3. Studies of Cancer in Experimental Animals

3.1 Inhalation exposure

There are no data relative to carcinogenicity by inhalation of cobalt metal, cobalt-metal powder or cobalt alloys.

3.1.1 *Mouse*

In a study undertaken by the National Toxicology Program (1998), groups of 50 male and 50 female $B6C3F_1$ mice, 6 weeks of age, were exposed to aqueous aerosols of 0, 0.3, 1 or 3 mg/m^3 cobalt sulfate heptahydrate (purity, ≈ 99%; mass median aerodynamic diameter (MMAD), 1.4–1.6 μm; geometric standard deviation (GSD), 2.1–2.2 μm) for 6 h per day on 5 days per week for 105 weeks. No adverse effects on survival were observed in treated males or females compared with chamber controls (survival rates: 22/50 (control), 31/50 (low dose), 24/50 (mid dose) or 20/50 (high dose) in males and 34/50, 37/50, 32/50 or 28/50 in females, respectively; survival times: 662, 695, 670 or 643 days in males and 694, 713, 685 or 680 days in females, respectively). Mean body weights increased in all treated females from week 20 to 105 and decreased in males exposed to the high dose from week 96 to 105 when compared with chamber controls. The incidence of neoplasms and non-neoplastic lesions of the lung is reported in Table 12. Exposure to cobalt sulfate heptahydrate caused a concentration-related increase in benign and malignant alveolar/bronchiolar neoplasms in male and female mice. All the alveolar/bronchiolar proliferative lesions observed within the lungs of exposed mice were typical of those arising spontaneously. However, exposure to cobalt did not cause an increased incidence of neoplasms in other tissues (National Toxicology Program, 1998; Bucher *et al*., 1999).

Table 12. Incidence of neoplasms and non-neoplastic lesions of the lung in mice in a 2-year inhalation study of cobalt sulfate heptahydrate

Lesions observed	No. of mice exposed to cobalt sulfate heptahydrate at concentrations (mg/m^3) of			
	0 (chamber control)	0.3	1.0	3.0
Males				
Total no. examined microscopically	50	50	50	50
Infiltration cellular, diffuse, histiocyte	1 (3.0)[a]	2 (3.0)	4 (2.3)	10[b] (1.5)
Infiltration cellular, focal, histiocyte	10 (2.7)	5 (2.6)	8 (3.0)	17 (2.7)
Bronchus, cytoplasmic vacuolization	0	18[b] (1.0)	34[b] (1.0)	38[b] (1.0)
Alveolar epithelium hyperplasia	0	4 (2.3)	4 (1.8)	4 (2.3)
Alveolar/bronchiolar adenoma	9	12	13	18[c]
Alveolar/bronchiolar carcinoma	4	5	7	11[c]
Alveolar/bronchiolar adenoma or carcinoma	11	14	19	28[b]
Females				
Total no. examined microscopically	50	50	50	50
Infiltration cellular, diffuse, histiocyte	0	0	0	4 (3.3)
Infiltration cellular, focal, histiocyte	2 (2.0)	5 (1.8)	7 (2.9)	10[c] (2.4)
Bronchus, cytoplasmic vacuolization	0	6[c] (1.0)	31[b] (1.0)	43[b] (1.0)
Alveolar epithelium hyperplasia	2 (1.5)	3 (1.3)	0	5 (2.0)
Alveolar/bronchiolar adenoma	3	6	9	10[c]
Alveolar/bronchiolar carcinoma	1	1	4	9[b]
Alveolar/bronchiolar adenoma or carcinoma	4	7	13[c]	18[b]

From National Toxicology Program (1998)

[a] Average severity grade of lesions in affected animals: 1, minimal; 2, mild; 3, moderate; 4, marked

[b] Significantly different ($p \leq 0.01$) from the chamber control group by the logistic regression test

[c] Significantly different ($p \leq 0.05$) from the chamber control group by the logistic regression test

3.1.2 *Rat*

In a study undertaken by the National Toxicology Program (1998), groups of 50 male and 50 female Fischer 344/N rats, 6 weeks of age, were exposed to aqueous aerosols of 0, 0.3, 1 or 3 mg/m^3 cobalt sulfate heptahydrate (purity, ≈ 99%; MMAD, 1.4–1.6 μm; GSD, 2.1–2.2 μm) for 6 h per day on 5 days per week for 105 weeks. No adverse effects on mean body weights nor on survival were observed in treated males or females compared with chamber controls (survival rates: 17/50 (control), 15/50 (low dose), 21/50 (mid dose) or 15/50 (high dose) in males and 28/50, 25/49, 26/50 or 30/50 in females, respectively; survival times: 648, 655, 663 or 643 days in males and 699, 677, 691 or 684 days in females, respectively). Exposure to cobalt sulfate heptahydrate caused a concentration-related increase in the incidence of benign and malignant alveolar bronchiolar neoplasms

in male and female rats and benign and malignant pheochromocytomas in female rats. However, exposure to cobalt sulfate did not cause an increased incidence of neoplasms in other tissues. The incidence of neoplasms and non-neoplastic lesions is reported in Table 13. In rats exposed to cobalt sulfate heptahydrate by inhalation, a broad spectrum of inflammatory and proliferative pulmonary lesions was observed. While many of these tumours were highly cellular and morphologically similar to those arising spontaneously, others, in contrast to those seen in mice, were predominantly fibrotic, squamous or mixtures of alveolar/bronchiolar epithelium and squamous or fibrous components. Benign neoplasms typical of those arising spontaneously were generally distinct masses that often compressed surrounding tissue. Malignant alveolar/bronchiolar neoplasms had similar cellular patterns but were generally larger and had one or more of the following histological features; heterogeneous growth pattern, cellular pleomorphism and/or atypia, and local invasion or metastasis. In addition to these more typical proliferative lesions, there were 'fibroproliferative' lesions ranging from less than 1 mm to greater than 1 cm in diameter. Small lesions with modest amounts of peripheral epithelial proliferation were diagnosed as atypical hyperplasia, while larger lesions with florid epithelial proliferation, marked cellular pleomorphism, and/or local invasion were diagnosed as alveolar/bronchiolar carcinomas. While squamous epithelium is not normally observed within the lung, squamous metaplasia of alveolar/bronchiolar epithelium is a relatively common response to pulmonary injury and occurred in a number of rats in this study. Squamous metaplasia consisted of small clusters of alveoli in which the normal epithelium was replaced by multiple layers of flattened squamous epithelial cells that occasionally formed keratin. One male and one female each had a large cystic squamous lesion rimmed by a variably thick band of friable squamous epithelium with a large central core of keratin. These lesions were diagnosed as cysts. In two exposed females, proliferative squamous lesions had cystic areas but also more solid areas of pleomorphic cells and invasion into the adjacent lung; these lesions were considered to be squamous-cell carcinomas. In all groups of male and female rats exposed to cobalt sulfate heptahydrate, the incidence of alveolar proteinosis, alveolar epithelial metaplasia, granulomatous alveolar inflammation and interstitial fibrosis was significantly greater than in the chamber controls. Exposure to cobalt sulfate heptahydrate caused a concentration-related increased incidence of benign and malignant pheochromocytomas in female rats. Although a very common spontaneous neoplasm in male Fischer 344/N rats, pheochromocytomas have a lower spontaneous occurrence in females. The marginally-increased incidence of pheochromocytomas in males was considered an uncertain finding because it occurred only in the group exposed to 1.0 mg/m^3 and was not supported by increased incidence or severity of hyperplasia (National Toxicology Program, 1998; Bucher *et al*., 1999).

Table 13. Incidence of neoplasms and non-neoplastic lesions of the lung in rats and of the adrenal medulla in female rats in a 2-year inhalation study of cobalt sulfate heptahydrate

Lesions observed	No. of rats exposed to cobalt sulfate heptahydrate at concentrations (mg/m^3) of			
	0 (chamber control)	0.3	1.0	3.0
Males				
Lung				
No. examined microscopically	50	50	48	50
Alveolar epithelium, hyperplasia	9 (1.8)[a]	20[b] (2.0)	20[b] (2.1)	23[c] (2.0)
Alveolar epithelium, hyperplasia, atypical	0	2 (3.0)	2 (3.0)	2 (4.0)
Metaplasia, squamous	0	1 (1.0)	4 (2.0)	2 (3.0)
Alveolar epithelium, metaplasia	0	50[c] (1.9)	48[c] (3.1)	49[c] (3.7)
Inflammation, granulomatous	2 (1.0)	50[c] (1.9)	48[c] (3.1)	50[c] (3.7)
Interstitium, fibrosis	1 (1.0)	50[c] (1.9)	48[c] (3.1)	49[c] (3.7)
Proteinosis	0	16[c] (1.4)	40[c] (2.3)	47[c] (3.4)
Cyst	0	0	0	1 (4.0)
Alveolar/bronchiolar adenoma	1	4	1	6
Alveolar/bronchiolar carcinoma	0	0	3	1
Alveolar/bronchiolar adenoma or carcinoma	1	4	4	7/50[b]
Females				
Lung				
No. examined microscopically	50	49	50	50
Alveolar epithelium, hyperplasia	15 (1.4)	7 (1.6)	20 (1.8)	33[c] (2.0)
Alveolar epithelium, hyperplasia, atypical	0	0	3 (3.7)	5[b] (3.2)
Metaplasia, squamous	0	1 (2.0)	8[c] (2.3)	3 (1.7)
Alveolar epithelium, metaplasia	2 (1.0)	47[c] (2.0)	50[c] (3.6)	49[c] (3.9)
Inflammation, granulomatous	9 (1.0)	47[c] (2.0)	50[c] (3.6)	49[c] (3.9)
Interstitium, fibrosis	7 (1.0)	47[c] (2.0)	50[c] (3.6)	49[c] (3.9)
Proteinosis	0	36[c] (1.2)	49[c] (2.8)	49[c] (3.9)
Cyst	0	0	1 (4.0)	0
Alveolar/bronchiolar adenoma	0	1	10[c]	9[c]
Alveolar/bronchiolar carcinoma	0	2	6[b]	6[b]
Alveolar/bronchiolar adenoma or carcinoma	0	3	15[c]	15[c]
Squamous-cell carcinoma	0	0	1	1
Alveolar/bronchiolar adenoma, alveolar/bronchiolar carcinoma or squamous-cell carcinoma	0	3	16[c]	16[c]

Table 13 (contd)

Lesions observed	No. of rats exposed to cobalt sulfate heptahydrate at concentrations (mg/m^3) of			
	0 (chamber control)	0.3	1.0	3.0
Adrenal medulla				
No. examined microscopically	48	49	50	48
Hyperplasia	8 (1.6)	7 (2.3)	11 (2.1)	13 (2.0)
Benign pheochromocytoma	2	1	3	8[b]
Benign, complex, or malignant pheochromocytoma	2	1	4	10[b]

From National Toxicology Program (1998)
[a] Average severity grade of lesions in affected animals: 1, minimal; 2, mild; 3, moderate; 4, marked
[b] Significantly different ($p \leq 0.05$) from the chamber control group by the logistic regression test
[c] Significantly different ($p \leq 0.01$) from the chamber control group by the logistic regression test

3.2 Intratracheal instillation

Rat

Steinhoff and Mohr (1991) reported on the exposure of rats to a cobalt–aluminium–chromium spinel (a blue powder [purity unspecified], with the empirical formula Co[II] 0.66, Al 0.7, Cr[III] 0.3, O 3.66, made of a mixture of CoO, $Al(OH)_3$ and Cr_2O_3 ignited at 1250 °C; 80% of particles < 1.5 μm). Groups of 50 male and 50 female Sprague-Dawley rats, 10 weeks of age, received intratracheal instillations of 10 mg/kg bw of the spinel in saline every 2 weeks for 18 treatments (then every 4 weeks from the 19th to the 30th treatment) for 2 years. Control groups of 50 males and 50 females received instillations of saline only and other control groups of 50 males and 50 females remained untreated. Animals were allowed to live until natural death or were killed when moribund. No appreciable difference in body weights or survival times was observed between the treated and control groups [exact survival data not given]. Alveolar/bronchiolar proliferation was observed in 0/100 untreated controls, 0/100 saline controls, and in 61/100 rats treated with the spinel. [The Working Group noted that the nature of the bronchoalveolar proliferation or possible association with inflammation was not described.] No pulmonary tumours were observed in 100 untreated or 100 saline controls. In the group that received the spinel, squamous-cell carcinoma was observed in one male rat and two females (Steinhoff & Mohr, 1991).

3.3 Intramuscular injection

Rat

In studies undertaken by Heath (1954, 1956), groups of 10 male and 10 female hooded rats, 2–3 months old, received a single intramuscular injection of 28 mg cobalt-metal powder (spectrographically pure, 400 mesh; 3.5 μm × 3.5 μm to 17 μm × 12 μm with large numbers of long narrow particles of the order of 10 μm × 4 μm) in 0.4 mL fowl serum into the thigh; a control group of 10 males and 10 females received fowl serum only. Average survival times were 71 weeks in treated males and 61 weeks in treated females; survival of controls was not specified. During the observation period of 122 weeks, 4/10 male and 5/10 female treated rats developed sarcomas (mostly rhabdomyosarcomas) at the injection site compared with 0/20 controls. A further group of 10 female rats received a single intramuscular injection of 28 mg cobalt-metal powder in 0.4 mL fowl serum; others received injections of 28 mg zinc powder (five rats) or 28 mg tungsten powder (five rats). Average survival time for cobalt-treated rats was 43 weeks. During the observation period of 105 weeks, sarcomas (mostly rhabdomyosarcomas) developed in 8/10 cobalt powder-treated rats; none occurred in the zinc powder- or tungsten powder-treated rats. No other tumours occurred in any of the cobalt-treated or other rats, except for one malignant lymphoma in a zinc-treated rat (Heath, 1954, 1956). [The Working Group noted the small number of animals and questioned the relevance of the route of administration.]

In a supplementary study, a group of 30 male hooded rats, 2–3 months of age, received a single intramuscular injection of 28 mg cobalt-metal powder (spectrographically pure [particle size unspecified]) in 0.4 mL fowl serum into the right thigh; a control group of 15 males received a single injection of fowl serum only. The rats were killed at daily intervals 1 to 28 days after injection. An extensive and continuing breakdown of the differentiated muscle fibres into free myoblasts, and the transformation of some of these myoblasts were described (Heath, 1960). [The Working Group questioned the relevance of the route of administration.]

In a series of three experiments, each of 80 female hooded rats, 7–9 weeks of age, received intramuscular injections of 28 mg of ‘wear’ particles obtained by grinding continuously artificial hip or knee prostheses in Ringer’s solution or synovial fluid in conditions simulating those occurring in the body. Prostheses were made of cobalt–chromium–molybdenum alloy (66.5% cobalt, 26.0% chromium, 6.65% molybdenum, 1.12% manganese). Particles (diameter, down to 0.1 μm [mostly 0.1–1 μm]) were injected in 0.4 mL horse serum and the rats were observed for up to 29 months [survival not specified]. No control group was reported. Sarcomas developed at the injection site in 3/16, 4/14 and 16/50 rats in the three series, respectively. Approximately half of the tumours were rhabdomyosarcomas; the remainder were mostly fibrosarcomas (Heath *et al.*, 1971; Swanson *et al.*, 1973).

3.4 Intramuscular implantation

3.4.1 *Rat*

As a follow-up to the studies by Heath *et al.* (1971) and Swanson *et al.* (1973) (see above), groups of female Wistar and hooded rats, weighing 190–310 and 175–220 g, respectively, received intramuscular implants of 28 mg coarse (100–250 μm diameter; 51 Wistar rats) or fine (0.5–50 μm diameter, 85% 0.5–5 μm; 61 Wistar and 53 hooded rats) particles of a dry powder, obtained by grinding a cobalt–chromium–molybdenum alloy (68% cobalt, 28% chromium, 4% molybdenum). The animals were observed for life. A sham-operated control group of 50 female Wistar rats was included. Survival at 2 years was: 11/51 rats receiving the coarse particles, 7/61 Wistar rats receiving the fine particles, 0/53 hooded rats receiving the fine particles and 5/50 Wistar controls. No tumour was noted at the implantation site of rats treated with either coarse or fine alloy particles nor in sham-operated control animals (Meachim *et al.*, 1982).

3.4.2 *Guinea-pig*

In a similar study in guinea-pigs (Meachim *et al.*, 1982), a group of 46 female Dunkin-Hartley guinea-pigs, weighing 550–930 g, received intramuscular implants of 28 mg powdered cobalt–chromium–molybdenum alloy (68% cobalt, 28% chromium, 4% molybdenum; particle diameter, 0.5–50 μm) and were observed for life; 12/46 animals were alive at 3 years. No control group was reported. No tumours were observed at the implantation site; nodular fibroblastic hyperplasia was observed at the implantation site in eight animals (Meachim *et al.*, 1982).

3.5 Subcutaneous implantation

Rat

Groups of five male and five female Wistar rats, 4–6 weeks of age, received subcutaneous implants of one pellet (approximately 2 mm in diameter) of a cobalt–chromium–molybdenum (Vitallium) alloy. The percentage composition of the metal constituents of the Vitallium alloy was not given. Animals were observed for up to 27 months [survival of animals receiving cobalt–chromium–molybdenum alloy not given]. No sarcomas developed in rats that received the pellets (Mitchell *et al.*, 1960).

3.6 Intra-osseous implantation

3.6.1 *Rat*

Groups of 10–17 male and 8–15 female Sprague-Dawley rats, 30–43 days of age, received implants of one of seven test materials containing cobalt alloyed with chromium and nickel, molybdenum, tungsten and/or zirconium, with traces of other elements (as small

rods, 1.6 mm diameter and 4 mm length, powders or porous compacted wire), in the femoral bone and were observed for up to 30 months. Groups of 13 male and 13 female untreated and sham-operated controls were available. Average survival time was more than 21 months. Sarcomas at the implant site were observed in 1/18 rats given cobalt-based alloy powder containing 41% cobalt, 3/26 rats (given a nickel–cobalt-based powder containing 33% cobalt and 3/32 rats given porous compacted wire containing 51% cobalt. No tumours were observed in two groups of 25 rats given rods containing 69 or 47% cobalt, in two groups of 26 rats given rods containing 0.11 or 33% cobalt, in two groups of 25 and 26 untreated rats nor in a group of 26 sham-treated control rats (Memoli *et al.*, 1986).

3.6.2 *Rabbit*

Two groups of 15–20 rabbits [strain, sex and age unspecified] received an implantation in the femoral cavity of metallic chromium dust or metallic cobalt dust [purity and particle size unspecified]. Physical examination by palpation and X-ray examination 3 years after implantation revealed no implantation-site tumour in survivors of the chromium-treated group nor in the six survivors of the cobalt-treated group (Vollman, 1938). In a follow-up study of survivors [number unspecified] at intervals up to 6 years after implantation, sarcomas were observed at the implantation site in three chromium-treated and two cobalt-treated rabbits (Schinz & Uehlinger, 1942). [The Working Group noted the limited reporting.]

3.7 Intraperitoneal injection

Rat

Groups of 10 male and 10 female Sprague-Dawley rats, 10 weeks of age, received three intraperitoneal injections at 2-month intervals of saline or cobalt–aluminium–chromium spinel powder (see Section 3.2) in saline (total dose, 600 mg/kg bw). Animals were allowed to live their natural lifespan or were sacrificed when moribund [survival not given]. Malignant peritoneal tumours occurred in 1/20 controls (histiocytoma) and 2/20 spinel-treated animals (one histiocytoma, one sarcoma) (Steinhoff & Mohr, 1991).

3.8 Intrarenal administration

Rat

Groups of female Sprague-Dawley rats, weighing 120–140 g, received a single injection of 5 mg metallic cobalt powder (20 rats) or cobalt sulfide powder (18 rats) [purity and particle size unspecified] suspended in 0.05 mL glycerine into each pole of the right kidney. A control group of 16 female rats received injections of 0.05 mL glycerine into each pole of the kidney. After 12 months, necropsies were performed on all rats; no tumours were observed in the kidneys of treated or control rats (Jasmin & Riopelle, 1976). [The Working

Group noted the short duration and inadequate reporting of the experiment and the unusual site of administration.]

3.9 Intrathoracic injection

Rat

Two groups of 10 female hooded rats, 2–3 months of age, received intrathoracic injections of 28 mg cobalt-metal powder (spectrographically pure; particle size, < 400 mesh; 3.5 μm × 3.5 μm to 17 μm × 12 μm, with many long narrow particles of the order of 10 μm × 4 μm) in serum [species unspecified] through the right dome of the diaphragm (first group) or through the fourth left intercostal space (second group) and were observed for up to 28 months. Death occurred within 3 days of the treatment in 6/10 rats injected through the diaphragm and in 2/10 rats injected through the intercostal space. The remaining rats in the first group (diaphragm) survived 11–28 months and in the second group (intercostal space), 7.5–17.5 months. Of the 12 rats that survived the injection, four developed intrathoracic sarcomas (three of mixed origin, including rhabdomyosarcomatous elements; one rhabdomyosarcoma arising in the intercostal muscles) (Heath & Daniel, 1962). [The Working Group noted the small numbers of animals and the questionable route of administration.]