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# Provisional Peer-Reviewed Toxicity Values for

# Vanadium and Its Soluble Inorganic Compounds Other Than Vanadium Pentoxide

(CASRN 7440-62-2 and Others)

Derivation of Subchronic and Chronic Oral RfDs

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## Commonly Used Abbreviations

| | |
|---|---|
| BMD | Benchmark Dose |
| IRIS | Integrated Risk Information System |
| IUR | inhalation unit risk |
| LOAEL | lowest-observed-adverse-effect level |
| $LOAEL_{ADJ}$ | LOAEL adjusted to continuous exposure duration |
| $LOAEL_{HEC}$ | LOAEL adjusted for dosimetric differences across species to a human |
| NOAEL | no-observed-adverse-effect level |
| $NOAEL_{ADJ}$ | NOAEL adjusted to continuous exposure duration |
| $NOAEL_{HEC}$ | NOAEL adjusted for dosimetric differences across species to a human |
| NOEL | no-observed-effect level |
| OSF | oral slope factor |
| p-IUR | provisional inhalation unit risk |
| p-OSF | provisional oral slope factor |
| p-RfC | provisional inhalation reference concentration |
| p-RfD | provisional oral reference dose |
| RfC | inhalation reference concentration |
| RfD | oral reference dose |
| UF | uncertainty factor |
| $UF_A$ | animal to human uncertainty factor |
| $UF_C$ | composite uncertainty factor |
| $UF_D$ | incomplete to complete database uncertainty factor |
| $UF_H$ | interhuman uncertainty factor |
| $UF_L$ | LOAEL to NOAEL uncertainty factor |
| $UF_S$ | subchronic to chronic uncertainty factor |

# PROVISIONAL PEER-REVIEWED TOXICITY VALUES FOR VANADIUM AND ITS SOLUBLE INORGANIC COMPOUNDS OTHER THAN VANADIUM PENTOXIDE (CASRN 7440-62-2 and others)

## Background

On December 5, 2003, the U.S. Environmental Protection Agency's (U.S. EPA) Office of Superfund Remediation and Technology Innovation (OSRTI) revised its hierarchy of human health toxicity values for Superfund risk assessments, establishing the following three tiers as the new hierarchy:

1) U.S. EPA's Integrated Risk Information System (IRIS).
2) Provisional Peer-Reviewed Toxicity Values (PPRTVs) used in U.S. EPA's Superfund Program.
3) Other (peer-reviewed) toxicity values, including
   - Minimal Risk Levels produced by the Agency for Toxic Substances and Disease Registry (ATSDR),
   - California Environmental Protection Agency (CalEPA) values, and
   - EPA Health Effects Assessment Summary Table (HEAST) values.

A PPRTV is defined as a toxicity value derived for use in the Superfund Program when such a value is not available in U.S. EPA's IRIS. PPRTVs are developed according to a Standard Operating Procedure (SOP) and are derived after a review of the relevant scientific literature using the same methods, sources of data, and Agency guidance for value derivation generally used by the U.S. EPA IRIS Program. All provisional toxicity values receive internal review by two U.S. EPA scientists and external peer review by three independently selected scientific experts. PPRTVs differ from IRIS values in that PPRTVs do not receive the multiprogram consensus review provided for IRIS values. This is because IRIS values are generally intended to be used in all U.S. EPA programs, while PPRTVs are developed specifically for the Superfund Program.

Because new information becomes available and scientific methods improve over time, PPRTVs are reviewed on a 5-year basis and updated into the active database. Once an IRIS value for a specific chemical becomes available for Agency review, the analogous PPRTV for that same chemical is retired. It should also be noted that some PPRTV documents conclude that a PPRTV cannot be derived based on inadequate data.

## Disclaimers

Users of this document should first check to see if any IRIS values exist for the chemical of concern before proceeding to use a PPRTV. If no IRIS value is available, staff in the regional Superfund and Resource Conservation and Recovery Act (RCRA) program offices are advised to carefully review the information provided in this document to ensure that the PPRTVs used are appropriate for the types of exposures and circumstances at the Superfund site or RCRA facility in question. PPRTVs are periodically updated; therefore, users should ensure that the values contained in the PPRTV are current at the time of use.

It is important to remember that a provisional value alone tells very little about the adverse effects of a chemical or the quality of evidence on which the value is based. Therefore, users are strongly encouraged to read the entire PPRTV document and understand the strengths and limitations of the derived provisional values. PPRTVs are developed by the U.S. EPA Office of Research and Development's National Center for Environmental Assessment, Superfund Health Risk Technical Support Center for OSRTI. Other U.S. EPA programs or external parties who may choose of their own initiative to use these PPRTVs are advised that Superfund resources will not generally be used to respond to challenges of PPRTVs used in a context outside of the Superfund Program.

**Questions Regarding PPRTVs**

Questions regarding the contents of the PPRTVs and their appropriate use (e.g., on chemicals not covered, or whether chemicals have pending IRIS toxicity values) may be directed to the U.S. EPA Office of Research and Development's National Center for Environmental Assessment, Superfund Health Risk Technical Support Center (513-569-7300), or OSRTI.

## INTRODUCTION

The U.S. Environmental Protection Agency's (U.S. EPA) Integrated Risk Information System (IRIS) (U.S. EPA, 2008) contains a file for vanadium pentoxide describing a chronic RfD and containing a message about assessing its carcinogenicity—but no chronic RfC. IRIS currently contains no files for elemental vanadium or other vanadium compounds. The Drinking Water Standards and Health Advisories list (U.S. EPA, 2006) does not include an RfD for any vanadium compound. The Health Effects Assessment Summary Table (HEAST; U.S. EPA, 1997) lists subchronic and chronic oral RfDs of $7 \times 10^{-3}$ mg/kg-day for vanadium and $2 \times 10^{2}$ mg/kg-day for vanadium sulfate. RfDs for both vanadium and vanadium sulfate were based on a chronic study in which rats were exposed to 5 mg/L vanadium as vanadyl sulfate for their lifetimes (Schroeder et al., 1970), as derived in U.S. EPA (1987). A total UF of 100 was used to derive the RfDs. The Agency for Toxic Substance and Disease Registry (ATSDR, 1992) derived an intermediate-duration oral minimal risk level (MRL) for vanadium of $3 \times 10^{-3}$ mg/kg-day based on a NOAEL of 0.3 mg/kg-day in a 3-month drinking water study in rats by Domingo et al. (1985); renal and respiratory effects (renal hemorrhagic foci and pulmonary vascular infiltration) were seen at higher doses (0.6 mg/kg-day). A total UF of 100, reflecting UFs of 10 each for interspecies extrapolation and intraspecies variability, was applied to the NOAEL. ATSDR (1992) does not derive a chronic oral MRL.

Neither IRIS (U.S. EPA, 2008) nor the HEAST (U.S. EPA, 1997) reports an RfC for vanadium. ATSDR (1992) derived an acute-duration inhalation MRL of 0.0002 mg/m$^3$ for vanadium based on a study of human exposure to vanadium pentoxide, but it does not provide inhalation MRLs for other vanadium compounds. The American Conference of Governmental Industrial Hygienists (ACGIH, 2007) lists a time weighted average-threshold limit value (TWA-TLV) of 0.05 mg $V_2O_5$/m$^3$ for vanadium pentoxide dust or fume (respirable fraction), with a "notice of intended change" to 0.02 mg V/m$^3$ (inhalable fraction) based on upper and lower respiratory tract irritation. The National Institute for Occupational Safety and Health (NIOSH, 2008) lists a recommended exposure limit (REL) of 0.05 mg V/m$^3$ for vanadium

pentoxide dust or fume as a 15-minute ceiling value. NIOSH includes a note that this REL applies to all vanadium compounds except vanadium metal and vanadium carbide—for which a REL of 1 mg/$m^3$ TWA and 3 mg/$m^3$ short-term exposure limit (STEL) applies (by analogy to ferrovanadium dust). The Occupational Safety and Health Administration (OSHA, 2008) permissible exposure limit (PEL) applicable to vanadium pentoxide is a ceiling of 0.1 mg $V_2O_5$/$m^3$ for fume and 0.5 mg $V_2O_5$/$m^3$ for dust.

An assessment of the carcinogenicity of vanadium is not available on IRIS (U.S. EPA, 2008), in the HEAST (U.S. EPA, 1997), or in the Drinking Water Standards and Health Advisories list (U.S. EPA, 2006). The Chemical Assessments and Related Activities (CARA) list (U.S. EPA, 1991, 1994) includes a Health Effects Assessment (HEA) for vanadium and compounds (U.S. EPA, 1987) that assigned vanadium to cancer weight-of-evidence Group D (Not Classifiable as to Human Carcinogenicity) based on inconclusive animal data (under U.S. EPA 1986 Guidelines for Carcinogen Risk Assessment). Vanadium has not been evaluated under the U.S. EPA (2005) Guidelines for Cancer Risk Assessment. Vanadium is not included in the 11th Report on Carcinogens available from the National Toxicology Program (NTP, 2005). The International Agency for Research on Cancer (IARC, 2008) has not evaluated vanadium for potential carcinogenicity. For vanadium pentoxide, ACGIH (2007) has posted notice of intended change in cancer notation from A4 (Not Classifiable) to A3 (Confirmed Animal Carcinogen).

To identify toxicological information pertinent to the derivation of provisional toxicity values, literature searches were conducted from 1960s through December 2007 using the following databases: MEDLINE, TOXLINE, BIOSIS, TSCATS1/2, CCRIS, DART/ETIC, GENETOX, HSDB, RTECS, and Current Contents (prior 6 months). Vanadium pentoxide (CASRN 1314-62-1) was excluded from the search because it has both an IRIS record and a separate PPRTV document. In addition to searching for vanadium and its subheadings in these databases, the following vanadium compounds were specifically included as search terms: vanadyl sulfate (CASRN 27774-13-6), sodium metavanadate (CASRN 13718-26-8), sodium orthovanadate (CASRN 13721-39-6), ammonium vanadate (CASRN 7803-55-6), vanadium sulfate (CASRN 16785-81-2), sodium hexavanadate (CASRN 12436-28-1), sodium tetravanadate (CASRN 1258-74-1), vanadious (4+) acid, disodium salt (CASRN 64082-34-4), vanadium dichloride (CASRN 10580-52-6), vanadium trioxide (CASRN 1314-34-7), and vanadium tetrachloride (CASRN 7632-51-1). Review documents by U.S. EPA (1987), ATSDR (1992), the World Health Organization (WHO, 1988, 2001), and Rydzynski (2001) were also consulted for relevant information. An updated literature search on PubMed was performed on August 17, 2009.

## REVIEW OF PERTINENT DATA

### Vanadium Compounds Assessed

As noted above, vanadium pentoxide is the subject of both an IRIS record and a separate PPRTV document, which should be used in the toxicity assessment of this particular vanadium compound.

Although vanadium has six oxidation states (-1, 0, +2, +3, +4, and +5), the most stable oxidation state is +4 (Rydzynski, 2001). In the environment, vanadium is bound to a variety of elements including oxygen, sodium, sulfur, and chloride; in commerce, vanadium is often used in an iron alloy (ferrovanadium) (Rydzynski, 2001). The literature searches identified toxicity data for the following inorganic compounds: vanadyl sulfate (+4), sodium metavanadate (+5), sodium orthovanadate (+5), and ammonium metavanadate (+5). Table 1 shows the CASRNs, molecular formulas, molecular weights, and vanadium mass fractions for these compounds. These compounds all exhibit some solubility in water (Rydzynski, 2001; ATSDR, 1992) and, thus, can be considered representative of soluble tetravalent and pentavalent vanadium compounds.

**Table 1. Soluble Inorganic Vanadium Compounds Considered in this PPRTV**

| Compound | Chemical Formula | Vanadium Valence | Molecular Weight (g/mol) | Vanadium Mass Fraction[a] |
|---|---|---|---|---|
| Vanadium | V | various | 50.94 | 1.0 |
| Vanadyl sulfate trihydrate | $VOSO_4 \cdot (H_2O)_3$ | +4 | 217.06 | 0.235 |
| Vanadyl sulfate pentahydrate | $VOSO_4 \cdot (H_2O)_5$ | +4 | 253.10 | 0.201 |
| Ammonium metavanadate or ammonium vanadate | $NH_4VO_3$ | +5 | 116.99 | 0.435 |
| Sodium metavanadate or sodium vanadate | $NaVO_3$ | +5 | 121.93 | 0.418 |
| Sodium orthovanadate or sodium vanadium oxide | $Na_3VO_4$ | +5 | 183.91 | 0.277 |

[a]Molecular weight of vanadium divided by molecular weight of compound.

In recent years, organic vanadium compounds have been synthesized in an effort to enhance the lipophilicity and biological uptake of vanadium for use in treating diabetes and/or cancer. Toxicity data for three organic vanadium compounds were located: bis(maltolato)oxyvanadium(IV) (BMOV), bis(ethylmaltolato)oxyvanadium(IV) (BEOV), and vanadyl acetyl acetonate. Because these compounds have been developed as pharmaceutical agents and are believed to have different absorption and/or toxicokinetic properties than soluble inorganic vanadium salts, they are not considered in this review.

There are three early studies of human exposure to vanadium (Curran et al., 1959; Dimond et al., 1963; and Sommerville and Davies, 1962) that employed compounds reported as "ammonium vanadyl tartrate" and "diammonium oxy-tartrato vanadate" or "diammonium vanado-tartrate." Information provided on the chemical form in the studies is limited to the names and the valence state (+4) for the latter compound (reported by Sommerville and Davies, 1962). Reliable chemical structures and valence states for these compounds have not been located; however, the tartrate component is an organic moiety. Given that the compounds administered in these studies are unknown, it is difficult to estimate vanadium doses from the reported doses of the compounds. Further, because the compounds used in these studies were likely organic in nature and may have exhibited different bioavailability than inorganic vanadium salts, these studies have been excluded from consideration in this review.

Oral exposures to either vanadyl or vanadate result in internal exposures to a mixture of vanadyl and vanadate complexes as a result of reduction/oxidation (redox) reactions that occur in the gastrointestinal tract as well as in the blood and tissues (Rydzynski, 2001; Etcheverry and Cortizo, 1998). Available information suggests that conditions in extracellular fluid favor the formation of vanadate, while intracellular (cytosolic) conditions favor the vanadyl redox state (Rydzynski, 2001). As a result of these physiological interconversions, there is no firm toxicological basis for distinguishing dose-response relationships for these two forms given the currently available data: while toxicology studies can be categorized based on whether humans or animals were exposed to vanadyl or vanadate compounds, target organs and tissues are likely exposed to a mix of these ions. For the purpose of this review, exposure to either the vanadyl or vanadate form is treated as biologically equivalent. Therefore, exposure estimates in all of the toxicity studies have been converted to equivalent vanadium doses for the purpose of dose-response assessment.

In summary, this PPRTV document applies to soluble inorganic vanadyl (+4) and vanadate (+5) compounds other than vanadium pentoxide, which is the subject of an IRIS review and separate PPRTV document. Data are not available to assess the toxicity of insoluble compounds or compounds in which vanadium exists in higher or lower valence states. Organic vanadium compounds are expected to exhibit different toxicokinetic properties than inorganic compounds and should be assessed independently if necessary. Finally, vanadyl and vanadate exposures are considered biologically equivalent (on the basis of equivalent vanadium dose) for the purpose of this review.

**Human Studies**

The possibility that vanadium may be an essential element for humans remains an unanswered question. Etcheverry and Cortizo (1998) reported that deficiencies in vanadium intake could be associated with alterations in bone structure and development, changes in plasma cholesterol, and changes in reproductive performance. However, WHO (2001) considered the issue unresolved and noted that, if vanadium is essential, required levels are very low (in the range of nanograms per day).

***Oral Exposure***

Fawcett et al. (1997) administered tablets of vanadyl sulfate trihydrate at a dose of 0.5 mg/kg-day (0.1 mg V/kg-day) for 12 weeks to weight trainers. The treatment and control groups each included 15 males and 5 females. The control group received a daily placebo. Subjects in the control and treatment groups were matched with respect to gender, age, height, weight, and weight-training program (e.g., intensity, schedule). Of those starting the study, 11 males and 4 females in the treatment group and 12 males and 4 females in the control group completed the study. There were two males that withdrew from the study because of self-reported side effects (tiredness and/or aggressiveness while weight training); these two subjects were unremarkable with respect to endpoints assessed in this study. There were four subjects that withdrew because of training-related injuries and three subjects withdrew for other reasons not related to health. Blood pressure was measured and blood samples collected periodically during the exposure period for evaluation of hematology (differential cell counts and blood viscosity tests) and serum chemistry (plasma alanine aminotransferase [ALT] and alkaline phosphatase [ALP], albumin, bilirubin, cholesterol, creatinine, high-density lipoprotein, total protein, triglyceride, and urea). No differences were observed between the treatment and control

groups for the following endpoints: body weight, systolic and diastolic blood pressure, hematology or serum chemistry (all data shown). Without corroborating information, the toxicological relevance of the self-reported symptoms of (tiredness and aggressiveness) is uncertain. The administered dose (0.1 mg V/kg-day) is considered a freestanding NOAEL with respect to the endpoints assessed in the study.

In a study designed to evaluate the safety of vanadyl sulfate as a diabetes treatment, Boden et al. (1996) administered 50 mg capsules of vanadyl sulfate twice daily (100 mg/day) for 4–8 weeks to four men and four women with noninsulin-dependent diabetes mellitus. The specific form of vanadyl sulfate was not reported; assuming vanadyl trihydrate, the corresponding dose of vanadium would be 0.34 mg V/kg-day in men and 0.39 mg V/kg-day in females of average body weight (70 kg and 60 kg, respectively). Of the eight patients, four men and two women were treated with placebo for 4 weeks after the end of vanadium treatment to provide reference data. Patients self-monitored their glucose using a glucometer and were examined weekly at a hospital, where blood was drawn for complete blood count, serum chemistry (glucose, insulin, blood urea nitrogen [BUN], fatty acids, vanadium content), liver and kidney function tests, and urinalysis (urinary nitrogen). Self-reported symptoms were recorded at that time. Glycemic control was assessed during and after the exposure period. Of the eight patients, four reported diarrhea with abdominal cramps and/or flatulence, one reported flatulence alone, and one reported slight nausea. Diarrhea lasted for 11 days in one patient but had abated after the first week in the others. Vanadyl sulfate treatment resulted in statistically significant ($p < 0.05$) decreases in fasting glucose concentration and hepatic glucose output during hyperinsulinemia. There were no effects on total body glucose uptake, glycogen synthesis, glycolysis, carbohydrate oxidation, or lipolysis during the euglycemic-hyperinsulenemic clamps. The study authors reported that weekly blood counts, urinalysis, and liver function tests were not affected by treatment (data not shown). A LOAEL of 0.34–0.39 mg V/kg-day is identified from these data based on gastrointestinal symptoms; no NOAEL is identified.

Goldfine et al. (2000) also investigated the use of vanadyl sulfate to treat noninsulin-dependent diabetes mellitus. Participants in the study were 16 diabetes patients (11 males and 5 females) between the ages of 18 and 65 who did not have active cardiovascular, pulmonary, renal, or hepatic disease. After 12 weeks of monitoring to derive baseline information, the subjects were given vanadyl sulfate by tablet at doses of 75, 150, or 300 mg/day for 6 weeks. Based on individual body weights reported in the study and assuming that the trihydrate form of vanadyl sulfate was used, doses are 0.12–0.23, 0.28–0.45, and 0.43–1.14 mg V/kg-day in the 75, 150, and 300 mg/day groups. Blood glucose was monitored throughout the study (other tests of glycemic control were also administered) and the patients were given physical examinations, blood tests (electrolytes, BUN, creatinine, complete blood count), liver and thyroid function tests and urine tests biweekly. To assess lipid peroxidation, levels of thiobarbituric acid-reactive substances in the serum were measured. Ambulatory blood pressure was measured 4 weeks after exposure was terminated. The patients were monitored for 2 additional weeks. Although patients exposed to the lowest dose range did not experience any gastrointestinal symptoms, several patients at the next dose reported complaints and all patients at the high dose reported cramping, abdominal discomfort, and/or diarrhea. The study authors reported that no other signs of toxicity were observed and blood tests and urinalysis did not indicate toxicity (data not shown). Systolic, diastolic, and mean arterial pressure were not changed by exposure nor was heart rate. Insulin sensitivity and glycemic control were not

dramatically improved in this study. A LOAEL of 0.28–0.45 mg V/kg-day is identified from this study based on gastrointestinal symptoms; the NOAEL is in the range of 0.12–0.23 mg V/kg-day.

Cusi et al. (2001) gave a group of 11 patients (four men and seven women, mean age 59 years) with type 2 diabetes doses of 150 mg vanadyl sulfate each day for 6 weeks after a 2-week period of exposure to increasing doses up to 150 mg/day (exposure regimen during run-up not reported). Assuming that vanadyl sulfate was in the trihydrate form, the estimated doses (during the 6-week period) are 0.5 mg V/kg-day in males and 0.6 mg V/kg-day in females (based on default body weights of 70 and 60 kg, respectively). Measures of glycemic control were assessed throughout the exposure period. Effects reported in the subjects included diarrhea (4/11) and abdominal discomfort (2/11). According to the authors, blood chemistry, complete blood count, and urinalysis were not affected by treatment (data not shown), nor was bone mineral density (measured in three subjects) or body weight. Measures of 24-hour ambulatory blood pressure and mean heart rate were not affected by treatment (data shown). Glycemic control was significantly improved. This study suggests a LOAEL of 0.5–0.6 mg V/kg-day based on gastrointestinal symptoms; a NOAEL could not be identified.

***Inhalation Exposure***

The few studies examining human exposure to vanadium compounds (other than vanadium pentoxide) via inhalation (Woodin et al., 2000; Sorensen et al., 2005; Zhou et al., 2007) do not specify the form of vanadium exposure; in these studies, coexposure to other compounds could not be ruled out. Woodin et al. (2000) found increases in self-reported upper and lower airway respiratory symptoms in 18 boilermaker workers exposed to vanadium compared with 11 utility worker control subjects. The study authors correlated these symptoms with estimated vanadium doses to the lung and upper airway in all but the highest exposure quartile; the authors attributed the high-dose reversal to a possible healthy worker effect. Sorensen et al. (2005) observed a positive association between levels of 7-hydro-8-oxo-2'-deoxyguanosine (a measure of DNA damage) in lymphocytes of 49 students in Copenhagen and concentrations of both vanadium and chromium in PM2.5 samples. Concentrations of platinum, nickel, copper, and iron were not related to the measures of DNA damage. Based on the English abstract of a paper published in Chinese, 106 workers with exposure to vanadium were reported to exhibit more negative moods as well as poorer performance on neurobehavioral tests (Santa Ana dexterity, Benton visual retention and pursuit aiming) than unexposed workers (Zhou et al., 2007). The average concentration of vanadium in the air of the exposed workers ranged from 0.034 to 0.805 mg/m$^3$; however, the form of vanadium is not specified. No further information is presented in the abstract.

A case report documented symptoms of metal-fume fever in a worker exposed to a vanadium catalyst, vanadyl pyrophosphate (Vandenplas et al., 2002). After exhibiting symptoms in the work environment, the individual was assessed by a physician under controlled conditions of exposure to the vanadium catalyst. Forced vital capacity and forced expiratory volume were decreased and fever and peripheral blood neutrophilia were observed. Concentrations of vanadium to which the individual was exposed in the workplace or under the challenge conditions were not reported.

**Animal Studies**

Only a few of the available laboratory animal studies provide information on the levels of vanadium in the basal diet and none of the studies considered dietary input to total vanadium dose. Kanisawa and Schroeder (1967), along with Schroeder et al. (1970), reported the concentration of vanadium in their basal diet as 3.2 mg V/kg food. Elfant and Keen (1987) reported a concentration of 1 mg V/kg in a "purified" diet. Finally, Scibior et al. (2006) measured the concentration of vanadium in their standard chow to be 0.45 mg V/kg. For a dietary concentration of 1 mg V/kg, the vanadium dose to rats and mice would be in the range of 0.1 to 0.2 mg V/kg-day (assuming default values for subchronic exposure in female Sprague-Dawley and B6C3F1 mice; U.S. EPA, 1988). This estimate may not be representative of all commercial laboratory animal feeds used in the studies included in this review. Because exposure to vanadium in the basal diet was not taken into account in any of the studies, doses reported in this review may be underestimated to some degree. Further, low-level exposure to vanadium among controls increases the uncertainty in findings of effect at doses near the estimated control dose.

***Oral Exposure***

**Subchronic Studies**—Domingo et al. (1985) exposed male Sprague-Dawley rats to sodium metavanadate for 12 weeks. A control group consisted of 10 rats given free access to drinking water without added vanadate. There were three treatment groups that consisted of 10 rats/group exposed to drinking water to which 5, 10, or 50 mg/L sodium metavanadate (2, 4, or 21 mg V/L) had been added. Vanadium doses estimated for this review based on reported water consumption and body weight (of the high-concentration group only) were 0.3, 0.6, and 3.0 mg V/kg-day. Body weight was measured weekly, while food consumption, water intake, and urine volume were assessed daily. At sacrifice, blood was collected from five rats for serum chemistry determinations (AST, ALT, total protein, bilirubin, creatinine, urea, uric acid, glucose, and cholesterol). Selected organs (liver, kidneys, heart, spleen, and lung) from all animals were weighed. Microscopic examination of the heart, kidney, liver, lung, spleen, and stomach was performed on 3 rats/group. Body-weight gain was significantly ($p < 0.05$) increased (42%) over controls in the high-dose group (3.0 mg V/kg-day) during the first 2 weeks of exposure, but not thereafter; actual body weights are not reported. Food and water intake were not affected in the high-dose group. The authors indicated that body weight, food consumption, and water intake were not affected in other treatment groups (data not shown). Urine volume was greater than controls in the high-dose group during the first month (58% to 2-fold higher; $p < 0.05$), but not during the remainder of the study. Compared to the control values, plasma protein, urea, and uric acid concentrations were significantly higher (31%, 28%, and 2-fold, respectively; $p < 0.05$) in the 3.0 mg V/kg-day treatment group but not in other treatment groups. Organ weights were not affected by treatment (data shown). The histopathology findings are summarized qualitatively as mild changes in the kidney (hemorrhagic foci in the corticomedullary region), spleen (hypertrophy and hyperplasia), and lungs (perivascular mononuclear cell infiltration). The authors reported that these changes occurred in all treatment groups, but they are described as "more evident" in the 3.0 mg V/kg-day treatment group. Incidences of these effects are not reported. Given the authors' report of histopathology and clinical chemistry findings in the low-dose group, even though only three animals were examined, 3.0 mg V/kg-day is considered to be a LOAEL.

A number of studies examined the beneficial effects of vanadium exposure on diabetic rats[1]. Most of the studies examined few or no toxicological endpoints and used doses of 10 mg V/kg-day or greater. Those studies that did examine a few toxicological endpoints, included a nondiabetic treatment group, and exposed the animals for at least 28 days are summarized in Table 2. The studies shown in the table indicate that doses of 12 mg V/kg-day and higher result in body weight reductions of at least 10%, often accompanied by marked reductions in fluid intake. The reduced fluid intake may reflect an organoleptic effect of vanadium compounds administered in drinking water. Although body-weight reductions can be related to reduced fluid intake, studies that have observed reduced body weight or body weight gain with dietary or gavage administration of vanadium (e.g., Sanchez et al., 1991, 1998, 1999; Paternain et al., 1990; Elfant and Keen, 1987) suggest that this may be a toxic effect of the element rather than resulting from reduced fluid intake. Thus, the body-weight decrement of at least 10% observed at a dose of 12 mg V/kg-day (Cam et al., 1993) indicates that this dose is a LOAEL.

Most of the studies that examined only effects in diabetic animals are not summarized here—primarily because the studies demonstrated improvements in diabetes-related effects, rather than any toxic effects of vanadium exposure. However, one study examining effects of vanadium exposure in diabetic animals bears special consideration because it identifies enhanced toxicity in the vanadium-treated animals when compared with both nondiabetic and diabetic controls. Domingo et al. (1991) exposed groups of 10 streptozotocin-induced diabetic male Sprague-Dawley rats to three different forms of vanadium: sodium metavanadate (150 mg/L), sodium orthovanadate (230 mg/L), and vanadyl sulfate pentahydrate (310 mg/L) in the drinking water for 28 days. Based on body weights and fluid intake measurements, the authors estimated vanadium doses of 22.7, 15.6, and 6.1 mg V/kg-day for vanadyl sulfate, sodium orthovanadate, and sodium metavanadate, respectively. Sodium chloride (80 mM) was added to the water to inhibit gastrointestinal effects of vanadium. Both diabetic and nondiabetic control groups (10/group) were included for comparison. Mortality, body weight, food and fluid intake and blood glucose were monitored throughout the exposure period. After exposure ended, blood samples were collected for analysis of hematocrit, glucose, urea, creatinine, AST and ALT.

[1]A chronic study that included a broader range of toxicological endpoints is discussed under Chronic Studies (published in three papers: Dai et al., 1994a,b; Dai and McNeill, 1994).

**Table 2. Studies of Effects in Streptozotocin-induced Diabetic and Nondiabetic Rats Exposed to Vanadium**

| Reference | Number and Sex of Rats | Vanadium Compound Administered | Duration | Dose[a] Vanadium (mg V/kg-day) | Significant Adverse Effects |
|---|---|---|---|---|---|
| Cam et al., 1993 | 11–16 males per group | Vanadyl sulfate in drinking water | 5 months | 12[b] (nondiabetic)<br><br>18–20 (diabetic) | Decreased body weight (14%), fluid intake (40%) and food intake (up to 10%) in treated nondiabetic rats relative to control nondiabetic rats. |
| Thompson et al., 1993 | 10–16 males per group | Vanadyl sulfate in drinking water | Up to 12 weeks | 36 (nondiabetic)<br><br>102 (diabetic) | Decreased body weight (30%), decreased fluid intake (54%) in treated nondiabetic rats relative to control nondiabetic rats.<br><br>Decreased body weight (11%) in treated diabetic rats relative to diabetic controls. |
| Yao et al., 1997 | 5–6 males per group | Vanadyl sulfate in drinking water | 7 weeks | 13 (nondiabetic)<br>24 or 29 (diabetic) | Decreased body-weight gain (14%), decreased fluid intake (34%) in treated nondiabetic rats relative to control nondiabetic rats. |
| Tunali and Yanardag, 2006; Akgün-Dar et al., 2007 | 5–13 males per group | Vanadyl sulfate via daily gavage | 60 days | 24 [b] | Lower body weight (11%), increased serum glucose and phospholipids, increased aortic lipid peroxidation, decreased stomach and aortic glutathione, decreased aortic diameter and aortic *tunica intima* thickness in treated nondiabetic rats relative to control nondiabetic rats.<br><br>Decreased tunica muscularis thickness (in aorta) in treated diabetic rats relative to both diabetic and nondiabetic controls. |

[a]Doses estimated by authors except where indicated.
[b]Doses estimated for this review based on default body weight and fluid intake (U.S. EPA, 1988). Cam et al. (1993) reported using the trihydrate form of vanadyl sulfate. Tunali and Yanardag (2006) and Akgün-Dar et al. (2007) did not report the form administered; it was assumed to be the trihydrate for the purpose of dose estimation.

In each of the groups exposed to sodium metavanadate and vanadyl sulfate, 3/10 rats died, while 2/10 diabetic rats treated with sodium orthovanadate died (Domingo et al., 1991). By comparison, no control nondiabetic rats died and 1/10 control diabetic rats died. Food and fluid intake in the groups exposed to sodium metavanadate and vanadyl sulfate were increased relative to the nondiabetic controls, but were lower than those of diabetic controls. Relative weight gain was significantly lower in diabetic controls than in nondiabetic controls (8.2% vs. 24% over study duration). However, the vanadium-treated rats lost weight over the exposure period (3.2%, 4.8%, and 7.2% losses in the groups exposed to vanadyl sulfate, sodium orthovanadate and sodium metavanadate, respectively; $p < 0.05$ relative to both diabetic and nondiabetic control groups). Thus, in this study, vanadium treatment enhanced the adverse effect of diabetes on body-weight gain. In addition, vanadium treatment (all forms) resulted in significantly ($p < 0.05$) higher serum urea concentrations relative to both diabetic and nondiabetic control groups. Treatment with vanadyl sulfate also increased the serum creatinine level relative to both control groups. This study suggests a LOAEL of 6.1 mg V/kg-day for body-weight losses in diabetic rats. Although mortality was observed in diabetic rats treated with vanadium (3/10 in the group exposed to 6.1 mg V/kg-day), it is not clear whether the deaths were attributable to the disease or the treatment; one death also occurred in the untreated diabetic group. A NOAEL cannot be determined.

A follow-up study assessing whether Tiron (sodium 4,5-dihydroxybenzene-1,3-disulfonate, a chelating agent) would mitigate the toxicity of vanadium in diabetic rats, provided some confirmation of these findings (Domingo et al., 1992). A group of 10 streptozotocin-induced diabetic rats was given sodium metavanadate at a concentration of 200 mg/L in the drinking water for 5 weeks, with or without Tiron; nondiabetic and diabetic control groups were included. The same parameters as in the earlier study were monitored. The authors estimated a vanadium dose of 23.2 mg V/kg-day in the group without Tiron exposure. As with the previous study, exposure to sodium metavanadate in diabetic rats resulted in body-weight loss (5%) while weight gains of 28% and 8.1% were seen in untreated nondiabetic and diabetic groups (respectively). The decrement was significantly different from both untreated groups at $p < 0.01$. In addition, serum urea was increased relative to both control groups (10.9 mmol/L vs. 6.3 and 8.2 mmol/L in nondiabetic and diabetic controls), while serum creatinine was not. Tiron administration did not ameliorate the effect of vanadium on body-weight gain, but did reduce serum urea concentrations. A LOAEL of 23.2 mg V/kg-day is identified from this study based on body-weight losses in treated diabetic rats.

A series of papers reported hematological effects of exposure to ammonium metavanadate (Gorski and Zaporowska, 1982; Zaporowska and Wasilewski 1989, 1990, 1991, 1992a,b; Zaporowska and Scibior, 1999). With few exceptions, the study protocols are largely the same. In most studies, 2-month old Wistar rats (either male or male and female) were exposed to ammonium metavanadate in drinking water provided ad libitum, typically for 4 weeks. Some studies examined the interaction of vanadium with another toxicant (ethanol or zinc), but some also provided data on exposure to the vanadium compound alone; in all cases, a single concentration of ammonium vanadate was used. Vanadium concentrations in the drinking water ranged from 50–300 mg/L, resulting in doses ranging from 7–29 mg V/kg-day in the various studies. Body weight, fluid intake, and food consumption were monitored during the exposure period. At sacrifice (at the end of exposure), the following hematological parameters were assessed: erythrocyte, reticulocyte, and total and differential leukocyte counts, hematocrit

[Hct], hemoglobin [Hgb], leukocyte composition in bone marrow and frequency of polychromatophilic erythrocytes in peripheral blood and bone marrow. A few other evaluations were conducted in individual studies. Zaporowska and Wasilewski (1992a) also examined the osmotic resistance of erythrocytes and the activities of glucose-6-phosphate dehydrogenase and lactate dehydrogenase in erythrocytes. Zaporowska and Scibior (1999) assessed the phagocytic activity of neutrophils and the activities of myeloperoxidase and alkaline phosphatase in the neutrophils. Based on the abstracts of papers published in Polish, Gorski and Zaporowska (1982) also examined the histopathology of liver and kidneys, and Zaporowska (1987) evaluated kidney histopathology.

Table 3 provides an overview of the study designs and results. Mortality occurred at doses of 13 mg/kg-day and higher in this series of papers. In general, the studies consistently demonstrated significantly depressed body-weight gain, food intake and fluid intake, decreased erythrocyte counts and hemoglobin concentrations and increased reticulocytes and polychromatophilic erythrocytes in exposed animals. Sporadic effects were observed on leukocytes or leukocyte composition and no effects on erythrocyte enzyme activities were reported. Abstracts from two studies (Gorski and Zaporowska, 1982; Zaporowska, 1987, both published in Polish) reported renal histopathology (parenchymatous degeneration with vacuolar degeneration and tubular casts) at doses of 9–29 mg V/kg-day, but the incidences of the renal effects are not given. Gorski and Zaporowska (1982) also reported parenchymatous degeneration of the liver. Neither study has been translated for this review. Taken together, these studies identify a FEL of 13 mg/kg-day based on mortality (Zaporowska and Wasilewski, 1992a).

In contrast to the other publications in this series, Zaporowska et al. (1993) used more than one concentration of ammonium metavanadate and also used lower doses that were not associated with mortality. Groups of 15–16 Wistar rats of each sex were given concentrations of 0, 10, or 50 mg V/L as ammonium metavanadate in drinking water for 4 weeks. Fluid intake was measured daily and body weight recorded weekly; based on these measures, the authors estimated doses of 1.2 or 5 mg V/kg-day in males and 1.5 or 7 mg V/kg-day in females. Food intake was also monitored daily during exposure. Blood was drawn (presumably at sacrifice at the end of exposure, although this is not specified) for hematology (erythrocyte count [RBC], leukocyte count [WBC], Hgb, Hct, leukocyte composition, polychromatophilic erythrocytes, and reticulocytes in peripheral blood) and erythrocyte enzyme activity determinations (catalase,glucose-6-phosphate dehydrogenase, lactate dehydrogenase and δ-aminolevulinic acid dehydratase). Malondialdehyde (MDA), glutathione (GSH) and L-ascorbic acid content of erythrocytes were also measured. At these doses, there was no mortality. Although body-weight gain was lower in exposed groups than in controls (as much as 9% lower at the high dose), the differences are not statistically significant. Food intake was not affected by treatment and fluid intake was decreased only in high-dose males (14% lower than controls, $p < 0.001$). Statistically significant—but modest—changes in erythrocyte count, hemoglobin concentration, and hematocrit are shown in Table 4. In addition to these changes, the percentage of reticulocytes was significantly increased at the high dose in both sexes (data presented graphically, $p < 0.05$). There was no effect on leukocyte composition or enzyme activity in erythrocytes. While MDA tended to be increased and GSH decreased in exposed animals, the changes are not statistically significant. However, the concentration of L-ascorbic acid in the plasma of male rats was reduced at both doses (24% and 37% below controls; $p < 0.05$). The high dose in this study is

**Table 3. Studies of Hematologic Effects in Rats Exposed to Ammonium Metavanadate in Drinking Water**

| Reference | Number and Sex of Rats | Conc. Vanadium (mg V/L) | Duration | Dose[a] (mg V/kg-day) | Significant Effects |
|---|---|---|---|---|---|
| Gorski and Zaporowska, 1982 Published in Polish. | 5–13 males per group | 0, 200 | 1, 2, or 3 months | 29[b] | Based on English abstract and tables only: decreased body-weight gain, decreased erythrocyte count, hemoglobin, and hematocrit; in "single cases," parenchymatous degeneration of liver and kidney, with vacuolar degeneration of kidney and tubular casts. |
| Zaporowska, 1987 Published in Polish. | 15 (sex not given) per group | 0, 50, 100, 200 | 4 weeks | 9, 12, 23[b] | Based on English abstract and tables only: decreased body-weight gain at high dose; "renal tubule cylinders" at mid- and high doses. |
| Zaporowska and Wasilewski, 1989 | 10–18 per sex per group | 0, 300 | 2, 4, or 8 weeks | 21–29 | Mortality[c] (6/16 and 2/14 males after 4 and 8 weeks; 2/13, 4/16, and 2/13 females after 2, 4, and 8 weeks), transient diarrhea in "some" rats, decreased body-weight gain, decreased food and water intake, decreased erythrocyte count and hemoglobin concentration, increased number polychromophilic erythroblasts. |
| Zaporowska and Wasilewski, 1990 | 10–21 per sex per group | 0, 300 | 4 weeks | 22–27 | Mortality[c] (6/21 males and 6/21 females), diarrhea, decreased body-weight gain, decreased food and water intake, decreased erythrocyte count, increased reticulocyte count, increased number polychromatophilic erythrocytes, decreased lymphocytes and plasma cells in bone marrow. |
| Zaporowska and Wasilewski, 1991 | 10–11 males per group | 0, 300 | 4 weeks | 20 | Decreased body-weight gain, fluid intake, food intake, erythrocyte count, and hemoglobin concentration. Increased reticulocytes and polychromatophilic erythrocytes in peripheral blood. |
| Zaporowska and Wasilewski, 1992a | 12–13 per sex per group | 0, 150 | 4 weeks | 13 | Mortality[c] (1/12 males); transient diarrhea (2 rats); decreased body-weight gain, food intake, fluid intake, erythrocytes, hemoglobin count; increased leukocyte count; decreased osmotic resistance of erythrocytes; increased reticulocytes, polychromatophilic erythrocytes, neutrophils and lymphocytes in peripheral blood. |
| Zaporowska and Wasilewski, 1992b | 12–14 per sex per group | 0, 300 | 4 weeks | 20–26 | Mortality[c] (2/13 males and 3/14 females); frequent diarrhea; decreased body-weight gain, food intake and fluid intake; decreased erythrocyte count and hemoglobin concentration; increased reticulocytes and polychromatophilic erythrocytes in peripheral blood and/or bone marrow. |
| Zaporowska and Scibior, 1999 | 10–13 males per group | 0, 150 | 4 weeks | 12 | Decreased body-weight gain, food intake and fluid intake; decreased phagocytic activity of neutrophils. |

[a]Doses estimated by authors based on fluid intake and body weight except where indicated
[b]Doses estimated for this review based on default body weight and fluid intake (U.S. EPA, 1988)
[c]No control animals died in any study

considered a LOAEL (5 mg V/kg-day in males and 7 mg V/kg-day in females) based on a 9% decrease in body-weight gain (albeit not significantly decreased from controls, and possibly related to reduced fluid intake) and modest hematology changes. The low dose (1.2 mg V/kg-day in males and 1.5 mg V/kg-day in females) is considered a NOAEL; the statistically significant hematology changes observed at this dose are not considered toxicologically significant.

**Table 4. Hematologic Effects in Rats Exposed to Ammonium Metavanadate for 4 Weeks[a]**

| Parameter | Control | 10 mg V/L | 50 mg V/L |
|---|---|---|---|
| ***Males*** | | **1.2 mg V/kg-day** | **5 mg V/kg-day** |
| Erythrocytes ($\times 10^{12}$/dm$^3$) | 8.32 ± 0.17 | 7.38 ± 0.20 [b] | 7.47 ± 0.27 [c] |
| Hemoglobin (mmol/L) | 9.37 ± 0.19 | 8.94 ± 0.28 | 8.65 ± 0.26 [c] |
| Hematocrit (%) | 0.48 ± 0.001 | 0.47 ± 0.004 [c] | 0.47 ± 0.003 [b] |
| ***Females*** | | **1.5 mg V/kg-day** | **7 mg V/kg-day** |
| Erythrocytes ($\times 10^{12}$/dm$^3$) | 8.24 ± 0.10 | 7.38 ± 0.14 [d] | 7.12 ± 0.17 [d] |
| Hemoglobin (mmol/L) | 9.41 ± 0.12 | 8.76 ± 0.30 | 8.72 ± 0.20 [c] |

[a]Zaporowska et al., 1993
[b]$p < 0.01$
[c]Significantly different from control, $p < 0.05$
[d]$p < 0.001$

In recent papers by the same group of investigators, sodium metavanadate was used as the test material in studies comparing the effects of vanadium alone or in combination with chromium or magnesium. Scibior (2005) administered sodium metavanadate in the drinking water to a group of 11 male Wistar rats at a concentration of 100 mg V/L; a group of 16 untreated rats served as controls. Food and fluid intake were measured daily and body weight recorded weekly during the 6-week exposure period. After exposure ended, blood was collected for hematology (RBC, Hct, Hgb, mean corpuscular volume [MCV], mean corpuscular hemoglobin [MCH], mean corpuscular hemoglobin concentration [MCHC], and WBC) and assessment of the total antioxidant status of erythrocytes. Based on measured body weight and fluid intake, the authors estimated the vanadium intake to be 8 mg V/kg-day. While total body-weight gain was reduced in the vanadium-exposed group (about 9% less than controls), the difference is not statistically significant. In treated rats, both food and fluid intake were reduced compared to controls (13% and 32% less than controls, respectively; $p < 0.05$). Modest—but statistically significant ($p < 0.05$)—effects observed with exposure include the following: increased erythrocyte count (in contrast to earlier studies that showed a decrease; 10% higher than controls) and decreased MCH (12% lower) and MCHC (4% lower). No other statistically significant effects were observed in the parameters evaluated. A LOAEL of 8 mg V/kg-day is identified for these data based on a 9% decrease in body-weight gain (albeit not significantly decreased from controls, and possibly related to reduced food and fluid intake) and hematology changes; no NOAEL can be determined.

Scibior et al. (2006) exposed male Wistar rats (12/group) to sodium metavanadate at a concentration of 0 or 125 mg V/L in the drinking water for 6 weeks. Based on fluid intake and body weight measurements, the authors estimated the average vanadium intake to be 11 mg V/kg-day in the exposed group. Evaluations were similar to those of previous studies and included body-weight gain, food and fluid intake, hematology (RBC, WBC, Hgb, Hct, MCV,

MCH, MCHC, and red-cell distribution width), leukocyte composition of peripheral blood smears, phagocytic activity of neutrophils in whole blood, erythrocyte concentrations of L-ascorbic acid and malondialdehyde, and the total antioxidant status of the plasma. In this study, significant ($p < 0.05$) effects of treatment included a 15% decline in body-weight gain, along with 6% and 30% decreases in food and fluid intakes (respectively). Erythrocyte count was decreased by 6%, while hemoglobin concentration was depressed by 10.6% compared to controls ($p < 0.05$). MCV and MCH were reduced by 4% and 6%, respectively ($p < 0.05$). Leukocyte count and leukocyte composition of peripheral blood were not affected by treatment. The plasma concentration of L-ascorbic acid was decreased (26%, $p < 0.05$), while malondialdehyde content of erythrocytes was increased (78%, $p < 0.05$). Based on data presented in tables, there are no statistically significant changes in Hct, MCHC, red-cell distribution width, or phagocytic activity of neutrophils with exposure. A LOAEL of 11 mg V/kg-day is identified for these data based on a 15% decrease in body-weight gain (possibly related to reduced food and fluid intake) and hematology changes; no NOAEL can be determined.

In contrast to the studies published by Zaporowska and collaborators, Dai et al. (1995) observed no effects on hematology parameters in groups of eight male Wistar rats exposed to ammonium metavanadate (140 mg/L) and vanadyl sulfate (260 mg/L) in the drinking water for 12 weeks. An additional group of eight rats received untreated water. Body weight, food intake, and fluid intake were measured before exposure and on Weeks 1, 2, 4, 8, and 12 of treatment. These data were used by the authors to estimate vanadium doses of 0.19 and 0.15 mmol V/kg-day for ammonium metavanadate and vanadyl sulfate, respectively; these values correspond to dose estimates of 9.7 and 7.6 mg V/kg-day, respectively. Blood samples were collected on the same schedule as body weight measurements for evaluation of Hct, Hgb, RBC, WBC, platelet count, differential leukocyte count, reticulocyte percentage and erythrocyte osmotic fragility tests. No other evaluations were performed. Vanadium in the drinking water led to significantly ($p < 0.05$) reduced fluid intake, regardless of the compound administered (data presented graphically). However, food intake and body weight were not affected by exposure and there was no statistically significant effect on any hematology parameter at any time (data shown graphically). This study identifies freestanding NOAELs of 9.7 and 7.6 mg V/kg-day (for ammonium metavanadate and vanadyl sulfate, respectively) for hematologic effects in male rats.

Adachi et al. (2000) exposed groups of seven female Wistar rats to sodium metavanadate in the diet for 10 weeks. Concentrations of 0, 50, or 100 ppm (0, 21, or 42 ppm V) were incorporated into the diet. Food intake and body weight were measured weekly; vanadium doses calculated for this review based on food intake (14 g/day) and body weight (0.260 kg) roughly estimated from graphical presentation of these data are 1.1 and 2.3 mg V/kg-day. After exposure was terminated, the animals were sacrificed and blood was collected for hematology (RBC, WBC, platelet count, reticulocyte count, Hgb, cell number, immunoglobulin levels) and serum chemistry (AST, ALT, cholinesterase [ChE], ALP). Thiobarbituric acid levels (a measure of lipid peroxidation) were determined in the liver, kidney, and spleen, while vanadium and metallothionein (a metal-binding protein) contents of the liver and kidney were also assayed. Histopathology was not assessed. Statistically significant decreases ($p < 0.05$) in body weight were observed at both doses after 3 weeks of exposure; however, the body-weight decrements at termination were less than 10% (approximately 5% and 7% lower than controls) at both doses.

Food intake was not affected by exposure. Hematology and serum chemistry data were presented graphically with statistical analysis of differences from control. ALT, ChE, and ALP levels were significantly ($p < 0.05$) decreased at both doses. Although AST levels were reduced by more than half at both doses, the difference was significantly different from control only at the high dose. A decrease in serum liver enzymes is not considered to be of toxicological significance. Hemoglobin content and hematocrit were slightly reduced at both doses ($p < 0.05$), but erythrocyte count was not affected. Based on visual inspection of the graphs, the Hgb decrease was about 4% at both doses, and Hct was decreased from about 51% to about 49%. Platelet and reticulocyte counts were increased, while leukocyte counts were decreased at the high dose only. The decrease in leukocytes was primarily a result of reduced lymphocyte counts, specifically B cells. Plasma levels of immunoglobulin G (IgG) and IgM were also reduced at the high dose. Lipid peroxidation, as measured by thiobarbituric acid content, was increased in the kidney at the high dose only. Metallothionein content of the kidney is very slightly statistically significantly ($p < 0.05$) higher in the exposed groups relative to controls; there was no difference in the liver. Given the minimal changes in hematology and small body-weight decrease (~7%), the high dose (2.3 mg V/kg-day) is considered a NOAEL.

Kasibhatla and Rai (1993) administered vanadium in drinking water to rabbits (strain and sex not given) in a study evaluating limited hematology parameters. Rabbits (4/dose) were exposed to concentrations of 0, 20, 40, or 80 ppm vanadium for 171, 171, 129, or 24 days, respectively. The test material was characterized as "metavanadate." These exposure levels correspond to doses of about 3.3, 6.7, and 13.8 mg V/kg-day based on measured body weights and default values for water intake (U.S. EPA, 1988). An untreated control group received tap water. Body weights were recorded at irregular intervals. Blood samples were collected periodically for evaluation of erythrocyte and leukocyte counts, hemoglobin concentration and packed cell volume. The authors reported clinical signs including diarrhea, conjunctivitis, weakness, white nasal secretions, and loss of appetite in exposed rabbits, but no information on incidences or doses is provided. Body weights were generally lower in the treated groups, but the authors' statistical analysis indicated significantly reduced body weights only in the low-dose group; thus, this finding appears to be spurious. The authors also reported statistically significant ($p < 0.05$) reductions in erythrocyte count, hemoglobin concentration, and packed cell volume in the treated animals. However, the hematology data show decreasing numbers of treated rabbits over time, without explanation. It is not clear whether the missing animals died or were otherwise removed from the study. The poor reporting in this study precludes determination of effect levels.

Steffen et al. (1981) observed increased blood pressure in renally compromised rats exposed to vanadium. Groups of 20 adult male uninephrectomized Sprague-Dawley rats were given rat chow containing 100-ppm vanadium and either tap water or a 1% solution of sodium chloride to drink for 9 weeks. Based on default values for food intake and body weight (U.S. EPA, 1988), the dose for this experiment was estimated to be 9 mg V/kg-day. Control groups of the same size were given untreated rat chow (which contained 0.3-ppm vanadium) with one of the two fluid options. Fluid intake, urine volume, and urinary sodium concentration were measured daily. Body weights and systolic blood pressure (measured by tail cuff) were measured weekly. Upon sacrifice at the end of exposure, heart weights were recorded. There was one rat exposed to vanadium and sodium chloride that died at Week 3 of exposure; cause of death was not noted. Body-weight gain was lower in the vanadium-treated groups than in the

corresponding control groups, with statistically significant ($p < 0.05$) reductions after the 4th week of treatment. Based on visual examination of the data presented graphically, body weights of the treated groups at termination were about 12% lower than corresponding control body weights. The authors reported that vanadium treatment did not alter water consumption, urine volume, or urinary excretion of sodium (data on sodium excretion shown) compared with corresponding control groups. In the vanadium-exposed group consuming tap water, blood pressure was increased over the tap-water control group after the 3rd week of exposure ($p < 0.05$). Blood pressure data are presented graphically; based on visual examination of the data, systolic blood pressure approached 150 mm Hg in the vanadium-tap water group, compared with a value of <130 mm Hg in the tap-water controls. Though blood pressure measures were higher in vanadium-treated rats consuming sodium chloride, the difference from the sodium chloride control group is not statistically significant. Heart weight was not affected by vanadium treatment (data not shown). These data suggest a LOAEL of 9 mg V/kg-day based on decreased body weight. Although one rat died in the first experiment, there are no other indications of severe toxicity that would suggest that the death was related to treatment.

Susic and Kentera (1986) assessed the effects of vanadium administration on pulmonary circulation in adult male Long-Evans rats. After 2 months of exposure to ammonium vanadate in the diet (300 ppm or about 130-ppm vanadium assuming that the administered form was ammonium metavanadate), pulmonary and systemic blood pressure and cardiac output were measured and pulmonary and systemic vascular resistances were calculated from these measurements. Blood pressure was measured directly using a femoral artery cannula in anaesthetized animals. Using default values for food consumption and body weight (U.S. EPA, 1988), this dietary concentration is estimated to result in a dose of about 12 mg V/kg-day. Arterial blood was collected for assessment of hematocrit (timing not reported), but the results are not reported. After the exposure period, the rats were sacrificed and hearts removed for determination of left and right ventricular weights. Body weight, heart rate, mean femoral artery pressure, cardiac output, and total peripheral resistance were not affected by exposure (data shown). Significant ($p < 0.05$) increases in right ventricular systolic and mean pressures, as well as the calculated pulmonary vascular resistance, were observed with exposure (data presented graphically). The right ventricles of exposed rats were slightly enlarged, as shown by increased relative weight compared to controls (5%, $p < 0.05$). These data suggest a LOAEL of 12 mg V/kg-day based on pulmonary hypertension.

Susic and Kentera (1988) compared the hypertensive effects of sodium metavanadate in normal and partially nephrectomized Long-Evans rats. Groups of 18–24 male rats were fed diets containing 0-, 300-, or 3000-ppm sodium metavanadate for 24 weeks. The authors estimated doses of 5 and 47 mg sodium metavanadate per rat per day, corresponding to doses of approximately 4.4 and 42 mg V/kg-day (assuming a body weight of 0.472 kg for male Long-Evans rats [U.S. EPA, 1988a]). A separate group of 38 rats was subjected to partial nephrectomy followed by exposure to either the control diet or a diet with 300-ppm sodium vanadate (calculated to deliver a dose of 4.5-mg sodium metavanadate per rat per day, or 4.0 mg V/kg-day). Measurements of systolic blood pressure, heart rate, and body weight were recorded biweekly and renal function (plasma creatinine concentration, 24-hour creatinine clearance, urinary sodium excretion, and urinary output) was assessed in eight randomly chosen rats per group during Weeks 5 and 6. After exposure was terminated, groups of six randomly selected rats per group were selected for determination of hematocrit as well as plasma and extracellular fluid volumes. The remaining animals were used for measurement of blood

pressure, cardiac output, and total peripheral resistance. The animals were then sacrificed for removal of hearts and measurement of left and right ventricular weights. Body weights were significantly lower at both doses in the nonnephrectomized rats ($p < 0.001$ by t-test performed for this review), but they did not exceed a 7% decrease from control body weight in either group. The authors indicated that food intake was not affected by exposure (data not shown). Graphical and tabular presentation of data indicated that systolic blood pressure, heart rate, and mean arterial pressure were unchanged by vanadium treatment in nonnephrectomized rats. Statistically significant ($p < 0.05$) changes observed in nonnephrectomized rats at the end of exposure included decreased cardiac output and increased total peripheral resistance at both doses and increased hematocrit and decreased extracellular fluid volume at the high dose (see Table 5). In partially nephrectomized rats, systolic blood pressure, mean arterial pressure, and total peripheral resistance were significantly increased by exposure; other parameters were not affected by exposure. The authors indicated that the increase in resistance resulted from a vasoconstrictive effect of vanadium. In rats with intact kidneys, the increased peripheral resistance was offset by a reduction in cardiac output and blood pressure remained stable. In partially nephrectomized rats, there was no compensatory reduction in cardiac output; thus, an increase in blood pressure was observed. Renal function was not modified by vanadium exposure in any of the groups of rats, based on the parameters measured (data shown). These data indicate a LOAEL of 4 mg V/kg-day based on increased blood pressure in partially nephrectomized rats. A NOAEL cannot be determined.

**Table 5. Significant Changes in Cardiovascular Parameters in Rats Exposed to Sodium Metavanadate for 24 Weeks[a]**

| Parameter | Control | 4 mg V/kg-day (300 ppm) | 47 mg V/kg-day (3000 ppm) |
|---|---|---|---|
| *Nonnephrectomized rats* | | | |
| Cardiac output (mL/min per 100 g) | 25.6 ± 1.2 | 22.2 ± 0.6[b] | 21.2 ± 0.9[b] |
| Total peripheral resistance (mm Hg/mL per min per 100g) | 4.44 ± 0.12 | 5.41 ± 0.23 [c] | 5.82 ± 0.31 [c] |
| Hematocrit | 41.9 ± 0.7 | 43.5 ± 0.5 | 45.5 ± 1.3[b] |
| Extracellular fluid volume (mL/100g) | 17.0 ± 0.3 | 15.9 ± 0.4 | 12.9 ± 0.3[d] |
| *Partially nephrectomized rats* | | | |
| Mean arterial pressure (mm Hg) | 112 ± 4 | 134 ± 3[d] | NA |
| Total peripheral resistance (mm Hg/mL per min per 100g) | 4.11 ± 0.29 | 5.15 ± 0.25[b] | NA |

[a]Susic and Kentera, 1988
[b]Significantly different from control, $p < 0.05$
[c]$p < 0.01$
[d]$p < 0.001$

Van Vleet et al. (1981; Van Vleet and Boon, 1980) exposed groups of six male pigs to ammonium metavanadate in feed (0 or 200 mg V/kg) for 10 weeks. The dose estimated for this review was 10 mg V/kg-day based on the average body weight reported in the study (12 kg) and assuming a feed consumption rate of 0.6 kg feed/day (Brooks et al., 1984; U.S. EPA, 1988). The authors indicated that food consumption was decreased in the treatment group relative to controls (data not reported); therefore, the calculated dose may overestimate the actual dose in the treatment group. Endpoints assessed include clinical signs, weekly body weight measurements, blood glutathione peroxidase activity, gross necropsy, and microscopic histopathology assessment of heart, kidney, liver, lung, skeletal muscle, stomach, and "other organs with lesions." There were two deaths in the treatment group (33% mortality): one death on Day 60 of

exposure and one on Day 65. Clinical signs observed in treated pigs (and not in controls) were emaciation, rough hair coats, diarrhea, and blood in feces (incidences not reported). Body weights were markedly lower in the treatment group compared to the control group (one-third to one-half of control values; significantly lower at $p < 0.05$) throughout the exposure period; this decrease may have been associated with the reduction in food consumption. Blood glutathione peroxidase concentrations were not different from controls. The histopathology assessment revealed no abnormalities in the control group and the following findings in the treatment group: ulceration of the large intestine (4/4 surviving pigs), bladder cystitis (2/4), periportal infiltration of mononuclear leukocytes in liver (3/4) and necrosis of the heart atria (2/4). The dose used in this study (10 mg V/kg-day) is a FEL based on mortality and emaciation.

**Chronic Studies**—There were three multiyear bioassays of vanadium published in the 1960s and 1970s that have been identified in the literature searches; none of the studies met current standards for assessment of chronic toxicity and/or carcinogenicity.

Kanisawa and Schroeder (1967) exposed white Swiss mice (53 treated, 198 controls; sex not specified) to vanadyl sulfate in the drinking water at a concentration of 5 mg V/L from birth until natural death. The dose estimated for this review is 1 mg V/kg-day based on default values for body weight and water intake (U.S. EPA, 1988). The group sizes are not specified. Survival and body weight were monitored. Upon death, the animals were examined for gross lesions and the heart, lung, kidney, liver, spleen, and abnormal organs were examined microscopically. The authors emphasized that the tumor data reflected only tumors visible under a magnifying lens since serial sections for histopathology evaluation were not performed. The authors reported that neither survival nor body weight were affected by vanadium treatment (data not shown). Tumor incidences were grouped across sex for reporting. Based on the tabulated results, exposure to vanadium did not increase the incidence of any individual tumor type or the total incidences of "pre-tumorous lesions," benign, or malignant tumors (grouped across target organ). However, statistical analysis of the individual tumor data is precluded by the absence of group size information. These data are not adequate to define effect levels for chronic exposure.

Schroeder et al. (1970) exposed Long-Evans rats to vanadyl sulfate in drinking water from weaning through natural death (up to 45 months in this study). The treatment group consisted of 61 female and 52 male rats that had free access to drinking water to which 5 mg/L vanadium was added. Controls (54 female, 52 males) were exposed to water without added vanadium. Doses estimated for this review based on reported body weights and default fluid intakes (U.S. EPA, 1988) are 0.7 and 0.9 mg V/kg-day in males and females, respectively. Body weight was measured weekly until 6 weeks of age and then monthly thereafter; at the same times, blood pressure was recorded and blood collected for assessment of serum glucose levels. Upon death, animals were necropsied, hearts were removed and weighed, and grossly visible tumors and other lesions were described. An outbreak of pneumonia during this study led to the deaths of 17 treated males, 17 treated females, 19 control males, and 12 control females; the timing of the outbreak was not reported. No differences were observed in the following endpoints: life span and longevity, body weight, blood pressure (measured with arterial cannula in anesthetized animals), urine protein and glucose, and gross tumor incidence (all data other than urine protein were shown). This study found significant ($p < 0.05$) differences between the treatment and control groups in the following endpoints: increased fasting plasma glucose concentrations (21%) in treated females, increased fasting plasma cholesterol concentrations

(18%) in treated males and decreased fasting cholesterol concentrations (41%) in treated females. Absolute and relative heart weights were 18 and 15% lower (respectively) in treated males relative to controls, while female heart weights were higher (4 and 5% higher for absolute and relative weights, respectively). No treatment-related increases in tumor formation were found. Microscopy was performed on "some" tissues; however, a comprehensive microscopy evaluation apparently was not performed or not reported. No microscopic lesions were reported for any animals, although the histological evaluations performed in this study were not adequate to detect any but the most severe lesions. However, a LOAEL of 0.7 mg V/kg-day can be established for increased fasting plasma glucose and cholesterol levels and decreased heart weights.

Using a study design similar to that above, Schroeder and Michener (1975) exposed groups of Swiss mice (54/sex) to vanadyl sulfate in drinking water (5 mg V/L) for their lifetimes; controls (54/sex) were given untreated water. The dose estimated for this review based on reported body weights and default estimates of fluid intake (U.S. EPA, 1988) was 1 mg V/kg-day in both sexes. The toxicological evaluations are the same as reported by Schroeder et al. (1970). Significant differences between the treatment and control groups included increased body weight in treated males and increased life span and longevity in treated males and females. A gross assessment of tumors and microscopy of "some" tissues revealed no treatment-related increases in tumor incidence. The limitations in the histological evaluations performed in this study preclude the identification of effect levels from these data.

Steffen et al. (1981) exposed groups of uninephrectomized rats (group sizes not reported) to dietary concentrations of 100- or 200-ppm vanadium (as sodium orthovanadate) for 56 weeks. Based on default values for food intake and body weight (U.S. EPA, 1988), the doses for this experiment are estimated to be 7 and 14 mg V/kg-day. Body weights and systolic blood pressure (measured by tail cuff) were measured weekly. Upon sacrifice at the end of exposure, heart weights were recorded, and tail artery norepinephrine content was measured. There were two rats given 14 mg V/kg-day that died "early in the experiment"; neither timing nor cause of death was reported (Steffen et al., 1981). In the 14 mg V/kg-day group, body weights were significantly ($p < 0.05$) below controls beginning at Week 20 of treatment; based on graphical presentation of the data, terminal body weight in this group was about 13% below that of controls. Body weight was not significantly different from controls in the low-dose group. In both groups of vanadium-treated rats, systolic blood pressure was significantly ($p < 0.05$) increased over controls, in a dose-dependent fashion, after the first 1–2 months of treatment. Increases of up to 10 and 25 mm Hg were seen in the low- and high-dose groups, respectively. Plasma vanadium concentration measured at sacrifice correlated strongly with the last measure of systolic blood pressure ($r = 0.71$, $p < 0.001$), bolstering evidence for the apparent relationship with exposure. The low dose (7 mg V/kg-day) is a freestanding LOAEL for increased blood pressure in uninephrectomized rats.

Dai et al. (1994a,b; Dai and McNeill, 1994) exposed groups of nondiabetic and diabetic (streptozocin-induced) male Wistar rats to vanadyl sulfate in drinking water for 1 year. The three publications each reported findings of different endpoints. A control group consisted of eight rats given free access to water without added vanadate. There were three treatment groups that consisted of 8 rats/group exposed to water to which vanadyl sulfate was added; the exposures (mg vanadyl sulfate/L) were as follows: treatment group 1500 mg/L for 52 weeks; treatment group 2500 mg/L for 1 week followed by 750 mg/L for 51 weeks; treatment group

3500 mg/L for 1 week followed by 750 mg/L for 1 week, followed by 1250 mg/L for 50 weeks. Food intake, fluid intake, and body weight were recorded every 3–5 weeks throughout the treatment period. On the basis of these measures, the authors estimated the doses of vanadyl sulfate to be 34, 54, and 90 mg/kg-day (8, 13, and 21 mg V/kg-day, using the molecular weight for the trihydrate form) in nondiabetic rats. In diabetic rats, vanadyl sulfate treatment was adjusted up or down in order to control blood glucose or prevent diarrhea and weight loss. The authors estimated vanadyl sulfate doses of 73 to 165 mg/kg-day (17 to 39 mg V/kg-day) at different time points in the diabetic rats. The general condition of the animals—especially the occurrence of diarrhea or cataracts—was assessed during treatment. Nonfasting blood glucose was measured weekly for the first month and then every 2–4 weeks thereafter. Fasting blood glucose, insulin, triglycerides and cholesterol were measured every 3 months during treatment. The following measurements were made after 3, 6, 9, and 12 months of exposure: blood pressure (measured with a tail cuff sensor in conscious animals), pulse rate, hematocrit and plasma concentrations of AST, ALT, and urea. Most animals were sacrificed after the exposure period; however, three control nondiabetic rats, eight treated nondiabetic rats, and five treated diabetic rats were monitored for 16 untreated weeks prior to sacrifice. At sacrifice, a hematology assessment (Hgb, RBC, total and differential WBC, platelet count, reticulocyte count) was conducted and the following organs were weighed and examined microscopically: adrenal, brain, heart, kidney, liver, lung, pancreas, spleen, testis, and thymus.

In nondiabetic rats, 1/8 animals treated at the highest dose died of unknown causes after 18 weeks of exposure (Dai et al., 1994a). Neither food nor fluid intake was significantly affected by exposure to vanadyl sulfate (data shown graphically). However, body weight gain was reduced in a dose-related manner in treated nondiabetic animals relative to control nondiabetic animals. Based on visual inspection of data presented graphically, the body weight decrements at termination were approximately 10% in the low- and mid-dose groups and 20% in the high-dose group; statistical analysis of the data was not presented. Other than body weight data, most information on the nondiabetic treated rats was pooled across the three treatment groups (the authors indicated that there were no differences among the three groups). Vanadyl sulfate treatment did not affect blood or plasma glucose levels, plasma triglycerides, or cholesterol levels, but significantly lowered plasma insulin levels compared with controls at Weeks 12 and 25 (data presented graphically; *p*-value not reported). No significant changes were observed in the treatment group relative to the control group for the following endpoints: systolic blood pressure, pulse rate, hematology endpoints and relative organ weights (Dai et al., 1994a; Dai and McNeill, 1994). Plasma ALT and urea concentrations are significantly ($p < 0.05$) higher (<2-fold higher based on data presented graphically) in the nondiabetic treatment group relative to the corresponding control group after 3 months of exposure but not after 6, 9, 12, or 16 months of exposure (data presented graphically).

Histopathology findings included a high incidence of glomerular and tubular degeneration and interstitial cell infiltration and fibrosis of the kidney in the nondiabetic control group: 3/5 (60%) at the end of exposure and 2/3 (66%) at 16 weeks postexposure, for a combined incidence of 5/8 (63%) for the two assessment times (Dai et al., 1994b). Despite this high incidence in controls, which was probably age- and/or husbandry-related, the treated animals (all three treatment groups pooled) had a higher incidence: 15/15 (100%, $p = 0.053$) at the end of the exposure, 7/8 (88%, $p = 0.049$) 16 weeks postexposure and a combined incidence of 22/23 (96%; $p = 0.043$) (based on Fisher exact test performed for this review). These results are consistent

with the higher plasma urea concentrations in the treatment group. No other histopathology findings are significantly increased with exposure to vanadyl sulfate. Based on the reduced body weight in the low-dose group (~10% lower than controls at termination), and possibly renal pathology, a LOAEL of 8 mg V/kg-day is identified for nondiabetic rats; no NOAEL can be determined.

Vanadyl sulfate treatment of diabetic rats improved or prevented a number of adverse effects seen in untreated diabetic rats, including: mortality; increased food and fluid intake; hypoinsulinemia; polydipsia; cataract formation; elevations of serum glucose, ALT, urea, triglycerides and cholesterol; bradycardia; decreased leukocyte count; increased relative organ weights and occurrence of megacolon (Dai et al., 1994a,b; Dai and McNeill, 1994). No improvement was seen in body-weight gain, which was markedly lower in both untreated and vanadyl sulfate-treated rats than in both control and treated nondiabetic rats. At the end of exposure, body weights were about 30% lower in both groups of diabetic rats when compared with nondiabetic controls (based on graphical presentation of data). Likewise, renal effects that were significantly increased in diabetic controls (compared with nondiabetic controls), including vacuolation of tubular epithelial cells and renal cell tumors, occurred at similar frequency in vanadyl sulfate-treated diabetic rats. As vanadium treatment was not associated with adverse effects in diabetic rats, the dose to this group (17 to 39 mg V/kg-day) is considered a NOAEL in diabetic rats.

Carmignani et al. (1991) exposed male Sprague-Dawley rats to sodium metavanadate in drinking water for 7 months beginning at weaning. Groups of 10 rats were exposed to water to which 0 or 100 mg V/L was added. The calculated dose was 12 mg V/kg-day, based on default values for fluid intake and body weight (U.S. EPA, 1988). At the end of exposure, blood pressure was measured (with an arterial cannula in anesthetized animals), urinalysis was performed on a 24-hour urine collection and both light and electron microscopic evaluation of the heart and kidney were performed. Systolic and diastolic blood pressure were significantly ($p < 0.05$) elevated in the treatment group compared to the control group (systolic: control 122 mmHg, treatment group 144 mmHg; diastolic: control 95 mmHg, treatment 115 mmHg), as was heart rate (control 239 beats per minute, treatment 288 beats per minute). According to the authors, the urinalysis revealed no difference in urine osmolarity, nitrogen, protein, or ionized calcium between the treatment and control group (data not shown). Urinary sodium and potassium excretion were significantly elevated (83% and >3-fold higher, respectively; $p < 0.05$) in the treatment group compared to the control group. The histopathology assessment revealed narrowing of the renal proximal tubules, which contained amorphous protein material and swollen mitochondria, in the treatment group. The incidences of these changes in treated and control animals are not reported. No changes were noted in the hearts of the treatment group relative to the control group. A LOAEL of 12 mg V/kg-day is identified based on the increased blood pressure and kidney histopathology. A NOAEL cannot be identified.

Investigators from the same laboratory (Boscolo et al., 1994) conducted further experiments with male Sprague-Dawley rats exposed to sodium metavanadate in drinking water. Groups of six rats were exposed to water containing 1, 10, or 40 mg V/L for 180, 210, and 210 days, respectively, in two experiments. Each experiment had a separate control group receiving untreated water for the same duration. Doses estimated for this review based on default estimates of fluid intake and body weight (U.S. EPA, 1988) were 0.12, 1.2, or 4.7 mg V/kg-day. The following endpoints were assessed: blood pressure (measured with an

arterial cannula in anesthetized animals); heart rate; plasma renin activity, plasma aldosterone, urinary kallikrein activity and urinary Kininase I and II activities (indicators of status of the renin-angiotensin-aldosterone system); urinalysis (creatinine, total nitrogen, proteins, sodium, potassium, and calcium); and microscopic examination of blood vessels, brain, heart, kidney, liver, and lung. Histochemical analysis of the Na+, K+-ATPase activity was assessed in the kidneys of high dose and control rats. Statistically significant changes in the measured parameters are shown in Table 6. Significantly higher ($p < 0.05$) systolic and diastolic blood pressures were observed in all treatment groups relative to the control group. The magnitude of the increase did not appear to be dependent on dose level. Plasma renin activity, plasma aldosterone concentration, and urinary kallikrein, Kininase I, and Kininase II were significantly elevated in the 1.2 and 4.7 mg V/kg-day treatment groups relative to controls, suggesting stimulation of the renin-angiotensin-aldosterone system at these exposure levels. In addition, Kininase I activity was doubled at 0.12 mg V/kg-day, although not statistically significant. In contrast, Kininase II activity and plasma aldosterone were significantly reduced at the low dose. The histological assessment revealed narrowing of the lumen and amorphous casts in renal proximal tubules and a decrease in histochemically detected $Na^+$, $K^+$-ATPase activity in injured tubules in the 4.7 mg V/kg-day treatment group. The authors also reported hydropic degeneration (swelling of the cells) in proximal, distal, and straight tubules. The incidences of the latter effect were not reported; however, the authors indicated that these changes were "less evident" at 1.2 mg V/kg-day and absent at 0.12 mg V/kg-day. These data suggest a LOAEL of 0.12 mg V/kg-day based on increased blood pressure (>20 mm Hg increase in both systolic and diastolic measures) and stimulation of the renin-angiotensin-aldosterone system. A NOAEL for increased blood pressure cannot be determined. However, a NOAEL for kidney effects (histopathology) is established at 0.12 mg V/kg-day, with a LOAEL at 1.2 mg V/kg-day.

**Table 6. Significant Effects on Cardiovascular Parameters in Male Rats Exposed to Sodium Metavanadate[a]**

| Parameter | Control[b] | 0.12 mg V/kg-day (180 days) | 1.2 mg V/kg-day (210 days) | 4.7 mg V/kg-day (210 days) |
|---|---|---|---|---|
| Systolic blood pressure (mm Hg) | 108 ± 5[c]<br>106 ± 7 | 130 ± 4[d] | 137 ± 5[d] | 132 ± 4[d] |
| Diastolic blood pressure (mm Hg) | 84 ± 4<br>85 ± 5 | 106 ± 3[d] | 112 ± 5[d] | 114 ± 7[d] |
| Plasma renin activity (ng/mL/h) | 13.4 ± 3.4<br>10.3 ± 2.7 | 10.6 ± 2.4 | 47.5 ± 14.9[d] | 40.6 ± 12.4[d] |
| Plasma aldosterone (pg/mL) | 264 ± 22<br>188 ± 57 | 158 ± 11[d] | 554 ± 160[d] | 265 ± 61 |
| Kallikrein (nM/mg creatinine) | 8.02 ± 1.90<br>8.43 ± 0.96 | 4.36 ± 0.60[d] | 13.67 ± 2.54[d] | 11.72 ± 0.80[d] |
| Kininase I (nM × $10^{-3}$ of hydrolyzed substrate/mg creatinine) | 27.6 ± 5.4<br>32.0 ± 4.2 | 56.8 ± 25.3 | 129.9 ± 14.9[d] | 156.8 ± 9.1[d] |
| Kininase II (nM x $10^{-3}$ of hydrolyzed substrate/mg creatinine) | 2.23 ± 0.33<br>1.83 ± 0.26 | 2.30 ± 0.31 | 2.63 ± 0.13[d] | 3.92 ± 4.08[d] |
| Urinary potassium excretion 11(mEq/g creatinine) | 113 ± 25<br>118 ± 14 | 106 ± 6 | 169 ± 18[d] | 221 ± 28[d] |

[a]Boscolo et al., 1994
[b]First result is for 180-day control group; second is for 210-day control group.
[c]Mean ± standard error of the mean
[d]Significantly different from corresponding control, $p < 0.05$

**Reproductive Studies—**Effects on reproductive success have been reported with preconception exposure to vanadium compounds. Domingo et al. (1986) administered daily gavage doses of 0, 5, 10, or 20 mg/kg-day sodium metavanadate (2.1, 4.2, or 8.4 mg V/kg-day) to male and female Sprague-Dawley rats (20/sex/dose). Male rats received daily doses for 60 days after which they were mated to female rats that had received the same doses 14 days prior to mating. Dosing of females continued through gestation. Half of the females were sacrificed on gestation day (GD) 14 for assessment of the number of corpora lutea, total implantations, resorptions, and living and dead fetuses. The remaining dams were continued on the exposure regimen through weaning of their pups (postnatal day [PND] 21). Evaluations of offspring included viability, body-weight gain, body and tail lengths and clinical signs on PND 1, 4, and 21. Results for pups were pooled across litters. Upon sacrifice of pups at weaning, the weights of heart, lungs, spleen, liver, kidneys, and testicles were recorded. The authors reported that maternal toxicity was not evident in the treated dams, but did not specify the endpoints measured to assess maternal effects. No significant differences between the treatment and control groups were observed in the various indicators of reproductive success assessed at sacrifice on GD 14 (data shown). A significant decrease ($p < 0.05$) in pup growth occurred in all treatment groups compared to the control group, as indicated by deficits in whole litter weight and pup body weight, head-to-rump length, tail length, and relative kidney and liver weights (organ-/body-weight ratios). Body weight per litter was significantly decreased in the high-dose group on PND 4 and the mid- and high-dose groups on PND 21. Table 7 shows the changes in pup growth parameters (pooled across litters) observed on PND 1, 4, and 21. At the

high dose, significant ($p < 0.05$) decreases in relative heart (males only) and spleen weights (both sexes) were also observed. These data suggest a developmental toxicity LOAEL of 2.1 mg V/kg-day based on growth retardation in pups; a developmental NOAEL was not identified. Due to the lack of information on maternal endpoints evaluated, effect levels for systemic toxicity cannot be determined

**Table 7. Significant Effects on Growth Parameters (Pooled Across Litters) in Pups of Dams Exposed to Sodium Metavanadate[a]**

| Parameter | Control | 2.1 mg V/kg-day | 4.2 mg V/kg-day | 8.4 mg V/kg-day |
|---|---|---|---|---|
| *Males* | | | | |
| Body weight PND 1 (g) | 7.9 ± 0.9(63)[b] | 7.0 ± 1.1[c] (57) | 6.5±0.9[c] (77) | 6.7±0.6[c] (48) |
| Body weight PND 4 (g) | 11.7 ± 1.3 (63) | 9.6 ± 1.8[c] (57) | 9.7 ± 1.2[c] (63) | 8.9 ± 0.8[c] (40) |
| Body weight PND 21 (g) | 42.0 ± 8.3(57) | 34.3 ± 7.9[c] (56) | 33.7 ± 10.8[c] (35) | 33.6 ± 7.6[c] (38) |
| Body length PND 1 (mm) | 56.8 ± 3.5 | 54.2 ± 3.6[d] | 53.4 ± 3.4[e] | 53.1 ± 3.0[e] |
| Body length PND 4 (mm) | 67.1 ± 3.4 | 62.0 ± 4.4[c] | 64.7 ± 3.6[c] | 62.2 ± 2.5[c] |
| Body length PND 21 (mm) | 119.1 ± 6.1 | 108.0 ± 10.0[c] | 102.8 ± 16.2[c] | 104.8 ± 10.8[c] |
| Tail length PND 4 (mm) | 30.4 ± 2.4 | 23.9 ± 3.4[c] | 25.8 ± 3.8[c] | 23.6 ± 2.3[c] |
| Relative liver weight (g/100g BW) | 5.12 ± 0.58 | 4.72 ± 0.56[d] | 4.63 ± 0.40[d] | 4.57 ± 0.54[d] |
| *Females* | | | | |
| Body weight PND 1 (g) | 7.6 ± 0.9 (54) | 6.8 ± 1.0[c] (58) | 6.4 ± 0.9[c] (62) | 6.5 ± 0.6[c] (43) |
| Body weight PND 4 (g) | 11.2 ± 1.9 (53) | 9.5 ± 1.6[c] (58) | 9.3 ± 1.4[c] (48) | 8.8 ± 1.1[c] (39) |
| Body weight PND 21 (g) | 41.0 ± 6.7 (51) | 32.5 ± 6.3[c] (53) | 29.7 ± 7.2[c] (20) | 32.1 ± 8.8[c] (38) |
| Body length PND 1 (mm) | 55.5 ± 3.4 | 53.6 ± 3.7[e] | 52.4 ± 3.9[c] | 52.0 ± 2.6[c] |
| Body length PND 4 (mm) | 65.5 ± 3.0 | 61.4 ± 3.8[c] | 63.0 ± 3.7[c] | 61.5 ± 3.3[c] |
| Body length PND 21 (mm) | 119.7 ± 6.9 | 105.5 ± 11.3[c] | 100.9 ± 11.7[c] | 104.4 ± 11.3[c] |
| Tail length PND 4 (mm) | 30.7 ± 2.4 | 25.1 ± 3.1[c] | 26.2 ± 3.8[c] | 24.3 ± 2.5[c] |
| Tail length PND 21 (mm) | 70.4 ± 8.0 | 66.3 ± 7.0[d] | 68.9 ± 9.5 | 61.0 ± 6.0[c] |
| Relative liver weight (g/100g BW) | 5.53 ± 0.45 | 5.04 ± 0.80[d] | 5.01 ± 0.75[d] | 4.72 ± 0.63[e] |
| Relative kidney weight (g/100g BW) | 1.56 ± 0.17 | 1.38 ± 0.22[d] | 1.45 ± 0.20[d] | 1.32 ± 0.16[e] |

[a]Domingo et al., 1986
[b]Mean ± SD (number of animals)
[c]$p < 0.001$
[d]Significantly different from control, $p < 0.05$
[e]$p < 0.01$

Llobet et al. (1993) exposed male Swiss mice to sodium metavanadate in drinking water for 64 days prior to mating for 4 days with unexposed females. There were four treatment groups that consisted of 24 mice per group given water to which 100, 200, 300, or 400 mg/L sodium metavanadate was added. The authors reported the doses as 20, 40, 60, or 80 mg/kg-day sodium metavanadate, which correspond to calculated vanadium doses of 8.4, 17, 25, or 33 mg V/kg-day. The control group consisted of 24 mice given water without added vanadate. Dams were killed 10 days after mating (GD 10–14) and their uteri were examined to evaluate pregnancy outcomes. Endpoints assessed included body weights; reproductive success, including the number of implantations, early or late resorptions and dead or live fetuses; testis and epididymis weights; and sperm counts, motility, and morphology. Body weights in the 33 mg V/kg-day group were significantly lower than in the control group (13%, $p < 0.05$). The absolute (but not relative) epididymis weight was reduced by treatment (12%, $p < 0.01$). There was no difference in the absolute or relative testis weight between the control and treatment groups. A lower number of successful impregnations occurred in the 25 and 33 mg V/kg-day dose groups compared to the control group (43.8% and 62.5%, respectively, compared with

81.3% in controls; $p < 0.01$). There were no differences in the number of resorptions or fetal mortality. Outcomes related to sperm included: lower spermatozoa counts in the 25 and 33 mg V/kg-day groups relative to the control group (44% and 31% lower, respectively); a lower spermatid count in the 33 mg V/kg-day group (30%, $p < 0.01$) and no significant difference in sperm motility or morphology between control and treatment groups. These data indicate a LOAEL of 25.1 mg V/kg-day based on reproductive effects in treated male mice (decreased spermatozoa counts and reduced fecundity); the NOAEL is 17 mg V/kg-day.

In a study comparing reproductive effects of vanadium in diabetic and nondiabetic rats, Ganguli et al. (1994a) administered concentrations of 0, 250, or 500 mg/L sodium orthovandate (~69 or 138 mg V/L) with 0.45% normal saline in the drinking water of female Sprague-Dawley rats. There were six groups of 15 rats/dose that were used (three groups each of nondiabetic and streptozocin-induced diabetic rats). The authors reported that the animals were mated to untreated males at the commencement of treatment (Day 1); however, the balance of the treatment regimen was not described, so the duration of treatment is not known. Body weight, fluid intake, and urine glucose were measured daily; however, data on body weight and fluid intake are not reported or described. In the absence of information on the treatment schedule, it is not possible to estimate doses with any degree of confidence. Pregnant dams were sacrificed one day after giving birth; those treated females that did not become pregnant or failed to deliver were sacrificed for examination of uteri and ovaries. At birth, the total number of pups and total body weight were recorded. In contrast to the findings discussed previously (Dai et al., 1994a,b; Dai and McNeill, 1994), vanadium was severely toxic to diabetic rats; 7/15 females exposed to 500 mg/L died before Day 15 of treatment and the remainder had severe diarrhea and lack of appetite; these animals were sacrificed humanely. Mortality also occurred at the low dose in diabetic rats (3/15). No deaths occurred in controls. In high-dose nondiabetic rats, moderate-o-severe diarrhea was observed in 12/15 rats; this effect was not reported in low-dose nondiabetic rats. The rate of conception was significantly ($p < 0.05$) reduced by vanadium exposure in both diabetic and nondiabetic rats. When compared with nondiabetic controls, the rate of conception is reduced by 13% and 20% at 250 and 500 mg/L (respectively) in nondiabetic rats and by 7%, 33%, and 47% in 0, 250, and 500 mg/L (respectively) diabetic groups. Ability to carry a pregnancy to term was also compromised by vanadium exposure, significantly so in the diabetic animals. Compared with nondiabetic controls, nondiabetic treated animals exposed to 250 and 500 mg/L were 30% and 84% (respectively) less likely to carry pregnancy to term. In diabetic animals, fewer than 10% of animals at the low dose carried pregnancy to term; as the high-dose group was sacrificed early, there are no data on this endpoint. Effect levels cannot be determined from these data since duration of treatment is unknown and doses could not be estimated.

Faria de Rodriguez et al. (1998a) conducted three experiments to evaluate the effects of exposure to vanadium on the development of the central nervous system in albino rats. This study was published in Spanish and translated for this review. Groups of four female rats were used in all experiments. In the first experiment, three groups were exposed to 0, 100, or 200 ppm ammonium metavanadate (43.5 or 87 ppm V) in the drinking water from weaning until mating; treatment was discontinued during mating and gestation. Doses estimated for this review based on default values of water intake and body weight (U.S. EPA, 1988) were 7 and 15 mg V/kg-day. From each group, two dams were sacrificed at GD 20, while the other two were allowed to deliver. Litters were sacrificed at birth for gross examination of external and

internal malformations and the CNS was removed for microscopic examination and histochemical assessment of glycosaminoglycans. In another experiment, two groups of neonates whose mothers had been exposed to 100-ppm ammonium metavanadate from 37 days of age until mating were exposed to concentrations of 0 or 100 ppm via lactation until weaning and then via drinking water until mating. As with the first experiment, half of each group was sacrificed at GD 20 and half after delivery; evaluation of litters was also the same. In the final experiment, newborn rats of untreated mothers were exposed via lactation and then via drinking water to 0 or 200 ppm ammonium metavanadate. All rats of the final experiment were permitted to deliver. Females in all of the control groups delivered litters averaging from 5–11 offspring each. All four rats exposed to 7 mg V/kg-day in the first experiment became pregnant, delivering an average of 11 offspring per litter. At 15 mg V/kg-day, one rat died, one delivered a litter of 11 offspring, and the other 2 did not become pregnant. In the second experiment, of four rats exposed to 7 mg V/kg-day from birth to mating, only two became pregnant and delivered litters, averaging six offspring each. Similarly, in the third experiment, exposure to 15 mg V/kg-day resulted in only 2/4 females delivering litters (4 and 10 offspring each). No gross external malformations were observed in any of the groups. Data on the microscopic examination of brains were grouped across the experiments, so a dose-response relationship could not be discerned. Of the 81 brains obtained from the offspring of treated animals, 13 exhibited unilateral hypoplasia of the olfactory bulb and one exhibited unilateral hypoplasia of the cerebral hemisphere; the remaining brains were characterized as normal. Microscopic effects on the olfactory bulbs (for example, thinning or disorganization of the glomerular layer) were also seen in the brains of animals with grossly observable effects; the incidences of specific effects were not reported. All brains of control offspring were normal both macroscopically and microscopically. Histochemical studies indicate that exposure to 15 mg V/kg-day increased the glucosaminoglycan content—specifically those of a low grade of sulfation. Effect levels cannot be determined from these data due to the lack of incidence data, the grouping of effect information across treatment groups, and incomplete reporting.

Faria de Rodriguez et al. (1998b) exposed male and female Swiss albino mice to ammonium metavanadate from birth until the animals were mated. This study was published in Spanish and translated for this review. The test compound was administered in drinking water to mothers so that the offspring were exposed via lactation until weaning, when they were continued on the same exposure via drinking water (0, 100, or 200 ppm) until mating. These concentrations correspond to 43.5 and 87 ppm vanadium or dose estimates of 15 and 30 mg V/kg-day (males) and 14 and 28 mg V/kg-day (females) based on default values[2] for body weight and water intake (U.S. EPA, 1988). The animals' body weight, body length, and tail length were measured weekly. At maturity, the males and females of each exposure group were mated with same-treated mice or cross-mated with untreated mice to evaluate separately the effects on each gender. Numbers of offspring, as well as weight and length of offspring, were assessed after successful mating. The authors reported the results of statistical analysis of the parameters, but did not report the data for any endpoints. In addition, results for the same exposure level were grouped across sex. The authors reported that there were no treatment-related differences in body weight of the parents. Body length of treated mice was significantly ($p < 0.05$) reduced with exposure to 28–30 mg V/kg-day, and tail length was significantly lower at both exposure levels when compared with control animals. In contrast, neither weight nor length of offspring was affected in any of the matings. Further, the number of

[2]Assuming body weight and water intake at weaning.

offspring was higher in the exposure groups than in the control group. Effect levels cannot be determined from these data due to inconsistent outcomes and poor reporting.

The same group of investigators conducted additional experiments on female Swiss albino mice (Nava de Leal et al., 1998). This study was also published in Spanish and translated for this review. Ammonium metavanadate was administered in drinking water at concentrations of 0, 100, or 200 ppm (0, 43.5, or 87 ppm vanadium) at various times as shown in Table 8. Dose estimates calculated for this review are 11 or 23 mg V/kg-day based on default values[3] for body weight and water intake (U.S. EPA, 1988). In each experiment, exposure was suspended during 8 days of mating with untreated males (ratio of 1 female to 2 males) and during gestation. After mating, the mice were housed individually and weighed twice weekly. Pregnant mice were allowed to deliver; pregnancy rates and number of offspring were recorded. Those mice that failed to become pregnant were sacrificed 21 days after mating for evaluation of the following parameters: uterine and ovarian weights; corpora lutea counts and histopathology examination of the ovaries. The reporting of results was limited by some inconsistencies and apparent typographical errors. As Table 8 shows, the pregnancy rate was significantly reduced from controls in 2/3 groups (C1 and F1C, but not A2) exposed to 23 mg V/kg-day but not in any group exposed to 11 mg V/kg-day. The absence of an effect on pregnancy rate in Group A2 (exposed to 23 mg V/kg-day from weaning until mating) contrasts with the findings in Group C1 (exposed to 11 mg V/kg-day until first mating and then to 23 mg V/kg-day from parturition until second mating) and suggests that cumulative exposure may be an important factor in the effects of vanadium on mating success. In the statistical analysis of litter sizes, groups exposed for different time periods to the same concentration were combined (details unclear). The results shown in Table 8 indicate that litter size is significantly smaller in mice exposed to 23 mg V/kg-day compared with controls (*p*-value not given). A similar approach was used to compare the numbers of corpora lutea; this analysis also showed a reduced average number of corpora lutea in mice exposed to 23 mg V/kg-day compared with untreated controls. Corpora lutea were counted only in mice that failed to become pregnant, which may have biased the findings.

[3]Assuming body weight and water intake for subchronic exposure.

**Table 8. Exposures, Group Sizes, and Pregnancy Outcomes Among Mice Exposed to Ammonium Metavanadate[a]**

| Group | Exposure Period | Dose (mg V/kg-day) | No. Mice | Pregnancy Rate (%) | Average Litter Size |
|---|---|---|---|---|---|
| A1 | Weaning to adulthood (mating) | 0<br>11 | 8<br>12 | NR[b]<br>50 | 13<br>13 |
| A2 | Weaning to adulthood | 0<br>23 | 8<br>12 | 50<br>66.6 | 9<br>8 |
| B | Weaning to adulthood | 0<br>11 | 8<br>12 | 75<br>83.3 | 11<br>9 |
| B1 | Second mating of Group B; no exposure between matings | 0<br>11 | 6<br>10 | 100<br>100 | 10<br>10 |
| F1B | Offspring of Group B | 0<br>11 | 9<br>26 | 66.6<br>73 | NR<br>NR |
| C | Weaning to adulthood | 0<br>11 | 8<br>12 | 75<br>83.3 | 9<br>10 |
| C1 | Mice Group C that successfully became pregnant; exposed from parturition until second mating | 0<br>23 | 6<br>10 | 100<br>20[c] | 9<br>3 |
| F1C | Offspring of Group C; exposed via lactation until weaning and drinking water until adulthood | 0<br>23 | 8<br>24 | 65<br>0[c] | 12<br>0 |

[a]Nava de Leal et al., 1998
[b]Not reported
[c]Significantly different from control, $p < 0.0001$

Microscopic examination of the ovaries from mice that failed to become pregnant showed histopathology associated with exposure to vanadium (Nava de Leal et al., 1998). Most (94%) samples of ovaries from control mice that failed to become pregnant were reportedly normal. In contrast, ovaries of mice exposed to 11 mg V/kg-day (Groups A1 and B) exhibited fewer follicles and/or follicular atresia (absence of follicles due to degeneration); the follicles that were seen were enlarged and conferred a "polycystic aspect" on the ovaries. Histopathology findings in the ovaries of mice exposed to 23 mg V/kg-day (Groups A2, C1, and F1C) were more pronounced, including absence of mature follicles and corpora lutea, marked follicular atresia, thickening of the external theca, loss of ovarian parenchymal architecture, cellular disaggregation, and cytoplasmic vacuolation in granulosa lutein cells. The authors reported the incidences of these findings in the ovaries of mice that did not become pregnant; however, the overall incidences of these effects in treated mice were not available, as histopathology was not assessed in mice that became pregnant. Ovarian histopathology changes in mice exposed to 11 mg V/kg-day suggest that this dose may be a LOAEL, despite the lack of effect on pregnancy success; however, the absence of data on overall incidences in the treated and control groups (including those that became pregnant), in addition to reporting problems, precludes definition of reliable effect levels for this study.

Morgan and El-Tawil (2003) also assessed the effects of vanadium exposure on reproductive success. Groups of 10 male and 20 female Sprague-Dawley rats were given ammonium metavanadate at concentrations of 0 or 200 mg/L (87 mg V/L) in the drinking water. Based on default values of water intake and body weight (U.S. EPA, 1988), the dose is estimated to be 28 and 30 mg V/kg-day in males and females, respectively. Exposed male rats were treated

for 70 days prior to mating with untreated females; exposed females were treated for 14 days premating and during mating, gestation, and lactation (total of 61 days). During premating, the estrous cycles of females were monitored. Maternal body weights were recorded at the end of gestation. Half of each group of females was sacrificed on GD 20, while the other half, along with their pups, was sacrificed after weaning on PND 21. Gravid uterine and placental weights were recorded. Males were sacrificed after mating for assessment of body, testes, epididymis, prostate, and seminal vesicle weights. Reproductive parameters assessed in the study included: gestation duration; signs of dystocia; numbers of corpora lutea, implantation sites, resorptions, pre- and postimplantation losses; live and dead fetuses; fetal body weight at birth and on PND 4, 7, 14, and 21 and fetal survival during lactation. During lactation, pups were examined for learning and memory responses; however, the specific methods and endpoints were not described. All pups were examined for gross malformations at sacrifice; two-thirds were examined for skeletal abnormalities and the remainder for visceral abnormalities. Exposure to ammonium metavanadate resulted in profound effects on reproductive success and offspring development, regardless of whether males or females were treated. Statistically significant adverse effects are reported for nearly every reproductive parameter assessed, including maternal body, uterine and placental weights; litter parameters; viability of offspring at birth; pup body weight during lactation and incidences of gross, visceral and skeletal malformations. In addition, fewer treated females exhibited normal estrous cycles; treatment of females also resulted in reduced survival and viability indices of offspring. Body weight of treated males was not affected, but testes, epididymis, prostate gland and seminal vesicle weights were significantly ($p < 0.05$) reduced by exposure. Few offspring were produced in the treated groups (20 and 35 in the offspring of treated males and females, respectively, compared with 216 controls). Those that were produced had a high frequency of gross, visceral, and skeletal anomalies. Data were reported using the fetus, rather than the litter, as the unit of statistical analysis, so it is not possible to assess the litter distribution of effects. These data suggest a freestanding LOAEL of 28 mg V/kg-day for reproductive toxicity in rats.

**Developmental Studies—**Elfant and Keen (1987) exposed groups of at least 14 pregnant Sprague-Dawley rats to diets containing 0- or 75-ppm vanadium (as sodium metavanadate) throughout pregnancy and lactation. Based on default values for body weight and food intake[4] (U.S. EPA, 1988), the dose of vanadium was around 7 mg/kg-day. Maternal weight and food intake were recorded daily. When the dams gave birth, live and dead pups were counted. Pup weights were recorded at birth and every second day thereafter until PND 21. Sacrifice of both dams and pups was performed at PND 21, whereupon brain, kidney, spleen, pancreas, heart, thymus, and testes were weighed. Liver samples were collected for analysis of lipid peroxidation products (reduced glutathione, thiobarbituric acid reactivity, and superoxide dismutase activity).

The authors reported that both food intake and body-weight gain were lower in the exposed dams (statistical analysis not reported); at parturition, the cumulative weight gain appeared to be about 25% lower in exposed animals relative to controls based on visual examination of body-weight gain data presented graphically (Elfant and Keen, 1987). Data on food intake are not reported. The percentage of pups born alive was smaller in exposed dams (about 80%) than in controls (about 90%) and survival to weaning was also lower (about 40% vs. about 70% in controls based on visual examination of data presented graphically and without

[4]Default values for body weight and food intake are uncertain estimates of weight and intake for pregnant animals, but they do provide an approximate estimate of dose in the absence of study-specific data on these parameters.

statistical analysis). The cumulative weight gain of the surviving pups was lower in exposed offspring; at weaning, mean body weights of exposed pups were about 34% lower than controls (data shown graphically and without statistical analysis). Pups of exposed dams were reported to exhibit diarrhea, seborrhea, lethargy, staggered gaits, and ocular exudate (incidences not reported). The relative weights of the liver, brain, and testes were higher in exposed vs. control pups (18%, 36%, and 15%, $p < 0.05$). Reductions in body-weight gain among exposed pups complicate the interpretation of these organ weight changes. Thiobarbituric acid reactivity was elevated in whole cell homogenates from the livers of both dams and pups exposed to vanadium; reduced glutathione was lower in exposed pups than in control pups but was not affected in dams. The latter findings suggest increases in lipid peroxidation with vanadium exposure that may contribute to developmental toxicity. These data indicate a maternal and developmental LOAEL of about 7 mg/kg-day based on reduced maternal food intake and weight gain, as well as reduced pup survival, body weight, growth and clinical signs in pups. A NOAEL cannot be identified.

The effects of sodium metavanadate on development were further studied by Paternain et al. (1987). Groups of 20 pregnant Sprague-Dawley rats were treated with sodium metavanadate via gavage at doses of 0, 5, 10, or 20 mg/kg-day during GD 6–15. Equivalent doses of vanadium were 2, 4, and 8 mg V/kg-day. On GD 20, the uteri were opened by Caesarean section for examination of corpora lutea, implantations, live and dead fetuses, and resorptions. Placental weights were recorded and fetal body weight, body length, and tail length were measured. Gross abnormalities were assessed in all fetuses; half were examined for skeletal abnormalities and half were examined for visceral anomalies. The paper does not report any evaluation of maternal toxicity parameters. At the high dose, fewer litters were produced than in controls or in other dose groups (14, 14, 12, and 8 in control, 2, 4, and 8 mg V/kg-day groups, respectively), but the decrease is not statistically significant. The numbers of resorptions were increased and numbers of live fetuses decreased at both 4 and 8 mg V/kg-day; however, these differences were also not statistically significant. A slight—but statistically significant—decrease in tail length was observed at 2 and 8 mg V/kg-day (4–5%, $p < 0.01$), but not at 4 mg V/kg-day; there was no apparent dose-response relationship. The authors reported that the incidences of skeletal and visceral abnormalities were not affected by treatment (data not shown). A higher percentage of fetuses in the high-dose group exhibited facial (18%) and dorsal (10%) hemorrhages when compared with controls (2% for facial and 2% for dorsal); however, a litter-based comparison between the groups is not presented. The authors characterized the 4 mg V/kg-day dose as a NOAEL for developmental effects on the basis of the hemorrhages observed at the high dose. However, the lack of information on the litter distribution of fetuses with hemorrhages precludes a reliable determination of effect levels from these data. Further, as maternal parameters were not evaluated, no determination of maternal effect levels can be made.

Paternain et al. (1990) administered gavage doses of 0, 37.5, 75, or 150 mg/kg-day vanadyl sulfate pentahydrate (7.5, 15, or 30 mg V/kg-day) to female Swiss mice on GD 6–15. The control group included 20 mice and the treatment groups consisted of 16 or 20 mice per group. Body weight and food consumption were recorded daily and observations for morbidity and mortality were also made daily. Dams were killed on GD 18 and fetuses harvested by Caesarean section; dams were then examined for gross pathology. The following litter parameters were evaluated: number of implants, number of resorptions, and number of live and dead fetuses. Fetal sex, weight, and length were noted. Pups were examined for external,

visceral, and skeletal abnormalities. Treatment did not result in mortality or clinical signs, and food consumption was not different between the treatment and control groups. A significant ($p < 0.05$) decrease in body-weight gain of the dams occurred in all treatment groups during the treatment period (46%, 53%, and 59% below controls at low, mid-, and high doses, respectively). At termination, body weights corrected for gravid uterine weights were reduced at 15 and 30 mg V/kg-day (16% below controls at both doses; $p < 0.05$). At these doses, absolute liver and kidney weights were also reduced proportionate to the body weight decrements. A significant ($p < 0.05$) increase in early resorptions occurred in all treatment groups relative to the control group (2–6 fold higher, without a clear dose-response relationship). Fetal body weights were significantly lower (13–21%, $p < 0.001$) in all treatment groups compared to the control group. The following external and internal soft-tissue abnormalities were observed at significantly elevated incidences (with litter as unit of statistical measure, $p < 0.05$) in fetuses of treated dams: hematomas of the dorsal area (all dose levels), hematomas of the facial area and neck (15 and 30 mg V/kg-day only), anophthalmia/microphthalmia (15 mg V/kg-day), cleft palate, and micrognathia (30 mg V/kg-day). The incidences of litters with external defects (grouped across type) were 2/20, 8/20, 11/20, and 17/20 in control, low, mid-, and high doses, respectively; these were significantly ($p < 0.05$) elevated above control at all dose levels. While the incidences of soft tissue abnormalities (exclusively hydrocephaly) were increased at the mid- and high-dose, the increases were not statistically significant. However, the incidence of skeletal defects were increased at all doses (4/20, 9/16, 15/20, 20/20 affected litters in control through high dose; $p < 0.05$ for all treatment groups). The skeletal abnormalities consisted of poorly ossified supraoccipital bone, carpus, tarsus and sternebrae, as well as bipartite sternebrae and irregular ribs. These data indicate a freestanding LOAEL of 7.5 mg V/kg-day for both maternal toxicity (reduced body-weight gain during treatment) and developmental toxicity (increased resorptions, skeletal malformations, and growth delays).

In a later study by the same laboratory, Sanchez et al. (1991) administered daily gavage doses of 0, 7.5, 15, 30, or 60 mg/kg-day sodium orthovanadate (equivalent to 0, 2.1, 4.2, 8.3, or 17 mg V/kg-day) to groups of 14–20 pregnant Swiss mice on GD 6–15. Maternal appearance, body weight and food consumption were recorded daily. The dams were sacrificed on GD 18 for evaluation of body weight, liver and kidney weights, gravid uterine weight, and uterine parameters (numbers of implants, early and late resorptions, live and dead fetuses). Live fetuses were weighed, sexed, and examined grossly for abnormalities; two-thirds were then prepared for skeletal examination and one-third for visceral examination. Exposure to doses of 8.3 or 17 mg V/kg-day proved to be lethal; 4/18 dams dosed at 8.3 mg V/kg-day died, while 17/19 given the high dose died. Body-weight gain during treatment was reduced at 8.3 mg V/kg-day (30% less than controls, $p < 0.01$) and not at lower doses. Food consumption was significantly ($p < 0.05$) reduced at the beginning of treatment at both 4.2 and 8.3 mg V/kg-day. Body weight at termination, corrected for gravid uterine weight, was unaffected at any dose. Relative kidney weight was slightly—but statistically significantly—increased at 8.3 mg V/kg-day; however, the body-weight reduction at this dose may have contributed to the increased relative kidney weight. Litter parameters were not affected by exposure; at 8.3 mg V/kg-day, one litter contained no viable implants, but the incidence of litters with resorptions was not significantly increased. External and visceral malformations were not increased in exposed groups relative to controls; however, the numbers of litters containing fetuses with incompletely ossified sacrococcygeal vertebrae, forelimb and hindlimb proximal phalanges were increased at 8.3 mg V/kg-day. The authors identified the low dose (2.1 mg V/kg-day) as a NOAEL for maternal toxicity,

presumably considering the decreased food consumption at 4.2 mg V/kg-day. Given the evidence for frank effects at the next higher dose (mortality at 8.3 mg V/kg-day), the decrease in food consumption is considered potentially indicative of toxicity and is used to define the 4.2 mg V/kg-day dose as a LOAEL. The authors considered the 4.2 mg V/kg-day dose to be a NOAEL for developmental effects. For the purpose of this review, the LOAEL for developmental toxicity is 8.3 mg V/kg-day based on increases in the incidence of litters with incomplete skeletal ossification.

Ganguli et al. (1994b) exposed female Sprague-Dawley rats to 250 mg/L sodium orthovanadate (~69 mg V/L) added to drinking water on GD 10–20. The study compared the effects of treatment in diabetic (streptozocin-induced) and nondiabetic rats. The doses (calculated for this review based on reported fluid intakes and estimated body weight[5] of 250 g) were 7.5 and 17 mg V/kg-day in the nondiabetic and diabetic treatment groups, respectively. The treatment groups consisted of 11 diabetic and 7 nondiabetic pregnant females; the control groups consisted of 6 diabetic and 5 nondiabetic pregnant females given water without the addition of vanadate. Endpoints examined in dams included blood and urine glucose concentrations and fluid intake. On GD 20, the animals were sacrificed; the number of live pups and the pups' weights were recorded. Maternal uteri, ovaries, and placentas were examined grossly. Vanadium treatment was lethal in diabetic pregnant rats; only 6/11 dams survived until termination. No deaths occurred in other groups. Intake of drinking water was significantly decreased by vanadium treatment in both nondiabetic and diabetic rats. Fluid intake in the nondiabetic treatment group was approximately half that of the corresponding control group; in diabetic treated rats, fluid intake was about one-third that of the diabetic controls, who had significantly higher water intake than nondiabetic controls. Blood glucose was significantly decreased in vanadium-treated diabetic rats ($p = 0.006$), but the levels were still above those of nondiabetic rats. Urine glucose was not affected by vanadium exposure. Statistical analysis is not reported, and only data on pooled litters are reported; thus, a statistical group comparison cannot be made. Nevertheless, the outcomes included a lower average number of live fetuses on GD 20 (6.71 vs. 9.6 in treated vs. control nondiabetic rats and 5.5 vs. 11.3 in treated vs. control diabetic rats) and lower average pup mass in nondiabetic rats (3.60 vs. 4.02 g in treated vs. control) but not in diabetic rats (statistical analysis not reported). These data suggest that 17 mg V/kg-day is an FEL based on maternal mortality in the treated diabetic rats. Other effect levels cannot be determined due to poor reporting of data and limited endpoints evaluated.

Poggioli et al. (2001) assessed the effects of prenatal and postnatal exposure to vanadyl sulfate on the growth and behavior of Wistar rats. Concentrations of 0 or 300 mg/L of vanadyl sulfate (corresponding to 70 mg V/L according to the authors) were administered in the drinking water along with 5 g/L NaCl to reduce gastrointestinal effects of vanadium. An untreated control group received water without vanadyl sulfate or NaCl. Dams were exposed beginning three days before the last day of pregnancy and continued until weaning; thus, the pups were exposed during 3 days of gestation and via lactation until weaning. Litters were culled to 8–10 pups 1 day after birth and at weaning the groups were again reduced to 10/sex/dose. After weaning, the pups were given the same drinking water as their mothers until they were 100 days of age. Body weight was recorded at regular intervals and food and water intake were measured at 2 months of age. Based on recorded water intake, the vanadium dose was estimated by the authors to be about 10 mg V/kg-day. Neurobehavioral assessments were performed at 1 month

[5]The starting weights were 210–230 g; however, ending body weights were not reported.

of age (locomotor activity and open field evaluation of ambulation, rearing, grooming and defecation) and 100 days of age (memory test assessing time spent exploring new and familiar objects). Survival to weaning was significantly reduced by vanadyl sulfate treatment when compared with either the NaCl or untreated controls (61% in treated vs. 100% and 94% in NaCl and untreated controls, respectively; $p < 0.0001$). Neither food nor water intake was affected by exposure. Body weights were significantly lower than untreated controls beginning at weaning (PND 25) in the vanadyl sulfate group. However, body weights were also reduced in the NaCl group, so the effect of vanadium exposure cannot be distinguished. Locomotor activity was not different among the groups (data shown). In contrast, the open field evaluation revealed significantly ($p < 0.05$) fewer outer ambulation (ambulation in the outer area of the cage), rearing and grooming events and increased defecation in treated male rats when compared with the NaCl group; the treated males also exhibited reduced rearing events compared with untreated controls. The memory test revealed similar impairment in both the NaCl and vanadium exposure groups, which the authors attributed to NaCl exposure rather than vanadium (as the effect was similar in both). A LOAEL of 10 mg V/kg-day is identified based on reduced survival to weaning; a NOAEL cannot be identified.

***Neurotoxicity***

Sanchez et al. (1998) exposed male Sprague-Dawley rats to daily gavage doses of sodium metavanadate at dose levels of 0, 1.7, 3.4, or 6.8 mg V/kg-day for 8 weeks (12 animals per group). Endpoints assessed include body-weight gain and two neurobehavioral assessments: open-field activity and active avoidance (electric shock with auditory and light stimulus as the conditioned stimulus). Body-weight gains were significantly lower (10% below controls at the end of the exposure period, $p < 0.05$) in the 6.8 mg V/kg-day group relative to the control group. Open-field activity was lower in the 3.4 and 6.8 mg V/kg-day groups relative to the control group—but only during the first of three testing sessions ($p < 0.05$, data presented graphically). Similarly, acquisition of the avoidance response to the conditioned stimulus was significantly lower in all treatment groups ($p < 0.05$; about one-half as many avoidance responses and latencies about twice that of the control group based on graphical presentation of the data)—but only during the last of three sessions. Neither parameter exhibited a clear dose-response relationship; the magnitude of change from control was similar at all doses.

These investigators also conducted a follow-up study designed to evaluate whether the chelating agent Tiron would ameliorate the effects of vanadium exposure on behavior (Sanchez et al., 1999). Groups of 10 male Sprague-Dawley rats were given daily gavage doses of water or aqueous sodium metavanadate at a dose of 6.84 mg V/kg-day for 8 weeks. There were two groups that were also given Tiron via i.p. injection at two different doses. Body weight was measured daily. After the end of exposure, open-field activity and active avoidance were assessed as in the previous study. The authors indicated that body weight was not affected by treatment (data not shown). Graphical presentation of the data indicated no effect of exposure on open field motor activity but significant ($p < 0.05$) inhibition of active avoidance. Administration of Tiron mitigated the effects of sodium metavanadate on both of these endpoints.

***Immunotoxicity***

The limited data on immunotoxicity of vanadium suggest little or no adverse effect on this endpoint. Alexandrova et al. (2002) assessed humoral and cellular immune responses in

BALB/c mice and Wistar rats (both sexes) exposed to ammonium vanadate. Exposure to ammonium vanadate in the drinking water (0.5 mg/L or 0.2 mg V/L) for 40 or 200 days (about 6 or 28 weeks) stimulated both humoral and immune responses, as measured by increases (above control values) in the number of antibody-synthesizing cells in the spleen after challenge with sheep erythrocytes, the titers of serum agglutinins and haemolysins (humoral response) and the migration of spleen cells and peritoneal macrophages in vitro (cellular response). In contrast to the results of Alexandrova et al. (2002), Sharma et al. (1981) observed a decrease (albeit not statistically significant) in antibody-producing cells in the spleen of male Swiss-Webster mice exposed to concentrations of 0, 1, 10, or 50 mg/L vanadium (as sodium orthovanadate) in the drinking water for up to 13 weeks. No treatment-related effects were observed on delayed hypersensitivity reaction and immunoglobulin levels (IgG, IgA, and IgM) were not affected by exposure. Both Alexandrova et al. (2002) and Sharma et al. (1981) observed increased DNA synthesis in splenic lymphocytes treated with vanadium and cultured in the presence of some mitogens (phytohemagglutinin and pokeweed) but not others (bacterial lipopolysaccharide), when compared with cells not treated with vanadium.

***Inhalation Exposure***

No subchronic or chronic animal studies of inhalation exposure to vanadium compounds (other than vanadium pentoxide) have been identified in the literature search.

**Other Studies**

***Toxicokinetics***

In the United States, exposure to vanadium primarily occurs through dietary sources. Estimates of the daily intake of vanadium in the diet are in the range of 10–30 μg V/day or 0.0001 to 0.0004 mg V/kg-day for an adult man (WHO, 2001). Few studies are available on the absorption of vanadium from the gastrointestinal tract in humans or experimental animals; however, existing data suggest a relatively low fractional absorption. WHO (2001) estimated the gastrointestinal absorption of vanadium to be about 3% of the administered dose based on animal studies. Therefore, a relatively small absolute difference in gastrointestinal absorption between rodents and humans could result in a large error in the equivalent dose extrapolation. There are no studies that allow a direct comparison of the absorption of vanadium when administered as vanadyl or vanadate compounds.

Once absorbed, vanadium is distributed primarily to the bone, with smaller amounts distributing to the kidney, liver, spleen, muscle, and testes (ATSDR, 1992; WHO, 2001; Rydzynski, 2001). Vanadium stored in bone is retained much longer than in other tissues, from which vanadium is rapidly excreted (ATSDR, 1992; Rydzynski, 2001). Urine appears to be the major excretory route for absorbed vanadium, while unabsorbed vanadium is excreted in the feces (ATSDR, 1992; WHO, 1988, 2001).

In blood, vanadyl and vanadate ions are interconverted through redox reactions that may involve glutathione, cysteine, ascorbate, and possibly other components of plasma and cytosol (Rehder and Jantzen, 1998). Vanadium in blood partitions between plasma and erythrocytes. In beagle dogs administered single intravenous injections of vanadyl sulfate or ammonium vanadate, approximately 30–45% of the vanadium in blood was associated with erythrocytes and approximately 80% of vanadium in serum was associated with transferrin (Harris et al., 1984). Albumin also participates as a protein ligand for vanadyl and vanadate in plasma

(Chasteen et al., 1986a,b). Vanadyl and vanadate form complexes with a variety of intracellular proteins including ATPases, calmodulin, kinases and phosphatases, ribonucleases and nucleic acids (Rehder and Jantzen, 1998). The redox state of the cytosol favors the intracellular reduction of vanadate to vanadyl, whereas the oxidation of vanadyl to vanadate is favored in plasma; the interconversion occurs in minutes (Etcheverry and Cortizo, 1998).

***Antineoplastic Studies***

Vanadium has been tested as an antineoplastic agent in animal models of colon, liver, and mammary carcinogenesis. All of the studies of this effect that were identified in the literature searches were conducted by a single laboratory. In all of the studies, vanadium was administered as ammonium monovanadate to rats at a concentration of 0.5 ppm in drinking water. Vanadium coadministration reduced the number of aberrant crypt foci (a preneoplastic lesion in colon cancer) in rats treated with 1,2-dimethylhydrazine and resulted in fewer colon tumors (Kanna et al., 2003, 2004, 2005). Mechanistic data collected in these studies showed that vanadium treatment reduced the number of DNA-protein cross-links and evidence of DNA damage in colon cells, reduced the PCNA index, decreased the frequency of chromosomal aberrations and increased glutathione S-transferase and cytochrome p450 levels when compared with rats treated with carcinogen alone (Kanna et al., 2003, 2004, 2005). Similar findings were observed in rat models of hepatocarcinogenesis. In rats treated with 2-acetylaminofluorene (2-AAF) or diethyl nitrosamine (DEN) and subsequently given vanadium, relative liver weight, incidence of gamma glutamyl transpeptidase (GGT)-positive foci, nodular incidence, number of liver nodules and multiplicity of nodules were reduced compared with treatment with the carcinogen alone (Chakraborty et al., 2005; 2006a,b,c; 2007a,b). Vanadium treatment reduced the frequency of modified DNA bases, DNA damage, and chromosomal aberrations; reduced the expression of metallothionein (a metalloprotein associated with neoplastic cell growth) and Ki-67 nuclear antigen; and increased the expression of p53 tumor suppressor (Chakraborty et al., 2005; 2006a,b,c; 2007a,b). Further evidence of a potential antineoplastic effect of vanadium was provided in studies of rat mammary carcinogenesis. Vanadium treatment reduced the incidence, total number, multiplicity and size of mammary tumors in rats pretreated with 7,12-dimethylbenz(a)anthracene (Ray et al., 2004; 2005a,b; 2006). Ray et al. (2006) used immunohistochemical analysis to show that vanadium exposure increased apoptosis in mammary tissues; p53 and Bax genes were upregulated, while the antiapoptotic protein Bcl2 was downregulated by vanadium. In studies performed in another laboratory, a vanadium-cysteine complex was effective in prolonging survival, reducing the rate of benzo(a)pyrene-induced leiomyosarcoma growth, and inducing some tumor remission when given to male rats beginning on the day a palpable tumor was observed (Evangelou et al., 1997; Liasko et al., 1998).

***Mechanistic***

Etcheverry and Cortizo (1998) reviewed the action of vanadium on cells in culture. Their review indicated that vanadate acts as an analogue of phosphate, resulting in the modification (stimulation or inhibition) of several enzymes involved in phosphate metabolism. In in vitro systems, vanadium compounds have been shown to inhibit Na+K+ ATPase, Ca2+ ATPase, H+K+ ATPase, H+-ATPase, K+ATPase, Ca+Mg+ATPase, dynein ATPase, actomysosin ATPase, protein tyrosine phosphatase, glutamine dehydrogenase, acid and alkaline phosphatases, glucose-6-phosphatase, phosphofructokinase, alanine aminotransferase, aspargine aminotransferase, ribonuclease, phosphodiesterase, phosphotyrosyl-phosphatase, while stimulating phospholipase C, adenyl cyclase, mitogen-activated protein kinases,

phosphatidylinositol 3-kinase, NADPH oxidase, glycogen synthase, lipoprotein lipase, and tyrosine kinase phosphorylase (Etcheverry and Cortizo, 1998; Rydzynski, 2001). In addition, vanadium is a strong mitogen, inducing cell proliferation in a number of different systems (including fibroblasts, Leydig cells, and bone cells); the mechanism for this effect may be related to the inhibition of protein tyrosine phosphatases (Etcheverry and Cortizo, 1998). The effects of vanadium on various enzymes, which, in turn, affect many systems, may be responsible for the diverse effects seen in vivo—including modulation of diabetes, renal effects, reproductive and developmental toxicity and cardiovascular effects. In a recent review, Coderre and Srivastava (2004) proposed a potential mechanism of action for the cardiovascular effects of vanadium. In the proposed scheme, vanadium inhibition of protein tyrosine phosphatases results in the intracellular release of calcium and activation of phosphatidylinositol 3-kinase (PI3K) and p38-mitogen activated protein kinase (p38 MAPK) signaling pathways; these effects, in turn, stimulate smooth muscle contraction and glucose uptake (Coderre and Srivastava, 2004). Vanadium causes contraction of several types of smooth muscles, including gastric and vascular smooth muscle (Coderre and Srivastava, 2004). The effects of vanadium on smooth muscle contraction and glucose uptake may help to explain the in vivo modulation of blood pressure by vanadium. The authors noted that vanadium has exerted both vasodilation and vasoconstriction effects in different systems; thus, the action of vanadium on blood pressure may vary with dose, duration, and model system (Coderre and Srivastava, 2004).

***Genotoxicity***

Genotoxicity testing of soluble inorganic vanadium salts have primarily given positive results for mutagenicity and clastogenicity (especially numerical chromosomal aberrations). In the *Bacillis subtilis* $Rec^-$ mutagenicity screening assay, ammonium metavanadate gave a positive result (greater inhibition of the $Rec^-$ strain than the wild type $Rec^+$ strain) at a concentration of 0.3 M (Kanematsu et al., 1980). However, spot mutation tests with *Escherichia coli* (B/r WP2 and WP2) and *Salmonella typhimurium* (TA1535, TA100, TA98, TA1537, and TA1538) were negative for this compound (Kanematsu et al., 1980). Ammonium metavanadate induced mitotic gene conversion and reverse point mutations in *Saccharomyces cerevisia* (strain D7) when tested at concentrations from 80–210 nM with and without S9 (Bronzetti et al., 1990). Greater numbers of conversions and mutations were observed in the absence of S9, suggesting that the metabolism of ammonium metavanadate may detoxify the compound. In a study of cultured Chinese hamster V79 and V79-derived *hprt*$^-$/*gpt*$^+$ transgenic G12 cells, ammonium metavanadate exposure resulted in weak, but concentration-related increases in *hprt* mutations in V79 cells and in *gpt* mutations in G12 variants when the cells were exposed for 24 hours at concentrations from 5–50 µM (Cohen et al., 1992; Klein et al., 1994). Owusu-Yaw et al. (1990) reported that vanadyl sulfate and ammonium metavanadate both induced significant ($p < 0.01$) increases in the frequency of sister chromatid exchanges (SCE) in Chinese hamster ovary (CHO) cells treated with and without S9. Concentrations resulting in increases in SCE were about 6 and 2 µg V/mL for vanadyl sulfate and ammonium metavanadate, respectively. These compounds also induced dose-related increases in the frequency of chromosomal aberrations at concentrations near those causing cytotoxicity. Cytotoxic concentrations ($TC_{50}$s) were 23 and 16 µg V/mL for vanadyl sulfate and ammonium metavanadate, respectively (Owusu-Yaw et al., 1990). In human lymphocytes cultured in vitro, sodium metavanadate, sodium orthovanadate and ammonium metavanadate and vanadyl sulfate resulted in increased frequencies of micronuclei and numerical chromosomal aberrations (primarily hypoploidy) at doses as low as 5 uM (Migliore et al., 1993). SCEs were induced at higher doses (Migliore et al., 1993).

In vivo studies in mice indicated that vanadyl sulfate (100 mg/kg body weight), sodium orthovanadate (75 mg/kg) and ammonium metavanadate (50 mg/kg) administered by gavage all increased the frequency of micronucleated polychromatic erythrocytes (2- to 3-fold increase over controls) (Ciranni et al., 1995). The frequencies of hypoploid (missing chromosomes) and hyperploid (having an excess of chromosomes) cells were also increased by both compounds. Only vanadyl sulfate exposure resulted in a statistically significant ($p < 0.05$) increase (up to 7-fold above control values) in structural chromosomal aberrations (Ciranni et al., 1995). Mice exposed for 5 months to sodium orthovanadate in drinking water were observed to exhibit statistically significant increases in bone marrow micronuclei (at exposure concentrations of 750 or 1500 mg/L) as well as evidence of DNA damage in splenocytes (measured by comet assay, at a concentration of 1500 mg/L)—but not in bone marrow cells, testis cells or epididymal sperm (Leopardi et al., 2005). In another study, oral exposure to drinking water containing vanadyl sulfate (2–1000 mg/L) did not increase the frequency of micronuclei in bone marrow polychromatic erythrocytes in male CD-1 mice exposed for 5 weeks (Villani et al., 2007). In reticulocytes from these same mice, the frequency of micronuclei was slightly increased at some exposure levels, but there was no dose-response relationship (Villani et al., 2007).

## DERIVATION OF PROVISIONAL SUBCHRONIC AND CHRONIC ORAL RfD VALUES FOR VANADIUM AND COMPOUNDS

Equal intakes of vanadium in any of the forms considered (vanadyl sulfate, sodium metavanadate, sodium orthovanadate, and ammonium metavanadate) were treated as toxicologically equivalent for the purpose of deriving provisional oral toxicity values on the following basis: (1) there is very little quantitative information about the gastrointestinal absorption of vanadium and no evidence that the absorption of vanadium will be substantially affected by the form of vanadium ingested for this set of compounds and (2) although there is evidence for pharmacologic specificity of the actions of vanadate and vanadyl ions in various biochemical systems, these forms are rapidly (within minutes) interconverted in the body in oxidation-reduction reactions that take place in the intracellular and extracellular compartments (Etcheverry and Cortizo, 1998; Mendz, 1998; Rydzynksi, 2001).

A total of four studies of humans exposed to vanadium compounds for brief durations (up to 12 weeks) are available; Table 9 provides an overview of the findings in these studies. Of these, three studies were of patients with diabetes. All of the studies used vanadyl sulfate in tablet form. Endpoints assessed in the studies included body weight, gastrointestinal symptoms, hematology, glycemic control, serum chemistry parameters, urinalysis, liver, kidney or thyroid function tests, and blood pressure. None of the studies reported significant effects on any endpoint other than gastrointestinal symptoms. Of particular note is the apparently normal kidney function and the absence of a blood pressure effect at daily doses as high as 0.5 to 1.1 mg V/kg-day. However, the exposure groups were small, no histopathology was possible, and often no referent population is included. While the individual studies are limited, the human studies collectively provide a short-term human LOAEL of approximately 0.3 mg V/kg-day in humans based on symptoms of gastrointestinal distress, including diarrhea, cramping, and discomfort. The NOAEL for these effects is approximately 0.1 mg V/kg-day based on the available studies. Gastrointestinal effects (severe diarrhea) have also been observed in rats

exposed to vanadium in drinking water (Zaporowska and Wasilewski, 1989, 1990, 1992a,b; Ganguli et al., 1994b); in rabbits exposed via drinking water (Khasibhatla and Rai, 1993) and in pigs exposed via the diet (Van Vleet et al., 1981; Van Vleet and Boon, 1980), providing support for the observed relationship between vanadium exposure and diarrhea in humans. The doses resulting in diarrhea in laboratory animals were in the 5–20 mg V/kg-day range. Studies in rats and mice indicate that vanadium exposure may be associated with effects on body weight, hematology, kidney function, blood pressure and reproduction. Animal studies that meet minimum criteria for possible use in deriving subchronic or chronic provisional RfDs (e.g., effect levels could clearly be identified) are summarized in Table 10. It should be noted that some of the LOAELs shown in Table 10 were identified for effects in partially nephrectomized rats (Steffen et al., 1981; Susic and Kentera, 1988) or in diabetic rats (Domingo et al., 1991, 1992).

**Table 9. Human Studies of Oral Exposure to Vanadium Compounds**

| Study Description | Dose (mg V/ kg-day) | Vanadium Form Administered | NOAEL (mg V/ kg-day) | LOAEL (mg V/ kg-day) | Responses at the LOAEL | Comments | Reference |
|---|---|---|---|---|---|---|---|
| Human, 4 M and 7 F Tablet, daily for 6 weeks after 2-week run-up | 0.5 (M)<br>0.6 (F) | Vanadyl sulfate (assumed trihydrate) | NA | 0.5 (M)<br>0.6 (F) | Gastrointestinal symptoms | Patients with type 2 diabetes | Cusi et al., 2001 |
| Human, 11 M and 5 F Tablet, daily for 6 weeks | 0.12–0.23<br>0.28–0.45<br>0.43–1.14 | Vanadyl sulfate (assumed trihydrate) | 0.12–0.23 | 0.28–0.45 | Gastrointestinal symptoms | Patients with type 2 diabetes | Goldfine et al., 2000 |
| Human, 12–13 M and 4 F Tablet, daily for 12 weeks | 0, 0.1 | Vanadyl sulfate trihydrate | 0.1 | NA | None | Weight trainers | Fawcett et al., 1997 |
| Human, 4 M and 4 F Tablet, daily for 4–8 weeks | 0.34 (M)<br>0.39 (F) | Vanadyl sulfate (assumed trihydrate) | NA | 0.34 (M)<br>0.39 (F) | Gastrointestinal symptoms | Patients with type 2 diabetes | Boden et al., 1996 |

Effects on blood pressure have been associated with vanadium exposure, although the available studies provide conflicting results. Boscolo et al. (1994) found a significant increase in systolic and diastolic blood pressure in rats exposed to 0.12, 1.2, or 4.7 mg V/kg-day as sodium metavanadate in the drinking water for 6 months. Carmignani et al. (1991) reported similar findings at a dose of 12 mg V/kg-day (as sodium metavanadate in drinking water). The increases in blood pressure are not corroborated by the Schroeder et al. (1970) chronic rat study or the Dai et al. (1994b) 52-week study in rats. Steffen et al. (1981) and Susic and Kentera (1988) reported increases in blood pressure in partially nephrectomized rats exposed to sodium orthovanadate and sodium metavanadate (respectively). In addition to these subchronic and chronic studies, a shorter-term study reported increased blood pressure in lean Zucker rats exposed to vanadium in the drinking water (about 10 mg V/kg-day) for 25 days (Hopfner et al., 1999). Several differences in the studies need to be taken into consideration in cross-study comparisons; Table 11 shows the major differences, which include the form of vanadium

**Table 10. Animal Studies of Oral Exposure to Vanadium Compounds**

| Study Description | Dose (mg V/kg-day) | Vanadium Form Administered | NOAEL (mg V/kg-day) | LOAEL (mg V/kg-day) | Responses at the LOAEL | Comments | Reference |
|---|---|---|---|---|---|---|---|
| *Shorter-term* | | | | | | | |
| Male Sprague-Dawley rats (10/group) were exposed via drinking water for 28 days | 0, 6.1, 15.6, 22.7 | Sodium metavanadate, sodium orthovanadate, vanadyl sulfate | NA | 6.1 | Body-weight loss in diabetic rats | No nondiabetic treatment group | Domingo et al., 1991 |
| Male Sprague-Dawley rats (10/group) were exposed via drinking water for 5 weeks | 0, 23.2 | Sodium metavanadate | NA | 23.2 | Body-weight loss in diabetic rats | No nondiabetic treatment group | Domingo et al., 1992 |
| Male and female Wistar rats (15–16/sex/group) were exposed via drinking water for 4 weeks | 0, 1.2, 5 (males) 0, 1.5, 7 (females) | Ammonium metavanadate | 1.2 (males); 1.5 (females) | 5 (males); 7 (females) | Reduced body weight (with reduced fluid intake) and hematology changes | | Zaporowska et al., 1993 |
| *Subchronic* | | | | | | | |
| Male Sprague-Dawley rats (20/group) were exposed via the diet for 9 weeks | 0, 9 | Sodium orthovanadate | NA | 9 | Decreased weight gain and increased blood pressure in uninephrectomized rats | | Steffen et al., 1981 |
| Male weanling pigs (6) were exposed via drinking water for 12 weeks | 0, 10 | Ammonium metavanadate | NA | 10 (FEL) | Emaciation and mortality | | Van Vleet, 1981 |
| Male Sprague-Dawley rats (10/group) were exposed via drinking water for 12 weeks | 0, 0.3, 0.6, 3.0 | Sodium metavanadate | NA (0.6, ATSDR, 1992) | 0.3 – 3.0 (indeterminate) | Mild changes in the kidney (hemorrhagic foci in the corticomedullary region), spleen (hypertrophy and hyperplasia) and lungs (perivascular mononuclear cell infiltration) | Occurring in all treatment groups, but "more evident" in the high-dose group. Clear AEL at 3 mg/kg-day. | Domingo et al., 1985 |
| Male Long-Evans rats (15/group) were exposed via the diet for 2 months | 0, 12 | Ammonium metavanadate | NA | 12 | Pulmonary hypertension | | Susic and Kentera, 1986 |
| Male Wistar rats (8/group) were exposed via drinking water for 12 weeks | 0, 7.7, 9.7 | Ammonium metavanadate, vanadyl sulfate | 7.7, 9.7 | NA | No effects on food intake, body weight, hematology | Fluid intake was reduced at this dose | Dai et al., 1995 |

**Table 10. Animal Studies of Oral Exposure to Vanadium Compounds**

| Study Description | Dose (mg V/kg-day) | Vanadium Form Administered | NOAEL (mg V/kg-day) | LOAEL (mg V/kg-day) | Responses at the LOAEL | Comments | Reference |
|---|---|---|---|---|---|---|---|
| Female Wistar rats (7/group) were exposed via the diet for 10 weeks | 0, 1.1, or 2.3 | Sodium metavanadate | 2.3 | NA | Small changes in hematology and body weight were not considered toxicologically significant | | Adachi et al., 2000 |
| Male Wistar rats (11–16/group) were exposed via drinking water for 6 weeks | 0, 8 | Sodium metavanadate | NA | 8 | Reduced body weight gain (possibly related to reduced food and fluid intake); hematologic effects | | Scibior, 2005 |
| Male Wistar rats (12/group) were exposed via drinking water for 6 weeks | 0, 11 | Sodium metavanadate | NA | 11 | Reduced body weight gain (possibly related to reduced food and fluid intake); hematologic effects | | Scibior et al., 2006 |
| *Intermediate* | | | | | | | |
| Male Long-Evans rats (12–24/group) were exposed via the diet for 24 weeks | 0, 4.4, 42 | Sodium metavanadate | NA | 4.4 | Increased blood pressure in partially nephrectomized rats | Blood pressure not affected in rats with intact kidneys | Susic and Kentera, 1988 |
| Male Sprague-Dawley rats (10/group) were exposed via drinking water for 7 months | 0, 12 | Sodium metavanadate | NA | 12 | Increased blood pressure, kidney histopathology | | Carmignani et al., 1991 |
| Male Wistar rats (12/group) were exposed via drinking water for 5 months | 0, 12 | Vanadyl sulfate | NA | 12 | Decreased body weight in treated nondiabetic rats relative to nondiabetic controls | | Cam et al., 1993 |
| Male Sprague-Dawley rats (6/group) were exposed via drinking water for 180 or 210 days | 0, 0.12, 1.2, 4.7 | Sodium metavanadate | NA | 0.12 | Increased blood pressure, stimulation of the renin-angiotensin-aldosterone system, and kidney histopathology | | Boscolo et al., 1994 |

**Table 10. Animal Studies of Oral Exposure to Vanadium Compounds**

| Study Description | Dose (mg V/kg-day) | Vanadium Form Administered | NOAEL (mg V/kg-day) | LOAEL (mg V/kg-day) | Responses at the LOAEL | Comments | Reference |
|---|---|---|---|---|---|---|---|
| *Chronic* | | | | | | | |
| Long-Evans rats were exposed via drinking water from weaning through natural death (up to 45 months) | 0, 0.7 (males ), d 0.9 (females) | Vanadyl sulfate | NA | NA | No effects observed | Histological evaluations inadequate to detect any but the most severe lesions; effect levels cannot be determined | Schroeder et al., 1970 |
| Male Sprague-Dawley rats (>20/group) were exposed via diet for 56 weeks | 0, 7, 14 | Sodium orthovanadate | NA | 7 | Increased blood pressure in uninephrectomized rats | | Steffen et al., 1981 |
| Male Wistar rats (8/group) were exposed via drinking water for 52 weeks | 0, 8, 13 or 21 | Vanadyl sulfate | NA | 8 | Reduced body-weight gain | Diabetic and nondiabetic rats | Dai et al., 1994a,b; Dai and McNeill, 1994 |
| *Reproductive* | | | | | | | |
| Male and female Sprague-Dawley rats (20/sex/group) were exposed via drinking water for 60 (M) or 14 (F) days premating and during gestation and lactation (F) | 0, 2.1, 4.2, 8.4 | Sodium metavanadate | NA | 2.1 (offspring) | Growth retardation in pups | Maternal effect levels could not be identified due to lack of information on endpoints assessed | Domingo et al., 1986 |
| Male and female Sprague-Dawley rats (10 M and 20 F/group) were exposed via drinking water for 70 days (M) or through premating, mating, gestation and lactation (61 days, F) | 0 or 28 (M) or 30 (F) | Ammonium metavanadate | NA | 28 | Effects on reproductive success, litter parameters, postnatal growth, male reproductive organ weights and skeletal malformations | | Morgan and El-Tawil, 2003 |
| Male Swiss mice (24/group) were exposed via drinking water for 64 days prior to mating | 8.4, 17, 25.1 or 33.4 | Sodium metavanadate | 17 | 25.1 | Decreased spermatozoa counts and reduced fecundity | | Llobet et al., 1993 |

**Table 10. Animal Studies of Oral Exposure to Vanadium Compounds**

| Study Description | Dose (mg V/kg-day) | Vanadium Form Administered | NOAEL (mg V/kg-day) | LOAEL (mg V/kg-day) | Responses at the LOAEL | Comments | Reference |
|---|---|---|---|---|---|---|---|
| *Developmental* | | | | | | | |
| Pregnant Sprague-Dawley rats (14/group) were exposed via the diet throughout pregnancy and lactation | 0, 7 | Sodium metavanadate | NA | 7 (maternal and develop-mental) | Reduced food intake and weight gain (maternal); reduced pup survival, body weight, growth and clinical signs (developmental) | | Elfant and Keen, 1987 |
| Pregnant Swiss mice (16–20/group) were exposed via daily gavage on GD 6–15 | 0, 7.5, 15.1 or 30.2 | Vanadyl sulfate pentahydrate | NA | 7.5 (maternal and develop-mental) | Reduced body-weight gain (maternal) Increased resorptions, growth deficits, external and skeletal abnormalities (developmental) | | Paternain et al., 1990 |
| Pregnant Swiss mice (14–18/group) were exposed via daily gavage on GD 6–15 | 0, 2.1, 4.2, 8.3 or 16.6 | Sodium orthovanadate | 2.1 (maternal) 4.2 (develop-mental) | 4.2 (maternal) 8.3 (develop-mental) | Reduced food consumption (maternal) Delayed skeletal ossification (developmental) | Maternal deaths occurred at 8.3 mg V/kg-day | Sanchez et al., 1991 |
| Male and female Wistar rats, (8–10/group) were exposed via drinking water from 3 days before birth until 100 days of age | 0, 10 | Vanadyl sulfate | NA | 10 | Reduced survival to weaning | | Poggioli et al., 2001 |

administered, the method of administration, the renal status of the affected animals, the strain of the affected animals and the method by which blood pressure was measured. All the blood pressure increases were from exposure to the vanadate; there were no blood pressure increases in the only two studies that used the vanadyl salt. Given the rapid interconversion of the two forms in plasma and cytosol, this discrepancy cannot be explained. Blood pressure was generally increased by 20–25 mm Hg over a 100-fold dose range within and among studies; this is particularly noted for the companion studies of Carmignani et al. (1991) and Boscolo et al. (1994) in which an interaction with thiopentane cannot be ruled out. Except for the shortest study of 25 days (Hopfner et al., 1999), there is no apparent exposure-duration effect on the magnitude of the blood pressure increase from exposure to vanadium for 9 to 56 weeks. No effects on blood pressure were observed in the human studies at doses as high as 0.5–1 mg V/kg-day (Boden et al., 1996; Fawcett et al., 1997; Goldfine et al., 2000; Cusi et al., 2001). Overall, these studies establish a NOAEL of at least 0.3 mg V/kg-day for blood pressure effects in humans for short-term exposure (6 weeks).

**Table 11. Comparison Among Studies in which Blood Pressure was Measured**

| Study | Observed Effect on Blood Pressure[a] | Magnitude of Effect (mm Hg) | Form and Method of Vanadium Administration | Renal Status and Strain of Affected Animals | Method of Blood Pressure Measurement |
|---|---|---|---|---|---|
| Boscolo et al., 1994 | Increase at ≥0.12 mg V/kg-day | 25 (not dose-related) | Na metavanadate in drinking water for 6 months | Intact Sprague-Dawley rats | Arterial cannula under thiopentane anesthesia |
| Carmignani et al., 1991 | Increase at 12 mg V/kg-day | 22 | Na metavanadate in drinking water for 7 months | Intact Sprague-Dawley rats | Arterial cannula under thiopentane anesthesia |
| Steffen et al., 1981 | Increase at 9 mg V/kg-day | 20 | Na metavanadate in diet for 9 weeks | Uninephrectomized Sprague-Dawley rats | Tail cuff in conscious animals |
| Steffen et al., 1981 | Increase at 7, 14 mg V/kg-day | 10, 25 | Na metavanadate in diet for 56 weeks | Uninephrectomized Sprague-Dawley rats | Tail cuff in conscious animals |
| Hopfner et al., 1999 | Increase at 10 mg V/kg-day | 15 | Na orthovanadate in drinking water for 25 days | Intact lean Zucker rats | Tail cuff in conscious animals |
| Susic and Kentera, 1988 | Increase at 4.4 mg V/kg-day | 22 | Na orthovanadate in diet for 24 weeks | Partially nephrectomized Long-Evans rats | Arterial cannula under nembutal anesthesia |
| Susic and Kentera, 1988 | None at 42 mg V/kg-day | 0 | Na orthovanadate in diet for 24 weeks | Intact Long-Evans rats | Arterial cannula under nembutal |
| Dai et al., 1994b | None at 21 mg V/kg-day | 0 | Vanadyl sulfate in drinking water for 1 year | Intact Wistar rats | Tail cuff in conscious animals |
| Schroeder et al., 1970 | None at 0.7 mg V/kg-day | 0 | Vanadyl sulfate in drinking water for 45 months | Intact Long-Evans rats | Anaesthetized animals; method not specified |

[a]Effect observed at lowest dose tested in all positive studies

Studies conducted by Susic and Kentera (1988) in which several cardiovascular endpoints were assessed provide some information as to why vanadium exposure may increase blood pressure in some animals and not in others. In rats with intact kidneys exposed to doses up to 42 mg V/kg-day, a vanadium-related increase in peripheral resistance was offset by a reduction in cardiac output and blood pressure remained stable (no effect on blood pressure was observed). In partially nephrectomized rats, there was no compensatory reduction in cardiac output; thus, an increase in blood pressure was observed (Susic and Kentera, 1988). Thus, one potential explanation as to why blood pressure was not increased in every study is that compensatory mechanisms may serve to modulate the effect on blood pressure. If so, then individuals with health conditions that compromise these compensatory mechanisms (e.g., impaired renal function) may be at greater risk from vanadium exposure, although this hypothesis must be considered somewhat speculative.

Limited mechanistic information also supports a potential relationship between vanadium exposure and blood pressure changes. Boscolo et al. (1994) showed that vanadium exposure can modify plasma levels of proteins involved in blood pressure homeostasis. In this study, exposure to sodium metavanadate at doses of 1.2 or 4.7 mg V/kg-day resulted in increases in plasma renin activity (an enzyme that converts angiotensin to angiotensin I, a precursor to the vasoconstrictor angiotensin II) and aldosterone (a hormone involved in salt:water balance), as well as increases in urinary excretion of kallikrein (an enzyme that releases vasodilating kinins from plasma proteins) and kininases I and II (enzymes that break down kinins). The effects on the renin-angiotensin-aldosterone system are consistent with the observed increases in blood pressure.

Several studies (Domingo et al., 1985; Gorski and Zaporowska, 1982; Zaporowska, 1987; Dai et al., 1994a,b; Dai and McNeill, 1994) have indicated that the kidney is a primary target organ of vanadium toxicity in male rats. Among these, the study identifying effects at the lowest dose was Domingo et al. (1985). This study reported histopathologic changes in kidneys of rats exposed to sodium metavanadate in drinking water at dosages of 0.3 mg V/kg-day and higher. However, as previously noted, this study is limited in that only three animals per exposure group were actually subjected to a histopathological assessment and the results are summarized without a qualitative or quantitative reporting of incidence and severity. As a result, it is difficult to verify that the observed effects were clearly increased by exposure. Boscolo et al. (1994) reported hydropic degeneration in the kidneys of rats exposed to sodium metavanadate at a dose of 1.2 mg V/kg-day (in drinking water) for 1 year, with additional histopathologic changes (narrowing of the lumen and appearance of amorphous casts in the renal proximal tubules) at the next higher dose (4.7 mg V/kg-day). The latter changes were also observed by Carmignini et al. (1991) at a drinking water dose of 12 mg V/kg-day for 1 year. Dai et al. (1994a,b; Dai and McNeill, 1994) reported an increased incidence of glomerular and tubular degeneration, with interstitial cell infiltration and fibrosis in the kidneys of rats exposed to vanadyl sulfate in the drinking water at doses of 8–21 mg V/kg-day for a year. Limited information provided in English abstracts of two Polish studies (Gorski and Zaporowska, 1982; Zaporowska, 1987) suggested renal histopathology in rats exposed to 12–29 mg V/kg-day as ammonium metavanadate. Of all of these studies, only Boscolo et al. (1994) identified an unequivocal NOAEL for kidney effects.

Clinical chemistry changes indicative of renal effects have also been reported in a number of animal studies, although similar changes have not been observed in human studies (Fawcett et al., 1997; Boden et al., 1996). Increases in plasma urea concentrations have been observed at a dose of 3.0 mg V/kg-day in rats treated with sodium metavanadate (Domingo et al., 1985) and at higher doses in a number of studies (Domingo et al., 1991, 1992; Dai et al., 1994a,b; Dai and McNeill, 1994). Serum creatinine was higher in diabetic rats exposed to 6.1–22.7 mg V/kg-day as sodium metavanadate (Domingo et al., 1991) but not in a follow-up study in which diabetic rats were exposed to 23.2 mg V/kg-day as sodium metavanadate (Domingo et al., 1992). Susic and Kentera (1988) observed no changes in indicators of renal function (plasma creatinine, 24-hour creatinine clearance, urinary sodium excretion, and urine output) in normal and partially nephrectomized Long-Evans rats exposed to 4.4 or 42 mg V/kg-day as sodium metavanadate. Boscolo et al. (1994) observed increased potassium excretion after rats were exposed to sodium metavanadate at 1.2 and 4.7 mg V/kg-day, but no changes in urinary creatinine, nitrogen, proteins, sodium, or calcium. It should be noted vanadium was administered in drinking water in all of the studies that indicated clinical chemistry changes related to renal function. A number of studies have shown reductions in fluid intake, including marked reductions at doses of ≥10 mg V/kg-day, when vanadium is incorporated into the drinking water of rats. Thus, the changes in renal function parameters may have been influenced to an unknown degree by decreases in fluid intake, particularly at the higher exposure levels. No changes in renal function were observed in the human studies at doses as high as 0.5–1 mg V/kg-day (Boden et al., 1996; Fawcett et al., 1997; Goldfine et al., 2000; Cusi et al., 2001). Overall, these studies establish a NOAEL of at least 0.3 mg V/kg-day for overt kidney effects in humans for short-term exposure (6 weeks).

Limited mechanistic information in animals also provides some support for potential renal toxicity after vanadium exposure. Adachi et al. (2000) measured higher levels of lipid peroxidation products in the kidneys of rats exposed to 2.3 mg V/kg-day. Boscolo et al. (1994) reported reductions in Na+ K+ ATPase in the kidneys of rats exposed to 4.7 mg V/kg-day as sodium metavanadate; vanadium is known to inhibit the sodium-potassium ATPase (Etcheverry and Cortizo, 1998; Rydzynski, 2001). In addition, studies of vanadium distribution after oral exposure indicate that higher levels of vanadium are observed in the kidneys than in other organs, providing support for this organ as a potential target of vanadium toxicity.

Available data also supports a finding of reproductive and developmental toxicity associated with vanadium exposure. Effects observed in the available studies (see Table 10), conducted in both rats and mice, include diminished fertility, reduced offspring viability, growth retardation of offspring and skeletal malformations (Morgan and El-Tawil, 2003; Poggioli et al., 2001; Llobet et al., 1993; Sanchez et al., 1991; Paternain et al., 1990; Elfant and Keen, 1987; and Domingo et al., 1986). In addition to the studies shown in the table, several other studies are not suitable for derivation of provisional toxicity values, but they do contribute to the overall database for reproductive and developmental toxicity. There were three studies published in Spanish that provide suggestive evidence that vanadium exposure (as ammonium metavanadate) may result in histopathologic changes in the ovaries (Nava de Leal et al., 1998), effects on the developing central nervous system (especially the olfactory bulbs; Faria de Rodriguez et al., 1998a) and growth delays (Faria de Rodriguez et al., 1998b). In a study with poorly-reported information on the treatment regimen,

Ganguli et al. (1994b) reported reduced rate of conception and reduced ability to carry pregnancy to term in rats exposed to sodium orthovanadate.

**Subchronic p-RfD**

Data pertinent to the derivation of a subchronic p-RfD for vanadium include short-term human studies, short-term (4–5 weeks) and subchronic animal studies, and reproductive and developmental toxicity studies. In addition, several studies of slightly longer duration (5–7 months) have some bearing on the subchronic p-RfD because of the kidney and blood pressure endpoints. Blood pressure and kidney effects were fairly common among the rat studies. Kidney toxicity was implied in the Domingo et al. (1985) 3-month study at doses as low as 0.3 mg/kg-day, although it was not clear as to whether this was a LOAEL; ATSDR (1992) determined that the 0.6 mg/kg-day exposure level was a NOAEL. Subsequent to that determination, Boscolo et al. (1994) found mild kidney lesions in rats after a 6-month exposure to 1.2 mg/kg-day, but none at 0.12 mg/kg-day. The most sensitive effect found by Boscolo et al. (1994) was increased blood pressure at 0.12 mg/kg-day; although a 6-month study is somewhat longer than subchronic, the findings are relevant to the subchronic p-RfD assessment because they establish a much lower LOAEL for this effect. All the kidney and blood-pressure effects occurred in male rats, but there was no direct indication that the kidney toxicity in any of these studies was a result of $\alpha_{2u}$-globulin accumulation. The relevant studies apparently did not test for the presence of $\alpha_{2u}$-globulin. However, as vanadium binds readily to proteins and is a protease inhibitor, accumulation of $\alpha_{2u}$-globulin in the proximal tubule cells, leading to tissue necrosis, is plausible. The hemorrhagic foci in the corticomedullary region described by Domingo et al. (1985) could indicate proximal tubule necrosis. However, the criteria for establishing an $\alpha_{2u}$-globulin mode of action have not been met. The human studies collectively identify a NOAEL of at least 0.3 mg V/kg-day for increased blood pressure and overt kidney toxicity, the most sensitive effects in rats; neither of these effects was observed for some subjects at dose levels of 0.5 to 1.1 mg V/kg-day. The human studies, however, were not considered for use in deriving the subchronic RfD. These studies are of short duration, used small numbers of subjects, and are not capable of detecting sub-clinical kidney damage—identifying a portal-of-entry effect (gastrointestinal distress) as the only adverse effect. Given no evidence of systemic effects in the human subjects, many of which were diabetic, the male rat may be particularly susceptible to kidney and blood pressure effects from vanadium exposure. Accordingly the increased blood pressure reported by Boscolo et al. (1994) at the lowest dose level (0.12 mg V/kg-day) is discounted as a basis for the pRfD, but the kidney effects remain relevant for consideration as the basis for the subchronic p-RfD.

The lowest reproductive/developmental toxicity LOAEL is 2.1 mg V/kg-day for growth retardation in the offspring of rats exposed prior to mating (Domingo et al., 1986); a NOAEL is not established. A clear dose-response relationship is reported in both sexes of offspring for a number of growth-related endpoints measured at several postnatal times. Benchmark dose modeling is rejected because the data were pooled across litters, BMD models could not be fit to most of the data, and it is not clear whether those endpoints that were fit successfully were the most sensitive. Therefore, only the LOAEL of 2.1 mg V/kg-day is considered as a potential POD for the subchronic p-RfD.

The NOAEL of 0.12 mg V/kg-day based on kidney histopathology at 1.2 mg V/kg-day in the 6-month rat study of Boscolo et al. (1994) provides the most appropriate basis for the

subchronic p-RfD. However, given exposure to vanadium in the diet, the NOAEL is adjusted upward by 0.1 mg/kg-day, which is the lower end of the range of likely dietary exposure discussed previously in this document. The subchronic p-RfD is derived as follows:

**Subchronic p-RfD** = NOAEL ÷ UF
= 0.22 mg V/kg-day ÷ 300
= **0.0007 mg V/kg-day or $7 \times 10^{-4}$ mg/kg-day**

The composite UF of 3000 is composed of the following:

- A full UF of 10 is used to account for interspecies extrapolation to account for potential pharmacokinetic and pharmacodynamic differences between rats and humans.
- A full UF of 10 is used to account for potentially susceptible individuals in the population in the absence of information on the variability of response to vanadium developmental toxicity in humans.
- A partial UF of 3 ($10^{0.5}$) is used to account for database deficiencies—in particular the lack of a reproductive toxicity study.

Confidence in the key study (Boscolo et al., 1994) is low. The study does not examine the factors that would determine whether the kidney effects in male rats were a result of $\alpha_{2u}$-globulin accumulation. Confidence in the database is medium. The toxicological database for oral exposure to vanadium includes human studies, several subchronic studies, several reproductive and developmental toxicity studies and limited studies of immunotoxicity and neurotoxicity. However, the majority of the subchronic studies evaluate limited endpoints; there are no comprehensive bioassays of subchronic duration. Although several studies reported kidney and blood pressure effects in male rats, none of them examined the factors that would determine whether the kidney effects were a result of $\alpha_{2u}$-globulin accumulation. The reproductive toxicity database does not include any adequate standard multigeneration studies. The available 2-generation studies (Faria de Rodriguez et al., 1998a; Nava de Leal et al., 1998) were limited by poor reporting or pooling of data across treatment groups; however, the results provided suggestive evidence for reproductive toxicity. Likewise, two short-term studies of neurotoxicity (Sanchez et al., 1998, 1999) provide suggestive evidence for an effect of vanadium exposure on avoidance response, but neither study conducted comprehensive tests of neurobehavioral endpoints. Low confidence in the subchronic p-RfD follows.

**Chronic p-RfD**

Kanisawa and Schroeder (1967) and Schroeder et al. (1970; Schroeder and Michener, 1975) conducted chronic mouse and rat studies; however, the histopathologic assessment in these studies included only gross morphologic evaluations after natural deaths of the animals and would not have detected more subtle histopathologic lesions, particularly kidney lesions. Thus, these studies are inappropriate as critical studies for the chronic p-RfD. The lowest LOAEL of the remaining relevant endpoints is 1.2 mg V/kg-day for kidney pathology in male rats after a 6-month exposure to sodium metavanadate in drinking water (Boscolo et al., 1994), the basis for the subchronic p-RfD. As the human studies would not have revealed subclinical tissue damage and were of short duration, chronic kidney damage would be of concern. Therefore, kidney toxicity is selected as the critical effect, with a LOAEL of

1.2 mg V/kg-day and NOAEL of 0.12 mg V/kg-day established in the Boscolo et al. (1994) rat study. As for the subchronic p-RfD, the NOAEL is adjusted to 0.22 mg/kg-day to account for dietary exposure. The chronic p-RfD is derived as follows:

**Chronic p-RfD** = NOAEL ÷ UF
= 0.22 mg V/kg-day ÷ 3000
= **0.00007 mg V/kg-day or $7 \times 10^{-5}$ mg/kg-day**

The composite UF of 3000 is composed of the following:

- A full UF of 10 is used to account for interspecies extrapolation to account for potential pharmacokinetic and pharmacodynamic differences between rats and humans.
- A full UF of 10 is used to account for potentially susceptible individuals in the population in the absence of information on the variability of human response to vanadium.
- A partial UF of 3 ($10^{0.5}$) is used to account for database deficiencies as per the subchronic p-RfD.
- A full UF of 10 is used to account for extrapolation to chronic exposure duration from a subchronic study.

Confidence in the key study (Boscolo et al., 1994) is low. The study focused on the blood pressure and kidney effects of vanadium; it did not address a comprehensive suite of endpoints. In addition, the issue of $\alpha_{2u}$-globulin accumulation was not addressed. Confidence in the database is medium as for the subchronic p-RfD. The chronic studies are of limited utility. Low confidence in the chronic p-RfD follows.

## FEASIBILITY OF DERIVING PROVISIONAL SUBCHRONIC AND CHRONIC INHALATION RfC VALUES FOR VANADIUM AND COMPOUNDS

There are no inhalation data with which to derive subchronic or chronic p-RfCs for vanadium compounds.

## PROVISIONAL CARCINOGENICITY ASSESSMENT FOR VANADIUM AND COMPOUNDS

There are no human data on the potential carcinogenicity of soluble inorganic vanadium compounds, nor are there adequate animal carcinogenicity bioassays; thus, under the U.S. EPA (2005) *Guidelines for Carcinogen Risk Assessment*, there is "*Inadequate Information to Assess* [the] *Carcinogenic Potential"* of vanadium. In early carcinogenicity bioassays of vanadium, no increases in tumor incidence were observed in rats or mice exposed chronically (Kanisawa and Schroeder, 1967; Schroeder et al., 1970; Schroeder and Michener, 1975). However, these studies are limited in several ways: there is limited histopathology evaluation,

and tumor findings are not reported by target organ. In addition, the study in rats (Schroeder et al., 1970) is hampered by significant animal loss due to a pneumonia outbreak. A number of studies in rats have indicated that vanadium may exert an antineoplastic effect in chemical carcinogenesis, reducing the number and/or incidence of leiomyosarcomas and tumors of the liver, colon, and mammary glands in rats (Evangelou et al., 1997; Liasko et al., 1998; Ray et al., 2004, 2005a,b, 2006; Chakraborty et al., 2005, 2006a,b,c, 2007a,b; Kanna et al., 2003, 2004, 2005). Mechanistic information supporting the potential antineoplastic effect includes evidence that vanadium can induce apoptosis in mammary tumor cells both in vitro and in vivo (Ray et al., 2006). Limited genotoxicity data have shown that vanadium can induce mutations in yeast and mammalian cells (Bronzetti et al., 1990; Cohen et al., 1992; Klein et al., 1994). In mammalian cells cultured in vitro, vanadium increased the SCE frequency at noncytotoxic concentrations (Owusu-Yaw et al., 1990). Vanadium has induced micronuclei and/or numerical chromosomal aberrations (hypoploidy or hyperploidy) in mice treated in vivo (Ciranni et al., 1995; Leopardi et al., 2005; Villani et al., 2007).

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