4. Other Relevant Data

4.1 Absorption, distribution, metabolism and excretion

The kinetics and effects of beryllium in humans and animals have been reviewed (Eisenbud, 1984; Skilleter, 1984; Cullen *et al.*, 1986; Reeves, 1986; Skilleter, 1986; Kriebel *et al.*, 1988b; Reeves, 1989; WHO, 1990; Deodhar & Barna, 1991; Haley, 1991).

4.1.1 *Humans*

After accidental exposure of 25 people to beryllium dust, the mean serum concentration of beryllium one day later was 3.5 ppb (μg/L); six days later, it had decreased to 2.4 ppb (Zorn *et al.*, 1986). In unexposed humans who had a mean blood beryllium concentration of 0.9 ng/g (ml), 33.2% of the beryllium in blood was associated with cellular constituents, 7.3% with low-molecular-weight compounds, 8.0% with prealbumin and 51.5% with γ-globulin (Stiefel *et al.*, 1980).

Subjects in the Beryllium Case Registry had elevated concentrations of beryllium in lung tissue (e.g. 0.32 μg/g in a metastinal node) more than 20 years after termination of short-term occupational exposure to beryllium (Sprince *et al.*, 1976).

4.1.2 *Experimental systems*

Retention of carrier-free ^{7}Be as chloride after oral dosage of RF mice, Sprague–Dawley rats, beagle dogs and *Macaca speciosa* monkeys was followed in urine excreted during the first two days. The authors estimated from counts in urine that the gastrointestinal absorption was about 0.6%; however, the urinary excretion of the three monkeys studied was reported to be 3.71% (Furchner *et al.*, 1973).

An early study on the kinetics of continuously inhaled beryllium sulfate in rats showed that the pulmonary burden of beryllium reached a plateau after about 36 weeks. After cessation of exposure, clearance was faster in males than in females. Beryllium was accumulated in tracheobronchial lymph nodes, where the concentration reached a peak at 52 weeks (Reeves & Vorwald, 1967). When rats were exposed to ^{7}Be as chloride and ^{4}Be as sulfate in aqueous aerosols by inhalation using nose-only exposure, 60% of the amount of beryllium deposited initially (the sum of the total body burden and the excreted amount) was found in the lungs and 13.5% in the skeleton (Zorn *et al.*, 1977).

When dogs inhaled aerosols of ^{7}Be as oxide calcined at 500 °C (low-fired) or 1000 °C (high-fired) through the nose, disappearance from the lungs followed first-order kinetics. The clearance half-time was 240 days for high-fired beryllium oxide and 64 days for the low-fired compound. Most of the beryllium in the body was located in the skeleton, tracheobronchial lymph nodes, liver and blood. During the first 32 days after exposure, 59% of the low-fired and 68% of the high-fired beryllium oxide was excreted through the gastrointestinal tract; by 180 days, 47% of the low-fired and 54% of the high-fired was excreted by that route and the balance *via* the kidneys (Finch *et al.*, 1990).

In rats, the clearance of inhaled beryllium oxide calcined at 1000 °C through the lungs showed two successive half-times: the first, comprising 30% of the initial lung burden, was

2.5 days, and the second (70%), 833 days. One to 63 days after exposure, a small fraction (0.58–1.73%) of the initial lung burden was observed in thoracic lymph nodes. About 15% was excreted in the faeces and 1.4% in the urine (Rhoads & Sanders, 1985). The clearance from the alveoli of inhaled beryllium oxide calcined at 1000 °C was faster in hamsters than in rats (Sanders *et al.*, 1975).

The disappearance of beryllium from the lungs of rats 3–171 days after exposure to 800 mg/m^3 metallic beryllium aerosol (mass median aerodynamic diameter, 1.4 μm; geometric mean standard deviation, 1.9) by nose-only inhalation once for 50 min was reported to fit best a first-order kinetic model with a half-time of 240 days (Haley *et al.*, 1990). In a carcinogenicity study (Wagner *et al.*, 1969), described in detail in section 3.1, rats, hamsters and squirrel monkeys were exposed by inhalation to ore dusts containing beryllium, beryl (containing 4.14% beryllium) and bertrandite (containing 1.4% beryllium). Increased concentrations of beryllium were detected in the skeleton, liver and lung after 6–12 months of exposure to beryl or bertrandite; exposure to beryl led to higher tissue concentrations than did exposure to bertrandite.

The highest concentrations of beryllium after an intramuscular injection of carrier-free ^{7}Be as chloride to rats were observed initially in the skeleton, liver, kidney, lungs and spleen; 56.3% of the dose injected was still at the site of injection after one day. During a 64-day follow-up, the skeleton and, to a lesser degree, spleen showed a constant increase, while there was a gradual decrease in the other organs; 20.5% of the dose injected was still at the site of injection (Crowley *et al.*, 1949). Accumulation in the liver, kidney, spleen and, especially, the skeleton was also observed seven days after an intravenous administration of ^{7}Be to rats and rabbits. In rats receiving ^{7}Be as sulfate, the liver and spleen contained appreciable amounts of beryllium; in animals receiving carrier-free ^{7}Be, a higher percentage was found in the skeleton. These differences were less marked in rabbits (Scott *et al.*, 1950).

Accumulation of beryllium in compact bone, liver and kidney was observed in dairy cows given carrier-free ^{7}Be as chloride orally or intravenously (Mullen *et al.*, 1972).

After intravenous injection of carrier-free ^{7}Be as chloride into rats in a solution at pH 2, 47% of the dose was excreted predominantly in the urine and 43% was detected in bone and bone marrow after 24 h; only 4% was detected in liver and 0.1% in spleen. When 1 μmol unlabelled beryllium chloride was added as carrier to the solution to be injected, the proportion found in the liver increased to 25% and that in spleen to 1%. At pH 6, 59% was found in the liver after administration of carrier-free ^{7}Be and 44% after addition of unlabelled beryllium chloride. Administration of labelled plus 0.15 μmol unlabelled beryllium chloride in citrate at pH 6 elicited similar responses to carrier-free ^{7}Be at pH 2, while labelled plus 0.3 μmol unlabelled beryllium hydroxide was accumulated strongly in the liver and spleen (Klemperer *et al.*, 1952).

The uptake of intravenously administered (20–800 μg/kg bw) beryllium phosphate was much more extensive in the liver and spleen (approximately 55% of the dose) than that of beryllium sulfate or citrate in mouse; the same phenomenon was observed in rats given a single dose (200 μg/kg bw). The uptake of the two soluble compounds was practically nil at dose levels up to 50 μg/kg, while uptake of the phosphate was independent of dose (Vacher *et al.*, 1974).

Beryllium phosphate and beryllium sulfate accumulated in both nonparenchymal and parenchymal cells of the liver after intravenous administration (Skilleter & Price, 1978). Beryllium oxide granules accumulated intracellularly in marrow throughout the skeletal system after intravenous administration to rabbits of beryllium oxide [method of preparation not given] (Fodor, 1977).

After an intraperitoneal or intravenous dose of carrier-free ^{7}Be as chloride, the disappearance of beryllium was best characterized by three consecutive half-times of 0.2–0.5, 6.3–21.7 and 50.9–52.4 days in mice, rats, dogs and *Macaca speciosa* monkeys (Furchner *et al.*, 1973).

Transplacental transfer of beryllium was demonstrated in mice after intravenous injection of beryllium chloride (Bencko *et al.*, 1979). Transport of ^{7}Be [chemical unspecified] across the rat placenta after intravenous injection was also reported (Schulert *et al.*, 1969).

An estimated 1% of a single oral dose of carrier-free ^{7}Be as chloride to a dairy cow was excreted in the milk within 91 h (Mullen *et al.*, 1972).

After an intravenous injection of beryllium sulfate to rats, most of the beryllium in plasma coeluted in Sephadex chromatography with phosphate and was attached to plasma globulins. A small part of the dose remained in a low-molecular-weight form (Vacher & Stoner, 1968). One-fourth to one-third of blood-borne beryllium in unexposed guinea-pigs and rats was bound to cellular constituents; this proportion was unchanged in animals exposed to beryllium by inhalation. In both exposed and unexposed guinea-pigs, the proportion bound to prealbumin was approximately 70%; in rats, it was 65% (Stiefel *et al.*, 1980). When beryllium chloride (10^{-4} mol/L) was dissolved in different plasma constituents at their normal plasma concentrations, only a very small proportion (generally less than 2.5%) remained dialysable; only citrate (62%), maleate (30%) and bicarbonate (10%) were significantly dialysable. Phosphate decreased the dialysable part of beryllium to 0.2%, and 4% of the added beryllium remained dialysable. It was concluded that at beryllium concentrations in excess of about 10^{-7} mol/L, most of the beryllium in plasma is nondialysable phosphate, and the small dialysable part is mainly citrate (Feldman *et al.*, 1953). In line with this finding, only 3% of beryllium sulfate added to serum *in vitro* traversed a dialysis membrane within 24 h (Reeves & Vorwald, 1961). A low-affinity binding site for beryllium was observed on the outer cell surface of human and guinea-pig lymphocytes; a binding site with a higher affinity was detected in the cell nucleus (Skilleter & Price, 1984).

After repeated intraperitoneal administrations to rats of beryllum sulfate, beryllium was concentrated in nuclei in the cells of the proximal convoluted tubuli (Berry *et al.*, 1987, 1989). In hepatocytes, beryllium was accumulated in lysosomes and nuclei (Levi-Setti *et al.*, 1988). After intravenous administration, the highest concentrations were observed in lysosomes; only at doses approaching the LD_{50} (corresponding to 2–83 μmol/kg bw beryllium sulfate) was there also accumulation in the nuclei in the liver (Witschi & Aldridge, 1968).

Beryllium showed affinity to nuclei isolated from rat liver *in vitro* (Witschi & Aldridge, 1968); it was not bound to DNA or histones (Witschi & Aldridge, 1968; Parker & Stevens, 1979) but to a highly phosphorylated non-histone protein fraction (Parker & Stevens, 1979).

4.2. Toxic effects

4.2.1 *Humans*

Exposure to beryllium compounds may cause an acute chemical pneumonitis, tracheobronchitis, conjunctivitis, dermatitis and chronic granulomatous pulmonary disease with systemic manifestations (Hardy & Tepper, 1959; Freiman & Hardy, 1970). The acute pulmonary disease was first described in Germany in 1933 (Weber & Engelhardt, 1933) and the chronic form in the USA in 1946 (Hardy & Tabershaw, 1946).

Acute beryllium disease, most frequently related to intense but brief exposure, consists of respiratory tract irritation and dermatitis, sometimes with conjunctivitis. The respiratory tract symptoms range from mild nasopharyngitis to a severe chemical pulmonitis, which may be fatal (Hardy & Tepper, 1959; Kriebel *et al.*, 1988b). In fatal cases, histopathological findings in the lungs have included interstitial oedema, cellular infiltration, elevated numbers of plasma cells, alveolar cell proliferation or desquamation and, sometimes, interalveolar oedema, hyaline membranes and organizing pneumonia (Freiman & Hardy, 1970).

Chronic beryllium disease is a systemic disorder with primary manifestations in the lung, characterized by a decrease in transfer factor with restrictive and obstructive ventilatory function. Histopathologically, the disease is characterized by non-caseating granuloma formation with giant cells, as in sarcoidosis, primarily seen in the lungs but also in other tissues. Chest radiography usually shows diffuse infiltrates and hilar adenopathy (Hardy & Tepper, 1959; Freiman & Hardy, 1970; Jones Williams, 1977; Kriebel *et al.*, 1988b). An improvement in lung function and even in lung radiographic findings was reported after a significant decrease in the air concentration of beryllium due to improved engineering and ventilation in plants (Sprince *et al.*, 1978).

Beryllium compounds known to cause beryllium-induced diseases include metallic beryllium (Jones Williams, 1977), beryllium alloys (Lieben *et al.*, 1964) and beryllium oxide fumes (Cullen *et al.*, 1987). The first cases of beryllium disease were identified in the fluorescent light-bulb industry (Hardy & Tabershaw, 1946), in which beryllium-containing phosphors (zinc beryllium manganese silicate), prepared by firing the individual oxides with silica, were used (Eisenbud & Lisson, 1983).

Although chronic beryllium disease has become rare since the adoption of stringent industrial hygiene measures, sporadic cases are still reported (Karkinen-Jääskeläinen *et al.*, 1982; Cullen *et al.*, 1987; Rossman *et al.*, 1988; Kreiss *et al.*, 1989; Newman *et al.*, 1989), e.g., among workers in a precious metal refinery, where exposure to beryllium did not exceed 2 $\mu g/m^3$ (Cullen *et al.*, 1987). A conspicuous feature of chronic beryllium disease is its occasional occurrence outside facilities in which beryllium compounds are used: Sterner and Eisenbud (1951) reported 10 cases among people who had never worked in a beryllium plant but who lived within 1 km of one; the best estimate of beryllium concentrations in the air in the area was 0.01–0.1 $\mu g/m^3$. In 1983, when the US registry for beryllium diseases contained 622 cases of chronic beryllium disease, 65 had had no occupational exposure to beryllium, 42 could be attributed to air pollution (41 occurred in the vicinity of two large production plants and one in a woman living near a fluorescent-lamp plant) and 23 to household exposure to dust brought home on work clothes (Eisenbud & Lisson, 1983).

In the cohort study based on the Beryllium Case Registry, reported in detail in section 2 (p. 68), the SMR for non-neoplastic respiratory diseases was 16.4 ($p < 0.001$) and that for non-neoplastic respiratory diseases (other than influenza and pneumonia), 32.1 ($p < 0.001$) (Infante *et al.*, 1980). In an updating of the cohort (Steenland & Ward, 1991), described in detail in section 2, the SMR for nonmalignant lung disease was 26.3 (95% CI, 20.6–33.1) for workers with less than four years of exposure and 45.8 (95% CI, 36.6–56.5) for workers with longer exposure.

In a cohort study of 9225 male workers employed in seven beryllium processing facilities in the USA (Ward *et al.*, 1992; described in section 2, p. 69), the SMR for pneumoconiosis and other respiratory diseases was 1.48 (95% CI, 1.21–1.80), that for diseases of the heart was 1.06 (1.00–1.12) and that for chronic and unspecified nephritis, renal failure and other renal sclerosis, 1.49 (1.00–2.12).

A nonsymptomatic form of chronic beryllium disease—typical granulomatous changes in transbronchial biopsy specimens with positive lymphocyte transformation tests—has been reported (Newman *et al.*, 1989).

Beryllium dermatitis may be a typical contact dermatitis, localized dermal ulceration or a subcutaneous granuloma. Ulceration of granulomas develops after a particle of a beryllium-containing substance is introduced into an abrasion, laceration or cut (Hardy & Tepper, 1959). People with beryllium-induced contact dermatitis react to patch testing (Curtis, 1951; DeNardi *et al.*, 1952). Patch testing may cause a flare of the dermatitis in sensitized people; it may also induce beryllium sensitivity (Curtis, 1951)

A role of immunological mechanisms in beryllium-induced chronic disease was originally proposed by Sterner and Eisenbud (1951). The condition has the features of a type IV cell-mediated hypersensitivity disorder, the beryllium acting as a hapten (Dayan *et al.*, 1990). Cell-free extracts of blood lymphocytes from people with experimentally induced, localized, dermal granulomatous beryllium lesions cultured in the presence of beryllium oxide contained migration inhibition factor, which inhibits the migration of guinea-pig peritoneal exudate cells (Henderson *et al.*, 1972). The factor was also produced by cell cultures originating from the blood of patients with chronic beryllium disease (Jones Williams *et al.*, 1972; Marx & Burrell, 1973). Lymphocytes from such patients responded to a beryllium oxide or beryllium sulfate challenge by blast transformation and increased thymidine incorporation (Hanifin *et al.*, 1970; Deodhar *et al.*, 1973). Proliferation of lymphocytes from patients with chronic beryllium disease in response to a challenge with beryllium sulfate or fluoride was more marked in lymphocytes obtained by bronchoalveolar lavage than in those harvested from circulating blood (Epstein *et al.*, 1982; Cullen *et al.*, 1987; Saltini *et al.*, 1989). The only lymphocytes obtained from bronchoalveolar lavage which proliferated were CD4+ (helper/inducer) T cells (Saltini *et al.*, 1989).

4.2.2 *Experimental systems*

When beryllium (as lactate or sulfate) was given intravenously to rats or rabbits at a dose of 0.5 or 0.75 mg/kg Be, death invariably followed within four days; the primary cause of death was liver damage and ensuing hypoglycaemia. In rabbits, but not in rats, convulsions were observed before death (Aldridge *et al.*, 1950).

A granulomatous lung disease, morphologically and immunologically similar to chronic beryllium disease in humans, was induced in beagle dogs by inhalation of beryllium oxide calcined at 500 °C, but not with beryllium oxide calcined at 1000 °C (Haley *et al.*, 1989).

Intratracheal instillation of 10 mg beryllium oxide (calcined at 560 °C) into male Hartley guinea-pigs of an inbred strain caused focal interstitial lymphomononuclear infiltrates in the lungs, which progressed to granulomatous lung lesions with fibrosis. Lymphocytes from the blood of these animals responded to beryllium sulfate *in vitro* by increased incorporation of tritiated thymidine (lymphocyte transformation test). The animals exhibited a positive reaction to intradermal beryllium sulfate. Intravenous or oral administration of beryllium sulfate before intratracheal instillation of beryllium oxide decreased the intensity of the pulmonary reaction; a similar effect was observed when the animals were treated with prednisone, L-asparaginase or cyclophosphamide. Splenic cells from animals with beryllium-induced lung disease given intraperitoneally to another group of animals of the same strain caused a similar disease and skin reactivity to beryllium sulfate. No lung disease, skin reactivity or reaction in the lymphocyte transformation test was induced by similar treatment of another inbred strain of guinea-pigs (Barna *et al.*, 1981).

In another study using the same responsive guinea-pig strain, lymphokine production by isolated lymph node cells from animals treated with beryllium oxide endotracheally and challenged with beryllium sulfate was demonstrated *in vitro*. The cells also secreted a factor that inhibited the migration of macrophages (Barna *et al.*, 1984).

Strain A (H-2^a haplotype) mice given an intratracheal instillation challenge of beryllium sulfate or beryllium oxide (calcined at 550 and 1100 °C) after immunization with beryllium sulfate had increased numbers of lymphocytes in bronchoalveolar lavage fluids two, four and eight weeks (months for the oxide) after the challenge. The cells were mainly CD4+ T lymphocytes. By four weeks, microgranulomas were observed in the lungs, which had developed into granulomatous lesions by eight weeks in the case of the sulfate. Such changes were not observed in mice not immunized with beryllium sulfate or in pretreated mice that were not challenged, nor in two strains of mice with different H-2 haplotypes [C57Bl/6(H-2^b) and BALB/c(H-2^d)] (Huang *et al.*, 1992).

In a descriptive toxicity study (see p. 86), male Fischer 344/N rats were exposed by nose only to 800 mg/m^3 metallic beryllium dust (mass median aerodynamic diameter, 1.4 μm) for 50 min, to give an initial lung burden of 625 μg. The animals were then followed for 171 days with timed terminations at 3, 7, 10, 14, 31, 59 and 115 days. Necrotizing, haemorrhagic pulmonitis and intra-alveolar fibrosis, followed by chronic inflammatory changes, were observed. The prevailing cell type obtained by bronchoalveolar lavage was neutrophils; few lymphocytes and no granulomas were observed (Haley *et al.*, 1990). Similarly, after a 1-h exposure of rats to 4.05 mg/m^3 Be as beryllium sulfate (mass median aerodynamic diameter, 1.9 μm), progressive focal interstitial pneumonitis, but no granulomatous disease, was observed; the gross histological picture was similar three weeks and 3, 6 and 12 months after the exposure (Sendelbach *et al.*, 1989).

Intratracheal instillation of beryllium sulfate after immunization with a subcutaneous injection of beryllium sulfate fortified with ovalbumin and Freund's adjuvant resulted in

granulomatous pulmonary disease in Fischer 344 rats within six weeks, accompanied by accumulation of both T and B lymphocytes in the lung tissue (Votto *et al.*, 1987).

In a carcinogenicity study (Wagner *et al.*, 1969; see section 3.1, p. 76), granulomatous lung lesions were observed in hamsters and rats exposed to bertrandite but not in those exposed to beryl ore. [It is not clear if the granulomas were morphologically similar to those observed in humans with chronic beryllium disease or to those in dogs and guinea-pigs after short-term exposure to beryllium oxide.]

The effect of beryllium sulfate (1-h exposure by inhalation; 13 mg/m^3 Be; particle mass median aerodynamic diameter, 1.9 μm) on cell kinetics was studied in rats and mice by autoradiographic determination of the proportion of tritium-labelled cells 90 min after intraperitoneal administration of tritiated thymidine (Sendelbach *et al.*, 1986). In rats, a strong proliferative response was seen, involving type II alveolar epithelial cells and interstitial and capillary endothelial cells. In mice, the proliferative response was weaker and was limited to alveolar macrophages and interstitial and endothelial cells.

Dietary administration of beryllium carbonate at 0.125–1% caused changes typical of rachitis in the skeleton of rats (Guyatt *et al.*, 1933).

Exposure of female rats by nose-only inhalation to beryllium oxide aerosol (mass median aerodynamic diameter, 1.10 μm; calcined at approximately 1000 °C [dust concentration and length of exposure not given]), to give an initial alveolar deposition of 30 μg beryllium, decreased alveolar clearance of subsequently administered plutonium oxide by up to 40% (Sanders *et al.*, 1975).

The concentration of beryllium sulfate required to decrease the viability of canine pulmonary alveolar macrophages *in vitro* by 50% was 0.11 mmol/L; the corresponding concentration for beryllium oxide calcined at 500 °C was 1.4 mmol/L, and that for beryllium oxide calcined at 1000 °C was 3.3 mmol/L. [Because of the limited solubility of beryllium sulfate in tissue culture media, it is not clear what proportion was truly in solution.] The solubility of the high-fired beryllium oxide in 100 ml 0.1 N hydrochloric acid was considerably lower than that of the low-fired compound. There was a similar tendency for differential solubility in simulated serum ultrafiltrate, which was not, however, significant (Finch *et al.*, 1988). Similar results were obtained in a study of cultured rat tracheal epithelial cells (Steele *et al.*, 1989).

Intravenous administration of beryllium sulfate at 30 μmol/kg bw to rats decreased the stimulation of thymidine incorporation into liver DNA after partial hepatectomy (Witschi, 1968); the decrease was accompanied by decreased activities of thymidine kinase, thymidylate kinase, thymidylate synthetase, deoxycytidylate deaminase and DNA polymerase (Witschi, 1970). No effect was observed on the incorporation of ^{14}C-orotic acid into RNA, the activity of RNA polymerase, incorporation of ^{14}C-leucine into histones or acetylation of histones (Marcotte & Witschi, 1972).

Addition of beryllium sulfate at 1–5 μmol/L increased ^{3}H-thymidine incorporation into splenic lymphocyte DNA by two to three fold (Price & Skilleter, 1985). This weak mitogenic effect was limited to B lymphocytes (Newman & Campbell, 1987). Beryllium sulfate, brought into solution as a sulfosalicylic acid complex, inhibited the growth of mouse fibroblasts in culture at concentrations higher than 10^{-5} mol/L (Rössner & Bencko, 1980).

Be^{2+} at a concentration of 0.1 mmol/L inhibited the proliferation of rat hepatocytes in culture induced by epidermal growth factor by 72%, but it did not affect the binding of growth factor to its receptors on the hepatocytes (Skilleter & Legg, 1989).

Beryllium fluoride complexes were bound to microtubules polymerized in the presence of glycerol from tubulin isolated from pig brain and stabilized the polymer formed (Carlier *et al.*, 1988, 1989). Divalent beryllium ($BeSO_4$), but not beryllium fluoride, stimulated microtubule-associated protein-dependent polymerization of tubulin purified from bovine brain and stabilized the polymer formed (Hamel *et al.*, 1991, 1992).

4.3 Reproductive and developmental effects

4.3.1 *Humans*

Kline *et al.* (1951) described the pregnancy of a 25-year-old woman who worked in a fluorescent-tube factory in 1942–44. She displayed signs of radiographic changes in lungs, cyanosis and dyspnoea in the seventh month of her second pregnancy in 1950. No beryllium was detected in a lung biopsy. The woman was treated with adrenocorticotrophic hormone and steroids and delivered a 2.75-kg child seven weeks later. Twenty-four-hour specimens of the urine of the infant collected on the second and third day after birth contained 0.4 and 0.015 μg Be. The child became severely hypoglycaemic after 48 h but was subsequently released from hospital.

Savitz *et al.* (1989) examined a subset of people covered by the 1980 US National Natality and Fetal Mortality Surveys for indications of adverse effects related to maternal or paternal occupational exposures to beryllium, as assessed from a job–exposure matrix. Paternal occupational exposure was associated with 3170 stillbirths, 552 preterm deliveries and 371 babies small for gestational age; the corresponding odds ratios (with 95% CI) were: 1.0 (0.7–1.3), 1.0 (0.5–2.0) and 0.9 (0.5–1.7), respectively. Maternal exposure to beryllium was not associated with these end-points.

4.3.2 *Experimental systems*

The effects of beryllium compounds on reproduction and prenatal development have been reviewed (Barlow & Sullivan, 1982). After oral exposure of male and female rats to a single intratracheal dose of 0.2 mg beryllium oxide (fired at 960 °C in one study and 500 °C in a second), no effect was noted in repeated breeding trials on fertility, postnatal viability or growth over 15 months. In fact, beryllium-treated rats tended to produce more litters over time than did controls (Clary *et al.*, 1975).

All offspring of Sprague–Dawley rats exposed intravenously to 0.316 mg/kg bw beryllium nitrate (one-tenth of the reported LD_{50}) on gestation day 1 died within two to three days after birth. Exposure to beryllium on day 11, but not on day 12, 13, 15 or 17 of gestation, resulted in death *in utero*; all pups in the other groups died within two to three days of delivery (Mathur *et al.*, 1987). [The Working Group noted the potential confounding effect of anaesthesia and surgery in the experimental design.]

4.4 Genetic and related effects

4.4.1 *Humans*

No data were available to the Working Group.

4.4.2 *Experimental systems* (see also Table 19 and Appendices 1 and 2)

(*a*) *Beryllium salts*

Beryllium sulfate was mutagenic to *Bacillus subtilis* in the *rec* assay, but no effect was seen using a higher dose of beryllium chloride. [The latter was actually a null effect, as no zone of inhibition was seen.] Both beryllium chloride and beryllium nitrate were mutagenic in a *rec* assay using spores of *B. subtilis*.

A null effect was also seen with beryllium sulfate in *Escherichia coli* in the pol^+/pol^- assay for DNA modifying effects. In a spot test using four strains of *E. coli* with different repair capacities, beryllium sulfate caused zones of inhibition of growth only in repair-deficient strains. The inhibition decreased with increasing pH, with little effect above pH 5–6. The authors suggested that beryllium interferes with use of exogenous orthophosphate rather than with DNA repair.

Beryllium chloride did not induce SOS repair, measured as λ prophage induction; no inhibition of growth was seen with continuous exposure to up to 5 mM, however, suggesting lack of uptake.

Beryllium sulfate was inactive in most bacterial mutagenesis assays. It did not induce point mutations in *Salmonella typhimurium* in the absence of metabolic activation in four laboratories. Negative results were found in the presence of various metabolizing systems, except in strain TA1535, in which equivocal results were obtained in the presence of some Aroclor-induced liver enzymes; however, no toxicity was seen, even at doses up to 5 mg/plate. Beryllium chloride and beryllium nitrate at similarly high doses were not mutagenic to *S. typhimurium*. Beryllium sulfate was not mutagenic to *S. typhimurium* in a plate incorporation assay, but it gave positive results in single fluctuation tests with *E. coli* and with one strain of *S. typhimurium*. Beryllium chloride induced a modest increase in the number of mutations in the *lac*I gene when grown with *E. coli*, but no clear dose–response relationship. It did not enhance the mutagenicity of ultraviolet radiation to *E. coli*, but it enhanced the mutagenicity of 9-aminoacridine to *S. typhimurium*.

Beryllium sulfate was not mutagenic when injected intraperitoneally to adult male Swiss–Webster mice in a host-mediated assay using *S. typhimurium* strains. It did not induce mitotic recombination in *Saccharomyces cerevisiae* D3 in the presence or absence of metabolic activation, and did not induce mutation in a host-mediated assay using the same strain.

Beryllium sulfate tetrahydrate did not induce unscheduled DNA synthesis in primary hepatocytes, as measured by autoradiographic light nuclear labelling; however, a dose of 10 mg/ml was reported to be toxic.

In the only study available, beryllium chloride was reported to increase the frequency of 8-azaguanine-resistant mutants in Chinese hamster V79 cells by a factor of about 6.

Beryllium chloride and beryllium nitrate induced sister chromatid exchange in the same cells. Beryllium sulfate also increased the frequency of sister chromatid exchange in cultured human lymphocytes and in Syrian hamster embryo cells. Studies on the ability of beryllium salts to induce chromosomal aberrations *in vitro* have had mixed results. Beryllium sulfate increased the frequency of chromatid aberrations in human lymphocytes in one of two studies, and a 21-fold increase was seen in the same study with Syrian hamster embryo cells. Higher doses of beryllium sulfate were nonclastogenic to Chinese hamster lung cells; however, toxicity was seen only at 2.5 mg/ml. It had little effect on chromosomes in Chinese hamster ovary cells, but fairly high concentrations enhanced the frequency of X-ray-induced chromatid-type exchanges. Extremely high concentrations of beryllium chloride caused chromosomal 'stickiness' in cultured peripheral lymphocytes of domestic pigs; chromosomal breakage was rare, whereas chromatid breaks were frequent.

Beryllium sulfate induced morphological transformation of Syrian hamster embryo cells and enhanced the transformation of the cells by simian adenovirus SA7 [no dose–response given]. In a comparative evaluation of in-vitro transformation systems, beryllium sulfate induced morphological transformation in BALB/3T3 cells, in Syrian hamster embryo cells and in Rauscher murine leukaemia virus-infected Fischer 344 rat embryo cells. [In none of the studies were transformed cells injected into suitable hosts to verify the occurrence of malignant transformation.]

In the only report of exposure *in vivo*, beryllium sulfate given by gavage at 50 and 80% of the four-day maximal tolerated dose did not induce micronuclei in the bone marrow of mice. A marked depression of bone-marrow erythropoiesis was observed, suggesting a toxic effect to the marrow.

(*b*) *Beryllium oxide*

This sparingly soluble compound did not induce differential toxicity in *B. subtilis*, mutation in two strains of *S. typhimurium* or sister chromatid exchange in Chinese hamster V79 cells.

Both single-strand breaks and morphological cell transformation were reported to be induced by low-fired beryllium oxide, but conflicting results were obtained for both end-points with high-fired beryllium oxide. [The data were not particularly convincing.]

Considerations with regard to genotoxic mechanisms

As pointed out in a review, beryllium is uniquely amphoteric among the alkaline earth elements. It can form positive and negative ions in acidic and basic media but not at neutrality, at which it forms poorly soluble particulates. Beryllium salts are readily precipitated in the tissues and are transported in blood predominantly as colloidal phosphate–hydroxide complexes weakly associated with plasma globulins; these may be taken up by macrophages. Cultured cells essentially accumulate only colloidal or particulate beryllium, by a temperature-dependent process deduced to be endocytosis. Macrophages, the cells most active in the endocytosis of particulate materials, appear to be those most sensitive to the cytotoxicity of beryllium (reviewed by Skilleter, 1984). Beryllium was toxic to mammalian cells only at concentrations at which a precipitate was seen in the culture

Table 19. Genetic and related effects of beryllium compounds

Test system	Result		Dose[a] (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Beryllium chloride				
PRB, λ Prophage induction, *Escherichia coli*	–[b]	0	45	Rossman *et al.* (1984)
BSD, *Bacillus subtilis rec* assay, differential toxicity	–	0	22.5	Nishioka (1975)
BSD, *Bacillus subtilis* (spores) *rec* assay, differential toxicity	+	0	84	Kuroda *et al.* (1991)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	0	NR	Ogawa *et al.* (1987)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	280	Kuroda *et al.* (1991)
SA2, *Salmonella typhimurium* TA102, reverse mutation	–	0	NR	Ogawa *et al.* (1987)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	0	NR	Ogawa *et al.* (1987)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	+[c]	0	450	Ogawa *et al.* (1987)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	0	NR	Ogawa *et al.* (1987)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	280	Kuroda *et al.* (1991)
SAS, *Salmonella typhimurium* TA2637, reverse mutation	–	0	NR	Ogawa *et al.* (1987)
SAS, *Salmonella typhimurium* TA2637, reverse mutation	+[c]	0	450	Ogawa *et al.* (1987)
ECK, *Escherichia coli* KMBL 3835 (*lac*I gene), forward mutation	+	0	0.09	Zakour & Glickman (1984)
EC2, *Escherichia coli* WP2, reverse mutation	–[d]	0	18	Rossman & Molina (1986)
G9H, Gene mutation, Chinese hamster lung V79 cells, *hprt* locus, *in vitro*	+	0	18	Miyaki *et al.* (1979)
SIC, Sister chromatid exchange, Chinese hamster lung V79 cells, *in vitro*	+	0	3.5	Kuroda *et al.* (1991)
CIA, Chromosomal aberrations, swine lymphocytes, *in vitro*	+	0	1.8	Vegni Talluri & Guiggiani (1967)
Beryllium nitrate				
BSD, *Bacillus subtilis* (spores) *rec* assay, differential toxicity	+	0	51	Kuroda *et al.* (1991)
SA0, *Salmonella typhimurium* TA100, reverse mutation (spot test)	–	0	900	Tso & Fung (1981)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	170	Kuroda *et al.* (1991)

Table 19 (contd)

Test system	Result		Dose[a] (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Beryllium nitrate (contd)				
SA9, *Salmonella typhimurium* TA98, reverse mutation	?	0	NR	Arlauskas *et al.* (1985)
SA9, *Salmonella typhimurium* TA98, reverse mutation	-	-	170	Kuroda *et al.* (1991)
SIC, Sister chromatid exchange, Chinese hamster lung V79 cells, *in vitro*	+	0	2.0	Kuroda *et al.* (1991)
Beryllium sulfate				
ECD, *Escherichia coli pol* A, differential toxicity (spot test)	-	0	28	Rosenkranz & Poirier (1979)
BSD, *Bacillus subtilis rec* assay, differential toxicity	+	0	90	Kada *et al.* (1980); Kanematsu *et al.* (1980)
ERD, *Escherichia coli rec* strains, differential toxicity	+	0	2.25	Dylevoi (1990)
SA0, *Salmonella typhimurium* TA100, reverse mutation	-	-	6	Simmon (1979a)
SA0, *Salmonella typhimurium* TA100, reverse mutation	-	-	14	Dunkel *et al.* (1984)[e]
SA0, *Salmonella typhimurium* TA100, reverse mutation	-	0	NR	Arlauskas *et al.* (1985)
***Salmonella typhimurium* TA100, reverse mutation (fluctuation)	+	0	4.5	Arlauskas *et al.* (1985)
SA0, *Salmonella typhimurium* TA100, reverse mutation	-	-	127	Ashby *et al.* (1990)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	-	-	10	Rosenkranz & Poirier (1979)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	-	-	6	Simmon (1979a)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	-	?[f]	0.9	Dunkel *et al.* (1984)[e]
SA5, *Salmonella typhimurium* TA1535, reverse mutation	-	0	NR	Arlauskas *et al.* (1985)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	-	-	127	Ashby *et al.* (1990)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	-	-	6	Simmon (1979a)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	-	-	14	Dunkel *et al.* (1984)[e]
SA7, *Salmonella typhimurium* TA1537, reverse mutation	-	0	NR	Arlauskas *et al.* (1985)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	-	-	127	Ashby *et al.* (1990)
SA8, *Salmonella typhimurium* TA1538, reverse mutation	-	-	10	Rosenkranz & Poirier (1979)
SA8, *Salmonella typhimurium* TA1538, reverse mutation	-	-	6	Simmon (1979a)

Table 19 (contd)

Test system	Result		Dose[a] (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Beryllium sulfate (contd)				
SA8, *Salmonella typhimurium* TA1538, reverse mutation	–	–	14	Dunkel *et al.* (1984)[e]
SA8, *Salmonella typhimurium* TA1538, reverse mutation	–	0	NR	Arlauskas *et al.* (1985)
SA8, *Salmonella typhimurium* TA1538, reverse mutation	–	–	127	Ashby *et al.* (1990)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	6	Simmon (1979a)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	14	Dunkel *et al.* (1984)[e]
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	0	NR	Arlauskas *et al.* (1985)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	127	Ashby *et al.* (1990)
SAS, *Salmonella typhimurium* TA1536, reverse mutation	–	–	6	Simmon (1979a)
ECW, *Escherichia coli* WP2 *uvr*A, reverse mutation	–	–	14	Dunkel *et al.* (1984)[e]
***, *Escherichia coli* WP2 *uvr*A, reverse mutation (fluctuation test)	?	0	NR	Arlauskas *et al.* (1985)
SCH, *Saccharomyces cerevisiae* D3, mitotic recombination	–	–	430	Simmon (1979b)
URP, Unscheduled DNA synthesis, primary rat hepatocytes	–	0	86	Williams *et al.* (1982)
SIS, Sister chromatid exchange, Syrian hamster embryo cells *in vitro*	+	0	0.05	Larramendy *et al.* (1981)
CIC, Chromosomal aberrations, Chinese golden hamster ovary cells *in vitro*	–	0	9	Brooks *et al.* (1989)
CIC, Chromosomal aberrations, Chinese hamster ovary cells *in vitro*	+[g]	0	9	Brooks *et al.* (1989)
CIC, Chromosomal aberrations, Chinese hamster lung cells *in vitro*	–	–	64	Ashby *et al.* (1990)
CIS, Chromosomal aberrations, Syrian hamster embryo cells *in vitro*	+	0	0.25	Larramendy *et al.* (1981)
TBM, Cell transformation, BALB/c 3T3 mouse cells *in vitro*	+	0	0.05	Dunkel *et al.* (1981)
TCS, Cell transformation, Syrian golden hamster embryo cells *in vitro*	+	0	0.016	Pienta *et al.* (1977)
TCS, Cell transformation, Syrian hamster embryo cells *in vitro*	+	0	0.13	DiPaolo & Casto (1979)

Table 19 (contd)

Test system	Result		Dose[a] (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Beryllium sulfate (contd)				
TRR, Cell transformation, RLV/Fischer rat embryo cells *in vitro*	+	0	0.005	Dunkel *et al.* (1981)
T7S, Cell transformation SA7/Syrian hamster embryo cells *in vitro*	+	0	5	Casto *et al.* (1979)
SHL, Sister chromatid exchange, human lymphocytes *in vitro*	+	0	0.05	Larramendy *et al.* (1981)
CHF, Chromosomal aberrations, human MRC5 fibroblasts *in vitro*	-	0	0.005	Paton & Allison (1972)
CHL, Chromosomal aberrations, human lymphocytes *in vitro*	+	0	0.25	Larramendy *et al.* (1981)
CHL, Chromosomal aberrations, human WI38 lymphocytes *in vitro*	-	0	0.009	Paton & Allison (1972)
HMM, Host-mediated assay, *Salmonella typhimurium* TA1530 in male Swiss–Webster mice	-		1.25, im or po	Simmon *et al.* (1979)
HMM, Host-mediated assay, *Salmonella typhimurium* TA1535 in male Swiss–Webster mice	-		103, im or po	Simmon *et al.* (1979)
HMM, Host-mediated assay, *Salmonella typhimurium* TA1538 in male Swiss–Webster mice	-		1.25, im or po	Simmon *et al.* (1979)
HMM, Host-mediated assay, *Saccharomyces cerevisiae* in mice	-		103, im or po	Simmon *et al.* (1979)
MVM, Micronucleus test, mouse bone marrow *in vivo*	-		116, po × 1	Ashby *et al.* (1990)
Beryllium oxide				
BSD, *Bacillus subtilis* (spores) *rec* assay, differential toxicity	-[h]	0	0.1	Kuroda *et al.* (1991)
SA0, *Salmonella typhimurium* TA100, reverse mutation	-[h]	-	0.08	Kuroda *et al.* (1991)
SA9, *Salmonella typhimurium* TA98, reverse mutation	-[h]	-	0.08	Kuroda *et al.* (1991)
DIA, DNA strand breaks, rat tracheal epithelial cells	+[i]	0	0.36	Steele *et al.* (1989)
DIA, DNA strand breaks, rat tracheal epithelial cells	?[j]	0	10	Steele *et al.* (1989)

Table 19 (contd)

Test system	Result		Dose[a] (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Beryllium oxide (contd)				
SIC, Sister chromatid exchange, Chinese hamster V79 lung cells *in vitro*	–[g]	0	0.03	Kuroda *et al.* (1991)
TCL, Cell transformation, rat tracheal epithelial cells *in vitro*	+[i]	0	0.1	Steele *et al.* (1989)
TCL, Cell transformation, rat tracheal epithelial cells *in vitro*	?[j]	0	10	Steele *et al.* (1989)

+, considered to be positive; (+), considered to be weakly positive in an inadequate study; –, considered to be negative; ?, considered to be inconclusive (variable responses in several experiments within an adequate study); 0, not tested

[a]LED, lowest effective dose; HID, highest ineffective dose. In-vitro tests, μg/ml; in-vivo tests, mg/kg bw. Doses given as concentration of element, not concentration of compound; im, intramuscularly; po, orally; NR, not reported

[b]Precipitate

[c]Comutation with 9-aminoacridine (100 μmol/plate) (not on profile)

[d]Comutation with ultraviolet radiation (not on profile)

[e]Results from four independent laboratories

[f]Negative in two laboratories, inconsistently positive in two laboratories

[g]Enhancement of effect of X irradiation (not on profile)

[h]BeO unspecified

[i]Low-fired oxide

[j]High-fired oxide

***Not displayed on profiles

medium (Rossman *et al.*, 1987). [The Working Group noted that the lack of toxicity of beryllium compounds in many studies of bacteria suggests lack of uptake.] In mammalian cells, intracellular transfer is from lysozyme to nucleus (reviewed by Skilleter, 1984).

Beryllium chloride (1–10 mM) increased misincorporation of nucleoside triphosphates during polymerization of poly-d(A–T) by *Micrococcus luteus* DNA polymerase (Luke *et al.*, 1975). In a similar system, beryllium chloride reduced the fidelity of DNA synthesis *in vitro* in the presence of avian myeloblastosis virus DNA polymerase, a synthetic prime template and complementary and noncomplementary nucleoside triphosphates. This effect was observed at concentrations at which even incorporation of complementary triphosphates was inhibited and was ascribed to the noncovalent binding of ionic divalent beryllium to DNA polymerase rather than to DNA (Sirover & Loeb, 1976). [It is not clear that such effects can occur within the cell, where the concentrations of Be^{2+} would probably be much lower; e.g. chromosomal aberrations have been reported at an extracellular concentration of < 5 μM.] The binding of beryllium by purified DNA is very weak ($K_a = 7 \times 10^3$/mol) (Truhaut *et al.*, 1968). It was reported in an abstract that beryllium can induce DNA–protein complexes (Kubinski *et al.*, 1977). [The Working Group considered that any 'genotoxic' effects of Be^{2+} are probably not caused by direct damage to DNA.]