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Provisional Peer Reviewed Toxicity Values for  
Vanadium pentoxide  
(CASRN 1314-62-1)

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## Acronyms and Abbreviations

bw	body weight
cc	cubic centimeters
CD	Caesarean Delivered
CERCLA	Comprehensive Environmental Response, Compensation and Liability Act of 1980
CNS	central nervous system
cu.m	cubic meter
DWEL	Drinking Water Equivalent Level
FEL	frank-effect level
FIFRA	Federal Insecticide, Fungicide, and Rodenticide Act
g	grams
GI	gastrointestinal
HEC	human equivalent concentration
Hgb	hemoglobin
i.m.	intramuscular
i.p.	intraperitoneal
IRIS	Integrated Risk Information System
IUR	inhalation unit risk
i.v.	intravenous
kg	kilogram
L	liter
LEL	lowest-effect level
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
m	meter
MCL	maximum contaminant level
MCLG	maximum contaminant level goal
MF	modifying factor
mg	milligram
mg/kg	milligrams per kilogram
mg/L	milligrams per liter
MRL	minimal risk level
MTD	maximum tolerated dose
MTL	median threshold limit
NAAQS	National Ambient Air Quality Standards
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

PBPK	physiologically based pharmacokinetic
ppb	parts per billion
ppm	parts per million
PPRTV	Provisional Peer Reviewed Toxicity Value
RBC	red blood cell(s)
RCRA	Resource Conservation and Recovery Act
RDDR	Regional deposited dose ratio (for the indicated lung region)
REL	relative exposure level
RfC	inhalation reference concentration
RfD	oral reference dose
RGDR	Regional gas dose ratio (for the indicated lung region)
s.c.	subcutaneous
SCE	sister chromatid exchange
SDWA	Safe Drinking Water Act
sq.cm.	square centimeters
TSCA	Toxic Substances Control Act
UF	uncertainty factor
µg	microgram
µmol	micromoles
VOC	volatile organic compound

## PROVISIONAL PEER REVIEWED TOXICITY VALUES FOR VANADIUM PENTOXIDE (CASRN 1314-62-1)

### Background

On December 5, 2003, the U.S. Environmental Protection Agency's (EPA's) 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. EPA's Integrated Risk Information System (IRIS).
2. Provisional Peer-Reviewed Toxicity Values (PPRTV) used in 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 EPA's Integrated Risk Information System (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 EPA IRIS Program. All provisional toxicity values receive internal review by two EPA scientists and external peer review by three independently selected scientific experts. PPRTVs differ from IRIS values in that PPRTVs do not receive the multi-program consensus review provided for IRIS values. This is because IRIS values are generally intended to be used in all 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 five-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 manuscripts 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 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 manuscript and understand the strengths and limitations of the derived provisional values. PPRTVs are developed by the EPA Office of Research and Development's National Center for Environmental Assessment, Superfund Health Risk Technical Support Center for OSRTI. Other 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 EPA Office of Research and Development's National Center for Environmental Assessment, Superfund Health Risk Technical Support Center (513-569-7300), or OSRTI.

## INTRODUCTION

Vanadium is a group Vb transition metal, which exists in several oxidation states from -2 to +5. Vanadium is commonly found in ores, tars, coals and oils and is used as an alloy in steel (WHO, 1988). Vanadium pentoxide ( $V_2O_5$ ) (Figure 1) is the most common form of vanadium used commercially. Occupational exposure to vanadium pentoxide is primarily by inhalation of dust generated during vanadium processing and fuel-oil ash during cleaning of oil-burning boilers and furnaces.



**Figure 1. Vanadium pentoxide structure**

The U.S. Environmental Protection Agency's (EPA) Integrated Risk Information System (IRIS) (U.S. EPA, 2007) lists a chronic reference dose (RfD) of 9E-03 mg/kg-day for vanadium pentoxide based on a 2.5-year dietary no-observed-adverse-effect level (NOAEL) of 17.85 ppm vanadium pentoxide (equivalent to 0.89 mg vanadium pentoxide/kg-day) for decreased hair cystine content reported in an unpublished study by Stokinger et al. (1953) (verification date 02/26/1986). IRIS does not list a chronic inhalation reference concentration (RfC) or cancer assessment for vanadium pentoxide, and currently contains no files for vanadium or other vanadium compounds (U.S. EPA, 2007). The Health Effects Assessment Summary Table (HEAST) (U.S. EPA, 1997) lists a subchronic RfD of 9E-03 mg/kg-day for vanadium pentoxide,

derived by adopting the chronic RfD from IRIS as the subchronic RfD (U.S. EPA, 1997). The Drinking Water Standards and Health Advisories list (U.S. EPA, 2004) does not report an RfD or carcinogenicity assessment for vanadium or vanadium compounds. The CARA list (U.S. EPA, 1991, 1994a) includes a Health and Environmental Effects Profile (HEEP) for Vanadium Pentoxide (U.S. EPA, 1985) that declined to derive toxicity values for vanadium pentoxide due to the weak database and a Health Effects Assessment (HEA) for Vanadium and Compounds (U.S. EPA, 1987) that derived the same RfD for vanadium pentoxide as that presented on IRIS. The Agency for Toxic Substance and Disease Registry (ATSDR, 1992) derived an intermediate-duration oral minimal risk level (MRL) for vanadium of  $3E-03$  mg/kg-day ( $3 \times 10^{-3}$  mg V/kg-day) based on NOAELs of 0.3 mg/kg-day for renal and respiratory effects (renal hemorrhagic foci and pulmonary vascular infiltration) seen at higher doses (0.57 mg/kg-day) in a 3-month study in rats exposed to sodium metavanadate in drinking water (Domingo et al., 1985). ATSDR (1992) did not derive a chronic oral MRL for vanadium due to lack of quantitative chronic exposure data. ATSDR (1992) did not consider data from the studies by Stokinger et al. (1953) useful for derivation of a chronic oral MRL, because data were “not available to determine the most sensitive end point.” IARC (2006) has classified vanadium pentoxide as a 2B carcinogen (possible human carcinogen). Vanadium exists in several different valence states, all of which are not equivalent toxicologically (IPCS, 2001). Therefore, the vanadium pentoxide assessment should not be applied to other vanadium compounds.

Literature searches for studies relevant to the derivation of provisional toxicity values for vanadium pentoxide (CASRN 1314-62-1) were conducted from 1986 to April 2007 in TOXLINE (supplemented with BIOSIS and NTIS updates), MEDLINE, TSCATS, RTECS, CCRIS, DART, EMIC/EMICBACK, HSDB, GENETOX and CANCERLIT, and Current Contents. The Environmental Health Criteria Document (WHO, 1988) and National Toxicology Program (NTP) status report (NTP, 2006) were also searched for relevant information.

## REVIEW OF PERTINENT LITERATURE

### Human Studies

**Oral.** No studies investigating the effects of acute, subchronic or chronic oral exposure to vanadium pentoxide in humans were identified.

**Inhalation.** Health effects of inhalation exposure to vanadium pentoxide and other vanadium compounds have been investigated since the early 1900s (Woodin et al., 1999; Lees, 1980). Numerous occupational and case studies report respiratory tract irritation, bronchitis (often called boiler makers' bronchitis), airway obstruction, chest pain, rhinitis, pharyngitis, laryngitis and conjunctivitis in workers exposed to fuel-oil ash containing vanadium during cleaning of oil-burning boilers (Woodin et al., 2000; Woodin et al., 1999; Hauser et al., 1995; Levy et al., 1984; Lees, 1980; Sjöberg, 1955; Williams, 1952) or to vanadium-containing dust during vanadium processing (Irsigler et al., 1999; Kiviluoto, 1980; Kiviluoto et al., 1979; Musk and Tees, 1982; Zenz et al., 1962; Vintinner et al., 1955). With the exception of the studies reviewed below, the chemical composition of fuel-oil ash particulate or vanadium dust (including identification of specific vanadium compounds), or exposure measurements for vanadium

pentoxide, were not reported; thus, limited information is available to define the exposure-response relationship between inhaled vanadium pentoxide and adverse respiratory effects in humans.

Sjöberg (1955) published seven case reports of respiratory symptoms, diagnosed as “vanadium bronchitis,” in workers following acute exposure to ash particles containing vanadium pentoxide during cleaning of oil-burning boilers. Vanadium pentoxide concentrations measured in two boilers on a single day during the cleaning process ranged from 2 to 85 mg/m<sup>3</sup>. Although specific exposure durations were not reported, in general, cleaning time for each boiler was 1-2 work days. Workers reported that respiratory symptoms, including cough, rhinitis, wheeze, sore throat and conjunctivitis, developed “after” exposure from boiler cleaning. Symptoms resolved within 2 weeks of exposure and re-developed in workers exposed during subsequent boiler cleanings. No additional information regarding the chemical composition of the fuel-oil ash was reported.

Severe respiratory tract irritation was reported in 74 of 100 workers exposed to vanadium pentoxide in fuel-oil ash during an oil-to-coal conversion of a power plant (Levy et al., 1984). Exposure occurred over a period of approximately 4 weeks, with typical exposure durations of 10 hours/day, 6 days/week. Eight air samples obtained from various parts of the boiler on a single day near the end of the 4-week exposure period revealed vanadium pentoxide concentrations ranging from 0.05 to 5.3 mg/m<sup>3</sup>; no additional air samples were obtained during the exposure period. Although a complete assessment of the ash composition was not conducted, levels of chromium, nickel and fumes of copper and iron oxide were reported as “within acceptable limits” (no measured levels were reported). Data on workers’ symptoms were collected by questionnaires distributed approximately 1 month after exposure had ceased. The most frequently reported symptoms were productive cough, sore throat, dyspnea on exertion and chest pain or discomfort. The onset of symptoms occurred within the first 2 weeks of exposure. Information regarding resolution of symptoms following cessation of exposure was not reported.

Zenz and Berg (1967) exposed nine volunteers to vanadium pentoxide dust for 8 hours to evaluate respiratory effects. Volunteers (gender not reported) were exposed to 0.1 mg/m<sup>3</sup> (n=2), 0.5 mg/m<sup>3</sup> (n=5) or 1 mg/m<sup>3</sup> (n=2) vanadium pentoxide in an environmental chamber; no control group or control exposures were included in this study. Particle size analysis revealed that 98% of particles had a diameter <0.5 µm. Post-exposure assessments of chest x-ray, blood, urine, nasal smear samples and pulmonary function were compared with baseline values determined for each subject prior to exposure. All subjects were observed for clinical symptoms for 11-19 months after exposure. Subjects exposed to 1 mg/m<sup>3</sup> vanadium pentoxide developed sporadic cough after 5 hours of exposure, which progressed to persistent cough during the last 3 hours of exposure and continued for 8 days. No other signs of respiratory irritation were observed. Results of pulmonary function tests and chest x-ray 1, 2 and 3 weeks after exposure were similar to baseline (data not reported). Hematology and urinalysis parameters were not affected by exposure (data not reported). Nasal smears obtained 24 hours, 72 hours and 1 week after exposure were negative for eosinophilia. Three weeks after the initial exposure, these subjects were accidentally exposed to a “heavy cloud” of vanadium pentoxide dust (concentration not reported) for 5 minutes. Within 16 hours of exposure, both subjects developed a “marked” productive cough with rales and expiratory wheeze, which continued for 1 week, although

pulmonary function test results were comparable to baseline (data not reported). Blood and nasal smear samples were negative for eosinophilia. Subjects in the 0.5 mg/m<sup>3</sup> exposure group developed a “loose” productive cough on the day after exposure, which lasted for 7-10 days. No additional symptoms were observed and post-exposure pulmonary function and laboratory tests were comparable to baseline results (data not reported). Subjects exposed to 0.1 mg/m<sup>3</sup> developed “considerable” mucus formation, which was easily cleared by coughing, within 24 hours after exposure, lasting for 4 days. No other symptoms or positive findings for pulmonary function or laboratory tests were observed. No treatment-related symptoms or clinical findings were reported for any subject during the 11-19 months post-treatment period.

## **Animal Studies**

### **Oral Subchronic Toxicity**

No animal studies that have comprehensively examined histopathological, biochemical and clinical endpoints of subchronic oral exposure were identified from the available literature. Mountain et al. (1953) evaluated the effects of subchronic exposure of rats to dietary vanadium pentoxide on body weight gain, erythrocyte count, hemoglobin and cystine content of hair. Groups of five male Wistar rats were fed diets containing 0, 25, 50, 500 or 1000 ppm of vanadium as vanadium pentoxide for 103 days (25 and 50 ppm groups; “low exposure” groups) or 75 days (500 and 1000 ppm groups; “high exposure” groups). After 35 days of treatment, dietary vanadium levels of the 25 and 50 ppm groups were increased to 100 and 150 ppm, respectively. At the end of treatment, body weight gain and cystine content of hair were measured in all groups, erythrocyte count and hemoglobin were measured in control and “low exposure” groups, and relative liver weight was measured in control and 500 ppm groups. Compared to control, body weight gain was increased in the 50 (54% increase) and 100 (45% increase) ppm groups and decreased in the 500 ppm group (66% decrease) and 1000 ppm group (details not reported); The increase in body weight gain at the low exposure levels was not explained and statistical significance was not reported. Relative liver weight in the 500 ppm group was significantly increased by 10% compared to control (data only reported for control and 500 ppm groups). Erythrocyte count and hemoglobin level were significantly decreased by 18 and 5%, respectively, in rats exposed to 100 ppm compared to control; no changes in hematological parameters were observed in the 50 ppm group (data not reported for 500 and 1000 ppm groups). Cystine content of hair was significantly decreased compared to control in all vanadium pentoxide treatment groups. Although the toxicological significance of decreased hair cystine content has not been established, the researchers speculate that vanadium may have inhibited enzymes, such as sulfur transferases, that decreased the availability of cystine for hair growth. On the basis of decreased hair cystine, potentially a result of enzyme inhibition, the lowest exposure of 50 ppm (5 mg/kg-day) is a LOAEL, although equivocal. Based on the low number of animals and the varied dose regimen, this study is judged to be inadequate for deriving a subchronic provisional RfD.

### **Oral Chronic Toxicity and Carcinogenicity**

Studies investigating the effects of chronic oral exposure of animals to vanadium pentoxide, including carcinogenicity studies, were not identified. According to the information

provided by IRIS (U.S. EPA, 2007), the unpublished 2.5-year dietary study on vanadium pentoxide in rats used as the basis of the chronic RfD (Stokinger et al., 1953) did not assess comprehensive toxicity endpoints. IRIS states that “the criteria used to evaluate vanadium toxicity were growth rate, survival, and hair cystine content.” According to IRIS, “the only significant change reported was a decrease in the amount of cystine in the hair of animals ingesting vanadium.” IRIS (U.S. EPA, 2007) did not report any additional information on the results of this unpublished study; thus, it does not appear that the carcinogenic potential of oral vanadium was evaluated. No additional oral chronic exposure studies in animals were identified in the literature search updates for this document.

### **Oral Reproductive and Developmental Toxicity**

Studies investigating the reproductive and developmental toxicity of subchronic or chronic oral exposure to vanadium pentoxide were not identified. There are several studies, however, addressing these endpoints following intraperitoneal administration of vanadium pentoxide in mice and rats.

### **Intraperitoneal Reproductive and Developmental Toxicity**

Male and female reproductive endpoints were evaluated in rats following prepubertal intraperitoneal administration of vanadium pentoxide (Altamirano et al., 1991). Newborn male and female rats were injected with 0 or 12.5 mg/kg vanadium pentoxide in saline on every second day from birth to age 21 days; groups sizes were 5 (treated males) or 9 (male and female controls and treated females). The males were sacrificed at 55 days of age and the females were sacrificed on the day of first vaginal estrus. Other groups of females were injected with 0 or 12.5 mg/kg-day vanadium pentoxide (n = 10 and 6, respectively) from age 21 days to the day of first vaginal estrus, at which time they were sacrificed. Reported endpoints in the males consisted of absolute weights of testis, prostate, seminal vesicles, adrenals, pituitary, thymus, liver, kidneys and submandibular glands. The only effects in treated males were statistically significant increases in seminal vesicle, thymus and submandibular gland weights (20.1, 29.5 and 19.2% higher than controls, respectively). Endpoints in the females included body weight, absolute organ weights (ovaries, uterus, adrenals, pituitary, thymus, liver, kidneys and submandibular glands), age at vaginal opening, number of ova in oviducts and ovulation rate. The only effects in treated females occurred in the group treated from 21 days of age; these consisted of statistically significant increases in body weight (12.5% higher than controls) and weights of thymus, submandibular gland and liver (31.1, 15.8 and 28.4%, respectively).

Developmental toxicity was evaluated in groups of 13 or 15 female CD-1 mice that were administered 0 or 8.5 mg/kg vanadium pentoxide in distilled water, respectively, by intraperitoneal injection on days 6-15 of gestation (Altamirano-Lozano et al., 1993). No maternal toxicity was reported (endpoints not specified). Developmental endpoints were assessed on gestation day 18 and included numbers of implants, resorptions and live fetuses, fetal weight and sex, and external malformations (all fetuses) and skeletal abnormalities (two-thirds of fetuses); fetal internal soft-tissue examinations do not appear to have been conducted. The treated group had statistically significant increases in the number of litters with abnormal fetuses (9/15 compared to 3/13 in controls), number of abnormal fetuses (15/149 compared to 3/124), and

number of fetuses with short limbs (8/149 compared to 0/124 in controls). Additionally, the numbers of ossification centers in forelimbs and hindlimbs were significantly reduced in the treated fetuses.

Fertility and sperm assessments were performed in male CD-1 mice following intraperitoneal administration of vanadium pentoxide (Altamirano-Lozano et al., 1996). In the fertility assessment, groups of 20 and 15 male mice were injected with 0 and 8.5 mg/kg vanadium pentoxide in saline, respectively, every 3 days for 60 days and mated 24 hours after the last injection. Statistically significant effects in the treated group included reduced fertility rate in the males (33% compared to 85% in controls), and reduced numbers of implantations sites and live fetuses and increased number of resorptions in the mated females. In the sperm assessment, 30 males were injected with 8.5 mg/kg vanadium pentoxide every 3 days for up to 60 days with groups of 5 evaluated after 10, 20, 30, 40, 50 or 60 days of treatment. Statistically significant effects included reduced sperm motility after  $\geq 10$  days, reduced sperm count and increased percentage of abnormal sperm after  $\geq 20$  days, decreased absolute testicular weight after  $\geq 50$  days (relative weight not reported), and decreased body weight after 60 days.

The results of three developmental studies published in the Chinese language are available only as English abstracts. Zhang et al. (1991) evaluated the developmental toxicity in NIH mice following intraperitoneal injection of 5 mg/kg-day vanadium pentoxide on days 1-5, 6-15, 7, 8, 9, 10, 11 or 14-17 of gestation. There were no adverse effects on preimplantation or implantation, teratogenicity, or premature births. Increased frequencies of resorption or fetal death were observed for gestation days 6-15, 7 and 14-17. Delayed ossification (sites not specified) was observed for gestation days 6-15, 8, 10 and 14-17. In a second study, Zhang et al. (1993a) evaluated developmental toxicity in Wistar rats following intraperitoneal injection of 0.33, 1.0 or 3.0 mg/kg-day on days 6-15 of gestation. Decreased placental weight and increases in embryo-fetus mortality and external or skeletal malformations (unspecified) occurred at 1.0 and 3.0 mg/kg-day. Maternal toxic symptoms (unspecified), decreased maternal weight gain during treatment and fetal growth retardation were observed at 3.0 mg/kg-day. In the third study, Zhang et al. (1993b) evaluated developmental toxicity in Wistar rats following intraperitoneal injection of vanadium pentoxide in doses of 3 mg/kg-day on days 6-15 of gestation or 5 mg/kg-day on days 9, 10, 11 or 9-12 of gestation. Effects in rats exposed on gestation days 6-15 and 9-12 included decreased maternal weight gain, increased fetal mortality, decreased fetal weight and crown-rump length, delayed ossification of unspecified bones, and increased incidences of subcutaneous hemorrhage, wavy ribs, and dilation of lateral ventricles and renal pelvis. Effects in rats exposed on a single day of gestation included subcutaneous hemorrhage and unspecified visceral anomalies following exposure on days 9, 10 and 11, and increased fetal mortality and delayed ossification of unspecified bones following exposure on day 10. Additional study details were not reported in the abstracts. Altogether, the three studies identified an intraperitoneal developmental-toxicity LOAEL for vanadium pentoxide of 1 mg/kg-day (Zhang et al., 1993a).

Although the IP studies show the potential of vanadium pentoxide to induce reproductive and developmental effects in rodents, the studies are of little use in the quantitation of vanadium pentoxide toxicity, as equivalent oral or inhalation exposures cannot be established.

## Inhalation Subchronic Toxicity

Subchronic toxicity of inhalation exposure to particulate aerosols of vanadium pentoxide was assessed in male and female rats (F344/N) and mice (B6C3F<sub>1</sub>) in a series of studies conducted as part of the NTP (2002) chronic exposure cancer bioassay. Standard toxicological assessments were conducted after exposure durations of 16 days and 3 months. In addition, 16-day exposure studies included immunotoxicity studies, cell proliferation studies and detailed histopathological evaluations to determine the time-to-onset and extent of early lung injury. The 3-month exposure studies also included evaluations of cardiovascular function, pulmonary function, sperm motility and vaginal cytology and analysis of pulmonary lavage fluid.

*NTP (2002) 16-Day Exposure Studies in Rats.* Groups of five male and five female rats were exposed (whole-body exposure) to particulate aerosols of vanadium pentoxide at concentrations of 0, 2, 4, 8, 16 or 32 mg/m<sup>3</sup> by inhalation, 6 hours per day, 5 days/week for 16 days (NTP, 2002). Particle size mean mass aerodynamic diameter and geometric standard deviation (MMAD±GSD) for each dose groups was as follows: 2 mg/m<sup>3</sup>=1.0±2.7; 4 mg/m<sup>3</sup>=1.2±2.8; 8 mg/m<sup>3</sup>=1.3±2.4; 16 mg/m<sup>3</sup>=1.2±2.8; 32 mg/m<sup>3</sup>=1.2±2.8. Three male rats in the 32 mg/m<sup>3</sup> group died on day 6 of exposure; researchers considered mortalities to be treatment-related. Although the cause of death was not explicitly stated, rats became emaciated, exhibited shallow, labored breathing, and had diarrhea. No deaths occurred in other treatment groups for male rats or in any group for female rats. During the first week of exposure, shallow, rapid respiration was observed in all rats in the 16 and 32 mg/m<sup>3</sup> exposure groups and red nasal discharge was observed in all rats in the 32 mg/m<sup>3</sup> exposure group. From exposure days 8 to 16, ocular and nasal discharge was observed in rats in the 16 mg/m<sup>3</sup> exposure group and rats in the 32 mg/m<sup>3</sup> groups became emaciated and had hunched or abnormal posture. Dose-related decreases in body weight gain and increases in lung weights (absolute and relative) were observed, as summarized in Table 1. Significant dose-related decreases in body weight gain were observed in males and females at concentrations of 8 mg/m<sup>3</sup> and greater. Absolute lung weight was significantly increased in male rats in the 16 mg/m<sup>3</sup> group and in female rats in the 2, 16 and 32 mg/m<sup>3</sup> groups. Relative lung weights were significantly increased in female rats in all vanadium pentoxide groups and in male rats exposed to concentrations of 4 mg/m<sup>3</sup> and above. No consistent changes were observed for absolute or relative weights of other organs. Other endpoints assessing general toxicity were not monitored in the 16-day study. No data on food or water consumption throughout the treatment period were reported.

To assess the onset and extent of early lung tissue changes from inhalation exposure to vanadium pentoxide, lung tissue was evaluated in additional groups of 40-60 female rats exposed to vanadium pentoxide (NTP, 2002). Rats were exposed to 0, 1, 2 or 4 mg/m<sup>3</sup> by inhalation for 6hours/day, 5 days/week for 16 days. On days 6 and 13, the lungs of 10 rats per group were evaluated for histopathological changes and cell proliferation was measured by incorporation of BrdU (Bromodeoxyuridine) implanted 140±3 hours earlier. Histopathology was also performed on lung tissue from four animals in each exposure group on days 1, 2, 5, 10 and 16 (data not presented in study report). Incidences of nonneoplastic lesion of the lung of female rats on days 6

<b>Table 1. Body Weight Gain and Lung Weight in Rats (F344/N) Exposed to Vanadium Pentoxide by Inhalation for 16 Days (Values are Means±Standard Error) (NTP, 2002)</b>						
Parameter	Exposure					
	Control	2 mg/m <sup>3</sup>	4 mg/m <sup>3</sup>	8 mg/m <sup>3</sup>	16 mg/m <sup>3</sup>	32 mg/m <sup>3</sup>
<b>Male Rats</b>						
Body weight gain during 16-day exposure period (g)	89±5	87±3	84±4	73±3 <sup>a</sup>	59±5 <sup>b</sup>	4±9 <sup>b</sup>
Absolute lung weight (g)	1.6±0.2	1.7±0.1	1.9±0.1	1.9±0.1	2.1±0.2 <sup>a</sup>	1.5±0.1
Relative lung weight	7.3±0.9	8.1±0.4	9.9±0.4 <sup>a</sup>	9.7±0.3 <sup>b</sup>	11.3±0.6 <sup>b</sup>	7.1±0.8 <sup>b</sup>
<b>Female Rats</b>						
Body weight gain during 16-day exposure period (g)	38±2	38±2	35±2	30±1 <sup>a</sup>	23±2 <sup>b</sup>	4±4 <sup>b</sup>
Absolute lung weight (g)	1.1±0.1	1.5±0.1 <sup>b</sup>	1.3±0.1	1.3±0.05	1.4±0.1 <sup>a</sup>	1.4±0.1 <sup>a</sup>
Relative lung weight	7.7±0.6	10.7±0.6 <sup>b</sup>	9.8±0.7 <sup>b</sup>	10.2±0.4 <sup>b</sup>	11.5±0.5 <sup>b</sup>	13.2±0.2 <sup>b</sup>

<sup>a</sup>Significantly different from control by William's test ( $p \leq 0.05$ )

<sup>b</sup>Significantly different from control by William's test ( $p \leq 0.01$ )

and 13 are summarized in Table 2. Hyperplasia of the alveolar and bronchiolar epithelium was observed in almost every rat exposed to 2 or 4 mg/m<sup>3</sup> for 6 or 13 days, and alveolar epithelial hyperplasia was observed in 3 of 10 animals exposed to 1 mg/m<sup>3</sup> for 13 days. Increased numbers of alveolar macrophages (histiocytic infiltrate) were observed in rats treated with 2 or 4 mg/m<sup>3</sup> for 6 days and in all vanadium pentoxide-exposed rats after 13 days of treatment. Minimal to mild interstitial inflammation was observed in all rats exposed to 2 or 4 mg/m<sup>3</sup> for 6 or 13 days, and in 3 of 10 and 8 of 10 rats exposed to 1 mg/m<sup>3</sup> for 6 or 13 days, respectively. On day 6, inflammation was characterized by small numbers of mononuclear cells localized primarily around blood vessels. On day 13, mononuclear cells were also observed in the septae of alveolar ducts. Minimal to mild interstitial fibrosis was observed in 6 of 10 animals exposed to 4 mg/m<sup>3</sup> for 13 days. A lowest-observed-adverse-effect level (LOAEL) of 1 mg/m<sup>3</sup> was identified for nonneoplastic lung lesions in female rats exposed to vanadium pentoxide by inhalation for 13 days; a 13-day NOAEL was not established. Since the severity of interstitial fibrosis was rated as minimal to mild, the LOAEL of 1 mg/m<sup>3</sup> is considered a minimal LOAEL.

Results of cell proliferation studies (measured by incorporation of BrdU) in rats exposed to 0, 1, 2 or 4 mg/m<sup>3</sup> vanadium pentoxide for days 6 and 13 are summarized in Table 3 (NTP, 2002). Cell turnover rates in the terminal bronchioles increased with increasing exposure concentration on days 6 and 13 (statistical significance not reported). Rates on day 13 were similar to those observed on day 6. In contrast, the incidence of alveolar cell proliferation in only the 4 mg/m<sup>3</sup> group was greater than that in the control group on day 6. By day 13, rates were increased in all groups of exposed rats, but not in a concentration-related manner.

<b>Table 2. Incidences of Nonneoplastic Lesions of the Lung in Female Rats (F344/N) Exposed to Vanadium Pentoxide by Inhalation for 6 or 13 Days (NTP, 2002)</b>				
<b>Endpoint</b>	<b>Number of Animals with Lesion<sup>a</sup></b>			
	<b>Control</b>	<b>1 mg/m<sup>3</sup></b>	<b>2 mg/m<sup>3</sup></b>	<b>4 mg/m<sup>3</sup></b>
<b>Day 6</b>				
Alveolar epithelium, hyperplasia	0	0	10 <sup>b</sup> (1.1)	8 <sup>b</sup> (1.4)
Bronchiole epithelium, hyperplasia	1 (1.0)	0	10 <sup>b</sup> (1.7)	10 <sup>b</sup> (1.8)
Histiocytic infiltrate	2 (1.0)	6 (1.3)	10 <sup>b</sup> (1.4)	10 <sup>b</sup> (1.8)
Inflammation	0	3 (1.0)	10 <sup>b</sup> (1.5)	10 <sup>b</sup> (2.5)
<b>Day 13</b>				
Alveolar epithelium, hyperplasia	0	3 (1.0)	10 <sup>b</sup> (1.0)	10 <sup>b</sup> (2.0)
Bronchiole epithelium, hyperplasia	0	0	10 <sup>b</sup> (1.0)	10 <sup>b</sup> (1.8)
Histiocytic infiltrate	0	10 <sup>b</sup> (1.3)	10 <sup>b</sup> (1.9)	10 <sup>b</sup> (2.2)
Inflammation	0	8 <sup>b</sup> (1.3)	10 <sup>b</sup> (1.7)	10 <sup>b</sup> (2.0)
Fibrosis	0	0	0	6 <sup>b</sup> (1.5)

<sup>a</sup>10 rats/treatment group; numbers in parentheses indicate average severity grade in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked

<sup>b</sup>Significantly different from control group by the Fisher exact test ( $p \leq 0.05$ )

<b>Table 3. Bromodeoxyuridine-Labeled Lung Nuclei in Female Rats (F344/N) Exposed to Vanadium Pentoxide by Inhalation for 6 or 13 days (NTP, 2002)</b>				
<b>Lung Location</b>	<b>Exposure Group<sup>a,b</sup></b>			
	<b>Control</b>	<b>1 mg/m<sup>3</sup></b>	<b>2 mg/m<sup>3</sup></b>	<b>4 mg/m<sup>3</sup></b>
<b>Terminal Bronchiole</b>				
Day 6	21.88±1.43	33.58±1.77	56.08±3.36	83.08±5.56
Day 13	24.24±1.12	45.91±2.04	62.72±2.04	91.96±4.65
<b>Alveoli/Alveolar Duct Area</b>				
Day 6	0.75±0.03	0.54±0.03	0.76±0.05	1.68±0.12
Day 13	0.83±0.03	1.82±0.07	1.72±0.10	1.56±0.07

<sup>a</sup>Data are given as the number of bromodeoxyuridine-labeled nuclei/mm basement membrane (mean±standard error)

<sup>b</sup>10 animals per exposure group

Additional groups of 22 male rats were exposed to 0, 4, 8 or 16 mg/m<sup>3</sup> for 16 days to assess the effects of treatment on pulmonary inflammation by analysis of pulmonary lavage fluid and systemic immunity as measured by pulmonary bactericidal activity (NTP, 2002). Analysis of bronchoalveolar lavage (BAL) fluid showed a localized inflammatory response in the lung of male rats (9-10 per exposure group) based on increases in cell number, protein, neutrophils and lysozymes. There was also a significant decrease in macrophages in lavage fluids of male rats exposed to 8 or 16 mg/m<sup>3</sup>. Data are summarized in Table 4. To assess pulmonary bactericidal activity, lungs from 12 male rats per dose group were exposed to viable radiolabeled

**Table 4. Pulmonary Lavage Parameters for Male Rats (F344/N) Exposed to Vanadium Pentoxide by Inhalation for 6 Hours/Day, 5 Days/Week for 16 Days (NTP, 2002)<sup>a</sup>**

Parameter and Species	Exposure Level			
	Control	4 mg/m <sup>3</sup>	8 mg/m <sup>3</sup>	16 mg/m <sup>3</sup>
Total cells (10 <sup>6</sup> )	11.07±1.9 (10)	18.61±6.6 <sup>b</sup> (10)	18.94±8.3 <sup>b</sup> (9)	21.70±7.7 <sup>b</sup> (10)
Macrophages (%)	98±2 (10)	91±8 (10)	78±14 <sup>b</sup> (10)	69±10 <sup>b</sup> (10)
Lymphocytes (%)	2±2 (10)	3±4 (10)	7±4 (10)	7±5 (10)
Neutrophils (%)	0±0 (10)	6±6 (10)	16±16 <sup>b</sup> (10)	25±12 <sup>b</sup> (10)
Lavage fluid protein (µg/mL)	117±32 (10)	221±23 <sup>b</sup> (10)	268±38 <sup>b</sup> (10)	253±38 <sup>b</sup> (10)
Lysozyme (µg/mL)	60±2 (10)	65±3 <sup>b</sup> (10)	65±5 <sup>b</sup> (10)	71±4 <sup>b</sup> (10)
Lysozyme (µg/µg protein)	0.54±0.14 (10)	0.30±0.03 <sup>b</sup> (10)	0.24±0.03 <sup>b</sup> (10)	0.28±0.04 <sup>b</sup> (10)

<sup>a</sup>Data are presented as means±standard deviations; numbers in parentheses are the number of animals per group

<sup>b</sup>Significantly different from control group ( $p \leq 0.05$ ) by Dunnett's test

[35S]-*Klebsiella pneumoniae* and evaluated for pulmonary bactericidal activity 3 hours after inoculation. No treatment-related effects were observed in male rats for any vanadium pentoxide group.

*NTP (2002) 16-Day Exposure Studies in Mice.* Groups of five male and five female mice were exposed (whole-body exposure) to vanadium pentoxide aerosol at concentrations of 0, 2, 4, 8, 16 or 32 mg/m<sup>3</sup> by inhalation, 6 hours per day, 5 days/week for 16 days (NTP, 2002). All male mice exposed to 32 mg/m<sup>3</sup> died or were sacrificed due to severe toxicity and one male exposed to 8 mg/m<sup>3</sup> died before completion of the study; researchers did not indicate if the death in the 8 mg/m<sup>3</sup> was considered related to treatment. Hypoactivity was observed in male and females in the 32 mg/m<sup>3</sup> group, with labored breathing observed in one female. Males in the 32 mg/m<sup>3</sup> group had hunched posture, and one was emaciated. Body weight gain and absolute and relative lung and liver weights of male and female mice are summarized in Table 5. Body weight gain over the 16-day treatment period was significantly reduced by 7% in male mice in the 16 mg/m<sup>3</sup> group and 28% in female mice in the 32 mg/m<sup>3</sup> compared to control. Absolute lung weights were significantly increased in a dose-dependent fashion in males exposed to 4 mg/m<sup>3</sup> or greater and relative lung weights were increased in males exposed to 2 mg/m<sup>3</sup> or greater. Absolute and relative lung weights were significantly increased in females in all exposure groups. Maximum increases in relative lung weights were 74% above control in males exposed to 16 mg/m<sup>3</sup> and 88% above control in females exposed to 16 mg/m<sup>3</sup>. Relative liver weights were increased in males and females exposed to 4 mg/m<sup>3</sup> and greater compared to control, with the maximum increase of 18% for males observed in the 16 mg/m<sup>3</sup> group and of 20% for females in the 32 mg/m<sup>3</sup> group. Absolute liver weights were increased in males in the 16 mg/m<sup>3</sup> group but decreased in females in the 32 mg/m<sup>3</sup> group. Mediastinal lymph nodes of several males and females exposed to 2 (females only), 4, 8 or 16 mg/m<sup>3</sup> were enlarged. While complete histopathology was not done, grossly enlarged nodes were confirmed histologically as lymphoid hyperplasia (data not presented in study report).

**Table 5. Body Weight Gain, Lung and Liver Weights in Mice (B6C3F<sub>1</sub>) Exposed to Vanadium Pentoxide by Inhalation for 16 Days (Values are Means±Standard Error) (NTP, 2002)**

Parameter	Exposure					
	Control	2 mg/m <sup>3</sup>	4 mg/m <sup>3</sup>	8 mg/m <sup>3</sup>	16 mg/m <sup>3</sup>	32 mg/m <sup>3</sup>
<b>Male Mice</b>						
Body weight gain during 16-day exposure period (g)	4.5±0.4	4.1±0.5	3.6±0.3	3.3±0.5	2.5±0.5 <sup>b</sup>	— <sup>c</sup>
Absolute lung weight (g)	0.2±0.01	0.2±0.01	0.3±0.01 <sup>b</sup>	0.3±0.01 <sup>b</sup>	0.3±0.01 <sup>b</sup>	— <sup>c</sup>
Relative lung weight	7.1±0.4	8.1±0.4 <sup>a</sup>	10.1±0.2 <sup>b</sup>	11.1±0.5 <sup>b</sup>	12.4±0.5 <sup>a</sup>	— <sup>c</sup>
Absolute liver weight (g)	1.56±0.05	1.54±0.03	1.69±0.06	1.66±0.02	1.70±0.05 <sup>a</sup>	— <sup>c</sup>
Relative liver weight	55.6±1.0	55.5±0.7	62.0±1.9 <sup>b</sup>	62.3±1.4 <sup>b</sup>	65.3±1.1 <sup>b</sup>	— <sup>c</sup>
<b>Female Mice</b>						
Body weight gain during 16-day exposure period (g)	3.5±0.3	4.1±0.4	2.4±0.4	1.5±0.4	2.4±0.3	-2.4±1.0 <sup>b</sup>
Absolute lung weight (g)	0.2±0.02	0.3±0.01 <sup>b</sup>	0.2±0.01 <sup>a</sup>	0.3±0.01 <sup>b</sup>	0.3±0.01 <sup>b</sup>	0.3±0.01 <sup>b</sup>
Relative lung weight	8.6±0.7	11.4±0.5 <sup>a</sup>	11.0±0.5 <sup>a</sup>	13.1±0.7 <sup>b</sup>	16.1±0.8 <sup>b</sup>	16.1±0.7 <sup>b</sup>
Absolute liver weight (g)	1.24±0.02	1.25±0.01	1.30±0.03	1.24±0.04	1.29±0.04	1.08±0.1 <sup>a</sup>
Relative liver weight	54.1±1.2	56.0±0.5	59.3±1.2 <sup>b</sup>	59.8±1.3 <sup>b</sup>	61.9±1.3 <sup>b</sup>	64.8±0.3 <sup>b</sup>

<sup>a</sup>Significantly different from control by William's test ( $p \leq 0.05$ )

<sup>b</sup>Significantly different from control by William's test ( $p \leq 0.01$ )

<sup>c</sup>Not measured; animals died prior to completion of treatment period

To assess the onset and extent of early lung tissue changes from inhalation exposure to vanadium pentoxide, lung tissue was evaluated in additional groups of 40-60 female mice that were exposed to vanadium pentoxide as part of the NTP (2002) 16-day study. Mice were exposed to 0, 2, 4 or 8 mg/m<sup>3</sup> by inhalation for 6 hours per day, 5 days/week for 16 days. On days 6 and 13, the lungs of 10 mice per group were evaluated for histopathological changes and cell proliferation measured by the incorporation of BrdU implanted 140±3 hours earlier.

Histopathology was also performed on lung tissue from four animals in each exposure group on days 1, 2, 5, 10 and 16 (data not presented in report). Incidences of nonneoplastic lung lesions in female mice on days 6 and 13 are summarized in Table 6. Hyperplasia of the alveolar and bronchiolar epithelium was observed in almost every exposed mouse, with severity generally increased with increasing exposure concentration and time. Hyperplasia was graded as minimal

<b>Table 6. Incidences of Nonneoplastic Lesions of the Lung in Female Mice (B6C3F<sub>1</sub>) Exposed to Vanadium Pentoxide by Inhalation for 6 or 13 Days (NTP, 2002)</b>				
<b>Parameter/Species</b>	<b>Number of Animals with Lesion<sup>a</sup></b>			
	<b>Control</b>	<b>2 mg/m<sup>3</sup></b>	<b>4 mg/m<sup>3</sup></b>	<b>8 mg/m<sup>3</sup></b>
<b>Day 6</b>				
Alveolar epithelium, hyperplasia	0	9 <sup>b</sup> (1.0)	10 <sup>b</sup> (1.1)	9 <sup>b</sup> (1.3)
Bronchiole epithelium, hyperplasia	0	10 <sup>b</sup> (1.0)	10 <sup>b</sup> (1.0)	9 <sup>b</sup> (1.3)
Histiocytic infiltrate	0	0	0	4 <sup>b</sup> (1.0)
<b>Day 13</b>				
Alveolar epithelium, hyperplasia	0	10 <sup>b</sup> (1.3)	10 <sup>b</sup> (2.0)	10 <sup>b</sup> (2.0)
Bronchiole epithelium, hyperplasia	0	10 <sup>b</sup> (1.3)	10 <sup>b</sup> (1.9)	10 <sup>b</sup> (1.6)
Histiocytic infiltrate	0	1 (1.0)	10 <sup>b</sup> (1.0)	10 <sup>b</sup> (1.1)
Inflammation	0	8 <sup>b</sup> (1.0)	10 <sup>b</sup> (2.0)	10 <sup>b</sup> (2.1)

<sup>a</sup>10 rats/treatment group; numbers in parentheses indicate average severity grade in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked

<sup>b</sup>Significantly different from control group by the Fisher exact test ( $p \leq 0.05$ )

to mild in all cases and involved the distal airways and alveolar ducts and alveoli. Alveolar macrophages (histiocytic infiltrate) were also observed in the alveoli of mice exposed to 8 mg/m<sup>3</sup> on days 6 and 13 and in mice exposed to 4 or 8 mg/m<sup>3</sup> on day 13. Minimal to mild interstitial inflammation, characterized by small numbers of mononuclear cells around vessels, small airways and into septae of alveolar ducts, was observed in most exposed mice after 13 days. The LOAEL for nonneoplastic lung lesion in female mice was 2 mg/m<sup>3</sup>; a NOAEL was not established. Since lesion severity was classified as minimal to mild, the LOAEL of 2 mg/m<sup>3</sup> is considered as minimal.

Results of cell proliferation studies (as measured by incorporation of BrdU) in mice exposed to 0, 2, 4 or 8 mg/m<sup>3</sup> vanadium pentoxide for days 6 and 13 are summarized in Table 7 (NTP, 2002). Cell turnover rates in the terminal bronchioles were increased for all treatment groups on day 6 and day 13.

Additional groups of 50 female mice were exposed to 0, 4, 8 or 16 mg/m<sup>3</sup> for 16 days to assess inflammatory and immune responses (NTP, 2002). Effects of treatment on pulmonary inflammation were evaluated by analysis of pulmonary lavage fluid and systemic immunity by pulmonary bactericidal activity analysis, influenza virus challenge, mixed lymphocyte culture response and cytotoxic T cell response after 16 days of exposure. Analysis of bronchoalveolar lavage (BAL) fluid showed a localized inflammatory response in the lung of female mice (9-10 per exposure group) based on increases in cell number, protein, neutrophils and lysozymes (Table 8). There was also a significant decrease in macrophages in lavage fluids of mice exposed to 8 or 16 mg/m<sup>3</sup>.

<b>Table 7. Bromodeoxyuridine-Labeled Lung Nuclei in Female Mice (B6C3F<sub>1</sub>) Exposed to Vanadium Pentoxide by Inhalation for 6 or 13 Days (NTP, 2002)</b>				
Lung Location	Exposure Group <sup>a, b</sup>			
	Control	2 mg/m <sup>3</sup>	4 mg/m <sup>3</sup>	8 mg/m <sup>3</sup>
<b>Terminal Bronchiole</b>				
Day 6	46.63±3.18	80.66±4.02	109.84±3.34	125.28±7.41
Day 13	44.37±2.42	75.45±3.67	92.59±4.38	63.02±3.22
<b>Alveoli/Alveolar Duct Area</b>				
Day 6	0.63±0.05	0.46±0.02	0.43±0.02	0.87±0.06
Day 13	0.49±0.03	1.15±0.05	2.01±0.10	2.32±0.08

<sup>a</sup>Data are given as the number of bromodeoxyuridine-labeled nuclei/mm basement membrane (mean±standard error)

<sup>b</sup>10 animals per exposure group

<b>Table 8. Pulmonary Lavage Parameters for Female Mice Exposed to Vanadium Pentoxide by Inhalation for 16 Days (NTP, 2002)<sup>a</sup></b>				
Parameter	Exposure Level			
	Control	4 mg/m <sup>3</sup>	8 mg/m <sup>3</sup>	16 mg/m <sup>3</sup>
Total cells (10 <sup>6</sup> )	3.42±3.15 (9)	9.03±4.98 (10)	12.38±7.20 <sup>b</sup> (10)	15.27±12.02 <sup>b</sup> (10)
Macrophages (%)	99±1 (10)	95±4 (10)	88±10 <sup>b</sup> (10)	80±10 <sup>b</sup> (10)
Lymphocytes (%)	1±1 (10)	5±4 <sup>b</sup> (10)	5±4 <sup>b</sup> (10)	5±3 <sup>b</sup> (10)
Neutrophils (%)	0±0 (10)	0±0 (10)	7±8 (10)	15±10 <sup>b</sup> (10)
Lavage fluid protein (µg/mL)	124±87 (10)	187±37 <sup>b</sup> (10)	207±41 <sup>b</sup> (10)	262±47 <sup>b</sup> (10)
Lysozyme (µg/mL)	20±3 (10)	29±5 <sup>b</sup> (10)	31±3 <sup>b</sup> (10)	29±4 <sup>b</sup> (10)
Lysozyme (µg/µg protein)	0.21±0.09 (10)	0.16±0.03 (10)	0.15±0.04 (10)	0.11±0.02 <sup>b</sup> (10)

<sup>a</sup>Data are presented as means±standard deviations; numbers in parentheses indicate the number of animals per group

<sup>b</sup>Significantly different from control group (p≤0.05) by Dunnett's test

To assess pulmonary bactericidal activity, lungs from 12 female mice per group were exposed to viable radiolabeled [35S]-*K. pneumoniae* and evaluated for pulmonary bactericidal activity 3 hours after inoculation. No treatment-related effects were observed for any vanadium pentoxide group. No treatment-related effects were observed in groups of 20 female mice per dose instilled intranasally with influenza virus and evaluated for moribundity for 14 days. Groups of eight female mice per dose were evaluated for mixed lymphocyte response to allogenic splenocytes and induction of cytotoxic T lymphocytes; no treatment-related effects were observed. Thus, results of immunotoxicity studies indicate that inhalation exposure of female mice to vanadium pentoxide at concentration up to 16 mg/m<sup>3</sup> for 16 days was not immunotoxic.

*NTP (2002) 3-Month Exposure Studies in Rats.* The 3-month exposure studies in F344/N rats were conducted to evaluate the cumulative toxic effects of subchronic inhalation exposure to

vanadium pentoxide (NTP, 2002). Groups of 10 male and 10 female rats were exposed (whole-body exposure) to aerosols of vanadium pentoxide at concentrations of 0, 1, 2, 4, 8 or 16 mg/m<sup>3</sup>, 6 hours per day, 5 days/week for 3 months. Particle size MMAD±GSD for each dose groups was as follows: 1 mg/m<sup>3</sup>=1.2±2.8; 2 mg/m<sup>3</sup>=1.1±2.8; 4 mg/m<sup>3</sup>=1.2±2.8; 8 mg/m<sup>3</sup>=1.0±2.8; 16 mg/m<sup>3</sup>=1.2±2.8. Additional groups of 10 male and 10 female rats were exposed to 4, 8 or 16 mg/m<sup>3</sup> for 12 (females) or 13 (males) weeks to investigate effects of exposure on cardiovascular function, pulmonary function and pulmonary inflammation. Clinical findings were recorded weekly and animals were weighed weekly and at the end of the study. Blood and urine were collected from core study rats at study termination and blood was collected for hematology and clinical chemistry determinations from cardiopulmonary physiology study rats on days 4 and 23. Necropsy and histopathological evaluations (light microscopy of comprehensive tissues) were performed on all core study animals and samples for sperm motility and vaginal cytology evaluations were collected from core study rats exposed to 0, 2 (male rats only), 4, 8 or 16 (female rats only) mg/m<sup>3</sup> at the completion of the study.

Seven male rats and three female rats exposed to 16 mg/m<sup>3</sup> vanadium pentoxide died during the study (NTP, 2002). Abnormal breathing, thinness, lethargy, abnormal posture and ruffled fur were observed in male and female rats exposed to concentrations of 8 mg/m<sup>3</sup> and above. Diarrhea and nasal/eye discharge were also observed in some rats exposed to 16 mg/m<sup>3</sup>. Weight gain and absolute and relative lung weights are summarized in Table 9. Weight gain over the 3-month treatment period was significantly decreased compared to control in males exposed to 4 (6% decrease), 8 (10% decrease) and 16 (60% decrease) mg/m<sup>3</sup> and in females exposed to 16 mg/m<sup>3</sup> (30% decrease). Absolute lung weights were significantly increased in males exposed to concentrations of 2 mg/m<sup>3</sup> and greater and in females exposed to 4 mg/m<sup>3</sup> and greater. Relative lung weights were significantly greater than control in males exposed to 2 (16% increase), 4 (30% increase), 8 (51% increase) or 16 (145% increase) mg/m<sup>3</sup> and in females exposed to 4 (19% increase), 8 (76% increase) or 16 (117% increase) mg/m<sup>3</sup>. Other organ weight differences were considered by the researchers to be related to body weight decreases.

Results of hematology assessments are presented in Table 10. Erythrocyte count was significantly increased in the 8 and 16 mg/m<sup>3</sup> groups and hematocrit was significantly increased in the 16 mg/m<sup>3</sup> group in male and female rats. Hemoglobin was significantly increased in females exposed to 16 mg/m<sup>3</sup> and somewhat elevated in males exposed to 16 mg/m<sup>3</sup>. Microscopic evaluation of the red blood cell morphology detected increased polychromasia and hypochromia in rats in the 16 mg/m<sup>3</sup> groups (data not presented). Significantly decreased mean cell hemoglobin concentrations were observed in males exposed to 8 and 16 mg/m<sup>3</sup> and in females exposed to 4, 8, and 16 mg/m<sup>3</sup>. Reticulocyte count was significantly increased in males and females exposed to 16 mg/m<sup>3</sup>. Mean cell volume was significantly decreased, indicative of microcytosis, in male rats at concentrations of 2 mg/m<sup>3</sup> and above and in female rats at concentrations of 4 mg/m<sup>3</sup> and above. The researchers state that the observed hematological changes, including erythrocytosis, are consistent with pulmonary lesions that reduce pulmonary oxygen transfer, resulting in tissue hypoxia and stimulation of erythropoiesis by increased renal production of erythropoietin. Erythrocyte microcytosis is consistent with ineffective erythropoiesis, suggestive of altered iron metabolism and heme/hemoglobin production.

**Table 9. Body Weight Gain, Lung and Liver Weights in Rats (F344/N) Exposed to Vanadium Pentoxide by Inhalation for 3 Months (Values are Means±Standard Error) (NTP, 2002)**

Parameter	Exposure					
	Control	1 mg/m <sup>3</sup>	2 mg/m <sup>3</sup>	4 mg/m <sup>3</sup>	8 mg/m <sup>3</sup>	16 mg/m <sup>3</sup>
<b>Male Rats</b>						
Weight gain during 3-month exposure period (g)	8.4±0.9	7.4±0.8	8.2±0.6	7.7±0.5	6.2±0.2 <sup>a</sup>	5.6±0.7 <sup>b</sup>
Absolute lung weight (g)	0.2±0.01	0.2±0.01	0.3±0.01 <sup>b</sup>	0.3±0.01 <sup>b</sup>	0.3±0.01 <sup>b</sup>	0.4±0.01 <sup>b</sup>
Relative lung weight	7.0±0.2	6.9±0.2	7.8±0.3	9.3±0.2 <sup>b</sup>	10.0±0.3 <sup>b</sup>	12.7±0.5 <sup>a</sup>
<b>Female Rats</b>						
Weight gain during 3-month exposure period (g)	9.7±1.0	10.0±1.0	8.1±0.4	5.8±0.5 <sup>b</sup>	6.1±0.4 <sup>b</sup>	5.4±0.3 <sup>b</sup>
Absolute lung weight (g)	0.2±0.01	0.3±0.01	0.3±0.01	0.3±0.02 <sup>b</sup>	0.4±0.02 <sup>b</sup>	0.4±0.02 <sup>b</sup>
Relative lung weight	8.1±0.59	8.8±0.3	9.7±0.5	13.2±0.9 <sup>b</sup>	13.2±0.6 <sup>b</sup>	16.3±0.5 <sup>b</sup>

<sup>a</sup>Significantly different from control by William's test ( $p \leq 0.05$ )

<sup>b</sup>Significantly different from control by William's test ( $p \leq 0.01$ )

**Table 10. Selected Hematology Parameters in Rats (F344/N) Exposed to Vanadium Pentoxide by Inhalation for 3 Months (NTP, 2002)<sup>a</sup>**

Parameter	Exposure					
	Control	1 mg/m <sup>3</sup>	2 mg/m <sup>3</sup>	4 mg/m <sup>3</sup>	8 mg/m <sup>3</sup>	16 mg/m <sup>3</sup>
<b>Male Rats</b>						
Number	9	9	10	9	10	3
Erythrocytes (10 <sup>6</sup> /μL)	9.2±0.1	9.0±0.1	9.1±0.1	9.3±0.2	9.7±0.2 <sup>b</sup>	15.1±0.3 <sup>c</sup>
Reticulocytes (10 <sup>6</sup> /μL)	0.2 ±0.02	0.22±0.03	0.19±0.02	0.23±0.03	0.25±0.02	0.8 ±0.08 <sup>b</sup>
Hematocrit (%)	48.5±0.6	47.7±0.5	47.6±0.6	48.7±0.9	49.9±0.7	71.2±2.8 <sup>b</sup>
Hemoglobin (g/dL)	15.8±0.1	15.5±0.1	15.5±0.2	15.9±0.2	16.1±0.2	20.4±0.8
Mean cell volume (fL)	52.9±0.2	52.9±0.1	52.3±0.1 <sup>b</sup>	52.2±0.2 <sup>b</sup>	51.3±0.2 <sup>c</sup>	46.8±1.0 <sup>c</sup>
Mean cell hemoglobin (pg)	17.3±0.2	17.2±0.1	17.1±0.1	17.1±0.02	16.5±0.2 <sup>c</sup>	13.4±0.4 <sup>c</sup>
<b>Female Rats</b>						
Number	10	10	9	10	10	6
Erythrocytes (10 <sup>6</sup> /μL)	8.0±0.1	7.8±0.1	8.1±0.2	8.3±0.1	8.6±0.1 <sup>b</sup>	12.5±0.34 <sup>c</sup>
Reticulocytes (10 <sup>6</sup> /μL)	0.15 ± 0.02	0.17 ± 0.01	0.17 ± 0.01	0.16 ± 0.02	0.17 ± 0.02	0.45 ± 0.08 <sup>c</sup>
Hematocrit (%)	45.8±0.5	44.3±0.4	46.1±1.2	46.4±0.4	47.2±0.6	60.8±1.4 <sup>c</sup>
Hemoglobin (g/dL)	15.5±0.2	15.0±0.1	15.5±0.2	15.6±0.1	15.8±0.1	18.2±0.3 <sup>c</sup>
Mean cell volume (fL)	56.9±0.1	56.9±0.1	56.6±0.1	55.8±0.1 <sup>c</sup>	55.0±0.2 <sup>c</sup>	48.7±0.6 <sup>c</sup>
Mean cell hemoglobin (pg)	19.3±0.2	19.3±0.2	19.0±0.2	18.7±0.2 <sup>c</sup>	18.5±0.2 <sup>c</sup>	14.6±0.3 <sup>c</sup>

<sup>a</sup>Values are means±standard error

<sup>b</sup>Significantly different from control (p≤ 0.05)

<sup>c</sup>Significantly different from control (p≤ 0.01)

Sporadic alterations in clinical chemistry and urinalysis variables were observed at various time-points in exposed males and females; however, no dose- or duration-related pattern of effect was observed. Sporadic changes in serum liver enzyme activities were not consistent with hepatocellular injury. Vanadium pentoxide exposure did not affect reproductive endpoints in males (sperm count, spermatid heads, sperm motility), but it did increase estrous cycle length by 10% in females exposed to 8 mg/m<sup>3</sup>, but not 16 mg/m<sup>3</sup>, and reduced the number of cycling females in surviving rats in the 16 mg/m<sup>3</sup> group (percent reduction not reported).

Complete histopathological assessments were performed on rats exposed to 0, 8 and 16 mg/m<sup>3</sup> for 3 months; except for nonneoplastic lesions of the lung and nose, findings were not considered related to treatment (NTP, 2002). Results of histopathological evaluations of lung and nasal tissue from male and female rats exposed to 1, 2, 4, 8 and 16 mg/m<sup>3</sup> for 3 months are summarized in Table 11. Significant increases in the incidences of epithelial hyperplasia of the lung were observed in male and female rats exposed to concentrations of 2 mg/m<sup>3</sup> or greater. Epithelial hyperplasia occurred in the distal airways and associated alveolar ducts and alveoli. The incidences of inflammation or fibrosis were significantly increased in males exposed to 2 mg/m<sup>3</sup> or greater and females exposed to 4 mg/m<sup>3</sup> or greater. The incidences of hyperplasia

**Table 11. Incidences of Selected Nonneoplastic Lesions of the Lung and Nose in Rats (F344/N) Exposed to Vanadium Pentoxide by Inhalation for 3 Months (NTP, 2002)**

Lesion Location and Type	Numbers of Animals with Lesions					
	Control	1 mg/m <sup>3</sup>	2 mg/m <sup>3</sup>	4 mg/m <sup>3</sup>	8 mg/m <sup>3</sup>	16 mg/m <sup>3</sup>
<b>Male Rats<sup>a</sup></b>						
<b>Lung</b>						
Epithelium, hyperplasia	0	0	10 <sup>b</sup> (2.0)	10 <sup>b</sup> (3.0)	10 <sup>b</sup> (3.6)	10 <sup>b</sup> (3.3)
Inflammation	0	0	9 <sup>b</sup> (1.0)	10 <sup>b</sup> (1.0)	10 <sup>b</sup> (1.6)	10 <sup>b</sup> (2.1)
Fibrosis	0	0	2 (1.0)	10 <sup>b</sup> (1.9)	10 <sup>b</sup> (3.2)	10 <sup>b</sup> (3.1)
Bronchiole, exudates	0	0	0	0	7 <sup>b</sup> (1.0)	8 <sup>b</sup> (1.4)
<b>Nose</b>						
Epithelium, hyperplasia	0	0	0	1 (1.0)	10 <sup>b</sup> (1.2)	10 <sup>b</sup> (2.0)
Epithelium, squamous metaplasia	0	0	0	1 (1.0)	10 <sup>b</sup> (1.2)	10 <sup>b</sup> (1.8)
Inflammation	0	0	0	0	0	7 <sup>b</sup> (1.6)
<b>Female Rats<sup>a</sup></b>						
<b>Lung</b>						
Epithelium, hyperplasia	0	0	10 <sup>b</sup> (1.3)	10 <sup>b</sup> (2.9)	10 <sup>b</sup> (3.5)	10 <sup>b</sup> (3.2)
Inflammation	0	0	0	10 <sup>b</sup> (1.0)	10 <sup>b</sup> (1.9)	10 <sup>b</sup> (1.2)
Fibrosis	0	0	0	10 <sup>b</sup> (1.0)	10 <sup>b</sup> (2.9)	10 <sup>b</sup> (3.2)
Bronchiole, exudates	0	0	0	0	10 <sup>b</sup> (1.0)	8 <sup>b</sup> (1.1)
<b>Nose</b>						
Epithelium, hyperplasia	0	0	0	10 <sup>b</sup> (1.0)	10 <sup>b</sup> (1.8)	10 <sup>b</sup> (2.7)
Epithelium, squamous metaplasia	0	0	0	8 <sup>b</sup> (1.0)	10 <sup>b</sup> (1.8)	10 <sup>b</sup> (2.8)
Inflammation	0	0	0	0	1 (1.0)	9 <sup>b</sup> (1.6)

<sup>a</sup>10 animals per treatment group; numbers in parentheses indicate average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked

<sup>b</sup>Significantly different from control by Fisher exact test ( $p \leq 0.01$ )

and metaplasia of the nasal respiratory epithelium were significantly increased in males exposed to 8 or 16 mg/m<sup>3</sup> and in females exposed to 4 mg/m<sup>3</sup> or greater. Nasal hyperplasia and metaplasia involved the respiratory epithelium covering the ventral portion of the nasal septum, the vomeronasal organ, and, to a lesser extent, the ventral lateral walls of the anterior portion of the nasal cavity. Inflammation of the nose was significantly increased in males and females exposed to 16 mg/m<sup>3</sup>.

Cardiopulmonary assessments were conducted in groups of 4-10 male and female rats exposed to 0, 4, 8 and 16 mg/m<sup>3</sup> for 3 months (NTP, 2002). No treatment-related changes in

cardiovascular function, as assessed by blood pressure (systolic, diastolic and mean), heart rate and electrocardiogram, were observed in rats exposed to 4 or 8 mg/m<sup>3</sup>. Decreased heart rate and diastolic, systolic and mean blood pressure observed in male and female rats exposed to 16 mg/m<sup>3</sup> were considered to be a reflection of the poor condition of the animals, coupled with an effect from anesthesia. Significant exposure-related decreases in pulmonary function (as assessed by respiratory rate, tidal and minute volume, expiratory resistance, vital and total capacity, diffusing capacity, and dynamic and peak compliance) were observed in male and female rats in all vanadium pentoxide groups tested. Observed changes in impaired capacity to diffuse carbon monoxide and reduced static and dynamic lung volumes at exposure concentrations of 4 mg/m<sup>3</sup> and greater are suggestive of a restrictive lesion. Changes in forced expiratory maneuvers in rats exposed to 16 mg/m<sup>3</sup> suggest the presence of an obstructive disease. It is not clear whether pulmonary function results are indicative of obstructive disease or merely reflect the deteriorating condition of the 16 mg/m<sup>3</sup> rats, since histopathological finding in lungs of rats exposed to 8 and 16 mg/m<sup>3</sup> were similar. Taken together, results of pulmonary function tests indicate that a restrictive injury was present in male and female rats exposed to 4 mg/m<sup>3</sup> or greater, while an obstructive lung injury may have been present in rats exposed to 16 mg/m<sup>3</sup>.

Pulmonary inflammation as assessed by analysis of pulmonary lavage fluid was evaluated in rats exposed to 0, 4, 8 and 16 mg/m<sup>3</sup> for 3 months (NTP, 2002). Concentration-related increases were observed in the total numbers of cells, lymphocytes, neutrophils and protein recovered in pulmonary lavage fluid from rats exposed to vanadium pentoxide at concentrations of 4 and 8 mg/m<sup>3</sup>, demonstrating a pulmonary inflammatory response in male and female rats. These endpoints also were increased in the 16 mg/m<sup>3</sup> group, but to a lesser extent, most likely due to the overt toxicity of vanadium pentoxide at this dose.

Results of this study show that inhalation exposure of male and female rats to vanadium pentoxide aerosol for 3 months produced adverse effects on the hematological system and the lung (NTP, 2002). Microcytic erythrocytosis, which was possibly secondary to impaired pulmonary function, was observed at concentrations of 2 mg/m<sup>3</sup> and greater in males and 4 mg/m<sup>3</sup> and greater in females. Absolute and relative lung weights were significantly increased compared to controls at concentrations of 4 mg/m<sup>3</sup> and greater in females and 2 mg/m<sup>3</sup> and greater and 4 mg/m<sup>3</sup> and greater, respectively, in males. The incidence of nonneoplastic lesions of the nose was increased in male and female rats at concentrations of 8 mg/m<sup>3</sup> and greater and 4 mg/m<sup>3</sup> and greater, respectively, and the incidence of nonneoplastic lesions of the lung was increased in male and female rats 2 mg/m<sup>3</sup> and greater. Results of pulmonary function tests consistent with restrictive lung disease were observed at concentrations of 4 mg/m<sup>3</sup> and greater. Based on decreased erythrocyte size in male rats and nonneoplastic lung lesions in male and female rats, the NOAEL and LOAEL values identified for 3-month inhalation exposure to vanadium pentoxide aerosols were 1 and 2 mg/m<sup>3</sup>, respectively.

*NTP (2002) 3-Month Exposure Studies in Mice.* Three-month exposure studies in B6C3F<sub>1</sub> mice were conducted to evaluate the toxicity of subchronic inhalation exposure to vanadium pentoxide (NTP, 2002). Groups of 10 male and 10 female mice were exposed (whole-body exposure) to aerosols of vanadium pentoxide at concentrations of 0, 1, 2, 4, 8 or 16 mg/m<sup>3</sup>, 6 hours per day, 5 days/week for 3 months. Particle size MMAD±GSD for each dose groups was as follows: 1 mg/m<sup>3</sup>=1.2±2.8; 2 mg/m<sup>3</sup>=1.1±2.8; 4 mg/m<sup>3</sup>=1.2±2.8; 8 mg/m<sup>3</sup>=1.0±2.9; 16 mg/m<sup>3</sup>=1.2±2.8.

Clinical findings were recorded weekly and animals were weighed weekly and at the end of the study. Necropsies were performed in all study animals. Histopathological examinations of lungs were performed in all mice in the 0, 1, 2, 4, 8 or 16 mg/m<sup>3</sup> groups and of thymus in all mice in the 0, 8 or 16 mg/m<sup>3</sup> groups. At the end of the 3-month exposure period, samples for sperm motility and vaginal cytology evaluations were collected from mice exposed to 0, 4, 8 or 16 mg/m<sup>3</sup>. Complete histopathological examination was performed in mice in the control and 16 mg/m<sup>3</sup> groups, although results were not reported. Assessments of cardiopulmonary function, pulmonary inflammation (analysis of pulmonary lavage), and hematological parameters were not conducted in mice.

One male mouse in the 16 mg/m<sup>3</sup> group died before the end of the study. The animal that died early appeared thin, but no other signs of toxicity were reported (NTP, 2002). No other treatment-related clinical findings were observed in any other mice in any treatment group. Weight gain and absolute and relative lung weights are summarized in Table 12. Weight gain over the 3-month treatment period was significantly decreased compared to control in males exposed to 8 (6% decrease) and 16 (10% decrease) mg/m<sup>3</sup> and in females exposed to 4 (11% decrease), 8 (10% decrease) and 16 (12% decrease) mg/m<sup>3</sup>. Absolute lung weights were significantly increased compared to control at concentrations of 2 mg/m<sup>3</sup> and higher in males and 4 mg/m<sup>3</sup> and higher in females. Relative lung weights were significantly greater than control in males exposed to 4 (33% increase), 8 (43% increase) or 16 (82% increase) mg/m<sup>3</sup> and in females exposed to 4 (62% increase), 8 (63% increase) or 16 (101% increase) mg/m<sup>3</sup>. Other organ weight differences were considered to be related to decreases in body weight by the researchers. The epididymal spermatozoal motility of males exposed to 8 or 16 mg/m<sup>3</sup> was significantly decreased by 13 and 5%, respectively. No treatment-related effects were observed for assessments of estrous cycle (estrous cycle length and number of cycling females).

Results of histopathological evaluations of lung tissue from male and female mice exposed to 0, 1, 2, 4, 8 and 16 mg/m<sup>3</sup> for 3 months are summarized in Table 13 (NTP, 2002). Epithelial hyperplasia was observed in male and female mice exposed to concentrations of 2 mg/m<sup>3</sup> and above, with lesion severity increasing with exposure concentration. Hyperplasia involved alveolar and, to a lesser extent, bronchiolar epithelium. Inflammation, which was characterized by multiple foci of a mixed cellular infiltrate oriented around blood vessels and bronchioles, was observed in male mice exposed to 4 mg/m<sup>3</sup> and above and in female mice exposed to 2 mg/m<sup>3</sup> and above. Infiltrate was composed primarily of macrophages with abundant cytoplasm and fewer lymphocytes and neutrophils. Histopathological evaluations of the thymus of male and female mice exposed to 0, 8 and 16 mg/m<sup>3</sup> for 3 months showed lymphoid depletion in mice exposed to 16 mg/m<sup>3</sup> (males: control, 0/9; 8 mg/m<sup>3</sup>, 0/8; 16 mg/m<sup>3</sup>, 2/7; females: 0/9, 0/9, 1/10).

Results of the 3-month inhalation study in mice indicate that the lung is the primary target organ for vanadium pentoxide toxicity (NTP, 2002). Based on increases in absolute lung weights at concentrations of 2 mg/m<sup>3</sup> and greater (males) and inflammation of the respiratory epithelium at concentrations of 2 mg/m<sup>3</sup> and greater (males and females), NOAEL and LOAEL values were identified as 1 and 2 mg/m<sup>3</sup>, respectively.

**Table 12. Body Weight Gain, Lung and Liver Weights in Mice (B6C3F<sub>1</sub>) Exposed to Vanadium Pentoxide by Inhalation for 3 Months (Values are Means±Standard Error) (NTP, 2002)**

Parameter	Exposure					
	Control	1 mg/m <sup>3</sup>	2 mg/m <sup>3</sup>	4 mg/m <sup>3</sup>	8 mg/m <sup>3</sup>	16 mg/m <sup>3</sup>
<b>Male Mice</b>						
Weight gain during 3-month exposure period (g)	8.4±0.9	7.4±0.8	8.2±0.6	7.7±0.5	6.2±0.2 <sup>a</sup>	5.6±0.7 <sup>b</sup>
Absolute lung weight (g)	0.2±0.01	0.2±0.01	0.3±0.01 <sup>b</sup>	0.3±0.01 <sup>b</sup>	0.3±0.01 <sup>b</sup>	0.4±0.01 <sup>b</sup>
Relative lung weight	7.0±0.2	6.9±0.2	7.8±0.3	9.3±0.2 <sup>b</sup>	10.0±0.3 <sup>b</sup>	12.7±0.4 <sup>b</sup>
<b>Female Mice</b>						
Weight gain during 3-month exposure period (g)	9.7±1.0	10.0±1.0	8.1±0.4	5.8±0.5 <sup>b</sup>	6.1±0.4 <sup>b</sup>	5.4±0.3 <sup>b</sup>
Absolute lung weight (g)	0.2±0.01	0.3±0.01	0.3±0.01	0.3±0.02 <sup>b</sup>	0.4±0.02 <sup>b</sup>	0.4±0.02 <sup>b</sup>
Relative lung weight	8.1±0.5	8.8±0.3	9.7±0.5	13.2±0.9 <sup>b</sup>	13.2±0.6 <sup>b</sup>	16.3±0.52 <sup>b</sup>

<sup>a</sup>Significantly different from control by William's test (p≤ 0.05)

<sup>b</sup>Significantly different from control by William's test (p≤ 0.01)

**Table 13. Incidences of Selected Nonneoplastic Lesions of the Lung in Mice (B6C3F<sub>1</sub>) Exposed to Vanadium Pentoxide by Inhalation for 3 Months (NTP, 2002)**

Lesion Type	Numbers of Animals with Lesions <sup>a</sup>					
	Control	1 mg/m <sup>3</sup>	2 mg/m <sup>3</sup>	4 mg/m <sup>3</sup>	8 mg/m <sup>3</sup>	16 mg/m <sup>3</sup>
<b>Male</b>						
Number	10	10	10	10	10	10
Inflammation	0	1 (1.0)	3 (1.0)	4 <sup>b</sup> (1.0)	10 <sup>c</sup> (2.0)	10 <sup>c</sup> (2.0)
Epithelium, hyperplasia	0	1 (1.0)	4 <sup>b</sup> (1.0)	5 <sup>b</sup> (1.0)	10 <sup>c</sup> (1.3)	10 <sup>c</sup> (3.0)
<b>Female</b>						
Number	10	9	10	9	10	10
Inflammation	0	1 (1.0)	7 <sup>c</sup> (1.0)	9 <sup>c</sup> (1.9)	10 <sup>c</sup> (1.9)	10 <sup>c</sup> (2.5)
Epithelium, hyperplasia	0	0	6 <sup>c</sup> (1.0)	9 <sup>c</sup> (1.5)	10 <sup>c</sup> (1.5)	10 <sup>c</sup> (2.5)

<sup>a</sup>Numbers in parentheses indicate average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked

<sup>b</sup>Significantly different from control by Fisher exact test, p≤ 0.05

<sup>c</sup>Significantly different from control by Fisher exact test, p≤ 0.01

*Knecht et al. (1985)*. The lung was also identified as a target organ for acute inhalation exposure to vanadium pentoxide. Sixteen male cynomolgus monkeys were exposed to aerosols of 0.5 or 5 mg/m<sup>3</sup> vanadium pentoxide by whole-body inhalation for 6 hours (Knecht et al., 1985), with exposures conducted at one-week intervals. Effects on airway function were evaluated by comprehensive pulmonary function tests (PFTs) in monkeys performed after exposure to 0.5 and 5 mg/m<sup>3</sup> and on pulmonary inflammation by analysis of bronchoalveolar lavage (BAL) fluid in monkeys performed after exposure to 5 mg/m<sup>3</sup>. Post-exposure values for PFTs and BAL were compared with baseline values determined for each animal prior to each exposure. Pulmonary function tests were not affected by acute exposure to 0.5 mg/m<sup>3</sup>. Significant changes in pulmonary function parameters compared to baseline values were observed following exposure to 5 mg/m<sup>3</sup> as follows: 16% increase in pulmonary resistance; 11% decrease in peak expiratory flow rate; 5-22% decreases in forced expiratory flow maneuvers; 33% increase in residual volume; and 24% increase in forced residual capacity. Results are consistent with air-flow limitation in both small peripheral and large central airways. A significant increase (approximately 87%; data presented graphically) in the total number of cells recovered in BAL fluid was observed 1 day after exposure to 5 mg/m<sup>3</sup> vanadium pentoxide. The increase in BAL fluid total cell number was primarily due to a marked increase (approximately 425%; data presented graphically) in the number of polymorphonuclear leukocytes. Results suggest that pulmonary inflammation and release of bronchoconstrictive mediators from inflammatory cells may play a role in vanadium pentoxide-induced air-flow restriction. An acute (single 6-hour exposure) LOAEL for vanadium pentoxide of 5 mg/m<sup>3</sup> for pulmonary function in monkeys was established in this study, with a NOAEL of 0.5 mg/m<sup>3</sup> evident.

*Knecht et al. (1992)*. Pulmonary reactivity was evaluated in adult male cynomolgus monkeys that were exposed to vanadium pentoxide dust aerosol by inhalation for 6 hours/day, 5 days/week for 26 weeks (Knecht et al., 1992). One control group exposed to filtered, conditioned air (n=8) and two exposed groups (n=8 each) receiving nominally equal weekly vanadium pentoxide exposures (concentration x time) with different exposure profiles were used. One exposed group (peak exposure group) received an actual concentration of 0.16 ( $\pm$ 0.01) mg/m<sup>3</sup> (0.1 mg/m<sup>3</sup> nominal) vanadium pentoxide on Mondays, Wednesdays and Fridays and 1.38 ( $\pm$ 0.07) mg/m<sup>3</sup> (1.1 mg/m<sup>3</sup> nominal) vanadium pentoxide on Tuesdays and Thursdays, and the other exposed group (constant exposure group) received a constant daily actual concentration of 0.57 ( $\pm$ 0.03) mg/m<sup>3</sup> (0.5 mg/m<sup>3</sup> nominal). The constant exposure regimen corresponded to a continuous exposure of 0.10 mg/m<sup>3</sup> after adjusting for exposure protocol (0.57 mg/m<sup>3</sup> x 6/24 x 5/7). The peak exposure regimen averaged to a slightly higher continuous exposure of 0.12 mg/m<sup>3</sup> after adjusting for exposure protocol. Provocation challenges consisting of single 6-hour exposures to 0.5 or 3.0 mg/m<sup>3</sup> vanadium pentoxide were used to compare pulmonary reactivity before and after the 26-week subchronic exposures. Vanadium pentoxide particle size was determined weekly during challenges and biweekly during exposures. Average particle size for the subchronic constant exposure group was 3.15  $\mu$ m (MMAD), with a GSD of 3.25  $\mu$ m. Particle sizes (MMAD $\pm$ GSD) for the peak exposure group were 3.17 $\pm$ 2.48 and 3.10 $\pm$ 2.45 for the 0.1 mg/m<sup>3</sup> and 1.1 mg/m<sup>3</sup> exposures, respectively. Pulmonary function tests, cytological and immunological analyses of blood and bronchoalveolar lavage fluid, and skin sensitivity tests were conducted before the pre- and post-exposure provocation challenges. Pulmonary function tests and bronchoalveolar lavage fluid analyses were also performed one day after the provocation challenges. Cytological endpoints included complete and differential blood cell

counts and leukotriene C<sub>4</sub> levels. Pulmonary function endpoints included total pulmonary resistance (RL), forced expiratory flow (FEF), forced vital capacity (FVC), residual volume (RV) and dynamic lung compliance (CL<sub>dyn</sub>). Immunological endpoints included total IgE, total IgG, albumin and total protein. The skin sensitivity tests assessed immediate and delayed responses to intradermal injections of vanadium pentoxide-monkey serum albumin conjugate. Respiratory distress, characterized by audible wheezing and coughing, occurred in 3/8 monkeys from the peak exposure group on peak exposure days during the first few weeks of the 26-week exposure; the responses developed within 3-4 hours of exposure and occasionally required early removal of the affected monkeys from the exposure chamber. The pre-exposure challenges produced an impairment in pulmonary function at 3.0 mg/m<sup>3</sup> characterized by airway obstructive changes (a 14% increase in RL and 13% decrease in FEV<sub>50</sub>/FVC accompanied by a 14% increase in RV and 3% decrease in FVC). Analysis of bronchoalveolar lavage fluid showed that the airway obstruction was accompanied by a significant influx of inflammatory cells (polymorphonuclear leukocytes) into the lung. Pulmonary function and other study endpoints were not significantly different between the three exposure groups (control, peak and constant) at either challenge concentration when the monkeys were rechallenged following subchronic exposure. The authors suggested that the absence of increased pulmonary reactivity to vanadium pentoxide following subchronic inhalation may be related to the development of tolerance. The study establishes a subchronic NOAEL<sub>[ADJ]</sub> of 0.10 mg/m<sup>3</sup> (continuous exposure) for pulmonary function. No subchronic LOAEL was indicated. However, an apparent acute, but reversible, LOAEL of 1.38 mg/m<sup>3</sup> is evidenced by the respiratory distress observed at that exposure level early in the study.

*Avila-Costa et al. (2004, 2005, 2006).* Results of three recent studies by Avila-Costa et al. (2004, 2005) suggest that inhalation exposure to vanadium pentoxide produces morphological changes to the central nervous system. Male CD-1 mice (n=48) were exposed to vanadium pentoxide by whole-body inhalation for 1 hour/day, 2 days/week for up to 8 weeks (Avila-Costa et al., 2004, 2005). Particle size was not reported in either study. The exposure concentration was reported as 0.02 M (Avila-Costa et al., 2004, 2005, 2006) or “1.4 mg/m<sup>3</sup>” (Avila-Costa et al., 2005). The same group of investigators (Gonzalez-Villalva et al., 2006; Mussali-Galante et al., 2005) using the same exposure protocol reported that the 0.02 M solution generated an average chamber concentration of 1.44 mg/m<sup>3</sup>, as vanadium metal (MW = 50.94). This exposure level corresponds to 5.13 mg/m<sup>3</sup> as vanadium pentoxide (MW = 181.9). The number of immunoreactive-TH<sup>+</sup> neurons in the substantia nigra region of the basal ganglia in the mesencephalon was measured at the end of each week of exposure and morphology of the blood-brain barrier was assessed after 8 weeks of exposure. No clinical signs of toxicity or other toxicological endpoints were reported in either study. A duration-dependent decrease in the number of immunoreactive-TH<sup>+</sup> neurons was observed from week 3 (decrease of approximately 30%; data presented graphically) through week 8 (decreased by approximately 63%; data presented graphically) of exposure (Avila-Costa et al., 2004). Morphological changes to the blood-brain barrier (cilia loss, cell sloughing and ependymal cell layer detachment) were observed after 8 weeks of exposure (Avila-Costa et al., 2005). Using a similar protocol, Avila-Costa et al. (2006) assessed the effects of vanadium pentoxide on memory and morphology of brain hippocampal neurons in male CD-1 mice that were exposed by whole-body inhalation for 1 hour/day, 2 days/week for up to 4 weeks. Groups of 6 exposed mice and 6 vehicle control mice (inhaling deionized water droplets) were evaluated after 24 hours and weekly for 4 weeks. No clinical signs or body weight changes were observed.

Spatial memory was tested using a modified Morris water maze task that was learned pre-exposure. Performance on this test, as assessed by latency (swimming time) to locate a hidden platform, was significantly impaired in the exposed mice at all time points in an increasing, time-related manner. Pyramidal neurons from the hippocampus CA1 region were evaluated for cytological and ultrastructural changes, because spatial memory mainly depends on this region of the brain. The cytological analysis assessed numbers of dendritic spines in the hippocampal cells; results showed a significant loss of dendritic spines in the exposed mice at all time points in an increasing time-related manner that correlated with the memory impairments. The ultrastructural analysis showed a significantly increased percentage of necrotic hippocampal cells at all time points that increased to a maximum of 33% after 4 weeks; other findings included hyperdense postsynaptic terminals and edema in mitochondria, dendrites, dendritic spines and presynaptic terminals. These three studies establish a LOAEL for morphological changes to the central nervous system accompanied by behavioural effects from short-term intermittent exposure to vanadium pentoxide at  $5.13 \text{ mg/m}^3$ .

*Gonzalez-Villalva et al. (2006)*. In another study by the same group as for the previously described studies (i.e., Avila-Costa et al., 2004, 2005, 2006), hematological effects of vanadium pentoxide were assessed in male CD-1 mice that were exposed by whole-body inhalation for 1 hour/day, 2 days/week for up to 12 weeks (Gonzalez-Villalva et al., 2006). A 0.02 M aqueous solution of vanadium pentoxide was aerosolized generating a reported average vanadium chamber concentration of  $1436 \text{ } \mu\text{g/m}^3$  ( $1.44 \text{ mg/m}^3$ ), corresponding to  $5.13 \text{ mg/m}^3$  as vanadium pentoxide. Groups of 8 exposed mice and 8 vehicle control mice (inhaling deionized water droplets) were evaluated after 24 hours and weekly for 12 weeks. Evaluations consisted of a complete blood count and morphological examination of platelets. Platelet count was significantly increased in the exposed mice on weeks 3-12; counts increased from week 3 to a maximum at week 9 and subsequently declined, but still remained above controls (quantitative data inadequately reported). The morphology examinations showed the presence of giant platelets at unspecified longer exposure times. The study establishes an apparent LOAEL for increased platelet count and altered platelet morphology from short-term intermittent exposure to vanadium pentoxide at  $5.13 \text{ mg/m}^3$ . A continuous exposure equivalent concentration cannot be estimated with any confidence, as the intermittency of the exposure protocol is extreme.

### **Inhalation Chronic Toxicity and Carcinogenicity**

*NTP (2002) 2-Year Exposure Studies in Rats*. The toxicity of chronic exposure to vanadium pentoxide was assessed in groups of 50 male and 50 female F344/N rats exposed (whole-body exposure) to particulate aerosols of vanadium pentoxide concentrations of 0, 0.5, 1 or  $2 \text{ mg/m}^3$  6 hours/day, 5 days/week for 104 weeks (Ress et al., 2003; NTP, 2002). Particle MMAD $\pm$ geometric standard deviation for each dose group was as follows:  $0.5 \text{ mg/m}^3=1.2\pm 2.9$ ;  $1 \text{ mg/m}^3=1.2\pm 2.9$ ;  $2 \text{ mg/m}^3=1.3\pm 2.9$ . Body weights and clinical findings were recorded throughout the exposure period. Necropsy and complete histopathological evaluation were performed on all animals. No clinical findings related to vanadium pentoxide exposure were observed. Mean body weights of females exposed to  $2 \text{ mg/m}^3$  were marginally less (3-6%; statistical significance not reported) than that of controls throughout the 2-year study; mean body weights of exposed male rats were similar to controls throughout the study. The number of male

and female rats surviving for the entire 104-week exposure period was low, but greater than that for control animals for all vanadium pentoxide groups (Table 14).

The incidences of nonneoplastic lesions of the respiratory tract in male and female rats are summarized in Table 14 (Ress et al., 2003; NTP, 2002). In male rats, the incidences of nonneoplastic lesions of the lungs (alveolar and bronchiole epithelium hyperplasia and alveolar histiocyte infiltration), larynx (inflammation and epiglottis degeneration, hyperplasia and squamous metaplasia) and nose (goblet cell hyperplasia) were significantly increased compared to control in all vanadium pentoxide exposure groups. In female rats, the incidences of nonneoplastic lesions of the lungs (interstitial fibrosis and alveolar histiocyte infiltration) and larynx (inflammation and epiglottis degeneration and hyperplasia) were significantly increased compared to control in all vanadium pentoxide exposure groups. In general, the incidences and severity ratings of respiratory lesions increased with exposure level. No treatment-related histopathological findings were observed in other tissues. The LOAEL of  $0.5 \text{ mg/m}^3$  was established for nonneoplastic lesions of the respiratory tract in male and female rats; a NOAEL was not identified.

The incidences of respiratory tumors in male and female rats exposed to vanadium pentoxide for 2 years are summarized in Table 15 (Ress et al., 2003; NTP, 2002). Compared to control, the incidences of alveolar/bronchiolar adenoma, alveolar/bronchiolar carcinoma or combined alveolar/bronchiolar adenoma or carcinoma were not significantly different compared to control (Poly-3 test) for male or female rats. NTP (2002) reports that the incidences of alveolar/bronchiolar adenoma in  $0.5$  and  $2 \text{ mg/m}^3$  males and  $0.5 \text{ mg/m}^3$  females, alveolar/bronchiolar carcinoma in  $0.5$  and  $2 \text{ mg/m}^3$  males, and combined alveolar/bronchiolar carcinoma in  $0.5$ ,  $1$  and  $2 \text{ mg/m}^3$  males and in  $0.5 \text{ mg/m}^3$  females exceed historical ranges for F344/N rats in inhalation chamber controls given NIH-07 diet, the same diet used in the NTP (2002) study (see Table 15 footnotes). Statistical comparisons of NTP (2002) tumor incidence data in rats and historical incidence data were not conducted. The researchers conclude that results of the NTP (2002) study were equivocal since the incidence of lung tumors in male and female rats could not be conclusively attributed to exposure to vanadium pentoxide. However, NTP (2002; Ress et al., 2003) also concluded that lung neoplasms were most likely related to vanadium pentoxide exposure, since tumor incidence in the NTP (2002) study exceeded that for historical controls.

*NTP (2002) 2-Year Exposure Studies in Mice.* The toxicity of chronic exposure to vanadium pentoxide was assessed in groups of 50 male and 50 female B6C3F<sub>1</sub> mice that were exposed (whole-body exposure) to particulate aerosols of vanadium pentoxide concentrations of 0, 1, 2 or  $4 \text{ mg/m}^3$ , 6 hours per day, 5 days/week, for 104 weeks (Ress et al., 2003; NTP, 2002). Particle MMAD±GSD for each dose group was reported as follows:  $1 \text{ mg/m}^3=1.3\pm2.9$ ;  $2 \text{ mg/m}^3=1.2\pm2.9$ ;  $4 \text{ mg/m}^3=1.2\pm2.9$ . Body weights and clinical findings were recorded throughout the exposure period. Necropsy and complete histopathological evaluation were performed on all animals. Many animals exposed to vanadium pentoxide were thin and exhibited abnormal breathing, particularly those exposed to 2 or  $4 \text{ mg/m}^3$  vanadium pentoxide (specific incidence data not reported). Mean body weights were generally less than control in males

**Table 14. Selected Nonneoplastic Lesions of the Respiratory System in Rats Exposed to Vanadium Pentoxide for 2 Years (NTP, 2002)**

Lesion Type and Location <sup>a</sup>	Exposure Group			
	Control	0.5 mg/m <sup>3</sup>	1 mg/m <sup>3</sup>	2 mg/m <sup>3</sup>
<b>Male Rats</b>				
Percent survival	40	58	52	54
<b>Lung</b>				
(Number of animals examined)	50	49	48	50
Alveolar epithelium, hyperplasia	7 (2.3)	24 <sup>b</sup> (2.0)	34 <sup>b</sup> (2.0)	49 <sup>b</sup> (3.3)
Bronchiole epithelium, hyperplasia	3 (2.3)	17 <sup>b</sup> (2.2)	31 <sup>b</sup> (1.8)	48 <sup>b</sup> (3.3)
Alveolar epithelium, squamous metaplasia	1 (1.0)	0	0	21 <sup>b</sup> (3.6)
Bronchiole epithelium, squamous metaplasia	0	0	0	7 <sup>b</sup> (3/7)
Inflammation, chronic active	5 (1.6)	8 (1.8)	24 <sup>b</sup> (1.3)	42 <sup>b</sup> (2.4)
Interstitial, fibrosis	7 (1.4)	7 (2.0)	16 <sup>c</sup> (1.6)	38 <sup>b</sup> (2.1)
Alveolus, histiocyte infiltration	22 (1.3)	40 <sup>b</sup> (2.0)	45 <sup>b</sup> (2.3)	50 <sup>b</sup> (2.1)
<b>Larynx</b>				
(Number of animals examined)	49	50	50	50
Inflammation, chronic	3 (1.0)	20 <sup>b</sup> (1.1)	17 <sup>b</sup> (1.5)	28 <sup>b</sup> (1.6)
Epiglottis epithelium, degeneration	0	22 <sup>b</sup> (1.1)	23 <sup>b</sup> (1.1)	33 <sup>b</sup> (1.5)
Epiglottis epithelium, hyperplasia	0	18 <sup>b</sup> (1.5)	34 <sup>b</sup> (1.5)	32 <sup>b</sup> (1.9)
Epiglottis epithelium, squamous metaplasia	0	9 <sup>b</sup> (1.7)	16 <sup>b</sup> (1.8)	19 <sup>b</sup> (1.9)
<b>Nose</b>				
(Number of animals examined)	49	50	49	48
Goblet cell, hyperplasia	4 (1.8)	15 <sup>b</sup> (1.8)	12 <sup>c</sup> (2.0)	17 <sup>b</sup> (2.1)
<b>Female Rats</b>				
Percent survival	28	40	34	30
<b>Lung</b>				
(Number of animals examined)	49	49	50	50
Alveolar epithelium, hyperplasia	4 (1.0)	8 (1.5)	21 <sup>b</sup> (1.2)	50 <sup>b</sup> (3.1)
Bronchiole epithelium, hyperplasia	6 (1.5)	5 (1.6)	14 <sup>c</sup> (1.3)	48 <sup>b</sup> (3.0)
Alveolar epithelium, squamous metaplasia	0	0	0	6 <sup>c</sup> (3.0)
Bronchiole epithelium, squamous metaplasia	0	0	0	1 (2.0)
Inflammation, chronic active	10 (1.5)	10 (1.1)	14 (1.2)	40 <sup>b</sup> (1.7)
Interstitial, fibrosis	19 (1.4)	7 <sup>b</sup> (1.3)	12 (1.6)	32 <sup>b</sup> (1.4)
Alveolus, histiocyte infiltration	26 (1.4)	35 <sup>c</sup> (1.3)	44 <sup>b</sup> (2.0)	50 <sup>b</sup> (1.9)
<b>Larynx</b>				
(Number of animals examined)	50	49	49	50
Inflammation, chronic	8 (1.8)	26 <sup>b</sup> (1.5)	27 <sup>b</sup> (1.3)	38 <sup>b</sup> (1.4)
Epiglottis epithelium, degeneration	2 (1.0)	33 <sup>b</sup> (1.2)	26 <sup>b</sup> (1.3)	33 <sup>b</sup> (1.5)
Epiglottis epithelium, hyperplasia	0	25 <sup>b</sup> (1.4)	26 <sup>b</sup> (1.3)	33 <sup>b</sup> (1.5)
Epiglottis epithelium, squamous metaplasia	2 (2.0)	7 (1.9)	9 (1.7)	16 <sup>b</sup> (1.4)
<b>Nose</b>				
(Number of animals examined)	50	50	50	50
Goblet cell, hyperplasia	12 (2.0)	19 (2.0)	16 (1.9)	30 <sup>b</sup> (2.0)

<sup>a</sup>Number of animals with lesion; numbers in parentheses indicate average severity grade of lesions in affected animals:

1=minimal, 2=mild, 3=moderate, 4=marked

<sup>b</sup>Significantly different from control by the Poly-3 test ( $p \leq 0.01$ )

<sup>c</sup>Significantly different from control by the Poly-3 test ( $p \leq 0.05$ )

<b>Table 15. Incidences of Respiratory Tumors in Rats Exposed to Vanadium Pentoxide for 2 Years (NTP, 2002)<sup>a</sup></b>				
<b>Tumor Type</b>	<b>Exposure Group</b>			
	<b>Control</b>	<b>0.5 mg/m<sup>3</sup></b>	<b>1 mg/m<sup>3</sup></b>	<b>2 mg/m<sup>3</sup></b>
<b>Male Rats</b>				
Number of animals examined	50	49	48	50
Alveolar/bronchoalveolar adenoma <sup>b</sup>	4 (8%)	8 (16%) <sup>c</sup>	5 (10%)	6 (12%) <sup>c</sup>
Alveolar/bronchoalveolar carcinoma <sup>d</sup>	0 (0%)	3 (6%) <sup>c</sup>	1 (2%)	3 (6%) <sup>c</sup>
Alveolar/bronchoalveolar adenoma or carcinoma <sup>e</sup>	4 (8%)	10 (20%) <sup>c</sup>	6 (12%) <sup>c</sup>	9 (18%) <sup>c</sup>
<b>Female Rats</b>				
Number of animals examined	49	49	50	50
Alveolar/bronchoalveolar adenoma <sup>f</sup>	0 (0%)	3 (6%) <sup>c</sup>	1 (2%)	0 (0%)
Alveolar/bronchoalveolar carcinoma	0 (0%)	0 (0%)	0 (0%)	1 (2%)
Alveolar/bronchoalveolar adenoma or carcinoma <sup>g</sup>	0 (0%)	3 (6%) <sup>c</sup>	1 (2%)	1 (2%)

<sup>a</sup>Numbers in parentheses indicate percent incidence; particle size mass mean aerodynamic diameter (MMAD±GSD): 0.5 mg/m<sup>3</sup>=1.2±2.9; 1 mg/m<sup>3</sup>=1.2±2.9; 2 mg/m<sup>3</sup>=1.3±2.9

<sup>b</sup>Historical incidence of alveolar/bronchoalveolar adenoma male F344/N rats fed in inhalation chamber controls given NIH-07 diet: range 0-10%

<sup>c</sup>Incidence exceeds historical control (statistical comparison between NTP (2002) data and historical data not conducted)

<sup>d</sup>Historical incidence of alveolar/bronchoalveolar carcinoma male F344/N rats fed in inhalation chamber controls given NIH-07 diet: range 0-4%

<sup>e</sup>Historical incidence of combined alveolar/bronchoalveolar adenoma or carcinoma male F344/N rats fed in inhalation chamber controls given NIH-07 diet: range 0-10%

<sup>f</sup>Historical incidence of alveolar/bronchoalveolar adenoma female F344/N rats fed in inhalation chamber controls given NIH-07 diet: range 0-4%

<sup>g</sup>Historical incidence of combined alveolar/bronchoalveolar adenoma or carcinoma female F344/N rats fed in inhalation chamber controls given NIH-07 diet: range 0-4%

exposed to 4 mg/m<sup>3</sup> (decreases of 5-15%) and in females for all exposure groups (1 mg/m<sup>3</sup>, decreases of 4-10%; 2 mg/m<sup>3</sup>, decreases of 14-20%; and 4 mg/m<sup>3</sup>, decreases of 4-19%) (statistical significance not reported). The number of mice surviving for the entire 104-week exposure period was similar to control for all exposure groups for female mice and for males in the 1 and 2 mg/m<sup>3</sup> groups, but survival was significantly decreased in males exposed to 4 mg/m<sup>3</sup> (Table 16).

The incidences of nonneoplastic lesions of the respiratory tract in male and female mice are summarized in Table 16 (Ress et al., 2003; NTP, 2002). In male mice, the incidences of nonneoplastic lesions of the lungs (hyperplasia of the alveolar and bronchiole epithelium, inflammation, alveolus histiocyte infiltration), larynx (squamous metaplasia of the epiglottis) and nose (olfactory and respiratory epithelium degeneration in males and olfactory epithelial degeneration and atrophy in females) were significantly increased compared to control in all

<b>Table 16. Selected Nonneoplastic Lesions of the Respiratory System in Mice Exposed to Vanadium Pentoxide for 2 Years (NTP, 2002)</b>				
<b>Lesion Type and Location<sup>a</sup></b>	<b>Exposure Group</b>			
	<b>Control</b>	<b>1 mg/m<sup>3</sup></b>	<b>2 mg/m<sup>3</sup></b>	<b>4 mg/m<sup>3</sup></b>
<b>Male Mice</b>				
Percent survival	78	66	72	50 <sup>b</sup>
<b>Lung</b> (Number of animals examined)	50	50	50	50
Alveolar epithelium, hyperplasia	3 (3.0)	41 <sup>c</sup> (2.2)	49 <sup>c</sup> (3.3)	50 <sup>c</sup> (3.9)
Bronchiole epithelium, hyperplasia	0	15 <sup>c</sup> (1.0)	37 <sup>c</sup> (1.1)	46 <sup>c</sup> (1.7)
Inflammation, chronic	6 (1.5)	42 <sup>c</sup> (2.4)	45 <sup>c</sup> (1.6)	47 <sup>c</sup> (2.0)
Alveolus, histiocyte infiltration	10 (2.4)	36 <sup>c</sup> (2.4)	45 <sup>c</sup> (2.6)	49 <sup>c</sup> (3.0)
Interstitial, fibrosis	1 (1.0)	6 (1.7)	9 <sup>c</sup> (1.2)	12 <sup>c</sup> (1.7)
<b>Larynx</b> (Number of animals examined)	49	50	48	50
Epiglottis epithelium, squamous metaplasia	2 (1.0)	45 <sup>c</sup> (1.0)	41 <sup>c</sup> (1.0)	41 <sup>c</sup> (1.0)
<b>Nose</b> (Number of animals examined)	50	50	50	50
Inflammation, suppurative	16 (1.3)	11 (1.4)	32 <sup>c</sup> (1.2)	23 <sup>b</sup> (1.3)
Olfactory epithelium, atrophy	6 (1.0)	7 (1.6)	9 (1.3)	12 (1.2)
Olfactory epithelium, degeneration	1 (1.0)	7 <sup>b</sup> (1.0)	23 <sup>b</sup> (1.1)	30 <sup>c</sup> (1.2)
Respiratory epithelium, degeneration	8 (1.1)	22 <sup>c</sup> (1.0)	38 <sup>c</sup> (1.2)	41 <sup>c</sup> (1.4)
<b>Bronchial Lymph Node</b> (Number of animals examined)	40	38	36	40
Hyperplasia	7 (2.1)	7 (2.4)	12 (2.1)	13 (2.2)

<b>Table 16. Selected Nonneoplastic Lesions of the Respiratory System in Mice Exposed to Vanadium Pentoxide for 2 Years (NTP, 2002)</b>				
<b>Lesion Type and Location<sup>a</sup></b>	<b>Exposure Group</b>			
	<b>Control</b>	<b>1 mg/m<sup>3</sup></b>	<b>2 mg/m<sup>3</sup></b>	<b>4 mg/m<sup>3</sup></b>
<b>Female Mice</b>				
Percent survival	76	64	60	64
<b>Lung</b> (Number of animals examined)	50	50	50	50
Alveolar epithelium, hyperplasia		31 <sup>c</sup> (1.6)	38 <sup>c</sup> (2.0)	50 <sup>c</sup> (3.3)
Bronchiole epithelium, hyperplasia	0	12 <sup>c</sup> (1.0)	34 <sup>c</sup> (1.0)	48 <sup>c</sup> (1.5)
Inflammation, chronic	0	37 <sup>c</sup> (1.3)	39 <sup>c</sup> (1.8)	49 <sup>c</sup> (2.0)
Alveolus, histiocyte infiltration	4 (1.0)	34 <sup>c</sup> (2.4)	35 <sup>c</sup> (2.4)	45 <sup>c</sup> (2.7)
Interstitial, fibrosis	0	1 (2.0)	4 <sup>b</sup> (2.5)	8 <sup>c</sup> (1.5)
<b>Larynx</b> (Number of animals examined)	50	50	49	50
Epiglottis epithelium, squamous metaplasia	0	39 <sup>c</sup> (1.0)	45 <sup>c</sup> (1.0)	44 <sup>c</sup> (1.1)
<b>Nose</b> (Number of animals examined)	50	50	50	50
Inflammation, suppurative	19 (1.1)	14 (1.2)	32 <sup>c</sup> (1.2)	30 <sup>c</sup> (1.3)
Olfactory epithelium, atrophy	2 (1.5)	8 <sup>b</sup> (1.3)	5 (1.0)	14 <sup>c</sup> (1.3)
Olfactory epithelium, degeneration	11 (1.2)	23 <sup>c</sup> (1.0)	34 <sup>c</sup> (1.2)	48 <sup>c</sup> (1.3)
Respiratory epithelium, degeneration	35 (1.3)	39 (1.5)	46 <sup>c</sup> (1.7)	50 <sup>c</sup> (1.8)
<b>Bronchial Lymph Node</b> (Number of animals examined)	39	40	45	41
Hyperplasia	3 (2.0)	13 <sup>c</sup> (1.8)	14 <sup>c</sup> (2.3)	20 <sup>c</sup> (2.3)

<sup>a</sup>Number of animals with lesion; numbers in parentheses indicate average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked

<sup>b</sup>Significantly different from control by the Poly-3 test ( $p \leq 0.05$ )

<sup>c</sup>Significantly different from control by the Poly-3 test ( $p \leq 0.01$ )

vanadium pentoxide exposure groups. In general, the incidences and severity ratings of lesions increased with exposure level. No treatment-related histopathological findings were observed in other tissues. The LOAEL of 1 mg/m<sup>3</sup> was established for nonneoplastic lesions of the respiratory tract in male and female mice; a NOAEL was not identified.

The incidences of tumors of the respiratory tract in male and female mice exposed to vanadium pentoxide for 2 years are summarized in Table 17 (Ress et al., 2003; NTP, 2002). The incidences of alveolar/bronchiolar adenoma, alveolar/bronchiolar carcinoma and combined alveolar/bronchiolar adenoma or carcinoma were significantly increased in all groups of exposed female mice. In male mice, the incidences of alveolar/bronchiolar carcinoma and combined alveolar/bronchiolar adenoma or carcinoma were significantly increased compared to control in all vanadium pentoxide groups and alveolar/bronchiolar adenoma was significantly increased in the 2 mg/m<sup>3</sup> group.

<b>Table 17. Incidences of Respiratory Tumors in Mice Exposed to Vanadium Pentoxide for 2 Years (NTP, 2002)</b>				
<b>Tumor Type<sup>a</sup></b>	<b>Exposure Group</b>			
	<b>Control</b>	<b>1 mg/m<sup>3</sup></b>	<b>2 mg/m<sup>3</sup></b>	<b>4 mg/m<sup>3</sup></b>
<b>Male Mice</b>				
Number of animals examined	50	50	50	50
Alveolar/bronchoalveolar adenoma <sup>b</sup>	13 (26%)	16 (32%)	26 <sup>c</sup> (53%) <sup>d</sup>	15 (30%)
Alveolar/bronchoalveolar carcinoma <sup>e</sup>	12 (24%)	29 <sup>c</sup> (58%) <sup>d</sup>	30 <sup>c</sup> (60%) <sup>d</sup>	35 <sup>c</sup> (70%) <sup>d</sup>
Alveolar/bronchoalveolar adenoma or carcinoma <sup>f</sup>	22 (28%)	42 <sup>c</sup> (84%) <sup>d</sup>	43 <sup>c</sup> (86%) <sup>d</sup>	43 <sup>c</sup> (86%) <sup>d</sup>
<b>Female Mice</b>				
Number of animals examined	50	50	50	50
Alveolar/bronchoalveolar adenoma <sup>g</sup>	1 (2%)	17 <sup>c</sup> (34%) <sup>d</sup>	23 <sup>c</sup> (46%) <sup>d</sup>	19 <sup>c</sup> (38%) <sup>d</sup>
Alveolar/bronchoalveolar carcinoma <sup>h</sup>	0 (0%)	23 <sup>c</sup> (46%) <sup>d</sup>	18 <sup>c</sup> (36%) <sup>d</sup>	22 <sup>c</sup> (44%) <sup>d</sup>
Alveolar/bronchoalveolar adenoma or carcinoma <sup>i</sup>	1 (2%)	32 <sup>c</sup> (64%) <sup>d</sup>	35 <sup>c</sup> (70%) <sup>d</sup>	32 <sup>c</sup> (64%) <sup>d</sup>

<sup>a</sup>Number of animals with tumor; numbers in parentheses indicate percent incidence; particle size mass mean aerodynamic diameter (MMAD±GSD): 1 mg/m<sup>3</sup>= 1.3±2.9; 2 mg/m<sup>3</sup>= 1.2±2.9; 4 mg/m<sup>3</sup>=1.2±2.9

<sup>b</sup>Historical incidence of alveolar/bronchoalveolar adenoma male B6C3F<sub>1</sub> mice fed in inhalation chamber controls given NIH-07 diet: range 8-36%

<sup>c</sup>Significantly different from control by the Poly-3 test (p≤0.01)

<sup>d</sup>Incidence exceeds historical control (statistical comparison with NTP (2002) tumor incidence not conducted)

<sup>e</sup>Historical incidence of alveolar/bronchoalveolar carcinoma male B6C3F<sub>1</sub> mice fed in inhalation chamber controls given NIH-07 diet: range 0-21%

<sup>f</sup>Historical incidence of combined alveolar/bronchoalveolar adenoma or carcinoma male B6C3F<sub>1</sub> mice fed in inhalation chamber controls given NIH-07 diet: range 14-42%

<sup>g</sup>Historical incidence of alveolar/bronchoalveolar adenoma female B6C3F<sub>1</sub> mice fed in inhalation chamber controls given NIH-07 diet: range 0-14%

<sup>h</sup>Historical incidence of alveolar/bronchoalveolar carcinoma female B6C3F<sub>1</sub> mice fed in inhalation chamber controls given NIH-07 diet: range 0-12%

<sup>i</sup>Historical incidence of combined alveolar/bronchoalveolar adenoma or carcinoma female B6C3F<sub>1</sub> mice fed in inhalation chamber controls given NIH-07 diet: range 4-16%

### **Inhalation Reproductive and Developmental Toxicity**

*Mussali-Galante et al. (2005)*. Effects of vanadium pentoxide on gamma-tubulin in testicular cells were assessed in male CD-1 mice that were exposed by whole-body inhalation for 1 hour/day, 2 days/week for up to 12 weeks (Mussali-Galante et al., 2005). A 0.02 M aqueous solution of vanadium pentoxide was aerosolized, generating a reported average vanadium chamber concentration of 1.44 mg/m<sup>3</sup>, as vanadium metal (MW = 50.94). This exposure level corresponds to 5.13 mg/m<sup>3</sup> as vanadium pentoxide (MW = 181.9). Groups of 3 exposed mice and 3 vehicle control mice (inhaling deionized water droplets) were evaluated weekly for 12 weeks. Gamma-tubulin is a key cellular protein involved in centrosome function and necessary for cell division. Immunohistochemistry was used to determine percentages of gamma-tubulin-immunopositive Sertoli, Leydig and germ cells. Vanadium exposure significantly decreased the percentage of immunopositive cells for all three testicular cell types beginning at week 2 or 3. The responses were duration-dependent with the lowest percentages of immunoreactive cells occurring at the end of exposure period; values at week 12 ranged from 1.2% for germ cells and 1.5% for Sertoli cells to 10.1% for Leydig cells (compared to 87-88% in controls).

*Fortoul et al. (2007)*. Ultrastructure of the seminiferous tubules was evaluated in the testes of male CD-1 mice that were exposed to vanadium pentoxide by whole-body inhalation for 1 hour/day, 2 days/week for up to 12 weeks (Fortoul et al., 2007). The exposure concentration was reported as 0.02 M, which apparently refers to the concentration of an aqueous solution of vanadium pentoxide that was aerosolized. Reports of other studies by the same group of investigators using the same exposure protocol indicate that the 0.02 M solution generated an average vanadium chamber concentration of 1.44 mg/m<sup>3</sup> (5.13 mg/m<sup>3</sup> as vanadium pentoxide) with 0.5-5 µm aerosol droplets (Avila-Costa et al., 2006; Gonzalez-Villalva et al., 2006; Mussali-Galante et al., 2005). Five exposed mice and three vehicle control mice (inhaling deionized water droplets) were sacrificed per week from the first to the 12<sup>th</sup> week of exposure. No overt toxicosis or changes in body or testicular weight were observed. Necrosis of the spermatogonium, spermatocytes and Sertoli cells occurred throughout most of the study. The most susceptible cells to necrosis appeared to be the spermatogonia. Spermatogonial mortality was increased during weeks 2-12 with a maximum at weeks 6-7 (40% necrotic cells). Spermatocyte and Sertoli cell mortality were increased during weeks 3-12 with maximums at weeks 5-6 (25% and 15% necrotic cells, respectively). Vacuolation occurred simultaneously with necrosis in the Sertoli cells. Testicular vanadium concentration was increased after the first week of exposure and remained stable throughout the exposure period; the average level was 33 times higher than controls.

Together the two studies (Mussali-Galante et al., 2005; Fortoul et al., 2007) establish a LOAEL for vanadium pentoxide for testicular effects from short-term intermittent exposure to 5.13 mg/m<sup>3</sup>. A continuous exposure equivalent concentration cannot be estimated with any confidence, as the intermittency of the exposure protocol is extreme.

## SUPPORTING STUDIES

**Oral Toxicokinetic** — Studies investigating the toxicokinetics of orally administered vanadium pentoxide in humans or animals were not identified.

**Inhalation Toxicokinetic** — The toxicokinetics of inhaled vanadium has been studied for several vanadium compounds; however, few studies have evaluated the toxicokinetics of inhaled vanadium pentoxide. Studies investigating the toxicokinetics of inhaled vanadium pentoxide in humans were not identified. Although occupational studies indicate that inhaled vanadium is absorbed, as indicated by an increase in blood and urine vanadium, studies do not identify the vanadium compounds that workers were exposed to or adequately quantify exposure (Barth et al., 2002; Kiviluoto et al., 1981).

Results of toxicokinetics studies of inhaled or intratracheally administered vanadium pentoxide in rats indicate that vanadium pentoxide is absorbed from the lung, undergoes a wide distribution and is eliminated primarily into the urine (Dill et al., 2004; NTP, 2002; Rhoads and Sanders, 1985). As part of the NTP (2002) cancer bioassay, lung and blood vanadium concentrations and lung clearance half-times of vanadium were determined in groups of five female F344/N rats and B6C3F1 mice exposed to 0, 1 (rats only), 2 or 4 (mice only) mg/m<sup>3</sup> for 6 hours/day, 5 days/week for 16 days. Tissue burden analyses and lung and blood vanadium

concentration analyses were performed immediately following exposure on day 16, and elimination of vanadium from blood and lung was determined up to 8 days after exposure. Blood vanadium concentrations in exposed and chamber control rats were highly variable. The small increases in blood vanadium concentrations in exposed rats indicate that either very little vanadium was absorbed or that vanadium was eliminated rapidly from the blood. No significant differences were observed in blood vanadium concentrations between exposed groups. In mice, blood vanadium concentrations in chamber control and exposed mice were below the limit of detection (value not reported) in 37 of 45 samples tested. In both species, lung burdens were proportional to exposure concentration, and lung vanadium concentrations were consistent with linear kinetics over the exposure range studied. Lung clearance half-times ranged from 4.42 to 4.96 days in rats and from 2.40 to 2.55 days in mice, and were not significantly different between exposure groups.

Dill et al. (2004) examined the lung deposition and clearance of inhaled vanadium pentoxide in rats and mice following chronic inhalation exposure. Groups of 3-5 female F344/N rats and B6C3F1 mice were exposed to vanadium pentoxide concentrations of 0, 0.5, 1 or 2 mg/m<sup>3</sup> and 0, 1, 2 or 4 mg/m<sup>3</sup>, respectively, by whole-body inhalation, 6 hours/day, 5 days/week for 1, 5 and 12 days and 1, 2, 6, 12 and 18 months. The same data were also reported by NTP (2002) as part of the cancer bioassay. Lung weights and lung vanadium burdens were determined in all animals at all time points, and blood vanadium concentrations were determined in up to five animals per group after 1, 2, 6, 12 and 18 months of exposure. Blood vanadium concentrations in exposed groups were significantly higher than in controls in both rats and mice, although it was not possible to determine if blood vanadium concentrations were proportional to exposure concentration due to the small sample size and small differences in blood concentrations between groups. Vanadium lung burden tended to be proportional to exposure concentration and reached steady-state levels within 1-2 months in mice and 6 months in rats in the lowest exposure group, but lung burden declined after 12 and 18 months in the mid- and high-dose group for both species. Declines in vanadium lung burden at higher doses were attributed to vanadium-induced pathological changes to the lung. Clearance of vanadium from the lung was faster for mice compared to rats, with modeled estimates for lung elimination half-life ranging from 6.26 to 13.9 days in mice and from 37.3 to 61.4 days in rats. Due to slower lung clearance in rats, lung retention after 18 months of exposure was higher for rats than mice.

Rhoads and Sanders (1985) investigated the absorption, distribution and excretion of a single dose of radiolabeled vanadium pentoxide (40 µg) administered by intratracheal instillation to young adult female rats of either the Wistar or Fischer strains. Vanadium in tissues was determined at several time-points during the first 24 hours and at 3, 5, 7 and 14 days after dosing. Clearance of vanadium from the lung exhibited a biphasic pattern, with a rapid initial phase (half-life = 11 minutes), followed by a slower phase (half-life = 1.9 days). Vanadium was distributed to liver, kidney, bone, blood, gastrointestinal tract and ovary, with peak levels reached in all tissues within 3 days. The highest peak was observed in bone (17.2% of administered dose), followed by blood (15.7% of administered dose). After 14 days, bone retained 12.7% and the "carcass" retained 40% of the administered dose. Less than 2% of the administered dose remained in each of the other tissues. Over the 14-day observation period, approximately 28% of the administered dose was eliminated in urine and 14% in the feces.

Whole body clearance exhibited biphasic kinetics, with a rapid initial phase (half-life = 11 hours), following by a slower phase (51 days).

### Genotoxicity

Results of *in vitro* assays of mutagenicity of vanadium pentoxide are summarized in Table 18. Vanadium pentoxide produced gene mutations in most bacterial test systems, although negative results were reported by NTP (2002) in a reverse mutation assay in *Salmonella typhimurium*. Negative results were also reported in a gene mutation assay in Chinese hamster V79 fibroblast cells (Zhong et al., 1994). Positive results were observed in cultured mammalian cells for DNA strand breaks, micronuclei formation, aneuploidy, altered mitosis, cell transformation and altered mitosis at concentrations that were not cytotoxic; negative results were reported for sister chromatid exchange and chromosomal aberrations.

Experimental data in animals provide conflicting evidence of genotoxicity following *in vivo* exposure to vanadium pentoxide. Vanadium pentoxide administered for 3 months by inhalation to male and female mice (1, 2, 4, 8 or 16 mg/m<sup>3</sup>) did not increase the frequency of micronucleated normochromatic erythrocytes in peripheral blood (NTP, 2002). Additional details of exposure are provided in the NTP (2002) study summary (see Animal Inhalation Subchronic Toxicity section).

Altamirano-Lozano et al. (1993, 1996, 1999). Genotoxicity was evaluated in male CD-1 mice following single intraperitoneal injections of 5.75, 11.5 or 23 mg/kg vanadium pentoxide (Altamirano-Lozano et al., 1993, 1996). Exposure caused no treatment-related effects on mitotic index, average generational time or sister chromatid exchanges in bone marrow cells (Altamirano-Lozano et al., 1993), although all doses induced DNA damage in testicular germ cells (Altamirano-Lozano et al., 1996). Altamirano-Lozano et al. (1999) assessed DNA damage in male CD-1 mice 24 hours following single intraperitoneal injections of 0, 23.0, 11.5 or 5.75 mg/kg vanadium pentoxide (corresponding approximately to the LD<sub>50</sub>, 1/2 LD<sub>50</sub> and 1/4 LD<sub>50</sub>, respectively). Comet test results show the number of cells with DNA damage (primarily single strand breaks and alkali labile damage) was increased in liver, kidney, lung, spleen and heart, although increases did not exhibit dose-dependence. No evidence of DNA damage was observed in bone marrow.

Evidence demonstrating mutagenic activity of vanadium pentoxide *in vivo* in humans is lacking. The *in vivo* genotoxicity of vanadium pentoxide in lymphocytes and whole blood leukocytes obtained from 49 male workers exposed to vanadium pentoxide at a processing plant was compared to 12 non-exposed controls (Ivancits et al., 2002). The average exposure duration for workers was 12.4 years. Measurements or estimates of worker exposure to vanadium pentoxide were not reported, although exposure to vanadium was confirmed through measurement of serum and urine vanadium. No significant differences between vanadium-exposed and control workers were observed for DNA stand breaks (as assessed by alkaline comet assay), 8-hydroxy-2'deoxyguanosine (an oxidized DNA base common indicative of oxidative stress) or the frequency of sister chromatid exchange.

**Table 18. Genotoxicity of Vanadium Pentoxide *In Vitro***

Test System	Endpoint	Results <sup>a</sup>		Reference
		With Activation	Without Activation	
<i>Bacillus subtilis</i> (recombinant repair phenotype -assay)	Recombination repair	+	+	Kada et al., 1980
<i>Escherichia coli</i> (reverse mutation)	Gene mutation	No data	+	Kanematsu et al., 1980
<i>Salmonella typhimurium</i> (reverse mutation)	Gene mutation	No data	+	Kanematsu et al., 1980
<i>S. typhimurium</i> (reverse mutation)	Gene mutation	-	-	NTP, 2002
Chinese hamster V79 fibroblast cell line	Gene mutation	No data	-	Zhong et al., 1994
Chinese hamster V79 fibroblast cell line	Mitosis	No data	+	Zhong et al., 1994
Chinese hamster V79 fibroblast cell line	Sister chromatid exchange	No data	-	Zhong et al., 1994
Chinese hamster V79 fibroblast cell line	Micronucleus formation	No data	+	Zhong et al., 1994
Human lymphocytes	Chromosomal aberrations	Not applicable	-	Roldan and Altamirano, 1990
Human lymphocytes	Sister chromatid exchange	Not applicable	-	Roldan and Altamirano, 1990
Human lymphocytes	Aneuploidy	Not applicable	+	Ramirez et al., 1997
Human lymphocytes	Polyploidy	Not applicable	+	Roldan and Altamirano, 1990
Human lymphocytes	DNA strand breaks	Not applicable	+	Rojas et al., 1996
Human lymphocytes	DNA strand breaks	Not applicable	+	Ivancsits et al., 2002
Human fibroblasts	DNA strand breaks	Not applicable	+	Ivancsits et al., 2002
Syrian hamster embryo cell	Cell transformation	Not applicable	+	Kerckaert et al., 1996

<sup>a</sup> - = negative; + = positive

### Other Potential Effects

Vanadium salts have emerged as potential agents in the treatment of diabetes due to their ability to lower blood glucose and mimic insulin activity. Several recent publications have reviewed the anti-diabetic action of numerous vanadium compounds, including inorganic vanadium salts, peroxovanadium complexes and organic vanadium compounds (Mukjerjee et al., 2004; Marzban and McNeill, 2003; Domingo, 2002; Cam et al., 2000; Srivasta, 2000). The glucose-lowering activity of vanadium compounds is attributed to vanadyl (the cationic form of vanadium), which is the predominant intracellular form of vanadium, rather than to specific vanadium compounds or complexes (Marzban and McNeill, 2003). Studies assessing the

anti-diabetic action of vanadium pentoxide were not located; therefore, the potential for vanadium pentoxide to lower blood glucose is unknown.

### **FEASIBILITY FOR DERIVING A PROVISIONAL SUBCHRONIC RfD FOR VANADIUM PENTOXIDE**

No studies investigating the effects of acute, subchronic or chronic oral exposure to vanadium pentoxide in humans were identified. No animal studies that have comprehensively examined histopathological, biochemical and clinical endpoints of subchronic oral exposure were identified. Mountain et al. (1953) evaluated the effects of exposure of rats to dietary vanadium pentoxide for 103 days on body weight gain, erythrocyte count, hemoglobin and cystine content of hair, but did not report any information on other endpoints. A chronic RfD of 9E-03 mg/kg-day for vanadium pentoxide is available on IRIS based on a 2.5-year dietary NOAEL of 17.85 ppm (equivalent to 0.89 mg/kg/day) for decreased hair cystine content in an unpublished study in rats by Stokinger et al. (1953); no effects on growth or survival were seen in this study. The lack of adequate subchronic oral data for humans or animals precludes derivation of a subchronic p-RfD for vanadium pentoxide. In the absence of a subchronic p-RfD, the chronic RfD of 9E-03 mg/kg-day can be applied to subchronic exposures, as is the standard practice.

### **DERIVATION OF PROVISIONAL SUBCHRONIC AND CHRONIC RfCs FOR VANADIUM PENTOXIDE**

#### **Provisional Subchronic RfC**

The available human and animal data identify the respiratory tract as the primary target for subchronic exposure to vanadium pentoxide. Irritation of the upper and lower respiratory tract has been reported in several acute and subchronic occupational and case studies of workers exposed to vanadium pentoxide in fuel-oil ash and vanadium dust (Woodin et al., 2000; Irsigler et al., 1999; Woodin et al., 1999; Hauser et al., 1995; Levy et al., 1984; Musk and Tees, 1982; Kiviluoto, 1980; Lees, 1980; Kiviluoto et al., 1979; Zenz et al., 1962; Sjöberg, 1955; Vintinner et al., 1955; Williams, 1952). Symptoms of irritation include bronchitis, airway obstruction, chest pain, rhinitis, pharyngitis, laryngitis and conjunctivitis. Although subchronic occupational exposure studies provide supportive evidence for the respiratory tract as a target for inhaled vanadium pentoxide, studies failed to adequately quantify vanadium pentoxide exposure. Furthermore, occupational exposures were most likely to a complex mix of chemicals, rather than to vanadium pentoxide only. Thus, the available occupational exposure studies are not suitable as the basis for the subchronic p-RfC. Results of an uncontrolled acute inhalation study in volunteers provide supportive evidence for the respiratory irritant effects of vanadium pentoxide, with symptoms of respiratory irritation (cough and increased mucous production) observed at a concentration of 0.1 mg/m<sup>3</sup> (Zenz and Berg, 1967). However, due to the lack of control exposure, short exposure duration (8 hours) and low number of subjects (n=2) studied, these data are not suitable for deriving the subchronic p-RfC.

Results of the NTP (2002) study in rats and mice provide evidence of toxicity to the upper and lower respiratory tract, including increased lung weight, inflammation, nonneoplastic lesions, and decreased pulmonary function following 13-day, 16-day or 3-month inhalation exposure to vanadium pentoxide. A significant increase in pulmonary inflammation and histiocytic infiltrate of minimal to mild severity was observed in female rats (assessments not made in male rats) exposed to vanadium pentoxide for 13 days, with a LOAEL of 1 mg/m<sup>3</sup>; a NOAEL was not established (Table 2). Similar results were observed for female mice (assessments not made in male mice) exposed for 13 days, with a LOAEL of 2 mg/m<sup>3</sup> for minimal to mild epithelial hyperplasia and inflammation; a NOAEL was not established (Table 6). Nonneoplastic lung lesions were observed in male and female rats and mice exposed for 3 months, with NOAEL and LOAEL values of 1 and 2 mg/m<sup>3</sup>, respectively, for minimal to mild epithelial hyperplasia (Tables 11 and 13). Significant exposure-related decreases in pulmonary function were observed in male and female rats, with a LOAEL of 4 mg/m<sup>3</sup> (pulmonary function not assessed in mice) (NTP, 2002). An acute exposure study in cynomolgus monkeys established NOAEL and LOAEL values of 0.5 and 5 mg/m<sup>3</sup>, respectively, for decreased pulmonary function (Knecht et al., 1985). Knecht et al. (1985) did not assess histopathological changes to the respiratory tract. Other targets identified from subchronic inhalation studies are the hematopoietic system and the central nervous system. Mild microcytosis was observed in male and female rats exposed to inhaled vanadium pentoxide for 3 months (Table 10); hematological endpoints were not examined in mice (NTP, 2002). The NOAELs for mild microcytosis were 1 mg/m<sup>3</sup> in male rats and 4 mg/m<sup>3</sup> in female rats. NTP (2002) stated that the observed erythrocytosis was consistent with tissue hypoxia resulting from reduced oxygenation caused by the pulmonary lesions. Consequently, the erythrocytosis is considered to be a secondary effect arising from the primary lung lesions. Avila-Costa et al. (2004, 2005, 2006) reported morphological changes in the substantia nigra region of the basal ganglia and the blood-brain barrier in male mice exposed to 1.4 mg/m<sup>3</sup> for up to 8 weeks; effects on central nervous system function or other comprehensive endpoints were not reported. This study did not provide any information on the exposure-response relationship for morphological changes to the central nervous system since only one exposure level was tested.

The most sensitive endpoints by species, gender and duration identified by acute and subchronic inhalation studies with vanadium pentoxide are summarized in Table 19. Adverse effects to the respiratory system were reported in rats and mice following exposure for 13 days, 16 days and 3 months (NTP, 2002) and in monkeys (Knecht et al., 1985), and humans (Zenz and Berg, 1967) following acute exposure. Adverse systemic effects identified were microcytic erythrocytosis in male rats exposed for 3 months (NTP, 2002) and morphological changes to the central nervous system (CNS) in male mice exposed for up to 8 weeks (Avila-Costa et al., 2004, 2005). Knecht et al. (1985) reported NOAEL and LOAEL values of 0.5 and 5 mg/m<sup>3</sup>, respectively, for respiratory effects; however, due to the short exposure duration (a single 6-hour inhalation exposure) and small number of animals studied, these data were not used for derivation of the subchronic p-RfC. The study in humans (Zenz and Berg, 1967) was not useful for derivation of the subchronic p-RfC due to the absence of control exposure; furthermore, subjective symptoms limit utility of the data. Morphological changes in the CNS reported by Avila-Costa et al. (2004, 2005) were not considered as the critical effect due to inconsistencies in reporting of the exposure concentration.

<b>Table 19. Most Sensitive Endpoints by Species, Gender and Duration Identified in Acute and Subchronic Inhalation Studies with Vanadium Pentoxide</b>					
<b>Species (sex)</b>	<b>Exposure Duration</b>	<b>Effect</b>	<b>NOAEL<sub>ADJ</sub> (mg/m<sup>3</sup>)</b>	<b>LOAEL<sub>ADJ</sub> (mg/m<sup>3</sup>)</b>	<b>Reference</b>
Monkey (male)	6 hours	Air-flow restriction (measured by pulmonary function tests)	0.5 <sup>a</sup>	5.0	Knecht et al., 1985
Human (not reported)	8 hours	Respiratory irritation, cough, mucus formation	—	0.1 <sup>a</sup>	Zenz and Berg, 1967
Rat (females)	13 days	Nonneoplastic lung lesions (histiocytic infiltrate, inflammation)	—	0.18	NTP, 2002
Mouse (females)	13 days	Lung inflammation	—	0.36	NTP, 2002
Mouse (both)	16 days	Relative lung weight	—	0.36	NTP, 2002
Mouse (males)	8 weeks	Morphological changes to CNS	—	5.6 <sup>b</sup>	Avila-Costa et al., 2004, 2005
Mouse (males)	12 weeks	Testicular effects	—	5.6 <sup>b</sup>	Mussali-Galante 2005; Fortoul et al., 2007
Rat (both)	3 months	Microcytic erythrocytosis, lung epithelial hyperplasia and inflammation	0.18	0.36	NTP, 2002
Mouse (both)	3 months	Inflammation of respiratory epithelium; increased absolute lung weights	0.18	0.36	NTP, 2002
Monkey (male)	6 months	No adverse effects	0.1	—	Knecht et al., 1992

<sup>a</sup> Single exposures not adjusted for continuous exposure

<sup>b</sup> Not adjusted for continuous exposure because of the highly intermittent exposure protocol (1 hr/day, 2 days/wk)

To determine the most sensitive endpoint for derivation of the subchronic p-RfC, human equivalent concentration (HEC) conversions were calculated for systemic effects (microcytic erythrocytosis) and respiratory tract effects in rats and mice reported in the 2-week and 3-month studies conducted by NTP (2002). Duration adjusted-concentrations (NOAEL<sub>[ADJ]</sub> and LOAEL<sub>[ADJ]</sub>) used to calculate HEC values are summarized in Table 20. Using the RDDR computer program, as specified in the RfC guidelines (U.S. EPA, 1994b), NOAEL<sub>[HEC]</sub>s and LOAEL<sub>[HEC]</sub>s (in mg vanadium pentoxide/m<sup>3</sup>) were calculated using mean body weights for males and females reported by NTP (2002) and the average particle size MMAD±GSD of 1.2±2.8 for rats and mice as reported by NTP (2002) for effects occurring in the lung or systemically. Based on results of the HEC conversions, the respiratory system was identified as the primary target for vanadium pentoxide toxicity. The most sensitive endpoint for respiratory effects within the subchronic exposure period is inflammation of the broncho-alveolar region of the lung in female rats exposed to vanadium pentoxide for 13 days, with a LOAEL<sub>[HEC]</sub> of 0.11 mg/m<sup>3</sup> (NTP, 2002). As discussed previously, based on the classification of lesion severity of minimal to mild, the LOAEL was considered minimal. As shown in Table 21, the incidence of

Species (sex)	Exposure Duration (days)	NOAEL (mg/m <sup>3</sup> )		LOAEL(mg/m <sup>3</sup> )		RDDR <sup>c</sup>	NOAEL <sub>[HEC]</sub> (mg/m <sup>3</sup> ) <sup>d</sup>	LOAEL <sub>[HEC]</sub> (mg/m <sup>3</sup> ) <sup>d</sup>
		Actual	Adjusted <sup>b</sup>	Actual	Adjusted <sup>b</sup>			
<b>Rat (females)</b>	<b>13</b>	—	—	<b>1</b>	<b>0.18</b>	<b>0.616</b>	—	<b>0.11</b>
Mouse (males)	13	—	—	2	0.36	1.340	—	0.48
Mouse (females)	13	—	—	2	0.36	1.145	—	0.41
Rat (males)	90	1	0.18	2	0.36	0.692	0.12	0.25
Rat (females)	90	1	0.18	2	0.36	0.616	0.11	0.22
Mouse (males)	90	1	0.18	2	0.36	1.340	0.24	0.48
Mouse (females)	90	1	0.18	2	0.36	1.145	0.21	0.41

<sup>a</sup> Generally bronchiolar or alveolar epithelial hyperplasia and inflammation

<sup>b</sup> Adjusted to continuous 24-hr/7-day exposures from 6-hr/5-day exposure protocol

<sup>c</sup> Regionally Deposited Dose Ratio per RfC methodology (U.S. EPA, 1994b)

<sup>d</sup> N(L)OAEL<sub>(ADJ)</sub> x RDDR

Parameter/Species	Number of Animals with Lesion <sup>a</sup>			
	Control	1 mg/m <sup>3</sup>	2 mg/m <sup>3</sup>	4 mg/m <sup>3</sup>
Histiocytic infiltrate	0	10 <sup>b</sup> (1.3)	10 <sup>b</sup> (1.9)	10 <sup>b</sup> (2.2)
Inflammation	0	8 <sup>b</sup> (1.3)	10 <sup>b</sup> (1.7)	10 <sup>b</sup> (2.0)

<sup>a</sup> 10 rats/treatment group; numbers in parentheses indicate average severity grade in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked

<sup>b</sup> Significantly different from control group by the Fisher exact test (p≤ 0.05)

inflammation was 100% or nearly 100% in all vanadium pentoxide groups; therefore, data were not suitable for benchmark dose (BMD) analysis. Thus, the NOAEL/LOAEL approach was used to identify the POD for derivation of the subchronic p-RfC. A subchronic p-RfC based on the most sensitive respiratory effect would be protective for microcytosis, which occurred at higher exposure levels than respiratory effects. The slightly longer-term 6-month NOAEL<sub>[ADJ]</sub> of 0.1 mg/m<sup>3</sup> in monkeys (Knecht et al., 1992) also supports the choice of the POD.

The LOEL<sub>[HEC]</sub> of 0.11 mg/m<sup>3</sup> calculated for nonneoplastic lesions of the lung (alveolar epithelial hyperplasia, histiocytic infiltrate and inflammation) in female rats (NTP, 2002) was used to derive the **subchronic p-RfC of 1E-04 mg/m<sup>3</sup>** as follows:

$$\begin{aligned}\text{subchronic p-RfC} &= \text{LOEL}_{[\text{HEC}]} \div \text{UF} \\ &= 0.11 \text{ mg/m}^3 \div 1000 \\ &= 0.0001 \text{ mg/m}^3 \text{ or } 1\text{E-}04 \text{ mg/m}^3\end{aligned}$$

The uncertainty factor (UF) of 1000 was composed of the following:

- A partial UF of 3 (10<sup>0.5</sup>) was applied for extrapolation from a minimal LOAEL to a NOAEL. Although 80-100% of treated animals were affected, the effects at the LOAEL were considered to be minimal biological effects based on the severity classification of minimal to mild.
- A default 10-fold UF for intraspecies differences was used to account for potentially susceptible individuals in the absence of quantitative information or information on the variability of response in humans. Individuals with pre-existing respiratory disorders may be more susceptible to inhaled vanadium pentoxide.
- The default UF of 3 (10<sup>0.5</sup>) when using the RfC dosimetry conversions (U.S. EPA, 1994b) was applied for interspecies extrapolation to account for potential pharmacodynamic differences between rats and humans. .
- The full default UF of 10 was used for database insufficiencies due to the lack of adequate inhalation developmental toxicity studies and a multi-generation reproduction study.
- The exposure duration in the critical study was only 13 days, which is less than subchronic. However, since the database included studies of subchronic duration, an additional UF to account for exposure duration is not required.

Confidence in the LOAEL for respiratory effects is high. Respiratory tract toxicity has been consistently reported in acute and subchronic exposure studies in mice, rats, monkeys and humans, with LOAEL values ranging from 0.1 to 5 mg/m<sup>3</sup>. Chronic inflammation of the lung was observed in rats at the same exposure level as the LOAEL (NTP, 2002). Confidence in the key study is high. NTP (2002) assessed comprehensive endpoints in an appropriate number of animals. Confidence in the experimental database is low. Although there is rigorous assessment of subchronic toxicity in two species (NTP, 2002), data on the developmental or reproductive toxicity of inhaled vanadium pentoxide are inadequate. There is evidence of testicular toxicity following inhalation exposure (Mussali-Galante 2005; Fortoul et al., 2007), but a definite LOAEL cannot be established given the intermittent exposure protocol. Reproductive and developmental effects were reported in rats and mice injected subcutaneously with higher doses (5 – 12.5 mg/kg), but an equivalent inhalation exposure cannot be determined from these studies (Altamirano et al, 1991; Altamirano-Lozano et al, 1993, 1996; Zhang et al. 1991, 1993a, 1993b). Overall confidence in the subchronic p-RfC is medium.

## Provisional Chronic RfC

The effects of chronic inhalation of vanadium pentoxide were assessed in a single 2-year exposure study in rats and mice (NTP, 2002). The 26-month single-exposure cynomolgus monkey study (Knecht et al., 1992) is the only other long-term study available. No additional chronic exposure studies in animals were identified. Similar to subchronic exposure studies, results of the NTP (2002) 2-year study identify the upper and lower respiratory tract as the target for chronic inhalation exposure to vanadium pentoxide. Other target organs were not identified in the chronic exposure studies conducted by NTP (2002). As discussed earlier, although irritation of the upper and lower respiratory tract has been reported in several acute and subchronic occupational and case studies of workers exposed to vanadium pentoxide in fuel-oil ash and vanadium dust, exposure was not sufficiently quantified; therefore, data are not suitable for deriving the p-RfC.

The NTP (2002) 2-year exposure study was chosen as the key study for the derivation of the p-RfC based on nonneoplastic lesions of the respiratory tract in male and female rats and mice. As summarized in Tables 14 and 16, numerous lesions of the upper and lower respiratory tract were observed in male and female rats and mice at the lowest exposure concentrations tested, yielding LOAELs of  $0.5 \text{ mg/m}^3$  in rats and  $1 \text{ mg/m}^3$  in mice; NOAELs were not identified. In rats exposed to  $0.5 \text{ mg/m}^3$ , lesions were observed in the nose, larynx, bronchioles and lung of males and the nose and larynx of females. In mice exposed to  $1 \text{ mg/m}^3$ , lesions were observed in the nose, bronchiole and lung of male mice and the nose, larynx, bronchioles and lung of female mice. To identify the most sensitive endpoint for derivation of the p-RfC, human equivalent concentration (HEC) conversions were calculated for each region of the respiratory tract in which lesions were observed at the lowest concentration tested (e.g.,  $\text{LOAEL}_{[\text{HEC}]}$ ). Duration adjusted-concentrations ( $\text{LOAEL}_{[\text{ADJ}]}$ ) used to calculate HEC values are summarized in Table 22. Using the RDDR computer program, as specified in the RfC guidelines (U.S. EPA, 1994b),  $\text{LOAEL}_{[\text{HEC}]}$ s (in  $\text{mg vanadium pentoxide/m}^3$ ) were calculated for each species and sex using mean body weights for males and females reported by NTP (2002) and the average particle size  $\text{MMAD} \pm \text{GSD}$  of  $1.2 \pm 2.9$  for rats and mice as reported by NTP (2002) study, with effects occurring in various regions of the respiratory tract (Table 22).

The Knecht et al. (1992) monkey study established a  $\text{NOAEL}_{[\text{ADJ}]}$  of  $0.1 \text{ mg/m}^3$ . This value is close to the  $\text{LOAEL}_{[\text{ADJ}]}$  of  $0.09 \text{ mg/m}^3$  for rats in the NTP (2002) study, bringing into consideration the relative HEC values. As there are no studies available to establish an RDDR for monkeys, dosimetry modeling cannot be used to establish an HEC. Therefore, the full 10-fold interspecies uncertainty factor would be applied to the  $\text{NOAEL}_{[\text{ADJ}]}$  if the monkey study was used as the basis for the RfC. The resulting RfC would be twice the RfC based on the  $\text{LOAEL}_{[\text{HEC}]}$  from the NTP (2002) study, so the monkey  $\text{NOAEL}_{[\text{ADJ}]}$  is not an appropriate choice for the POD.

As shown in Table 22, the lowest  $\text{LOAEL}_{[\text{HEC}]}$  of  $0.016 \text{ mg/m}^3$  was observed for nonneoplastic lesions of the larynx of female rats, indicating that the larynx was the most sensitive target for chronic inhalation exposure to vanadium pentoxide. Thus, nonneoplastic lesions of the larynx in the female rat were chosen as the critical effect for the basis of the p-RfC.

**Table 22. LOAEL Values, Expressed in Terms of HEC, for Nonneoplastic Lesions of Various Regions of the Respiratory Tract in Rats and Mice Exposed to Vanadium Pentoxide by Inhalation for 2 Years (NTP, 2002)**

Species (Sex)	LOAEL (mg/m <sup>3</sup> )	LOAEL <sub>[ADJ]</sub> <sup>a</sup> (mg/m <sup>3</sup> )	Lesion Location	RDDR	LOAEL <sub>[HEC]</sub> <sup>b</sup> (mg/m <sup>3</sup> )
Rat (Male)	0.5	0.09	lung	0.398	0.036
	0.5	0.09	bronchiole	2.328	0.210
	0.5	0.09	larynx and nose	0.340	0.031
Rat (Female)	0.5	0.09	lung	0.414	0.037
	0.5	0.09	<b>larynx</b>	0.182	<b>0.016</b>
Mouse (Male)	1	0.18	lung	0.975	0.176
	1	0.18	bronchiole	3.089	0.556
	1	0.18	nose	0.338	0.061
Mouse (Female)	1	0.18	lung	0.942	0.170
	1	0.18	bronchiole	2.993	0.539
	1	0.18	lung and bronchiole	0.313	0.258
	1	0.18	larynx and nose	1.433	0.056

<sup>a</sup>LOAEL<sub>[ADJ]</sub> = LOAEL x 6/24 x 5/7

<sup>b</sup>LOAEL<sub>[HEC]</sub> = LOAEL<sub>[ADJ]</sub> x RDDR

Incidence data for lesions of the larynx in female rats are summarized in Table 23. To determine the point of departure for derivation of the p-RfC, benchmark dose modeling was conducted for two lesions of the larynx, inflammation and epithelial hyperplasia, in the female rat. Degeneration of the epiglottis epithelium was not selected for BMD modeling because the incidence of this lesion did not exhibit dose-dependence, with the same incidence observed in the low and high dose groups. Epithelial squamous metaplasia was not selected for BMD modeling

**Table 23. Incidences of Nonneoplastic Lesions of the Larynx in Female Rats Exposed to Vanadium Pentoxide by Inhalation for 2 Years (NTP, 2002)**

Lesion Type <sup>a</sup>	HEC (mg/m <sup>3</sup> )			
	Control	0.016 mg/m <sup>3</sup>	0.033 mg/m <sup>3</sup>	0.067 mg/m <sup>3</sup>
Number of animals examined	50	49	49	50
Inflammation, chronic	8	26 <sup>b</sup>	27 <sup>b</sup>	38 <sup>b</sup>
Epiglottis, epithelial hyperplasia	0	25 <sup>b</sup>	26 <sup>b</sup>	33 <sup>b</sup>
Epiglottis, epithelial degeneration	2	33 <sup>b</sup>	36 <sup>b</sup>	33 <sup>b</sup>
Epiglottis, epithelial squamous metaplasia	2	7	9	16 <sup>b</sup>

<sup>a</sup>Number of animals with lesion

<sup>b</sup>Significantly different from control by the Poly-3 test, p ≤ 0.01

because the incidence of this lesion was not significantly different from control at the low- and mid-dose groups; thus, other lesions of the larynx were more sensitive endpoints. In all vanadium pentoxide groups, lesion severity was classified as minimal to mild. Duration-adjusted exposure concentrations ( $\text{Conc}_{[\text{ADJ}]}$ ) of 0.09, 0.18 and  $0.37 \text{ mg/m}^3$ , corresponding to nominal exposure concentrations of 0.5, 1 and  $2 \text{ mg/m}^3$ , were calculated as follows:

$$\text{Conc}_{[\text{ADJ}]} = \text{Conc} \times 6/24 \times 5/7$$

HECs were calculated by multiplying  $\text{Conc}_{[\text{ADJ}]}$  by the RDDR of 0.182 for lesions of the larynx in female rats.

Modeling was performed using the Benchmark Dose Modeling Software (BMDS; Version 1.3.1) developed by the National Center for Environmental Assessment (U.S. EPA, 2000). In accordance with the U.S. EPA (2000) BMD methodology, the default benchmark response (BMR) of 10% increase in extra risk was used as the basis for the BMD ( $\text{BMD}_{10}$ ), with the  $\text{BMDL}_{10}$  represented by the 95% lower confidence limit on the  $\text{BMD}_{10}$ . All available dichotomous models were fit to the incidence data for chronic inflammation and epithelial hyperplasia of the epiglottis (Table 23). Goodness-of-fit was evaluated using the Chi-square statistic calculated by the BMDS program. Acceptable global goodness of fit was a p-value greater than or equal to 0.1. Models that did not meet this criterion were eliminated from consideration. Local fit was evaluated visually on the graphic output, by comparing the observed and estimated results at each data point. The model with the lowest Akaike's information criteria (AIC) value was considered to provide a superior fit.

Results of the BMDS modeling for chronic inflammation of the larynx are summarized in Table 24. As assessed by the chi-square goodness-of-fit test, several models in the software provided adequate fits to the data. The log-logistic model was determined to be the best-fitting model, as indicated by the lowest AIC (Table 24 and Figure 2), with a  $\text{BMDL}_{10}$  of  $0.0022 \text{ mg/m}^3$ .

Results of the BMDS modeling for epithelial hyperplasia of the epiglottis are summarized in Table 25 and Figure 3. As assessed by the chi-square goodness-of-fit test, four of the models provided adequate fits ( $\chi^2$  p-value  $\geq 0.1$ ). Three of the models (gamma, multi-stage and Weibull), however, are mathematically equivalent (as an exponential distribution) as the power (or slope) parameter was estimated at the constrained lower bound of 1. The  $\text{BMDL}_{10}$  of  $0.0022 \text{ mg/m}^3$  from the log-logistic model was selected based on the lowest AIC value (Table 24).

Benchmark dose modeling of incidence data for chronic inflammation of the larynx and epithelial hyperplasia of the epiglottis yielded the same  $\text{BMD}_{10}$  of  $0.003 \text{ mg/m}^3$  and  $\text{BMDL}_{10}$  of  $0.0022 \text{ mg/m}^3$ . The **provisional RfC of  $0.000007 \text{ mg/m}^3$  or  $7\text{E}-06 \text{ mg/m}^3$**  was derived as follows:

$$\begin{aligned} \text{p-RfC} &= \text{BMDL}_{10} \div \text{UF} \\ &= 0.0022 \text{ mg/m}^3 \div 300 \\ &= 0.000007 \text{ mg/m}^3 \text{ or } 7\text{E}-06 \text{ mg/m}^3 \end{aligned}$$

**Table 24. Goodness of Fit Statistics and BMD<sub>10</sub>s and BMDL<sub>10</sub>s From Models Fit to Incidence Data for Chronic Inflammation of the Larynx of Female Rats Exposed to Vanadium Pentoxide by Inhalation for 2 Years (NTP, 2002)**

Model	Degrees of Freedom	$\chi^2$ Test Statistic	$\chi^2$ p-Value <sup>a</sup>	AIC	BMD <sub>10</sub> (mg/m <sup>3</sup> )	BMDL <sub>10</sub> (mg/m <sup>3</sup> )
Gamma <sup>b</sup>	2	3.54	0.1702	241.742	0.00526062	0.00404152
Logistic	2	7.36	0.0253	245.803	0.00978952	0.00806489
<b>Log-Logistic<sup>c,d</sup></b>	<b>2</b>	<b>1.67</b>	<b>0.4348</b>	<b>239.886</b>	<b>0.00314813</b>	<b>0.00216334</b>
Multistage 1-degree <sup>e</sup>	2	3.54	0.1702	241.742	0.00526063	0.00404152
Probit	2	7.36	0.0252	245.792	0.00971167	0.00811435
Log-probit <sup>d</sup>	2	5.95	0.0511	244.055	0.00919646	0.00699817
Weibull <sup>b</sup>	2	3.54	0.1702	241.742	0.00526061	0.00404152

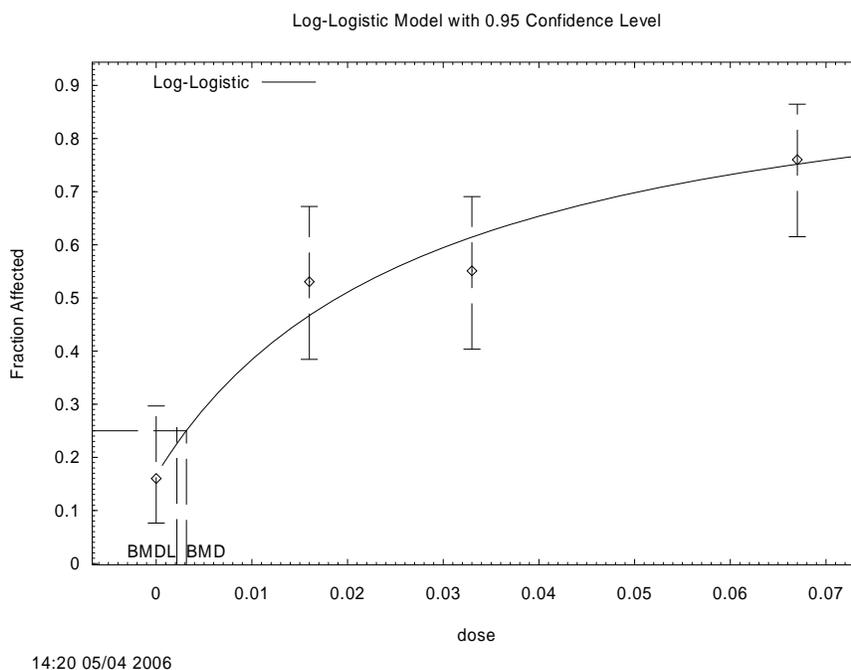
<sup>a</sup>Values <0.1 fail to meet BMDS goodness-of-fit criteria

<sup>b</sup>Power restricted to  $\geq 1$ ; lower bound on parameter estimate hit

<sup>c</sup>Best-fitting model as assessed by the lowest-AIC criterion

<sup>d</sup>Slope restricted to  $\geq 1$ ; lower bound on parameter estimate hit

<sup>e</sup>Betas restricted to  $\geq 0$ ; lowest degree polynomial model with an adequate fit is reported



BMDs and BMDLs indicated are for a 10% extra risk and are in units of mg/m<sup>3</sup>

**Figure 2. Observed and Predicted Incidences of Inflammation of the Larynx in Female Rats Exposed to Vanadium Pentoxide by Inhalation for 2 Years by NTP (2002)**

**Table 25. Goodness of Fit Statistics and BMD<sub>10</sub>s and BMDL<sub>10</sub>s From Models Fit to Incidence Data for Epithelial Hyperplasia of the Epiglottis in Female Rats Exposed to Vanadium Pentoxide by Inhalation for 2 Years (NTP, 2002)**

Model	Degrees of Freedom	$\chi^2$ Test Statistic	$\chi^2$ p-Value <sup>a</sup>	AIC	BMD <sub>10</sub> (mg/m <sup>3</sup> )	BMDL <sub>10</sub> (mg/m <sup>3</sup> )
Gamma <sup>b</sup>	3	14.11	0.0028	214.607	0.00461527	0.00384345
Logistic	2	25.06	0.0000	237.359	0.012132	0.0101008
<b>Log-Logistic<sup>c,d</sup></b>	<b>3</b>	<b>4.42</b>	<b>0.2191</b>	<b>206.048</b>	<b>0.00272969</b>	<b>0.00205055</b>
Multistage 1-degree <sup>e</sup>	3	14.11	0.0028	214.607	0.00461527	0.00384345
Probit	2	24.99	0.0000	236.771	0.0117506	0.00992799
Log-probit <sup>d</sup>	3	19.14	0.0003	218.709	0.00757035	0.00635086
Quantal-linear	3	14.11	0.0028	214.607	0.00461528	0.00384345
Quantal-quadratic	2	33.14	0.0000	246.358	0.0205956	0.0172045
Weibull <sup>b</sup>	3	14.11	0.0028	214.607	0.00461528	0.00384345

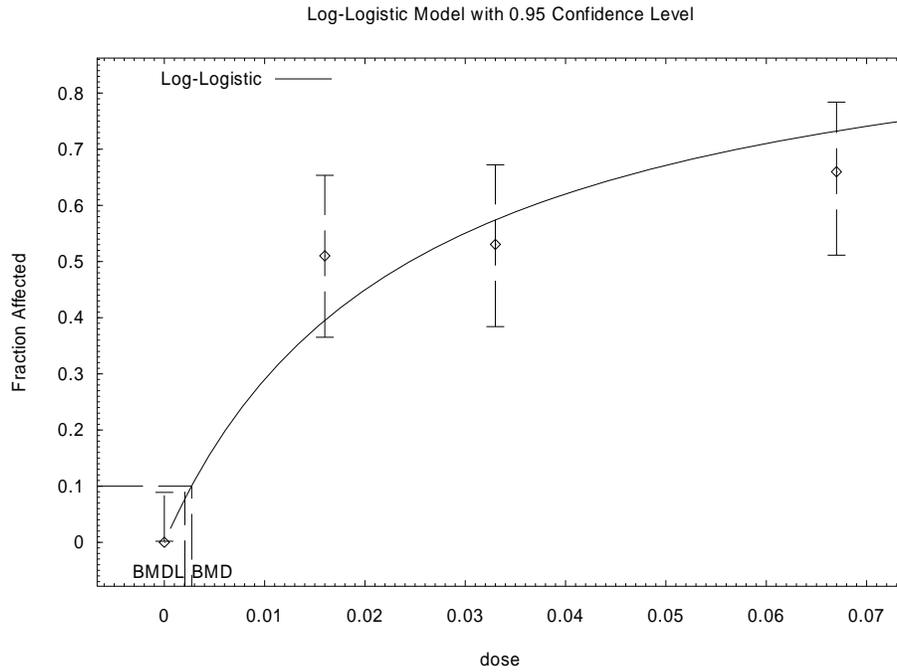
<sup>a</sup>Values <0.1 fail to meet conventional goodness-of-fit criteria

<sup>b</sup>Power restricted to  $\geq 1$

<sup>c</sup>Best-fitting model

<sup>d</sup>Slope restricted to  $\geq 1$

<sup>e</sup>Betas restricted to  $\geq 0$ ; degree polynomial model with the best fit is reported



BMD and BMDL indicated are for a 10% extra risk and are in units of mg/m<sup>3</sup>

**Figure 3. Observed and Predicted Incidences of Epithelial Hyperplasia of the Epiglottis in Female Rats Exposed to Vanadium Pentoxide by Inhalation for 2 Years by NTP (2002)**

The uncertainty factor of 300 was composed of the following:

- A default 10-fold UF for intraspecies differences was used to account for potentially susceptible individuals in the absence of quantitative information or information on the variability of response in humans. Individuals with pre-existing respiratory disorders may be more susceptible to inhaled vanadium pentoxide.
- A partial UF of 3 ( $10^{0.5}$ ) was applied for interspecies extrapolation to account for potential pharmacodynamic differences between rats and humans. Converting the rat data to human equivalent concentrations by the dosimetric equations accounts for pharmacokinetic differences between rats and humans; thus, it was not necessary to use the default UF of 10 for interspecies extrapolation (U.S. EPA, 1994b).
- The full default UF of 10 was used for database insufficiencies due to the lack of adequate inhalation developmental toxicity studies and a multi-generation reproduction study.
- The POD was determined by benchmark dose analysis; therefore, a UF to extrapolate from a minimal LOAEL to a NOAEL was not necessary.

Confidence in the key study is high. NTP (2002) assessed comprehensive endpoints in an appropriate number of animals. Confidence in the experimental database is medium, since the toxicity of chronic inhalation exposure to vanadium pentoxide was rigorously assessed in two rodent species by NTP (2002). A long-term, although less than chronic, NOAEL was established in monkeys, as well. Respiratory tract toxicity has been consistently reported in acute, subchronic and chronic exposure studies in mice, rats, monkeys and humans, although little quantitative data are available in humans. Data on the developmental or reproductive toxicity of inhaled vanadium pentoxide are inadequate. There is evidence of testicular toxicity following inhalation exposure (Mussali-Galante 2005; Fortoul et al., 2007), but a definite LOAEL cannot be established given the intermittent exposure protocol. Reproductive and developmental effects were reported in rats and mice injected subcutaneously with higher doses (5 – 12.5 mg/kg), but an equivalent inhalation exposure cannot be determined from these studies (Altamirano et al, 1991; Altamirano-Lozano et al, 1993, 1996; Zhang et al. 1991, 1993a, 1993b). Overall confidence in the p-RfC is medium.

## **PROVISIONAL CARCINOGENICITY ASSESSMENT FOR VANADIUM PENTOXIDE**

### **Weight of Evidence Descriptor**

Under the 2005 Guidelines for Carcinogen Risk Assessment (U.S. EPA, 2005a), the available evidence for inhalation exposure to vanadium pentoxide is suggestive of carcinogenic potential. In the NTP (2002) study, there was some evidence of carcinogenic activity in male rats but equivocal evidence in female rats. Although the incidence of bronchoalveolar tumors in vanadium-pentoxide-treated rats was not significantly increased compared to control, tumor incidence was elevated relative to historical control in most treatment groups in male rats and

some treatment groups in female rats (Table 15). There was clear evidence of carcinogenesis in both male and female mice based on the increased incidence of alveolar/bronchiolar tumors (NTP, 2002; Ress et al., 2003). No studies evaluating the carcinogenic potential in humans exposed to inhaled vanadium pentoxide were identified. No studies suitable for evaluation of the oral carcinogenic potential for vanadium pentoxide were located. As a whole, the evidence is not strong enough to support a classification of “likely to be carcinogenic to humans.”

### **Mode of Action Discussion**

The U.S. EPA (2005a) Guidelines for Carcinogen Risk Assessment defines mode of action as a sequence of key events and processes, starting with the interaction of an agent with a cell, proceeding through operational and anatomical changes, and resulting in cancer formation. Examples of possible modes of carcinogenic action include mutagenic, mitogenic, anti-apoptotic (inhibition of programmed cell death), cytotoxic with reparative cell proliferation and immunologic suppression. Available evidence suggests that bronchoalveolar tumors observed in animals following inhalation exposure to vanadium pentoxide may arise from genetic mechanisms, although it is possible that tumors result from a proliferative response to injury in the respiratory tract.

### **Hypothesized Mutagenic Mode of Action**

**Key Events** — The precise mechanism of vanadium pentoxide-induced carcinogenicity has not been fully determined. There is evidence that vanadium pentoxide is capable of eliciting genotoxic effects (Table 18). Although results of *in vitro* studies have yielded mixed results, in general, genotoxic effects have been observed in bacterial and mammalian cell systems. In cultured hamster fibroblasts, positive effects have been observed for mitosis and micronucleus formation, although negative effects were reported for sister chromatid exchange and gene mutation (Zhong et al., 1994). In cultured human fibroblasts, vanadium pentoxide induced DNA strand breaks (Ivancsits et al., 2002). Thus, genotoxicity has been observed in *in vitro* test systems of target organ cells. Experimental data in animals provide conflicting evidence of genotoxicity following *in vivo* exposure to vanadium pentoxide. DNA damage (primarily single strand breaks and alkali labile damage) was increased in liver, kidney, lung, spleen and heart 24 hours following single intraperitoneal injections of vanadium pentoxide to mice (Altamirano-Lozano et al., 1999). Vanadium pentoxide administered for 3 months by inhalation to male and female mice (1, 2, 4, 8 or 16 mg/m<sup>3</sup>) did not increase the frequency of micronucleated normochromatic erythrocytes in peripheral blood (NTP, 2002), although genotoxic effects on cells of the respiratory tract were not assessed. Evidence demonstrating mutagenic activity of vanadium pentoxide *in vivo* in humans is lacking.

**Strength, Consistency, Specificity of Association** — Genotoxic activity has been demonstrated in cultured hamster and human fibroblasts and in several tissues, including lung, following single parenteral exposure of mice (Altamirano-Lozano et al., 1999), providing evidence of genotoxicity in target organ cells. Although Altamirano-Lozano et al. (1999) show evidence of genotoxicity in several tissue types, carcinogenicity following inhalational exposure to vanadium pentoxide has only been observed in the respiratory tract. Data demonstrating

genotoxicity following inhalation exposure to vanadium pentoxide in animals or humans are lacking.

**Dose-Response Concordance** — A dose-response concordance has not been established between the development of bronchoalveolar tumors and mutagenesis following inhalation exposure to vanadium pentoxide. Although DNA damage was increased in liver, kidney, lung, spleen and heart of mice exposed to single parenteral doses of vanadium pentoxide, effects did not exhibit dose-dependence (Altamirano-Lozano et al., 1999).

**Temporal Relationships** — Genotoxicity was observed in liver, kidney, lung, spleen and heart 24 hours after a single parenteral exposure (Altamirano-Lozano et al., 1999), indicating that a mutagenic mechanism could be the initiating event in the development of respiratory tumors following chronic inhalation exposure (Ress et al., 2003; NTP, 2002).

**Biological Plausibility and Coherence** — Based on DNA damage observed in the lung of mice following single parenteral exposure to vanadium pentoxide (Altamirano-Lozano et al., 1999) and results of *in vitro* studies in cultured hamster and human fibroblasts (Ivancsits et al., 2002; Zhong et al., 1994), mutagenicity is plausible as the mode of action for bronchoalveolar tumors. However, no direct evidence is available linking mutagenesis in respiratory cells to the development of bronchoalveolar tumors following chronic inhalation exposure to vanadium pentoxide.

### **Hypothesized Nonmutagenic Mode of Action**

**Key Events** — It is generally accepted that sustained cell proliferation in response to cell death from toxicity or other causes is a significant risk factor for cancer. One possible nonmutagenic mode of vanadium carcinogenic action is stimulation of cell proliferation (mitogenic) and cytotoxicity with subsequent reparative cell proliferation (cytotoxic). Regeneration of respiratory epithelial cells following injury from inhaled vanadium has the potential to produce carcinogenesis as a result of replication errors becoming fixed mutations before DNA repair can be completed.

Subchronic and chronic inhalation studies in rats and mice provide evidence that vanadium causes cell injury with subsequent reparative cell proliferation, suggesting that nonmutagenic actions may be involved in the development of bronchoalveolar tumors (NTP, 2002). Pulmonary inflammation and bronchoalveolar epithelial hyperplasia were observed following exposure of mice to inhaled vanadium pentoxide for 6 and 13 days (Tables 6 and 8). In addition, increases in cell proliferation, as measured by incorporation of BrdU, were observed in mice exposed for 6 and 13 days (Tables 3 and 6). Nonneoplastic lesions indicative of cell damage and proliferation, including chronic inflammation, hyperplasia and fibrosis, were observed throughout the respiratory tract of mice exposed to inhaled vanadium pentoxide for 3 months or 2 years, demonstrating damage and repair in the region of tumor development (Tables 13, 16 and 17). These observations demonstrate that cell injury in the respiratory tract may have preceded the development of respiratory tumors in mice. Although results of the 2-year cancer bioassay in rats were equivocal, bronchoalveolar cell damage and proliferation were also observed in subchronic and chronic inhalations studies in rats (NTP, 2002). Vanadium pentoxide

has been shown also to induce mediators of inflammatory responses and fibrogenesis (Pierce et al., 1996; Bonner et al., 1998; Silbajoris et al., 2000) and to induce oxidative stress by generation of reactive oxygen species (reviewed by Valko et al., 2006). All of these observations further strengthen the evidence for a MOA for vanadium pentoxide based on stimulation of cell proliferation.

**Strength, Consistency, Specificity of Association** — Inhaled vanadium pentoxide produces cell damage with subsequent reparative cell proliferation in the respiratory tract of rats and mice and bronchoalveolar carcinomas and adenomas in mice (NTP, 2002). Subchronic and chronic studies identify the respiratory tract as the primary target organ for subchronic exposure and the only target organ for chronic exposure to inhaled vanadium pentoxide (NTP, 2002). Data demonstrating carcinogenesis of inhaled vanadium pentoxide in humans are lacking.

**Dose-Response Concordance** — Exposure of mice to inhaled vanadium pentoxide for 13 days or 3 months produced respiratory tract damage at the lowest concentrations tested, yielding LOAELs of 2 and 1 mg/m<sup>3</sup>, respectively; NOAEL values were not established (NTP, 2002). A significant increase in the incidence of bronchoalveolar adenomas and carcinomas in was observed at all exposure levels tested (1, 2 and 4 mg/m<sup>3</sup>). Thus, cell damage and proliferation and tumors are observed at similar exposure levels. However, since NOAEL values were not established in the subchronic and chronic exposure studies, it is not possible to determine if cancer develops at doses below those producing cell damage. The 2-year cancer bioassay in rats failed to clearly demonstrate tumorigenesis, although cell damage and proliferation were observed in the 16-day, 3-month and 2-year studies at the lowest concentrations tested (NTP, 2002).

**Temporal Relationships** — Histopathological findings of the 16-day, 3-month and 2-year inhalation studies in mice and rats show that inhaled vanadium pentoxide induces cell damage and a reparative proliferative response throughout the entire respiratory tract (NTP, 2002). Lesions progress from inflammation and increased cell proliferation following 16-day exposure, to hyperplasia following 3-month exposure and to fibrosis and tissue remodeling after 2-year exposure. The finding that bronchoalveolar tumors are found subsequent to tissue damage and repair is consistent with the hypothesis that vanadium pentoxide acts through a mode of action dependent on cell toxicity (NTP, 2002).

**Biological Plausibility and Coherence** — It is generally accepted that sustained cell proliferation in response to cytotoxicity can be a significant risk factor for cancer (Correa, 1996). Sustained cytotoxicity and regenerative cell proliferation may result in the perpetuation of mutations (spontaneous or directly or indirectly induced by the chemical), resulting in uncontrolled growth. It is also possible that continuous proliferation may increase the probability that damaged DNA will not be repaired. Reparative proliferation alone is not assumed to cause cancer. Tissues with naturally high rates of turnover do not necessarily have high rates of cancer, and tissue toxicity in animal studies does not invariably lead to cancer. Nevertheless, regenerative proliferation associated with persistent cytotoxicity appears to be a risk factor of consequence.

## Conclusions

The available evidence for the MOA for vanadium pentoxide tumorigenicity is insufficient but there is some support for both a mutagenic MOA and a MOA dependent on cellular cytotoxicity and reparative regeneration. *In vitro* and *in vivo* studies provide evidence that vanadium pentoxide is capable of eliciting genotoxic and mutagenic effects in mammalian respiratory cells; however, direct evidence linking mutagenicity to respiratory cells following inhalation exposure is lacking. Results of the 16-day, 3-month and 2-year inhalation studies in mice (NTP, 2002) are consistent with the hypothesis that vanadium pentoxide acts through a mode of action dependent on cellular toxicity, based on the observations that cytotoxicity and reparative proliferation occur following subchronic exposure and bronchoalveolar tumors are produced at exposure levels that produce cytotoxicity and reparative proliferation. However, dose-response data in mice for damage/repair and tumor development are limited since NOAEL values were not established. Although evidence is generally supportive of mode of actions involving mutagenicity or cellular toxicity and repair, there is insufficient evidence to support these hypotheses; thus, a linear approach was taken to calculate the inhalation cancer unit risk in accordance with the default recommendation of the 2005 Guidelines for Carcinogen Risk Assessment (U.S. EPA, 2005a). It is possible that a mixed MOA, involving both MOAs discussed above, or an undetermined MOA, may be responsible for tumor induction. The use of the default procedures for age-adjustment of unit risk for chemicals with a mutagenic MOA to account for possible age-dependence of carcinogenic potency is not recommended (U.S. EPA, 2005b).

## Quantitative Estimates of Carcinogenic Risk

According to the 2005 Guidelines for Carcinogen Risk Assessment (U.S. EPA, 2005a), dose-response assessment estimates for potential risks to humans are recommended for agents considered “Carcinogenic to Humans” and “Likely to Be Carcinogenic to Humans.” However, when the weight of evidence descriptor is “Suggestive Evidence of Carcinogenic Potential,” a dose-response assessment may be useful for some purposes “when the evidence includes a well-conducted study.” Thus, a quantitative analysis of cancer risk is justified for inhaled vanadium pentoxide on the basis that the NTP (2002) cancer bioassay in mice was a well-conducted study, assessing comprehensive toxicological endpoints for lifetime exposure in an adequate number of animals. Risk assessors should be reminded that there is only suggestive evidence of human carcinogenicity from vanadium pentoxide exposure so there is uncertainty associated with quantitation of the database.

**Oral Exposure.** No human or animal studies examining the carcinogenicity of vanadium pentoxide following oral exposure were located. Therefore, derivation of an oral slope factor is precluded.

**Inhalation Exposure.** The NTP (2002) 2-year carcinogenicity study in mice was used for the derivation of an inhalation unit risk, based on the dose-response relationship for alveolar/bronchiolar (A/B) neoplasms (adenoma and carcinoma). Exposure concentrations in these studies were adjusted to continuous exposure as follows:

$$\text{Conc}_{[\text{ADJ}]} = \text{Conc} \times 6/24 \times 5/7$$

This adjustment resulted in duration-adjusted concentrations of 0, 0.18, 0.37 and 0.74 mg/m<sup>3</sup> for the control, 1, 2 and 4 mg /m<sup>3</sup> groups, respectively. Using the RDDR computer program, as specified in the RfC guidelines (U.S. EPA, 1994b), HECs (in mg/m<sup>3</sup>) were calculated at each exposure level for male and female mice using mean body weights for males and females reported by NTP (2002) and the average particle size MMAD±GSD of 1.2±2.9 for rats and mice as reported by NTP (2002) study for effects occurring in the thoracic region of the respiratory tract. HECs were calculated by multiplying Conc<sub>[ADJ]</sub> by the RDDR for male and female mice (Table 26).

<b>Table 26. Human Equivalent Concentrations (HEC) Corresponding to Adjusted Exposure Concentrations for Thoracic Region of the Respiratory Tract in Mice</b>			
<b>Species and Sex<sup>a</sup></b>	<b>RDDR</b>	<b>Conc<sub>[ADJ]</sub> (mg/m<sup>3</sup>)</b>	<b>HEC (mg/m<sup>3</sup>)</b>
Male mice	1.481	0.18	0.267
		0.37	0.548
		0.74	1.096
Female mice	1.433	0.18	0.258
		0.37	0.530
		0.74	1.060

<sup>a</sup>Default body weight values for chronic exposure for male and female Fisher rats and B3C6F1 mice listed in the RfC guidelines (U.S EPA, 1994b): male mice = 37.3g; female mice = 35.3 g

Modeling was performed using the Benchmark Dose Modeling Software (BMDS; Version 1.3.1) developed by the National Center for Environmental Assessment (U.S. EPA, 2000). Predicted concentrations associated with a 10% extra risk (BMD<sub>10</sub>) were calculated, with the BMDL<sub>10</sub> represented by the 95% lower confidence limit on the BMD<sub>10</sub>. The multi-stage cancer model was fit to the incidence data for tumors (combined alveolar/ bronchoalveolar adenomas and carcinomas) in mice; males and females were modeled separately (Table 27). In accordance with the U.S. EPA (2000) BMD methodology, the default benchmark response (BMR) of 10% increase in extra risk was used as the basis for the BMD, with the BMDL represented by the 95% lower confidence limit on the BMD. Models were run using the default restrictions on parameters built into the BMD software. Goodness-of-fit was evaluated using the Chi-square statistic calculated by the BMDS program. Acceptable global goodness of fit was a p-value greater than or equal to 0.1. The multi-stage model failed to fit adequately all but the male mice data with the high dose dropped (Table 28). Therefore, all other BMDS dichotomous models were fit to each of the data sets.

<b>Table 27. Incidences of Respiratory Tumors in Male and Female Mice Exposed to Vanadium Pentoxide for 2 Years (NTP, 2002)</b>				
<b>Male Mice <sup>a</sup></b>	<b>HEC</b>			
	<b>Control</b>	<b>0.267 mg/m<sup>3</sup></b>	<b>0.548 mg/m<sup>3</sup></b>	<b>1.096 mg/m<sup>3</sup></b>
Alveolar/bronchoalveolar adenoma or carcinoma	22/50	42/50 <sup>b</sup>	43/50 <sup>b</sup>	43/50 <sup>b</sup>
<b>Female Mice <sup>a</sup></b>	<b>HEC</b>			
	<b>Control</b>	<b>0.258 mg/m<sup>3</sup></b>	<b>0.530 mg/m<sup>3</sup></b>	<b>1.060 mg/m<sup>3</sup></b>
Alveolar/bronchoalveolar adenoma or carcinoma	1/50	32/50 <sup>b</sup>	35/50 <sup>b</sup>	32/50 <sup>b</sup>

<sup>a</sup>Number of animals with tumor

<sup>b</sup>Significantly different from control by the Poly-3 test ( $p \leq 0.01$ )

**Table 28. Goodness of Fit Statistics and BMD<sub>10</sub>s and BMDL<sub>10</sub>s from Models Meeting Goodness-of-Fit Criteria for Incidence Data for Combined Bronchoalveolar Adenomas and Carcinomas in Male and Female Mice Exposed to Vanadium Pentoxide by Inhalation for 2 Years (NTP, 2002)**

Model	Degrees of Freedom	$\chi^2$ Test Statistic	$\chi^2$ p-Value <sup>a</sup>	AIC	BMD <sub>10</sub> (mg/m <sup>3</sup> )	BMDL <sub>10</sub> (mg/m <sup>3</sup> )
<b>Male Mice – All Doses Included</b>						
Log-Logistic <sup>b</sup>	2	3.65	0.1610	200.956	0.0213865	0.0123449
<b>Male Mice – High Dose Dropped</b>						
Gamma <sup>c</sup>	1	2.65	0.1033	159.738	0.0338493	0.0243233
Log-Logistic <sup>b</sup>	1	0.68	0.4093	157.726	0.015405	0.00836071
Multi-Stage 1-Degree <sup>d</sup>	1	2.65	0.1033	159.738	0.0338494	0.0243233
Multi-Stage 2-Degree <sup>d</sup>	1	2.65	0.1033	159.738	0.0338494	0.0243233
Probit	1	4.53	0.0333	161.775	0.0528617	0.0418641
Log-Probit <sup>b</sup>	1	2.48	0.1156	159.466	0.058643	0.0406918
Quantal-Linear	1	2.65	0.1033	159.738	0.0338494	0.0243233
Weibull <sup>e</sup>	1	2.65	0.1033	159.738	0.033849	0.0243233
<b>Female Mice – High Dose Dropped</b>						
Gamma <sup>b</sup>	1	4.44	0.0350	144.58	0.0373539	0.0301586
Logistic	1	17.95	0.0000	160.278	0.0991538	0.0801981
Log-Logistic <sup>b,c</sup>	1	1.07	0.3004	141.289	0.0204481	0.0141445
Multi-Stage 1-Degree <sup>d</sup>	1	4.44	0.0350	144.58	0.0373539	0.0301586
Multi-Stage 2-Degree <sup>e</sup>	1	4.44	0.0350	144.58	0.0373539	0.0301586
Probit	1	17.62	0.0000	159.488	0.0962905	0.0792222
Log-Probit <sup>b</sup>	1	4.54	0.0332	144.589	0.0664798	0.053212
Quantal-Linear	NA <sup>e</sup>	NA	NA	NA	NA	NA
Quantal-Quadratic	1	23.11	0.0000	161.631	0.128423	0.11419
Weibull <sup>f</sup>	NA	NA	NA	NA	NA	NA

<sup>a</sup>Values >0.1 meet conventional goodness-of-fit criteria

<sup>b</sup>Slope restricted to  $\geq 1$

<sup>c</sup>Power restricted to  $\geq 1$

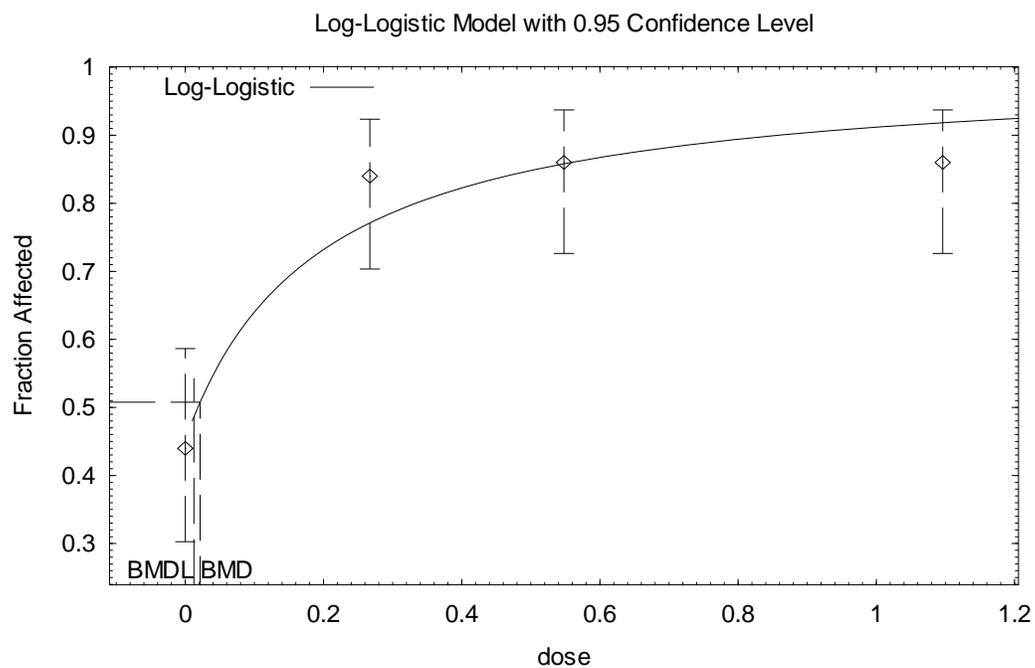
<sup>d</sup>Betas restricted to  $\geq 0$

NA = Not applicable; model failed to converge

The BMDS modeling results for models meeting goodness-of-fit criteria are summarized in Table 28. For incidence data in male mice, the log-logistic model was the only model that met goodness-of-fit criteria when all three vanadium pentoxide groups were included, predicting BMD<sub>10</sub> and BMDL<sub>10</sub> values of 0.021 and 0.012 mg/m<sup>3</sup>, respectively (Figure 4). Since the response did not increase monotonically with the high dose included, the impact of dropping the high dose was explored. When the high dose was dropped, several models fit the incidence data for male mice, with BMD<sub>10</sub> and BMDL<sub>10</sub> values ranging from 0.015 to 0.058 mg/m<sup>3</sup> and from 0.008 to 0.042 mg/m<sup>3</sup>, respectively. The log-logistic model also yielded the best fit (i.e., lowest AIC) to tumor incidence in males when the high dose was dropped (BMDL<sub>10</sub> of 0.008 mg/m<sup>3</sup>).

None of the dichotomous models fit tumor incidence data for female mice when all three vanadium pentoxide groups were included. One model (log-logistic) fit the incidence data for female mice when the high dose was dropped, predicting BMD<sub>10</sub> and BMDL<sub>10</sub> values of 0.020 and 0.014 mg/m<sup>3</sup>, respectively (Figure 5). The BMDL<sub>10</sub> of 0.012 derived for males including all doses was similar to that of 0.014 for females with the high dose dropped. The lower BMDL<sub>10</sub> of 0.012 for male mice was selected as the point of departure (POD) for derivation of the inhalation unit risk.

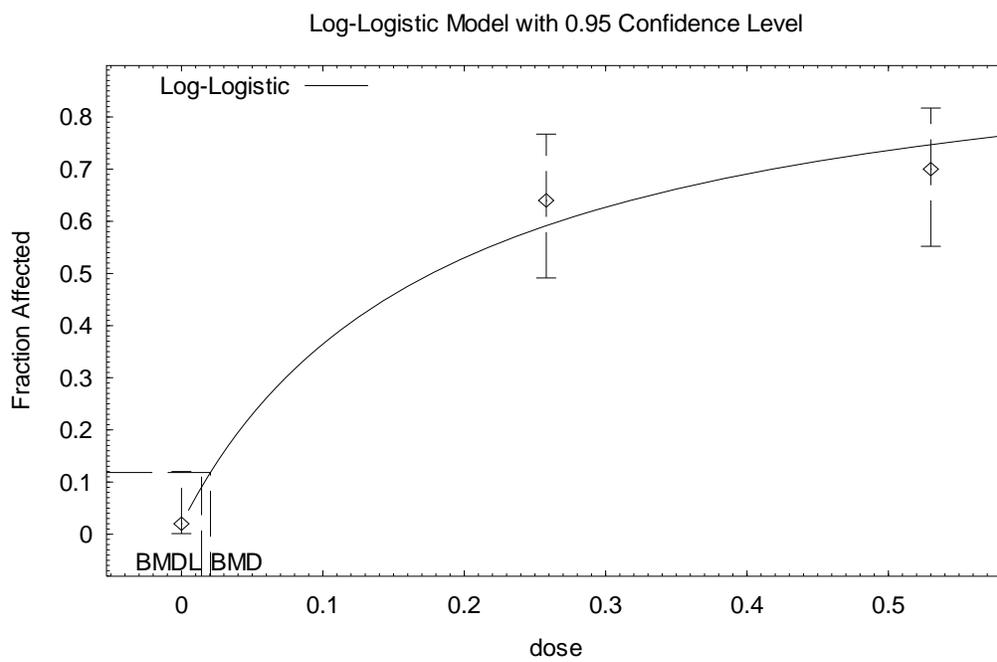
The **inhalation cancer unit risk of 8.3 (mg/m<sup>3</sup>)<sup>-1</sup>** was calculated by dividing 0.1 by the human equivalent BMDL<sub>10[HEC]</sub> of 0.012 mg/m<sup>3</sup>. Continuous lifetime exposure concentrations of vanadium pentoxide that correspond with specified risk levels are shown in Table 29.



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BMD and BMDL indicated are for a 10% extra risk and are in units of mg/m<sup>3</sup>

**Figure 4. Observed and Predicted Incidences of Combined Bronchoalveolar Adenomas and Carcinomas in Male Mice Exposed to Vanadium Pentoxide by Inhalation for 2 Years by NTP (2002)**



**Figure 5. Observed and Predicted Incidences of Combined Bronchoalveolar Adenomas and Carcinomas in Female Mice Exposed to Vanadium Pentoxide by Inhalation for 2 Years by NTP (2002) (High Dose Dropped)**

**Table 29. Continuous Lifetime Exposure Concentrations Corresponding to Specified Cancer Risk for Vanadium Pentoxide**

Risk <sup>a</sup>	Exposure Concentration
1x10 <sup>-4</sup> Risk	1.2x10 <sup>-5</sup> mg/m <sup>3</sup>
1x10 <sup>-5</sup> Risk	1.2x10 <sup>-6</sup> mg/m <sup>3</sup>
1x10 <sup>-6</sup> Risk	1.2x10 <sup>-7</sup> mg/m <sup>3</sup>

<sup>a</sup>Extra risk due to vanadium pentoxide exposure

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