (Paschin *et al.*, 1981). In the same animals, dominant lethal effects were produced at 2 mg/kg bw (21 doses) and 20 mg/kg bw (single dose) (Paschin *et al.*, 1982) but not at 0.5-1.5 mg/kg bw (single dose) (Paschin *et al.*, 1981). At 20 mg/kg bw, potassium dichromate reduced the rate of pregnancies in BALB/c mice (Léonard & Deknudt, 1981 [Abstract]; Deknudt, 1982 [Abstract]) but failed to produce dominant lethal effects (Léonard & Deknudt, 1981 [Abstract]).

Calcium chromate, strontium chromate, basic zinc chromate

Calcium chromate, strontium chromate and the industrial product, basic zinc chromate or zinc yellow [$ZnCrO_4.Zn(OH)_2$ plus 10% CrO_3], are generally completely dissolved in the media used in short-term tests. The results obtained in a variety of experimental systems thus virtually overlap with those reported for highly soluble chromium[VI] compounds.

Calcium chromate failed to induce an SOS response in strain PQ37 of *E. coli* (Brams *et al.*, 1987). In contrast, it was active in a differential killing assay in *E. coli* WP2, using the triple mutant CM871 (*uvrA*⁻ *recA*⁻ *lexA*⁻) (De Flora *et al.*, 1984a), and in the *trp*⁻ → *trp*⁺ reversion test with strains WP2 (Venitt & Levy, 1974) and WP2*uvrA* (Dunkel *et al.*, 1984). In the *his*⁻ → *his*⁺ reversion test in *S. typhimurium*, positive results were reported with calcium chromate (Petrilli & De Flora, 1977; De Flora, 1981a; Bennicelli *et al.*, 1983; Haworth *et al.*, 1983; De Flora *et al.*, 1984a, 1987b; Dunkel *et al.*, 1984; Petrilli *et al.*, 1985; Venier *et al.*, 1985b), strontium chromate (Venier *et al.*, 1985b) and zinc yellow (Petrilli & De Flora, 1978b; De Flora, 1981a). The potency, spectrum of sensitivity of *S. typhimurium* strains and behaviour in the presence of in-vitro metabolic systems were comparable to those reported for highly soluble chromium[VI] compounds. However, toxic effects of calcium chromate hampered the detection of forward and back mutations in the DNA of phage T4 grown in *E. coli* BB (Corbett *et al.*, 1970).

Calcium chromate induced 'petite' mutants in mitochondria of 19 haploid strains of *Saccharomyces cerevisiae* (Egilsson *et al.*, 1979) and produced differential chromosome breakage in excision-repair-deficient females of *Drosophila melanogaster* (*mei-9*ᵃ test), with complete loss of the X or Y and partial loss of the Y chromosome (Zimmering, 1983).

Zinc yellow produced alterations of the mitotic cycle in human epithelial-like heteroploid HEp-2 cells (Levis & Majone, 1981). Calcium chromate stimulated DNA repair replication in Syrian hamster embryo primary cells, as evaluated by caesium chloride gradient density sedimentation (Robison *et al.*, 1984). It also produced DNA single-strand breaks, DNA interstrand and DNA-protein cross-links (alkaline elution technique) in mouse embryo C3H10T1/2 cells, Chinese hamster CHO cells and human osteosarcoma cells, the maximal sensitivity being recorded in early S-phase (Sugiyama *et al.*, 1986a,b); human cells were more sensitive than mouse or hamster cells (Sugiyama *et al.*, 1986b). In Chinese hamster CHO cells, calcium chromate produced single-strand breaks, induced alkali-labile sites (Cantoni & Costa, 1984) and, as assessed by alkaline sucrose gradient, decreased the DNA molecular weight (Robison *et al.*, 1982). DNA cross-links were more pronounced and only partially repaired in a repair-deficient line (EM9) as compared with CHO wild-type cells (AA8). Conversely, repair of single-strand breaks was similar in the two cell lines (Christie *et al.*, 1984). Calcium chromate produced strand breaks, detected by nucleoid gradient sedimentation, when applied to intact cells, but no breakage was observed when nucleoids were exposed directly to chromium[VI] (Robison *et al.*, 1984).

Calcium chromate induced forward mutation at the thymidine kinase locus in mouse lymphoma L5178Y cells, with no change (Myhr & Caspary, 1988) or decreased activity (McGregor *et al.*, 1987; Mitchell *et al.*, 1988) in the presence of rat

liver post-mitochondrial supernatant. This salt induced dose-dependent cytotoxicity and forward mutation to 6-thioguanine resistance in Chinese hamster CHO cells but no mutation to ouabain resistance in the same cells or in mouse embryo C3H10T1/2 cells (Patierno *et al.*, 1988).

The frequency of sister chromatid exchange was increased in Chinese hamster CHO cells by all three compounds (Table 26). In contrast to nickel, no predominance of sister chromatid exchange was observed in heterochromatic regions (Sen & Costa, 1986). Chromosomal aberrations, with a random distribution of chromosomal damage, were produced by calcium chromate and zinc yellow (Table 27). Aberrant division patterns and spindle modifications were also caused by calcium chromate in human skin fibroblasts (Nijs & Kirsch-Volders, 1986).

Several authors reported that calcium chromate could determine cell transformation *in vitro* in different systems: anchorage-independent growth of Syrian hamster BHK fibroblasts in carboxymethylcellulose, after several passages of treated cells (Fradkin *et al.*, 1975) or in soft agar (Bianchi *et al.*, 1983; Hansen & Stern, 1985), with an enhancing effect in the presence of NTA (Lanfranchi *et al.*, 1988), attachment-independence of virus-infected rat embryo 2 FR_4 50 cells (Traul *et al.*, 1981) [see General Remarks for concern about this assay], morphological transformation of mouse BALB/3T3 cells, R-MulV-infected rat embryo cells and Syrian hamster embryo primary cells (Dunkel *et al.*, 1981); and enhancement of morphological transformation in the same cells by the simian adenovirus SA7 (Casto *et al.*, 1979). As reported for potassium dichromate, calcium chromate did not induce morphological transformation in mouse embryo C3H10T1/2 cells (Patierno *et al.*, 1988).

Conflicting results were reported in studies *in vivo* with calcium chromate. It increased the frequency of sister chromatid exchange in bone-marrow cells of intraperitoneally injected Chinese hamsters (Kaths, 1981), whereas it failed to increase micronuclei in bone-marrow polychromatic erythrocytes of intraperitoneally injected BALB/c mice (Fabry, 1980) or Chinese hamsters (Kaths, 1981), and did not induce dominant lethal mutations in BALB/c mice (Léonard & Deknudt, 1981 [Abstract]).

Zinc chromate, barium chromate, lead chromate and derived pigments (chromium orange, chromium yellow and molybdenum orange)

An extensive data base is available concerning chromium[VI] compounds with poor solubility under the conditions used in experimental systems. These are zinc chromate, chromium orange or basic lead chromate [($PbCrO_4.PbO$)], molybdenum orange ($PbCrO_4.PbSO_4.PbMoO_4$), barium chromate, chromium yellow ($PbCrO_4.PbSO_4.SiO_2.Al_2O_3$) and lead chromate, which is one of the most insoluble salts. As is to be expected, their activity in short-term tests was related to the availability of chromate to target cells, which was often achieved by artificial solubiliza-

tion in acids or alkali, except in mammalian cells, where some penetration of insoluble compounds is likely to occur by phagocytosis.

Lead chromate did not induce differential killing in *E. coli* W3110 or P3478 (*polA*⁻), even when dissolved in sodium hydroxide (Nestmann *et al.*, 1979); this result parallels those reported with soluble chromium[VI] compounds in *polA*⁻ strains. It was equally toxic in WP2 and in CM871 (*uvrA*⁻ *recA*⁻ *lexA*⁻), unless dissolved in NTA (Venier *et al.*, 1987). Lead chromate did not elicit the SOS response in *E. coli* PQ37, unless it was solubilized by NTA (Venier *et al.*, 1989).

Lead chromate reverted *E. coli* ($trp^- \rightarrow trp^+$), when assayed in a fluctuation test after preliminary solubilization in sodium hydroxide (Nestmann *et al.*, 1979) and in both the spot test and a fluctuation test when dissolved in NTA (Venier *et al.*, 1987). In the $his^- \rightarrow his^+$ reversion test in *S. typhimurium*, zinc chromate was active in aqueous medium, and its mutagenicity was increased in the presence of sodium hydroxide or NTA (Venier *et al.*, 1985b). Chromium orange was mutagenic when spotted directly on the centre of agar plates and also became active in the plate test when dissolved in sodium hydroxide (Petrilli & De Flora, 1978b; De Flora, 1981a) or in NTA (Venier *et al.*, 1985b; Loprieno *et al.*, 1985). Likewise, molybdenum orange was mutagenic when spotted in solid form and in the plate test when dissolved in sodium hydroxide (De Flora, 1981a). Barium chromate was inactive unless dissolved in NTA (Venier *et al.*, 1985b). Lead chromate, tested following solubilization in acid or alkali, was mutagenic to the same strains that are sensitive to soluble chromium[VI] compounds (Nestmann *et al.*, 1979; Petrilli & De Flora, 1982). When tested in aqueous suspension, it was not mutagenic, but mutagenic chromate was released when it was dissolved in sodium hydroxide or NTA (Loprieno *et al.*, 1985; Venier *et al.*, 1985b, 1987). Its inactivity in strain TA102 was unaffected by the presence of oil dispersants (De Flora *et al.*, 1985a). Highly insoluble chromium yellow was inactive even when spotted in solid form; it became mutagenic in the plate test only when dissolved in sodium hydroxide (De Flora, 1981a; Petrilli & De Flora, 1982). In the gal^+/gal^- forward mutation test in strain K-12/343/113 (λ) of *E. coli*, lead chromate was inactive even when dissolved in sodium hydroxide (Nestmann *et al.*, 1979).

Lead chromate, dissolved in hydrochloric acid, induced mitotic recombination in strain D5 of *Saccharomyces cerevisiae*; the effect was decreased in the presence of rat liver post-mitochondrial supernatant (Nestmann *et al.*, 1979). It induced sex-linked recessive lethal mutations in *Drosophila melanogaster* only when dissolved in NTA (Costa *et al.*, 1988).

Alterations in the mitotic cycle were induced by the lead chromate-containing pigments, chromium orange, molybdenum orange and chromium yellow, in human epithelial-like heteroploid HEp-2 cells following a 48-h incubation in cell growth medium (Levis & Majone, 1981). Lead chromate, even when dissolved in sodium

hydroxide, did not induce DNA fragmentation in Chinese hamster CHO cells, as evaluated by alkaline sucrose gradient (Douglas *et al.*, 1980), and it was not mutagenic in these cells, as evaluated in both 6-thioguanine- and ouabain-resistant systems; it did not induce ouabain or 6-thioguanine resistance in mouse embryo C3H10T1/2 cells (Patierno *et al.*, 1988). In the *hprt* assay in Chinese hamster V79 cells, lead chromate gave negative results both for 8-azaguanine resistance (Newbold *et al.*, 1979) and 6-thioguanine resistance, unless it was dissolved in NTA (Celotti *et al.*, 1987).

In aqueous suspension, all of these poorly soluble chromium[VI] compounds induced sister chromatid exchange in mammalian cells (Table 26). In human peripheral blood lymphocytes, lead chromate also induced micronuclei, with an enhancing effect following addition of an equimolar concentration of NTA (Montaldi *et al.*, 1987b). Aqueous suspensions of lead chromate, of all three derived pigments and of zinc chromate were clastogenic in mammalian cells (Table 27).

Zinc chromate and lead chromate induced anchorage-independent growth of Chinese hamster BHK fibroblasts in the soft agar assay (Hansen & Stern, 1985). [See General Remarks, p. 44, for concern about this assay.] Only lead chromate (which was phagocytized) induced morphological transformation in mouse embryo C3H10T1/2 cells, which contrasted with the lack of transforming ability of potassium dichromate, calcium chromate and strontium chromate observed in the same study (Patierno *et al.*, 1988). Both lead chromate (Casto *et al.*, 1979; Hatch & Anderson, 1986) and zinc chromate (Casto *et al.*, 1979) enhanced viral transformation in Syrian hamster embryo primary cells.

Lead chromate increased the frequency of micronuclei in polychromatic erythrocytes and decreased the polychromatic/normochromatic erythrocyte ratio in bone-marrow cells of intraperitoneally treated C57Bl/6N mice (Watanabe *et al.*, 1985).

Chromyl chloride

Chromyl chloride [Cl_2CrO_2], a volatile liquid chromium[VI] compound, reverted *his*$^-$ *S. typhimurium* in the plate test; its potency, the spectrum of sensitivity of bacterial strains and the attenuating effect of rat liver post-mitochondrial supernatant were similar to those seen for soluble chromium[VI] compounds. Moreover, as assessed by suitable modifications of the standard *Salmonella* test, its vapours were also mutagenic (De Flora *et al.*, 1980; De Flora, 1981a).

(iv) *Other chromium compounds*

The water-soluble chromium[II] salt, chromous chloride [$CrCl_2$], which readily oxidizes to chromium[III] in contact with air, induced infidelity of DNA synthesis, with poly[d(A-T)] as a template in the presence of avian myeloblastosis virus DNA polymerase (Sirover & Loeb, 1976). Chromium[II] was inactive, however, in

all assays with cellular systems, including production of DNA fragmentation in Novikoff ascites hepatoma cells (Wedrychowski *et al.*, 1986a), of chromosomal aberrations and sister chromatid exchange in Syrian hamster embryo primary cells (Tsuda & Kato, 1977), and of aneuploidy in human skin fibroblasts (Nijs & Kirsch-Volders, 1986).

Chromium carbonyl [$Cr(CO)_6$], a hexacoordinated compound with oxidation state 0 (dissolved in ether due to its insolubility in water), was inactive in a differential killing test in *E. coli* (WP2 *vs.* WP67 and CM871) (De Flora *et al.*, 1984a) and in the reversion test in various *his*$^-$ *S. typhimurium* strains (De Flora, 1981a; De Flora *et al.*, 1984a).

In contrast to a purple, anionic chromium[III]-glutathione complex, a green sodium chromium[V]-glutathione complex ($Na_4(GSH)_4Cr(V).8H_2O$) cleaved super-coiled DNA of the bacteriophage PM_2 (Kortenkamp *et al.*, 1989). Similarly, in contrast to chromium[VI] and [III], the chromium[V] complex *trans*-bis[2-ethyl-2-hydroxybutanoato(2-)]oxochromate[V] cleaved covalently closed, circular plasmid puc9 DNA. In addition, it reverted strain TA100 of *S. typhimurium* with a potency comparable to that of potassium dichromate (Farrell *et al.*, 1989).

3.3 Other relevant data in humans

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

(i) *Chromium[III] compounds*

More than 99% of administered chromium was recovered in faeces following oral administration of chromic chloride to humans; about 94% was recovered after duodenal administration. In both cases, about 0.5% was excreted in urine (Donaldson & Barreras, 1966). After exposure to chromium[III] by inhalation, urinary concentrations of chromium were somewhat increased, indicating respiratory absorption (Aitio *et al.*, 1984; Foa *et al.*, 1988). Pulmonary uptake of chromium[III] is influenced by the nature of the compound; uptake and excretion of chromium[III] lignosulfonate dust by industrial workers was similar to that of water-soluble chromium[VI] (Kiilunen *et al.*, 1983). A study of tannery workers indicated two half-times — one in the order of hours, the other in the order of several days — for urinary excretion of chromium[III] (Aitio *et al.*, 1988).

After one volunteer had immersed his hand in tanning liquor for 1 h, monitoring of blood and urine for 24 h failed to detect dermal absorption of chromic sulfate (Aitio *et al.*, 1984). However, a fatal chromium intoxication, due to skin absorption, was described after accidental submersion of a worker in hot (70°C) chromic sulfate tanning liquor (Kelly *et al.*, 1982).

(ii) *Chromium[VI] compounds*

Following oral administration of sodium chromate in tracer doses to humans, faecal excretion of chromium indicated that about 10% of the administered dose had been absorbed from the gastrointestinal tract. After duodenal administration, approximately half of the administered radioactivity appeared to have been absorbed on the basis of faecal excretion, while 10% appeared in the urine during the first 24 h. Reduction of chromium[VI] to the trivalent form was demonstrated (Donaldson & Barreras, 1966). Circadian monitoring showed post-meal peaks of chromium[VI] reducing activity that may correspond to several tens of milligrams per day (De Flora *et al.*, 1987a).

Correlation between respiratory exposure to chromium[VI] and urinary excretion of chromium has been demonstrated in welders and in workers in the plating industry (Lindberg & Vesterberg, 1983; Aitio *et al.*, 1988). The respiratory uptake rate is unknown, but it depends on the solubility of the chromium compound (for review, see Aitio *et al.*, 1988). Chromium[VI] is reduced in the lower respiratory tract by the epithelial lining fluid and by pulmonary alveolar macrophages. At equivalent numbers of cells, the reducing efficiency of alveolar macrophages by biochemical mechanisms was significantly greater in smokers than in nonsmokers (Petrilli *et al.*, 1986c).

In contrast to chromium[III], which is bound to plasma proteins such as transferrin, chromium[VI] entering the blood stream is taken up selectively by erythrocytes, reduced, and bound predominantly to haemoglobin (Gray & Sterling, 1950; Aaseth *et al.*, 1982; Kitagawa *et al.*, 1988; see also the section on genetic and related effects). Reduction of chromium[VI] during transport in the blood is consistent with the finding that chromium is present in urine only in its reduced form (Mertz, 1969; Nomiyama *et al.*, 1980).

Aitio *et al.* (1988) reviewed the results of biological monitoring of chromium exposure to estimate biological half-times for excretion; the most data were available for manual metal arc stainless-steel welders exposed to soluble chromium[VI]. Three half-times — 7 h, 15-30 days and three to five years — were identified. The best estimates for the sizes of the different compartments are 40%, 50% and 10%, respectively. Lindberg and Vesterberg (1983) also found a correlation between exposure and urinary excretion of chromium in platers.

Retention of chromium on the skin was observed following topical application of sodium chromate (Baranowska-Dutkiewicz, 1981).

(*b*) *Toxic effects*

In adults, the lethal oral dose of chromates is considered to be 50-70 mg/kg bw. The clinical features of acute poisoning are vomiting, diarrhoea, haemorrhagic diathesis and blood loss into the gastrointestinal tract, causing cardiovascular shock

(Sharma *et al.*, 1978; World Health Organization, 1988). If the patient survives for more than about eight days, the major effects are liver necrosis and tubular necrosis of the kidneys (World Health Organization, 1988).

Chronic ulcers of the skin and acute irritative dermatitis have been reported consistently in workers exposed to chromium-containing materials (World Health Organization, 1988). Chromates and chromium[VI] released from alloys and chromium-plated objects have been associated with the induction of allergic contact dermatitis. It is generally assumed that chromium[VI] is necessary for the sensitization, while both chromium[VI] and chromium[III] may cause dermatitis in sensitized individuals (see review by Haines & Nieboer, 1988). Intracellular reduction of chromium[VI] to the trivalent form seems to be a prerequisite for the effect (Polak *et al.*, 1973). In a study conducted in Finland, 2% of men and 1.5% of women showed a positive patch-test reaction to potassium dichromate (Pelkonen & Fräki, 1983). Chromium ulcers and chromate dermatitis have been reported in people in numerous occupations that involve manual handling of products containing chromium (Pedersen, 1982; Burrows, 1983; Polak, 1983; Nieboer *et al.*, 1984). The role of chromium[III] compounds in causing skin ulcers and acute irritative dermatitis is unclear (World Health Organization, 1988).

Inhalation of chromium[VI] compounds may give rise to necrosis in the nasal septum, leading to perforation. Lindberg and Hedenstierna (1983) found nasal irritation in chrome plating workers exposed by inhalation to chromium trioxide (> 1 $\mu g/m^3$ Cr) and nasal perforation in two-thirds of workers with exposure to peak levels above 20 $\mu g/m^3$ Cr. Decreased respiratory function has been reported in platers exposed to chromates (Bovet *et al.*, 1977; Lindberg & Hedenstierna, 1983). Similar effects have been observed in welders and ferrochromium workers, although the role of chromium is uncertain as such persons have mixed exposures (World Health Organization, 1988).

Bronchial asthma may occur as a result of inhalation of chromate dust or chromium trioxide fumes (Meyers, 1950). Asthma among chromium platers, welders and ferrochromium workers has been reported to be due to exposure to chromates, among other compounds (Haines & Nieboer, 1988).

Franchini *et al.* (1978) reported on the excretion of β-glucuronidase, protein and lysozyme in the urine of 99 workers exposed to chromium compounds. No abnormal level was found among 39 stainless-steel welders; eight of 36 workers using special electrodes when welding on armoured steel had increased urinary levels of β-glucuronidase, and three of these workers had proteinuria. Among 24 workers engaged in chrome plating, nine had increased β-glucuronidase levels and four had elevated levels of protein in urine. The increased excretion of enzymes found in these workers was corroborated by exposing rats to potassium dichromate by sub-

cutaneous injection (1.5 mg/kg bw as a single injection or 0.3 mg/kg bw every other day for two weeks); furthermore, a correlation between chromium in the renal cortex and an increase in chromium clearance was reported. Verschoor *et al.* (1988) investigated a number of parameters of kidney function in chrome platers, welders, boiler-makers and an unexposed reference group. Urinary chromium values ranged from 0.3 to 62 μg/g creatinine (0.1-2 μg/g among controls). Renal function was not related to urinary chromium or to chromium clearance, but chromium clearance was increased in the two groups with the highest exposure (platers and welders).

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

In a review, Clarkson *et al.* (1985) found no report in the literature of an effect of any chromium compound on reproduction or prenatal development in humans.

(*d*) *Genetic and related effects*

The studies described below are summarized in Appendix 1 to this volume.

(i) *Chromium[III] compounds*

In a comparison of 17 healthy tannery workers with continuous exposure for 13.4 ± 8.2 years to chrome alum and 13 external employees matched for social status, age, sex and years of service, no increase in the frequency of chromosomal aberrations was seen (Hamamy *et al.*, 1987). Average chromium levels of exposed persons were 0.12 μg/l plasma and 0.14 μg/l urine; these values were not considered to be different from those of controls. The level of chromium in air ranged from 15 (day) to 47 (night) μg/m³. [The Working Group noted that exposure was estimated by correlation with a parallel study.] When the data were analysed according to smoking habit, workers who smoked had higher frequencies of chromosome-type aberrations per cell (0.035) than either nonsmoking workers (0.011; $p < 0.01$) or control smokers (0.016; $p < 0.05$). The authors commented that the values for controls were relatively high in comparison with those in other cytogenetic studies reported in the literature.

In the study described below, enhanced levels of chromosomal aberrations, correlated with exposure duration, were observed in workers exposed to 'chromoxide' (Bigaliev *et al.*, 1977a). [The Working Group was unclear whether or not this was a chromium[III] compound.]

(ii) *Chromium[VI] compounds*

Bigaliev *et al.* (1977a) examined peripheral lymphocytes from 132 workers in chromium production who were exposed to one of five chromium compounds and compared them with 37 healthy, unexposed workers. Significant increases in the

frequency of chromosomal aberrations over control values of 1.88 ± 0.74% metaphases with aberrations were observed, as follows: monochromate (sodium chromate), with a dose-related trend (correlation with exposure duration) ranging from 3.6 to 8.2% aberrant metaphases; sodium chrompik (sodium dichromate), with a dose-related trend ranging from 4.5 to 5.7% aberrant metaphases; potassium dichromate, with a dose-related trend ranging from 3.6 to 9.0%; chromoxide (as reported in the preceding section), with a dose-related trend ranging from 4.5 to 7.2%; and chromanhydride (chromium trioxide), with a dose-effect trend ranging from 5.4 to 9.4%. [The Working Group noted that no information was provided on exposure levels or on selection criteria, but the overall sample size was large.] When chromosomal aberrations were examined in detail (Bigaliev *et al.*, 1977b,c; Bigaliev, 1981), increased frequencies were found for single and double fragments, for translocations and for aneuploidy, consisting mainly of chromosome loss. Dose-responses were observed overall, and for each type of damage. In a later study (Bigaliev *et al.*, 1979), elongated cell-cycle times were seen for cultured peripheral lymphocytes from a group of chromium workers with five or more years' exposure, compared with a control group registered at the city blood transfusion station. An effect of duration of exposure was reported in a further analysis (Bigaliev *et al.*, 1977c; Bigaliev, 1981).

Several studies have been carried out on chromium platers. Increased frequencies of sister chromatid exchange were found in a study of male chromium platers exposed to chromium trioxide fumes (Stella *et al.*, 1982). Mean sister chromatid exchange values of 8.08 ± 2.67 ($p < 0.001$) were observed in exposed workers *versus* 6.31 ± 1.56 in ten healthy male donors aged 20-35 who had not been exposed to ionizing radiation. The authors noted particularly that the seven youngest workers, although the most recently engaged in chromium plating, showed significantly increased sister chromatid exchange frequencies. An effect of age on the induction of sister chromatid exchange was noted in the control group. [The Working Group noted that details were not provided on exposure, or on confounding factors].

Sarto *et al.* (1982) analysed sister chromatid exchange and chromosomal aberrations in peripheral blood lymphocytes of chrome platers in four factories in the same region, grouped by type of exposure and factory: groups 1 (eight persons) and 2 (nine persons) used a 'bright plating' process and were exposed to chromium trioxide and nickel; groups 3 (12 persons) and 4 (nine persons) used a 'hard plating' process and were exposed only to chromium trioxide. Controls were 35 healthy male sanitary workers who had not been exposed to occupational or diagnostic ionizing radiation for at least five years and had not knowingly been exposed to either occupational mutagens or mutagenic drugs. Their mean ages and smoking habits were similar to those of the exposed workers. The average ages in the four exposed groups were 39, 42, 24 and 34 years, respectively; urinary chromium levels (μg/g

creatinine) in the four groups were 5.1 ± 1.8, 7.1 ± 3.3, 11.8 ± 8.7 and 6.8 ± 3.7, respectively, *versus* 1.9 + 1.4 for controls. Sister chromatid exchange frequencies in the 'hard plating' groups were increased ($p < 0.001$) from 6.60 ± 0.80% in controls to 8.30 ± 1.80%; however, when the values were analysed by age, a significant increase in sister chromatid exchange was observed only in the group of younger workers (group 3). A correlation was observed between sister chromatid exchange frequency and both age and urinary chromium levels (more sister chromatid exchange in younger workers with higher levels of chromium). A significant increase in the frequency of chromosomal aberrations, mostly of the chromosome type, was observed, from 1.7% of metaphases in controls to 3.8 ($p < 0.001$) in 'bright' platers and 2.8 ($p < 0.01$) in 'hard' platers. Chromatid-type aberrations were observed only in the 'bright' platers. The correlation between urinary chromium levels and chromosomal aberrations was poor.

No increase in the frequency of sister chromatid exchange was observed in a group of 24 male chromium platers exposed to chromium in air for 0.5-30.5 (mean, 11.6 ± 7.5) years, when compared with a group of office workers matched for sex, age and smoking habit (Nagaya, 1986). Smokers and nonsmokers were analysed separately for each group, and a smoking-related increase in the frequency of sister chromatid exchange was observed for both exposed (smokers, 10.7 ± 1.7%; nonsmokers, 9.0 ± 1.0%) persons and controls (smokers, 10.6 ± 2%; nonsmokers, 8.9 ± 1.2%). No correlation was seen between sister chromatid exchange frequencies and urinary chromium levels (13.1 ± 16.7 μg/l for exposed persons, none detected for controls). In a further study of a larger group (Nagaya *et al.*, 1989), essentially the same results were obtained. The authors speculated that the chromium exposure may have been too low to affect circulating lymphocytes. [The Working Group noted that high control values were observed in both studies.]

Choi *et al.* (1987) compared two groups of metal platers, consisting of seven workers in chromium surface treatment (group 1) and 25 workers in chromium plating (group 2), with 15 non-plating workers matched for age, sex and length of career. Exposures to chromium in air and urine were 0.027 (0.021-0.034) mg/m^3 and 24.0 ± 7.8 μg/l, respectively, for group 1, and 0.008 (0.005-0.012) mg/m^3 and 15.2 ± 5.9 μg/l, respectively, for group 2. Sister chromatid exchange frequency was increased from 3.6 ± 1.5 (controls) to 6.9 ± 1.8 ($p < 0.05$) in group 1 and to 5.4 ± 2.1 ($p < 0.05$) in group 2. A dose-effect relationship was observed with urinary chromium levels ($p < 0.01$). No effect of smoking was observed in exposed workers or controls.

Deng *et al.* (1983, 1988) observed significant increases in the frequencies of sister chromatid exchange and of chromosomal aberrations (gaps, breaks, fragments; 5.7% *versus* 0.8% in controls) in lymphocytes of seven chromium platers. Details of the study are provided in the monograph on nickel and nickel compounds, p. 389.

Several studies of occupational exposures to chromium during welding are described in the monograph on welding, pp. 487-489. Both enhancement (Koshi *et al.*, 1984) and lack of enhancement (Husgafvel-Pursiainen *et al.*, 1982; Littorin *et al.*, 1983) of sister chromatid exchange and chromosomal aberrations were reported in exposed workers.

As reported in an abstract (Imreh & Radulescu, 1982), 18 workers in a bichromate producing plant with a mean duration of exposure of 21.3 years (19-26 years) showed significantly elevated frequencies of chromosomal and chromatid-type aberrations and micronuclei when compared with eight mechanics from the same plant and with 34 healthy external controls. Sister chromatid exchange frequencies were not significantly greater than in the mechanics.

3.4 Case reports and epidemiological studies of carcinogenicity to humans

Epidemiological studies on chromium have been reviewed extensively (see, e.g., Sunderman, 1976; Norseth, 1980; Anon., 1981; Norseth, 1981; Sunderman, 1984, 1986; Adachi & Takemoto, 1987; Fan & Harding-Barlow, 1987; Hayes, 1988; World Health Organization, 1988; Yassi & Nieboer, 1988). Epidemiological studies on welders exposed to chromium and its compounds are summarized in the monograph on welding (see pp. 489-505).

Epidemiological studies of cancer in workers in industries in which exposure to chromium compounds could occur are summarized in Tables 28-31. Standardized mortality ratios (SMRs) and confidence intervals (CIs), assuming Poisson distribution, are given in square brackets when they were calculated by the Working Group.

(*a*) *Chromate production*

(i) *Case reports*

Many case reports of lung cancer have been published in relation to work in chromate production. Many of these were reviewed by a Working Group for the IARC (1980a); further case reports were made by Pfeil (1935), Alwens and Jonas (1938), Zober (1979), Hyodo *et al.* (1980), Abe *et al.* (1982), Tsuneta (1982) and Nishiyama *et al.* (1985, 1988). After having seen five cases of gastrointestinal cancer among 44 deceased chromate workers, Teleky (1936) drew attention to the possibility that chromate exposure could also be associated with an increased risk for cancer of the gastrointestinal tract.

(ii) *Epidemiological studies*

The Working Group considered six studies covering several partially overlapping populations in seven plants producing chromate from chemical-grade chromite ore (Brinton *et al.*, 1952) in the USA; the degree of overlap could not be ascertained.

Table 28. Epidemiological studies of cancer in workers in chromate-producing industries

Study population	Reference population	Cancer of respiratory organs			Cancer at other sites			Reference
		Site	Number	Estimated relative risk	Site	Number	Estimated relative risk	
Seven US chromate plants; active employees 1930–47; 193 deaths	Male oil refinery workers, 1933–38	Respiratory system	42	20.7	Digestive system Oral region (also included in respiratory system)	13 3	2.0 5.4*	Machle & Gregorius (1948)
Seven US chromium plants; active employees 1940–50; 5522 person-years	US male white, non-white	Respiratory system, except larynx	10 white 16 non-white	14.3* 80.0*	Other sites	6 (whole cohort)	1.0 ns	Brinton *et al.* (1952); Gafafer (1953)
Health survey, 897 workers	Boston X-ray survey	Bronchogenic/lung	10	53.6 (prevalence ratio)				Gafafer (1953)
Three US plants; men employed 1937–40, surveyed 1941–60	Cancer mortality; US males 1950, 1953, 1958	Respiratory (160–164)	69 (2 maxillary sinus)	9.4*	Digestive system	16	1.5 ns	Taylor (1966); Enterline (1974)
290 cases near US chromium plant	Random sample of hospital admissions	Lung	11[a]	∞				Baetjer (1950)
US chromate plant; employed one or more years 1931–37; all jobs related to exposure to soluble and insoluble chromium; lifetime exposure in months calculated	No independent comparison group	Lung	41					Mancuso & Hueper (1951); Mancuso (1975)

Table 28 (contd)

Study population	Reference population	Cancer of respiratory organs			Cancer at other sites			Reference
		Site	Number	Estimated relative risk	Site	Number	Estimated relative risk	
US chromate plant; 2101 (restricted to 1803) workers initially employed three or more months 1945–74; status 1977 (88.5% complete); population working in new and/or old production sites	Baltimore City; mortality	Lung	59	2.0*	Digestive system Other	13 14	0.60 0.40	Hayes *et al.* (1979)
Three UK chromate factories; men employed 1949–55	Cancer mortality, England and Wales	Lung	12	3.6*	All other sites	No increase		Bidstrup & Case (1956)
Same UK factories as studied by Bidstrup & Case (1956); 1948–77; 2715 males	Cancer mortality, England, Wales and Scotland	Lung	116	2.4*	Other sites Nasal cancer	80 2	1.2 ns 7.1*	Alderson *et al.* (1981)
Two FRG chromate plants; 1140 male workers employed more than one year 1934–79	Mortality, North Rhine Westphalen	Lung	51	2.1*	Stomach	12	0.94 ns	Korallus *et al.* (1982)
Tokyo chromium manufacture; 896 production workers, 1918–78	Age–, cause-specific mortality, Japanese males	Respiratory cancers	31 (6 sino-nasal)	9.2*	Stomach	11	1.0	Satoh *et al.* (1981)
		1–10 years' exposure	5	4.2*				
		11–20 years' exposure	9	7.5*				
		≥21 years' exposure	17	17.5*				

Table 28 (contd)

Study population	Reference population	Cancer of respiratory organs			Cancer at other sites			Reference
		Site	Number	Estimated relative risk	Site	Number	Estimated relative risk	
273 chromate producers in Japan; 1947–73; observed 1960–82	Age-, cause-specific mortality, Japanese males	Lung	25 (plus 1 maxillary sinus)	18.3*	Digestive system	6	0.9	Watanabe & Fukuchi (1984)
540 Italian chromate producers employed 10 years or more, 1948–85	Italian cause-specific death rates	Lung Highly exposed	14 6	2.2* 4.2*	Larynx Pleura	3 3	2.9 30*	De Marco *et al.* (1988)

[a]In comparison with internal reference population

*Significant at 95% level

ns Nonsignificant

Table 29. Epidemiological studies of cancer in workers in chromate–pigment industries

Study population	Reference population	Cancer of respiratory organs			Cancer at other sites			Reference
		Site	Number	Estimated relative risk	Site	Number	Estimated relative risk	
Norwegian chromium pigment production since 1948; 133 workers of whom 24 over 3 years' employment to 1972	Cancer incidence, Norway 1955–76	Lung	6 (one case with <3 years' employment)	44 67 (10 years' latency)	Gastrointestinal Nasal cavity	3 1	6.4 -	Langård & Norseth (1975, 1979); Langård & Vigander (1983)
UK chromate pigment factories: A, lead & zinc chromate; B, lead & zinc chromate; C, lead chromate; followed up to 1981	Mortality, England and Wales	Lung A (1932–54) B (1948–67) C (1946–60)	 21 11 7	 2.2* 4.4* 1.1 ns				Davies (1978, 1979, 1984a)
French lead and zinc chromate manufacturers; 251 males employed 6 months or more, 1958–77	Standard death rates, northern France 1958–77	Lung	11	4.6*				Haguenoer *et al.* (1981)
German and Dutch manufacturers of zinc and lead chromates; 978 workers followed up for 15 076 person–years	Local death rates, FRG and the Netherlands	Lung	19	2.0*				Frentzel-Beyme (1983)

Table 29 (contd)

Study population	Reference population	Cancer of respiratory organs			Cancer at other sites			Reference
		Site	Number	Estimated relative risk	Site	Number	Estimated relative risk	
US lead and zinc chromate production workers employed ≥1 month 1940–69; 1181 white, 698 non-white; followed up to end of 1982	Mortality, US whites and non-whites	Lung (30–year latency)	24	1.4 ns	Stomach	6	1.8 ns	Sheffet *et al.* (1982); Hayes *et al.* (1989)
		< 1 year exposure	3	1.4**				
		1–9 years' exposure	3	2.0**				
		≥10 years' exposure	6	3.2**				

** p for trend < 0.01

ns Nonsignificant

Table 30. Epidemiological studies of cancer in workers in chromium–plating industries

Study population	Reference population	Cancer of respiratory organs			Cancer at other sites			Reference
		Site	Number	Estimated relative risk	Site	Number	Estimated relative risk	
54 UK chromium-plating plants; 1056 male platers	1099 non-exposed males in plants and in two nonplating industries	Lung	24	1.4 ns	All sites Gastrointestinal Other sites	44 8 12	1.7* 1.5 ns 1.9 ns	Royle (1975a,b)
Japanese chromium platers; 952 workers with > 6 months' exposure	Platers not exposed to chromium and clerical workers	Lung	0	–	All sites	5	0.5 ns	Okubo & Tsuchiya (1977, 1979, 1987)
US workers in diecasting and Ni–Cr–plating plant, 1974–78	US national mortality statistics	Lung men women	 28 10	 1.9* 3.7*	Stomach Larynx Lymphosarcoma	4 2 2	2.5 ns 3.3 ns 2.9 ns	Silverstein *et al.* (1981)
Nine plants, Parma, Italy, 116 'thick' and 62 'thin' platers; employed more than 1 year 1951–81	Mortality, Italy	Lung	3	3.3* (4.3* for 'thick' platers)	All sites	8	1.9	Franchini *et al.* (1983)
UK chromium platers; 2689 (1288 men, 1401 women) first employed 1946–75; observed 1946–83	Mortality, England and Wales	Lung men women Nasal cavity (men and women) Larynx men women	 63 9 3 3 0	 1.6* 1.1 ns 10* 3.0 ns –	Stomach (men and women) Liver men women	25 4 0	1.5 ns 6.7* –	Sorahan *et al.* (1987)

* Significant at 95% level
ns Nonsignificant

Table 31. Epidemiological studies of cancer in workers in ferrochromium industries

Study population	Reference population	Cancer of respiratory organs			Cancer at other sites			Reference
		Site	Number	Estimated relative risk	Site	Number	Estimated relative risk	
USSR ferrochromium alloy industry; 1955-69	Mortality, general population of municipality	Lung (men)	Not given	4.4-6.6* by age	All sites (men) Oesophagus (men)	Not given Not given	0.5-3.3* 2.0*-11.3*	Pokrovskaya & Shabynina (1973)
Swedish ferrochromium plant; ferroalloy; 1876 workers for 1 or more years 1930-75; traced by parish lists and cancer registry	County or national statistics; classification of work areas by Cr[III] and Cr[VI]	Lung All workers Maintenance workers Arc workers	 7 4 (2 mesotheliomas) 2 (1 mesothelioma)	 1.2 ns 4.0* 1.0 ns	Prostate (all workers)	23	1.2 ns	Axelsson *et al.* (1980)
Norwegian ferrochromium and ferrosilicon; 1235 male workers employed 1928-65	General population; internal comparison with unexposed	Lung (ferrochromium workers)	10	1.5 ns	All sites Kidney Prostate Stomach (ferrochromium workers)	132 5 12 7	0.8 ns 2.7 ns 1.5 ns 1.4 ns	Langård *et al.* (1980, 1989)

*Significant at 95% level

ns, Nonsignificant

Machle and Gregorius (1948) reported high proportionate mortality from respiratory cancer among male workers at the seven chromate-producing plants in the USA: between 1930 and 1947, the annual death rate from respiratory cancer was 2.63/1000, as compared with a frequency of 0.09/1000 in a comparison group from an oil refinery in 1933-38. [The Working Group noted that the age structures of the two populations were not given.]

Brinton *et al.* (1952) and Gafafer (1953) conducted a mortality study (a US Public Health Service study) of male workers in the seven chromate manufacturing plants during 1940-50 with 5522 person-years of membership in sick-benefit associations for persons 15-74 years old, not including workers who had terminated employment with the chromate industry and those who had died more than one year after the onset of disability. Comparison was made to age- and race-specific US male mortality rates during 1940-48. Ten deaths from cancer of the respiratory system (except larynx) were observed among white employees (SMR, 1429 [95% CI, 685-2627]). Among non-white employees, 16 deaths from cancer at this site were found (SMR, 8000 [95% CI, 4573-12 991]). For the entire study group, six deaths from cancers at all other sites were observed [SMR, 95.2; 95% CI, 35-207]. [The Working Group noted that the SMR for lung cancer may have been biased, because of the exclusion of terminated and retired workers and of those who did not belong to the sick-benefit plan.] A health survey of 897 workers gave a prevalence ratio of 53.6 for bronchogenic cancer in chromate workers compared to persons who had undergone a chest X-ray survey for lung cancer (Gafafer, 1953).

Enterline (1974) reanalysed data from a study by Taylor (1966) of 1212 male workers who had been employed in three of the US plants for three months or longer for the period 1937-60. The study cohort, constructed from earnings reports in old age and survivors disability insurance records, was restricted to men born after 1889. Vital status was ascertained through 1960 by searching the death claim files of the records; death certificates were subsequently obtained for workers for whom death claims had been filed. Age-specific mortality figures for US males in the calendar years 1950, 1953 and 1958 were used as reference. A total of 69 deaths from cancers of the respiratory system (ICD codes 160-164) was observed (SMR, 943 [95% CI, 733-1193]), two of which were from maxillary sinus cancer; the author regarded this rate as greatly elevated. Furthermore, a small excess of deaths from cancer of the digestive system was observed (16 deaths; SMR, 153 [95% CI, 88-249]).

In a study of medical records from two hospitals in Baltimore, MD, USA, near a chromate-producing plant, Baetjer (1950) found that 11 (3.8%) of 290 male lung cancer patients admitted in 1925-48 had had exposure to chromates, whereas no chromate-exposed worker was found among a 'random' sample of 725 other hospital admissions. Ten of the 11 cases had worked in the local chromate production

plant and one in an electrical company. Occupational history was derived only from records.

Mancuso (1975) reported on a cohort recruited from a US chromate-producing plant that had been investigated earlier (Mancuso & Hueper, 1951). In the earlier report, six lung cancer deaths were observed, giving a relative risk of 15; using hygiene data collected in 1949, cumulative exposures to soluble, insoluble and total chromium, combined with length of exposure, were computed for each worker in the cohort. The second analysis was confined to the 41 deaths from lung cancer that occurred in persons first employed between 1931 when the plant started operation and 1937 and followed through 1974, and rates were computed using direct standardization, with the entire plant population as the standard. Mortality from lung cancer was associated with cumulative exposure to insoluble chromium, to soluble chromium and to total chromium. [The Working Group noted that the three classes of exposure were highly correlated and the risks of exposure to soluble and insoluble chromium could not be distinguished.]

Hayes *et al.* (1979) studied workers at a chromate production plant in Baltimore, MD, USA, which had been partly renovated in 1950-51 and 1960 to reduce exposure to chromium dusts. The study cohort consisted of 2101 workers with more than 90 days of work experience, first employed between 1945 and 1974, and followed through July 1977; vital status was ascertained for 75% on an individual basis and for another 14% on a group basis. SMRs for 1803 hourly employees were calculated on the basis of expected values derived from the age-, race- and time-specific mortality rates for Baltimore City males. There were 404 deaths from all causes (SMR, 92). The overall SMR for cancer of the trachea, bronchus and lung (ICD code 162) was 202, based on 59 observed deaths (95% CI, 155-263). Workers hired between 1945 and 1949, before the plant was renovated, who had been employed for fewer than three years, had an SMR for lung cancer of 180 (95% CI, 110-270), based on 20 observed deaths, whereas workers with three or more years of employment hired in that period had an SMR of 300 (95% CI, 160-520), based on 13 observed deaths. For workers hired in 1950-59, when part of the plant had better environmental controls, similarly elevated risks were seen, based on 12 and nine cases for short-term and long-term employment, respectively. No case of lung cancer was detected in 1960-74 after the plant had been renovated, but, as the authors noted, the latent period is too short for an adequate assessment of risk for cancer at this site. Additional case-control analyses were performed to determine whether specific work areas were associated with lung cancer hazard. Controls who had died from causes other than cancer were matched individually by race, date of hire, age at initial employment and duration of employment to the 66 hourly or salaried employees who had died from lung cancer. A significant ($p < 0.05$) elevation in risk for lung cancer was found for employees who had worked in the 'special products' and dich-

romate areas, where soluble chromium[VI] compounds were produced and packaged (relative risks, 2.6 and 3.3, respectively).

On the basis of data from the previous study and the results of 555 air samples analysed in 1945-50, Braver *et al.* (1985) studied the relationship between exposure to chromium[VI] and occurrence of lung cancer. The authors reported a dose-response relationship with cumulative exposure. [The Working Group noted that the association appeared to be due predominantly to duration of exposure and not to estimated level of exposure, which did not vary substantially.]

A total of 723 chromate production workers from three factories in the UK who were interviewed and radiographed were followed up in 1949-55 by Bidstrup and Case (1956), who reported significantly higher than expected lung cancer mortality: 12 deaths [SMR, 364; 95% CI, 188-635] (based on age-adjusted rates for England and Wales). The average duration of exposure was 12.2 years; 165 (22.8%) persons had worked for more than 20 years (Bidstrup, 1951). For cancers at other sites, the observed and expected numbers of deaths did not differ significantly.

Alderson *et al.* (1981) studied 2715 chromate production workers with more than one year of work experience between 1948 and 1977 and who had undergone at least one X-ray of the lungs, 79 (2.9%) of whom were lost to follow-up, at the same three UK factories studied by Bidstrup and Case (1956). The percentage of heavy smokers was reported to be lower among the workers than among males in England and Wales [numbers not given]. During the study period, 602 deaths occurred (SMR, 135 [95% CI, 125-146]), 116 of which were from lung cancer (SMR, 242 [95% CI, 200-290]). Two deaths from nasal cancer were observed in one factory; 0.28 would have been expected for the whole cohort (SMR, 714 [95% CI, 87-2580]).

Korallus *et al.* (1982) identified 1140 workers who had been employed for one year or more at two chromate-producing plants in the Federal Republic of Germany. The study subjects were active workers and pensioners who had been hired before 1948 or workers hired thereafter. Vital status was ascertained from personnel documents and from population registries until 1979. Cause of death was determined from medical records and, in some cases, from death certificates. The SMR for respiratory cancer (ICD 8, 160-163) was 210 [95% CI, 156-276]. A total of 20 deaths from bronchial carcinomas (and one laryngeal carcinoma) was seen in one factory (SMR, 192 [95% CI, 119-294], and 30 deaths, all from bronchial carcinoma, in the second (SMR, 224 [95% CI, 151-319]). The author noted difficulties in the ascertainment of cause of death and of comparability with the standard population.

Satoh *et al.* (1981) studied 896 men who had been engaged in manufacturing chromium compounds for one or more years in a factory in the Tokyo, Japan, area between 1918 and 1975. The workers were observed from 1918 through 1978 or to death; vital status could not be ascertained for an additional 165 retired workers. The authors stated that 84% of the chromium compounds manufactured between

1934 and 1975 were hexavalent compounds and 16% trivalent compounds. The expected numbers of deaths were based on age- and cause-specific mortality rates for Japanese males. Between 1950 and 1978, 120 deaths (SMR, 90) were observed, 31 of which were from respiratory cancer [SMR, 923; 95% CI, 627-1310]; 25 of these were from lung cancer and six from sinonasal cancer. No other cancer occurred in excess. When the population was subdivided by duration of work, there were five cases of respiratory cancer in the group with one to ten years of exposure [SMR, 423; 95% CI, 138-989], nine in the group with 11-20 years' exposure [SMR, 748; 95% CI, 343-1424] and 17 in the group with more than 21 years of exposure [SMR, 1747; 95% CI, 1021-2806].

Watanabe and Fukuchi (1984) reported in an abstract a mortality study of 273 workers employed in 1947 or later at a chromate-producing factory in Japan for at least five years until 1973, previously studied by Ohsaki *et al.* (1974, 1978). The population was observed from January 1960 to December 1982. Expected numbers of deaths were based on age-, year- and cause-specific deaths rates for the Japanese male population. Sixty deaths from all causes were observed; 33 from all cancers, of which 25 were from lung cancer (SMR, 1832 [95% CI, 1190-2714]) and six from cancer of the digestive organs [SMR, 88; 95% CI, 32-192]; one cancer of the maxillary sinus was seen.

In an Italian cohort study of 981 chromate production workers employed for one year or more in 1948-85 (De Marco *et al.*, 1988), analysis was limited to the 540 workers followed up for ten years or more. Cause-specific death rates in Italy were used as a reference level. The SMR for lung cancer was 217 (14 deaths; 95% CI, 118-363), and there were three deaths each from cancers of the pleura and larynx. Among a subgroup of workers with heavy exposure to hexavalent chromium compounds (on the basis of job histories), the SMR for lung cancer was 420 (six deaths [95% CI, 154-193]).

(*b*) *Production of chromate pigments*

(i) *Case reports*

Newman (1890) reported the first case of cancer in a 'chrome worker', which was an adenocarcinoma of the anterior half of the left nostril in a 47-year-old male worker who had had perforation of his nasal septum for 20 years; the patient had been exposed to chrome pigments. Since that time, there have been a number of case reports of lung cancer in workers involved in production of chromate pigments (Gross & Kölsch, 1943; Letterer *et al.*, 1944; Langård & Kommedal, 1975; Zober, 1979; Rivolta *et al.*, 1982).

(ii) *Epidemiological studies*

Langård and Vigander (1983) followed up 133 workers for 1953-80, who had been employed in a small Norwegian company producing chromate pigments in

1948-72, previously studied by Langård and Norseth (1975). The work force was exposed to zinc chromate from 1951; a small number of workers had also been exposed to lead chromate between 1948 and 1956. While past levels of exposure to hexavalent chromium are unknown, exposures to chromates as chromium measured in 1973 ranged from 0.01 to 1.35 mg/m^3 (Langård & Norseth, 1979). One case of lung cancer occurred among 109 workers with less than three years of employment prior to 1972. Six cases of lung cancer occurred in a subpopulation of 24 workers with more than three years of work experience prior to 1972 [giving a standardized incidence ratio (SIR) of 4444 (95% CI, 1631-9674) on the basis of national incidence rates among males]. More than ten years after first exposure, the SIR was 6667 [95% CI, 2447-14 510] on the basis of national reference rates. Only 18 workers had worked at the plant for more than five years, and all six cases belonged to this subgroup. One of the cases had worked in the production of zinc chromate as well as lead chromate, while five cases had worked in the production of zinc chromate only. A previous follow-up had found one case of cancer of the nasal cavity, one of cancer of the prostate and three of cancer of the gastrointestinal tract (one cancer of the pancreas, one stomach cancer and one cancer of the large intestine) (Langård & Norseth, 1979). The three latter cases occurred in the subgroup of 24 workers employed for more than three years before 1972 [SMR, 638; 95% CI, 0.6-8.8].

Davies (1978, 1979, 1984a) studied mortality among 1002 male workers at three factories in the UK where chromate pigments were manufactured. Production of lead chromate[VI] occurred in all factories; workers in two of the factories (A and B) were additionally involved in manufacturing zinc chromate[VI] until 1964 and 1976, respectively. Small amounts of barium chromate were produced in factory A from 1942, and small amounts of strontium chromate were produced in factory B from the early 1950s to 1968. Factory A closed in 1982 and factory B in 1978. Exposure levels were classified only as high, medium or low. The 1984 report extended the follow-up from the 1930s or 1940s to the end of 1981. The expected numbers were based on calendar time period-, sex- and age-specific mortality rates for England and Wales. An excess of lung cancer appeared in two groups of workers assigned to high and medium exposure: factory A, those entering before 1955 (21 cases; SMR, 222 [95% CI, 138-340]) and factory B, those entering before 1968 (11 cases; SMR, 440 [95% CI, 220-787]). In workers with low exposure to zinc and lead chromates in factories A and B, seven lung cancer deaths were observed [SMR, 101; 95% CI, 41-208]. In factory C, where only lead chromate was produced, seven lung cancer deaths were observed [SMR, 109; 95% CI, 44-224], and the highest ratio was found for one to 29 years of follow-up of a group of 33 men among early entrants with high and medium exposure (three cases [SMR, 357; 95% CI, 74-1044]). The author indicated that moderate or heavy exposure to zinc chromate may give rise to

a high risk for developing lung cancer, and that relatively mild or short-term exposure may not constitute a measurable lung cancer hazard.

Davies (1984b) also studied a subgroup of 57 workers involved in the production of lead chromate pigments from lead nitrate in the same three factories, who had been reported to the work inspectorate to have lead poisoning, mostly between 1930 and 1945. Mortality was observed through 1981, giving 1585 person-years of observation. Four deaths from lung cancer (SMR, 145 [95% CI, 40-370]) were observed. [The Working Group noted that this small sample of workers might have been highly selected.]

Haguenoer *et al.* (1981) reported deaths among a cohort of 251 workers in a factory manufacturing zinc and lead chromate pigments in France who had been employed for more than six months between 1 January 1958 and 31 December 1977. Fifty deaths occurred, the specific cause of which was known from medical records for 30. Expected numbers were derived from death certificates. Among the 30 deaths, there were 11 confirmed lung cancer deaths (SMR, 461; 95% CI, 270-790). The mean time from first employment until detection of cancer was 17 years, and the mean duration of employment among cases was 15.3 years. [The Working Group noted that cause of death was ascertained from different sources for observed and expected cases.]

Frentzel-Beyme (1983) studied mortality among men employed for more than six months in three factories in the Federal Republic of Germany and two factories in the Netherlands that produced lead and zinc chromate pigments. The total number of study participants was 1396. Regional death rates in the two countries were used to estimate expected figures. In an analysis of 978 men with exposure beginning before 1965, 117 deaths were observed [SMR, 96], of which 19 were from lung cancer [SMR, 204; 95% CI, 123-319].

Hayes *et al.* (1989) followed-up a cohort studied by Sheffet *et al.* (1982) consisting of 1879 male employees of a New Jersey (USA) lead and zinc chromate pigment production factory who had been employed for at least one month between January 1940 and December 1969; they were observed from 1940 to 1982. US age- and calendar-specific death rates for white and nonwhite men were used as reference. Vital status was ascertained for 1737 workers (92%). Airborne chromium concentrations were measured during later years, giving estimates of > 0.5 mg/m^3 for exposed jobs and of > 2 mg/m^3 for highly exposed jobs; the ratio of lead chromate:zinc chromate in the working atmosphere was reported to be about 9:1, and low levels of nickel may have been present. The SMR for all cancers was 93 (101 deaths; 95% CI, 76-113). Among a total of 41 lung cancer deaths (SMR, 116; 95% CI, 83-158), 24 occurred among workers exposed to chromate dusts (SMR, 143). The SMR for lung cancer among men who had not worked in chromium-exposed jobs was 92 (17 deaths; 95% CI, 53-147), and that for men who had worked for less than one year was 93 (seven

deaths; 95% CI, 37-192). For those with cumulative exposure to chromate dusts of one to nine years, the SMR was 176 (nine deaths; 95% CI, 80-334), and for ten or more years, 194 (eight deaths; 95% CI, 83-383). When accounting for 30 years since first employment among men with more than ten years' exposure, the SMR rose to 321 (95% CI, 117-698), based on six cases. In jobs with exposure to chromate dusts, a nonsignificant excess of cancer of the digestive tract was found; for stomach cancer, the SMRs were 149, 185 and 214 for those with less than one, one to nine and more than ten years' exposure, respectively.

(*c*) *Chromium plating*

(i) *Case reports*

Cases of lung cancer have also been reported among chromium platers (Bařík *et al.*, 1958; Kleisbauer *et al.*, 1972; Korallus *et al.*, 1974b,c; Michel-Briand & Simonin, 1977; Takemoto *et al.*, 1977; Sano, 1978; Zober, 1979; Brochard *et al.*, 1983; Kim *et al.*, 1985).

(ii) *Epidemiological studies*

Royle (1975b) conducted a mortality study among past and current workers with three months or more of consecutive employment in 54 chromium-plating plants in Yorkshire, UK. The study covered 1238 chromium-plating workers (1056 men, 182 women), 142 of whom had died by 31 May 1974. A control population of 1284 manual workers (1099 men, 185 women) was drawn from non-chromium-plating departments of the largest firms and from the past and current work force of two industrial companies located in the same geographic region. The control subjects were matched individually to the platers by sex, age, date when last known to be alive and, for current workers, smoking habits. The study population represented 91% of the total exposed population and 93% of the eligible controls. Compared with the controls, chromium platers experienced a significant excess proportion of deaths from total cancer: 51/142 *versus* 24/104 in men and women combined ($p < 0.01$). The excess was statistically significant only for individuals who had been platers for more than one year. In male chromium platers, 24 lung cancer deaths (ICD codes 162, 163) out of a total of 130 deaths were observed *versus* 13/96 among controls (nonsignificant). Cancer of the gastrointestinal tract and of 'all other sites' also occurred in excess in men, but the differences were not significant: 8/130 deaths from gastrointestinal cancers among exposed *versus* 4/96 in controls; 12/130 deaths from cancers of 'all other sites' in exposed *versus* 5/96 in controls. The smoking habits of platers and controls were similar. A higher proportion of controls had worked in asbestos processing (8.3% of controls *versus* 3.6% of platers); more platers had worked in coal mines, foundries, potteries, cotton manufacture and flax and hemp mills (Royle, 1975a). [The Working Group noted that past exposure to asbestos among the controls might have led to some underestimation of the lung cancer risk

in the exposed group, and that the method of analysis used made the study difficult to interpret.]

Okubo and Tsuchiya (1977, 1979) reported results from a mortality study among 952 chromium platers in Tokyo, Japan. The cohort was constructed from records for 1970-76 of the Tokyo Health Insurance Society of the Plating Industry, and consisted of chromium platers (889 men, 63 women) who were born prior to 31 May 1937, had more than six months of work experience in chromium plating and had a work history record. Vital status was ascertained from a questionnaire sent to the management of the plating firms and, for retired workers, by contacting family registers; persons whose vital status was unknown were assumed to be alive. The expected number of deaths was derived from age-, sex- and year-specific death rates for the Tokyo general population. Twenty-one deaths from all causes were observed in chromium platers [SMR, 55; 95% CI, 34-83]. No case of lung cancer occurred, although 1.2 would have been expected in men. These results were reiterated in a 99% follow-up of a subgroup reported in an abstract (Okubo & Tsuchiya, 1987). [The Working Group questioned the completeness of assembling the cohort, the low age structure of the population and the limited period of follow-up.]

Silverstein *et al.* (1981) performed a proportionate mortality study in a group of hourly employees and retirees with at least ten years of service in a die-casting and nickel- and chromium-electroplating plant in the USA. The 238 subjects who had died between January 1974 and December 1978 were included in the study. Causes of death as stated on death certificates were compared with US national mortality rates. A total of 53 deaths from cancer were observed (proportionate mortality ratio (PMR), 135 [95% CI, 101-176]) among white men and 23 among white women (PMR, 127 [95% CI, 81-191)]. The study revealed 28 lung cancer deaths (PMR, 191 [95% CI, 127-276]) in white men and ten among white women (PMR, 370 [95% CI, 178-681]). Smoking habits were not known. Four deaths from stomach cancer (PMR, 254 [95% CI, 69-648]), two from laryngeal cancer (PMR, 330 [95% CI, 40-1184] and two from lymphosarcoma and reticulosarcoma (PMR, 285 [95% CI, 35-1032]) occurred in white men. A case-control analysis of the lung cancer deaths, using deaths from cardiovascular disease as controls, tested the association of cancer with duration of work in different work sites, without considering possible confounders. An association was seen (odds ratio, 9.2; $p = 0.04$) for white men with more than five years' work in a department which, prior to 1971, was one of the major die-casting and plating areas in the plant. The authors noted that, although the population had been exposed primarily to chromium[VI], they had also been exposed to nickel compounds and may have been exposed to polycyclic aromatic hydrocarbons and metal fumes during die-casting.

Franchini *et al.* (1983) reported cancer mortality in a group of 178 Italian chromium electroplaters, 62 of whom were 'bright' (thin plating) and 116 of whom were

'hard' (thick plating) platers, and who had worked for at least one year in one of nine plants between 1951 and 1981. In 1980, exposure to chromium averaged 7 μg/m³ air as chromium trioxide near the plating baths and 3 μg/m³ in the middle of the room; measurements of urinary chromium showed that hard platers were more heavily exposed than bright platers: the median level of chromium in the urine of hard platers was 23.1 μg/g creatinine in 1974-76 and 5.7 μg/g creatinine in 1980-81. The SMR for deaths from all causes was 97 (15 deaths [95% CI, 55-163]); there were eight deaths from malignant tumours (SMR, 191; [95% CI, 82-375]) and three from lung cancer [SMR, 333; 95% CI, 69-974]. Seven of the cancer deaths occurred among hard platers [SMR, 259; 95% CI, 105-534] as did all three of the lung cancers [SMR, 429; 95% CI, 88-1252].

Sorahan *et al.* (1987) reported the mortality experience of 2689 chromium platers (1288 men, 1401 women) in the UK observed from January 1946 to December 1983 who were involved mainly in 'bright' (thin) plating of bumpers and overriders, initially reported by Waterhouse (1975). Scattered sampling of exposure had taken place before 1973, showing air concentrations of chromium trioxide up to 8.0 mg/m³, while the median values were 'nondetectable' or 'trace'; after 1973, measurements generally showed levels of chromium below 50 μg/m³. The cohort comprised workers employed in 1946-75 with more than six months' employment as a (chromium) electroplater. Death rates were compared with those of the general population of England and Wales. All members of the cohort had had at least some exposure to chromium but also some exposure to nickel chloride and nickel sulfate. A total of 213 cancer deaths (SMR, 130 [95% CI, 113-148]) and 72 lung cancer deaths (SMR, 150 [95% CI, 117-189]) were observed in men and women combined; 63 lung cancer deaths occurred in men (SMR, 158 [95% CI, 121-202]) and nine in women (SMR, 111 [95% CI, 46-261]). When the figures for each sex were combined and account was taken of time from first employment, the highest SMRs were 342 [95% CI, 182-585] after ten to 14 years and 245 [95% CI, 127-428] after 15-19 years of work at the chromium baths. Overall, three deaths (two in men, one in women) from cancer of the nose and nasal cavities occurred (SMR, 1000 [95% CI, 206-2922]); all three persons had been exposed to chromium for one to two years, while the third had also worked for 13 years plating nickel. There were 25 deaths from stomach cancer (SMR, 154 [95% CI, 100-228]), but this excess occurred only in men. Four deaths from cancer of the liver were observed in men (SMR, 667 [95% CI, 182-1707]) but none in women. In an analysis of data on first job held, the SMR for lung cancer was 199 (46 deaths [95% CI, 146-266] for men first employed as chrome bath workers and 101 (17 deaths [95% CI, 59-161]) for chromium workers who were first employed at other work sites. The authors reported that only 11% of workers had had periods of work at both the chrome baths and other chrome work. Although a

significant association was found between work at chrome baths and death from lung cancer, no such association was found with work at nickel baths (Burges, 1980).

In a case-control study in Denmark of 326 cases of laryngeal cancer and 1134 controls (Olsen & Sabroe, 1984), two of the cases occurred among male chromium platers, yielding a standardized incidence odds ratio of 110 (95% CI, 30-360).

(*d*) *Production of ferrochromium alloys*

Pokrovskaya and Shabynina (1973) studied a cohort of male and female factory workers engaged in chromium ferroalloy production between 1955 and 1969 in the USSR. Workers were reported to be exposed to chromium[VI] and chromium[III] compounds as well as benzo[*a*]pyrene. Death certificates were obtained from the municipal vital statistics office, and comparison was made with city mortality rates by sex and by ten-year age group. Access to complete work histories made it possible to exclude from the control cohort subjects who had been exposed to chromium in other plants. Male chromium workers aged 50-59 experienced significant [$p = 0.001$] increases in death rates from all malignancies, from lung cancer and from oesophageal cancer, as compared with deaths rates in the municipal population. The relative risk for lung cancer in men was reported to range from 4.4 in the 30-39-year age group to 6.6 ($p = 0.001$) in the 50-59-year age group. A large proportion of the cases of lung cancer among workers exposed to high concentrations of dust (cinder pit workers, metal crushers, smelter workers), including workers who were not exposed to benzo[*a*]pyrene in areas of furnace charge and finished products preparation. [The Working Group noted that the numbers of workers and the numbers of cancers by specific site were not reported.]

Axelsson *et al.* (1980) studied employees at a ferrochromium plant in Sweden producing ferrochromium alloys by furnace reduction of chromite ore, quartz, lime and coke; the study was restricted to all 1876 men employed for at least one year during the period 1 January 1930 to 31 December 1975 and alive in 1951. Records were available for all employees who had worked since 1913. Individuals were categorized according to length and place of work in the factory. Death certificates (1951-75) were obtained from the national Central Bureau of Statistics and incident cancer cases (1958-75) from a manual search of Cancer Registry files. Expected numbers of cancer deaths and incident cases were calculated assuming a 15-year latent period from onset of employment. The estimated levels of chromium metal plus chromium[III] in the work atmosphere ranged from 0 to 2.5 mg/m^3, and those for chromium[VI] from 0 to 0.25 mg/m^3. There were 87 cases of cancer in the period 1958-75 [SIR, 101; 95% CI, 81-125], of which seven were cancers of the trachea, bronchus, lung and pleura [SIR, 119; 95% CI, 48-245]. Among 641 arc furnace workers, who were considered as being most likely to have encountered exposure to chromium[III] and [VI], there were two cases of cancer at these sites [SIR, 95; 95%

CI, 12-344], one of which was a pleural mesothelioma. Among 326 maintenance workers, there were four cases of cancer at these sites [SIR, 400; 95% CI, 109-1024], two of which were mesotheliomas. Asbestos had been used in the factory.

Langård *et al.* (1980, 1990) studied male workers at a ferrochromium and ferrosilicon production plant in Norway, primarily to explore the hypothesis that chromium[III] might be carcinogenic to humans. Workers with one year or more of work were included. Hygiene studies in the plant in 1975 indicated the presence of chromium[III] and [VI] in the work environment; the atmosphere contained a mean of 0.01-0.29 mg/m^3 chromium, 11-33% of which was water-soluble chromium[VI]. The 1980 study comprised 976 workers with first employment before 1960 and alive in 1953; in the 1990 report, the cohort also included those with first employment before 1965 (to make a total of 1235 workers). In the latter report, 357 deaths from all causes were observed (SMR, 81 [95% CI, 73-90]). The SIR for all cancers was 84 (132 observed; 95% CI, 70-100); the total number of lung cancers was 17 (SIR, 88 [95% CI, 56-123]). Among the 379 ferrochromium workers, there were ten cases of lung cancer [SIR, 154; 95% CI, 74-283], 12 of the prostate [SIR, 151 [95% CI, 78-262] and five of the kidney [SIR, 273; 95% CI, 89-638]. The excess of lung cancer in ferrochromium workers was higher in the 1980 study (seven cases; SMR, 226 [95% CI, 91-466]).

(*e*) *Other industrial exposures to chromium*

In an exploratory proportionate mortality study, Tsuchiya (1965) investigated the occurrence of cancer in 1957-59 in about 200 Japanese companies with more than 1000 employees each. A total of 492 cancer deaths occurred among 1 200 000 workers during that period. The 22 lung cancer deaths that occurred among workers in industries handling chromium compounds were compared with the Japanese mortality rate for 1958 [SMR, 220; 95% CI, 138-333]. The author pointed out that because a person had handled chromium or nickel in a factory did not necessarily imply that he had been exposed to these elements. [The Working Group noted that the design of the study did not exclude selection bias, and that exposures to chromium and a variety of carcinogens were not mutually exclusive.]

Dalager *et al.* (1980) carried out a proportionate mortality study on a group of spray painters using zinc chromate primer paints in the maintenance of aircraft at two US military bases. Spray painting was carried out mainly in air-conditioned booths, but without respirators. The study cohort consisted of 977 white male workers who had spray painted for at least three months and who had terminated employment within a ten-year period prior to 31 July 1959. The relative 'frequency' of causes of death through 1977 was generated by comparing the observed number of cases with the expected relative frequency in the white US male population. There were 202 deaths among the spray painters; 50 had died of cancer (PMR, 136 [95%

CI, 101-179]), 21 of whom had respiratory cancer (ICD 160-164; PMR, 184 [95% CI, 114-282]). The proportionate cancer mortality rate for respiratory cancer was 146 (not significant).

Bertazzi *et al.* (1981) studied the causes of death in 1954-78 for 427 workers who had been employed for at least six months between 1946 and 1977 in a plant producing paints and coatings, including chromate[VI] pigments. They found 18 deaths due to cancer *versus* 9.8 expected on the basis of national rates; there were eight lung cancer deaths, giving SMRs of 227 [95% CI, 156-633] based on local rates and 334 [95% CI, 106-434] on the basis of national rates. The authors were unable to differentiate between exposures to different paints and coatings; they stated that the primary exposure was to chromate[VI] pigments but that there was low exposure to asbestos.

Cornell and Landis (1984) studied the causes of death for 851 men who had worked in 26 US nickel/chromium foundries between 1968 and 1979 and compared them with the mortality experience of US males and of a control group of foundry workers not exposed to nickel/chromium. Sixty deaths were from lung cancer *versus* 56.9 expected in the general population; a total of 103 deaths from all other neoplasms was observed with 118.0 expected. No death from nasal cancer was observed.

Stern *et al.* (1987) followed up 9365 workers from two chrome leather tanneries in Minnesota and Wisconsin, USA, from identification of the cohort in 1940 through to December 1982. Follow-up was 95% complete. By that time, 1582 deaths had occurred, giving a SMR of 89. The SMRs for cancer of the lung, trachea and bronchus (ICD 162-163) were low in both tanneries (18 deaths; SMR, 67; 95% CI, 40-106 and 42 deaths; SMR, 93; 95% CI, 67-126) in comparison with expected rates in the respective states. [The Working Group noted that exposure to chromium was low and occurred in only a small subgroup of the workers.]

Hernberg *et al.* (1983a,b) conducted a joint Danish-Finnish-Swedish case-control study among 167 living cases of cancer of the nasal or paranasal sinuses diagnosed between 1 July 1977 and 31 December 1980, who were individually matched for country, age and sex with patients with colonic or rectal cancer. Cases and controls were interviewed by telephone. Patients who had had work-related exposures during the ten years before occurrence of the illness were excluded. Sixteen patients, many of whom were included within the category 'stainless steel welding' and 'nickel', *versus* six controls reported exposure to chromium (odds ratio, 2.7; 95% CI, 1.1-6.6). Among 21 cases categorized as having been exposed to nickel and/or chromium, including the above cases, only two had been exposed to chromium only: one spray painter (chromates) and one steel worker.

In a case-control study in North Carolina and Virginia, USA, of 160 patients (93 men, 67 women) with cancers of the nasal cavity and paranasal sinuses diag-

nosed between 1970 and 1980, Brinton *et al.* (1984) found chromium/chromate exposure to be 5.1 times more frequent among male cases than among 290 hospital controls, based on five exposed male cases. The authors stated that the excess was associated mainly with use of chromate products in the building industry and in painting.

A hospital-based case-control study in Norway of 176 incident male lung cancer cases was performed by Kjuus *et al.* (1986). Cases were recruited between 1979 and 1983, and 176 age- and sex-matched control subjects were recruited from the same hospitals. Seven cases and six controls had been exposed to chromium and nickel compounds (welding excluded) for more than three years. The risk ratio, adjusted for smoking, was 1.4 (95% CI, 0.4-4.4).

Rafnsson and Jóhannesdóttir (1986) followed up 450 Icelandic men born between 1905 and 1945 who were licensed as masons (cement finishers). Nine deaths from cancer of the lung, trachea and bronchus (ICD 162, 163) were found (SMR, 314; 95% CI, 143-595). The eight men who had been licensed for 20 years had a SMR of 365 (95% CI, 158-720). The concentration of chromium[VI] in Icelandic cement in 1983 was 5.8-9.5 mg/kg; however, masons also work with other substances. [The Working Group noted that respiratory exposure to chromates would have been very low, suggesting that the excess may have been due to other factors.]

In an extended case-control study, Claude *et al.* (1988) further examined the possible relationship between work-related exposure and bladder cancer proposed by Claude *et al.* (1986). A total of 531 male cases were recruited from hospitals in the Federal Republic of Germany between 1977 and 1985 and were compared with sex- and age-matched controls recruited mainly from urological hospital wards. Exposure to chromium/chromate was reported for 52 cases *versus* 24 controls (odds ratio, 2.2; 95% CI, 1.4-3.5). The corresponding figures for spray painting were 49 *versus* 17 (odds ratio, 2.9; 95% CI, 1.7-4.9). Details were not given on the extent to which spray painting included exposure to chromium-containing paints. After adjustment for smoking, the rate ratio estimates for duration of exposure to chromium/chromate were (number of cases/controls in parentheses); one to nine years, 1.2 (10/8); ten to 19 years, 1.0 (9/7); 20-29 years, 2.0 (11/5); and $\geq$30 years, 3.0 (26/8), which gives a *p*-value for trend of 0.009. The corresponding rate ratios for spray painting were: 4.7 (13/2), 8.4 (8/1), 2.0 (14/9) and 2.4 (17/8). [The Working Group noted that the possibility of recall bias was high, since the risk ratios for 24/25 exposures exceeded unity.]

(*f*) *Environmental exposure to chromium*

The possible relation between environmental exposure to chromium and mortality from lung cancer was studied by Axelsson and Rylander (1980) in a population-based study among people living close to two Swedish ferrochromium smelt-

ers. Air concentrations of chromium near the smelter were 100-400 ng/m^3. The lung cancer mortality rates in the two communities where the smelters were located were 253 per million ($p < 0.05$) and 161 per million, respectively, as compared with the county rate of 194 per million during the entire period studied (1961-75).