Opinion of the Scientific Panel on Dietetic Products, Nutrition and Allergies on a request from the Commission related to the Tolerable Upper Intake Level of Vanadium

(Request No EFSA-Q-2003-018)

(adopted on 19 February 2004)

SUMMARY

The European Food Safety Authority is asked to derive an upper level for the intake of vanadium from food that is unlikely to pose a risk of adverse health effects.

Vanadium has not been shown to be essential for humans.

Orally administered vanadium compounds produce adverse effects on kidneys, spleen, lungs and blood pressure in rats and show reproductive and developmental toxicity in rats and mice. In humans, gastrointestinal disturbances have been reported. The available data are inadequate, however, to derive a tolerable upper intake level.

The intake of vanadium from normal food is estimated to be of the order of 10-20 µg/day. This daily intake is at least three orders of magnitude below the lowest doses reported to cause adverse effects. In the case of supplements used by athletes and body builders, however, the intake can be similar to the doses causing adverse effects in rats and humans. Therefore, a risk can be expected to result from the prolonged ingestion of such supplements.

KEY WORDS

Vanadium, tolerable upper intake level, food safety.

BACKGROUND


In addition, and as announced in its White Paper on Food Safety, the Commission aims to put forward a proposal for harmonising legislation concerning the addition of vitamins and minerals to foods.

With a view to provide scientific support to the European Commission’s legislative work in this field, the Scientific Committee on Food (SCF) issued, from October 2000 to April 2003, a series of opinions on tolerable upper intake levels of individual vitamins and minerals and

safety factors in relation to their use in fortified foods and food supplements (available on the Internet at: http://europa.eu.int/comm/food/fs/sc/scf/out80_en.html).

The SCF opinions covered 22 out of the 29 nutrients, which were considered to be within their mandate for this task. The SCF did not have sufficient time to adopt opinions for the following vitamins and minerals: vitamin C, chloride, fluoride, iron, phosphorus, potassium and sodium. In addition, during the decision making process for the adoption of Directive 2000/46/EC on food supplements the Parliament requested that boron, nickel, silicon, vanadium and tin should be allowed to be used in food supplements. Therefore, the European Food Safety Authority is asked to provide scientific opinions on the remaining 12 vitamins and minerals in accordance with the present terms of reference.

TERMS OF REFERENCE

With respect to the outstanding 12 vitamins and minerals, the European Food Safety Authority is asked 1) to review the upper levels of daily intakes that are unlikely to pose a risk of adverse health effects; 2) to provide the basis for the establishment of safety factors, where necessary, which would ensure the safety of fortified foods and food supplements containing the aforementioned nutrients.

ASSESSMENT

1. INTRODUCTION

Vanadium is widely distributed in the earth’s crust. It occurs naturally in the form of about 70 minerals, but does not occur as metallic vanadium. In its compounds, it forms different oxidation states, the most common being +3, +4, and +5.

2. NUTRITIONAL BACKGROUND

2.1 Food levels and dietary intake

Among foods examined for vanadium by atomic absorption spectroscopy, most concentrations were within a range from 1-30 µg/kg fresh weight. Fats and oils, fruits and vegetables contained the least vanadium, ranging from 1-5 µg/kg. Whole grains, seafood, meats and dairy products generally contained 5-30 µg/kg. Dillseeds and black pepper contained the most vanadium, 431 and 987 µg/kg, respectively (Myron et al., 1977).

The daily dietary intake was estimated to be of the order of a few tens of µg (WHO, 1996). Total diet studies resulted in an average intake of 13 µg/day in the UK (Evans et al., 1985) and an estimated dietary intake in the range of 6-18 µg/day for different age-sex-groups of adults and 6.5-11 µg/day for infants, children, and adolescents in the USA (Pennington and Jones, 1987).

In drinking water supplies in the USA, 91% of samples had below 10 µg vanadium/L, the maximum concentration was 70 µg/L and the average 4.3 µg/L (Durfor and Becker, 1963).
26% of tap water samples of 34 areas in the USA contained vanadium at concentrations of 1.3-33 µg/L with a mean of 4.85 µg/L (Greathouse and Craun, 1978).

According to data provided by the manufacturers of vitamin and mineral supplements sold in the UK and representative of at least 70% of the UK market, the highest level of vanadium in multiple-nutrient products is 25 µg/tablet or capsule (EGVM, 2003). Weight training athletes are reported, however, to use up to 60 mg/day of vanadyl sulphate, equivalent to 18.6 mg vanadium/day, to improve performance (Barceloux, 1999). Vanadium supplements aimed at body builders are reported to be available at doses between 7.5 and 10 mg/day (EGVM, 2003).

2.2 Nutritional requirements and intake recommendations

Vanadium has not been shown to be essential for humans and has no nutritional value. Therefore, nutritional requirements or intake recommendations have not been established. The SCF stated that evidence supporting the essentiality of vanadium has yet to be established (SCF, 1993). A maximum intake level of 1.8 mg vanadium/day for adults is recommended by FNB and EGVM stated that there are insufficient data to establish a safe upper level (FNB, 2001; EGVM, 2003).

2.3 Deficiency

Some signs of vanadium deficiency in goats and rats have been reported (WHO, 1996). In humans, however, vanadium deficiency has not been identified.

3. BIOLOGICAL CONSIDERATIONS

3.1 Function

A number of vanadium dependent enzymes have been found in lower organisms, such as bacteria and algae. In higher animals and humans, however, no specific biochemical function has yet been identified for vanadium. Nevertheless, the possibility has been considered that vanadium might play a role in the regulation of some enzymes, such as the Na⁺/K⁺-exchanging ATPase, phosphoryl-transfer enzymes, adenylate cyclase and protein kinases. Therefore, its role in hormone, glucose, lipid, bone and tooth metabolism has also been discussed (WHO, 1996).

Vanadium compounds have been shown to mimic the action of insulin in isolated cell systems, animal models and diabetic patients. Therefore, their use in the therapy of diabetes mellitus has been considered (Shechter, 1990; Shamberger, 1996).

Vanadium has also been suggested as an aid in body building, but there is no evidence that it is effective (Fawcett et al., 1996).

3.2 Absorption, distribution, metabolism and excretion

The low concentration of vanadium normally present in urine compared with the daily intake and the faecal levels indicate, that less than 5% of ingested vanadium is absorbed (WHO, 1996). The results of animal studies are in general in agreement with this conclusion. Uptake
of radioactive vanadium pentoxide (V₂O₅) given orally to rats was 2.6% of the administered dose (Conklin et al., 1982). Other studies in rats have indicated that amounts greater than 10% can be absorbed from the gastrointestinal tract under some conditions (Bogden et al., 1982; Wiegmann et al., 1982).

Absorbed vanadium is transported in the serum mainly bound to transferrin. Extracellular vanadium is present in the form of vanadate (5+) and intracellular vanadium most likely in the vanadyl (4+) form. After administration by different routes to rats, the highest amounts were found in lungs (after intratracheal installation), bone, kidneys, liver and spleen. Studies on rats and mice showed a three-compartment model for elimination with plasma half-times of 15 minutes, 14 hours and 8.5 days (Lagerkvist et al., 1986).

3.3 Normal levels in human tissues and fluids

In contrast to earlier assumptions based on colorimetric determinations, Byrne and Kosta (1978) estimated the total body pool to be only 100 to 200 µg vanadium. Normal concentrations in human blood serum have been reported to be in the range of 0.02-0.94 µg/L (Cornelis et al., 1981). Thus, serum vanadium values above 1 µg/L probably indicate excessive exposure (WHO, 1996).

4. HAZARD IDENTIFICATION

This section is limited to oral toxicity data with the exception of carcinogenicity. Other toxicological aspects of vanadium, in particular hazards from inhalative exposure and aspects of occupational medicine, have been reviewed elsewhere (Browning, 1969; EPA, 1977; NIOSH, 1977; NRCC, 1980; MAK, 1984; Lagerkvist et al., 1986; WHO, 1988 and 2001).

4.1 Effects on cell cycle and proliferation

Vanadium compounds elicit several biological effects, affecting cell cycle, signalling pathways, and cell survival in vitro and in vivo.

Induction of apoptosis and p53 activation were observed in murine epidermal JB6 P+ cells treated with sodium metavanadate (2.24 µg/mL) or vanadyl sulphate (3.26 µg/mL) (Ye et al., 1999; Huang et al., 2000).

In newborn rat brain cells, sodium metavanadate induced apoptosis and the activation of extracellular-signal regulated kinases (Luo et al., 2003).

In vivo, a marked increase of apoptotic cells was observed in lung cells of BALB/cJ mice treated orally with sodium metavanadate (50 µg/mouse). In general the addition of NADPH, sodium formate or superoxide dismutase enhanced vanadate-induced apoptosis, which was inhibited by catalase or deferoxamine, suggesting a role for oxidative species in this process (Wang et al., 2003).

In the human breast cancer cell line Mcf-7, vanadate stimulated cell proliferation and activated oestrogen receptor-α (ER-α), inducing the expression of oestrogen-regulated genes (Martin et al., 2003).
Exposure to noncytotoxic levels of vanadate stimulated interleukin-8 production in human promyelocyte cells (Sonoda et al., 1997), and inhibited tyrosine phosphatases in the human bronchial epithelial cell line BEAS 2B, resulting in increased levels of tyrosine phosphorylation (Samet et al., 1999) and activation of mitogen activated protein kinases (MAP kinases) (Samet et al., 1998).

In the human alveolar epithelial cancer cell line A549, exposure to vanadate led to cell growth arrest at G2/M and caused up-regulation of cell growth regulatory proteins (p21, phospho cdc2 and cdc25) and activation of MAP kinases. Different reactive oxygen species were shown to affect specific MAP kinases and cell cycle regulatory proteins (Zhang et al., 2003).

4.2 Acute toxicity

The oral acute toxicity of vanadium varies with the species and the nature of the compound. In general, vanadium is said to be better tolerated by the rat and the mouse than by larger animals including the rabbit and horse (WHO, 1988). Acute vanadium poisoning in animals is characterized by marked nervous disturbance, haemorrhagic enteritis and a fall of temperature. Death is preceded by paralysis of hind legs, laboured respiration and convulsions (Browning, 1969).

In rats of unknown strain, vanadium pentoxide and ammonium metavanadate have been reported to have an oral LD$_{50}$ of 10.4 and 18.3 mg/kg body weight, equivalent to 5.8 and 8.0 mg vanadium/kg body weight, respectively (Massmann, 1956). In male Sprague-Dawley rats, sodium metavanadate and vanadyl sulphate pentahydrate were less toxic with oral LD$_{50}$ values equivalent to 41 mg vanadium/kg body weight and 90.3 mg vanadium/kg body weight, respectively. In male Swiss mice, the LD$_{50}$ values of these compounds were equivalent to 31 mg vanadium/kg body weight and 94 mg vanadium/kg body weight, respectively (Llobet and Domingo, 1984).

4.3 Subacute/Subchronic toxicity

Groups of 10 male Sprague-Dawley rats received 0, 5, 10, and 50 mg/L sodium metavanadate in drinking water for a period of three months. The concentrations were equivalent to approximately 0.8, 1.5 and 7.7 mg vanadium/kg body weight/day as calculated by FNB (2001). Appearance, behaviour, food and water consumption, growth, mortality and the weights of liver, kidneys, heart, spleen and lungs were not affected. The histopathological examination of organs in 3 rats per group, however, showed mild lesions in kidneys (corticomedullar microhaemorrhagic foci), spleen (hypertrophy and hyperplasy in the white pulp) and lungs (mononuclear cell infiltration, mostly perivascular), more evident in animals receiving the highest dose. These lesions are described as dose-dependent, their significance, however, is not clearly specified. The vanadium concentrations in a number of organs were elevated at the highest dose and in kidneys and spleen at the dose of 10 mg/L. In addition, significantly increased plasma concentrations of urea and uric acid were found at the highest dose level (Domingo et al., 1985).

Groups of 12 male streptozotocin-induced diabetic rats were exposed to drinking water containing NaCl (80 mM) and sodium metavanadate (150 mg/L), sodium orthovanadate (230 mg/L) or vanadyl sulphate pentahydrate (310 mg/L) for 28 days. The groups were compared to controls, either diabetic or non-diabetic, receiving drinking water containing NaCl (80 mM) only. At the ingested doses, equivalent to 6.1, 15.6 and 22.7 mg vanadium/kg body...
weight/day, the daily food and fluid intake as well as the blood glucose levels were significantly reduced relative to the diabetic controls, with sodium metavanadate being the most effective compound tested. Signs of toxicity including decreased weight gain, increased serum concentrations of urea and creatinine and some deaths were observed in all vanadium-treated animals (Domingo et al., 1991).

Groups of 10 male Sprague-Dawley rats received drinking water with 0 and 100 mg vanadium/L as sodium metavanadate for 7 months. Heart rate and systolic and diastolic blood pressure were increased significantly in the vanadate exposed animals. Vanadate strongly increased also the levels of urinary Na\(^+\) and K\(^+\) and affected the kidneys, where the lumen of the proximal tubule cells was narrowed and contained amorphous protein material (Carmignani et al., 1991).

Groups of 6 male weaning Sprague-Dawley rats were given sodium metavanadate in drinking water at concentrations of 0, 10 and 40 mg vanadium/L for 210 days and 0 and 1 mg vanadium/L for 180 days. All vanadium exposed rats showed a significant increase in systolic and diastolic blood pressure, but not of heart rate and cardiac inotropism. These changes were not dose-dependent. Rats exposed to 40 mg/L had effects on kidney morphology (narrowed proximal tubules containing amorphous material and hydropic degeneration in proximal, distal and straight tubules). Such abnormalities were less evident in rats treated with 10 mg/L and absent in those treated with 1 mg/L of vanadium. At 10 and 40 mg/L, plasma renin, urinary kallikrein and urinary kininase I and II activities as well as urinary potassium were increased (Boscolo et al., 1994).

Groups of 5 male unilateral-nephrectomized Sprague-Dawley rats received normal rat chow containing 0.3 mg vanadium/kg and normal rat chow supplemented with sodium orthovanadate, equivalent to 100 mg vanadium/kg diet, and drank either tap water or a 1% solution of sodium chloride for a period of 9 weeks. In a second experiment, groups of 9 male unilateral-nephrectomized Sprague-Dawley rats drank tap water and ate normal rat chow or normal rat chow supplemented with sodium orthovanadate, equivalent to 100 or 200 mg vanadium/kg diet for a period of 56 weeks. In the vanadate exposed rats drinking tap water, systolic blood pressure gradually increased over several weeks and then was sustained in a dose-related manner. This effect was correlated positively with plasma vanadium levels from 0.04 to 0.27 µg/mL and associated with an increased heart-to-body-weight ratio (Steffen et al., 1981).

Groups of about 12 two-month old Wistar rats of both sexes received 0 and 150 mg vanadium/L as ammonium metavanadate in their drinking water for a period of 4 weeks, equivalent to 0 and 13 mg vanadium/kg body weight/day, respectively. A small decrease in food and water consumption and body weight gain was observed in treated animals with transient diarrhoea in two cases. In the peripheral blood, a significant decrease in erythrocyte count and haemoglobin level and significant increases in the percentage of reticulocytes and polychromatophilic erythrocytes and in the neutrophilic granulocyte and lymphocyte count were noted. Some inhibitory influence on the phagocytic activity of granulocytes in females was also observed (Zaporowska and Wasilewski, 1992).

Groups of about 15 two-month old Wistar rats of both sexes received 0, 10 and 50 mg vanadium/L as ammonium metavanadate in their drinking water for a period of 4 weeks, equivalent to 0, 1.5 and 5 to 6 mg vanadium/kg body weight/day, respectively. The erythrocyte count was significantly decreased at both dose levels associated with a significant
decrease of the haemoglobin level and increased percentage of reticulocytes in the peripheral blood at the highest dose level. In addition, the L-ascorbic acid level in the plasma of males was significantly decreased at both dose levels (Zaporowska et al., 1993).

In a study with non-diabetic and streptozotocin-diabetic male Wistar rats, groups of 8-32 animals were untreated or exposed to vanadyl sulphate hydrate for 52 weeks at doses of 500 mg/L stepwise increased to 1250 or 1500 mg/L (Dai et al., 1994a), equivalent to 8-36 mg vanadium/kg body weight/day (Dai et al., 1995). The treatment caused significant decreases in body weight gain and plasma insulin level in non-diabetic rats (Dai et al., 1994b), but did not produce persistent impairment of hepatic or renal function and significant changes in organ weights, gross and microscopic findings in either non-diabetic or diabetic animals. However, increased amounts of vanadium were retained in various organs for months after cessation of treatment in the following rank order: bone > kidneys > testis > liver > pancreas > brain (Dai et al., 1994a). In contrast to other studies, systolic blood pressure, pulse rate and selected haematological indices were not significantly changed (Dai and McNeill, 1994).

In another study, groups of 8 male Wistar rats were exposed to ammonium metavanadate (10 mg vanadium/kg body weight/day), vanadyl sulphate hydrate (8 mg vanadium/kg body weight/day) and bis (maltolato)oxovanadium (IV) (9 mg vanadium/kg body weight/day) for 12 weeks. Again, there were no significant differences of haematological parameters between controls and exposed animals (Dai et al., 1995).

4.4 Chronic toxicity/Carcinogenicity

Only limited carcinogenicity studies by oral route on vanadyl sulfate are presently available.

Groups of 23 male and 29 female Charles River CD mice were given 5 mg/L vanadium as vanadyl sulfate in the drinking water from weaning until natural death. The diet fed to the mice for the first 6 months contained 1.4 mg/kg vanadium and thereafter 3.2 mg/kg vanadium, on a wet basis. Dead animals were autopsied, gross lesions noted and abnormal tissues sectioned for microscopic analysis. No effects in terms of growth, survival, longevity, increased incidence of tumours or other pathological signs were observed (Schroeder and Balassa, 1967).

Groups of 50 Long-Evans rats of each sex received drinking water containing 5 mg/L vanadium as vanadyl sulfate from the time of weaning until natural death. The diet contained 3.2 mg/kg vanadium. Seventeen animals of each sex were killed by an epidemic of virulent pneumonia and removed from the series. No toxicity was observed. Growth, survival and incidence of visible tumours at necropsy were not significantly different from the controls (Schroeder et al., 1970).

Groups of 54 males and 54 females of Swiss mice of the Charles River CD strain were given 5 mg/L vanadium as vanadyl sulfate in the drinking water for life span. Body weights were examined at intervals and animals that died naturally were dissected, gross tumours detected and sections made of heart, lung, liver, kidney and spleen for microscopic examination. Males given vanadium had significantly higher body weights than controls and longevity was increased by vanadium in both sexes. A slight increase in the number of tumour-bearing female mice was not considered relevant (Schroeder and Mitchener, 1975).
The results of these studies are hampered by strong limitations such as: a non-standard protocol; only one single dose level; no signs of toxicity; high content of vanadium in the diet (more than half of that compared with the vanadium concentration in the drinking water); high spontaneous total incidence of tumours. Therefore, the results of these studies should be considered inconclusive.

Recently, the results of 2-year standard bioassays with vanadium pentoxide particles in F344/N rats and B6C3F1 mice exposed by inhalation have been made available (NTP, 2002).

Groups of 50 male and 50 female rats were exposed to particulate aerosols of vanadium pentoxide at concentrations of 0, 0.5, 1 or 2 mg/m\(^3\) by inhalation, 6 hours per days, 5 days per week for 104 weeks. Survival and body weights were generally similar to those of the controls; only mean body weights in females exposed to 2 mg/m\(^3\) were lower than those of the controls. In male rats, the incidences of lung alveolar/bronchiolar neoplasms (adenomas or carcinomas) often exceeded the historical control ranges: adenomas (4/50, 8/49, 5/48, 6/50); carcinomas (0/50, 3/49, 1/48, 3/50); adenomas/carcinomas (4/50, 10/49, 6/48, 9/50). Some tumours also occurred, at lower extent, in the females: adenomas (0/49, 3/49, 1/50, 0/50); adenomas plus 1 carcinoma at the top dose (0/49, 3/49, 1/50, 1/50). Non-neoplastic lesions in the respiratory tract (e.g. hyperplasia, inflammation, fibrosis) were also observed, generally increased at higher concentrations. The NTP conclusions were: “some evidence of carcinogenic activity” in male rats, and “equivocal evidence of carcinogenic activity” in female rats.

Groups of 50 male and 50 female mice were exposed to particulate aerosols of vanadium pentoxide at concentrations of 0, 1, 2 or 4 mg/m\(^3\) by inhalation, 6 hours per day, 5 days per week for 104 weeks. Survival of males at the top concentration was significantly less than that of controls. Mean body weights of 4 mg/m\(^3\) males and all exposed groups of females were generally less than in controls. The incidences of lung alveolar/bronchiolar neoplasms were significantly increased in all exposed groups both in males and in females. Males: adenomas (13/50, 16/50, 26/50, 15/50); carcinomas (12/50, 29/50, 30/50, 35/50); adenomas/carcinomas (22/50, 42/50, 43/50, 43/50). Females: adenomas (1/50, 17/50, 23/50, 19/50); carcinomas (0/50, 23/50, 18/50, 22/50); adenomas/carcinomas (1/50, 32/50, 35/50, 32/50). Non-neoplastic lesions were also observed with increased severity at higher concentrations. The NTP conclusion was: “clear evidence of carcinogenic activity” in male and female mice.

Based on these inhalation studies, vanadium pentoxide has been evaluated by IARC as “possibly carcinogenic to humans” (Group 2B) on the basis of “sufficient evidence” in experimental animals, in the absence of human data (IARC Monographs, Vol. 86, 2003). The relevance of the results of the NTP inhalation studies in rats and mice for oral ingestion of vanadium pentoxide or other vanadium compounds is unclear.

4.5 Genotoxicity

4.5.1 In vitro studies in bacteria

In a screening of metal compounds with a semiquantitative spot test procedure without metabolic activation, vanadium pentoxide (+5) and ammonium metavanadate (+5) were reported not to be mutagenic in the Escherichia coli strains B/r and WP2, and in Salmonella enterica var. Typhimurium TA1535, TA1537, TA1538, TA98 and TA100 (Kanematsu et al.,
In the same study, vanadium oxydichloride, vanadium pentoxide and ammonium metavanadate were evaluated as positive in the rec assay in *Bacillus subtilis*.

Ammonium metavanadate was found to be weakly mutagenic in *Salmonella enterica var. Typhimurium* TA1535 in a plate incorporation assay with a modified Vogel-Bonner medium, inducing a doubling of revertants at the highest dose tested (43.3 µg/plate, without S9) (Arlauskas *et al.*, 1985).

Negative results were obtained in the standard *Salmonella enterica var. Typhimurium* reversion test (Ames test) with vanadium pentoxide at concentrations from 0.03 to 333 µg/plate in strains TA97, TA98, TA100, TA102, and TA1535, with or without Aroclor induced rat or hamster liver S9 (NTP, 2002).

4.5.2 In vitro studies in eukaryotic cells

Mitotic gene conversion and point mutations were induced in *Saccharomyces cerevisiae* strain D7 by ammonium metavanadate at concentrations from 9.4 to 24.6 µg/mL (Bronzetti *et al.*, 1990). Positive results were also obtained in the same test system with vanadyl sulfate (+4) at concentrations from 26.1 to 68.5 µg/mL, only in experiments with cells from logarithmic growth phase (Galli *et al.*, 1991). In the same study, both ammonium metavanadate (0.47-0.58 µg/mL) and vanadyl sulfate (1.22-1.63 µg/mL) induced mitotic aneuploidy in the *S. cerevisiae* strain D6.1M.

In Chinese hamster V79 cells, no induction of forward mutations at the hp3 locus was observed after treatment with vanadyl sulfate (+4) (0.081-0.81 µg/mL), both in the presence or in the absence of metabolic activation (Galli *et al.*, 1991), or after treatment with vanadium pentoxide (1-3 µg/mL) in the absence of metabolic activation (Zhong *et al.*, 1994). In experiments without exogenous metabolic activation, ammonium metavanadate, at low non-cytotoxic concentrations (up to 0.58 ng/mL), increased the frequency of mutations at the hp3 locus in V79 cells, and, at higher concentrations (0.585 µg/mL), at the bacterial gpt gene in transgenic G12 cells (Cohen *et al.*, 1992).

Vanadium trioxide (+3), vanadyl sulfate and ammonium metavanadate, at concentrations respectively of 0.1-1 µg/mL, 0.5-6 µg/mL and 0.5-4 µg/mL, induced significant increases in SCE frequencies in Chinese hamster ovary cells (CHO-K1). The genotoxic activities of ammonium metavanadate and vanadyl sulphate were decreased in the presence of hepatic S9 (Owusu-Yaw *et al.*, 1990). In the same study, all three vanadium compounds induced significant increases of structural chromosomal aberrations, with a prevalence of breaks and exchanges, after a 2-hour treatment with 4, 8, 16 µg/mL (ammonium metavanadate), 6, 12, 24 µg/mL (vanadyl sulphate), and 12, 18 µg/mL (vanadium trioxide), both in the absence and in the presence of metabolic activation.

In another study, no significant increase in sister chromatid exchange (SCE) frequency was observed in Chinese hamster V79 cells after 24h treatment with vanadium pentoxide at concentrations up to 4 µg/mL. However, a dose-related increase of endoreduplication and micronucleated cells containing CREST-positive micronuclei was observed, indicating an aneugenic effect (Zhong *et al.*, 1994).
No induction of structural chromosome aberrations and SCE was observed in human lymphocytes treated for 48h with 2, 4 and 6 µg/mL of vanadium pentoxide in the absence of metabolic activation (Roldán and Altamirano, 1990). However, a statistically significant increase in the frequency of polyploid cells was reported at all dose levels.

A study by Migliore et al. (1993), investigated the ability of sodium ortho-(0.41-13.12 µg/mL) and metavanadate (0.28-8.96 µg/mL), ammonium metavanadate (0.29-9.36 µg/mL) and vanadyl sulphate (0.41-13.04 µg/mL) to produce structural and numerical chromosome aberrations, micronuclei and SCE in human lymphocytes in vitro. Treatments with vanadium compounds did not induce any significant increase in the frequency of cells with structural chromosomal aberrations. On the other hand, statistically significant increases in SCE rates were observed with all compounds at the two highest concentrations tested, and large increases in the incidence of micronuclei from 1.64 µg/mL onwards. The characterization of micronuclei by fluorescence in situ hybridization showed that centromeres were present in a large fraction of micronuclei, indicating an aneugenic potential for these vanadium compounds. The same authors also demonstrated the preferential loss of acrocentric and sex chromosomes after treatment of human lymphocytes in vitro with vanadyl sulphate and sodium orthovanadate (Migliore et al., 1995 and 1999).

Immunostaining of the spindle apparatus using an anti-β-tubulin antibody, and an in vitro assay measuring the polymerization/depolymerization of purified tubulin, confirmed that vanadium pentoxide at doses as low as 1.82 ng/mL affect spindle organization and function in human lymphocytes through the disruption of microtubules (Ramírez et al., 1997).

In a recent study, Rodríguez-Mercado et al. (2003) evaluated the genotoxicity of vanadium tetraoxide (+4) in human peripheral blood cells in vitro. A dose-related increase of structural chromosome aberrations and SCE, as well as an inhibition of mitotic and replicative index, were observed in cultures treated with vanadium tetraoxide (+4) (2-16 µg/mL, without metabolic activation). On the other hand, no increase in comet test parameters was observed after short (2 h) treatments with V$_2$O$_4$, possibly because of the limited ability of V+4 to cross cell membrane.

In other tests for DNA damage using single cell gel electrophoresis (comet assay), vanadium pentoxide induced DNA single strand breaks and/or alkali labile sites in non-stimulated human leukocytes at doses from 0.54 µg/mL or more. On the other hand, in stimulated lymphocytes only the highest concentration tested (540 µg/mL) induced a significant increase in DNA damage (Rojas et al., 1996).

In another study, orthovanadate significantly increased comet tail length in non-stimulated human lymphocytes (0.25-0.5 µg/mL) and in cultured human fibroblasts (from 0.025 µg/mL); moreover, co-exposure of human fibroblasts to vanadate (0.025 µg/mL) and UV or bleomycin resulted in non-repairable DNA double-strand breaks (Ivancsits et al., 2002).

The induction of morphological transformation by vanadium +5 and +4 has been studied in mouse BALB 3T3 cells. Ammonium and sodium vanadate (+5) showed transforming activity at concentrations of 0.351 µg/mL or higher, while vanadyl sulphate (+4) was ineffective (Sabbioni et al., 1991 and 1993).
Sodium orthovanadate (0.033-1.14 µg/mL) was assayed for cell transformation in Syrian hamster embryo cells. A marked increase in cell transformation was noted only at the highest dose, without any effect on cloning efficiency (Rivedal et al., 1990; Kerckaert et al., 1996).

Finally, ammonium metavanadate, added at the concentration of 0.585 µg/mL, amplified the yields of type II and type III foci in methylcholanthrene-initiated C3H/10T1/2 cells (Parfett and Pilon, 1995).

### 4.5.3 In vivo studies

Treatment of Drosophila larvae with vanadyl sulphate (163-407 µg/mL) induced somatic mutations, detected as aberrant red sectors in the w+/w adult eye (Barrera Ferrer and Villalobos Cabrera, 1998).

Single cell gel electrophoresis (comet assay) was used to assess the ability of V₂O₅ to induce DNA damage in vivo in six different organs (liver, kidney, lung, spleen, heart and bone marrow) of CD-1 mice. V₂O₅ was given by intraperitoneal injection at 5.75, 11.5 and 23 mg/kg body weight (LD₅₀) 24 h before sacrifice. A significant, although not dose-dependent, increase of DNA migration was observed in kidney, liver, heart and lung cells, whereas only slight damage was induced in spleen cells, and no effect in bone marrow (Altamirano-Lozano et al., 1999). With the same protocol significant, dose-related increase of DNA migration was observed in testicular cells with the lowest dose assayed (5.75 mg/kg) onwards (Altamirano-Lozano et al., 1996).

Vanadyl sulphate, sodium orthovanadate and ammonium metavanadate were tested for the induction of micronuclei, and structural and numerical chromosome aberrations in bone marrow of male CD-1 mice. Micronuclei and hyperploidy were induced by all compounds when given by single intragastric administration at 100, 75 and 50 mg/kg body weight, respectively, but only vanadyl sulphate induced a significant increase of structural chromosomal aberrations at the dose of 100 mg/kg body weight, corresponding approximately to 1/5 of the LD₅₀ (Ciranni et al., 1995).

No increase in the frequency of micronucleated normochromatic erythrocytes, and no alteration of the PCE/NCE ratio, were seen in peripheral blood samples of male and female B6C3F1 mice exposed for 3 months by inhalation to 1-16 mg/m³ vanadium pentoxide (NTP, 2002).

Sodium orthovanadate, administered intraperitoneally to ICR female mice during oocyte maturation at doses of 0, 5, 15, and 25 mg/kg body weight, induced different cytogenetic abnormalities in oocytes and in bone marrow cells. In oocytes, vanadate induced premature anaphase, whereas in bone marrow sodium orthovanadate increased tetraploidy, hyperploidy and premature centromere separation (Mailhes et al., 2003).

### 4.5.4 Summary of genotoxicity test results

Overall, genotoxicity test results suggest that vanadium compounds do not induce gene mutations in bacterial cells as well as, with the possible exception of ammonium metavanadate, in mammalian cells. There is clear evidence that pentavalent and tetravalent forms of vanadium produce aneuploidy in vitro and in vivo, very likely through interference with microtubule assembly and spindle formation (Winkelhaus and Hauser, 1997; Ochi,
2002), as well as polyploidy, endoreduplication and other aneugenic-related effects. The production of micronuclei, in vitro and/or in vivo, appears to be caused by chromosome loss (aneuploidy), and not by chromosome-breaking mechanism (clastogenicity).

Less clear is the evidence that vanadium compounds can produce structural chromosomal aberrations, although vanadium tetraoxide was positive in vitro, and vanadyl sulphate was weakly positive in vivo.

The DNA damaging activity, observed in vitro and/or in vivo by the Comet assay, is probably due to reactive oxygen species generated through a Fenton-like reaction (Sakurai, 1994; Shi et al., 1996; Altamirano-Lozano, 1998).

In conclusion, the nature of the genotoxicity database indicates that indirect, threshold-based modes of action are probably responsible of the aneugenic and DNA damaging activity of vanadium compounds.

4.6 Reproductive and developmental toxicity

Groups of 20 female Sprague-Dawley rats were administered intragastrically 0, 5, 10 and 20 mg sodium metavanadate/kg body weight/day, equivalent to about 0, 2, 4, and 8 mg vanadium/kg body weight/day for 14 days before mating with groups of 20 Sprague-Dawley males which had received the same doses for 60 days. No significant adverse effects were observed on numbers of corpora lutea, implantations, live and dead foetuses, or resorptions. However, the average body weight/litter of the rat pups nursed by vanadium-treated mothers was significantly reduced on post-natal day 21 at 10 and 20 mg/kg body weight/day. In addition, average body weight, body length and tail length as well as relative organ weights of liver, kidneys and spleen showed significant decreases even at the lowest dose (Domingo et al., 1986).

Groups of 20 pregnant rats were given intragastrically doses of 0, 5, 10 and 20 mg sodium metavanadate/kg body weight/day on days 6-14 of gestation. The highest dose equivalent to about 8 mg vanadium/kg body weight/day was found to be embryotoxic but was not embryolethal or teratogenic (Paternain et al., 1987).

Groups of 16 to 20 pregnant Swiss mice were administered doses of 0, 37.5, 75 and 150 mg/kg body weight/day of vanadyl sulphate pentahydrate, equivalent to about 0, 7.5, 15 and 30 mg vanadium/kg body weight/day once daily by gavage on gestational days 6-15. Maternal toxicity was observed as evidenced by reduced weight gain, reduced body weight and decreased absolute liver and kidney weights at 75 and 150 mg/kg body weight/day. The number of total implants, live and dead foetuses, late resorptions, the sex ratio and the post-implantation losses were not significantly different from the controls. However, there was a significant increase in the number of early resorptions per litter, a reduction of body weights and body length, an increase of the number of stunted foetuses and an increased incidence of poorly ossified skeletal elements at all dose levels. In addition, an increase of external malformations was seen with cleft palates and micrognathia as the major gross malformations at 30 mg vanadium/kg body weight/day (Paternain et al., 1990).

Groups of 14-20 Swiss mice received sodium orthovanadate by gavage at doses of 0, 7.5, 15, 30, and 60 mg/kg body weight/day on days 6-15 of pregnancy. Maternal toxicity was observed at 30 and 60 mg/kg body weight/day and reduced food consumption and weight gain.
at 15 and 30 mg/kg body weight/day. Embryolethality and teratogenicity were not observed, but foetal toxicity in the form of a significant delay in the ossification process of skeletal districts was seen at 30 mg/kg body weight/day. The no observed adverse effect level (NOAEL) for maternal toxicity was 7.5 mg/kg body weight/day, equivalent to about 2 mg vanadium/kg body weight/day, and the NOAEL for developmental toxicity was 15 mg/kg body weight/day, equivalent to about 4 mg vanadium/kg body weight/day, under the conditions of this study (Sanchez et al., 1991).

Groups of 24 male Swiss mice received for 64 days sodium metavanadate in the drinking water at dosages of 0, 20, 40, 60 and 80 mg/kg body weight/day, equivalent to about 0, 8, 17, 25 and 33 mg vanadium/kg body weight/day. At the end of the exposure period, each group was divided into two subgroups: 8 animals were used for a mating trial and 16 animals for testes pathology and sperm examination. A significant decrease in the pregnancy rate was observed at 60 and 80 mg/kg body weight/day. Decreased body and epididymis weight was observed at 80 mg/kg body weight/day. Spermatozoa counts were significantly reduced at 60 and 80 mg/kg body weight/day, but the sperm motility was unaffected. The NOAEL in this study was 40 mg/kg body weight/day, equivalent to about 17 mg vanadium/kg body weight/day (Llobet et al., 1993).

4.7 Human data

Twelve patients, 10 of whom had coronary heart disease and 9 were hypercholesterolaemic, were treated orally with diammonium vanadotartrate (25 mg, 3 times daily for 2 weeks, increased to 125 mg daily during the following fortnight and maintained for a further 5 months in 10 patients). Persistent abdominal pain, anorexia, nausea and loss of weight were reported in 5 patients. A green tongue appeared in 5 men and one other developed pharyngitis with marginal ulceration of the tongue (Somerville and Davies, 1962).

Six patients received ammonium vanadyl tartrate in increasing doses between 25 and 125 mg/day for 45 to 68 days. All patients experienced gastrointestinal difficulties with cramps and diarrhoea including one patient who did not receive more than 50 mg/day, equivalent to about 0.17 mg vanadium/kg body weight/day. Doses of 50 mg/day or more resulted also in a green tint on the tongue. No effects were seen at 25 mg/day in 4 subjects, equivalent to approximately 0.08 mg vanadium/kg body weight/day (Dimond et al., 1963).

125 mg sodium metavanadate/day, equivalent to 0.83 mg vanadium/kg body weight/day, administered orally to 10 diabetic patients for two weeks resulted in an improvement of insulin sensitivity in non-insulin- and insulin-dependent diabetes mellitus patients and a decrease in serum cholesterol levels. The major adverse side effect was gastrointestinal intolerance including mild diarrhoea in 4 subjects. No evidence of toxicity was detected by screening electrolytes, blood urea nitrogen and creatinine, by liver and thyroid function studies, urine analysis and a complete blood count (Goldfine et al., 1995).

Gastrointestinal effects including nausea, diarrhoea, abdominal cramps and flatulence were reported in patients with non-insulin dependent diabetes mellitus given 100 mg vanadyl sulphate/day for a few weeks, equivalent to approximately 0.52 mg vanadium/kg body weight/day (Cohen et al., 1995; Boden et al., 1996; Halberstam et al., 1996).

In a double blind, placebo-controlled trial, vanadyl sulphate capsules were given to 11 male and 4 female weight training athletes at mean daily doses of 9 mg vanadium, i.e. 0.12 mg
vanadium/kg body weight/day, for a period of 12 weeks. 5 subjects in the treatment group and 4 in the placebo group withdrew for various reasons. Body weight and blood pressure remained stable during the trial. No treatment effects were seen on haematocrit, blood viscosity, haematological and biochemical indices, compared to the placebo group (Fawcett et al., 1997).

DNA strand breaks, 8-OHdG, and SCE were analysed in blood leukocytes and lymphocytes of 46 male workers employed in a vanadium factory (vanadium concentration in serum 2.18-46.35 µg/L), and compared with 12 non-exposed controls. No exposure-related increase in genotoxic or oxidative DNA damage was observed (Ivancsits et al., 2002).

CONCLUSIONS

1. DOSE RESPONSE ASSESSMENT AND DERIVATION OF A TOLERABLE UPPER INTAKE LEVEL (UL)

According to the available subacute and subchronic studies with rats, orally administered vanadium compounds produced adverse effects on kidneys, spleen, lungs and blood pressure. In one study, effects occurred even at 0.8 mg vanadium/kg body weight/day after administration for a period of 3 months (Domingo et al., 1985). A NOAEL cannot be derived from these studies.

Similarly, there is no NOAEL for developmental toxicity in rats. Toxic effects of sodium metavanadate were seen in the offspring of rats nursed by vanadium-treated mothers at and above doses of 2 mg vanadium/kg body weight/day (Domingo et al., 1986). In mice, however, NOAELs for maternal toxicity of about 2 mg vanadium/kg body weight/day and for developmental toxicity of about 4 mg vanadium/kg body weight/day were reported (Sanchez et al., 1991).

An adequate evaluation of the carcinogenic potential of vanadium by the oral route is not possible, due to strong limitations of the presently available feed studies. The relevance of the NTP inhalation studies in rats and mice for oral ingestion of vanadium is unclear.

There is clear evidence that both pentavalent and tetravalent vanadium compounds induce aneuploidy in vitro due to interference with the spindle apparatus. These forms, as well as trivalent vanadium, can also produce DNA damage in vitro and/or \( \text{V}_2\text{O}_3 \) in vivo, probably due to reactive oxygen species. Overall, the available data suggest that vanadium compounds do not produce gene mutations. The evidence for them also being able to produce structural chromosomal aberrations is unclear.

Clinical experience in humans is limited to studies with small numbers of volunteers and patients. In these studies, gastrointestinal disturbances including diarrhoea and abdominal cramps were observed as major side effects. The lowest dose of a vanadium compound reported to cause such an effect, was equivalent to about 0.2 mg vanadium/kg body weight/day (Dimond et al., 1963).

On the basis of these limited dose-response data, a tolerable upper intake level cannot be derived.
2. **RISK CHARACTERIZATION**

Vanadium has not been shown to be essential for humans.

Studies in humans have shown that the oral intake of vanadium compounds can cause gastrointestinal disturbances. In addition, vanadium compounds affect kidneys and other organs of rats at relatively low doses and have adverse effects at higher doses on the reproduction and the development of the offspring of rats and mice. These effects have not been demonstrated in humans, but there is no evidence that they cannot occur in humans.

The mean dietary intake of vanadium is about of 10-20 µg/person/day or 0.2-0.3 µg/kg body weight/day. This daily intake is at least three orders of magnitude below the lowest doses reported to cause adverse effects in rats (800 µg vanadium/kg body weight/day) and in humans (about 200 µg vanadium/kg body weight/day).

The available data, however, are inadequate to define the highest level of oral intake that can be regarded as tolerable. In the case of supplements taken by athletes and body builders, with daily doses up to 18 mg vanadium/person/day, equivalent to about 300 µg vanadium/kg body weight/day, the intake is similar to the doses reported to cause gastrointestinal effects in humans and kidney lesions in rats. Therefore, a risk of adverse effects could be expected to result from the prolonged ingestion of such supplements.

**REFERENCES**


PANEL MEMBERS


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