4. Other Data Relevant to an Evaluation of Carcinogenicity and its Mechanisms

4.1 Deposition, retention, clearance and metabolism

Vanadium pentoxide (V_2O_5) is a poorly soluble oxide which, in water or body fluids, releases some vanadium ions which may speciate either in cationic (VO_2^+) or anionic (HVO_4^{2-}) forms [at physiological pH: $H_2VO_4^-$].

Toya *et al*. (2001) showed that vanadium pentoxide powder (geometric mean diameter, 0.31 μm) was eight times more soluble in an artificial biological fluid (Gamble's solution) than in water.

Elimination from the lung, and distribution to and elimination from tissues, is partly a function of solubility. Sodium vanadate is more soluble than vanadium pentoxide and is consequently cleared more rapidly from the lung (Sharma *et al*., 1987).

Vanadium (V) is reduced to vanadium (IV) in humans and other mammals. It is considered to be an essential element in chickens, rats and probably humans (Nielsen, 1991; French & Jones, 1993; Crans *et al*., 1998; Hamel, 1998; National Toxicology Program, 2002). The main source of vanadium intake for the general human population is food (see also Section 1.3.5).

4.1.1 *Humans*

Zenz and Berg (1967) studied responses in nine human volunteers exposed to 0.2 mg/m^3 vanadium pentoxide (particle size, 98% < 5 μm) for 8 h in a controlled environmental chamber. The highest concentration of vanadium was found in the urine (0.13 mg/L [2.6 μM/L]) 3 days after exposure; none of the volunteers had detectable concentrations 1 week after exposure.

Pistelli *et al*. (1991) studied 11 vanadium pentoxide-exposed workers 40–60 h after they had removed ashes from boilers of an oil-fired power station. Seven of the workers were smokers compared with eight of 14 controls. Vanadium concentrations in urine were determined by AAS and ranged between 1.4 and 27 μg/L in the exposed group. Four of the controls had detectable concentrations of vanadium in the urine (range, 0.5–1.0 μg/L).

Hauser *et al.* (1998) determined concentrations of vanadium by means of GF-AAS in the urine of workers overhauling an oil-fired boiler where concentrations of vanadium pentoxide in the air ranged from 0.36 to 32.2 μg/m^3 (mean, 19.1 μg/m^3). On the first day of work on the overhaul, the mean vanadium concentrations in urine were 0.87 mg/g creatinine before a shift and 1.53 mg/g creatinine after a shift. However, the vanadium concentrations in the start-of-shift urine samples on the last Monday of the study were not significantly different from the start-of-shift concentrations on the previous Saturday, a time interval of about 38 h between the end of exposure and sample collection. Spearman rank correlation between start-of-shift concentration of vanadium in urine and concentration of vanadium in workplace dust during the previous day was not strong (r = 0.35) due to incomplete and insufficient information on respirator usage as noted by the authors. These data support a rapid initial clearance of inhaled vanadium occurring on the first day of work followed by a slower clearance phase that was not complete 38 h after the end of exposure (Hauser *et al.*, 1998).

Kucera *et al*. (1998) analysed vanadium in biological samples from workers engaged in the production of vanadium pentoxide by a hydrometallurgical process and occupationally non-exposed controls. Average exposure time was 9.2 years (range, 0.5–33 years). Concentrations of vanadium in workplace air samples were high (range, 0.017–4.8 mg/m^3). Concentrations of vanadium in the blood of a subsample of workers was 12.1 ± 3.52 μg/L (geometric mean ± GSD) compared with 0.055 ± 1.41 μg/L among the non-exposed controls. Vanadium concentrations in morning urine were 29.2 ± 3.33 μg/L in exposed workers and 0.203 ± 1.61 μg/L for the non-exposed. The finding of high concentrations in morning urine is compatible with the fact that long-term exposure results in vanadium accumulation in the bone from which it can be released slowly.

Vanadium pentoxide was found to be rapidly absorbed following inhalation exposure, but poorly through dermal contact or when ingested as ammonium vanadyl tartrate (Dimond *et al.*, 1963; Gylseth *et al.*, 1979; Kiviluoto *et al.*, 1981; Ryan *et al*., 1999). When given orally, 0.1–1% is absorbed from the gut, although absorption of more soluble vanadium compounds is greater. About 60% of absorbed vanadium is excreted in the urine within 24 h (McKee, 1998). Based on samples from autopsies, vanadium was found to be distributed to

the lungs and the intestine. It was not detected in heart, aorta, brain, kidney, ovary or testes, although detection methods were reported to be insensitive (Schroeder *et al.*, 1963; Ryan *et al*., 1999).

Using AAS, Fortoul *et al*. (2002) analysed vanadium concentrations in lung tissue samples from autopsies of Mexico city residents in the 1960s and 1990s (n = 39 and 48, respectively). Vanadium concentrations were 1.04 ± 0.05 μg/g in lung samples from the 1960s and 1.36 ± 0.08 μg/g in samples from the 1990s, indicating an increase in ambient exposure to vanadium.

4.1.2 *Experimental systems*

(*a*) *In-vivo studies*

Absorption of vanadium compounds after oral administration is known to be strongly affected by such dietary components as type of carbohydrate, fibre protein concentration, other trace elements, chelating agents and electrolytes (Nielsen, 1987). Associated pathology or physiological state may also affect vanadium absorption and hence may render a consistent determination of a lethal dose (e.g. LD_{50}) by the oral route very difficult (Thompson *et al*., 1998).

In general, the absorption, distribution and elimination of vanadium pentoxide and other vanadium compounds are similar. There are, however, variations depending on the solubility of the administered compound, the route of exposure and the form of vanadium administered (National Toxicology Program, 2002).

(i) *Inhalation studies*

Mice

In a National Toxicology Program tissue burden study (2002), male and female $B6C3F_1$ mice were exposed to 1, 2, or 4 mg/m^3 vanadium pentoxide by inhalation for 104 weeks (for details, see Section 3.1.1). Tissue burden analyses were performed on days 1, 5, 12, 26, 54, 171, 362 and 535 after the start of treatment. Lung weights increased throughout the study, most markedly in the group exposed to the highest concentration. The mean lung weights of the two lower-dose groups were similar. Lung vanadium burden increased roughly in proportion to the exposure concentration, with strong indications of linear toxicokinetics. As with the rats (see below), lung burdens in the mice did not reach a steady state in the groups exposed to 2 and 4 mg/m^3; they peaked near day 54 (at 5.9 and 11.3 μg, respectively), and then declined until day 535. In the low-dose group (1 mg/m^3), the lung burden reached a steady state around day 26 at a level of 3 μg vanadium. The same toxicokinetic model could be applied to both mice and rats (see below), with an initial deposition rate increasing with increasing exposure concentration, and a decline in deposition rate over the course of the study. In the group exposed to 4 mg/m^3, the deposition rate decreased from 0.62 to 0.27 μg/day between day 1 and day 535 and in the group exposed to 2 mg/m^3 it decreased from 0.41 to 0.22 μg/day. However, in the group exposed to the lowest dose there was a minimal decline in deposition rate between

days 1 and 535 (0.31 to 0.26 μg/day). Lung clearance half-lives in mice were 6, 11 and 14 days for the 1, 2 and 4 mg/m^3 exposure groups, respectively. Total vanadium lung doses were estimated to have been 153, 162 and 225 μg, respectively, while normalized lung doses were 153, 80.9 and 56.2 μg vanadium per mg vanadium pentoxide per m^3 exposure. On day 535, mice had retained approximately 2–3% of the total estimated lung doses (National Toxicology Program, 2002).

In an inhalation model described by Sánchez *et al.* (2003; abstract only), male CD-1 mice were exposed to an aerosol of 0.02 M vanadium pentoxide for 2 h twice a week for 4 weeks. Concentrations of vanadium (determined by AAS) in lung, liver, kidney, testes and brain increased after the first week of inhalation in all the organs examined and remained at almost the same values at the end of the fourth week. The organ with the highest concentrations of vanadium was the liver followed by the kidney. The lowest concentrations were found in testes. However, at the fourth week, a decrease in concentrations of vanadium was observed in the kidney.

Rats

In a study undertaken by the National Toxicology Program (2002), blood and lung concentrations, lung clearance half-life of vanadium, and the onset and extent of vanadium pentoxide-induced lung injury were determined in female Fischer 344 rats exposed to 0, 1 or 2 mg/m^3 vanadium pentoxide for 16 days. Lung weights of exposed rats were significantly greater than those of control animals on days 0, 1 and 4 post-exposure but were similar on day 8 post-exposure. There was little difference in lung weights between exposed groups. AUC analysis showed that lung burdens were proportional to exposure concentration throughout the recovery period. The results suggested linear toxicokinetics. Lung clearance half-lives during the 8-day recovery period were similar among exposed groups (range, 4.42–4.96 days). Concentrations of vanadium in blood were similar among exposed groups, but several orders of magnitude lower than the concentrations in lung tissue, and showed only marginal increases with increasing exposure doses.

In the 2-year inhalation study (National Toxicology Program, 2002), tissue burden analyses were performed on female Fischer 344 rats on days 1, 5, 12, 26, 54, 173, 360 and 540 after the start of exposure to 0.5, 1 or 2 mg/m^3 vanadium pentoxide. Lung weights increased throughout the study, with similar increases in the two lower-dose groups. When lung burden data were integrated over all time points, they did appear to be approximately proportional to exposure concentrations. During the two years, lung burdens in the two higher-dose groups (1 and 2 mg/m^3) did not reach a steady state, but showed an increase until day 173 followed by a decline until day 542. In contrast, the lung burden in the group exposed to 0.5 mg/m^3 increased with time and reached a steady state at 173 days. The data fitted a model in which the rate of deposition of vanadium in the lung decreased with time, while the initial deposition rates increased with the exposure concentration. Between days 1 and 542, the calculated deposition rate decreased from 0.41 to 0.25 μg/day in the 1-mg/m^3 exposure group and from 0.68 to 0.48 μg/day in the 2-mg/m^3 exposure group. There was no such change in deposition rate in the group

exposed to the lowest dose (approximately 0.22 μg/day). These results are likely to be explained by altered pulmonary function in the higher-dose groups, resulting in lung clearance rates that were lower than in the low-dose group. Lung clearance half-lives were 37, 59 and 61 days for the high, medium and low exposure groups, respectively, i.e. much longer than in the 16-day study (see above). Apparently, vanadium is cleared more rapidly from the lungs of rats exposed to vanadium pentoxide for short periods of time or at low concentrations repeatedly for longer periods. From the deposition curves over the 542 days of the study, the estimated total vanadium lung doses were 130, 175 and 308 μg for the 0.5-, 1- and 2-mg/m^3 exposure groups, respectively. Normalized lung doses (μg vanadium/mg vanadium pentoxide per m^3) were not constant but decreased with increasing exposure, i.e., 260, 175 and 154 μg per mg/m^3 for low, medium and high dose groups, respectively. This decrease was due to the reduced deposition of vanadium with increasing exposure concentration. Rats retained approximately 10–15% of the estimated lung dose on day 542. Concentrations of vanadium in blood were much lower than in lung and were only marginally higher in exposed rats than in controls. Vanadium concentrations in blood of exposed animals peaked on days 26 or 54, then declined throughout the rest of the study. Because the changes were small, it was difficult to distinguish between decreased absorption from the lung, resulting from reduced deposition, and increased elimination from the blood (National Toxicology Program, 2002).

Kyono *et al.* (1999) showed that the health status of the lung influences the deposition and retention of vanadium. In an experimental model for nickel-induced bronchiolitis in rats, bronchiolitic rats and control animals were exposed to vanadium pentoxide (2.2 mg/m^3; MMAD, 1.1 μm) for 5 h. The vanadium content in the lungs of controls was higher (about 100%) than in bronchiolitic rats after 1 day of exposure, but 2 days later the retention was 20% in controls and 80% in bronchiolitic rats. Elimination of vanadium was found to be much slower in bronchiolitic rats.

(ii) *Intratracheal instillation*

Several studies have shown that after intratracheal instillation of vanadium pentoxide in rats there was generally a rapid initial clearance of up to 50% during the first hour, a second phase with a half-life of about 2 days and a third phase during which vanadium remained in the lung for up to 63 days (Oberg *et al*., 1978; Conklin *et al*., 1982; Rhoads & Sanders, 1985).

(iii) *Oral administration*

Administration of vanadium pentoxide by gavage resulted in absorption of 2.6% of the dose through the gastrointestinal tract 3 days after the treatment (Conklin *et al.*, 1982). Distribution was mainly to bone, liver, muscle, kidney, spleen and blood. Chronic treatment with inorganic vanadium salts or organic vanadium has been shown to result in significant accumulation in the bone, spleen and kidney (Mongold *et al*., 1990; Thompson & McNeil, 1993; Yuen *et al.*, 1993).

Studies with non-diabetic and streptozotocin-diabetic rats given vanadyl sulfate in their drinking-water (0.5–1.5 mg/mL) for 1 year showed concentrations of vanadium to be in the following order [of distribution]: bone > kidney > testis > liver > pancreas > plasma > brain. Vanadium was found to be retained in these organs 16 weeks after cessation of treatment while the concentrations in plasma were below the limits of detection at this time (Dai *et al*., 1994).

(*b*) *Cellular studies*

Edel and Sabbioni (1988, 1989) showed accumulation of vanadium in hepatocytes and kidney cells (in the nucleus, cytosol and mitochondria) in rats exposed to vanadium as radioactive ^{48}V (V) pentavanadate ions and ^{48}V (IV) tetravalent ions by intratracheal instillation, oral administration or intravenous injection.

Cell cultures (human Chang liver cells, bovine kidney cells), incubated in medium supplemented with vanadium in the form of vanadate, have been shown to accumulate this element in the nucleus and mitochondria (Bracken *et al*., 1985; Stern *et al*., 1993; Sit *et al*., 1996). In BALB/3T3 C1A31-1-1 cells incubated in the presence of sodium vanadate and vanadyl sulfate, the cellular retention of both compounds was similar. After exposure to a non-toxic dose (1 μM for 48 and 72 h), nearly all vanadium was present in the cytosol, but at a toxic dose (10 μM for 48 and 72 h), 20% of the vanadium was found in cellular organelles (Sabbioni *et al*., 1991).

4.2 Toxic effects

4.2.1 *Humans*

In humans, acute vanadium poisoning can manifest itself in a number of symptoms including eye irritation and tremors of the hands (Lewis, 1959). In addition, a greenish colouration of the tongue has been observed in humans exposed to high concentrations of vanadium pentoxide and is probably due to the formation of trivalent and tetravalent vanadium complexes (Wyers, 1946). The green colour disappears within 2–3 days of cessation of exposure (Lewis, 1959).

(*a*) *Studies with volunteers*

Zenz and Berg (1967) studied the effects of vanadium pentoxide in nine male volunteers exposed in an inhalation chamber to concentrations of vanadium pentoxide of 0.1, 0.25, 0.5 or 1.0 mg/m^3 (particle size, 98% < 5 μm) for 8 h, with follow-up periods of 11–19 months. Acute respiratory irritation was reported, which subsided within 4 days after exposure (see also Section 4.1.1).

No skin irritation was reported in 100 human volunteers after skin patch testing with 1, 2 and 10% vanadium pentoxide in petrolatum (Motolese *et al*., 1993).

(*b*) *Studies of workers exposed to vanadium*

There is an extensive published literature concerning the development of 'boilermakers bronchitis' in persons cleaning boilers in which fuel oils containing high concentrations of vanadium were used (Hudson, 1964; Levy *et al.*, 1984). The clinical picture is characterized by dyspnoea which is largely reversible. Levy *et al*. (1984) studied 100 workers exposed to vanadium pentoxide (0.05–5.3 mg/m^3) during the conversion of a utility company power plant and found severe respiratory tract irritation in 74 individuals. Expiratory flow rates and forced vital capacity were decreased in about 50% of a subsample (35 individuals) of the workers studied.

Eye irritation has been reported in workers exposed to vanadium (Lewis, 1959; Zenz *et al*., 1962; Lees, 1980; Musk & Tees, 1982). Skin patch testing in workforces produced two isolated reactions (but none in unexposed volunteers; see Section 4.2.1). The underlying reason for the skin responses in these workers is unclear (Motolese *et al*., 1993).

Lewis (1959) investigated 24 men exposed to vanadium pentoxide for at least 6 months from two different centres, and age-matched with 45 control subjects from the same areas. Exposure to vanadium pentoxide was between 0.02 and 0.92 mg/m^3. In the exposed group, 62.5% complained of eye, nose, and throat irritation (6.6% in control), 83.4% had a cough (33.3% in control), 41.5% produced sputum (13.3% in control), and 16.6% complained of wheezing (0% in control). Physical findings included wheezes, rales, or rhonchi in 20.8% (0% in controls), hyperaemia of the pharynx and nasal mucosa in 41.5% (4.4% in controls), and 'green tongue' in 37.5% (0% in controls).

Zenz *et al*. (1962) reported on 18 workers exposed to varying concentrations of vanadium pentoxide dust (mean particle size, < 5 μm) in excess of 0.5 mg/m^3 during a pelletizing process. Three of the men most heavily exposed developed symptoms, including sore throat and dry cough. Examination of each on the third work day revealed markedly inflamed throats and signs of intense persistent coughing, but no evidence of wheezing. The three men also reported 'burning eyes' and physical examination revealed slight conjunctivitis. Upon resumption of work after a 3-day exposure-free period, the symptoms returned within 0.5–4 h, with greater intensity than before, despite the use of respiratory protective equipment. After the process had been operating for 2 weeks, all 18 workers, including those primarily assigned to office and laboratory duties, developed symptoms and signs to varying degrees, including nasopharyngitis, hacking cough, and wheezing. This study confirms that vanadium pentoxide exposure can produce irritation of the eye and respiratory tract.

Lees (1980) reported signs of respiratory irritation (cough, respiratory wheeze, sore throat, rhinitis, and nosebleed) and eye irritation in a group of 17 boiler cleaners. As there was no control group and it was unclear whether there was exposure to compounds other than vanadium, no conclusions can be drawn regarding the cause or significance of these symptoms. However, the findings are compatible with those of other studies on inhalation of vanadium pentoxide.

Huang *et al.* (1989) conducted a clinical and radiological investigation of 76 workers who had worked in a ferrovanadium factory for 2–28 years. In the exposed group, out of 71 workers examined, 89% had a cough (10% in controls), expectoration was seen in 74% (15% in controls), 38% were short of breath (0% in controls), and 44% had respiratory harshness or dry sibilant rale (0% in controls). In 66 of the exposed group examined, hyposmia or anosmia was reported in 23% (5% in controls), congested nasal mucosa in 80% (13% in controls), erosion or ulceration of the nasal septum in 9% (0% in controls), and perforation of the nasal septum in one subject (1.5%) (0 in controls). Chest radiographs of all 76 exposed subjects revealed 68% with increased, coarsened, and contorted bronchovascular shadowing (23% in controls). [While exposure to vanadium compounds may have contributed to the clinical findings and symptoms reported, no firm conclusion can be drawn from this study in this regard, as mixed exposures are likely to have occurred.]

A prospective study (Hauser *et al*., 1995a) of pulmonary function in 26 boiler workers exposed to fuel oil ash showed decreased FEV_1 (forced expiratory volume in 1 s) values which were associated with PM_{10} exposure but not with vanadium exposure. There was no post-exposure change in non-specific airway responsiveness. Hauser *et al*. (1995b) used nasal lavage analysis to study upper airway responses in 37 utility workers exposed to fuel oil ash. Responses were examined in relation to vanadium concentrations and PM_{10} particles using personal samplers. A significant increase in polymorphonuclear cells in nasal lavage was observed in samples from nonsmokers but not in smokers, suggesting that exposure to vanadium dust is associated with upper airway inflammation. In both nonsmokers and smokers, a dose–response relationship between adjusted polymorphonuclear cell count and either PM_{10} or respirable vanadium dust exposure could not be found.

Woodin *et al*. (1998) studied the effects of vanadium exposure/PM_{10} concentrations in 18 boilermakers engaged in a utility boiler conversion; 11 utility workers acted as controls. The nasal lavage technique was used at various time points and interleukins (IL-6, IL-8), eosinophilic cationic protein (ECP) and myeloperoxidase (MPO) were investigated as biomarkers. Increases were observed in IL-8 and MPO concentrations but not IL-6 and ECP concentrations, in the exposed workers. The authors concluded that the changes observed in the upper airways were related to increased PM_{10} and upper airway vanadium concentrations. Subsequent studies in workers exposed to vanadium-rich fuel-oil ash (Woodin *et al*., 2000) also demonstrated lower (72% versus 27% for controls) and upper (67% versus 36% for controls) airway symptoms.

While the majority of the above studies have noted reversibility of these acute pulmonary effects, asthma [now possibly labelled ‘reactive airways disfunction syndrome’] has been reported to develop as a sequela to high, acute exposure to vanadium in some exposed workers (Musk & Tees, 1982).

(*c*) *Environmental exposure*

A single epidemiological study has been conducted (Lener *et al.*, 1998) assessing individual exposure in the general population to dusts generated by a plant processing vanadium-rich slag. It was estimated that an area with a radius of 3 km was exposed to the dust

from the plant in Mnisek in the Czech Republic. The population in this area at the time of the study was 4850. The two-year study concentrated on three groups of 10–12-year-old schoolchildren: 15 children (11 boys, four girls) from the localities of Cisovice and Lisnice (Group A), the area potentially most affected by the emission of vanadium; 28 children (14 boys, 14 girls) from the locality of Mnisek (Group B), an area of medium exposure; and 32 children (17 boys, 15 girls) from the locality of Stechovive (Group C), a control area not affected by any emission from vanadium production. Vanadium concentrations in venous blood, hair and fingernail clippings were determined. The mean vanadium concentration in blood was 0.10 ± 0.07 μg/L in the exposed Group A (Group B data not given) and 0.05 ± 0.05 μg/L in the control group. In hair, the concentrations were 96 ± 42 μg/kg and 181 ± 114 μg/kg in the exposed groups A and B, respectively, compared with 69 ± 50 μg/kg in controls. Concentrations in fingernails were 189 ± 41 μg/kg and 186 ± 38 μg/kg in the exposed groups A and B, respectively, compared with 109 ± 68 μg/kg in the controls. Vanadium concentrations in blood, hair and fingernails were elevated in children living close to the plant. In group B, those with parent(s) working at the plant had higher vanadium concentrations in hair than those whose parent(s) did not, suggesting a secondary exposure in the home from dust transferred on working clothes.

Health status of the children in the study was assessed based on haematological parameters, specific immunity, cellular immunity and cytogenetic analysis. Children from the exposed groups A and B had lower red blood cell counts and lower concentrations of serum and salivary secretory IgA than control group, and a seasonal decrease in IgG. Marked differences between exposed and control groups were seen in natural cell-mediated immunity, with significantly higher mitotic activity of T-lymphocytes in children living in the immediate vicinity of the plant. A higher incidence of viral and bacterial infections was registered in children from the exposed area. However, the study could not control for confounding by exposures to compounds other than vanadium. Cytogenetic analysis revealed no genotoxic effects (see Section 4.4.1). The overall conclusion was that long-term exposure to vanadium had no negative impact on health; the differences observed were within the range of normal values in all cases (Lener *et al*., 1998).

4.2.2 *Experimental systems*

(*a*) *In-vivo studies*

(i) *General toxicity*

The acute toxicity of vanadium is low when given orally, moderate when inhaled and high when injected. As a rule, the toxicity of vanadium increases as its valency increases, with vanadium (V), as in vanadium pentoxide, being the most toxic form (Lagerkvist *et al*., 1986; WHO, 1988; National Toxicology Program, 2002).

Studies in animals have shown that equivalent doses of vanadium pentoxide are better tolerated by small animals, including rats and mice, than by larger animals, such as rabbits

and horses (Hudson, 1964). The LD_{50} of vanadium pentoxide is highly species-dependent (Table 6). Differences in diet and route of vanadium administration may contribute to these discrepancies.

Table 6. Acute toxicity values for vanadium pentoxide in experimental animals

Species	Route of administration	Dose or concentration/ exposure	Parameter[a]	Reference
Mouse	Oral	23 mg/kg bw	LD_{50}	Lewis (2000)
	Subcutaneous	10 mg/kg bw	LD_{50}	Lewis (2000)
	Subcutaneous	87.5–117.5 mg/kg bw	LD	Hudson (1964)
	Subcutaneous	102 mg/kg bw	LD_{100}	Venugopal & Luckey (1978)
Rat	Oral	10 mg/kg bw	LD_{50}	Lewis (2000)
	Inhalation	70 mg/m^3/2 h	LC_{LO}	Lewis (2000)
	Subcutaneous	14 mg/kg	LD_{50}	Lewis (2000)
	Intraperitoneal	12 mg/kg bw	LD_{50}	Lewis (2000)
Guinea-pig	Subcutaneous	20–28 mg/kg bw	LD	Hudson (1964)
Rabbit	Intravenous	1–2 mg/kg bw	LD	Hudson (1964)
	Intravenous	10 mg/kg	LD_{LO}	Lewis (2000)
	Inhalation	205 mg/m^3/7 h	LC_{100}	Sjöberg (1950)
	Subcutaneous	20 mg/kg	LD_{LO}	Lewis (2000)
Cat	Inhalation	500 mg/m^3/23 min	LC_{LO}	Lewis (2000)

[a] LD_{100}: dose which is lethal to 100% of the animals; LD_{50}, dose which is lethal to 50% of the animals; LC_{100}, concentration in air which is lethal to 100% of the animals; LC_{LO}, lethal concentration low: the lowest concentration in air which is lethal to animals; LD, lethal dose

Ammonium metavanadate given to six weanling pigs at a dose of 200 mg/kg of feed (200 ppm) for 10 weeks was found to suppress growth and increase mortality (Van Vleet *et al*., 1981). In contrast, ammonium metavanadate was not markedly toxic when 200 mg/kg of feed (200 ppm) (approximately equivalent to 6.6 mg/kg bw) or less were fed to growing lambs for 84 days (Hansard *et al*., 1978).

(ii) *Respiratory effects*

Inhalation exposure

Male CD-1 mice exposed by inhalation to vanadium pentoxide (0.01-M and 0.02-M solution as aerosol, for 1 h) developed an increased mitochondrial matrix density and distorted nuclear morphology in non-ciliated bronchiolar Clara cells (Sánchez *et al*., 2001; abstract only).

In rats and mice exposed to vanadium pentoxide at concentrations up to 16 mg/m^3 for 3 months, inflammation and epithelial hyperplasia were observed in the nose and lung of rats and in the lung of mice at exposures ≥ 2 mg/m^3. Non-neoplastic lesions in the nose

and lung of rats were noted at all doses, and rats exposed to ≥ 4 mg/m^3 developed fibrosis (National Toxicology Program, 2002).

In addition, decreases in heart rate and in diastolic, systolic and mean blood pressure were seen in male and female F344/N rats exposed to 16 mg/m^3. These effects were not attributed to a direct cardiotoxic action of vanadium pentoxide but were considered to reflect the poor condition of the animals coupled with an effect of the anaesthesia (used to facilitate implantation of electrodes for electrocardiogram measurements). The overall pulmonary changes indicated the presence of restrictive lung disease in both sexes exposed to vanadium pentoxide concentrations of ≥ 4 mg/m^3, while an obstructive lung disease may have been present in the group exposed to 16 mg/m^3 (National Toxicology Program, 2002).

In a two-year study, F344/N rats and B6C3F$_1$ mice (50 animals per sex and per species) were exposed to vanadium pentoxide at concentrations of 0, 0.5, 1 or 2 (rats only), 1, 2 or 4 (mice only) mg/m^3, by inhalation for 2 years. Non-neoplastic proliferative and inflammatory lesions of the respiratory tract were observed in both species at increasing frequency with increased exposure concentration (see Tables 3.1.1 and 3.1.2, Section 3) (National Toxicology Program, 2002; Ress *et al.* 2003). The main differences observed between acute (3 months) and chronic (2 years) effects of exposure to vanadium pentoxide were the development by 2 years of chronic inflammation of the bronchi, septic bronchopneumonia, interstitial infiltration and proliferation, and emphysema (National Toxicology Program, 2002).

When rabbits were exposed to vanadium pentoxide by inhalation (8–18 mg/m^3, 2 h per day, 9–12 months) and rats to vanadium pentoxide condensation aerosol (3–5 mg/m^3, 2 h per day every 2 days, 3 months) or vanadium pentoxide dust (10–40 mg/m^3, 4 months), similar respiratory effects (sneezing, nasal discharge, dyspnoea and tachypnea) were produced in both species, which in some cases included attacks of bronchial asthma and a haemorrhagic inflammatory process (Roshchin, 1967b, 1968, cited by WHO, 1988).

In studies carried out by Sjöberg (1950), rabbits exposed to vanadium pentoxide dust (205 mg/m^3) developed tracheitis, pulmonary oedema and bronchopneumonia and died within 7 h. In another experiment, repeated inhalation of vanadium pentoxide (20–40 mg/m^3, 1 h per day, for several months) by rabbits produced chronic rhinitis and tracheitis, emphysema, patches of lung atelectasis and bronchopneumonia.

When adult male cynomolgus monkeys were exposed by inhalation to 0.5 or 5.0 mg/m^3 vanadium pentoxide dust aerosol for 1 week, significant air flow limitation was produced only at the 5.0 mg/m^3 dose in both central and peripheral airways, without changes in parenchymal function. However, analysis of BALF showed a significant increase in the absolute number and relative percentage of polymorphonuclear leukocytes, indicating that vanadium pentoxide induced pulmonary inflammatory effects (Knecht *et al*., 1985). In a study conducted to evaluate changes in pulmonary reactivity resulting from repeated vanadium pentoxide inhalation through the use of provocation challenges, and after different subchronic exposure regimens, one group of monkeys (n = 8) was exposed by inhalation (6 h per day, 5 days per week, for 26 weeks) to 0.1 mg/m^3 vanadium pentoxide on Mondays, Wednesdays and Fridays, with a twice-weekly peak exposure of

1.1 mg/m^3 on Tuesdays and Thursdays, and another group (n = 8) was exposed to a constant daily concentration of 0.5 mg/m^3; a control group (n = 8) received filtered, conditioned air. Pre-exposure challenges with vanadium pentoxide induced airway obstruction with a significant influx of inflammatory cells into the lung in both subchronic exposure groups. Inhalation of vanadium pentoxide with intermittent high exposure concentrations did not produce an increase in pulmonary reactivity to vanadium pentoxide, and cytological, immunological and skin test results indicated the absence of allergic sensitization (Knecht *et al*., 1992).

Intratracheal exposure

Zychlinski *et al.* (1991) investigated the toxic effects of vanadium pentoxide in rats exposed intratracheally to 0.56 mg vanadium pentoxide/kg bw once a month for 12 months. Body weight gain of exposed animals slowed following the 10th treatment when compared with control animals. Lung weights were significantly greater than in controls, but other organ weights were unchanged. The glucose concentrations in blood of treated animals were slightly decreased whereas total cholesterol concentrations were reduced markedly. In parallel to this in-vivo study, in-vitro experiments with isolated untreated rat lung microsomes and mitochondria in the presence of reduced nicotinamide adenine dinucleotide phosphate (NADPH) were performed to investigate the mechanism of the chronic toxic effects of vanadium. The results showed that vanadium(V) undergoes one-electron redox cycling (enzymatic reduction) in rat lung biomembranes and that non-enzymatic reoxidation of vanadium(IV) initiates lipid peroxidation under aerobic conditions. It was postulated that free-radical redox cycling of vanadium may be responsible for the observed pulmonary toxicity.

When female CD rats were instilled intratracheally with 42 or 420 µg/kg bw vanadium pentoxide and followed from 1 h to 10 days, pulmonary inflammation was induced in a dose-dependent manner, but neutrophil influx was not detected until 24 h after exposure. Expression of mRNA for two cytokines, macrophage inflammatory protein-2 (MIP-2) and KC protein was also detected in the bronchoalveolar macrophages (Pierce *et al*., 1996).

Bonner *et al*. (2000) reported that two weeks after a single intratracheal instillation of 1 mg/kg bw vanadium pentoxide, male Sprague-Dawley rats developed constrictive airway pathology including airway smooth muscle cell thickening, mucous cell metaplasia and fibrosis.

Evaluating the effects of a single intratracheal dose of residual oil fly ash in rats, Dreher *et al*. (1997), Kodavanti *et al*. (1998) and Silbajoris *et al*. (2000) concluded that vanadium compounds were the major toxic component inducing pulmonary injury, activation of alveolar macrophages and inflammatory changes. In addition, Silbajoris *et al*. (2000) described the induction of some mitogen-activated protein (MAP) kinases in the alveolar epithelium of the animals.

Rice *et al*. (1999) instilled Sprague-Dawley rats intratracheally with 1 mg/kg bw vanadium pentoxide and found proliferation of myofibroblasts, indicating pulmonary fibrosis. Toya *et al*. (2001), using the same model, found that intratracheal instillation

with 0.88, 3.0 or 13.0 mg/kg bw vanadium pentoxide for 4 weeks induced pathological lung lesions that developed dose-dependently, and were characterized by exudative inflammation, injury of alveolar macrophages, and swelling and mucous degeneration of the broncho-bronchiolar epithelium.

(iii) *Hepatic effects*

In mice exposed to vanadium pentoxide (0.02 M inhaled for 30 min), fatty degeneration, extramedullary haematopoietic activity and neutrophilic infiltration around the central veins were detected in the liver (Acevedo-Nava *et al*., 2001; abstract only).

In rats and rabbits, fatty changes with necrosis in the liver and a drastic reduction in liver tissue respiration have been observed as a result of long-term exposure to vanadium pentoxide by inhalation (10–70 mg/m^3, 2 h per day, 9–12 months) (Roshchin, 1968, cited by Lagerkvist, 1986). Livers and kidneys of rats treated with vanadium(V) showed an electron paramagnetic resonance signal characteristic of vanadium(IV) (Johnson *et al*., 1974).

The bioenergetic functions of liver mitochondria have been studied *in vivo* and *in vitro* following acute and chronic exposure of rats to vanadium pentoxide via the respiratory tract or exposure of isolated rat liver mitochondria to various vanadium pentoxide concentrations. *In vivo*, the mitochondrial respiration with glutamate (as nicotinamide adenine dinucleotide (NAD)-linked substrate) or succinate (as flavine adenine dinucleotide (FAD)-linked substrate) was inhibited significantly when compared with control animals. No inhibition was found with ascorbate as cytochrome c-linked substrate. The same effects were observed *in vitro*. These combined effects provide evidence that vanadium(V) acts as an inhibitor of respiration in rat liver mitochondria. It was postulated that significant amounts of vanadium(V) accumulated in the intermembrane space of liver mitochondria of exposed rats. The enzymatic process of detoxification, by reduction of vanadium(V) in the tissue, may be insufficient to prevent the deleterious action of this compound on liver mitochondria (Zychlinski & Byczkowski 1990).

(iv) *Renal effects*

Glomerular hyperaemia and necrosis of convoluted tubules in the kidney were observed in some early studies of acute toxicity of vanadium compounds in various mammalian species (Hudson, 1964; Pazhynich, 1966; WHO, 1988).

Intraperitoneal administration of sodium orthovanadate to rats resulted in inhibition of tubular reabsorption of sodium and hypokalaemic distal renal tubular acidosis with increased urinary pH (Bräunlich *et al*., 1989; Dafnis *et al*., 1992). Vanadium, in the form of ammonium metavanadate injected subcutaneously into rats, was found to be toxic to the kidney at doses of 0.6 and 0.9 mg/kg bw per day for 16 days. Histological changes were observed, including necrosis, cell proliferation and fibrosis. Vanadium was shown to be more toxic for the kidneys in rats when given by a parenteral route (Al-Bayati *et al*., 1989).

Chronic treatment of rats with vanadyl sulfate has been shown to result in significant accumulation of the element in the kidneys (Mongold *et al.*, 1990; Thompson & McNeill, 1993); however, most is probably bound to small peptides or macromolecules in the form

of vanadyl and thus is not available as vanadate, a more potent inhibitor of Na^+/K^+-ATPases (Cantley *et al.*, 1977; Rehder, 1991; Thompson *et al.*, 1998).

(v) *Nervous system effects*

Neurophysiological effects have been reported following acute exposure (by oral administration and subcutaneous injection) of dogs and rabbits to vanadium oxides and salts (vanadium trioxide, vanadium pentoxide, vanadium trichloride and ammonium metavanadate). These effects included disturbances of the central nervous system, such as impaired conditioned reflexes and neuromuscular excitability (Roshchin, 1967a). The animals behaved passively, refusing to eat, and lost weight. In cases of severe poisoning, diarrhoea, paralysis of the hind limbs and respiratory failure were followed by death (Hudson, 1964; Roshchin, 1967b, 1968).

In a study reported by Seljankina (1961 cited by Lagerkvist *et al.*, 1986 and WHO, 1988), solutions of vanadium pentoxide were administered orally to rats and mice at doses of 0.005–1 mg/kg bw per day for periods ranging from 21 days at the higher concentrations to 6 months at the lower concentrations. A dose of 0.05 mg/kg bw was found to be the threshold dose for functional disturbances in conditioned reflex activity in both mice and rats. Repeated exposure to aqueous solutions (0.05–0.5 mg/kg bw per day, for 80 days) of vanadium pentoxide impaired conditioned reflex mechanisms in rats.

In male CD-1 mice exposed by inhalation to 0.02 M vanadium pentoxide 2 h twice a week for 4 weeks, Golgi staining revealed a drastic reduction in dendritic spines in the striatum compared with controls, showing that the inhalation of vanadium causes severe neuronal damage in the corpus striatum (Montiel-Flores *et al*., 2003; abstract only). Using the same inhalation model, after 12 weeks of exposure, a decrease in dendritic spines of granule cells of the olfactory bulb was observed (Mondragón *et al*., 2003; abstract only). In addition, ultrastructural modifications in nuclear morphology of these cells were evident, Golgi apparatus was dilated and an increase in lipofucsin granules was observed, as well as necrosis of some cells (Colin-Barenque *et al*., 2003; abstract only). In the cerebellum, necrosis and apoptosis of the Purkinje and granule cell layers were seen (Meza *et al*., 2003; abstract only).

(vi) *Cardiovascular system effects*

Perivascular swelling, as well as fatty changes in the myocardium, were observed by Roshchin (1968, cited by WHO, 1988) following chronic exposure of rats and rabbits to vanadium pentoxide (10–70 mg/m^3, 2 h per day, 9–12 months) by inhalation.

(vii) *Skeletal alterations*

The effect of vanadium pentoxide on bone metabolism has been investigated in weanling rats. Vanadium pentoxide (10.0–200.0 µmol/kg bw [1.8–36.4 mg/kg bw]) was administered orally for 3 days. Low doses (10–100 µmol/kg bw [1.8–18 mg/kg bw]) caused increases in alkaline phosphatase activity and DNA content in the femoral diaphysis, indicating that vanadium pentoxide may play a role in the enhancement of bone

formation *in vivo*. However, high doses (over 150 μmol/kg bw [27 mg/kg bw] had toxic inhibitory effects (Yamaguchi *et al*., 1989).

(viii) *Immunological effects*

In the National Toxicology Program study (2002), a localized inflammatory response was seen in the lungs of male F344/N rats and female B6C3F$_1$ mice exposed by inhalation to 4, 8, or 16 mg/m^3 vanadium pentoxide in a 16-day study. Increases in cell numbers, protein, neutrophils and lysozymes in BALF were observed but the number of macrophages in lavage fluids of male rats and female mice exposed to 8 or 16 mg/m^3 was decreased. No effects were seen on systemic immunity in rats and mice.

When weanling and adult ICR mice were given 6 mg/kg bw vanadium pentoxide by gavage (5 days per week for 6 weeks), an increase in the number of leukocytes and plaque-forming cells, as well as enhanced phytohaemagglutinin responsiveness, increased spleen weight and depression of phagocytosis were observed in treated mice. In Wistar rats given vanadium pentoxide in drinking-water (1 or 100 mg/L for 6 months), the higher dose resulted in increased spleen weight and concanavalin-A responsiveness; a depression of phagocytosis was found in a dose-dependent manner. These results suggest activation of T- and B-cell immune responses (Mravcová *et al.*, 1993).

(ix) *Biochemical effects*

Chakraborty *et al*. (1977) gave male albino rats vanadium pentoxide orally at a dose of 3 mg/kg bw five times a week for the first week and 4 mg/kg bw for a further 2 weeks and found that it induced histological and enzymatic alterations including inhibition of biosynthesis, enhanced catabolism and increased use of L-ascorbic acid in the liver and kidney tissues of the rats.

(*b*) *In-vitro studies*

(i) *Organ culture*

Garcia *et al.* (1981) found that treatment with vanadium pentoxide (10^{-5}–10^{-2} M [1.82–1820 μg/mL]) produced dose-dependent contractions of the rat vas deferens organ cultures *in vitro*; a response that could be associated with the inhibition of Na^+/K^+-ATPase activity.

Schiff and Graham (1984) used organ cultures of hamster trachea to study the in-vitro effects of vanadium pentoxide (0.1, 1, 10 or 100 μg/mL) and oil-fired fly ash (10, 50, 100 or 250 μg/mL) on mucociliary respiratory epithelium following exposure for 1 h per day for 9 consecutive days. Vanadium pentoxide was found to decrease ciliary activity and produce ciliostasis in tracheal ring explants. The degree of change depended on the concentration and length of exposure; early morphological alterations consisted of vacuolization of both nuclei and cytoplasm of tracheal epithelium cells.

Preincubation of rat kidney brush border membrane vesicles with 1 mM [182 μg/mL] vanadium pentoxide for 8 h significantly inhibited citrate uptake in a time-dependent manner. This effect was attributed to a direct interaction of vanadium with the sodium

citrate cotransporter. The results suggest that vanadium pentoxide has nephrotoxic potential (Sato *et al*., 2002).

(ii) *Cell culture*

In cultures of bovine alveolar macrophages, Fisher *et al.* (1986) found that vanadium pentoxide was the most cytotoxic compound when compared with other metals or metalloids (zinc oxide, nickel sulfide, manganese oxide, sodium arsenite, sodium selenite) tested. Vanadium caused a reduction in phagocytosis by macrophages to 50% of control values after incubation for 20 h at a concentration of 0.3 μg/mL, but this concentration was also associated with a substantial (59%) loss of macrophage viability. The authors concluded that their results confirmed those of previous studies (Waters *et al.*, 1974) which demonstrated that vanadium is a unique macrophage toxicant.

Vanadium(V) and related compounds are known to exert potent toxic effects on a wide variety of biological systems. One of the pathways of vanadium(V) toxicity is thought to be mediated by oxygen-derived free radicals (Zychlinski *et al*., 1991; Shi *et al*., 1997; Ding *et al*., 1999).

Parfett and Pilon (1995) evaluated the effects of promoters such as vanadium compounds on oxidative stress-regulated gene expression and promotion of morphological transformation in C3H/10T1/2 cells. Promoters which elevate intracellular oxidant levels can be distinguished by a spectrum of induced gene expression which includes the oxidant-responsive murine proliferin gene family. Proliferin transcription was found to be induced 20-fold by 5 μM [0.9 μg/mL] vanadium pentoxide. Another pentavalent vanadium, ammonium metavanadate (5 μM [0.6 μg/mL]), added as promoter in two-stage morphological transformation assays, amplified yields of Type II and Type III foci in monolayers of 20-methylcholanthrene-initiated C3H/10T1/2 cells. These results suggest that pentavalent vanadium compounds could promote morphological transformation in these cells by creating a cellular state of oxidative stress, which induces the expression of proliferin. Proliferation of MCF-7 cells was found to be stimulated after 4-day treatments with 0.5–2 μM vanadium(V); the effect reached a plateau at 1 μM vanadium, declined at 3 μM and disappeared at 5 μM (Auricchio *et al.*, 1995; 1996).

To determine the effect of vanadium pentoxide on the release of two major immunoregulatory cytokines, mouse macrophage-like WEHI-3 cells were treated *in vitro* (Cohen *et al.*, 1993). Vanadium pentoxide decreased the release of IL-1 and TNFα stimulated with lipopolysaccharide endotoxin. Spontaneous release of the IL-1/TNF-regulating prostanoid prostaglandin E_2 (PGE_2) was significantly increased by the highest concentration of ammonium metavanadate tested, although lipopolysaccharide endotoxin-stimulated PGE_2 production was unaffected. These results showed that pentavalent vanadium could alter the host's immunocompetence. In another study with WEHI-3 cells treated with 100 μM or 100 nM vanadium pentoxide or ammonium metavanadate, the capacity of macrophage-like cells to bind and respond to interferon γ was altered (Cohen *et al*., 1996).

When mice and rat hepatocytes or human Hep G2 cells were treated *in vitro* with vanadium pentoxide (1, 10 or 100 μM), gene expression (after 2-h treatment) and

secretion of IL-8, MIP-2 chemokines and TNFα (after 18-h treatment) were increased. The induction of IL-8 and MIP-2 secretion was inhibited by antioxidants such as tetramethylthiourea and *N*-acetylcysteine, showing that the events responsible for this gene expression involve cellular redox changes (Dong *et al*., 1998). Vanadium pentoxide caused a several-fold increase in heparin-binding epidermal growth factor-like growth factor (HB-EGF) mRNA expression and protein in normal human bronchial epithelial cells and increased the release of HB-EGF mitogenic activity of these cells (Zhang *et al.*, 2001a).

Wang and Bonner (2000) showed that vanadium pentoxide activated extracellular signal-regulated kinases 1 and 2 (ERK-1/2) in rat pulmonary myofibroblasts. This activation was an oxidant-dependent event and required components of an epidermal growth factor-receptor signalling cascade.

Ingram *et al*. (2003) showed that vanadium pentoxide stimulated HB-EGF mRNA expression and hydrogen peroxide production by human lung fibroblasts. Both vanadium pentoxide and hydrogen peroxide activated ERK-1/2 and p38 MAP kinases. Inhibitors of these two kinase-pathways significantly reduced both vanadium and H_2O_2-induced HB-EGF expression. These data indicate that vanadium upregulates HB-EGF via ERK and p38 MAP kinases.

Evidence suggests that some forms of vanadium (sodium metavanadate, peroxovanadate and pervanadate) or vanadium-containing particles from environmental and occupational sources can trigger or potentiate apoptosis. The pentavalent form of vanadium has been shown to cause apoptosis in a JB6 P^+ mouse epidermal cell line (Cl 41) and in lymphoid cell lines, but may be anti-apoptotic in others such as malignant glioma cells (Hehner *et al.*, 1999; Chin *et al.*, 1999; Huang *et al*., 2000; Chen *et al*., 2001).

Rivedal *et al*. (1990) found that vanadium pentoxide exposure for 5 days promoted the induction of morphological transformation of hamster embryo cells pre-exposed to a low concentration of benzo[*a*]pyrene for 3 days. However, when vanadium pentoxide (0.25, 0.50 or 0.75 μg/mL) was tested in the Syrian hamster embryo (SHE) assay, the results were negative after a 24-h exposure, but significant morphological transformation was produced after a 7-day exposure. This pattern of response (24-h SHE negative/7-day SHE positive) has been seen with other chemicals (i.e., 12-*O*-tetradecanoylphorbol 13-acetate, butylbenzyl phthalate, methapyrilene) that have tumour promotion-like characteristics (Kerckaert *et al*., 1996a,b).

(iii) *Cell-free systems*

In cell-free systems, vanadium(V) caused the oxidation of thiols, including GSH and cysteine, and induced the formation of thiyl radicals (Shi *et al*., 1990; Byczkowski & Kulkarni, 1998). It has been shown that depletion of GSH not only decreases the antioxidant defence in the cytosol, but also prevents regeneration of a vital lipid-soluble antioxidant, α-tocopherol, thereby increasing the vulnerability of phospholipid-rich biomembranes to oxidative stress and lipid peroxidation (Byczkowski & Kulkarni, 1998).

Vanadium can inhibit a variety of enzymes such as heart adenyl cyclase and protein kinase, ribonucleases, phosphatases, and several adenosinetriphosphatases (ATPases), but it can stimulate a number of others. The enzymes inhibited include phosphoenzyme ion-transport ATPases, acid and alkaline phosphatases, Na^++K^+ATPase, H^++K^+ATPase, phosphotyrosyl protein phosphatase, dynein (contractile protein ATPase associated with microtubules of cilia and flagella), myosin ATPase, phosphofructokinase, adenylate kinase and cholinesterase (Nechay, 1984; WHO, 1988).

Vanadium(V) appears to undergo a redox cycling when the inner mitochondrial membrane permeability barrier to vanadate polyanions is broken. It has been proposed that vanadium(V) stimulates the oxidation of NAD(P)H by biological membranes and amplifies the initial generation of $O_2^{-\bullet}$ produced by membrane-associated NAD(P)H oxidase. This stimulatory effect is due to interaction of vanadium(V) with $O_2^{-\bullet}$ but not with the membrane-associated enzymes (Liochev & Fridovich, 1988).

Using ESR spin trapping, Shi and Dalal (1992) demonstrated that rat liver microsomes/NADH, in the absence of exogenous H_2O_2, generated hydroxyl ($^\bullet$OH) radicals from the reduction of vanadium(V) via a Fenton-like mechanism. This radical generation may play a role in vanadium(V)-induced cellular injury.

4.3 Reproductive and developmental effects

4.3.1 *Humans*

No data were available to the Working Group.

4.3.2 *Experimental systems*

(*a*) *In-vivo studies*

Several studies describe the reprotoxic (male or female reproductive capability) and developmental (teratological) effects of vanadium pentoxide (Lagerkvist *et al*., 1986; Domingo, 1994; Leonard & Gerber, 1994; Domingo, 1996; Leonard & Gerber, 1998; National Toxicology Program, 2002).

(i) *Toxicokinetics in pregnant animals*

Li *et al*. (1991) treated non-pregnant and pregnant Wistar rats with 5 mg/kg vanadium pentoxide intraperitoneally and reported the tissue distribution of this compound. Non-pregnant rats had significant concentrations of vanadium in kidney, ovary, uterus and liver, suggesting that female genital organs are important target organs in the distribution of vanadium. Treatment of pregnant rats gave similar results, including the presence of vanadium in the placenta. The authors suggested that vanadium could pass the blood–placenta barrier.

Zhang *et al*. (1991a) analysed the passage of vanadium across the placenta into the embryo/fetus of pregnant Wistar rats at different times after different dose regimens: 4 h

after treatment with a single intraperitoneal injection of vanadium pentoxide (5 mg/kg bw) on day 12 of gestation; 1, 4, 24 or 48 h after a single treatment (5 mg/kg bw) on days 16–18 of gestation; or 120 h after the final treatment with 0.33, 1 or 3 mg/kg bw given daily on days 6–15 of gestation. The concentrations of vanadium in maternal blood, placenta and fetus were elevated after these different treatments in comparison with those of the respective untreated groups. The vanadium concentration in fetuses increased with increasing doses, suggesting that the embryo/fetus accumulated vanadium (Zhang *et al*., 1991a).

(ii) *Effects on reproductive organs and fertility*

Male CD-1 mice were treated intraperitoneally with 8.5 mg/kg bw vanadium pentoxide once every 3 days for 60 days. Groups of five animals were killed every 10 days after the beginning of treatment. Twenty-four hours after the last injection, the males were mated with untreated females. A decrease in fertility rate, implantations, live fetuses and fetal weight, and an increase in the number of resorptions/dam was observed. In males, sperm count and motility were impaired as treatment advanced and the presence of abnormal sperm was observed on days 50 and 60 of treatment (Altamirano-Lozano & Alvarez-Barrera, 1996; Altamirano-Lozano *et al.*, 1996).

In a National Toxicology Program study (2002), reduced epididymal sperm motility was observed in $B6C3F_1$ mice exposed to vanadium pentoxide by inhalation (8- and 16 mg/m^3 dose groups) for 3 months. There were no effects on estrous cycle parameters in females. No effects were seen on reproductive parameters in male and female F344/N rats exposed by inhalation to 4, 8 or 16 mg/m^3 vanadium pentoxide (National Toxicology Program, 2002).

To evaluate the effect of vanadium pentoxide on the newborn rats, Altamirano *et al*. (1991) injected 12.5 mg/kg bw vanadium pentoxide intraperitoneally into male and female prepubertal CII-ZV rats every 2 days (from birth to 21 days), and into female rats from day 21 to the day of the first vaginal estrus. No changes in vaginal opening nor in the estrous cycle were observed in either prepubertal or adult female rats; however, the ovulation rate was reduced in the treated adult females. No differences were observed in the weights of ovaries, uterus, adrenal gland or pituitary gland, compared to those of untreated rats; the weights of thymus, liver, kidneys and submandibular glands of newborn treated females were similar to those of controls. However, when treatment began at 21 days of age, an increase in the weight of thymus, submandibular glands and liver was observed. In male prepubertal rats, an increase was observed in the weight of seminal vesicles, thymus and submandibular glands but not of testis and prostate of animals treated with vanadium from birth to 21 days. The results indicate that, as observed with other metals, the toxicological effects of vanadium pentoxide differ in males and females, with toxicity in prepubertal rats being higher in males than in females.

(iii) *Developmental effects*

To evaluate the effects of vanadium pentoxide on the embryonic and fetal development of mice, Wide (1984) injected pregnant albino NMRI mice via the tail veins with

1.5 mM/animal [273 µg/animal ~ 10 mg/kg bw] vanadium pentoxide on day 3 or day 8 of gestation. All animals were killed 2 days before parturition (17th day of pregnancy) and fetuses were dissected and examined. Treatment with vanadium pentoxide on day 8 of gestation did not induce teratogenic effects but reduced fetal skeletal ossification.

In a study of the developmental toxicity of vanadium pentoxide, Zhang *et al*. (1991b) injected pregnant female NIH mice intraperitoneally with 5 mg/kg bw vanadium pentoxide per day on different days of gestation (days 1–5, 6–15, 7, 8, 9, 10, 11 or 14–17 of pregnancy). No effects on pre-implantation were found, nor malformations nor premature birth. However, an increased frequency of resorptions or fetal death was observed in animals treated on days 7, 6–15, and 14–17 of gestation. Delayed skeletal ossification was noted in mice treated on days 6–15, 8, 10 and 14–17 of gestation. The authors suggested that vanadium pentoxide acted as a weak developmental toxicant but not a teratogen.

To evaluate the teratogenic effects of vanadium pentoxide, female CD-1 mice were injected intraperitoneally once daily on days 6–15 of gestation with 8.5 mg/kg bw. Vanadium did not cause significant adverse effects on the number of live and dead fetuses (including resorptions) nor on fetal implants; however, a decrease in fetal weight and a delay in skeletal ossification were observed. Limb shortening was the most frequent alteration. No maternal toxicity was detected (Altamirano-Lozano *et al*., 1993).

In female Wistar rats exposed to 0.33, 1 or 3 mg/kg bw vanadium pentoxide from days 6–15 of gestation, the highest dose was toxic. Increased fetal mortality and external or skeletal malformations with delay in ossification were also observed (Zhang *et al.*, 1993a). Similar results were found in one further study in Wistar rats (Zhang *et al*., 1993b).

(*b*) *In-vitro studies*

Li *et al.* (1995) investigated the toxicological effects of vanadium pentoxide (0.125, 0.25, 0.5, 2 or 3 mM) in rat Leydig cells *in vitro* and found no obvious relationship between testosterone secretion and the concentration of vanadium. The authors concluded that Leydig cells are not a target for vanadium pentoxide. This is in agreement with results of in-vivo studies previously reported by Altamirano *et al.* (1991) who had shown that the weight of the testis and prostate were not increased after vanadium treatment of rats (see Section 4.3.2(ii)).

Altamirano-Lozano *et al.* (1997, 1998a) tested the reprotoxic effects of various metal compounds on boar spermatozoa *in vitro*. Sperm were exposed to vanadium pentoxide (5.5, 16.5, 27.5, 55, 110 or 220 µM) and motility was analysed 0, 1, 2, 3, 4, 5 and 6 h after treatment. A dose- and time-dependent reduction in sperm motility was observed, in accordance with results obtained *in vivo* in mice by the same group (Altamirano-Lozano *et al.*, 1996).

4.4 Genetic and related effects

4.4.1 *Humans*

Lener *et al.* (1998) studied children exposed to vanadium in air in an area close to a plant processing vanadium-rich slag (see Section 4.2.3). Group A comprised 15 children from the area potentially most affected by vanadium emissions; Group B, 28 children from an area of medium exposure; and Group C, 32 children was the control group. No significant induction of chromosomal aberrations was found in the lymphocytes of exposed children (1.2 ± 1.2 in Group A; 1.3 ± 1.1 in Group B) compared with the control group (0.95 ± 0.97). Sister chromatid exchange was analysed in exposed children (4.6 ± 1.0 in Group A; 4.6 ± 0.87 in Group B) but no data were available from controls. However, the authors concluded that these results revealed no genotoxic effects of vanadium exposure.

Only one in-vivo study of the genotoxic action of vanadium pentoxide in adult humans has been reported. Ivancsigts *et al*. (2002) studied the effect of occupational exposure to vanadium pentoxide by measuring DNA strand breaks using the single-cell gel electrophoresis assay 'Comet Assay', formation of 8-hydroxy-2′-deoxyguanosine, and the frequency of sister chromatid exchange in whole blood or lymphocytes of 49 male workers in a vanadium-processing factory. Although there was significant vanadium uptake (mean vanadium concentration in serum, 5.38 μg/mL), no increase in cytogenetic end-points nor in oxidative DNA damage was observed in the cells from these workers.

4.4.2 *Experimental systems*

(*a*) *Biochemical assays*

Effects of vanadium compounds on DNA-metabolizing enzymes have been reported by Sabbioni *et al*. (1983). Vanadate(V) ions (10^{-7}–10^{-3} M) inhibited calf thymus terminal deoxynucleotidyl transferase (with an apparent Ki of 2.5 μM) and the catalytic activity of mammalian DNA polymerase α (at I_{50} of 60 μM), while bacterial DNA polymerase-I was inhibited when the concentration was increased to about 0.5 mM.

(*b*) *Mutagenicity* (see Table 7)

(i) *In-vitro studies*

The mutagenicity of vanadium compounds has been reviewed (Graedel *et al*., 1986; Léonard & Gerber, 1994; Altamirano-Lozano *et al*., 1998b; Léonard & Gerber, 1998; National Toxicology Program, 2002).

The majority of the results of mutagenic activity of vanadium have been shown in *Escherichia coli* and *Salmonella typhimurium* (Hansen & Stern, 1984; Graedel *et al.*, 1986; Leonard & Gerber, 1994); there is one study only with exogenous metabolic activation (National Toxicology Program, 2002).

Early studies demonstrated that vanadium pentoxide was more genotoxic in recombination-repair-deficient (rec^-) strains of *Bacillus subtilis* than in the wild-type rec^+

Table 7. Genetic and related effects of vanadium pentoxide

Test system	Result[a]		Dose[b] (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Escherichia coli, spot test B/r WP2try$^-$, WP2hcr$^-$try$^-$	–	NT	0.5 M	Kanematsu *et al*. (1980)
Escherichia coli, WP_2, WP_2uvrA, CM_{891}, reversion assay	+	NT	1200 µg/plate	Si *et al.* (1982)[c]
Escherichia coli, ND160 and MR102, frameshift mutation	–		1200 µg/plate	Si *et al.* (1988)[c]
Bacillus subtilis, M45 recombination-repair-deficient (rec$^-$)	+	NT	0.5 M	Kanematsu & Kada (1978); Kada *et al*. (1980); Kanematsu *et al*. (1980)
Bacillus subtilis H17 (rec$^+$) and M45 (rec$^-$) recombination-repair-deficient	+	NT	100 000	Sun (1996)
Salmonella typhimurium, TA100, TA1535, TA1537, TA1538, (his$^-$)	–	NT	0.5 M	Kanematsu *et al*. (1980)
Salmonella typhimurium, TA100, TA98, TA102, TA1535 reverse mutation	–	–	333 µg/plate	National Toxicology Program (2002)
Salmonella typhimurium, TA97, TA98, TA100, TA102 reverse mutation	–	NT	200 µg/plate	Zen *et al.* (1988)[c]
Gene mutation, 6-thioguanine resistant mutation, Chinese hamster lung fibroblast cell line (V79) *in vitro*	–		4	Zhong *et al*. (1994)
Sister chromatid exchanges, Chinese hamster lung fibroblast cell line (V79) *in vitro*	–		4	Zhong *et al*. (1994)
Micronucleus formation in binucleated cells, cytochalasin-B assay, Chinese hamster lung fibroblast cell line (V79) *in vitro*	+	NT	1	Zhong *et al*. (1994)
Numerical chromosomal aberrations, endoreduplication, Chinese hamster lung fibroblast cell line (V79) *in vitro*	+		1	Zhong *et al*. (1994)

Table 7 (contd)

Test system	Result[a]		Dose[b] (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Numerical chromosomal aberrations, aneuploidy, kinetochore staining of micronuclei in binucleated cells, Chinese hamster lung fibroblast cells line (V79) *in vitro*	+		1	Zhong et al. (1994)
DNA strand breaks, alkaline 'Comet Assay', human lymphocytes *in vitro*	+	NT	0.3 µM	Rojas *et al*. (1996a, b)
Inhibition of double-strand DNA breaks repair, alkaline and neutral 'Comet Assay', human fibroblasts *in vitro*	+		UV (4.8 kJ/m^2) + V_2O_5 0.5 µM	Ivancsists *et al*. (2002)
	+		Bleomycin (1 µg/mL) + V_2O_5 0.5 µM	
Sister chromatid exchanges, human lymphocytes *in vitro*	–	NT	47 M	Sun *et al.* (1989)[c]
Sister chromatid exchanges, human lymphocytes *in vitro*	–	NT	6	Roldán & Altamirano (1990)
Sister chromatid exchanges, human lymphocytes *in vitro*	+	NT	4[d]	Roldán-Reyes *et al*. (1997)
Structural chromosomal aberrations, human lymphocytes *in vitro*	–	NT	6	Roldán & Altamirano (1990)
Numerical chromosomal aberrations, polyploidy, human lymphocytes *in vitro*	+	NT	2	Roldán & Altamirano (1990)
Aneuploidy, FISH centromeric probes, human lymphocytes *in vitro*	+	NT	0.001 µM	Ramírez *et al*. (1997)
Inhibition of microtubule polymerisation, immunostaining, human lymphocytes *in vitro*	+	NT	0.1 µM	Ramírez *et al*. (1997)
Chromosomes associated and satellite association, human lymphocyte *in vitro*	+	NT	4	Roldán & Altamirano (1990)

Table 7 (contd)

Test system	Result[a]		Dose[b] (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
DNA strand breaks, alkaline 'Comet Assay', in several organs of CD-1 mice *in vivo*	+		5.75 ip	Altamirano-Lozano *et al*. (1996, 1999)
DNA synthesis, inhibition assay, mice testes, spleen, liver and lymphocytes *in vivo*	–		58.4 po	Zen *et al.* (1988)[c]
Sister chromatid exchanges, CD-1 mice, bone marrow, *in vivo*	-		23 ip	Altamirano-Lozano *et al*. (1993); Altamirano-Lozano & Alvarez-Barrera (1996)
Micronucleus formation, 615 and Kunming albino mice, bone marrow, *in vivo*	+		0.17 ip	Si *et al.* (1982)[c]
Micronucleus formation, 615 and Kunming albino mice, bone marrow, *in vivo*	+		0.25 sc	Si *et al.* (1982)[c]
Micronucleus formation, 615 and Kunming albino mice, bone marrow, *in vivo*	+		0.5 mg/m^3, inhal.	Si *et al.* (1982)[c]
Micronucleus formation, Kunming albino mice, bone marrow, *in vivo*	–		11.3 po	Sun *et al.* (1989)[c]
Micronucleus formation, Kunming albino pregnant mice, fetal liver, maternal bone marrow, maternal spleen, *in vivo*	+		0.2–5 ip[e]	Liu *et al.* (1992)[c]
Micronucleus formation, B6C3F1mice, peripheral blood erythrocytes, *in vivo*	–		16 mg/m^3, inhal.	National Toxicology Program (2002)
Structural chromosomal aberrations, CD-1 mice, bone marrow, *in vivo*	–		23 ip	Altamirano-Lozano & Alvarez-Barrera (1996)

Table 7 (contd)

Test system	Result[a]		Dose[b] (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Structural chromosomal aberrations, albino rat, bone marrow cells, *in vivo*	?		4 po	Giri *et al*. (1979)
Dominant lethal mutations, CD-1 mice *in vivo*	+		8.5 ip	Altamirano-Lozano *et al.* (1996)
Dominant lethal mutations, CD-1 mice *in vivo*	–		4 sc	Si *et al.* (1982)[c]

FISH, fluorescence in-situ hybridization

[a] +, positive; –, negative; (+), weak positive; NT, not tested;?, inconclusive

[b] LED, lowest effective dose; HID, highest ineffective dose; in-vitro tests, μg/mL, except where stated otherwise; in-vivo tests, mg/kg bw per day; po, orally, by gavage; sc, subcutaneously; ip, intraperitoneally; inhal., by inhalation

[c] Cited in Sun (1996)

[d] Combined with 20 μg of caffeine

[e] LED not given

(Kanematsu & Kada, 1978; Kanematsu *et al*., 1980). However, vanadium pentoxide was not mutagenic in several strains of *E. coli* or *S. typhimurium*. But Si *et al.* (1982) (cited by Sun *et al.*, 1996) demonstrated that vanadium pentoxide induced reverse mutations in *E. coli* WP2, WP2uvrA and CM-981, but not frameshift mutations in strains ND-160 or MR102. This compound showed negative results in *S. typhimurium* strains TA100, TA1535, TA1537, TA1538, TA97, and TA98.

Bis(cyclopentadienyl)vanadium chloride (1 to 33 μg/plate) was mutagenic or weakly mutagenic in strains TA97 and TA100 without exogenous metabolic activation system, but not mutagenic in strains TA1535 and TA98 with or without metabolic activation (Zeiger *et al.*, 1992).

In another series of studies, vanadium pentoxide (0.33 to 333.00 μg/plate) was not mutagenic in *S. typhimurium* strains TA97, TA98, TA100, TA102 or TA1535, with or without induced rat or hamster liver S9 enzymes (National Toxicology Program, 2002).

No increase in the frequency of micronucleated normochromatic erythrocytes was seen in peripheral blood samples from male or female $B6C3F_1$ mice exposed to vanadium pentoxide by inhalation in concentrations up to 16 mg/m^3 for 3 months. Furthermore, no effect was seen in the ratio of polychromatic erythrocytes/normochromatic erythrocytes in peripheral blood, indicating a lack of toxicity to the bone marrow by vanadium pentoxide (National Toxicology Program, 2002).

[The Working Group was aware of positive results on induction of mitotic recombination by vanadium pentoxide in *Drosophila*; the data were reported in BSc and MSc theses].

In Chinese hamster lung fibroblast cell lines, vanadium pentoxide induced endoreduplication and micronuclei which were shown to be kinetochore-positive, but did not induce gene mutation nor sister chromatid exchange.

In human lymphocytes cultured *in vitro*, positive genotoxic effects of vanadium pentoxide were demonstrated for the induction of DNA damage with the alkaline 'Comet Assay' (two studies from the same laboratory), sister chromatid exchange when the compound was given in combination with caffeine (one study out of three), chromosomes associated, satellite associations and polyploidy with Hoechst staining (a single study), aneuploidy with fluorescence in-situ hybridization staining and inhibition of microtubule polymerization with immunostaining (a single study).

Vanadium pentoxide was shown to inhibit repair of double-strand breaks induced in human fibroblasts by UV radiation or bleomycin in both the neutral and alkaline comet assays.

(ii) *In-vivo studies*

In CD-1 mice, induction of DNA damage by vanadium pentoxide administered intraperitoneally was demonstrated with the alkaline 'Comet Assay' in several organs. In the same mouse strain, a lack of sister chromatid exchange and chromosomal aberrations was reported in bone marrow; however, dominant lethal effects were observed after intraperitoneal injection of vanadium pentoxide (8.5 mg/kg bw).

In 615 and Kunming albino mice, micronuclei were induced in bone marrow by vanadium pentoxide administered by inhalation, by subcutaneous injection or by intraperitoneal injection. The results were negative following oral administration. Micronuclei were also seen in fetal liver after intraperitoneal injection of vanadium pentoxide into pregnant mice. No induction of dominant lethals was observed.

A single in-vivo study of the induction of chromosomal aberrations in albino rats was inconclusive (number of animals not reported).

(*c*) *Genetic changes in vanadium pentoxide-induced tumours*

In a National Toxicology Program study (2002), male and female B6C3F$_1$ mice were exposed by inhalation to 1, 2, or 4 mg/m^3 vanadium pentoxide for 2 years (see Section 3.1.1). The lung carcinomas that developed as a result of this exposure showed a high frequency of K-*Ras* mutation, loss of heterozygosity in the region of the K-*Ras* gene on chromosome 6 and activation of MAP kinase (Zhang *et al.*, 2001b; Devereux *et al.*, 2002; National Toxicology Program, 2002). The authors concluded that these genetic alterations played an important role in vanadium pentoxide-induced lung carcinogenesis. On the other hand, there was no evidence of overexpression of mutant p53 suggesting no evidence of a role for altered p53 function in the lung carcinomas due to exposure to vanadium pentoxide (Devereux *et al.*, 2002; National Toxicology Program, 2002).

4.5 Mechanistic considerations

Vanadium pentoxide is considered to induce oxidative damage leading to DNA alkali-labile sites and DNA strand breakage.

Inhibition of microtubule polymerization may explain the aneugenic effects of vanadium pentoxide. Whether these spindle disturbances are related to oxidative damage or to direct interaction with vanadium cations is unclear. Indirect effects of vanadium pentoxide through inhibition of various enzymes involved in DNA synthesis and DNA repair also contribute to its genotoxicity.

Induction of dominant lethal mutations in mice may result from one, or a combination, of the modes of action mentioned above.