TOXICOLOGICAL REVIEW

OF

VANADIUM PENTOXIDE (V_2O_5)

(CAS No. 1314-62-1)

In Support of Summary Information on the Integrated Risk Information System (IRIS)

September 2011

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AIC	Akaike Information Criterion
ATSDR	Agency for Toxic Substances and Disease Registry
BAL	bronchiolar lavage
BALT	bronchus-associated lymphoid tissue
BMC	benchmark concentration
BMCL	benchmark concentration (lower limit)
$BMCL_{[HEC]}$	benchmark concentration (lower limit) adjusted for dosimetric differences across species to humans
BMD	benchmark dose
BMDL	benchmark dose (lower limit)
BMDS	benchmark dose software
BMR	benchmark response
bw	body weight
CASRN	Chemical Abstracts Service Registry Number
cc	cubic centimeters
CD	caesarean delivered
CERCLA	Comprehensive Environmental Response, Compensation and Liability Act of 1980
CL_{dyn}	dynamic lung compliance
CBMN Cyt	cytokinesis-block micronucleus cytome
CNS	central nervous system
cu.m	cubic meter
DAF	dosimetric adjustment factor
EGF	epidermal growth factor
ERK	extracellular signal-regulated protein kinase
FEF	forced expiratory flow
FIFRA	Federal Insecticide, Fungicide, and Rodenticide Act
FVC	forced vital capacity
g	grams
GI	gastrointestinal
GSD	geometric standard deviation
HED	human equivalent dose
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
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
LOD	limit of detection

LOH	loss of heterozygosity
m	meter
MAP	mitogen-activated protein
MCA	3-methylcholanthrene
MCL	maximum contaminant level
MF	modifying factor
mg	milligram
mg/kg	milligrams per kilogram
mg/L	milligrams per liter
MMAD	median aerodynamic diameter
MMP	matrix metalloproteinases
MOA	mode of action
MPO	myeloperioxidase
MRL	minimal risk level
MSW	Multistage Weibull
MTD	maximum tolerated dose
NAAQS	National Ambient Air Quality Standards
NMMAPS	National Morbidity, Mortality, and Air Pollution Study
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
NTP	National Toxicology Program
OSF	oral slope factor
OSHA	Occupational Safety and Health Administration
PBPK	physiologically based pharmacokinetic
PDGF	platelet-derived growth factor
PFT	pulmonary function test
PHA	phytohemagglutinin responsiveness
PM	particulate matter
PMN	polymorphonuclear leukocyte
POD	point of departure
ppb	parts per billion
ppm	parts per million
PPRTV	Provisional Peer Reviewed Toxicity Value
PWM	pokeweed mitogen responsiveness
RBC	red blood cell(s)
RCRA	Resource Conservation and Recovery Act
RDDR	regional deposited dose ratio (for the indicated lung region)
RfC	inhalation reference concentration
RfD	oral reference dose
RGDR	regional gas dose ratio (for the indicated lung region)
RL	total pulmonary resistance
ROFA	residual oil fly ash
RSV	respiratory syncytial virus
RV	residual volume
SCE	sister chromatid exchange
SD	standard deviation
SDNN	standard deviation of the normal-to-normal intervals

SDWA	Safe Drinking Water Act
SE	standard error
sq.cm.	square centimeters
TLV	threshold limit value
TWA	time-weighted average
TSCA	Toxic Substances Control Act
UF	uncertainty factor
µg	microgram
µmol	micromoles
VOC	volatile organic compound

FOREWORD

The purpose of this Toxicological Review is to provide scientific support and rationale for the hazard and dose-response assessment in IRIS pertaining to chronic exposure to vanadium pentoxide. It is not intended to be a comprehensive treatise on the chemical or toxicological nature of vanadium pentoxide, and does not address other vanadium compounds.

The intent of Section 6, *Major Conclusions in the Characterization of Hazard and Dose Response*, is to present the major conclusions reached in the derivation of the reference dose, reference concentration and cancer assessment, where applicable, and to characterize the overall confidence in the quantitative and qualitative aspects of hazard and dose response by addressing the quality of data and related uncertainties. The discussion is intended to convey the limitations of the assessment and to aid and guide the risk assessor in the ensuing steps of the risk assessment process.

For other general information about this assessment or other questions relating to IRIS, the reader is referred to EPA's IRIS Hotline at (202) 566-1676 (phone), (202) 566-1749 (fax), or hotline.iris@epa.gov (email address).

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1. INTRODUCTION

This document presents background information and justification for the Integrated Risk Information System (IRIS) Summary of the hazard and dose-response assessment of vanadium pentoxide. IRIS Summaries may include oral reference dose (RfD) and inhalation reference concentration (RfC) values for chronic and other exposure durations, and a carcinogenicity assessment.

The RfD and RfC, if derived, provide quantitative information for use in risk assessments for health effects known or assumed to be produced through a nonlinear (presumed threshold) mode of action (MOA). The RfD (expressed in units of mg/kg-day) is defined as an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime. The inhalation RfC (expressed in units of mg/m^3) is analogous to the oral RfD, but provides a continuous inhalation exposure estimate. The inhalation RfC considers toxic effects for both the respiratory system (portal-of-entry) and for effects peripheral to the respiratory system (extrarespiratory or systemic effects). Reference values are generally derived for chronic exposures (up to a lifetime), but may also be derived for acute (≤24 hours), short-term (>24 hours up to 30 days), and subchronic (>30 days up to 10% of lifetime) exposure durations, all of which are derived based on an assumption of continuous exposure throughout the duration specified. Unless specified otherwise, the RfD and RfC are derived for chronic exposure duration.

The carcinogenicity assessment provides information on the carcinogenic hazard potential of the substance in question, and quantitative estimates of risk from oral and inhalation exposures may be derived. The information includes a weight-of-evidence judgment of the likelihood that the agent is a human carcinogen and the conditions under which the carcinogenic effects may be expressed. Quantitative risk estimates may be derived from the application of a low-dose extrapolation procedure. If derived, the oral slope factor is a plausible upper bound on the estimate of risk per mg/kg-day of oral exposure. Similarly, an inhalation unit risk is a plausible upper bound on the estimate of risk per $\mu g/m^3$ air breathed.

Development of these hazard identification and dose-response assessments for vanadium pentoxide has followed the general guidelines for risk assessment as set forth by the National Research Council (1983). EPA Guidelines and Risk Assessment Forum technical panel reports that may have been used in the development of this assessment include the following: *Guidelines for the Health Risk Assessment of Chemical Mixtures* (U.S. EPA, 1986b), *Guidelines for Mutagenicity Risk Assessment* (U.S. EPA, 1986a), *Recommendations for and Documentation of Biological Values for Use in Risk Assessment* (U.S. EPA, 1988), *Guidelines for Developmental Toxicity Risk Assessment* (U.S. EPA, 1991), *Interim Policy for Particle Size and Limit Concentration Issues in Inhalation Toxicity* (U.S. EPA, 1994a), *Methods for Derivation of*

Inhalation Reference Concentrations and Application of Inhalation Dosimetry (U.S. EPA, 1994b), *Use of the Benchmark Dose Approach in Health Risk Assessment* (U.S. EPA, 1995), *Guidelines for Reproductive Toxicity Risk Assessment* (U.S. EPA, 1996), *Guidelines for Neurotoxicity Risk Assessment* (U.S. EPA, 1998), *Science Policy Council Handbook*: *Risk Characterization* (U.S. EPA, 2000b), *Benchmark Dose Technical Guidance Document* (U.S. EPA, 2000a), *Supplementary Guidance for Conducting Health Risk Assessment of Chemical Mixtures* (U.S. EPA, 2000c), *A Review of the Reference Dose and Reference Concentration Processes* (U.S. EPA, 2002), *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a), *Supplemental Guidance for Assessing Susceptibility from Early-Life Exposure to Carcinogens* (U.S. EPA, 2005b), *Science Policy Council Handbook: Peer Review* (U.S. EPA, 2006b), *A Framework for Assessing Health Risks of Environmental Exposures to Children* (U.S. EPA, 2006a), and *Recommended Use of Body Weight 3/4 as the Default Method in Derivation of the Oral Reference Dose* (U.S. EPA, 2011).

The literature search strategy employed for vanadium pentoxide was based on the chemical name, the Chemical Abstracts Service Registry Number (CASRN), and multiple common synonyms. Any pertinent scientific information submitted by the public to the IRIS Submission Desk was also considered in the development of this document. Primary, peer-reviewed literature identified through August 2011 was included where that literature was determined to be critical to the assessment. The relevant literature included publications on vanadium pentoxide, which were identified through Toxicology Literature Online (TOXLINE), PubMed, the Toxic Substance Control Act Test Submission Database (TSCATS), the Registry of Toxic Effects of Chemical Substances (RTECS), the Chemical Carcinogenesis Research Information System (CCRIS), the Developmental and Reproductive Toxicology/Environmental Teratology Information Center (DART/ETIC), the Hazardous Substances Data Bank (HSDB), the Genetic Toxicology Data Bank (GENE-TOX), Chemical abstracts, and Current Contents. Other peer-reviewed information, including health assessments developed by other organizations, review articles, and independent analyses of the health effects data were retrieved and may be included in the assessment where appropriate.

Portions of this document were developed under a Memorandum of Understanding with the Agency for Toxic Substances and Disease Registry (ATSDR) as part of a collaborative effort in the development of human health toxicological assessments.

2. CHEMICAL AND PHYSICAL INFORMATION

As an element, vanadium (V) exists in several oxidation states, from −1 to +5. Vanadium (Chemical Abstracts Service Registry Number [CASRN] 7440-62-2) is a soft silver-gray metal commonly found in ores, tars, coals, and oils and is used as an alloy in steel (WHO, 1988). The focus of this Toxicological Review is vanadium pentoxide (CASRN 1314-62-1), but a short description of the chemistry of vanadium and related compounds is given below for clarification.

The chemistry of vanadium is complex; the valence state of vanadium can shift depending on pH and other factors. In the body, there is an interconversion of two oxidation states of vanadium, the tetravalent form, vanadyl (V^{4+}), and the pentavalent form, vanadate (V^{5+}). Vanadate is considered more toxic than vanadyl because vanadate is reactive with several enzymes and is a potent inhibitor of the Na+K+-ATPase of plasma membranes (Patterson et al., 1986; Harris et al., 1984).

Generally, the V^{3+} and V^{4+} valence states predominate in body tissues while V^{5+} predominates in plasma (IPCS, 2001). Vanadium pentoxide (V_2O_5), sodium metavanadate ($NaVO_3$), sodium orthovanadate (Na_3VO_4), and ammonium metavanadate (NH_4VO_3) all contain vanadium in the +5 oxidation state. Of these compounds, V_2O_5 is the only compound that is covalently bonded.

The physicochemical properties of vanadium compounds differ, which determines their solubility under different pH conditions and their accessibility and availability in biological systems [reviewed in Assem and Levy (2009)]. An acidic pH favors the tetravalent state (V^{4+}) keeping it as vanadyl, while the pentavalent state (V^{5+}) as vanadate (Crans et al., 2004) is prevalent under alkaline conditions. In the case of oral ingestion, vanadium compounds are exposed to a range of pH solutions in the digestive tract starting at the stomach (pH typically between 1 and 3.5), followed by the small intestine (pH around 8). Bruyere et al. (1999) state that at pH between 1.3 and 3.3, the predominant form of vanadium is VO^{2+} and at higher pH, the form is $VO(OH)_3$. When the pH is high, V^{5+} (e.g., $VO_4(OH)_3$) predominates and polymerized vanadium is predominant ($H_nV_{10}O_{29}^{(n-6)}$) (Bruyère et al., 1999). At physiological pH, vanadium compounds have been shown to exist in monomeric tetravalent $[VO(OH)_3]^-$ and dimeric $[(VO)_2(OH)_5]^-$ forms, as well as pentavalent ($H_2VO_4^-$) forms [reviewed in Assem and Levy (2009)]. Thus, the valence of a vanadium compound will depend on the pH.

The solubility of different vanadium compounds in water between 20 and 25°C differs among valences as shown in Table 2-1 (HSDB, 2008; WHO, 2001). Elemental vanadium (V^0) is insoluble in water. The tetravalent (V^{4+}) compound vanadyl sulfate ($VOSO_4$) is highly soluble 534.64 g/L (Rahman and Skyllas-Kazacos, 1998), while the pentavalent (V^{5+}) vanadium compounds such as vanadium pentoxide (V_2O_5) is less soluble (8 g/L). Other vanadium compounds such as sodium metavanadate ($NaVO_3$), sodium orthovanadate (Na_3VO_4), and ammonium metavanadate (NH_4VO_3) have solubilities of 211 g/L, 100 g/L, and 58 g/L,

respectively (WHO, 2001). Furthermore, the rate of dissolution (which is distinct from solubility —an equilibrium or thermodynamic parameter) of various vanadium compounds can vary, resulting in different concentrations of specific forms of vanadium. The various forms of vanadium may be absorbed differently, which could result in different physiological effects. For example, V^{5+} compounds can mimic phosphate and inhibit phosphatases (Assem and Levy, 2009).

Table 2-1. Valence states and water solubility of various vanadium compounds

Vanadium compound	Formula	CASRN	Valency	Solubility (g/L) at 20–25°C (HSDB, 2008; WHO, 2001)
Vanadium	V	7440-62-2	0	Insoluble
Vanadium pentoxide	V_2O_5	1314-62-1	+5	8
Sodium m-vanadate	$NaVO_3$	13718-26-8	+5	211
Sodium o-vanadate	Na_3VO_4	13721-39-6	+5	100
Ammonium m-vanadate	NH_4VO_3	7803-55-6	+5	58 (WHO, 2001); 5.2 at 15°C (HSDB, 2008)
Vanadium oxytrichloride	$VOCl_3$	7727-18-6	+5	Soluble, decomposes in presence of moisture into vanadic acid and HCl.
Vanadyl sulfate	$VOSO_4$	27774-13-6	+4	535 at 20°C (Rahman and Skyllas-Kazacos, 1998)
Vanadium tetrachloride	VCl_4	7632-51-1	+4	Decomposes
Vanadyl oxydichloride	$VOCl_2$	10213-09-9	+3	Decomposes
Vanadium trioxide	V_2O_3	1314-34-7	+3	Slightly soluble

Source: Adapted from Assem and Levy (2009).

This Toxicological Review focuses exclusively on vanadium pentoxide (V_2O_5, CASRN 1314-62-1) (Figure 2-1), the most common form of vanadium used commercially. Vanadium pentoxide exists in the pentavalent state as a yellow-red powder (Table 2-2) (OSHA, 2007).

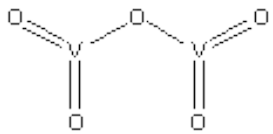

Figure 2-1. Vanadium pentoxide structure.

Table 2-2. Chemical and physical properties of vanadium pentoxide

Characteristic	Information	Reference
Chemical name	Vanadium pentoxide	
Synonym(s)	Vanadium oxide, vanadic anhydride dust, divanadium pentaoxide, divanadium pentoxide, vanadium pentaoxide	OSHA (2007)
Chemical formula	V_2O_5	CAS
CASRN	1314-62-1	CAS
Molecular weight	181.9	
Color	Yellowish-red powder Yellow to rust-brown orthorhombic powder Yellow-orange powder or dark gray flakes dispersed in air	OSHA (2007); O'Neil (2001); NIOSH (2005)
Melting point	690°C	OSHA (2007)
Boiling point	1750°C	OSHA (2007)
Density at 18°C	3.357	ChemFinder.com; HSDB (2008); Lewis (1997).
Odor threshold:	Odorless	OSHA (2007); NIOSH (2005)
Solubility: Water Organic solvents	 8 g/L (20°C) 10 g/L (20°C) Soluble in alkalis, concentrated acids, insoluble in alcohol	OSHA (2007); ChemFinder.com; O'Neil (2001); HSDB (2008)
Vapor pressure	0 mm Hg	HSDB (2008)
Specific gravity	3.4 g/cm^3	NTP (2008)
Flash point	Not applicable, noncombustible	OSHA (2007)
Conversions: ppm to mg/m^3 mg/m^3 to ppm	 1 ppm = 7.44 mg/m^3 1 mg/m^3 = 0.134 ppm	 Calculated Calculated

3. TOXICOKINETICS

The toxicokinetics of vanadium pentoxide has been investigated in the few studies described below and as reviewed by Cooper (2007) The toxicokinetics of several other vanadium compounds has been evaluated in animal models and is reviewed elsewhere (Mukherjee et al., 2004; Barceloux, 1999; Sabbioni et al., 1996). Vanadium pentoxide is rapidly absorbed following inhalation and oral exposures through the lungs and the gastrointestinal tract, respectively, although the amount absorbed through the gastrointestinal tract is low. Laboratory animal studies show that vanadium pentoxide is distributed primarily to the bone, lungs, liver, and kidney following inhalation and oral exposures. Elimination of vanadium pentoxide, which has been studied only following inhalation exposure, occurs primarily through the urine.

3.1. ABSORPTION

3.1.1. Inhalation Exposure

Several occupational studies indicate that absorption can occur in humans following inhalation exposure. An increase in urinary vanadium levels was found in workers occupationally exposed to <1 ppm (<7.44 mg/m^3) of vanadium compounds, including vanadium pentoxide (Orris et al., 1983; Kiviluoto et al., 1981a; Gylseth et al., 1979; Lewis, 1959), with the majority excreted in urine within 1 day after long-term or moderate exposure to vanadium dust (Kiviluoto et al., 1981a). The vanadium concentration in serum was higher than the nonoccupationally exposed controls following exposure to vanadium pentoxide dust (Kiviluoto et al., 1981b).

Indirect evidence for absorption of vanadium in animals is indicated in studies involving inhalation exposure or intratracheal administration. In rats and mice exposed to 0.28–2.2 mg vanadium/m^3 as vanadium pentoxide[1] for 16 days or 2 years (6 hours/day, 5 days/week), marginal increases in blood vanadium levels were observed, suggesting that vanadium pentoxide was poorly absorbed or rapidly cleared from the blood (Dill et al., 2004; NTP, 2002). In the 2-year studies by NTP (2002), the increases in blood vanadium levels were concentration-related, although not statistically significant. Intratracheal studies suggest that vanadium pentoxide is readily absorbed through the lungs. The greatest absorption of a radioactive dose, ^{48}V, was found to occur 5 minutes after administration in albino rats (gender not specified) (Roshchin et al., 1980). Most of the vanadium, that is, 80 and 85% of the tetravalent (V^{4+}) and pentavalent (V^{5+}) forms of vanadium, respectively, cleared from the lungs 3 hours after intratracheal exposure in male albino rats (Edel and Sabbioni, 1988). After 3 days, 90% of

[1]Many studies describe exposures in terms of concentration of vanadium, particularly when describing exposure to mixtures. When possible, a concentration is given as amounts of vanadium pentoxide. As listed here (mg vanadium/m^3 as vanadium pentoxide) shows exposure was to vanadium pentoxide, with data shown here in concentration of vanadium.

vanadium pentoxide was eliminated from the lungs of female rats following intratracheal instillation (Conklin et al., 1982). In an intratracheal instillation study in female Fischer rats, 50% was cleared in 18 minutes, and the rest within a few days (Rhoads and Sanders, 1985).

Wallenborn et al. (2007) analyzed the metal components of a complex particulate matter (PM) mixture and tracked the absorption of the various metals in different tissues following a single intratracheal dose in rats. Healthy male Wistar rats were instilled with a single intratracheal dose of combustion-derived PM containing a moderate amount of transition metals, including vanadium. The composition of vanadium in the PM was 62.95 μg/mg, with 7.18 μg/mg in the water soluble fraction, 26.50 μg/mg in the acid-soluble fraction, and 29.27 μg/mg in the insoluble fraction. According to calculations, of the 196.63 μg/rat of vanadium instilled (theoretical), 110.32 μg/rat was measured in the lung 4 hours postinstillation and 62.76 μg/rat was measured in the lung 24 hours postinstillation. In the plasma and lung, vanadium was significantly elevated 4 hours postinstillation (130,000 ng V/g lung tissue, and 350 ng V/g plasma) compared to 24 hours (60,000 ng V/g lung tissue and 110 ng V/g plasma, respectively) suggesting rapid uptake of water-soluble vanadium. The vanadium component of PM was tracked to the lung, plasma, heart, and liver and was significantly increased compared to controls at both 4 and 24 hours postinstillation compared to saline controls. This study enabled changes in component metals of a complex mixture to be detected in various organs and provides evidence that metals can dissociate from PM and translocate to various target organs, depending on solubility (Wallenborn et al., 2007).

3.1.2. Oral Exposure

No studies were available in the published literature regarding the rate and extent of absorption in humans after oral exposure to vanadium pentoxide.

Data suggest that vanadium is rapidly absorbed but the level of absorption through the gastrointestinal tract of animals is low. Vanadium was reported in tissues and urine of male albino rats within hours after a single oral dose (Edel and Sabbioni, 1988), suggesting that it is rapidly absorbed. Young rats that consumed vanadium in drinking water and feed were found to have higher vanadium levels in tissue samples 21 days after birth than they did 115 days after birth (Edel et al., 1984). The data suggest a higher absorption of vanadium in young animals due to a greater nonselective permeability of the undeveloped intestinal barrier. Thus, age of the rodents appears to be important in determining the absorption of vanadium in the gastrointestinal tract. Less than 0.1% of an intragastric dose was detectable in the blood of albino rats at 15 minutes postexposure, and less than 1% at 1 hour (Roshchin et al., 1980). Similarly, only 2.6% of an orally administered radiolabeled dose of vanadium pentoxide was absorbed 3 days after exposure in female Fischer rats (Conklin et al., 1982).

3.1.3. Dermal Exposure

No specific studies were available in the published literature regarding absorption in humans or animals after dermal exposure to vanadium pentoxide. However, absorption by this route, however, is generally considered to be very low (WHO, 1988). Because vanadium is a metal with low solubility, absorption through the skin is thought to be minimal.

3.1.4. Other Routes of Exposure

No studies were available in the published literature regarding the nature and extent of absorption in humans through other routes of exposure to vanadium pentoxide.

3.2. DISTRIBUTION

Distribution has been determined from autopsy cases with unknown routes of exposure. Vanadium has been detected in the lung (in 52% of the cases) and intestine (in 16% of the cases) of humans with no known occupational exposure using autopsy data, as reviewed in Schroeder et al. (1963). In the gastrointestinal tract, vanadium was found primarily in the ileum (37%), cecum (45.1%), sigmoid colon (15.9%), and rectum (26.2%). Most positive samples had 0.01 µg or less per g of tissue. The heart, aorta, brain, kidney, muscle, ovary, and testes were found to have no detectable vanadium concentrations.

3.2.1. Inhalation Exposure

There are limited data on the distribution of vanadium in workers; serum vanadium levels in workers were highest within a day after inhalation exposure followed by a rapid decline in levels upon cessation of exposure (Kiviluoto et al., 1981b; Gylseth et al., 1979). Analytical studies have shown low levels of vanadium in human kidneys and liver, with even less in the brain and heart, and in milk. Higher levels were detected in hair, bone, and teeth (Byrne and Kosta, 1978). Inhalation exposure and intratracheal administration studies have examined the distribution of vanadium in rodents. In F344/N rats chronically exposed to 0.56 or 1.1 mg vanadium/m^3 as vanadium pentoxide (6 hours/day, 5 days/week), vanadium lung burdens peaked after 173 days of exposure and declined until 542 days; lung levels never reached steady state (NTP, 2002). In contrast, lung burdens appeared to reach steady state by exposure day 173 in rats exposed to 0.28 mg vanadium/m^3 (NTP, 2002). Similarly, lung burdens did not reach steady state in B6C3F_1 mice exposed to 1.1 or 2.2 mg vanadium/m^3 as vanadium pentoxide, 6 hours/day and 5 days/week for 542 days (NTP, 2002). Rather, lung burdens peaked near day 54 and declined through day 535. Steady state was achieved in mice exposed to 0.56 mg vanadium/m^3 during the first 26 days of exposure.

Vanadium is found to have a two-phase lung clearance after a single acute exposure in both male Wistar rats and female Fischer rats (Rhoads and Sanders, 1985; Oberg et al., 1978). The initial phase is rapid with a large percentage of the absorbed dose distributed to most organs and blood 24 hours postexposure, followed by a slower clearance phase. Vanadium is

transported mainly in the plasma. It is found in appreciable amounts in the blood initially and only at trace levels 2 days after exposure (Roshchin et al., 1980). The pentavalent and tetravalent forms of vanadium compounds were found to have similar distribution patterns in male albino Sprague-Dawley rats (Edel and Sabbioni, 1988). Three hours after exposure to the pentavalent or tetravalent form, 15–17% of the absorbed dose was found in the lung, 2.8% in the liver, and 2% in the kidney (Edel and Sabbioni, 1988). After intratracheal instillation of pentavalent vanadium, retention of vanadium was observed in the lungs, liver, kidneys, bone, testes, and spleen with clearance at different time points postexposure with little to no retention observed in the stomach, intestines, heart, or trachea (Edel and Sabbioni, 1988). This distribution is similar to the distribution observed following inhalation and oral exposures.

3.2.2. Oral Exposure

No studies were available in the published literature regarding distribution in humans after oral exposure to vanadium pentoxide.

Acute studies with rats showed the highest vanadium concentration in the bones. Male rats had approximately 0.05% of the administered ^{48}V in bones, 0.01% in the liver, and <0.01% in the kidney, blood, testis, or spleen after 24 hours (Edel and Sabbioni, 1988). Other authors who found that the bone had the greatest concentration of radiolabeled vanadium, followed by the kidney (Roshchin et al., 1980), noted similar findings. Conklin et al. (1982) reported that after 3 days, 25% of the absorbed vanadium pentoxide was detectable in the bones and blood of female Fischer rats.

Oral exposure for an intermediate duration produced the highest accumulation of vanadium in the kidney. In young male Sprague-Dawley rats at 3 weeks of age, the kidneys, heart, and lungs had the highest levels immediately following exposure (Edel et al., 1984). Vanadium in the kidney, liver, and lung decreased significantly at 115 days of age. An accumulation in muscle and fat was related to the growing mass of the tissues with age. The higher levels of vanadium in the young rat tissues might be due to the higher retention capacity of the undeveloped tissues, or to greater permeability of the intestinal wall. Adult rats exposed to 5 or 50 ppm vanadium in drinking water for 3 months had the highest vanadium levels in the kidney, followed by bone, liver, and muscle (Parker and Sharma, 1978). The retention in bone could have been due to phosphate displacement. All tissue levels plateaued during the third week of exposure. A possible explanation for the initially higher levels in the kidney during intermediate-duration exposure is the daily excretion of vanadium in the urine. When the treatment is stopped, levels decrease in the kidney. At the cessation of treatment, vanadium mobilized rapidly from the liver and slowly from the bones. Other tissue levels decreased rapidly after oral exposure was discontinued. Thus, vanadium was retained much longer in the bones (Edel et al., 1984; Parker and Sharma, 1978).

Radike et al. (2002) assessed the distribution of various metals, including vanadium, in female B6C3F$_1$ mice. Mice ingested either (1) a metal mixture containing chromium (Cr), cadmium (Cd), arsenic (As), nickel (Ni) and vanadium (V) in drinking water; or (2) a metal mixture containing Cr, Cd, As, Ni, and V in NIH-31 feed. In water and feed, the calculated vanadium concentration in the mixture was 45 ppm and 1.105 ppm, respectively. Measured vanadium levels in the small intestine were 10 ppm at 5 weeks and 14 ppm at 8 weeks, and were significantly higher compared to controls than any other metal constituent in the small intestine. Vanadium pentoxide is distributed primarily to bone (10–25% of administered oral dose), liver (~5%), and kidney (~4%). In addition, vanadium levels in the kidneys and the femur were significantly greater than in controls at 4, 8, 12, 16, and 24 weeks following oral dosing. Vanadium levels in small intestine and kidneys were lower in mice given vanadium as part of a heterogeneous metal mixture in feed versus water (Radike et al., 2002).

3.2.3. Dermal Exposure

No studies were available in the published literature regarding distribution in humans or animals after dermal exposure to vanadium.

3.2.4. Other Routes of Exposure

After intraperitoneal administration to rats, vanadium is distributed to all organs. After 24 hours, the highest concentrations were found in the bones and kidney, although initial levels were highest in the kidney (Roshchin et al., 1980; Sharma et al., 1980).

3.3. METABOLISM

Vanadium is an element, and as such, is not metabolized. In the oxygenated blood, it circulates as a polyvanadate (isopolyanions containing pentavalent vanadium) but in tissues, it is retained mainly as the vanadyl cation (cationic form of tetravalent vanadium). Depending on the availability of reducing equivalents (such as reduced glutathione [GSH], NADPH, and NADH) and oxygen, vanadium can be reduced, reoxidized, or undergo redox cycling (Byczkowski and Kulkarni, 1992).

3.4. ELIMINATION AND EXCRETION

3.4.1. Inhalation Exposure

Occupational studies showed that urinary vanadium levels significantly increased in vanadium pentoxide-exposed workers (Orris et al., 1983; Kiviluoto et al., 1981a; Gylseth et al., 1979; Zenz et al., 1962; Lewis, 1959). Male and female workers exposed to 0.1–0.19 mg/m^3 vanadium in a manufacturing company had significantly higher urinary levels (20.6 µg/L) than the nonoccupationally exposed control subjects (2.7 µg/L) (Orris et al., 1983). The correlation between ambient vanadium levels and urinary levels of vanadium is difficult to determine from these epidemiological studies (Kiviluoto et al., 1981b). In most instances, no other excretion

routes were monitored. Analytical studies have shown very low levels in human milk (Byrne and Kosta, 1978). Evidence from animal studies supports the occupational findings. Vanadium administered intratracheally to rats was reported to be excreted predominantly in the urine (Oberg et al., 1978) at levels twice those found in the feces (Rhoads and Sanders, 1985). Three days after intratracheal exposure to radiolabeled vanadium pentoxide, 40% of the recovered ^{48}V dose was cleared in the urine, while 30% remained in the bones and 2–7% remained in the lungs, liver, kidneys, or blood (Conklin et al., 1982).

In female rats exposed to 0.56 or 1.1 mg vanadium/m^3 as vanadium pentoxide for 16 days (6 hours/day, 5 days/week), lung clearance half-times during an 8-day recovery period were 4.42 and 4.96 days, respectively (NTP, 2002). In mice similarly exposed to 1.1 or 2.2 mg vanadium/m^3 as vanadium pentoxide, lung clearance half-times were 2.55 and 2.40 days, respectively (NTP, 2002). In contrast to the 16-day exposure data, the lung clearance half-times in female rats exposed to 0.28, 0.56, or 1.1 mg vanadium/m^3 for 2 years (6 hours/day, 5 days/week) were 37.3, 58.6, and 61.4 days, respectively (NTP, 2002). In mice, the half-times were 6.26, 10.7, and 13.9 days at 0.56, 1.1, and 2.2 mg vanadium/m^3 exposure levels (NTP, 2002).

After intratracheal instillation of pentavalent vanadium, clearance from lungs was initially rapid (3 hours) but with some vanadium (2% original dose) remaining at 12 days postexposure. All other tissues eliminated 98–99% of the original dose by 3 hours postexposure (Edel and Sabbioni, 1988). Epidemiological studies and animal studies suggest that elimination of vanadium following inhalation exposure occurs primarily through urination.

3.4.2. Oral Exposure

No studies were available in the published literature regarding excretion in humans or laboratory animals after oral exposure to vanadium pentoxide.

3.4.3. Dermal Exposure

No studies were available in the published literature regarding excretion in humans or laboratory animals after dermal exposure to vanadium pentoxide.

3.4.4. Other Routes of Exposure

No studies were available in the published literature regarding excretion in humans or laboratory animals after other routes of exposure to vanadium pentoxide.

3.5. PHYSIOLOGICALLY BASED PHARMACOKINETIC (PBPK) MODELS

No PBPK models for vanadium pentoxide are available.

4. HAZARD IDENTIFICATION

4.1. STUDIES IN HUMANS–EPIDEMIOLOGY, CASE REPORTS, CLINICAL CONTROLS

4.1.1. Oral Exposure

Few relevant studies investigating the effects of acute, subchronic, or chronic oral exposure to vanadium pentoxide in humans were identified in the peer-reviewed literature. One study, Kucera et al. (1992), measured vanadium in the hair and blood of children exposed to vanadium by accidental drinking of contaminated water near a vanadium pentoxide plant. Vanadium levels in the hair did not differ significantly for the control and exposed groups. An increase in vanadium concentrations was found in the blood of exposed children (median: 0.078 μg/L) compared to control children (median: 0.042 μg/L). No exposure-response relationship could be quantified. These results suggest that vanadium levels in blood, but not hair, are a sensitive or suitable indicator of environmental exposure.

4.1.2. Inhalation Exposure

Health effects of inhalation exposure to vanadium pentoxide include respiratory tract irritation, bronchitis (often called boilermakers' bronchitis), airway obstruction, chest pain, rhinitis, pharyngitis, laryngitis, and conjunctivitis in workers exposed to vanadium-containing dust during vanadium pentoxide processing (Irsigler et al., 1999; Musk and Tees, 1982; Kiviluoto et al., 1981a; Kiviluoto, 1980; Kiviluoto et al., 1979; Zenz et al., 1962; Sjoberg, 1956, 1951) or to fuel-oil ash containing vanadium during cleaning and maintenance of oil-burning boilers (Kim et al., 2004; Hauser et al., 2001; Woodin et al., 2000; Woodin et al., 1999; Woodin et al., 1998; Hauser et al., 1995a, b; Levy et al., 1984; Ross, 1983; Lees, 1980; Sjoberg, 1955; Williams, 1952). The literature includes one report of a controlled human exposure study involving inhalation of pure vanadium pentoxide dust (Zenz and Berg, 1967). Descriptions of these studies are found in Table 4-1. While exposures in the vandadium pentoxide production facilities were to vanadium pentoxide in dust, exposures to residual oil fly ash (ROFA) involved a mixture of pollutants including elemental vanadium, vanadium oxides, vanadium pentoxide, vanadium sulfates, particulate matter (PM), and other metal constituents (Hauser et al., 1995b). Vanadium pentoxide is a major constituent of ROFA (2.5–40% by weight, as reported in Navarro et al. , 2007), and the health effects models evaluated in the studies of boilermakers focused on vanadium content of respirable PM. Therefore, these papers are integral to assessing the nature and scope of the health response to vanadium pentoxide.

More recently, epidemiology studies on the metals content of ambient PM found that communities in the United States with higher vanadium content in PM have higher PM-related risk of mortality or hospitalizations for cardiovascular or respiratory disease (Bell et al., 2009;

Patel et al., 2009; Dominici et al., 2007; Lagorio et al., 2006; Lippmann et al., 2006) (described in Appendix D). Because exposures to vanadium, not vanadium pentoxide, were evaluated in the studies of ROFA or PM air pollution, information to characterize the exposure-response relationship between inhaled vanadium pentoxide alone and adverse health effects in humans is limited. Moreover, both ROFA and PM are mixtures, containing several other components that also could contribute to observed health effects.

4.1.2.1. *Controlled Human Exposure Study*

A report of a controlled human exposure study indicated that an 8-hour exposure to pure vanadium pentoxide dust concentrations between 0.1 and1 mg/m^3 resulted in a productive cough and wheeze that persisted for several days (Zenz and Berg, 1967). Volunteers (gender not reported) were exposed to 0.1 mg/m^3 (n = 2), 0.25 mg/m^3 (n = 5) or 1 mg/m^3 (n = 2) vanadium pentoxide in an environmental chamber for 8 hours; no filtered air exposure was included in this study. Particle-size analysis revealed that 98% of particles had a diameter <0.5 µm. Postexposure assessments of chest x-ray, blood, urine, nasal smear samples, and pulmonary function were similar to baseline values determined for each subject prior to exposure (data not reported). No treatment-related symptoms or clinical findings were reported for any subject during the 11- to 19-month posttreatment period.

4.1.2.2. *Occupational Exposure during Vanadium Pentoxide Mining and Processing*

Respiratory and other symptoms have been documented in case reports and two cross-sectional studies among workers employed at facilities producing and processing vanadium pentoxide (Irsigler et al., 1999; Musk and Tees, 1982; Kiviluoto et al., 1981b; Kiviluoto, 1980; Kiviluoto et al., 1979; Zenz et al., 1962; Sjoberg, 1956, 1951). Sjöberg (1951) reviewed earlier reports of symptoms among workers exposed to vanadium pentoxide and described symptoms among 36 employees (foremen, workers, builders/repairers) at a vanadium pentoxide production factory in Falun, Sweden that began operation in 1946. The employees experienced symptoms on one or multiple occasions while under his medical surveillance between 1947 and 1950. Symptoms generally persisted an average of 13 days. Air samples in different parts of the vanadium pentoxide facility were found to contain dust concentrations of 0.6–86.9 mg/m^3 during pulverization of iron ore slag with a vanadium content of 4.8−7.5%. A significant proportion of the dust consisted of small, respirable particles (22% < 8 µm, 39% < 12 µm). Although vanadium pentoxide concentrations in air were not reported, vanadium was detected in the blood and urine of 23 and 27 individuals, respectively.

Sjöberg (1951) reported the main findings of his medical surveillance, including those of a thorough examination of the cohort in October 1948, comparing them to an external referent population. The referent population comprised 703 workers from mines and sawmills in northern Sweden that were followed during the same period and were examined using the same methods. The authors assumed that the comparison population was exposed only to inert dust

but no sampling data were reported. The main symptoms of the upper respiratory tract were nasal irritation or nasal catarrh (inflammation of mucus membranes; 42% versus 20% among unexposed) and throat dryness and pain (86% versus 8% among unexposed). These symptoms were reported to be more prevalent at the final examination in 1948 and included acute and chronic pathological changes in the nose and pharynx. Lower respiratory tract symptoms included cough with no sputum (61%), cough with sputum (39%), wheezing (86%), and shortness of breath (75%). In the comparison group, prevalence of coughing and shortness of breath was 4% and 24%, respectively. Bronchoscopy, measured in five individuals, revealed no severe changes in four cases and mild chronic bronchitis in one person. Spirometric measures among the exposed and referent groups were similar. A larger decrease in hemoglobin levels (7.8 ± 2.36% compared to 3.0 ± 2.07%) and red blood cell (RBC) count was observed among the exposed, particularly among workers who were permanently or frequently employed at the plant during the observation period, although levels remained within normal limits. The authors did not observe leucopenia or eosinophilia in blood samples. Two additional published case summaries described symptoms similar to those reported by Sjöberg (1955, 1951) among workers after the start of new plant operations producing vanadium pentoxide pellets (n = 18) (Zenz et al., 1962) or refining vanadium pentoxide (n = 4) (Musk and Tees, 1982). Both exposures were to dry vanadium pentoxide powder at high concentrations (>0.5 mg/m^3).

Kiviluoto et al. (1981a; 1981b; 1980; 1979) published a series of reports regarding an investigation in 1975 of respiratory symptoms and clinical findings among 63 employees (process workers, repairmen, foremen, and a laboratory worker) at a factory making vanadium pentoxide from magnetite ore. A referent group of 63 men living in the same area was selected from workers at the magnetite ore mine (concentrating plant, the mine, the repair shop, and the office) and matched for age (within 2 years) and smoking habit (within five cigarettes per day). Vanadium concentrations in the breathing zone during March–May 1976 were 0.028 mg/m^3 (on a time-weighted average [TWA] basis) with a range of 0.002–0.42 mg/m^3. Air monitoring results were not reported for the referent population. Urinary vanadium concentrations among the exposed averaged 0.26 ± 0.17 μmol/L (18-hour excretion), while concentrations among the referent group did not exceed the limit of detection (< 0.04 μmol/L).

Clinical assessments were conducted by health personnel with no knowledge of exposure status. Workers who reported wheezing were twice as likely to work in the vanadium factory ($p < 0.05$) (Kiviluoto, 1980). Prevalence of nasal catarrh, cough, phlegm, or other respiratory symptoms did not differ between the exposed and referent groups. Spirometric measurements (FVC, FEV_1, adjusted for height), obtained at the end of workers' summer holidays, did not differ between the exposed and referent groups ($p > 0.01$) and were not related to duration of exposure to vanadium dust ($p > 0.1$). Inflammation also was observed in nasal smears; these results are discussed in the section on immunological endpoints (Appendix D). Nonfasting serum chemistry parameters, analyzed in May–June, 1975, when vanadium concentrations were

higher (0.2−0.5 mg/m^3) were compared between 16 exposed and 16 referent subjects. Among the several serum chemistry parameters tested, the difference between the exposed and referent groups for serum albumin, chloride, urea, bilirubin, and conjugated bilirubin were statistically significant, although no values were outside the range of reference values. Hematological results (nonfasting) for 63 exposed and 16 referents, analyzed in March–May, 1976, when vanadium concentrations were lower (0.01−0.04 mg/m^3), did not vary by exposure group ($p > 0.05$). In addition, no differences were noted for serum cholesterol, serum triglyceride, or leukocyte differential. In conclusion, this cross-sectional study found a higher prevalence of wheeze and some markers of inflammation in nasal smears among an occupational population producing vanadium pentoxide with TWA concentrations between 0.002 and 0.42 mg/m^3. An effect on nonfasting serum chemistry and hematological markers was indicated at higher concentrations between 0.2 and 0.5 mg/m^3.

Irsigler et al. (1999) evaluated the clinical histories of 40 men, employed at a vanadium pentoxide production plant in South Africa, who were referred by the plant's medical staff for more detailed medical assessment because of persistent respiratory symptoms (cough, breathing difficulty, wheezing) between October 1995 and October 1997. Twelve men, aged 19–60 years with bronchial hyperresponsiveness to inhaled histamine or exercise challenge (out of 40 men referred) were selected for analysis along with 12 men, aged 24–54 years, who were referred and did not have bronchial hyperresponsiveness, matched by age and smoking. All were free of current symptoms and had no previous history of asthma when they began employment at the plant. Vanadium pentoxide concentrations in air from area samples were <0.15 mg/m^3 in the mills, kiln, leaching, and pollution control areas; 1.53 mg/m^3 in the fusion precipitation area; and 0.057 mg/m^3 in the ferrovanadium area. Vanadium pentoxide in spot urine samples was detected in 10 of the 12 workers with bronchial reactivity (5.2–180 µg/g creatinine) and was above a level considered to be toxic in 3 individuals (>50 µg/g creatinine). Levels in the 12 referent men ranged between 12.0 and 55 µg/g creatinine, with one person having >50 µg/g creatinine. Concentrations of sulfur dioxide and ammonia were above their recommended occupational limits in the kiln area. Among nine subjects who returned for a follow-up examination after 5 to 23 months with no vanadium pentoxide exposure, eight still exhibited bronchial reactivity. Work tasks did not appear to differ between the two groups. This small study did not inform understanding of what aspects of vanadium pentoxide exposure influence the prevalence of bronchial reactivity. Although the group with bronchial reactivity had two subjects with high urine vanadium levels, the data reported by the authors did not indicate that urine vanadium levels or job sites varied by bronchial reactivity status and were not analyzed statistically. Also, the sample size was not adequate to determine an association of vanadium pentoxide exposure with bronchial reactivity.

4.1.2.3. *Occupational Exposure during Cleaning and Maintenance of Oil-Fired Boilers*

Vanadium pentoxide is present in significant amounts along with other vanadium oxides, vanadium sulfate, and metals in ash that accumulates in oil- and coal-fired boilers, and in other fuel types used in boilers. Several reports of case histories and epidemiologic studies of boilermakers involved in the construction, cleaning, and maintenance of oil-fired boilers have described upper and lower respiratory symptoms similar to those reported among workers processing vanadium pentoxide (Kim et al., 2004; Hauser et al., 2001; Woodin et al., 2000; Woodin et al., 1999; Woodin et al., 1998; Hauser et al., 1995a, b; Levy et al., 1984; Ross, 1983; Lees, 1980; Sjoberg, 1955; Williams, 1952). Additional health parameters have been investigated, including pulmonary function (Woodin et al., 1999; Hauser et al., 1995a; Levy et al., 1984; Lees, 1980); biomarkers of inflammation in nasal fluid (Woodin et al., 1998; Hauser et al., 1995b); and autonomic cardiac function (Magari et al., 2002). Studies have investigated acute effects among workers that cleaned or overhauled boilers over a period of a few days to several weeks and chronic conditions among boilermakers who had worked in that occupation for several years.

Several case summaries described the health response of workers cleaning oil-fired boilers in Great Britain (Ross, 1983; Williams, 1952), Sweden (Sjoberg, 1955), and Canada (Lees, 1980). Two case series reports that reported measurements of vanadium pentoxide in the air within the boilers described respiratory symptoms, including rhinorrhoea, sneezing, eye irritation, sore throat, and chest pain that began within 1 to 12 hours, with subsequent onset of a dry cough, becoming paroxysmal and productive in some workers, wheezing, dyspnea upon exertion, and fatigue after 6–24 hours (Sjoberg, 1955; Williams, 1952). Bronchial irritation, bronchitis, and the development of rales in regions of the lung of some workers also was reported. Symptoms persisted for 3 days to 1 week after exposure was ended. Concentration of vanadium pentoxide particles (10−20 μm in diameter) in the air inside the boilers during cleaning was 17–85 mg/m^3.

In 1981, the Occupational Safety and Health Administration conducted an investigation of work-related bronchitis among 100 boilermakers exposed to vanadium pentoxide during an oil-to-coal conversion of a utility company power plant in western Massachusetts (Levy et al., 1984). The conversion occurred over approximately 6 weeks, October 15–November 30, with most of the men working 10-hour days, 6 days per week. Air samples obtained in the boiler at approximately 4 weeks during the conversion indicated vanadium pentoxide fume concentrations of 0.05–5.3 mg/m^3. Concentrations of chromium, nickel, and copper and iron oxide fumes were stated to be within acceptable limits. Nitrogen dioxide and hydrogen sulfide were not detected. Low concentrations of carbon dioxide (<5 ppm) and ozone (<0.1 ppm) were measured. Sulfur dioxide (<1 ppm) was measured in the boiler during welding operations and outside the boiler (1–35 ppm) when expansion joints were cut with a torch.

In early December, investigators distributed a questionnaire to all 100 workers through the union president and responses were received from 55 men over the next 2 months. All respondents, aged 23–60 years, reported symptoms, includingcough with sputum (85%), sore throat (76%), dyspnea on exertion (71%), chest pain or discomfort (65%), headache (56%), runny nose or sneezing (56%), wheezing (55%) and tiredness (51%). The median time to onset was 7 days with clustering at 0–4 days and 6–8 days. When the questionnaires were completed, symptoms had resolved or were improving in 41 of the 55 respondents. Although three-fourths of the respondents stated that they had used a respirator over half the time when in the boiler, more than half stated that the respirator used was a paper mask. Respondents had been working as boilermakers a median of 10 years. A marked deficit in pulmonary function, particularly in FEF, was observed in some workers. Pulmonary function was assessed, however, in only 60% of respondents by several different health providers. The symptoms and effects on lung function are consistent with previous reports about health effects among boilermakers (Lees, 1980; Sjoberg, 1955; Williams, 1952). However, this study is limited by a lack of baseline information on health status or comparison to a comparable occupational group, a relatively low response among the exposed workers, and data collection days to weeks after exposure was discontinued.

A subsequent study of lung function among boilermakers overhauling an oil-fired boiler, which measured baseline lung function and used a more detailed exposure assessment, did not observe an association with respirable vanadium dust concentrations (Hauser et al., 1995a). A total of 36 of 80 eligible workers with an average of 16.9 years on the job completed a baseline test, and 26 completed a postexposure test. Investigators developed daily exposure estimates for each subject based on work diaries detailing tasks and locations and personal sampling. Time-weighted average (1–10 hours) sampling was available for 15% of the total number of study days and these data were applied to the task/location information to assign exposure levels for PM_{10} and vanadium dust for each worker. Change in lung function between the baseline and postexposure tests 4 weeks later, adjusted for the average of the pre- and postexposure values, was inversely associated with peak PM_{10} in multiple linear regression models adjusting for age and current smoking status. However, mean, peak, and day-of respirable vanadium dust concentrations, were not associated with any spirometric indices (the data were not presented). PM_{10} and vanadium dust exposure also were not related to bronchial reactivity as measured with methacholine challenge tests before and after the overhaul. The authors noted that the concentrations of vanadium dust were low, averaging between 2.2 and 31.3 $\mu g/m^3$, and the variation in the range of concentrations might not have been wide enough to detect a relationship with lung function in this small sample. Alternatively, a different constituent in PM_{10} might have caused the deficits in lung function.

Woodin et al. (2000; 1999; 1998) described in a series of reports a prospective clinical study that evaluated health measures among 18 boilermakers and compared them to 11 utility workers involved in the overhaul of a large, oil-fired boiler over a 6-week period from mid-May

1995 to late June 1995. The men had volunteered for the study and had no allergic symptoms 2 weeks prior to or during the overhaul. Vanadium and PM_{10} exposures were calculated for each subject for each workday using information on task duration and location and use of personal protective equipment from job diaries, and vanadium and PM_{10} concentrations from personal exposure monitors in the breathing zone. Utility workers did not enter the boiler during the overhaul. Vanadium levels before the boiler work were comparable for boilermakers and utility workers (geometric mean (SD – standard deviation) $\mu g/m^3$: 1.2 (1.4), and 1.1 (1.2), respectively]. During the boiler work, vanadium levels rose to a geometric mean (SD) of 8.9 (2.3) $\mu g/m^3$ inside the boiler but did not change appreciably outside the boiler [geometric mean (SD) $\mu g/m^3$: 1.4 (1.6) ($p < 0.001$)]. Exposure estimates were adjusted for the type of protective gear worn and its duration to calculate individual daily dose. The daily dose to the upper and lower airway was estimated using values for minute volume, penetration and deposition rates, and particle size.

Incidence of upper airway symptoms (nasal congestion/irritation, throat irritation) was 67% (12/18) among boilermakers and 36% (4/11) among utility workers. The incidence of lower airway symptoms (chest tightness, wheeze, cough, and sputum production) was 72% (13/18) among boilers and 27% (3/11) among utility workers. The workers recorded symptoms five times per day in a log and scored them for severity with a numerical score from 0 to 3. The highest severity score for each day was used in the analysis. Robust regression models of lower airway maximum severity scores and average symptom frequency in relation to quartiles of lung vanadium dose indicated a dose-related increase. Maximum lower airway severity scores were increased by 0.47 ($p = 0.01$), 0.86 ($p < 0.01$), and 0.24 (0.10) in quartiles of lung vanadium doses 2, 3, and 4 compared to 1. Average lower airway symptom frequency was increased by 0.19 ($p = 0.02$), 0.39 ($p < 0.01$), and 0.14 (0.07) in quartiles of lung vanadium doses 2, 3, and 4 compared to 1. The regression models were adjusted for current smoking.

Lung function was assessed on three occasions: before the overhaul, during the overhaul (before the shift on the last day), and 2 weeks after the work ended (Woodin et al., 1999). No changes in the four airflow measures, FEF_{25}, FEF_{50}, FEF_{75}, and MMEF, were observed over the course of the study. Change in lung function from the beginning to the end of the overhaul was not associated with upper or lower airway dose levels of either vanadium or PM_{10} when assessed in linear regression models adjusting for smoking and age. In addition, mean dose estimates were not different between individuals who experienced a loss of either FEV_1 or FVC > 100 mL or those who experienced no change or an increase (two-sample t-test).

Boilermakers were exposed to higher PM_{10} concentrations compared to utility workers both before (geometric mean [SD]: 0.40 (1.60) versus 0.10 [2.70], $p < 0.05$) and during the overhaul (geometric mean {SD]: 0.47 (1.90) versus 0.13 (4.00), $p < 0.001$). In contrast to the elevations in vanadium concentrations measured during the overhaul, PM_{10} concentrations did not increase appreciably ($p > 0.05$). During the boiler overhaul, ozone concentrations increased

somewhat outside the boiler, but did not change inside the boiler. The authors reported that levels of other metals (cadmium, chromium, manganese, lead, arsenic, and nickel) were low. All samples were 1–3 orders of magnitude below the 1996 TLV, and no significant changes were observed during the overhaul.

Reductions in lung function over 2 years were investigated by Hauser et al. (2001) in a longitudinal study of boilermaker construction workers exposed to combustion particles from multiple sources, including power plants (oil, coal, natural gas), trash incinerators, paper mill incinerators, and other industrial sources with boiler, vessels, and tanks requiring maintenance and repair. A total of 118 boilermakers from Local 29 of the International Brotherhood of Boilermakers, Iron Shipbuilders, Blacksmiths, Forgers and Helpers (81% of those contacted) were followed between 1997 and 2000. Participants completed spirometry after some days off work, a modified American Thoracic Society questionnaire on respiratory symptoms, and a work history questionnaire at baseline, with two annual follow-up visits. Baseline FEV_1 and FVC were 90% and 94% of predicted. The nine participants who were lost to follow-up had a lower mean baseline FEV_1 compared to those who remained in the study (84% compared to 91.2% predicted). The number of years worked as a boilermaker was a statistically significant predictor of annual FEV_1 (−33.5 mL/years worked [95% CI: −45.9, −21.1]). In generalized estimating equation models adjusting for age, baseline FEV_1, and cigarette smoking status, the number of hours worked at gas-fired power plants in the previous year was inversely related to annual FEV_1 (−9.8 mL/100 hours worked [95% CI: −16.0, −3.5). Adjusted models analyzing the number of hours worked at oil- and coal-fired power plants also showed FEV_1 reductions but the associations were not statistically significant. This study provides evidence of long-term declines in lung function among boilermakers exposed to combustion particles from several fuel types. The study did not estimate exposure to individual substances, however, and no conclusions can be drawn regarding a role for vanadium compounds.

The effect of occupational $PM_{2.5}$ concentrations and metals components on cardiac autonomic function during a work shift was studied among a panel of 39 boilermaker construction workers with an average of 13 years (0-40 years) in that occupation {Magari, 2002, 34813}. Metals concentrations over an 8- to 10-hour work shift were determined from particle samples (<2.5 μm) collected using personal monitors. Vanadium concentrations were skewed with a mean of 0.76 ± 1.96 $\mu g/m^3$ and a median of 0.13 ± 1.96 $\mu g/m^3$ (range 0–11.62). Fifteen of forty-eight personal samples were above the LOD for vanadium (0.00859 $\mu g/m^3$). Average $PM_{2.5}$ concentrations were 1.16 ± 1.61 $\mu g/m^3$ with a median of 0.56 ± 1.96 $\mu g/m^3$ (range 0.09–7.76). Vanadium concentrations during the shift were associated with a 3.98-msec (95% CI: 1.64, 6.32) per $\mu g/m^3$ increase in heart rate variability estimated from the mean of the 5- minute average SD of the normal-to-normal intervals (SDNN) in mixed effects regression models adjusted for smoking status, age, and mean heart rate. Lead also was associated with an increase

in SDNN index (11.3 msec/μg/m^3, 95% CI: 2.88, 19.73).[2] Vanadium concentrations were not correlated with either lead or $PM_{2.5}$, indicating that these pollutants were not likely to be confounders for the association of heart rate variability with vanadium. No associations with heart rate variability were observed for the other analyzed metals, including nickel, chromium, manganese, or copper. The increase in heart rate variability associated with vanadium and lead concentrations could have resulted from the temporal framework chosen for the analysis (e.g., averages over the work shift). Indeed, a subsequent study examining changes in a different index of autonomic cardiac function, rMSSD (square root of the mean squared differences of successive intervals), averaged over the nighttime hours (0:00–7:00), found an inverse association with average work-shift concentrations of the $PM_{2.5}$ metal, manganese (Cavallari et al., 2008). The biological significance of the association of vanadium with increases in heart rate variability is unclear. All-cause mortality has been associated with decreased heart rate variability measured at baseline in longitudinal studies (Dekker et al., 1997). The effect on heart rate variability, however, indicates that vanadium exposure might alter autonomic function. Alternatively, the observed association could have been due to chance.

In summary, symptoms of the upper and lower respiratory tract, including headache, runny nose or sneezing, sore throat, cough with sputum, dyspnea on exertion, chest pain or discomfort, wheezing, and tiredness, were reported in case-series, cross-sectional, and panel studies of workers employed at vanadium pentoxide production facilities (Ross, 1983; Musk and Tees, 1982; Kiviluoto, 1980; Kiviluoto et al., 1979; Zenz et al., 1962; Sjoberg, 1951) and of boilermakers exposed to vanadium compounds in residual oil fuel ash over a few days to several weeks (Woodin et al., 2000; Levy et al., 1984; Ross, 1983; Musk and Tees, 1982; Kiviluoto, 1980; Lees, 1980; Kiviluoto et al., 1979; Zenz et al., 1962; Sjoberg, 1956, 1955; Williams, 1952; Sjoberg, 1951). Some symptoms began after a few hours, while the onset of other symptoms occurred after 1 to several days. Vanadium pentoxide concentrations of particle size less than 5 microns in production facilities where the studies were conducted varied by work location, ranging from 0.06 mg/m^3 in the ferrovanadium area to 1.53 mg/m^3 in the fusion precipitation area at one facility (Irsigler et al., 1999). Concentrations of vanadium pentoxide inside the boiler during a cleaning operation were 85 mg/m^3 (Sjoberg, 1955) and ranged from 0.05–5.3 mg/m^3 during conversion of a power plant from coal to oil (Levy et al., 1984).

Although a few of the case reports of workers exposed to ROFA during the cleaning or conversion of boilers at electrical generating facilities reported measurements of vanadium pentoxide, the case-control and panel studies evaluated symptoms and other health effects in relation to ambient concentrations or biomarker concentrations of vanadium. Therefore, relying on these studies to draw conclusions regarding the hazard of vanadium pentoxide is not possible

[2]These effect estimates for vanadium and lead were reported as such in the abstract and in Table 4, but were transposed in the text of the results.

to. However, the findings of these studies are consistent with studies of workers in vanadium pentoxide production. The estimated dose of vanadium in the nose was associated with upper airway symptom severity scores (nasal congestion/irritation, throat irritation) during a boiler overhaul with geometric mean respirable (<10 μm) vanadium concentrations of 8.9 ± 2.3 $\mu g/m^3$ (0.009 mg/m^3) (Woodin et al., 2000). The estimated dose of vanadium in the lung was associated with increases in lower airway symptom frequency and severity (chest tightness, wheeze, cough, and sputum production).

Vanadium pentoxide concentrations in vanadium pentoxide production facilities were not associated with pulmonary function deficits in the one epidemiological investigation that included a referent group (Kiviluoto, 1980; Kiviluoto et al., 1979)1981. Pulmonary function was measured after a 2- to 4- week summer holiday and changes across the shift or during the work week were not assessed. Among boilermakers, pulmonary function declines (FEV_1, FVC, $FEF_{25\%–75\%}$) over the course of a boiler overhaul were observed, but no associations with vanadium measures were found among the studies with more systematic and detailed reporting of methods and results (Woodin et al., 1999; Hauser et al., 1995c; Levy et al., 1984; Lees, 1980). The authors reported that concentrations of respirable vanadium dust were relatively low and insufficient variation in exposure might have precluded detection of an association with the small changes in pulmonary function that occurred during the overhaul. Uncertainties in the exposure estimates, particularly inside the boiler, also might have prevented detection of associations between vanadium exposure and pulmonary function. The cohort had worked in this occupation an average of 20 years and could represent a healthy, less susceptible population. In addition to vanadium, boilermakers are exposed to increased levels of other metals during boiler overhauls including nickel, chromium, and manganese (Liu et al., 2005). The observed pulmonary function declines among boilermakers might be explained by exposure to other ROFA constituents.

Table 4-1. Studies of Vanadium Pentoxide Exposure: Occupational Case Studies, Epidemiology Studies, and Controlled Human Exposure

Study design, time period; Participants	Exposure assessment and exposure levels	Outcome definition and primary results	Reference
Controlled human exposure; 9 healthy volunteers, aged 27–44 yr No previous history of respiratory disease or known exposure to pulmonary irritants 2 participants smoked cigarettes, <1 pack/day	Pure vanadium pentoxide dust, 98% < 5 microns in diameter 0.1 (N = 2), 0.25 (N = 5) and 1 mg/m^3 (N = 2) for 8 hr Blood vanadium not detected Urine vanadium after 3 d: max 0.013 mg/100 mL, after 7 d: not detected Fecal vanadium: max 0.003 mg/gm, after 2 wk not detected No filtered air exposure	Two subjects exposed (inadvertently) to 1 mg/m^3 developed coughing after 5 hr, coughing persisted for 8 d, no other signs of irritation Lung function tests (FVC, FEV_1, MEF, MMF, MMET, FIVC) at baseline (three occasions), after 8-h exposure, and once/week for 3 wk— no difference noted (data not presented) No alteration of white blood cell counts, differential cell patterns, or urinalyses (data not presented) No eosinophilia in nasal smears at 24 hr, 72 hr, or 1 wk later Accidental re-exposure to dust cloud for 5 min caused coughing with sputum within 16 hr, rales and wheezes within 2 d, with normal lung function 0.25 mg/m^3 for 8 hr (N = 5) Productive cough by next morning with no other symptoms, resolved after 1 wk to 10 d. Subjects continued normal activities, differential white blood counts were normal. Pulmonary function tests were not changed (data not presented) 0.1 mg/m^3 for 8 hr (N = 2) Mucus within 24 hr with coughing, subsiding after 72 hr and resolved after 4 d	Zenz and Berg (1967)

Table 4-1. Studies of Vanadium Pentoxide Exposure: Occupational Case Studies, Epidemiology Studies, and Controlled Human Exposure

Study design, time period; Participants	Exposure assessment and exposure levels	Outcome definition and primary results	Reference
Case series, 1947–1950 36 employees (foremen, workers, builders/repairers) at a V_2O_5 production factory in Falun, Sweden that began operation in 1946; compared to 703 workers from mines and sawmills in northern Sweden Referent group followed during same period using same methods	Concentration in dust during pulverization of iron ore slag: 0.6–86.9 mg/m^3 with vanadium content of 4.8–7.5%; 22% < 8microns, 39% < 12 microns Pattern of exposure not described Vanadium detected in blood (N = 29) and urine (N = 27) of exposed, not detected among unexposed	Main symptoms: All in series had symptoms at least once; Symptoms lasted an average of 13 d *Severe irritation of the upper respiratory tract: nasal irritation; inflammation/catarrh (exposed 42%, unexposed 20%); throat dryness and pain (exposed 86%, unexposed 8%), cough with no sputum (22/36, 4% among unexposed) *Lower respiratory tract symptoms: cough with sputum (14/36), wheezing (31/36), shortness of breath (27/36, 24% among unexposed) *Other common symptoms: palpitation of heart on exertion, weakness, and fatigue. Other signs/symptoms: *Bronchopneumonia in four cases, pneumonia in one case; *Skin disease (N = 6): papular eruption on the face, hands, dorsa of feet. Positive reaction to patch test in one case with eczematous lesions. *Tremor for a short period (N = 1). *Greenish black discoloration of the tongue (N = 1) *Decrease in hemoglobin levels (7.8 ± 2.36% compared to 3.0 ± 2.07%) and red blood cell count *No signs of chronic changes in the lung reported Spirometry: Exposed / Unexposed VC 4.84 ± 0.13 / 4.49 ± 0.07 RC 1.70 ± 0.08 / 1.56 ± 0.04 TC 6.54 ± 0.16 / 6.05 ± 0.09	Sjöberg (1956, 1951)

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Table4-1. Studies of Vanadium Pentoxide Exposure: Occupational Case Studies, Epidemiology Studies, and Controlled Human Exposure

Study design, time period; Participants	Study design, time period; Participants	Study design, time period; Participants	Study design, time period; Participants
Case series, 2 weeks during 1961 18 men, aged 21–55 yr, assigned tasks in the pelletizing of vanadium pentoxide in a pilot-plant operation	Exposure to pure vanadium pentoxide powder during processing; highest exposure during unloading, mulling, and pelletizing Possible exposure to vanadium pentoxide fumes during high temperature pelletizing Concentrations of vanadium pentoxide in air calculated as >0.5 mg/m^3, mean particle size <5 microns Urinary vanadium detected in 12 men	Most highly exposed experienced symptoms by end of first work day (N = 3) Burning eyes, sore throat, and dry cough. Symptoms by end of day 3: Slight conjunctivitis, inflamed throat, intense persistent coughing. After 3 d of no exposure, symptoms recurred with greater severity after work resumed with respirator use. Cough accompanied by acute diffuse rales. Clinical symptoms in all workers (persisting for up to 2 wk): Conjunctivitis, nasopharyngitis, hacking cough, fine rales, and wheezing (N = 16) Upper respiratory irritation (N = 2) White blood cell counts elevated (N = 9 of 17)—persisted up to 2 wk in some "Skin itch" complaints (N = 4) No spirometric changes attributed to exposure Chest x-rays were normal in all 18 men	Zenz et al. (1962)
Case Reports 4 workers at new vanadium pentoxide refinery in Western Australia, aged 22, 24, 41, and 52 yr, all smokers Examined 1 (N = 3) and 6 (N = 1) wk after exposure	Exposure to dust from dry ammonium vanadate powder 24-hr urinary vanadium (N = 1) 24 hr after maintenance work discontinued: 0.003 mg	Respiratory symptoms reported: Within hours: headache, green discoloration of the tongue, stuffy nose, lethargy, cough with sputum Within 3 d: wheezing, papular and cough with sputum Within 1 d to 2 wk: sore throat, hoarse voice, exertional dyspnea, wheezing Symptoms resolved within 1–4 wk after exposure ceased Pulmonary function at 1 wk (N = 3): mild to moderately severe airflow obstruction, Bronchial hyperreactivity to histamine (N = 2) One case with family history of asthma and positive skin prick tests, exhibited prolonged asthma symptoms that persisted until 8 wk after exposure ceased.	Musk and Tees (1982)
Case reports: 8 maintenance workers at large oil-fired boiler working 12 hr/d, 7 d/wk	One of 2 random samples from deadspace contained 0.6% vanadium pentoxide by weight; 3 samples inside boiler contained 14.2, 10.0, and 5.8% by weight No protective equipment worn Urinary vanadium concentration 0.05–11.0 mg/L	Symptoms reported in seven of eight workers: sore throat, running nose, cough with sputum, breathlessness and wheeze, sore and watering eyes, and blepharitis Persistent upper respiratory irritation in two workers, and eye irritation and blepharitis in one.	Ross (1983)

Table4-1. Studies of Vanadium Pentoxide Exposure: Occupational Case Studies, Epidemiology Studies, and Controlled Human Exposure

Study design, time period; Participants	Study design, time period; Participants	Study design, time period; Participants	Study design, time period; Participants
Case series; October 1995–October 1997 12 men, aged 19–60 yr, employed at a vanadium pentoxide production plant in South Africa who visited the plant's medical station for treatment of respiratory symptoms, were referred for more detailed assessment because of persistence of symptoms, and who were found to have bronchial hyperresponsiveness to inhaled histamine or exercise challenge (out of 40 men referred); compared to 12 of the 40, aged 24–54, who were referred and did not have bronchial hyperresponsiveness, matched by age and smoking	V_2O_5 concentration in air by plant site: Mills, kiln, leaching, and pollution control: <0.15 mg/m^3, fusion precipitation: 1.53 mg/m^3, ferrovanadium: 0.057 mg/m^3 Urine V_2O_5 (µg/g creatinine): 5.2–180 in 10 of 12 reactive subjects; 180 µg/g (N = 1) and 86.6 µg/g (N = 1) creatinine, nondetectable (N = 2) 12–55 µg/g creatinine in nonreactive subjects, detected in all subjects	Histamine PC20 FEV_1 – Dose resulting in a decrease of 20% or greater; PC20 > 8 mg/mL defined as normal Exercise challenge: free treadmill running indoors for 6 min. Positive test defined as a decrease of 200 mL or 15% or greater fall in FEV_1 Bronchial reactivity persisted in eight of nine workers who returned for testing after 5–23 mo without exposure Six reactive workers worked in the fusion precipitation area and three others worked in all areas Five non reactive workers worked in the fusion precipitation area Differences in biomarker levels or job site were not analyzed statistically in relation to bronchial reactivity status The authors concluded that alternate explanations were not the reason for the bronchial reactivity observed, including atopy, viral upper respiratory tract infection, or smoking, as similar prevalence was noted in reactive and nonreactive groups.	Irsigler et al. (1999)

Table4-1. Studies of Vanadium Pentoxide Exposure: Occupational Case Studies, Epidemiology Studies, and Controlled Human Exposure

Study design, time period; Participants	Study design, time period; Participants	Study design, time period; Participants	Study design, time period; Participants
Cross-sectional, prevalence, 1975 63 of 79 employees (process workers, repairmen, foremen, and a laboratory worker) at a factory making vanadium pentoxide from magnetite ore. All employed at factory ≥4 mo and not on holiday or sick leave, aged 19–52 yr Referent group: 63 men living in same area selected from workers at magnetite ore mine (concentrating plant, the mine, the repair shop, and the office), matched for age (within 2 yr), smoking habit (within 5 cigarettes/day), aged 20–52 yr	Vanadium dust concentration at various sites measured on 8 d over 2 shifts in March–May 1976 (LOD: 0.002 mg/m^3, CV: 6% at 0.05 mg/m^3 Respirable fraction (≤5 μm)–20% TWA (mg/m^3) Breathing zone (112 samples, 58 workers) whole shift: 0.028 (0.002–0.42) Breathing zone grinding (2 samples, 1 worker, 1-hr): 1.7 (0.25–4.7) Stationary samples whole shift (80 samples, 15 workers): 0.012 (0.002–0.043) Packing smelt (7 samples, 1 worker, 1-hr): 0.13 (0.02–0.37) Grinding room (1 sample, 1 worker): 2.3 (1–2.3) Serum vanadium (0.22 ± 0.14 μmol/L) Urinary vanadium Exposed: 0.26 ± 0.17 μmol/L, 18 hour excretion Referent <0.04 μmol/L LOD Average duration of exposure: 10.8 yr Previous concentrations in air averaged 0.2–0.5 mg/m^3 1970–1975 Analyses were conducted “blind” to exposure status	Prevalence of symptoms: Nasal catarrh cough, phlegm, or other: no difference, McNemar χ^2test Wheezing more prevalent in exposed ($p < 0.05$) Pathological changes in upper respiratory tract: cytological and histological samples Nasal smears: number of neutrophils increased (N = 45 pairs) Exposed 16 (29%) Referent 1 (2%), $p < 0.001$ McNemar’s paired test Biopsies of nasal mucosa Number of plasma cells increased (N = 57 pairs) Exposed 15 (26%) Referent 4 (7%), $p < 0.05$) Number of round cells increased (N = 56 pairs) Exposed 21 (38%) Referent 10 (18%), $p < 0.05$ No changes in number of eosinophils in cytological and histological samples in exposed during 7–11 mo of exposure Histological analysis: in exposed, subepithelial lymphocyte and plasma cell infiltration, frequent papillarity in the mucous membrane surface and hyperemia Spirometry (FVC, FEV_1) expressed at BTPS. Used highest value of five attempts. Measured at the end of workers’ summer holidays (2–4 wk) Paired t-test of $FEV_1/Height^2$: no difference between exposed and referent for FEV_1 or FVC ($p < 0.1$) Nonfasting chemical analysis, 16 exposed to high levels (0.2–0.5 mg/m^3) compared to 16 referents during May and June 1975: statistically significant differences between exposed and referent for serum albumin, chloride, urea, bilirubin, and conjugated bilirubin. Differences were slight and authors concluded not clinically significant Hematological analysis, 63 exposed, 22 referent, during March–May 1976 during low exposure (0.01-0.04 mg/m^3): no differencs No differences for serum cholesterol or serum triglyceride or leukocyte differential	Kiviluoto et al. (1979); Kiviluoto (1980); Kiviluoto et al. 1981a

Table4-1. Studies of Vanadium Pentoxide Exposure: Occupational Case Studies, Epidemiology Studies, and Controlled Human Exposure

Study design, time period; Participants	Study design, time period; Participants	Study design, time period; Participants	Study design, time period; Participants
Studies of ROFA Exposure: Occupational Case Studies and Epidemiology Studies			
Case reports, eight male workers engaged in cleaning oil-fired boilers in Great Britain, 1946–1949 Ages 26–43 yr Examinations before and after cleaning jobs of approx. 1 week	Concentration of dust particles by diameter (mg/m^3), 3 samples in superheater during cleaning µm — mg/m^3 — % 0.15–1.0 — 0.36 — 93.6 1.0–5.5 — 4.09 — 6.14 5.5–11 — 7.86 — 0.26 Vanadium in air (mg/m^3) Superheater chamber 40.2 Superheater chamber 17.2 Combustion chamber 58.6 Urinary vanadium (24-h collection) N = 1: 0.4 and 0.07 µg/mL N = 2: trace N = 5: <LOD LOD: 0.02 mg/specimen	Symptoms: Immediate onset (within 30 min to 12 hr) Rhinorrhoea, sneezing, watering of the eyes, sore throat, and pain behind sterum Delayed onset (6–24 hr) Dry cough becoming paroxysmal and productive in some workers (N = 3), wheezing, severe dyspnea upon climbing, lassitude, depression Greenish-black coating on tongue Generalized bronchospasm and bronchitis (N = 2) Development of rales in right auxillary region of lung (N = 2) Slight hand tremor (N = 2) No cardiac abnormalities, no change in blood pressure Symptoms persisted for 3 d to 1 wk after exposure ceased	Williams (1952)
Case studies, 7 men engaged in cleaning oil-fired boilers at the Västerås generating station in Sweden, 1951–1953	Vanadium pentoxide in air, 2 samples in oil-fired boiler during cleaning: 85 and 57 mg/m^3, 10–20 microns Exposure during cleaning 1–2 d, 8 hr	Vanadium bronchitis diagnosed Common symptoms: Eye irritation (N = 3) Nose irritation (N = 7) Throat irritation (N = 6) Bronchial irritation (N = 6) Cough (N = 6) Wheezing in chest (N = 5) Dyspnea (N = 1) Fatigue (N = 5) Palpitation of heart (N = 2)	Sjoberg (1955)

Table4-1. Studies of Vanadium Pentoxide Exposure: Occupational Case Studies, Epidemiology Studies, and Controlled Human Exposure

Study design, time period; Participants	Study design, time period; Participants	Study design, time period; Participants	Study design, time period; Participants
Case studies; clinical evaluation of 17 men occupationally exposed cleaning bottom ash from the boiler of an oil-fired electricity generating station, ages not stated, Year not stated Demographic information was not provided No previous history of symptoms and no positive patch skin tests at baseline	Vanadium content of bottom ash and crusted deposits was 15.3% and 24.2–35%, respectively. Personal sampling at breathing zone (N = 4): mean TWA dust <10 microns: 523 µg/m^3; total dust concentration: >26 µg/m^3 Men wore cartridge filter type respirators (peak leakages 9%) Urinary vanadium concentration < LOD (40 µg/L) (N = 16) 280 µg/L in one worker with no respirator	Symptoms recorded on the day after completion of the cleaning operation: (N = 17) Cough with sputum: 77% Respiratory wheeze: 53% Sore throat: 41% Rhinitis: 29% Headache: 18% Dry cough: 18% Eye irritation: 12% Tongue discoloration: 6% Epistaxis: 6% Itch or rash: 0 Coarse rhonchi audible in those with wheeze, widespread rales found in two men Symptoms resolved in 4–6 d Reductions in FVC or FEV_1 as percent of baseline did not fall below 87% Pumonary function: difference between mean baseline and lowest mean values during days 1–4 and day 8, paired t-test FVC: 5.3 ± 0.74 vs. 4.7 ± 0.38 ($p < 0.05$) FEV: 4.5 ± 0.38 vs. 3.9 ± 0.4 ($p < 0.001$) FMF (l/s) reduction: 1.16 ± 0.82 (23%), range 0.33 2.4 (9–31% decrease) Lowest values occurred on day 2 but had not recovered on day 8	Lees (1980)

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Table4-1. Studies of Vanadium Pentoxide Exposure: Occupational Case Studies, Epidemiology Studies, and Controlled Human Exposure

Study design, time period; Participants	Study design, time period; Participants	Study design, time period; Participants	Study design, time period; Participants
Case studies: 8 maintenance workers at large oil-fired boiler working 12 hours/day, 7 days/week	One of two random samples from deadspace contained 0.6% vanadium pentoxide by weight; three samples inside boiler contained 14.2, 10.0, and 5.8% by weight. No protective equipment worn Urinary vanadium concentration 0.05–11.0 mg/L	Symptoms reported in seven of eight workers: sore throat, running nose, cough with sputum, breathlessness and wheeze, sore and watering eyes, and blepharitis Persistent upper respiratory irritation in two workers, and eye irritation and blepharitis in one.	Ross (1983)
OSHA investigation of 100 boilermakers exposed during a coal to oil conversion of a utility company power plant in western MA, aged 23–60 yr. Oct ober 15–November 30, 1981 Questionnaires and clinical assessment in December 1981; 55/100 returned questionnaire; clinical data from examinations of 70 workers analyzed Investigation conducted days after exposure had ceased	Concentration of vanadium pentoxide measured on November 17: In boiler: V_2O_5 0.05–5.3 mg/m^3 (N = 8) Chromium, nickel, and fumes of copper and iron oxide within "acceptable" limits, No CO_2 or hydrogen sulfide detected, CO_2 < 5 ppm, ozone < 0.1 ppm, SO_2 < 0.1 ppm Urinary vanadium (N = 3) by AAS (LOD 10 µg/L): Not detected	100% reported symptoms (N = 55) Cough with sputum (85%) Sore throat (76%) Dyspnea on exertion (71%) Chest pain or discomfort (65%) Headache (56%) Runny nose or sneezing (56%) Wheezing (55%) Tiredness (51%) Time to onset of symptoms: Median 7 d, clustering at 0–4 d and 6–8 d At questionnaire, symptoms were resolved or improving in 41 workers Pulmonary function tests (measured by study and private physicians) (N = 33): FVC median 87% of predicted (5 of 27 < 80%) FEV_1 median 93% predicted (8/27 < 80%) FEV_1/FVC median 79% predicted $FEF_{25–75\%}$ median 57% predicted (N = 24) (4/24 > 80%) Median $FEF_{25–75\%}$ was not correlated with history of cigarette smoking, although smoking history was not obtained for 31%.	Levy et al. (1984)

Table4-1. Studies of Vanadium Pentoxide Exposure: Occupational Case Studies, Epidemiology Studies, and Controlled Human Exposure

Study design, time period; Participants	Study design, time period; Participants	Study design, time period; Participants	Study design, time period; Participants
Cross-sectional, clinical 50 male boilermakers and utility workers at a local electrical company; 37 with baseline and postexposure lavage data and no cold or flu symptoms during previous 2 weeks.	Exposure determined for each of 3 d between baseline nasal lavage and after exposure using: Personal sampling (37% of exposure days), self-administered work diary for each day listing tasks (welding, cutting, and grinding), and specific location (boiler, airheater, and condenser). Data from personal air samples (1- to 10-h TWA) were used to assign exposure levels to each of 29 task and location categories. PM_{10} (1- to 10-hr TWA): 50–4,510 μg/m^3. Respirable vanadium dust 0.10–139.2 μg/m^3.	Differential cell count for PMNs, eosinophils, and epithelial cells/mL of recovered lavage fluid. Percent of total cells. Adjusted by dividing the change in PMN or epithelial cell counts by the mean of baseline and postexposure cell counts (expressed as percent). Adjusted mean change in PMNs/mL (SD): 40% (100%), range −98% to 200%, $p < 0.05$. Smokers Non-smokers ΔPMN_{adj} (%) −0.1 (86.6)50.3 (102) Δ%PMNs(%) –0.98 (15.5) 4.56 (28.5) $\Delta Epithelial_{adj}$ (%) 53.0 (78.7)20.6 (82.1) Δ%Epithelial (%) 14.0 (27.3)−3.9 (29.1) Adjusted change in PMN, change in % PMNs, change in epithelial cell counts, or change in % epithelial cells regressed on each of five exposure indices for PM_{10} or vanadium dust were not significantly associated in regression models stratified by smoking status. Large variation in change in cell count parameters	Hauser et al. (1995a)

Table4-1. Studies of Vanadium Pentoxide Exposure: Occupational Case Studies, Epidemiology Studies, and Controlled Human Exposure

Study design, time period; Participants	Study design, time period; Participants	Study design, time period; Participants	Study design, time period; Participants
Prospective study, clinical 18 boilermakers (workers inside boiler) and 11 utility workers (lower exposure, outside boiler) involved in overhaul of a large, oil-fired boiler; 6 weeks, mid-May 1995–late June 1995. White men, mean age (range): boilermakers: 37 (26–61 yr); utility workers: 35 (30–55 yr) % smokers: boilermakers: 39% utility workers: 17% No colds or flu symptoms during the study period	Vanadium concentration in nasal lavage (NL) fluid (median [N, minimum, maximum]) Boilermakers: 0.5 (15, 0.5, 9.7), 4.7 (15, 0.5, 102.9), 0.5 (6, 0.5, 2.7) at baseline, during, and 2 wk after the overhaul, respectively, $p < 0.05$ (NL2 vs. NL1 or NL3). Utility workers: 0.5 (8, 0.5, 3.8), 0.5 (8, 0.5, 8.0), <LOD at baseline, during, and after the overhaul, respectively. Boilermakers compared to utility workers (geometric mean, [GSD]) Vanadium before: 1.2 (1.4) vs. 1.1 (1.2) mg/m^3 During: 8.9 (2.3) vs. 1.4 (1.6) mg/m^3, Difference between groups, $p < 0.01$; Difference before vs. after, $p < 0.001$ PM_{10} before: 0.40 mg/m^3 (1.6) vs. 0.10 mg/m^3 (2.7), $p < 0.05$ During: 0.47 mg/m^3 (1.9) vs. 0.13 mg/m^3 (2.4), $p < 0.001$ Ozone before: 4.5 ppb (1.2) vs. 1.6 ppb (1.5), $p < 0.05$ During: 3.7 ppb (2.2) vs. 4.8 ppb (2.2), $p > 0.05$.	Nasal lavage was conducted on three occasions prior to the work shift, approx. 12 hr after the previous day's exposure Cytokine and enzyme levels in nasal lavage fluid Interleukin-8 (pg/mL) Boilermakers / Utility workers Before: 93.7 (22.6–235.0) / 69.2 (24.6–104.5) During: 4.7 (0.5–102.9)* / 0.5 (0.5–8.0) After: 0.5 (0.5–2.7) / <LOD Myeloperoxidase (ng/mL) Boilermakers / Utility workers Before: 22.7 (2.0–72.8) / 25.6 (10.1–47.6) During: 33.9 (2.0–103.0)* / 27.2 (4.9–66.2) After: 24.2 (3.9–58.1) / 25.6 (4.9–51.7) *Difference from before or after sample, $p < 0.05$ Authors concluded that boilermakers experienced an upper airway inflammatory response while working inside the boiler containing fuel-oil ash. PM_{10}, other metals, and ozone considered potential confounders. However, only vanadium levels changed during study, other metals and ozone concentrations were low. IL-6 and ECP levels not changed. No association between vanadium concentration and levels of MPO or IL-8. No association between cytokine or enzyme levels and smoking status or age.	Woodin et al. (1998)

Table4-1. Studies of Vanadium Pentoxide Exposure: Occupational Case Studies, Epidemiology Studies, and Controlled Human Exposure

Study design, time period; Participants	Study design, time period; Participants	Study design, time period; Participants	Study design, time period; Participants
Prospective study, clinical 18 boilermakers (workers inside boiler) and 11 utility workers (lower exposure, outside boiler) involved in overhaul of a large, oil-fired boiler; 6 weeks, mid-May 1995–late June 1995 White men, age range: boilermakers: 26–61 yr; utility workers: 30–55 yr Mean length of employment as boilermaker: 20.5 yr (3-39) No colds or flu symptoms during the study period Workers recorded symptoms in log 5 times per day, graded for severity (0–3). 50% of participants returned for spirometry 2 weeks after overhaul Data collection was over 90% complete	Vanadium and PM_{10} exposure calculated for each subject on each work day using job diaries and data from personal exposure monitors with adjustments for type of protective gear worn and duration. Samplers were located in the breathing zone, 10- to 12-hr samples. Daily dose to upper and lower airway estimated using values for minute volume, penetration rates, deposition rates, and particle size. Exposure was estimated for subjects who did not wear personal monitors from experience of people who did wear a monitor and worked in same area. Levels of cadmium, chromium, manganese, lead, arsenic, and nickel were low, all samples 1–3 orders of magnitude below 1996 TLV, no significant changes during the overhaul.	Highest severity score for each day used in analysis. Upper airway symptoms (nasal congestion/irritation, throat irritation) Boilermakers — Utility workers Incidence: 12/18 (67%) — 4/11 (36%) Severity Score (Mean) Before: 0.40* — 0.23 During: 0.43* — 0.18 Lower airway symptoms: (chest tightness, wheeze, cough, and sputum production) Boilermakers — Utility workers Incidence: 13/18 (72%) — 3/11 (27%) Severity Score (Mean) Before: 0.65** — 0.11 During: 0.85*** — 0.01 *Difference between groups, $p < 0.05$ **Difference between groups, $p < 0.01$ ***Difference between groups, $p < 0.0001$ Symptom severity score and average symptom frequency associated with quartiles of lung vanadium dose and nasal PM_{10} dose in a dose-related manner in regression models adjusted for current smoking. Generally the response in the highest quartile was lower than that in the third quartile. Robust linear regression with cluster option to account for multiple symptom reports by individuals Pulmonary function: highest of three acceptable curves used in analysis, repeated measures analysis of variance across 3 test days. No change over time for airflow measures (FEF_{25}, FEF_{50}, FEF_{75}, and MMEF) mean (SD) — FEV_1 (l) — FVC (l) Before: 3.73 (0.61) — 5.01 (0.67) During: 3.76 (0.54) — 4.94 (0.61) After: 3.65 (0.42) — 4.92 (0.55) % predicted FEV_1: 7 subjects showed decreases > 100 mL during overhaul, 11 showed no change or increases. % predicted FVC: 8 subjects showed decreases > 100 mL during overhaul, 10 showed no change or an increase. No association of having a FEV_1 or FVC decrement > 100 mL with PM_{10} or vanadium exposure; Estimated dose of PM_{10} or vanadium in nose or lung was not associated with change in FEV_1 or FVC during overhaul in multiple regression models adjusting for smoking status or age.	Woodin et al. (2000; 1999); same study as Woodin et al. (1998)

Table4-1. Studies of Vanadium Pentoxide Exposure: Occupational Case Studies, Epidemiology Studies, and Controlled Human Exposure

Study design, time period; Participants	Study design, time period; Participants	Study design, time period; Participants	Study design, time period; Participants
Panel study among boilermakers overhauling an oil-powered boiler; spirometry before and after 4 weeks of work; 36 of 80 eligible completed baseline test, 26 completed postexposure spirometry, 24 completed a postexposure challenge test The 26 subjects represent a self-selected subgroup with higher average FEV_1 and FVC % predicted and higher PC12-FEV_1 (less reactive airways) compared to the 10 without postexposure spirometry—a healthier subset of the initial study population Healthy worker effect—workers had a long duration of employment Mean age (range): 42.5 yr (27–60) Average of 16.9 yr as boilermaker (6 mo–35 yr)	Exposure estimated for each subject for each day based on work diary (task/location) and personal sampling (1- to 10-hr TWA available for 15% of total number of study days). Three Exposure indices: Average, peak (1- to 10-hr TWA), and concentration on the day of postexposure test Vanadium concentrations in PM_{10} Average 12.2 ± 9.1 μg/m^3 (2.2–31.3) Peak 20.2 ± 11.4 μg/m^3 (2.2–32.2) Day-of 12.1 ± 10.9 (1.6–31.1) Authors noted concentrations of vanadium low, variation in exposure small, and sample size was low.	Spirometry: Baseline (at least 1 d prior to start of overhaul): For analysis, used highest of three acceptable and reproducible lung function values. Methacholine challenge tests: concentration of methacholine that caused a 15% fall in FEV_1. ΔFEV_1adj: ΔFEV_1 divided by average of pre- and postexposure FEV_1 Spirometric indices showed statistically significant decreases for ΔFEV_1 (mean of –140 ± 160 mL; range: −390 to 420), $\Delta FEV_{25\%}$, $\Delta FEV_{50\%}$, $\Delta FEV_{25–75\%}$ (mean of –270 ± 450 mL/s; range: −1,170 to 870), and ΔFVC (mean of –140 ± 200 mL; range: −580 to 320), but not $\Delta FEV_{75\%}$ after exposure Multiple regression analysis (adjusting for age and smoking status): peak PM_{10} inversely associated with adjusted ΔFEV_1 ($p = 0.03$), $\Delta FEV_{25\%}$, ($p = 0.07$), $\Delta FEV_{50\%}$, ($p = 0.01$), ΔFVC ($p = 0.01$), but not $\Delta FEV_{25–75\%}$, ($p = 0.23$) and $\Delta FEV_{75\%}$ ($p = 0.43$) Peak, average, and day-of respirable vanadium dust not associated with adjusted spirometric indices. The 10 workers without postexposure spirometry had worked fewer years at their present job (12.5 ± 8.3) compared to those with postexposure spirometry (18.7 ± 8.0), $p < 0.05$, and had a lower % predicted preexposure FEV_1 and FVC.	Hauser et al. (1995a)

Table4-1. Studies of Vanadium Pentoxide Exposure: Occupational Case Studies, Epidemiology Studies, and Controlled Human Exposure

Study design, time period; Participants	Study design, time period; Participants	Study design, time period; Participants	Study design, time period; Participants
2-yr longitudinal study 118 boilermakers exposed to combustion particles and metals through building, maintaining, and repairing oil-, coal-, and gas-fired boilers at power plants, boilers and other vessels at nuclear plants, incinerators at trash dumps and paper mills 81% of 145 invited Recruited through Local 29 of the International Brotherhood of Boilermakers, Iron Shipbuilders, Blacksmiths, Forgers and helpers between December 1997 and March 1998 Male, Caucasian (97%) Mean age 42.6 yr (10.5–56.5), 37 (31.4%) current smokers	Detailed work history ascertained through self-administered questionnaire completed 6 mo after enrollment, and at 1- and 2-yr follow-ups. Recall assisted through providing union records of work dates, hours, and locations. Hours worked per year at each plant type (oil, coal, natural gas, trash, tree bark/sap and nuclear). Approximately 70% worked at ≥ types of plants. Respirator use was 38.4 and 31.8% for years 1 and 2 at gas-fired plants, 48.7 and 63.7% at oil-fired plants, and 59 and 66.9% at coal-fired plants. Exposure to vanadium pentoxide was not estimated.	Annual decline in lung function: Spirometry measurements at boilermakers union hall (Quincy, MA) or an apprentice training site (Portland, ME). Testing 9 am to 2 pm, between December and March to control seasonal variation. Largest value of three acceptable curves Data analysis using generalized estimating equation regression; Maximum FEV_1 at each visit in relation to hours worked during the previous year at each plant type. Baseline FEV_1 90% of predicted Annual FEV_1 (mL/yr) adjusting for age, smoking, and baseline FEV_1: Duration of employment as boilermaker (years worked): −33.5 (95% CI: −45.9, −21.1) Gas-fired plant work hours: −9.8 (95% CI: −16.0, −3.5) Oil-fired plant work hours: −12.1 (95% CI: −24.7, 0.6) Coal-fired plant work hours: −11.9 (95% CI: −30.4, 6.7) Annual FEV_1 mL/Ever worked at specific fuel type Gas-fired plant: −99.7 (95% CI: −154.8, −44.5) Oil-fired plant: −77.6 (95% CI: −151.1, −4.1) Coal-fired plant: −73.4 (95% CI: −128.8, −18.0) Association in model of ever working at a gas-fired plant remained statistically significant with adjustment for percentage of time wearing a respirator.	Hauser et al. (2001)
Panel study; 39 apprentice and journeyman boilermaker construction workers; average age 38 yr (18–59 yr), average duration of employment 13 yr (0–40 yr) Particulate matter concentrations and heart rate monitored during 8- to 10-hr workshift	Metals concentrations over an 8- to 10-hr work shift were determined from particle samples (<2.5 µm) collected using personal monitors. Vanadium concentrations (corrected for blank filter metal content) were skewed, mean 0.76 ± 1.96 µg/m^3, median 0.13 ± 1.96 µg/m^3 (range 0–11.62). 15/48 personal samples above LOD for vanadium (0.00859 µg/m^3). Average $PM_{2.5}$ concentrations 1.16 ± 1.61 µg/m^3, median 0.56 ± 1.96 µg/m^3 (range 0.09–7.76). Vanadium concentrations were not correlated with either lead or $PM_{2.5}$	Heart rate was monitored using a five-lead Holter monitor. Heart rate variability was estimated as the mean of 5-min average SD of the normal-to-normal intervals (SDNN). Mixed effects regression models included random effect for each study subject and fixed covariates for smoking status, age, and mean heart rate. SDNN increase (index per µg/m^3) Vanadium: 3.98-msec (95% CI: 1.64, 6.32) Lead: 11.3 msec/µg/m^3 (95% CI: 2.88, 19.73). $PM_{2.5}$: −0.77 msec/µg/m^3 (95% CI: −2.36, 2.81) No associations with heart rate variability were observed for the other analyzed metals, including nickel, chromium, manganese, or copper.	Magari et al. (2002)

Table4-1. Studies of Vanadium Pentoxide Exposure: Occupational Case Studies, Epidemiology Studies, and Controlled Human Exposure

Study design, time period; Participants	Study design, time period; Participants	Study design, time period; Participants	Study design, time period; Participants
Prospective 5-day study of 20 boilermakers during overhaul of power plant boilers (74% participation), 18–59 yr of age, with mean 21.7 ± 12.9 yr of boilermaking experience June 1999 10/20 were current cigarette or cigar smokers Demographic characteristics did not vary significantly between participants and nonparticipants ($p > 0.25$)	Personal samplers (PEMs) worn during workshift, 8-hr TWA $PM_{2.5}$ Total metal concentration including metal oxides on particulate filter. Analyzed for vanadium, chromium, manganese, nickel, copper, and lead. Metal concentration and metal content (metal mass/total $PM_{2.5}$ mass). 8-hr TWA concentration, μg/m^3 (Q_{25}–Q_{75}) (n = 39 samples) Total $PM_{2.5}$ mass: 440 (290–760) Vanadium: 1.23 (0.47–3.53) Chromium: 0.11 (0.05–0.27) Manganese: 0.81 (0.12–2.67) Nickel: 0.96 (0.31–1.88) Copper: 0.98 (0.25–2.53) Lead: 0.17 (0.04–0.53) Correlations between metals: $0.52 < r < 0.92$, $p < 0.001$	Urinary μg 8-OHdG/grams creatinine (controls for variation in urine dilution) Preshift urine samples collected after average of 2 d away from work Mean preshift 8-OHdG concentration (SE): 13.26 (1.04) μg/g creatinine Mean cross-shift change: 1.88 (0.74) μg/g creatinine, $p = 0.02$ No difference by smoking status Coefficient for linear mixed regression model for 8-OHdG levels and metal, adjusted for urinary cotinine levels, chronic bronchitis status, and age: μg/g creatinine per 1 mg/m^3 increase in metal 8-hr TWA: $PM_{2.5}$: 1.67 (95% CI: 0.21, 3.14) Vanadium: 0.23 (95% CI: 0.04. 0.42) Chromium: 3.08 (95% CI: –0.50. 6.67) Manganese: 0.47 (95% CI: 0.05, 0.89) Nickel: 0.33 (95% CI: 0.01, 0.64) Copper: 0.20 (95% CI: −0.07, 0.46) Lead: 1.67 (95% CI: 0.02, 3.26) Vanadium, Manganese, and Nickel also were significantly related to 8-OHdG levels when expressed as proportion of total $PM_{2.5}$ mass. Regression models adjusted for tobacco smoking, chronic bronchitis status (yes/no), and age. 4/20 had chronic bronchitis	Kim et al. (2004)

4.2. SUBCHRONIC AND CHRONIC STUDIES AND CANCER BIOASSAYS IN ANIMALS – ORAL AND INHALATION

4.2.1. Oral Exposure

4.2.1.1. *Subchronic Studies*

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 1,000 ppm of vanadium (in vanadium pentoxide) for 103 days (25- and 50-ppm groups; "low-exposure" groups) or 75 days (500- and 1,000-ppm groups; "high-exposure" groups). After 35 days of treatment, dietary vanadium levels of the low-exposure groups were increased to 100 and 150 ppm, resulting in average daily doses of 0, 74.5, 116, 500, and 1,000 ppm (0, 5.9, 9.2, 39, or 79 mg/kg-day)[3] of vanadium resulting in a doses of 0, 10.5, 16.4, 69.6, or 141.0 mg/kg-day of vanadium pentoxide.[4] At the end of treatment, body weight gain, liver weight, and cystine content of hair were measured in all groups; erythrocyte count and hemoglobin level were measured in control and low-exposure groups; and relative liver weight was measured in control and 69.6 mg/kg-day groups. Average body weights of animals at the conclusion of the study were not reported, although average weight gain in grams per rat was reported. Compared to control, average body weight gain was increased in the 10.5 mg/kg-day and 16.4 mg/kg-day groups (54 and 45% increase, respectively) and decreased in the 69.6 mg/kg-day group (66%) and 141.0 mg/kg-day group (no gain in body weight over the study period). The increase in body weight gain at the low exposure levels was not explained and statistical significance or SDs were not reported for any result. Relative liver weight in the 69.6 mg/kg-day group was statistically significantly increased compared to control, reported as a ratio of liver weight/body weight (3.86 compared to 3.51, $p < 0.05$, F ratio in analysis of variance). Data on relative liver weight were not reported for other dose groups. A dose-dependent decrease in erythrocyte count (12.8 and 21.3%) was observed over the duration of the study, in rats exposed to 10.5 mg/kg-day and 16.4 mg/kg-day vanadium pentoxide, respectively, compared to controls (3.8% decrease) (Table 4-2). A 20–30% decrease in erythrocyte count is considered biologically significant; no statistical analysis was reported by the study authors, however, and no measure of variance (standard error or SD) was given for the means. Data were not reported for high dose groups.

[3]Calculation: mg/kg-day = ppm(mg of compound per kg food) × mg food consumed per day × 1/kg body weight [using reference food consumption rate of 0.0217 kg/day (U.S. EPA, 1988) and average body weight of 0.275 kg for male rats (Mountain et al., 1953)].

[4]Conversion from mg/kg-day of vanadium to amount vanadium pentoxide: [(mg/kg-day vanadium)(molecular weight of vanadium pentoxide, 181.9)]/(2 × molecular weight of vanadium, 101.9).

Hemoglobin levels decreased 4.6% and 10.5%, respectively, in the 10.5 and 16.4 mg/kg-day groups over the duration of the study compared to 3.9% in controls. Cystine content of hair significantly decreased in a non-dose-dependent manner in all vanadium pentoxide treatment groups compared to controls, with the exception of the lowest exposure group. The biological significance of decreased hair cystine content is not established, although the researchers speculated that vanadium might have inhibited enzymes, such as sulfotransferases, that decreased the availability of cystine for hair growth. This study observed potentially dose-related changes in erythrocytes, body weight gain, and liver weight in treated animals. No statistical analysis was performed for the decrease in erythrocytes and body weight gain, however, and no degree of variance was reported (precluding statistical analysis for this review). Compared to hematological data for Wistar rats (Charles River Laboratories, 2008; Wright et al., 1983), however, the observed decrease in erythrocyte counts observed in this study is outside of the reference range for the historical controls and thus is believed to be a clinically significant finding. Based on decreased red blood cell count, the NOAEL and LOAEL values identified following oral exposure to vanadium pentoxide are 10.5 and 16.4 mg/kg-day, respectively.

Table 4-2. Hematological results of oral vanadium pentoxide exposure in Wistar rats

	Control	10.5 mg/kg-d	16.4 mg/kg-d
Red Cell Count (M/mm^3)[a]			
Start	8.0	7.8	8.0
Finish (103 d)	7.7	6.8	6.3
Percent change between start and finish of expt (%)	3.8	12.8	21.3
Hemoglobin, %			
Start	15.6	15.2	15.3
Finish (103 d)	15.0	14.5	13.7
Percent change between start and finish of expt (%)	3.9	4.6	10.5

[a]M/mm^3 = million cells per millimeter.
Source: Mountain et al. (1953).

4.2.1.2. *Chronic Studies*

A 2.5-year dietary study on vanadium in rats (strain not described) was previously used as the basis of the chronic reference dose (RfD) (Stokinger et al., 1953). The results were summarized in *Patty's Industrial Hygiene and Toxicology*, 3rd ed. (1981). In this chronic study, an unspecified number of rats was exposed to dietary levels of 10 or 100 ppm vanadium (about 17.9 or 179 ppm vanadium pentoxide; 1.41 and 14. 1 mg/kg-day)[5] for 2.5 years. Endpoints

[5]Converted to ppm vanadium pentoxide by [(ppm vanadium)(MW vanadium pentoxide, 181.9)]/(2 × MW vanadium, 101.9). No information was given on average rat weights so values cannot be converted directly to mg/kg-day. Conversions are based on data from a subchronic study from the same group (Mountain et al., 1953). Calculation: mg/kg-day = ppm(mg of compound per kg food) × mg food consumed/day × 1/kg body weight [using reference food consumption rate of 0.0217 kg/day (U.S. EPA, 1988) and average body weight of 0.275 kg for male rats (Mountain et al., 1953)].

evaluated were limited to growth rate, survival, and hair cystine content. The study did not assess comprehensive toxicity endpoints. Hair cystine content was significantly decreased in exposed animals, compared to controls but no values were given. This study reports the oral NOAEL upon which an RfD can be based as 17.9 ppm (1.41 mg/kg-day) vanadium pentoxide. The biological significance of decreased hair cystine is unclear, however, and is not specific to vanadium pentoxide exposure. No additional oral chronic exposure studies in animals were identified in the published literature.

4.2.2. Inhalation Exposure

4.2.2.1. *Subchronic Studies*

NTP conducted 3-month exposure studies in F344/N rats to evaluate the cumulative toxic effects of subchronic inhalation exposure to vanadium pentoxide (NTP, 2002). Chemical identity and purity of vanadium pentoxide was evaluated prior to the beginning and following the conclusion of all assays. Particle size given in mass median aerodynamic diameter (MMAD) ± geometric standard deviation (GSD) for each dose group was as follows: 1 mg/m^3 = 1.2 ± 2.8; 2 mg/m^3 = 1.1 ± 2.8; 4 mg/m^3 = 1.2 ± 2.8; 8 mg/m^3 = 1.0 ± 2.9; 16 mg/m^3 = 1.2 ± 2.8. 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^3, 6 hours per day, 5 days per week for 3 months. Additional groups of 10 male and 10 female rats were exposed to 4, 8, or 16 mg/m^3 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. Blood was also collected from cardiopulmonary physiology study rats on days 4 and 23 for hematology and clinical chemistry determinations. Necropsy and histopathological evaluations (light microscopy of comprehensive tissues)[6] were performed on all main study rats exposed to 0, 2 (male rats only), 4, 8, or 16 (female rats only) mg/m^3 at the completion of the study. Sperm motility and vaginal cytology evaluations were analyzed from all core study rats.

Seven male rats and three female rats exposed to 16 mg/m^3 vanadium pentoxide died during the study (NTP, 2002). Abnormal breathing, emaciation, lethargy, abnormal posture, and ruffled fur were observed in male and female rats exposed to concentrations of 8 mg/m^3 and higher.

[6]Complete histopathology was performed on 0, 8 (rats only), and 16 mg/m^3 rats and mice. In addition to gross lesions and tissue masses, the following tissues were examined in the 3-month studies: adrenal gland, bone with marrow, brain, clitoral gland, esophagus, gallbladder (mice only), heart and aorta, large intestine (cecum, colon and rectum), small intestine (duodenum, jejunum, and ileum), kidney, larynx, liver, lung and mainstream bronchi, lymph nodes (mandibular, mediastinal, mesenteric, and bronchial), mammary gland (except male mice), nose, ovary, pancreas, parathyroid gland, pituitary gland, preputial gland, prostate gland, salivary gland, skin, spleen, stomach (forestomach and glandular), testis (with epididymis and seminal vesicle), thymus, thyroid gland, trachea, urinary bladder, and uterus. The lung of rats and mice and nose of rats in all remaining exposure groups and the thymus in 8 mg/m^3 mice were also examined.

Diarrhea and nasal/eye discharge were also observed in some rats exposed to 16 mg/m^3. Weight gain and absolute and relative lung weights are summarized in Table 4-3. 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^3 and in females exposed to 16 mg/m^3 (30% decrease). Absolute lung weights were significantly increased in males exposed to concentrations of 2 mg/m^3 and greater and in females exposed to 4 mg/m^3 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^3 and in females exposed to 4 (19% increase), 8 (76% increase) or 16 (117% increase) mg/m^3. Other organ weight differences were considered to be related to body weight decreases.

Table 4-3. Body weight gain and lung weights in rats (F344/N) exposed to vanadium pentoxide by inhalation for 3 months (values are means ± standard error)

Parameter	Exposure					
	Control	1 mg/m^3	2 mg/m^3	4 mg/m^3	8 mg/m^3	16 mg/m^3
Male Rats						
Weight gain during 3-month exposure period (g)	197 ± 6	202 ± 5	180 ± 5	173 ± 8[a]	161 ± 5[a]	1 ± 9[a]
Absolute lung weight (g)	2.38 ± 0.17	2.56 ± 0.11	2.65 ± 0.07	2.93 ± 0.09[a]	3.26 ± 0.13[a]	1.98 ± 0.10[b]
Relative lung weight	6.77 ± 0.36	7.40 ± 0.28	7.83 ± 0.19 [a]	8.88 ± 0.22[a]	10.20 ± 0.30[a]	16.60 ± 0.33[a]
Female Rats						
Weight gain during 3-month exposure period (g)	87 ± 3	88 ± 4	96 ± 4	83 ± 3	77 ± 4	25 ± 7[a]
Absolute lung weight (g)	1.65 ± 0.11[c]	1.58 ± 0.04	1.92 ± 0.12[b]	1.95 ± 0.08[b,c]	2.16 ± 0.06[a]	2.16 ± 0.12[a]
Relative lung weight	8.37 ± 0.58[c]	7.84 ± 0.16	9.23 ± 0.53	10.00 ± 0.38[b,c]	11.48 ± 0.33[a]	18.15 ± 1.06[a]

[a]Significantly different from control by William's or Dunnett's test ($p \leq 0.01$)
[b]Significantly different from control by William's or Dunnett's test ($p \leq 0.05$)
[c]n = 9

Source: NTP (2002).

Results of hematology assessments following 3 months of inhalation exposure are presented in Table 4-4. Erythrocyte count was significantly increased in the 8 and 16 mg/m^3 groups and hematocrit was significantly increased in the 16 mg/m^3 group in male and female rats. Hemoglobin was increased significantly only in females exposed to 16 mg/m^3. Microscopic evaluation of the RBC morphology detected increased polychromasia and hypochromia in rats in the 16 mg/m^3 groups (data not presented). Significantly decreased mean cell hemoglobin

concentrations were observed in males exposed to 8 and 16 mg/m^3 and in females exposed to 4, 8, and 16 mg/m^3. Reticulocyte count was significantly increased in males and females exposed to 16 mg/m^3. Mean cell volume was significantly decreased, indicative of microcytosis, in male rats at concentrations of 2 mg/m^3 and above and in female rats at concentrations of 4 mg/m^3 and above. 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 4-4. Selected hematology parameters in rats (F344/N) exposed to vanadium pentoxide by inhalation for 3 months[a]

Parameter	Exposure					
	Control	1 mg/m^3	2 mg/m^3	4 mg/m^3	8 mg/m^3	16 mg/m^3
Male Rats						
Number	9	9	10	9	10	3
Erythrocytes (10^6/µL)	9.2 ± 0.1	9.0 ± 0.1	9.1 ± 0.1	9.3 ± 0.2	9.7 ± 0.2[b]	15.1 ± 0.3[c]
Reticulocytes (10^6/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 [b]
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[b]
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[b]	52.2 ± 0.2[b]	51.3 ± 0.2[c]	46.8 ± 1.0[c]
Mean cell hemoglobin (pg)	17.3 ± 0.2	17.2 ± 0.1	17.1 ± 0.1	17.1 ± 0.02	16.5 ± 0.2[c]	13.4 ± 0.4[c]
Female Rats						
Number	10	10	9	10	10	6
Erythrocytes (10^6/µL)	8.0 ± 0.1	7.8 ± 0.1	8.2 ± 0.2	8.3 ± 0.1	8.6 ± 0.1[b]	12.5 ± 0.34[c]
Reticulocytes (10^6/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[c]
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[c]
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[c]
Mean cell volume (fL)	56.9 ± 0.1	56.9 ± 0.1	56.6 ± 0.1	55.8 ± 0.1[c]	55.0 ± 0.2[c]	48.7 ± 0.6[c]
Mean cell hemoglobin (pg)	19.3 ± 0.2	19.3 ± 0.2	19.0 ± 0.2	18.7 ± 0.2[c]	18.5 ± 0.2[c]	14.6 ± 0.3[c]

[a]Values are means ± standard error
[b]Significantly different from control ($p \leq 0.05$)
[c]Significantly different from control ($p \leq 0.01$)

Source: NTP (2002).

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. Occasional 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^3, but not to 16 mg/m^3, and reduced the number of cycling females in surviving rats in the 16 mg/m^3 group (percent reduction not reported) (NTP, 2002).

Complete histopathological assessments were performed on rats exposed to 0, 8, and 16 mg/m^3 for 3 months; only nonneoplastic lesions of the lung and nose were 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^3 for 3 months are summarized in Table 4-5. 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^3 or greater, compared to controls. Epithelial hyperplasia occurred in the distal airways and associated alveolar ducts and alveoli. Inflammation and fibrosis were significantly increased in males (2 mg/m^3 or greater) and females (4 mg/m^3 or greater). In the nasal compartment, incidences of hyperplasia and metaplasia of the respiratory epithelium were significantly increased in males exposed to 8 or 16 mg/m^3 and in females exposed to 4 mg/m^3 or greater. Nasal hyperplasia and metaplasia was localized to respiratory epithelium on 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. Nasal inflammation was significantly increased in males and females exposed to 16 mg/m^3.

Cardiopulmonary assessments were conducted in groups of 4-10 male and female rats exposed to 0, 4, 8, and 16 mg/m^3 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^3. Decreased heart rate and systolic, diastolic, and mean blood pressure observed in male and female rats exposed to 16 mg/m^3 were considered to be a reflection of the poor condition of the animals, and complicated by 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 at all concentrations of vanadium pentoxide-exposed male and female rats. Observed changes in impaired capacity to diffuse carbon monoxide and reduced static and dynamic lung volumes at exposure concentrations of 4 mg/m^3 and greater suggest a restrictive lesion. Changes in forced expiratory maneuvers in rats exposed to 16 mg/m^3 suggest the presence of an obstructive disease.

Table 4-5. Incidences of selected nonneoplastic lesions of the lung and nose in rats (F344/N) exposed to vanadium pentoxide by inhalation for 3 months

Endpoint	Numbers of Animals with Lesions (Avg. Severity Score)					
	Control	1 mg/m^3	2 mg/m^3	4 mg/m^3	8 mg/m^3	16 mg/m^3
Male Rats[a]						
Lung						
Epithelium, hyperplasia	0	0	10[b] (2.0)	10[b] (3.0)	10[b] (3.6)	10[b] (3.3)
Inflammation	0	0	9[b] (1.0)	10[b] (1.0)	10[b] (1.6)	10[b] (2.1)
Fibrosis	0	0	2 (1.0)	10[b] (1.9)	10[b] (3.2)	10[b] (3.1)
Bronchiole, exudates	0	0	0	0	7[b] (1.0)	8[b] (1.4)
Nose						
Epithelium, hyperplasia	0	0	0	1 (1.0)	10[b] (1.2)	10[b] (2.0)
Epithelium, squamous metaplasia	0	0	0	1 (1.0)	10[b] (1.2)	10[b] (1.8)
Inflammation	0	0	0	0	0	7[b] (1.6)
Female Rats[a]						
Lung						
Epithelium, hyperplasia	0	0	10[b] (1.3)	10[b] (2.9)	10[b] (3.5)	10[b] (3.2)
Inflammation	0	0	0	10[b] (1.0)	10[b] (1.9)	10[b] (1.2)
Fibrosis	0	0	0	10[b] (1.0)	10[b] (2.9)	10[b] (3.2)
Bronchiole, exudates	0	0	0	0	10[b] (1.0)	8[b] (1.1)
Nose						
Epithelium, hyperplasia	0	0	0	10[b] (1.0)	10[b] (1.8)	10[b] (2.7)
Epithelium, squamous metaplasia	0	0	0	8[b] (1.0)	10[b] (1.8)	10[b] (2.8)
Inflammation	0	0	0	0	1 (1.0)	9[b] (1.6)

[a]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
[b]Significantly different from control by Fisher exact test ($p \leq 0.01$)

Source: NTP (2002).

Pulmonary function results might indicate obstructive disease or could reflect the deteriorating condition of the 16 mg/m^3 rats, given that histopathological finding in lungs of rats exposed to 8 and 16 mg/m^3 were similar. Taken together, results of pulmonary function tests (PFTs) indicate that a presence of restrictive injury in male and female rats exposed to concentrations of 4 mg/m^3 or greater, while an obstructive lung injury could have been present in rats exposed to 16 mg/m^3.

Bronchoalveolar lavage (BAL) fluid was analyzed for markers of pulmonary inflammation in rats exposed to 0, 4, 8 and 16 mg/m^3 for 3 months (NTP, 2002). Concentration-related increases were observed in the total numbers of cells, lymphocytes,

neutrophils, and protein recovered in BAL fluid from rats exposed to vanadium pentoxide at concentrations of 4 and 8 mg/m^3, demonstrating a pulmonary inflammatory response in male and female rats. These endpoints also were increased in the 16 mg/m^3 group, but to a lesser extent, and vanadium pentoxide was overtly toxic 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^3 and greater in males and 4 mg/m^3 and greater in females. Absolute and relative lung weights were significantly increased compared to controls at concentrations of 4 mg/m^3 and greater in females and 2 mg/m^3 and greater and 4 mg/m^3 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^3 and greater and 4 mg/m^3 and greater, respectively, and the incidence of nonneoplastic lesions of the lung was increased in male and female rats at 2 mg/m^3 and greater. Results of PFTs consistent with restrictive lung disease were observed at concentrations of 4 mg/m^3 and greater. Based on decreased erythrocyte size in male rats and nonneoplastic lung lesions and increased lung weight in male (epithelial hyperplasia and inflammation of the lung) and female (epithelial hyperplasia) rats, the NOAEL and LOAEL values identified for 3-month inhalation exposure to vanadium pentoxide aerosols were 1 and 2 mg/m^3, respectively.

NTP conducted a 3-month exposure study in B6C3F$_1$ mice 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 vanadium pentoxide aerosols at concentrations of 0, 1, 2, 4, 8, or 16 mg/m^3, 6 hours per day, 5 days per week for 3 months. Particle size given in mass median aerodynamic diameter (MMAD) ± geometric standard deviation (GSD) for each dose group was as follows: 1 mg/m^3 = 1.2 ± 2.8; 2 mg/m^3 = 1.1 ± 2.8; 4 mg/m^3 = 1.2 ± 2.8; 8 mg/m^3 = 1.0 ± 2.9; 16 mg/m^3 = 1.2 ± 2.8. Clinical findings were recorded weekly. Animals were weighed weekly and at the end of the study. All study animals were necropsied. Histopathological examinations of lungs were performed in all mice in the 0, 1, 2, 4, 8, or 16 mg/m^3 groups and of thymus in all mice in the 0, 8, or 16 mg/m^3 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^3. Complete histopathological examination was performed in mice in the control and 16 mg/m^3 groups. 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^3 group died before the end of the study. Other than appearing thin, 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 4-6. 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^3 and in females exposed to 4 (11% decrease), 8 (10% decrease) and 16 (12% decrease) mg/m^3. Absolute lung weights were significantly increased compared to control at concentrations of 2 mg/m^3 and higher in males and 4 mg/m^3 and higher in females. Relative lung weights were significantly greater than the control in males exposed to 4 (33% increase), 8 (43% increase), or 16 (82% increase) mg/m^3 and in females exposed to 4 (62% increase), 8 (63% increase), or 16 (101% increase) mg/m^3. 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^3 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^3 for 3 months are summarized in Table 4-7 (NTP, 2002). Epithelial hyperplasia was observed in male and female mice exposed to concentrations of 2 mg/m^3 and above; lesion severity increased with increasing exposure concentration. Hyperplasia involved alveolar and, to a lesser extent, bronchiolar epithelium. Inflammation was characterized by multiple foci of a mixed cellular infiltrate oriented around blood vessels and bronchioles and was observed in male mice exposed to 4 mg/m^3 and above and in female mice exposed to 2 mg/m^3 and above. Infiltrate was composed primarily of macrophages with abundant cytoplasm and fewer lymphocytes and neutrophils. Histopathological evaluations ofthe thymus of male and female mice exposed to 0, 8, and 16 mg/m^3 for 3 months showed lymphoid depletion in mice exposed to 16 mg/m^3 (males: control, 0/9; 8 mg/m^3, 0/8; 16 mg/m^3, 2/7; females: 0/9, 0/9, 1/10).The lung was identified as the most sensitive target organ in the 3-month inhalation study in mice (NTP, 2002). Based on increases in absolute lung weights at concentrations of 2 mg/m^3 and greater (males) and hyperplasia of the respiratory epithelium at concentrations of 2 mg/m^3 and greater (males and females), NOAEL and LOAEL values were identified as 1 and 2 mg/m^3, respectively.

In a study using cynomolgus monkeys, weekly provocation challenges (single 6-hour exposures to 0.5 or 3.0 mg/m^3) with inhaled vanadium pentoxide aerosol for 6 weeks produced statistically significant pulmonary responses, prior to a subchronic exposure (6 hours per day, 5 days per week for 26 weeks) (Knecht et al., 1992). The subchronic exposure was divided into three groups; one group (n = 8) was exposed to filtered, conditioned air and two exposed groups(n = 8 each) received nominally equal weekly vanadium pentoxide exposures (concentration × time) with different exposure profiles. The peak exposure group received an actual concentration of 0.16 ± 0.01 mg/m^3 (0.1 mg/m^3 nominal) vanadium pentoxide on Mondays, Wednesdays, and Fridays and 1.38 ± 0.07 mg/m^3 (1.1 mg/m^3 nominal) vanadium pentoxide on Tuesdays and Thursdays.

Table 4-6. Body weight gain and lung weights in mice ($B6C3F_1$) exposed to vanadium pentoxide by inhalation for 3 months (values are means ± standard error)

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

[a]Significantly different from control by William's test ($p \leq 0.05$)
[b]Significantly different from control by William's test ($p \leq 0.01$)

Source: NTP (2002).

Table 4-7. Incidences of selected nonneoplastic lesions of the lung in mice ($B6C3F_1$) exposed to vanadium pentoxide by inhalation for 3 months

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

[a]Numbers in parentheses indicate average severity grade of lesions in affected animals: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked
[b]Significantly different from control by Fisher exact test, $p \leq 0.05$
[c]Significantly different from control by Fisher exact test, $p \leq 0.01$

Source: NTP (2002).

The constant exposure group received a constant daily actual concentration of 0.57 ± 0.03 mg/m^3 (0.5 mg/m^3 nominal). The constant exposure regimen corresponded to a continuous

exposure of 0.10 mg/m^3 after adjusting for exposure protocol (0.57 mg/m^3 × 6/24 × 5/7). The peak exposure regimen averaged to a slightly higher continuous exposure of 0.12 mg/m^3 after adjusting for exposure protocol. 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 µm (MMAD), with a GSD of 3.25 µm. Particle sizes (MMAD ± GSD) for the peak exposure group were 3.17 ± 2.48 and 3.10 ± 2.45 for the 0.1 mg/m^3 and 1.1 mg/m^3 exposures, respectively. PFTs, cytological and immunological analyses of blood and bronchiolar lavage fluid, and skin sensitivity tests were conducted before the pre- and postexposure provocation challenges. Immunological analyses are described in Appendix D. PFTs and bronchiolar lavage fluid analyses were also performed 1 day after the provocation challenges. Cytological endpoints included complete and differential blood cell counts and leukotriene C_4 levels. Pulmonary function endpoints included total pulmonary resistance (RL), forced expiratory flow (FEF), FVC, residual volume (RV), and dynamic lung compliance (CL_{dyn}). Respiratory distress, characterized by audible wheezing and coughing, occurred in three of eight 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 or 4 hours of exposure and occasionally required early removal of the affected monkeys from the exposure chamber. Impaired pulmonary function accompanied preexposure provocation challenges with vanadium pentoxide at 3.0 mg/m^3 and was characterized by a 14% increase in RL and 13% decrease in FEV_{50}/FVC accompanied by a 14% increase in RV and 3% decrease in FVC. 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 might be attributed to the development of tolerance. The study establishes a subchronic $NOAEL_{[ADJ]}$ of 0.10 mg/m^3 (continuous exposure) for pulmonary function. No subchronic LOAEL was established. An apparent acute, but reversible, LOAEL of 1.38 mg/m^3, however, is established based on the respiratory distress observed at 1.38 mg/m^3 at an early time point.

Hematological effects of vanadium pentoxide were assessed in male CD-1 mice that were exposed by whole-body inhalation for 1 hour per day, 2 days per week for up to 12 weeks (González-Villalva et al., 2006). A 0.02 M aqueous solution of vanadium pentoxide was aerosolized, generating a reported average vanadium of 1,436 µg/m^3 (1.44 mg/m^3 vanadium), as measured by filters following the 12-week exposure. This study did not provide reliable exposure information, thus exposure concentrations in mg/m^3 could not be determined. Groups of eight exposed mice and eight 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 312; counts increased from week 3 to a maximum at week 9 and subsequently declined, but 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 2.56 mg/m^3. A continuous exposure equivalent concentration cannot be estimated with any confidence, as the intermittency of the exposure protocol is extreme.

4.2.2.2. *Chronic Studies*

The toxicity of chronic inhalation exposure to particulate aerosols of vanadium pentoxide was assessed in groups of 50 male and 50 female F344/N rats exposed (whole body exposure) at concentrations of 0, 0.5, 1, or 2 mg/m^3 for 6 hours per day, 5 days per week for 104 weeks (Ress et al., 2003; NTP, 2002). Particle MMAD ± GSD for each dose group was reported as follows: 1 mg/m^3 = 1.3 ± 2.9; 2 mg/m^3 = 1.2 ± 2.9; 4 mg/m^3 = 1.2 ± 2.9. Body weights and clinical findings were recorded throughout the exposure period. Necropsy and comprehensive histopathological evaluation[7] were performed on all animals. No clinical findings related to vanadium pentoxide exposure were observed. Mean body weights of females exposed to 2 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 percent survival of male and female rats for the entire 104-week exposure period ranged from 52 to 58% in male rats and 30 to 40% in female rats. The percent survival of controls was 40% for male rats and 28% for female rats (Table 4-8). No infection was reported in the study that might account for these survival rates but the rates are comparable to historical rates of survival for male and female F344/N rats.

[7]In addition to gross lesions and tissue masses, the following tissues were examined in the 2-year bioassay: adrenal gland, bone with marrow, brain, clitoral gland, esophagus, gallbladder (mice only), heart and aorta, large intestine (cecum, colon and rectum), small intestine (duodenum, jejunum, and ileum), kidney, larynx, liver, lung and mainstream bronchi, lymph nodes (mandibular, mediastinal, mesenteric, and bronchial), mammary gland (except mail mice), nose, ovary, pancreas, parathyroid gland, pituitary gland, preputial gland, prostate gland, salivary gland, skin, spleen, stomach (forestomach and glandular), testis (with epididymis and seminal vesicle), thymus, thyroid gland, trachea, urinary bladder, and uterus.

Table 4-8. Selected nonneoplastic lesions of the respiratory system in F344/N rats exposed to particulate aerosols of vanadium pentoxide for 2 years

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

Table 4-8. Selected nonneoplastic lesions of the respiratory system in F344/N rats exposed to particulate aerosols of vanadium pentoxide for 2 years

Endpoint[a]	Exposure Group			
	Control	0.5 mg/m^3	1 mg/m^3	2 mg/m^3
Nose				
Number of animals examined	50	50	50	50
Goblet cell, hyperplasia	13 (2.0)	19 (2.0)	16 (1.9)	30[b] (2.0)

[a]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
[b]Significantly different from control by the Poly-3 test ($p \leq 0.01$)
[c]Significantly different from control by the Poly-3 test ($p \leq 0.05$)
[d]Nonneoplastic lesions observed at time of sacrifice; percent survival consistent with historical controls for F344 rats in NTP studies.
Source: NTP (2002).

The incidences of nonneoplastic lesions of the respiratory tract in male and female rats are summarized in Table 4-8 (Ress et al., 2003; NTP, 2002). In male rats, the incidences of nonneoplastic lesions of the lungs (alveolar and bronchiolar epithelial hyperplasia and alveolar histiocyte infiltration), larynx (inflammation and epiglottis degeneration, hyperplasia and squamous metaplasia), and nose (goblet cell hyperplasia) were significantly increased compared to controls 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 the 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. A LOAEL of 0.5 mg/m^3 was established for nonneoplastic lesions of the respiratory tract in male and female rats; a NOAEL was not identified.

The NTP (2002) and Ress et al. (2003) studies also conducted analysis of neoplasms in rats exposed to vanadium pentoxide by inhalation for 2 years, using the protocol described above. Compared to concurrent controls, the incidences of alveolar/bronchiolar adenoma, alveolar/bronchiolar carcinoma or combined alveolar/bronchiolar adenoma, or carcinoma were not significantly different (Poly-3 test) for male or female rats Table 4-9. Compared to historical controls,[8] alveolar/bronchiolar adenoma in 0.5 and 2 mg/m^3 males (n = 8 and 6, respectively) and 0.5 mg/m^3 females (n = 3), alveolar/bronchiolar carcinoma in 0.5 and 2 mg/m^3 males (n = 3 and 3, respectively), and combined alveolar/bronchiolar carcinoma in 0.5, 1, and 2 mg/m^3 males

[8]The historical control databases were different for male and female F344/N rats. Historical control male F344/N rats were fed the NTP-2000 diet, while the historical control female F344/N rats were fed the NIH-07 diet and included a larger database.

(n = 10, 6 and 9, respectively) and in 0.5 mg/m^3 females (n = 3) were statistically significantly different (see Table 4-9 footnotes).

Table 4-9. Incidences of respiratory tumors in rats exposed to vanadium pentoxide in a 2-year inhalation study[a]

Tumor Type	Exposure Group				
	Historical Control	Control	0.5 mg/m^3	1 mg/m^3	2 mg/m^3
Male Rats					
Number of animals examined	1054	50	49	48	50
Alveolar/bronchiolar adenoma[b]	18 (10%)	4 (8%)	8 (16%)[c]	5 (10%)	6 (12%)[c]
Alveolar/bronchiolar carcinoma[d]	8 (4%)	0 (0%)	3 (6%)[c]	1 (2%)	3 (6%)[c]
Alveolar/bronchiolar adenoma or carcinoma[e]	26 (10%)	4 (8%)	10 (20%)[c]	6 (12%)[c]	9 (18%)[c]
Female Rats					
Number of animals examined	1050	49	49	50	50
Alveolar/bronchiolar adenoma[f]	12 (4%)	0 (0%)	3 (6%)[c]	1 (2%)	0 (0%)
Alveolar/bronchiolar carcinoma	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (2%)
Alveolar/bronchiolar adenoma or carcinoma[g]	14 (4%)	0 (0%)	3 (6%)[c]	1 (2%)	1 (2%)

[a]Numbers in parentheses indicate percent incidence; particle size mass mean aerodynamic diameter ± geometric standard deviation (MMAD ± GSD): 0.5 mg/m^3 = 1.2 ± 2.9; 1 mg/m^3 = 1.2 ± 2.9; 2 mg/m^3 = 1.3 ± 2.9.
[b]Historical incidence of alveolar/bronchiolar adenoma male F344/N rats fed in inhalation chamber controls given NTP-2000 diet.
[c]Incidence exceeds historical control (statistical comparison between NTP (2002) data and historical data not conducted).
[d]Historical incidence of alveolar/bronchiolar carcinoma of male F344/N rats fed in inhalation chamber controls given NTP-2000 diet.
[e]Historical incidence of combined alveolar/bronchiolar adenoma or carcinoma male F344/N rats fed in inhalation chamber controls given NTP-2000 diet.
[f]Historical incidence of alveolar/bronchiolar adenoma female F344/N rats fed in inhalation chamber controls given NIH-07 diet.
[g]Historical incidence of combined alveolar/bronchiolar adenoma or carcinoma female F344/N rats fed in inhalation chamber controls given NIH-07 diet.

Source: NTP (2002).

NTP (2002) and Ress et al. (2003) concluded that exposure to vanadium pentoxide caused alveolar and bronchiolar adenomas and carcinomas in male rats because incidence exceeded historical controls. The marginal increase in lung neoplasms observed in female rats was statistically significant only in the 0.5 mg/m^3 exposure group. This increase was not definitively attributed to vanadium pentoxide exposure because the tumors were observed only at the lowest dose and no dose response was evident.

NTP (2002) and Ress et al. (2003) also reported the toxicity of chronic exposure to vanadium pentoxide in mice. Groups of 50 male and 50 female B6C3F$_1$ mice were exposed (whole-body exposure) to vanadium pentoxide particulate aerosol concentrations of 0, 1, 2, or

4 mg/m^3, 6 hours per day, 5 days per week, for 104 weeks (Ress et al., 2003; NTP, 2002). Particle MMAD ± GSD for each dose group was reported as follows: 1 mg/m^3 = 1.3 ± 2.9; 2 mg/m^3 = 1.2 ± 2.9; 4 mg/m^3 = 1.2 ± 2.9. Body weights and clinical findings were recorded throughout the exposure period. Necropsy and comprehensive histopathological evaluation were performed on all animals and analysis of both nonneoplastic and neoplastic lesions was performed. Many mice exposed to vanadium pentoxide were thin and exhibited abnormal breathing, particularly those exposed to 2 or 4 mg/m^3 vanadium pentoxide (specific incidence data not reported). Mean body weights were generally less than control in males exposed to 4 mg/m^3 (decreases of 5-15%) and in females for all exposure groups (1 mg/m^3, decreases of 4-10%; 2 mg/m^3, decreases of 14-20%; and 4 mg/m^3, decreases of 4-19%) (Statistical significance was not reported). The number of mice surviving for the entire 104-week exposure period was similar to control (78% for male mice and 76% for female mice) for all exposure groups for female mice and for males in the 1 and 2 mg/m^3 groups, but survival was significantly decreased in male mice exposed to 4 mg/m^3 (50% survival rate) (Table 4-10).

Table 4-10. Selected nonneoplastic lesions of the respiratory system in B6C3F$_1$ mice exposed to vanadium pentoxide in a 2-year inhalation study

Endpoint[a]	Exposure Group			
	Control	1 mg/m^3	2 mg/m^3	4 mg/m^3
Male Mice				
Percent survival	78	66	72	50[b]
Lung				
Number of animals examined	50	50	50	50
Alveolar epithelium, hyperplasia	3 (3.0)	41[c] (2.2)	49[c] (3.3)	50[c] (3.9)
Bronchiole epithelium, hyperplasia	0	15[c] (1.0)	37[c] (1.1)	46[c] (1.7)
Inflammation, chronic	6 (1.5)	42[c] (1.5)	45[c] (1.6)	47[c] (2.0)
Alveolus, histiocyte infiltration	10 (2.4)	36[c] (2.4)	45[c] (2.6)	49[c] (3.0)
Interstitial, fibrosis	1 (1.0)	6 (1.7)	9[c] (1.2)	12[c] (1.7)
Larynx				
Number of animals examined	49	50	48	50
Epiglottis epithelium, squamous metaplasia	2 (1.0)	45[c] (1.0)	41[c] (1.0)	41[c] (1.0)
Nose				
Number of animals examined	50	50	50	50
Inflammation, suppurative	16 (1.3)	11 (1.4)	32[c] (1.2)	23[b] (1.3)
Olfactory epithelium, atrophy	6 (1.0)	7 (1.6)	9 (1.3)	12 (1.2)
Olfactory epithelium, degeneration	1 (1.0)	7[b] (1.0)	23[b] (1.1)	30[c] (1.2)
Respiratory epithelium, degeneration	8 (1.1)	22[c] (1.0)	38[c] (1.2)	41[c] (1.4)
Bronchial Lymph Node				
Number of animals examined	40	38	36	40
Hyperplasia	7 (2.1)	7 (2.4)	12 (2.1)	13 (2.2)
Female Mice				
Percent survival	76	64	60	64
Lung				
Number of animals examined	50	50	50	50
Alveolar epithelium, hyperplasia	0	31[c] (1.6)	38[c] (2.0)	50[c] (3.3)
Bronchiole epithelium, hyperplasia	0	12[c] (1.0)	34[c] (1.0)	48[c] (1.5)
Inflammation, chronic	0	37[c] (1.3)	39[c] (1.8)	49[c] (2.0)
Alveolus, histiocyte infiltration	4 (1.0)	34[c] (2.4)	35[c] (2.4)	45[c] (2.7)

Table 4-10. Selected nonneoplastic lesions of the respiratory system in B6C3F$_1$ mice exposed to vanadium pentoxide in a 2-year inhalation study

Endpoint[a]	Exposure Group			
	Control	1 mg/m^3	2 mg/m^3	4 mg/m^3
Interstitial, fibrosis	0	1 (2.0)	4[b] (2.5)	8[c] (1.5)
Larynx				
Number of animals examined	50	50	49	50
Epiglottis epithelium, squamous metaplasia	0	39[c] (1.0)	45[c] (1.0)	44[c] (1.1)
Nose				
Number of animals examined	50	50	50	50
Inflammation, suppurative	19 (1.1)	14 (1.2)	32[c] (1.2)	30[c] (1.3)
Olfactory epithelium, atrophy	2 (1.5)	8[b] (1.3)	5 (1.0)	14[c] (1.3)
Olfactory epithelium, degeneration	11 (1.2)	23[c] (1.0)	34[c] (1.2)	48[c] (1.3)
Respiratory epithelium, degeneration	35 (1.3)	39 (1.5)	46[c] (1.7)	50[c] (1.8)
Bronchial Lymph Node (Number of animals examined)	39	40	45	41
Hyperplasia	3 (2.0)	13[c] (1.8)	14[c] (2.3)	20[c] (2.3)

[a]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
[b]Significantly different from control by the Poly-3 test ($p \leq 0.05$)
[c]Significantly different from control by the Poly-3 test ($p \leq 0.01$)

Source: NTP (2002).

The incidences of nonneoplastic lesions of the respiratory tract in male and female mice are summarized in Table 4-10 (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, and alveolus histiocyte infiltration), larynx (squamous metaplasia of the epiglottis), and nose (olfactory and respiratory epithelium degeneration) were significantly increased compared to control in all vanadium pentoxide exposure groups. In female mice, the incidences of nonneoplastic lesions of the lungs (hyperplasia of the alveolar and bronchiole epithelium, inflammation, and alveolus histiocyte infiltration), larynx (squamous metaplasia of the epiglottis), and nose (olfactory epithelium atrophy and degeneration and hyperplasia) were significantly increased compared to control in all vanadium pentoxide exposure groups. Incidences of interstitial fibrosis were significantly increased in male and female mice exposed to 2 or 4 mg/m^3. In general, the incidences and severity ratings of lesions increased with exposure level and matched the types of lesions observed in rats. No treatment-related histopathological findings were observed in other tissues. The LOAEL of 1 mg/m^3 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 4-11 (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 treatment groups and alveolar/bronchiolar adenoma was significantly increased in the 2 mg/m^3 group.

Table 4-11. Incidences of respiratory tumors in B6C3F$_1$ mice exposed to vanadium pentoxide in the 2-year inhalation study

Tumor Type[a]	Exposure Group				
	Historical Control	Control	1 mg/m^3	2 mg/m^3	4 mg/m^3
Male Mice					
Number of animals examined	1071	50	50	50	50
Alveolar/bronchiolar adenoma[b]	201(19%)	13 (26%)	16 (32%)	26[c] (53%)	15 (30%)
Alveolar/bronchiolar carcinoma	97(9%)	12 (24%)	29[c] (58%)	30[c] (60%)	35[c] (70%)
Alveolar/bronchiolar adenoma or carcinoma	285 (26.8%)	22 (28%)	42[c] (84%)	43[c] (86%)	43[c] (86)
Female Mice					
Number of animals examined	1075	50	50	50	50
Alveolar/bronchiolar adenoma	67 (6.3%)	1 (2%)	17[c] (34%)	23[c] (46%)	19[c] (38%)
Alveolar/bronchiolar carcinoma	43 (–3.9%)	0 (0%)	23[c] (46%)	18[c] (36%)	22[c] (44%)
Alveolar/bronchiolar adenoma or carcinoma	109 (–10.1%)	1 (2%)	32[c] (64%)	35[c] (70%)	32[c] (64%)

[a]Number of animals with tumor; numbers in parentheses indicate percent incidence; particle size mass mean aerodynamic diameter + geometric standard deviation (MMAD ± GSD): 1 mg/m^3= 1.3 ± 2.9; 2 mg/m^3 = 1.2 ± 2.9; 4 mg/m^3 = 1.2 ± 2.9.
[b]Historical incidence of alveolar/bronchiolar adenoma male B6C3F$_1$ mice fed in inhalation chamber controls given NIH-07 diet.
[c]Significantly different from control by the Poly-3 test ($p \leq 0.01$).

Source: NTP (2002).

A recent study has examined the effect of vanadium pentoxide exposure by aspiration (Rondini et al., 2010). Rondini et al. (2010) examined the induction of pulmonary inflammation and tumor promotion in three different mice strains (A/J, BALB/c, and C57BL/6J) following oropharyngeal aspiration exposure. This study was designed to test the hypothesis that vanadium pentoxide acts as a tumor promoter in exposed rodents. Three mouse strains were used to further understand potential susceptibility to these effects. These particular mouse strains were selected because of their known differential susceptibility to chronic pulmonary inflammation and carcinogenesis: A/J mice are sensitive, BALB/C are intermediate, and C57BL/6J are resistant. The experiment was designed to measure vanadium pentoxide tumor promotion following tumor initiation by 3-methylcholanthrene (MCA). All experimental mice were exposed to MCA (10 μg/g bw in corn oil; intraperitoneal injection) in week 1, followed by 5 weekly aspirations of either vanadium pentoxide (4 mg/kg) or PBS. Tumor incidence was measured at 20 weeks post-MCA exposure. Statistically significant lung tumor increases were observed in A/J and BALB/C mice as compared to the MCA-treated control ($p \leq 0.05$; Table 4-12). Differences were also observed between strains, with A/J mice showing increased tumorigenicity in response to

vanadium pentoxide. In the absence of MCA, vanadium pentoxide was not sufficient to initiate tumorigenesis in this study. C57BL/6J had no tumors following exposure (data not shown). To evaluate the strain differences in inflammation, mice were aspirated with vanadium pentoxide (4 mg/kg bw) four times weekly, with BALF collected at 6 hours, 1 days, 3 days, 6 days, and 21 days following the last aspiration. Cellular infiltrates and protein content were compared, and lungs were snap-frozen for histopathology. Increased pulmonary inflammation and hyperpermeability was increased in all exposed strains in a pattern similar to that of tumor induction, with greater increases observed in A/J mice than in BALB/C. C57BL/6J showed the smallest increases in all parameters compared to all mouse strains. All results returned to baseline at 21 days postexposure. The results of PMN increases were confirmed by histopathology in A/J and C57BL/6J mice. Further analysis was performed to measure inflammatory chemokine production (KC, MIP-2, MCP-1) and transcription factor activity (NFkB, c-Fos) and signaling pathway activation (MAPK). Like the increased inflammatory markers above, increased levels of KC and MCP-1 were observed in A/J and BALB/C mice as compared to the C57BL/6J mice. Similar strain differences were observed for the transcription activity of NFkB and c-Fos and the MAPK signaling activity in A/J mice as compared to C57BL/6J (BALB/C were not analyzed).

Table 4-12. Lung tumor multiplicity in MCA-treated mice exposed to vanadium pentoxide by pharyngeal aspiration (Rondini et al., 2010) [a,b]

	PBS Control	Vanadium Pentoxide
A/J	3.3 ± 0.75 (n = 4)	10 ± 1.4 (n = 15)
BALB/C	0.78 ± 0.28 (n = 8)	2.2 ± 0.36 (n = 12)

[a]No tumors were observed in C57BL/6J mice.
[b]Number of animals for each treatment in parentheses.

Source: Rondini et al. (2010).

In summary, the identified noncancer health effects following occupational exposure via inhalation to vanadium pentoxide in humans include respiratory irritation, cough, and bronchitis; inhalation exposure in animals results in multiple health effects, including pulmonary inflammation, lung and nasal hyperplasia, and pulmonary fibrosis. Vanadium pentoxide inhalation exposure for 2 years was associated with a wide spectrum of nonneoplastic pulmonary lesions in both ratsand mice, ranging from hyperplasia to inflammation, fibrosis, and metaplasia. Lesions were detected in the lung, larynx, and nose in both rats and mice exposed to vanadium pentoxide. Bronchial lymph node changes were detected in mice exposed to vanadium pentoxide. No human epidemiology studies that examined carcinogenesis following exposure to vanadium pentoxide are available. The evidence of carcinogenicity in male and female mice exposed to vanadium pentoxide is clear, however, and some evidence indicates carcinogenicity

in male rats, based on observations of alveolar and bronchiolar neoplasms that exceeded historical controls in groups exposed to vanadium pentoxide (NTP, 2002). A more recent study has also shown lung tumor promotion in sensitive mouse strains (Rondini et al., 2010).

4.3. REPRODUCTIVE/DEVELOPMENTAL STUDIES—ORAL, INHALATION, INTRAPERITONEAL AND INJECTION

4.3.1. Oral Studies

Vanadium pentoxide delivered orally to weanling rats (10–200 µmol/kg) for 3 days produced a significant increase in alkaline phosphatase activity and DNA content in the diaphysis of femoral bones, suggesting that vanadium pentoxide might be linked to bone formation in the developing rat (Yamaguchi et al., 1989). Yamaguchi et al. (1989) examined the potential effects of vanadium pentoxide on bone metabolism. Weanling male Wistar rats (n = 5 per group) were exposed to 1.8-36.4 mg/kg (10–200 µmol/kg)[9] vanadium pentoxide orally 1 hour following an oral injection of 15.3 µmol Zn/100g) three times at 24-hour intervals. The highest dose vanadium pentoxide tested (200 µmol/kg) led to death in four of nine rats (cause of death not described). Authors state that administration of zinc prevented death in co-exposed animal. All rats were killed 24 hours following the last vanadium administration, and blood was immediately removed by cardiac puncture. Statistically significant increases in calcium (27.3–36.4 mg/kg; $p < 0.05$) and decreases in phosphorus (36.4 mg/kg; $p < 0.01$) were observed in serum from high-dose rats. Administration of zinc completely prevented these serum changes. Femurs also were immediately removed. The diaphysis and epiphysis were used for alkaline phosphatase measures (right femur) and DNA content analyses (left femur). Alkaline phosphatase activity was significantly increased at the lowest dose tested (1.8 mg/kg; $p < 0.05$) and peaked at 3.6 mg/kg. Further increasing doses led to decreases in alkaline phosphatase activity. A similar pattern was observed for bone DNA content, with statistically significant increases at 1.8–18.1 mg/kg ($p < 0.05$), but decreased at higher doses. Although the authors state the interaction of vanadium and zinc led to an increase in bone calcium, based on the data presented in the figures in this publication, vanadium administration did not lead to alterations of bone calcium. Overall, this study shows an increase in both DNA content and alkaline phosphatase activity in weanling male rats following oral administration of vanadium pentoxide, suggesting a possible role of vanadium in increased bone growth.

Mravcova et al. (1993)assessed the extent of vanadium pentoxide accumulation in the bones of rats following 6-month exposure (full study description is presented in Appendix D). Vanadium accumulated in the epiphyseal cartilage of the tibia in rats. Significantly higher concentrations of vanadium accumulated in the tibia and incisors of weanling rats compared to adults. No dose response data for these endpoints were reported.

[9]Conversion factor: µmol = 0.1819 mg

4.3.2. Inhalation Studies

Investigations of the reproductive and developmental toxicity of subchronic or chronic inhalation exposure to vanadium pentoxide are limited to two studies. Mussali-Galante et al. (2005), used immunohistochemistry to assess the amount of gamma tubulin accumulating within somatic and testicular germ cells. Mussali-Galante et al. (2005) exposed 60 male CD-1 mice to inhaled vanadium pentoxide (0.02 M, apparently aqueous solution containing aerosolized vanadium pentoxide) for 1 hour twice a week for 12 weeks. Avila-Costa et al. (2004), investigators from the same laboratory, used the same protocol and reported that droplets of the vanadium pentoxide mixture had average diameters of 0.5–5 µm. Thirty-six control animals inhaled only vehicle (deionized water). Groups of three exposed animals and three control animals were sacrificed each week for 12 weeks. Results indicated accumulation of vanadium pentoxide in testes (Mussali-Galante et al., 2005). Gamma tubulin was significantly decreased in testicular samples exposed to vanadium pentoxide compared to control, starting after the first week of exposure. Changes in gamma tubulin suggest changes in microtubule-involved function, such as cell division, which can affect spermatogenesis. Responses were duration dependent, with the lowest percentages of immunoreactive cells occurring at the end of the 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) (Mussali-Galante et al., 2005).

Fortoul et al. (2007) analyzed testes for ultrastructural changes, testosterone concentration, and vanadium tissue concentration, using the same protocol as that reported above (Mussali-Galante et al., 2005). No overt toxicity or changes in body or testicular weight were observed. Histopathological analysis revealed necrotic cell death in spermatocytes (25%) and Sertoli cells (15%) at weeks 5 and 6 in vanadium-exposed animals. Spermatocytes exhibited cytoplasmic vacuolation, nuclear distortion, and intercellular edema in response to vanadium pentoxide exposure. Spermatogonia (40% necrosis during weeks 6 and 7) were the most susceptible cell type, followed by spermatocytes and Sertoli cells. Moreover, vanadium pentoxide concentrations increased dramatically after 1 week of exposure and remained consistently elevated (average concentration 0.05 µg/g dry tissue in controls, 1.63 µg/g dry tissue in exposed animals, 33 times higher). Concentrations of testosterone were highly variable and not statistically significant.

Vanadium pentoxide exposure did not affect reproductive endpoints in male rats (sperm count, spermatid heads, and sperm motility). It did, however, increase estrous cycle length by 10% in female rats exposed to 8 mg/m^3, but not to 16 mg/m^3, and reduced the number of cycling females in surviving rats in the 16 mg/m^3 group (percent reduction not reported). The epididymal spermatozoal motility of male mice exposed to 8 or 16 mg/m^3 was significantly decreased by 13 and 5%, respectively. No treatment-related effects were observed for assessments of estrous cycle in female mice (estrous cycle length and number of cycling females) (NTP, 2002).

4.3.3. Intraperitoneal and Injection Studies

Male and female reproductive endpoints were evaluated in young rats following intraperitoneal (i.p.) 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 other day from birth to age 21 days; groups sizes were five (treated males) or nine (male and female controls and treated females). Males were sacrificed at 55 days of age and 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 testes, 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 evaluated 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; the effects consisted of statistically significant increases in body weight (14.3% higher than controls) and increased weights of thymus, submandibular gland, and liver (31.1, 15.8 and 28.4% above control weights, respectively).

Fertility and sperm assessments were also performed in CD-1 mice following i.p. 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 three days for 60 days and were mated 24 hours after the last injection. Statistically significant effects in the treated group included reduced fertility rate in males (33% compared to 85% in controls), reduced numbers of implantation sites (average = 5.8 compared to 10.88 in controls), reduced numbers of live fetuses (3.4 compared to 10.53 in controls), and increased number of resorptions per dam (2.00 compared to 0.24 average resorptions in controls). In the sperm assessment, 20 males were injected with 8.5 mg/kg vanadium pentoxide every 3 days for up to 60 days with groups of five evaluated after 10, 20, 30, 40, 50, or 60 days of treatment. Statistically significant effects included reduced sperm motility on or after day 10, reduced sperm count and increased percentage of abnormal sperm after day 20, decreased absolute testicular weight after day 50 (relative weight not reported), and decreased body weight after day 60.

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 i.p. injection on days 6–15 of gestation (Altamirano-Lozano et al., 1993). No maternal toxicity was reported (endpoints not specified). Developmental toxicity endpoints were assessed on gestation day 18. Endpoints included number of implants, resorptions, and live fetuses. For all fetuses, weight,

sex, and external malformations were noted. For two-thirds of the fetuses, skeletal abnormalities were also recorded. 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 in controls), 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.

Zhang et al. (1991) evaluated the developmental toxicity in NIH mice following i.p. 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, developmental toxicity, 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. (1993b) evaluated developmental toxicity in Wistar rats following i.p. 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. (1993a) evaluated developmental toxicity in Wistar rats following i.p. 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 available. Overall, the three studies identified an i.p. developmental toxicity LOAEL for vanadium pentoxide of 1 mg/kg-day (Zhang et al., 1991a, 1993a,b).

One study examined the effects of vanadium pentoxide (1.1 mg/kg in distilled water) following tail vein injection in a total of 20 pregnant NMRI mice (data pooled from three studies with 6–10 mice each) (Wide, 1984). Injections were performed before implantation or on gestation day 3 or 8, and animals were euthanized on gestation day 17 (2 days before parturition. Preimplantation exposure had no effect on the fetuses relative to number per litter, weight, or external and internal morphology. Exposure to vanadium pentoxide on gestation day 3 or 8 did not lead to significant changes in resorption frequencies, fetal weights, or frequencies of fetal hemorrhages as compared to controls. The number of fetuses defined as having less mature

skeletons[10] by the authors, however, was significantly greater in mice given vanadium pentoxide on gestation day 8, but not gestation on gestation day 3 ($p < 0.001$, χ^2 test). Although the i.p. and tail-vein injection studies show the potential of vanadium pentoxide to cause reproductive and developmental effects in rodents, the studies are not useful for quantification of vanadium pentoxide toxicity, as equivalent oral or inhalation exposures cannot be established.

4.4. OTHER DURATION – OR ENDPOINT—SPECIFIC STUDIES

Several studies determined LD_{50} values for vanadium pentoxide following acute and short-term exposure. Among rats and mice, oral LD_{50} values ranged from 10 to 137 mg/kg body weight (Yao et al., 1986 as cited in WHO, 2001; IARC, 2006; Lewis et al., 2000). Acute inhalation studies determined an LC67 of 1,440 mg/m^3 in rats (U.S. EPA, 1992) as well as clinical signs of respiratory toxicity at 5 mg/m^3 in cynomolgus monkeys. Additionally, acute and short-term studies have evaluated immune system and nervous system toxicities. These studies are described in detail in Appendix D.

4.5. MECHANISTIC DATA AND OTHER STUDIES IN SUPPORT OF THE MODE OF ACTION FOR PULMONARY FIBROSIS AND CANCER

Noncancer effects in the lung range from histiocytic infiltration and alveolar inflammation to hyperplasia of alveolar epithelium and pulmonary fibrosis. These endpoints exist in a plausible biological response continuum—from inflammation to reparative hyperplasia to fibrosis. These effects also display a temporal and dose-response continuum, ranging from inflammatory and hyperplastic responses that occur at earlier time points (16 days) and at lower doses (2 mg/m^3) to fibrosis that occurs at later time points (3 months) and at higher doses (4 mg/m^3). Inflammation and hyperplasia are biologically relevant as precursor events to pulmonary fibrosis. Several investigators have systematically investigated the molecular mechanisms underlying vanadium pentoxide-induced pulmonary inflammation and fibrosis.

The evidence for mutagenicity in humans is limited (Ivancsits et al., 2002; Ehrlich et al., 2008; Kleinsasser et al., 2003). Few studies have examined genotoxicity in humans in vivo, with equivocal results. Studies have demonstrated a genotoxic effect of vanadium pentoxide on human cells in vitro (Ramirez et al., 1997; Rojas et al., 1996; Roldan and Altamirano, 1990). Based on these studies, vanadium pentoxide-induced mutagenicity could occur at doses higher than those measured in these occupational exposures, could be tissue specific, and could be associated with oxidative stress rather than direct DNA damage. In vitro tests in bacterial and yeast systems provide mixed evidence of vanadium pentoxide-induced mutagenicity. In general, classic gene mutation assays were negative, as were tests that assessed sister chromatid exchange

[10]No ossification of three of four elements examined (supraoccipital bone, sternum, metatarsalia, and all caudal vertebrae).

(SCE) and other chromosomal aberrations. DNA strand breaks (Ivancsits et al., 2002; Rojas et al., 1996) and micronucleus formation (Zhong et al., 1994) were indicated in some studies in cultured cells but depended on cell type. Fibroblasts appear to be more sensitive to vanadium exposure in vitro than are blood cells. Similarly, experimental data from animal studies is equivocal. NTP (2002) reported that the frequency of micronucleated normochromatic erythrocytes in peripheral blood was not increased in exposed mice compared to control mice. Several studies by Altamirano-Lozano et al. (1999; 1996; 1993), however, have noted DNA damage in specific target tissues in vanadium pentoxide-treated mice, although these studies used intraperitoneal injection as the route of exposure. These studies are described in detail in Appendix D.

4.6. SYNTHESIS OF MAJOR NONCANCER EFFECTS

4.6.1. Oral

Only one study was identified in humans exposed to vanadium pentoxide by the oral route of exposure (Kucera et al., 1992). In this study, vanadium levels in the hair and blood of children exposed to vanadium, by drinking contaminated water near a vanadium pentoxide plant, were measured. However, no exposure-response relationship could be quantified. Limited animal studies are available examining toxicity following oral exposure to vanadium pentoxide. Table 4-13 presents a summary of the noncancer results for the subchronic and chronic oral studies of vanadium pentoxide toxicity in experimental animals.

The only acute studies available report an oral LD_{50} value that ranges from ~10-137 mg/kg body weight in rats and an oral LD_{50} value of 64 mg/kg body weight in mice, depending on the source (Yao et al., 1986 as cited in WHO, 2001). Clinical signs of toxicity included lethargy, excessive tearing (lacrimation), and diarrhea. Histopathological analysis revealed liver necrosis and swelling of renal tubules. No acute dose-response studies are available for any animal species.

Data on toxicity following subchronic oral exposure to vanadium pentoxide are limited to one study (Mountain et al., 1953). The primary noncancer health effects of subchronic oral exposure in animals include changes in relative liver weight, decreased erythrocyte and hemoglobin counts, and decreased hair cystine content in rats (Mountain et al., 1953). Mountain et al., (1953) observed decreased erythrocyte count and hemoglobin counts at ≥ 16.4 mg/kg-day. These effects were correlated, so observed decreases could not be considered as separate effects. The study authors also reported increased relative liver weights, but this endpoint was only measured in the control and the high dose group (69.6 mg/kg-day) and thus no dose-response could be established. Changes in hair cystine levels were observed at doses ≥ 1.41 mg/kg-day. However, the biological significance of this effect is unknown, as changes in hair cystine content can be considered a potential biomarker of exposure, rather than an adverse effect.

Table 4-13. Summary of noncancer results of repeat-dose studies for oral exposure of experimental animals to vanadium pentoxide

Species	Gender	Avg. Daily Dose (mg/kg-d)	Exposure Duration and Route	Response at LOAEL	NOAEL (mg/kg-d)	LOAEL (mg/kg-d)	Comments	Reference
Wistar rat (n = 5 per group)	Male	0, 10.5, 16.4, 69.6, 141.0 mg/kg-d (corresponds to 0, 74.5,[a] 116.1,[b] 500, and 1,000 ppm)	103 d in food	Decreased erythrocyte count	10.5	16.4	Dietary exposure was increased at day 35 of study (from 25 to 100 and from 50 to 150 ppm) (See notes below)	Mountain et al. (1953)
		0, 10.5, 16.4, 69.6, 141.0 mg/kg-d (corresponds to 0, 74.5,[a] 116.1,[b] 500, and 1,000 ppm)	103 d in food	Decreased relative liver weight	--	69.6		
		0, 10.5, 16.4, 69.6, 141.0 mg/kg-d (corresponds to 0, 74.5,[a] 116.1,[b] 500 and 1000 ppm)	103 d in food	Decreased hair cystine	10.5	16.4		
Rat (strain and number not reported)	Male	1.41 and 14.1 mg/kg-d (corresponds to 17.9 and 179 ppm)	2.5 yr in food	Decreased hair cystine	1.41	14.1	Study published in *Patty's Industrial Hygiene and Toxicology* 3rd ed. (1981); Original data not available.	Stokinger et al. (1953)

[a] Represents an average dose based on 25 ppm for 35 days and 100 ppm for 68 days.
[b] Represents an average dose based on 50 ppm for 35 days and 150 ppm for 68 days.

Chronic animal studies evaluating effects of oral exposure to vanadium pentoxide are limited. Stokinger et al.(1953) (as cited in Clayton and Clayton, 1981) reported significantly decreased hair cystine levels (no details were provided regarding the percent decrease or dose response) in exposed rats. Comprehensive toxicity endpoints were not evaluated, and the number and strain of rats used were not reported in the study. The biological relevance of decreased hair cystine levels is unknown. The study authors suggested that decreased hair cystine could be related to poor nutritional status, as a result of an aversion to vanadium

pentoxide in the feed, rather than directly to vanadium exposure. These decreases may also reflect changes to enzymatic pathways.

4.6.2. Inhalation

Table 4-14 presents a summary of the noncancer effects observed following inhalation exposure to vanadium pentoxide in animals. Details of the available human studies are available in Section 4.1 and Table 4-1. The identified noncancer health effects following occupational exposure to vanadium pentoxide in humans include respiratory irritation, airway obstruction, chest pain, bronchitis, and similar effects. Inhalation exposure in animals results in multiple health effects, including pulmonary inflammation, lung and nasal hyplerplasia, pulmonary fibrosis, changes in nervous system structure and function, and reproductive/developmental effects. Animal studies have identified the lung as the most sensitive organ to vanadium pentoxide-induced toxicity. Other animal studies have also reported effects on the nervous system, reproductive system and the developing fetus, and immune system following inhalation exposure to vanadium pentoxide.

The primary noncancer health effects identified following acute inhalation exposure in humans are respiratory irritation, cough, and mucus formation. A human controlled exposure study (n = 9) performed by Zenz and Berg (1967) reported respiratory irritation, cough, and mucus formation in humans exposed to vanadium pentoxide for 8 hours. Levy et al.(1984) described respiratory irritation in 100 workers who were reportedly exposed to 0.05–5.3 mg/m^3 vanadium for 10 hours per day, 6 days per week, for 4 weeks. This study did not include a referent group, and exposure to vanadium pentoxide could not be directly correlated to effects. Respiratory and other symptoms have been documented among workers employed at facilities producing and processing vanadium pentoxide (Kiviluoto et al., 1981a; (Irsigler et al., 1999; Musk and Tees, 1982; Kiviluoto, 1980; Kiviluoto et al., 1979; Zenz et al., 1962; Sjoberg, 1956, 1951). Similar effects were observed in occupational studies of boilermakers involved in the construction, cleaning, and maintenance of oil-fired boilers (Kim et al., 2004; Hauser et al., 2001; Woodin et al., 2000; 1999; 1998; Hauser et al., 1995b, a; Levy et al., 1984; Ross, 1983; Lees, 1980; Sjoberg, 1955; Williams, 1952).

Observed respiratory effects in humans are supported by the available literature evaluating toxicity in animals. Specifically, male monkeys exposed to vanadium pentoxide at 0.5 or 5.0 mg/m^3 for 6 hours (Knecht et al., 1985) were shown to have restriction of air flow at high dose. WHO (2001) also reported that a 1-hour inhalation exposure to vanadium pentoxide dust in rats led to an LC_{67} of 1.44 mg/L (1,440 mg/m^3). Clinical signs of toxicity included respiratory difficulty, increased respiratory tract mucus production, and irritation of the eyes, nose, and throat. Short-term inhalation exposure in animals exposed to vanadium pentoxide for 3 months also increased pulmonary inflammation and relative lung weight in rodents (NTP, 2002).

NTP (2002) evaluated pulmonary and nasal endpoints in both male and female rats and mice after a 3-month exposure to vanadium pentoxide at 0, 1, 2, 4, 8, and 16 mg/m^3. Lung inflammation, lung hyperplasia, and increased relative lung weight were observed at the low doses in rats and mice. In addition, rats exhibited bronchiolar exudates, microcytic erythrocytosis, lung fibrosis, and nasal lesions. Body weight loss and increased absolute lung weight were reported in mice. Rats appear to be more sensitive to inhalation exposure to vanadium pentoxide than mice following subchronic exposure to vanadium pentoxide, based on the occurrence of a wider variety of nonneoplastic lesions throughout the respiratory tract.

Knecht et al. (1992) also observed that inhaled vanadium pentoxide leads to decreased pulmonary function in male monkeys exposed to vanadium pentoxide at 0.1, 0.5, or 1.1 mg/m^3 for 26 weeks (6 hours per day, 5 days per week). However, effects on pulmonary function parameters were reversible and were not replicated following subsequent challenge.

NTP (2002) also reported nonneoplastic pulmonary lesions in male and female rats and mice exposed to vanadium pentoxide via inhalation for 2 years. Specifically, increased alveolar and bronchiolar epithelial hyperplasia in male rats; alveolar histiocyte infiltration, laryngeal inflammation, and epiglottis epithelial degeneration, hyperplasia, and squamous metaplasia in male and female rats; and goblet cell hyperplasia in nasal compartments in male rats was observed. Based on these findings, the study LOAEL was 0.5 mg/m^3 based on nonneoplastic lesions of the lungs, larynx, and nose. Increased incidences of pulmonary inflammation and hyperplasia, nasal olfactory or respiratory epithelium degeneration, and epiglottis metaplasia were reported in both male and female mice. The lung fibrosis and nasal inflammation were noted at ≥2 mg/m^3 in both sexes. In general, the incidence and severity of pulmonary lesions increased with increasing dose. No treatment-related histopathological lesions were observed in other evaluated tissues. Interstitial fibrosis was significantly elevated in exposed male and female mice compared to controls at 2 and 4 mg/m^3. A LOAEL of 1.0 mg/m^3 was identified based on these nonneoplastic lesions of the lungs, larynx, and nose. Increased pulmonary irritation was also observed following pharyngeal aspiration in three mouse strains (Rondini et al., 2010).

Other effects observed following inhalation exposure to vanadium pentoxide included CNS, hematological, and male reproductive effects. Neurotoxicity also has been observed in mice following a single inhalation exposure. Avila-Costa et al. (2006) noted significant changes in brain morphology and impairment of memory in male CD-1 mice compared to controls following a single 1-hour exposure to 0.02 M (2.5 mg/m^3 vanadium) inhaled vanadium pentoxide. Selected hematologic parameters (number of erythrocytes and reticulocytes and percent hematocrit) were significantly altered in male and female rats at the highest dose level tested following subchronic exposure to vanadium pentoxide (NTP, 2002). Other subchronic studies have also reported increased platelet counts (González-Villalva et al., 2006). Reproductive effects due to vanadium pentoxide inhalation exposure included morphological

changes to spermatogonia, spermatocytes, and Sertoli cells and testicular malformation (Fortoul et al., 2007; Mussali-Galante et al., 2005; NTP, 2000). Estrous cycle length was increased in female rats but not in mice following inhalation exposure at high doses, while decreased spermatozoal motility was observed in male mice but not in male rats at high doses (NTP, 2002).

Table 4-14. Summary of the noncancer results for the subchronic and chronic inhalation exposure studies of vanadium pentoxide toxicity in experimental animals

Species (sex)	Exposure (mg/m^3)	Response at LOAEL	NOAEL (mg/m^3)	LOAEL (mg/m^3)	Comments	Reference
Male CD-1 mice (n = 48)	2.56 mg/m^3 1 hour, 2 times/week for 8 weeks	Morphological changes to CNS (cilia loss, increased cell sloughing, ependymal cell layer detachment, decreased dendritic spines in hippocampus and substantia nigra, increased cell loss, decreased performance in Morris water maze)	-	2.56	No clinical signs of toxicity or other toxicologic endpoints were reported.	Avila-Costa et al. (2006; 2005; 2004)
Male CD-1 mice (n = 8)	2.56 mg/m^3 1 hour/day, 2 days/week for 12 weeks	Increased platelet counts and altered platelet morphology	-	2.56	None	González-Villalva et al. (2006)
Male CD-1 mice (n = 60)	2.56 mg/m^3 1 hour/day, 2 days/week for 12 weeks	Decreased gamma globulin in testes, increased cell death in spermatogonia	-	2.56	None	Mussali-Galante et al. (2005); Fortoul et al. (2007)
Male Cynomolgus Monkey (n = 8/group)	0.1, 0.5, or 1.1 mg/m^3 6 hours/day, 5 days/week for 26 weeks	Impaired pulmonary function	0.10 (continuous exposure)	not established	Exposures occurred on alternate days for two sets of animals (some received 0.1 or 1.1 mg/m^3 on alternate days, while a second group was exposed to a constant concentration (0.5 mg/m^3) for all 5 days. Pulmonary function parameters were reversible and did not reappear following subsequent challenge.	Knecht et al. (1992)

Table 4-14. Summary of the noncancer results for the subchronic and chronic inhalation exposure studies of vanadium pentoxide toxicity in experimental animals

Species (sex)	Exposure (mg/m^3)	Response at LOAEL	NOAEL (mg/m^3)	LOAEL (mg/m^3)	Comments	Reference
F344/N rat (n = 10/sex/ group)	0, 1, 2, 4, 8, 16 mg/m^3 6 hours/day, 5 days/week for 3 month	Lung epithelium hyperplasia and inflammation, increased relative lung weight, altered levels of hematological parameters in males. Lung epithelium hyperplasia and increased absolute lung weight in females.	1.0	2.0	None	NTP (2002)
$B6C3F_1$ mice (n = 10/sex/group)	0, 1, 2, 4, 8, 16 mg/m^3 6 hours/day, 5 days/week for 3 months	Increased absolute lung weight and lung epithelium hyperplasia in males. Lung epithelium hyperplasia and inflammation in females.	1.0	2.0	None	
F344/N rat (n = 50/sex/group)	0, 0.5, 1, 2 mg/m^3 6 hours per day, 5 days per week for 2 years	Effects were observed in the lungs (alveolar and bronchiolar epithelial hyperplasia and alveolar histiocyte infiltration), larynx (inflammation and epiglottis degeneration, hyperplasia and squamous metaplasia), and nose (goblet cell hyperplasia) in males. Effects were observed in the lungs (interstitial fibrosis and alveolar histiocyte infiltration) and larynx (inflammation and epiglottis degeneration and hyperplasia) in females.	–	0.5	No other clinical findings or altered survival.	NTP (2002)
$B6C3F_1$ mice (n = 50/sex/group)	0, 1, 2, 4 mg/m^3 6 hours per day, 5 days per week for 2 years	Effects were observed in the lungs (alveolar and bronchiolar epithelial hyperplasia, inflammation, alveolus histiocyte infiltration in both males and females), larynx (squamous metaplasia of the epiglottis in both males and females), and nose (olfactory and respiratory epithelium degeneration in males and olfactory epithelial degeneration and atrophy in females)	–	1.0	Mice reported as thin, survival significantly decreased in male mice at 4 mg/m^3.	

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4.6.3. Mode of Action Information

Currently, evidence is insufficient to establish the mode of action (MOA) for vanadium pentoxide noncancer toxicity. The limited data to inform the mechanisms of various noncancer health effects (pulmonary toxicity, neurotoxicity, and reproductive toxicity) following inhalation exposure to vanadium pentoxide are summarized below.

4.6.3.1. *Pulmonary Toxicity*

Evidence suggests that a potential mechanism of action underlying the formation of pulmonary fibroproliferative lesions may involve inflammation, leading to a regenerative hyperplastic response (i.e., presence of mitogens and observed smooth muscle thickening). Oxidative stress also has been implicated. Oxidative stress induced directly or indirectly by vanadium pentoxide may work in combination with vanadium pentoxide-induced inflammatory responses to activate signaling molecules such as ERK1/2 and p38 kinase that lead to induction of growth factors and generation of fibrotic lesions. These potential modes of action are supported by a limited number of mechanistic analyses of inflammation and fibrosis following exposure to vanadium pentoxide.

Inflammation. Pierce et al. (1996) identified proinflammatory cytokines associated with vanadium pentoxide exposure. Evidence suggests that oxidative stress may be involved in vanadium pentoxide-induced pulmonary injury. All species of vanadium can participate in redox cycling and generate reactive oxygen species (Carter et al., 1997). Intracellular and extracellular hydrogen peroxide production increases significantly after 18-hour exposure to vanadium pentoxide compared to controls (Antao-Menezes et al., 2008). Vanadium pentoxide also induced significant IFN-β expression after 18 and 24 hours, however this expression was inhibited by catalase, an inhibitor of hydrogen peroxide. Oxidative stress was also a suggested mechanism underlying STAT-1 activation. Addition of either an NAPDH inhibitor or a xanthine oxidase inhibitor ablated STAT-1 activation in cells exposed to vanadium pentoxide (Antao-Menezes et al., 2008). Moreover, spontaneous hydrogen peroxide generation by fibroblasts was depleted within minutes by addition of vanadium pentoxide (Ingram et al., 2003). In addition, vanadium can contribute to inhibition of protein tyrosine phosphatases through the generation of reactive oxygen species (Zhang et al., 2001). Hydrogen peroxide and vanadium pentoxide could have reacted to form peroxovanadium intermediates or ROS, or both, that led to the production of HB-EGF in fibroblasts. Oxidative stress could also be inducing ERK and p38 kinase leading to the production of HB-EGF (Ingram et al., 2003) or by ROS-mediated competitive inhibition of protein tyrosine phosphatases (Zhang et al., 2001)(Samet et al., 1999). Gene array analysis and subsequent confirmation by PCR revealed that various oxidative stress genes such as superoxide dismutase (SOD2), pipecolic acid oxidase (PIPOX), and oxidative stress response (OXR1) were altered by vanadium pentoxide exposure (Ingram et al., 2007). Thus vanadium pentoxide-mediated production of ROS might lead to oxidative stress and induce downstream signaling

events that result in activation of mitogens and proinflammatory cytokines that contribute to the formation of fibroproliferative lesions in the lung.

Fibrosis. Bonner et al. (1998) has published numerous studies describing the mechanism underlying the formation of fibroproliferative lesions in response to vanadium pentoxide exposure. Specifically, smooth muscle thickening and increased collagen deposition were observed beneath ciliated epithelial cells in vanadium pentoxide-exposed male Sprague-Dawley rats (Bonner et al., 1998). Additionally, proliferating myofibroblasts were the principal cell type that contributed to the observed fibrosis (Bonner et al., 2000). Using both in vitro and in vivo models, Rice et al. (1999) showed that inhibition of autophosphorylation of tyrosine kinases reduced vanadium pentoxide-induced pulmonary fibrosis, thus implicating the tyrosine kinases as key signaling mediators underlying the mechanism of PDGF release and ultimately, fibrinogenesis.

Zhang et al. (2001) and Ingram et al. (2003) identified a second mitogen growth factor similar to heparin-binding epidermal growth factor (HB-EGF) as an important mediator of vanadium pentoxide-induced injury in vitro in normal human bronchial epithelial cells (NHBECs). Further, two signaling molecules (ERK and the p38 subunit of MAP kinase) were activated in response to vanadium pentoxide (Ingram et al., 2003). Gene array analysis confirmed the importance of HB-EGF and IL-8 in vanadium pentoxide-induced lung injury and identified several new candidate genes, including growth factors (VEGF, CTGF), chemokines (CXCL9, CXCL10), oxidative response genes (SOD2, PIPOX, OXRI), and DNA-binding proteins (GAS1, STAT1) (Ingram et al., 2007).

Bonner et al. (2002) also has reported that fibroproliferative lesions are resolved and repair is initiated in response to vanadium pentoxide. Their work illustrates that mice deficient in prostaglandins (PG), such as the enzyme cyclooxygenase (COX)-2, are protected from vanadium pentoxide-induced fibroproliferative lesions and indicates the potentially important role of cyclooxygenases in mitigating vanadium pentoxide induced injury. Antao-Menezes et al. (2008) characterized the role of STAT-1 in vanadium pentoxide pulmonary fibrosis. Their work identified Interferon-beta (IFN-β) as a mediator of vanadium pentoxide-induced STAT-1 activation in normal human lung fibroblasts, and linked STAT-1 activation with STAT-1-dependent production of a chemokine, CXCL10, that was identified in the Ingram et al. (2007) gene array analysis. Thus, fibroblasts appear to synthesize IFN-β that activates STAT-1. STAT-1 activation simultaneously causes growth arrest and increases levels of CXCL10, which then diminish fibrinogenesis, in a negative feedback loop. In summary, vanadium pentoxide-stimulated production of IFN-β activates signaling pathways that lead to the resolution of fibrosis after vanadium pentoxide-induced injury.

4.6.3.2. *Neurotoxicity*

The mechanism(s) underlying nervous system toxicity in response to vanadium pentoxide is not well characterized. A duration-dependent decrease in the number of immunoreactive TH+ neurons and morphological changes to the blood-brain barrier were observed in response to vanadium pentoxide. Blood-brain barrier disruption has been suggested to be related to brain region-specific changes in metalloproteinases (MMP-2 and MMP-9) that have been observed following vanadium pentoxide exposure (Colin-Barenque et al., 2008); more work, however, is needed to characterize these findings fully.

4.6.3.3. *Reproductive Toxicity*

Limited studies of vanadium pentoxide have examined reproductive and developmental toxicity. Short-term oral exposures in weanling rats demonstrated a potential effect on bone growth as measured by serum calcium concentrations and bone alkaline phosphatase activity (Yamaguchi et al., 1989). Excess vanadium pentoxide was found to accumulate in the testes following inhalation exposure (Mussali-Galante et al., 2005). Moreover, significant decreases in gamma globulin were observed in testicular samples exposed to vanadium pentoxide (Mussali-Galante et al., 2005). Decreased gamma-globulin levels could lead to changes in microtubule formation that would impact spermatogenesis. Injection studies described by Wide (1984) demonstrated decreased ossification in fetuses exposed to vanadium pentoxide in utero. Determination of a specific MOA for reproductive toxicity is not possible due to the limited studies examining these effects.

4.7. EVALUATION OF CARCINOGENICITY

4.7.1. Summary of Overall Weight of Evidence

Under the U.S. EPA *Guidelines for Carcinogenic Risk Assessment* (U.S. EPA, 2005a), vanadium pentoxide is "likely to be carcinogenic to humans" by the inhalation route of exposure based on alveolar/bronchiolar tumors in male and female mice and rats following inhalation exposure to vanadium pentoxide for 2 years (Ress et al., 2003; NTP, 2002). NTP (2002) reported statistically significant and dose-related increased incidence of alveolar/bronchiolar tumors in male and female mice. Although the incidence of bronchiolar 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 (see Table 4-9). No other tumor type was significantly increased in either rats or mice in this study. These results are supported by a recent study by Rondini et al. (2010), which also showed increased lung tumors in male mice (A/J, BALB/C) following exposure to vanadium pentoxide along with an initiator (MCA).

U.S. EPA's *Guidelines for Carcinogenic Risk Assessment* (2005a) indicates that, for tumors occurring at a site other than the initial point of contact, the weight of evidence for

carcinogenic potential could apply to all routes of exposure that have not been adequately tested at sufficient doses. An exception occurs when there is convincing information (e.g., toxicokinetic data) that absorption does not occur by other routes. Information available on the carcinogenic effects of vanadium pentoxide via the inhalation route is limited to examination of the respiratory tumors. Information on the carcinogenic effects of vanadium pentoxide via the oral and dermal routes in humans or animals is absent. Based on the observance of only portal-of-entry tumors (respiratory tumors) following inhalation exposure, and in the absence of information to establish an MOA, this cancer descriptor applies only to the inhalation route of exposure. Therefore, vanadium pentoxide is "likely to be carcinogenic to humans" by the inhalation route of exposure, and the database has "inadequate information to assess carcinogenic potential" via the oral or dermal route.

4.7.2. Synthesis of Human, Animal, and Other Supporting Evidence

Few studies are available that assess the carcinogenic potential of vanadium pentoxide. Although both epidemiology and laboratory animal studies show similar respiratory tract toxicity, currently no epidemiology studies are available in the published literature examining carcinogenicity.

Only two published laboratory animal studies provide evidence for the carcinogenic potential of vanadium pentoxide (Rondini et al., 2010; NTP, 2002). Vanadium pentoxide has been shown to induce pulmonary tumors following inhalation exposure NTP (2002). F344/N rats (50 per sex per group) and B6C3F$_1$ mice (50 per sex per group) were exposed to vanadium pentoxide particles for 6 hours per day, 5 days per week, for 2 years. Rats were exposed to 0.5, 1.0, or 2.0 mg/m^3 of vanadium pentoxide, with mice exposed to 1.0, 2.0, or 4.0 mg/m^3 of vanadium pentoxide. Lung tumors were observed in male and female rats, but incidence exceeded historical controls only in male rats (Table 4-9). Both male and female mice showed statistically significant increases in lung tumors as compared to controls ($p \leq 0.01$). These increases were observed in both sexes at all doses, with 50% of the male mice in the highest exposure group dying before the end of the study. Survival rates in all other exposed groups for both rats and mice were not significantly different from controls. Both rats and mice showed other lesions of the respiratory tract, including inflammation, fibrosis, and hyperplasia (Table 4-8 and Table 4-10). Decreased body weight gain was observed as early as 3 months postexposure in the high dose groups of all exposed animals.

Along with the NTP study (2002), a recent study by Rondini et al. (2010) examined tumor promotion of vanadium pentoxide in three different mice strains. Lung tumors were observed in two of three mouse strains 20 weeks after MCA tumor initiation, followed by exposure (only males exposed) to vanadium pentoxide (4 mg/kg, 5 times weekly) (Table 4-12). A/J and BALB/C male mice showed increases in lung tumors following exposure to both MCA

(initiator) and vanadium pentoxide, but not vanadium pentoxide alone, suggesting vanadium pentoxide may act as a tumor promoter.

4.7.3. Mode of Action Information

Alveolar/bronchiolar tumors were observed in both mice and rats following inhalation exposure to vanadium pentoxide. In vitro tests in bacterial and yeast systems provide mixed evidence of vanadium pentoxide-induced mutagenicity. In general, gene mutation assays were negative, as were tests that assessed SCE and other chromosomal aberrations. DNA strand breaks (Ivancsits et al., 2002; Rojas et al., 1996) and micronucleus formation (Zhong et al., 1994) were reported in some studies in cultured cells but depended on cell type. Information is insufficient to establish a MOA for bronchiolar tumors observed in animals following inhalation exposure to vanadium pentoxide.

4.8. SUSCEPTIBLE POPULATIONS AND LIFE STAGES

4.8.1. Possible Childhood Susceptibility

There are no reports of childhood susceptibility due to exposure to vanadium pentoxide. There are also no reports indicating increased susceptibility in the developing fetus but data on developmental effects of vanadium pentoxide are very limited (see discussion of data gaps below). Young rats that consumed vanadium in the drinking water and feed were found to have higher tissue vanadium levels 21 days after birth than they did 115 days after birth (Edel et al., 1984). The data suggest higher absorption of vanadium in young animals due to a greater nonselective permeability of the undeveloped intestinal barrier. Thus, age of the rodents appears to play an important role in the absorption of vanadium in the GI tract. Mravcova et al. (1993) assessed the extent of vanadium pentoxide accumulation in the bones of rats following a 6-month exposure. Vanadium accumulated in the epiphyseal cartilage of the tibia in rats with significantly higher concentrations of vanadium in the tibia and incisors of weanling rats compared to adults. No dose-response data for these endpoints were reported.

4.8.2. Possible Gender Differences

Reports of gender differences are limited to the carcinogenicity data from NTP (2002) where clear evidence of carcinogenicity was reported in male and female mice exposed to vanadium pentoxide. Some evidence of carcinogenicity was reported in male rats, based on observations of alveolar and bronchiolar neoplasms that exceeded historical controls in groups exposed to vanadium pentoxide. The number of neoplasms in female rats was not higher than that observed in historical controls and, thus, a relationship between neoplasms and vanadium pentoxide could not be established in female rats (NTP, 2002)(Ress, 2003). Therefore, increased tumor incidence in rats is equivocal overall but the lack of any increase in females suggests a gender-related increase in susceptibility in males. Whether this observed difference is applicable

to humans is unknown. There are no reported differences for response to vanadium pentoxide between genders for either animals or humans for any noncancer endpoint.

4.8.3. Other Susceptible Populations

No data exist on the role of genetic polymorphisms in differentially susceptible human populations in response to vanadium pentoxide exposure. In mice, research suggests that all strains have an inflammatory response to vanadium pentoxide exposure, but the severity of inflammation varies greatly from strain to strain. Such variability in response suggests that a genetic component contributes to the severity of vanadium pentoxide-induced pulmonary inflammation and tumorigenicity in mice (Rondini et al., 2010). The NTP study (2002) used $B6C3F_1$ mice in all exposure protocols for 16-day, 3-month, and 2-year exposure studies. Pulmonary fibrosis was observed in this hybrid strain.

Kyono et al. (1999) used a rat model of acute bronchiolitis (Br) to investigate whether animals with preexisting lung conditions would be differentially susceptible to inhaled vanadium pentoxide. Compared to exposed normal rats, Br rats exhibited delayed recovery from preexisting lesions and exacerbated lung inflammation. Sensitive rats also showed reductions in the deposition and clearance rates of inhaled particles.

Rondini et al. (2010) examined the effect of exposure to vanadium pentoxide in three mouse strains of varying susceptibility to lung cancer (A/J, BALB/C, and C57BL/6J) in an initiation/promotion model (see full study description in Appendix D). Three mouse strains were used to further understand potential susceptibility to these effects. These particular mouse strains were selected because of their known differential susceptibility to chronic pulmonary inflammation and carcinogenesis: A/J mice are sensitive, BALB/C are intermediate, and C57BL/6J are resistant. Statistically significant lung tumor increases were observed in A/J and BALB/C mice as compared to the MCA-treated control ($p \leq 0.05$; Table 4-12). Differences were also observed between strains, with A/J mice showing increased tumorigenicity in response to vanadium pentoxide. In the absence of MCA, vanadium pentoxide was not sufficient to initiate tumorigenesis in this study. C57BL/6J had no tumors following exposure (data not shown).

Overall, the differential inflammatory responses observed in the three strains of mice appear to positively correlate with increased levels of chemokines, such as keratinocyte-derived chemokine (KC) and monocyte chemotactic protein-1 (MCP-1), increased binding of transcriptional factors NFκB and AP-1 (c-Fos), sustained activation of MAP kinases (MAPKs), and extracellular signal-regulated kinases 1 and 2 (ERK 1/2), suggesting inflammation as a major response in mice. Turpin et al. (2010) examined pulmonary inflammation and fibrosis following intranasal aspiration exposure to vanadium pentoxide with and without RSV exposure (see full study description in Appendix D). In this study, vanadium pentoxide exposure also caused a significant increase in cell proliferation in the airways and lung parenchyma, lung mRNAs for TGF-β-1, CTGF, PDGF-C, Col1A2, and mRNAs for IFN-α and -β, and IFN-inducible

chemokines CXCL9 and CXCL10 compared to controls. Pre- or posttreatment with RSV caused a significant reduction in all mRNAs. Together, results from this study showed that vanadium pentoxide induces inflammatory and fibrogenic response in mouse lung, and these effects were suppressed by RSV infection. Yu et al. (2011) demonstrated increased mucin production from the airway epithelium in female AKR mice following laryngeal aspiration of vanadium pentoxide (4 mg/kg bw). These results were supported by in vitro studies showing increased gene expression for genes related to mucin production following exposure to vanadium pentoxide.

5. DOSE-RESPONSE ANALYSIS

5.1. ORAL REFERENCE DOSE (RfD)

5.1.1. Choice of Principal Study and Critical Effect with Rationale and Justification

Two human studies are available which measured cystine levels in hair and fingernails and vanadium levels in urine (Kucera et al., 1994) or blood (Kucera et al., 1992) following oral exposure to vanadium pentoxide. Kucera et al. (1994) detected vanadium in urine of workers from a Czechoslovakian vanadium pentoxide production plant; these urinary levels of vanadium, however, are suspected of having resulted from multiple exposure routes. Kucera et al. (1992) measured vanadium in the hair and blood of children and in the blood of adults potentially exposed through ingestion of vanadium-contaminated drinking water (concentration range: 0.001–0.1 mg/L). Vanadium concentrations in water supply wells exceeded the maximum permissible limit in drinking water (0.01 mg/L) with the contamination continuing over 2 years. Significantly increased vanadium concentrations were found in blood of exposed children compared to unexposed children and adults, whereas vanadium levels in hair of exposed children (adults not measured) were no different from the control group. No exposure-response relationship could be determined for either endpoint. Changes in hair cystine levels have not been associated with adverse health effects. Additional studies of workers occupationally exposed to vanadium pentoxide exist, although these workers are presumed to have been exposed by multiple routes, and inhalation was likely the primary route of exposure (Kiviluoto et al., 1981b)(Irsigler et al., 1999; Musk and Tees, 1982; Kiviluoto, 1980; Kiviluoto et al., 1979; Zenz et al., 1962; Sjoberg, 1956, 1951).

The results of a 2.5-year dietary study on vanadium in rats (strain not described) (Stokinger et al., 1953) were summarized in *Patty's Industrial Hygiene and Toxicology*, 3rd ed. (1981). This study did not assess comprehensive toxicity endpoints, and details of the study are limited in the available publication. Although hair cystine content was stated to be significantly decreased in exposed animals compared to controls, no quantitative data were presented. The biological significance of decreased hair cystine is unclear, however, and this effect is known to be caused by other chemical exposure. Due to these limitations, this study was not selected for the principal study. No additional oral chronic exposure studies in animals were identified in the published literature.

Mountain et al. (1953) evaluated the effects of subchronic oral vanadium pentoxide exposure in laboratory animals. Male Wistar rats were exposed to vanadium pentoxide for 103 days using average daily doses of 0, 10.5, 16.4, 69.6, 141.0 mg/kg-day vanadium pentoxide in feed. Changes were observed in body weight gain, erythrocyte count, hemoglobin, and cystine content of hair. Other endpoints of toxicity were not reported.

The study authors reported increased body weight gain in the low exposure groups (0–16.4 mg/kg-day) and decreased body weight gain in the highest exposure group (141.0 mg/kg-day). No explanation, statistical analysis, or measures of variability (standard error [SE] or standard deviation [SD]), however, accompanied these data. Cystine content of hair was significantly decreased compared to control at doses ≥16.4 mg/kg-day vanadium pentoxide. Alterations in hair cystine levels can be associated with dietary changes or altered health status (Kleinfeld et al., 1961). The authors speculated that vanadium may have inhibited enzymes, such as sulfur transferases, that decreased the availability of cystine for hair growth. Data to support a relationship between decreased hair cystine levels and adverse health outcomes, however, are not available in the published literature. Thus, the biological significance of decreased hair cystine content is unclear.

Mountain et al. (1953) also reported relative liver weight increases and decreases in erythrocytes and hemoglobin levels. Mean relative liver weights, reported as a ratio of liver weight to body weight, were statistically significantly elevated above controls at 69.6 mg/kg-day (i.e., 3.51 ± 0.06 versus 3.86 ± 0.07). Liver weight data were not reported for other doses. Apparent dose-related decreases in red blood cell (RBC) count (21.3%) and hemoglobin concentration (10.5%) were observed in the 10.5 and 16.4 mg/kg-day vanadium pentoxide dose groups compared to controls (Table 5-1). Study authors performed no statistical analysis on these data and reported no measure of variance, precluding independent statistical analysis. The effects on RBC count and hemoglobin concentration observed in this oral study are consistent with the hematological effects observed in a 3-month inhalation study of vanadium pentoxide in rats (NTP, 2002). Decreases in mean cell volume (MCV) and mean cell hemoglobin (MCH) accompanied by erythrocyte microcytosis suggested altered iron metabolism and heme/hemoglobin production following vanadium pentoxide inhalation exposure. Based on the dose-related decreases in RBC count in the oral study supported by the hematological effects observed in the inhalation study, decreased RBC count was considered adverse and selected as the critical effect.

Although a dose-related decrease in RBC count was observed at 10.5 and 16.4 mg/kg-day vanadium pentoxide (12.8% and 21.3%, respectively) in the Mountain et al. (1953) study, the magnitude of this change was considered biologically significant at 16.4 mg/kg-day, which EPA identified as the lowest-observed-adverse-effect level (LOAEL). The lowest dose of 10.5 mg/kg-day was identified by EPA as a no-observed-adverse-effect level (NOAEL).

Mountain et al., (1953) was selected as the principal study for the derivation of the RfD. This study utilized four dose groups and an untreated control group with five animals per group and evaluated several hematological endpoints. Limitations of the study include lack of evaluation of a comprehensive number of toxicological endpoints. The study authors reported dose dependent, statistically significant decreases in RBC counts (Table 5-1) following inhalation exposure to vanadium pentoxide which was selected as the critical effect for the

derivation of the RfD. Specifically, the percent change at the low and high dose was 12.8 and 21.3%, respectively compared to 3.8% in the controls. The percent change in RBC count was considered adverse and thus was selected as the critical effect for the derivation of the RfD.

Table 5-1. Hematological results of oral vanadium pentoxide exposure in rats

	Control	10.5 mg/kg-d	16.4 mg/kg-d
Red Blood Cell Count (M/mm^3)			
Start	8.0	7.8	8.0
Finish (103 d)	7.7	6.8	6.3
Percent change between start and finish of experiment (%)	3.8	12.8	21.3
Hemoglobin, %			
Start	15.6	15.2	15.3
Finish (103 d)	15.0	14.5	13.7
Percent change between start and finish of experiment (%)	3.9	4.6	10.5

Source: Mountain et al. (1953).

5.1.2. Methods of Analysis—Including Models (e.g., PBPK and BMD)

The most sensitive endpoint following oral exposure to vanadium pentoxide is decreased RBC count with a NOAEL of 10.5 mg/kg-day vanadium pentoxide (Mountain et al., 1953). Because the study authors reported the decrease in RBC count as a mean with no measure of variability (SE or SD), this continuous endpoint could not be subjected to benchmark dose (BMD) modeling. Therefore, the NOAEL of 10.5 mg/kg-day is identified as the point of departure (POD) for use in deriving the RfD for vanadium pentoxide.

In EPA's guidance document *Recommended Use of Body Weight$^{3/4}$ as the Default Method in Derivation of the Oral Reference Dose* (U.S. EPA, 2011), the Agency endorses a hierarchy of approaches for converting doses administered orally to laboratory animal species to human equivalent oral exposures in deriving the RfD, with the preferred approach being physiologically-based toxicokinetic modeling. An alternate approach includes using chemical-specific information in the absence of a complete physiologically-based toxicokinetic model. In lieu of a toxicokinetic model or chemical-specific data to inform the generation of human equivalent oral exposures, EPA endorses body weight scaling to the ¾ power (i.e., $BW^{3/4}$) as a default to extrapolate toxicologically equivalent doses of orally administered agents from laboratory animals to humans for the purpose of deriving an RfD. When $BW^{3/4}$ scaling is used in deriving the RfD, EPA also advocates a reduction in the interspecies uncertainty factor from 10 to 3, as $BW^{3/4}$ scaling addresses predominantly toxicokinetic (and some toxicodynamic) aspects of the UF_A.

Statements in the guidance raise some important uncertainties in applying allometric scaling, and more specifically $BW^{3/4}$ scaling, when trying to extrapolate across different sized

individuals within a species (e.g., between neonates and adults) or across individuals in different lifestages between species (e.g., between fetal rats and adult humans). Furthermore, the data on which to base a default allometric scaling factor for converting the administered dose in a laboratory animal in a different lifestage to a comparable dose in an adult human are sparse, and thus more uncertain. For these reasons, a $BW^{3/4}$ scaling factor would not be applied as a default approach (in combination with a reduced default UF for interspecies extrapolation) when extrapolating from developmental effects in laboratory animals to adult humans when deriving the RfD.

No physiologically based toxicokinetic modeling information exists for vanadium pentoxide. The selected critical effect (decreased RBC counts) is associated with the parent compound, is not considered a portal-of-entry effect, and was observed in mature rats. Therefore, consistent with U.S. EPA guidance (U.S. EPA, 2011), the POD identified in rats (i.e., 10.5 mg/kg-day) is converted to a human equivalent dose (HED) through application of a dosimetric adjustment factor (DAF) derived as follows (Table 5-2):

$$DAF = (BW_a^{1/4} / BW_h^{1/4}),$$

where:

DAF = dosimetric adjustment factor
BW_a = animal body weight
BW_h = human body weight

Using a BW_a of 0.25 kg for rats and a BW_h of 70 kg for humans, the resulting DAF is 0.244. Applying this DAF to the POD identified in rats yields an HED of 2.56 mg/kg-day as follows:

HED = laboratory animal dose (mg/kg-day) × DAF
= 10.5 mg/kg-day × 0.244
= 2.56 mg/kg-day

Table 5-2. Human equivalent dose conversion by $BW^{3/4}$ for RfD derivation[a]

Species	$BW_a^{1/4} / BW_h^{1/4}$ = DAF	HED	UF	RfD(mg/kg-d)
Rat (0.25kg)	$0.25kg^{1/4} \div 70kg^{1/4} = 0.244$	10.5 mg/kg-d × 0.244 = 2.56 mg/kg-d	Total = 3000 UF_A = 3 UF_H = 10 UF_S = 10 UF_D = 10	2.56 ÷ 3000 = 8.5×10^{-4}

[a] Using the BW_a of 0.25 kg for rats and BW_h of 70 kg for humans, and multiplying it by the NOAEL of 10.5 mg/kg-d from Mountain et al. (1953).

5.1.3. RfD Derivation—Including Application of Uncertainty Factors

The NOAEL of 10.5 mg/kg-day for decreased RBC count in male rats (Mountain et al., 1953) was used as the POD to derive an RfD. The uncertainty factors, selected based on EPA's *A Review of the Reference Dose and Reference Concentration Processes* (U.S. EPA, 2002; Section 4.4.5), address five areas of uncertainty resulting in a composite UF of 3000. This composite uncertainty factor was applied to the selected POD to derive an RfD.

An interspecies uncertainty factor, UF_A, of 3 ($10^{0.5}$ = 3.16, rounded to 3) was applied to account for uncertainty in extrapolating from laboratory animals to humans in the absence of information to characterize the toxicokinetic or toxicodynamic differences between rats and humans after oral vanadium pentoxide exposure. For vanadium pentoxide, toxicokinetic uncertainty was accounted for by calculating an HED through application of a DAF as outlined in the U.S. EPA guidance on the use of $BW^{3/4}$ scaling in the derivation of the oral RfD (U.S. EPA, 2011). As the toxicokinetic differences are thus accounted for, only the toxicodynamic uncertainty remains, and a UF_A of 3 is retained to account for this residual uncertainty.

An intraspecies uncertainty factor, UF_H, of 10 was applied to account for potentially susceptible individuals in the absence of data evaluating the variability of response to oral vanadium pentoxide exposure in the human population.

A subchronic-to-chronic uncertainty factor, UF_S, of 10 was applied for extrapolation from a subchronic to chronic study because a subchronic study was used for the POD.

A LOAEL-to-NOAEL uncertainty factor, UF_L, of 1 was applied because a NOAEL was identified from the principal study and used as the POD (U.S. EPA, 2002).

A database uncertainty factor, UF_D, of 10 was applied to account for database deficiencies due to the lack of a developmental toxicity study and a multigenerational reproductive toxicity study for vanadium pentoxide by the oral route. Studies using alternate routes of exposure (intraperitoneal) have indicated adverse reproductive and developmental effects in response to vanadium pentoxide, including statistically significant increases in seminal vesicle, thymus, and submandibular gland weights in male mice and body weight, thymus, submandibular gland, and liver weights in female mice (Altamirano et al., 1991) as well as reduced fertility (Altamirano-Lozano et al., 1996). No physiologically based toxicokinetic models are available for conducting a route-to-route extrapolation.

Therefore, the RfD for vanadium pentoxide is calculated as follows:

$$\begin{aligned} \text{RfD} &= \text{NOAEL}_{\text{HED}} \div \text{UF} \\ &= 2.56 \text{ mg/kg-day} \div 3{,}000 \\ &= 0.000085 \text{ mg/kg-day or } 9 \times 10^{-4} \text{ mg/kg-day} \end{aligned}$$

Note: Because vanadium exists in several valence states, all of which are not equivalent toxicologically (WHO, 2001), the values generated here apply to vanadium pentoxide and should not be applied to other vanadium compounds.

5.1.4. Previous RfD Assessment

U.S. EPA previously derived an RfD of 9×10^{-3} mg/kg-day for vanadium pentoxide in 1987 based on a 2.5-year dietary NOAEL of 0.89 mg/kg-day vanadium pentoxide for decreased hair cystine content [U.S. EPA (1987); Stokinger et al. (1953) reported in *Patty's Industrial Hygiene and Toxicology*, 3rd Ed. (1981)]. Limited details were provided, and this study is not available for analysis. The rats (number and species unspecified) were exposed to dietary levels of vanadium pentoxide (0.89 or 8.9 mg/kg-day for 2.5 years) and assessed for growth rate, survival, and hair cystine content. Of the endpoints reported in the book chapter, decreased hair cystine was selected as the critical effect with a NOAEL of 0.89 mg/kg-day. Upon further analysis as described in Section 5.1.1, this study was determined to be inadequate for use in deriving an RfD.

5.2. INHALATION REFERENCE CONCENTRATION (RfC)

5.2.1. Choice of Principal Study and Critical Effect with Rationale and Justification

The available human and animal data identify the respiratory tract as the primary target for chronic inhalation exposure to vanadium pentoxide. Irritation of the upper and lower respiratory tract has been reported in several acute, subchronic, and chronic 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., 1995b; Levy et al., 1984; Musk and Tees, 1982; Kiviluoto, 1980; Lees, 1980; Kiviluoto et al., 1979; Zenz et al., 1962; Lewis, 1959; Sjoberg, 1955; Vintinner et al., 1955; Williams, 1952). These results are supported by effects observed in rodents, including inflammation, hyperplasia, and fibrosis. Although subchronic occupational exposure studies provide supportive evidence for the respiratory tract as a target for inhaled vanadium pentoxide, studies often failed to quantify vanadium pentoxide concentration as a constituent in an inhaled mixture. Thus, the available occupational exposure studies are not suitable as the basis for the RfC.

The toxicity database for inhalation exposure in laboratory animals includes two chronic studies (NTP, 2002; Knecht et al., 1992). This study was not selected as the principal study. Although this study demonstrated statistically significant pulmonary responses in monkeys following provocation challenge exposure to vanadium pentoxide, an absence in increased pulmonary reactivity with subchronic exposure to vanadium pentoxide was observed, suggesting that the monkeys may have developed a tolerance to treatment due to the experimental design of this study.

Results of the subchronic and chronic NTP (2002) studies in F344/N rats and $B6C3F_1$ mice provide evidence of toxicity to the upper and lower respiratory tract, including increased lung weight, inflammation, histological lesions, and decreased pulmonary function following 3-month and 2-year inhalation exposures to vanadium pentoxide. (see Sections 4.2.2.1 and 4.2.2.2). Specifically, effects observed in rats at the low dose (2 mg/m^3) and greater included,

increased lung weights (both males and females), hematological effects (males), and lung effects (epithelium hyperplasia in males and females and inflammation in males) following exposure to vanadium pentoxide for 3 months. Based on decreased erythrocyte size in male rats and increased lung weights (males) and nonneoplastic lung lesions in males (epithelial hyperplasia and inflammation in the lung) and females (epithelial hyperplasia) rats, the NOAEL and LOAEL values identified for the 3-month inhalation exposure to vanadium pentoxide aerosols in rats were 1 and 2 mg/m^3, respectively. Erythrocytosis was observed in male and female rats exposed to inhaled vanadium pentoxide for 3 months; hematological endpoints were not examined in mice (NTP, 2002). The erythrocytosis was considered to be a secondary effect arising from the primary lung lesions and was not considered as a critical effect. Effects observed in mice at the low dose and greater included, increased lung weight (males) and lung effects (epithelium hyperplasia in both males and females) following exposure to vanadium pentoxide for 3 months. Based on increases in absolute lung weights (males) and hyperplasia of the respiratory epithelium at concentrations of 2 mg/m^3 and greater (males and females), NOAEL and LOAEL values identified for the 3-month inhalation exposure to vanadium pentoxide aerosols in mice as of 1 and 2 mg/m^3, respectively Other observed endpoints include increased lung bronchiolar exudate, lung fibrosis, altered nasal morphology, and increased nasal inflammation. Significant exposure-related decreases in pulmonary function were also observed in male and female rats at doses of > 4 mg/m^3 (NTP, 2002). Abnormal breathing, emaciation, and lethargy were observed in male and female rats exposed to concentrations of 8 mg/m^3 or higher. Final body weight was statistically significantly decreased as compared to the respective controls at 16 mg/m^3 in male rats (60%) and male mice (10%); 8 mg/m^3 in male rats (10%) and mice (6%); 16 mg/m^3 in female rats (30%) and female mice (12%); 8 mg/m^3 in female mice (10%); and 4 mg/m^3 in female mice (11%).

Similarly, in the chronic 2 year study by NTP (2002), effects were observed on the lung, larynx, and nasal tissues. Specifically, in rats the incidences of nonneoplastic lesions of the lungs (alveolar and bronchiolar epithelial hyperplasia [males], interstitial fibrosis[females] and alveolar histiocyte infiltration[both males and females]); larynx (inflammation and epiglottis degeneration and hyperplasia [males and females] and squamous metaplasia [males]); and nose (goblet cell hyperplasia [males]) effects were significantly increased compared to controls 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. A LOAEL of 0.5 mg/m^3 was established for nonneoplastic lesions of the respiratory tract in male and female rats; a NOAEL was not identified. In mice, the incidences of nonneoplastic lesions of the lungs (hyperplasia of the alveolar and bronchiole epithelium, inflammation, and alveolus histiocyte infiltration); larynx (squamous metaplasia of the epiglottis); and nose (olfactory and respiratory epithelium degeneration[males and females] and atrophy [females]) were significantly increased compared

to control in all vanadium pentoxide exposure groups. Incidences of interstitial fibrosis were significantly increased in male and female mice exposed to 2 or 4 mg/m^3. In general, the incidences and severity ratings of lesions increased with exposure level and matched the types of lesions observed in rats. No treatment-related histopathological findings were observed in other tissues. The LOAEL of 1 mg/m^3 was established for nonneoplastic lesions of the respiratory tract in male and female mice; a NOAEL was not identified.Other studies identified morphological changes in the central nervous system (CNS) of male mice exposed to vanadium pentoxide for up to 8 weeks (Avila-Costa et al., 2005; Avila-Costa et al., 2004). Avila-Costa et al. (2005; 2004), also 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 V/m^3 for up to 8 weeks; effects on CNS function or other comprehensive endpoints were not reported. Using the same dose regimen (1.4 mgV/m^3 twice per week for up to 1 month) Avila-Costa et al. (2006) reported a time-dependent loss of dendritic spines, necrotic-like cell death, and morphological changes to the hippocampal region and that these changes might be related to the associated spatial memory loss. Morphological changes in the CNS reported by Avila-Costa et al. (2006; 2005; 2004) were not considered as the critical effect due to lack of information on the exposure-response relationship for morphological changes to the CNS, as only one exposure level was tested. Moreover, the lung appears to be the most sensitive target to inhalation exposure to vanadium pentoxide.

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, histological lesions, and decreased pulmonary function following 3-month inhalation exposure to vanadium pentoxide. Although body weights and a complete necropsy and histological analysis were performed, adverse effects in other target organs were not identified in the chronic exposure study. The series of effects described in the NTP (2002)bioassay and supporting studies from the available inhalation database reflect a dose-response with increase in severity with vanadium pentoxide concentration, and a progression of respiratory effects (infiltration of macrophages, inflammation, hyperplastic response, fibrosis). Based on the dose-response and temporal relationship of effects throughout the course of the 2-year study, and the concordance with effects observed in humans, the NTP (2002) study is selected as the critical study. Specifically, the NTP (2002) chronic study is well-designed and well-reported utilizing 50 animals per dose group, 3 dose groups, and includes an evaluation of numerous toxicological endpoints. As with the subchronic exposure studies, results of this 2-year inhalation study identify the upper and lower respiratory tract as the target for chronic inhalation exposure to vanadium pentoxide. The nasal and laryngeal lesions reported in NTP (2002) are among the most sensitive effects observed and were observed in both sexes of rats and mice (Table 5-3). In addition, irritation of the upper and lower respiratory tract has been reported in several occupational and case studies of workers exposed for days or weeks to vanadium pentoxide in fuel-oil ash and vanadium dust

(Irsigler et al., 1999; Musk and Tees, 1982; Kiviluoto et al., 1981a; Kiviluoto et al., 1981b; Kiviluoto, 1980; Kiviluoto et al., 1979; Zenz et al., 1962; Sjoberg, 1956, 1951). Therefore, NTP (2002) was selected as the principal study, with inflammation of the larynx in female rats considered adverse and selected as the critical effect.

5.2.2. Methods of Analysis

To analyze the concentration-response effect of vanadium pentoxide, the reported concentrations of vanadium pentoxide were converted to human equivalent concentrations (HEC) prior to any modeling (Table 5-4 and Appendix B). The *Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry* (hereafter referred to as the RfC Methodology) recommends converting the adjusted POD ($POD_{[ADJ]}$) to an HEC (U.S. EPA, 1994b). The RfC Methodology separates gases into three categories based on their water solubility and reactivity with tissues in the respiratory tract, and recommends the use of regional deposited dose ratios (RDDRs) for converting to HECs for particles (e.g., vanadium pentoxide). RDDRs were calculated for rats with the RDDR computer program (U.S. EPA, 1994b) using mean body weights for male and female rats and the average particle-size mass median aerodynamic diameter (MMAD) ± geometric standard deviation (GSD) of 1.24 ± 1.89 for rats as reported by NTP (2002). HEC conversions (in mg vanadium pentoxide/m^3) were calculated by multiplying the duration-adjusted exposure concentrations ($Conc_{[ADJ]}$) by the pulmonary RDDR for lesions in the lung, or the extrathoracic RDDR for lesions in the larynx and nose, and are summarized in Table 5-4. $Conc_{[ADJ]}$ of 0.09, 0.18, and 0.36 mg/m^3, corresponding to nominal exposure concentrations of 0.5, 1, and 2 mg/m^3, were calculated to account for continuous ambient exposure:

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

HECs were calculated by multiplying $Conc_{[ADJ]}$ by the extrathoracic RDDR for lesions of the larynx in female rats (Appendix B, Table B-5).

To determine the POD for derivation of the RfC, BMD modeling was conducted on lesions observed in both male and female rats in the NTP study (2002) (chronic lung inflammation, alveolar epithelium hyperplasia, chronic inflammation of the larynx, respiratory epithelial hyperplasia of the larynx and nose) with the best-fitting model selected for each endpoint (Appendix B).

As shown in Table 5-5, the lowest benchmark concentration ($BMCL_{[HEC]}$) of 0.003 mg/m^3 was observed for chronic inflammation of the larynx of female rats, indicating that the larynx was the most sensitive target for chronic inhalation exposure to vanadium pentoxide. A 53, 55, and 76% increase in the incidence of inflammation in the larynx compared to 16% in controls was observed at the low, mid, and high doses, respectively and was considered adverse. Thus, it was chosen as the POD for the basis of the RfC calculation.

Table 5-3. Selected nonneoplastic lesions of the respiratory system in F344/N rats exposed to particulate aerosols of vanadium pentoxide for 2 years

Endpoint [a]	Exposure Group			
	Control (% incidence)	0.5 mg/m^3 (% incidence)	1 mg/m^3 (% incidence)	2 mg/m^3 (% incidence)
Male rats				
Percent survival (%)	40	58	52	54
Lung				
Number of animals examined	50	49	48	50
Alveolar epithelium, hyperplasia	7 (14)	24[b] (49)	34[b] (71)	49[b] (98)
Bronchiole epithelium, hyperplasia	3 (6)	17[b] (35)	31[b] (65)	48[b] (96)
Inflammation, chronic active	5 (10)	8 (16)	24[b] (50)	42[b] (84)
Interstitial, fibrosis	7 (14)	7 (14)	16[c] (33)	38[b] (76)
Alveolus, histocyte infiltration	22 (44)	40[b] (82)	45[b] (94)	50[b] (100)
Larynx				
Number of animals examined	49	50	50	50
Inflammation, chronic	3 (6)	20[b] (40)	17[b] (34)	28[b] (56)
Epiglottis epithelium, degeneration	0 (0)	22[b] (44)	23[b] (46)	33[b] (66)
Epiglottis epithelium, hyperplasia	0 (0)	18[b] (36)	34[b] (68)	32[b] (64)
Epiglottis epithelium, squamous metaplasia	0 (0)	9[b] (18)	16[b] (32)	19[b] (38)
Nose				
Number of animals examined	49	50	49	48
Goblet cell, hyperplasia	4 (8)	15[b] (30)	12[c] (24)	17[b] (35)
Female rats				
Percent survival (%)	28	40	34	30
Lung				
Number of animals examined	49	49	50	50
Alveolar epithelium, hyperplasia	4 (8)	8 (16)	21[b] (42)	50[b] (100)
Bronchiole epithelium, hyperplasia	6 (12)	5 (10)	14[c] (28)	48[b] (96)
Inflammation,chronic active	10 (20)	10 (20)	14 (28)	40[b] (80)
Interstitial, fibrosis	19 (39)	7[b] (14)	12 (24)	32[b] (64)
Alveolus, histocyte infiltration	26 (53)	35[c] (71)	44[b] (88)	50[b] (100)
Larynx				
Number of animals examined	50	49	49	50
Inflammation, chronic	8 (16)	26[b] (53)	27[b] (55)	38[b] (76)
Epiglottis epithelium, degeneration	2 (4)	33[b] (67)	26[b] (53)	40[b] (80)
Epiglottis epithelium, hyperplasia	0 (0)	25[b] (51)	26[b] (53)	33[b] (66)
Epiglottis epithelium, squamous metaplasia	2 (4)	7 (14)	9 (18)	16[b] (32)

Table 5-3. Selected nonneoplastic lesions of the respiratory system in F344/N rats exposed to particulate aerosols of vanadium pentoxide for 2 years

Endpoint [a]	Exposure Group			
	Control (% incidence)	0.5 mg/m^3 (% incidence)	1 mg/m^3 (% incidence)	2 mg/m^3 (% incidence)
Nose				
Number of animals examined	50	50	50	50
Goblet cell, hyperplasia	13 (26)	19 (38)	16 (32)	30[b] (60)

[a]Number of animals with lesions; numbers in parentheses indicate percent incidence compared to control.
[b]Significantly different from control by the Poly-3 test, $p \leq 0.01$.
[c]Significantly different from control by the Poly-3 test, $p \leq 0.05$.
Source: NTP (2002).

Table 5-4. Human equivalent concentrations of vanadium pentoxide in the 2-year inhalation studies in F344/N rats

Concentration as reported (mg/m^3)	Continuous exposure adjustment factor[a]	RDDR[b]		HEC[c] (mg/m^3)	
		Extrathoracic	Pulmonary	Extrathoracic	Pulmonary
Male rats (F344/N)					
0	0.179	0.516	0.496	0.00	0.00
0.5	0.179	0.530	0.494	0.05	0.04
1	0.179	0.520	0.495	0.09	0.09
2	0.179	0.503	0.498	0.18	0.18
Female rats (F344/N)					
0	0.179	0.263	0.524	0.00	0.00
0.5	0.179	0.259	0.524	0.02	0.05
1	0.179	0.263	0.524	0.05	0.09
2	0.179	0.245	0.524	0.09	0.19

[a]Continuous exposure adjustment factor = (6/24) × (5/7); animals were exposed to vanadium pentoxide 6 hr/d, 5 d/wk.
[c]Refer to Appendix Table B-4.
[d]Human equivalent concentration = concentration as reported × continuous exposure adjustment factor × RDDR.
Source: NTP (2002).

Table 5-5. Candidate PODs for vanadium pentoxide derived from NTP studies (2002) through BMDS modeling

Endpoint	Selected model[a]	BMR (extra risk)	HEC[b]	
			BMC (mg/m^3)	BMCL[c] (mg/m^3) (candidate POD)
Male F344/N Rats				
Lung				
Alveolar epithelium hyperplasia	Probit	0.1	0.016	0.013
Chronic active inflammation	Logistic	0.1	0.035	0.029
Larynx				
Chronic inflammation	Log-Logistic	0.1	0.017	0.012
Respiratory epithelium, epiglottis, hyperplasia	Log-Logistic	0.1	0.008	0.006
Nose				
Goblet cell, respiratory epithelium, hyperplasia	Log-Logistic	0.1	0.044	0.026
Female F344/N Rats				
Lung				
Alveolar epithelium hyperplasia	Gamma	0.1	0.076	0.063
Chronic active inflammation	Multistage (Stage3)	0.1	0.080	0.048
Larynx				
Chronic inflammation	Log-Logistic	0.1	0.005	0.003
Respiratory epithelium, epiglottis, hyperplasia	Log-Logistic	0.1	0.004	0.003
Nose				
Goblet cell, respiratory epithelium, hyperplasia	Multistage (Stage2)	0.1	0.038	0.014

[a]Selected model is the best-fitting model for the dataset based on the draft Benchmark Dose Technical Guidance (2000a).
[b]HEC = human equivalent concentration.
[c]BMCL = the lower bound of benchmark concentration (BMC) at 95% confidence level.

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 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.

Modeling was performed using the Benchmark Dose Modeling Software (BMDS; Version 2.1.2) (U.S. EPA, 2000a). Biological and statistical considerations were taken into account in the selection of a benchmark response (BMR) level for all datasets. In the absence of information indicating what magnitude of inflammatory changes in the larynx and epiglottis are considered biologically significant, the BMR of 10% increase in extra risk was used as the basis

for the benchmark concentration (BMC), with the $BMCL_{10}$ represented by the 95% lower confidence limit on the BMC_{10} (U.S. EPA, 2000a). Following the model selection steps outlined in the draft Benchmark Dose Technical Guidance (U.S. EPA, 2000a), the best-fitting model was selected. Biological and statistical considerations were taken into account in the selection of a BMR level for this dataset. Statistically, a 10% level of response is intended to represent a response level near the lower range of detectable observations in typical studies conducted with 50 animals per dose group (U.S. EPA, 2000a).

Results of the BMDS modeling for chronic inflammation of the larynx in female rats are summarized in Table 5-6 and for epiglottis hyperplasia in Table 5-7. As assessed by the χ^2 goodness-of-fit test, several models demonstrated adequate goodness of fit (p-value ≥ 0.1) and good visual fit (Appendix B). In accordance with the draft *Benchmark Dose Technical Guidance* (U.S. EPA, 2000a), the LogLogistic model was selected as the best-fitting model. The BMC_{10} and $BMCL_{10}$ were estimated as 0.005 and 0.003 mg/m^3.

Table 5-6. Benchmark modeling results for incidence of larynx chronic inflammation in female rats

Model[a]	Goodness of fit				BMR	HEC[c]	
	p-Value	AIC[b]	Largest scaled residual	Scaled residual of interest		BMC (mg/m^3)	BMCL[d] (mg/m^3)
Log-Logistic	0.46	242.0	−0.88	−0.13	Extra Risk 10%	0.005	0.003
Log-Probit	0.27	243.6	−0.89	−0.02		0.003	0.000
Gamma	0.19	243.7	1.58	−0.57		0.007	0.006
Multistage[e] (Stage1)							
Weibull							
Probit	0.03	247.5	1.99	1.99		0.013	0.011
Logistic	0.03	247.6	1.97	1.97		0.014	0.011

[a]Selected (best-fitting) model shown in first row, in boldface type.

Table 5-7. Benchmark modeling results for incidence of larynx respiratory epithelium, epiglottis, hyperplasia in female rats

Model[a]	Goodness of Fit				BMR	HEC[c]	
	p-Value	AIC[b]	Largest scaled residual	Scaled residual of interest		BMC (mg/m^3)	BMCL[d] (mg/m^3)
LogLogistic	0.30	205.3	1.53	0.000	Extra Risk 10%	**0.004**	**0.003**
LogProbit	0.78	204.2	−0.57	0.000		0.000	failed
Gamma	0.01	212.3	2.85	0.000		0.006	0.005
Multistage[e] (Stage1)							
Weibull							
Probit	0.00	234.6	3.258	3.258		0.016	0.013
Logistic	0.00	235.3	−3.295	3.174		0.016	0.014

[a]Selected (best-fitting) model shown in first row, in boldface type.
[b]AIC = Akaike Information Criterion.
[c]HEC = human equivalent concentration.
[d]BMCL = the lower bound of BMC at 95% confidence level.
[e]For Multistage model, up to three stages were tested. Only the model with the lowest AIC and lowest stage is presented here.
Source: NTP (2002).

5.2.3. RfC Derivation—Including Application of Uncertainty Factors

BMD modeling of incidence data for chronic inflammation of the larynx in female rats yielded a $BMCL_{10}$ of 0.003 mg/m^3. The uncertainty factors, selected based on EPA's *A Review of the Reference Dose and Reference Concentration Processes* (U.S. EPA, 2002; Section 4.4.5), address five areas of uncertainty resulting in a composite UF of 300. This composite uncertainty factor was applied to the selected POD to derive an RfC.

An interspecies uncertainty factor, UF_A of 3 ($10^{1/2}$ = 3.16, rounded to 3) was applied to account for uncertainty in characterizing the toxicokinetic or toxicodynamic differences between rats and humans following inhalation exposure to vanadium pentoxide. In this assessment, toxicokinetic uncertainty was accounted for by the calculation of an HEC by applying a DAF as outlined in the RfC methodology (U.S. EPA, 1994b). As the toxicokinetic differences are thus accounted for, only the toxicodynamic uncertainties remain, and a UF of 3 is retained to account for this residual uncertainty.

An intraspecies uncertainty factor, UF_H, of 10 was applied to account for potentially susceptible individuals in the absence of data evaluating variability of response to inhalation of vanadium pentoxide. Considering the pulmonary effects of vanadium pentoxide, individuals with preexisting respiratory disorders might be more susceptible to inhaled vanadium pentoxide.

A LOAEL-to-NOAEL uncertainty factor, UF_L, of 1 was applied because the current approach is to address this factor as one of the considerations in selecting a BMR for BMD modeling (U.S. EPA, 2000a, 1994b). In this case, a BMR of 10% increase in the incidence of chronic inflammation of the larynx and epithelial hyperplasia of the epiglottis was selected assuming it represents a minimal, biologically significant change.

A subchronic-to-chronic uncertainty factor, UF_S, of 1 was applied to account for extrapolation from a subchronic to chronic exposure duration because the RfC was derived from a study of chronic duration.

A database uncertainty factor, UF_D, of 10 wasapplied to account for database deficiencies due to the lack of a developmental toxicity study and a multigeneration reproductive study for vanadium pentoxide by the inhalation route. Studies using alternative routes of exposure (intraperitoneal) have indicated adverse reproductive and developmental effects in response to vanadium pentoxide, including statistically significant increases in seminal vesicle, thymus, and submandibular gland weights in male mice and body weight, thymus, submandibular gland, and liver weights in female mice (Altamirano et al., 1991) as well as reduced fertility (Altamirano-Lozano et al., 1996). No pharmacokinetic models are available for conducting a route-to-route extrapolation.

The chronic RfC for vanadium pentoxide is calculated as follows:

$$\begin{aligned} \text{RfC} &= \text{BMCL}_{10} \div \text{UF} \\ &= 0.003 \text{ mg/m}^3 \div 300 \\ &= 0.00001 \text{ mg/m}^3 \text{ or } 1 \times 10^{-5} \text{ mg/m}^3 \end{aligned}$$

Note: Because vanadium exists in several valence states, all of which are not equivalent toxicologically (WHO, 2001), the values generated here apply to vanadium pentoxide and should not be applied to other vanadium compounds.

5.2.4. Previous RfC Assessment

An inhalation assessment for vanadium pentoxide was not previously available in IRIS.

5.3. UNCERTAINTIES IN THE ORAL REFERENCE DOSE (RfD) AND THE INHALATION REFERENCE CONCENTRATION (RfC)

The following discussion identifies uncertainties associated with the quantification of the RfD and RfC for vanadium pentoxide that are not accounted for by the application of the uncertainty factors. As presented earlier in this section, EPA standard practices and RfC guidance (U.S. EPA, 2002, 1995, 1994a, b) were followed in applying a UF approach to a POD for both the RfD and RfC (see Sections 5.1.3 and 5.2.3). Factors accounting for uncertainties associated with several steps in the analyses were adopted to account for extrapolation from an

animal study to human exposure and to a diverse human population of varying susceptibilities, for extrapolation from subchronic to chronic exposure duration, and for database deficiencies.

The RfD was derived based on the critical effect (decreased red blood cells) in rats exposed to vanadium pentoxide in the diet in a subchronic study (Mountain et al., 1953). Although limited human data are available, decreased red blood cells in this study following exposure to vanadium pentoxide might suggest anemia as a potential effect of exposure. A NOAEL-to-LOAEL approach was used for derivation of the chronic RfD, as the data were not amenable for Benchmark Dose Modeling. There is no physiologically based toxicokinetic modeling information; however, based on EPA's *Recommended Use of Body Weight$^{3/4}$ as the Default Method in Derivation of the Oral Reference Dose* (U.S. EPA, 2011), the POD identified in rats (i.e., decreased red blood cells) was converted to an HED by applying a dosimetric adjustment factor. Statements in this guidance document raise some important uncertainties in applying allometric scaling, and more specifically $BW^{3/4}$ scaling, when trying to extrapolate across different-sized individuals within a species (e.g., between neonates and adults) or across individuals in different lifestages between species (e.g., between fetal rats and adult humans). Furthermore, the data on which to base a default allometric scaling factor for converting the administered dose in a laboratory animal in a different lifestage to a comparable dose in an adult human are sparse, and thus more uncertain. However, given that the Mountain et al. (1953) study was performed on adult male rats and decreased red blood cells are not a portal-of-entry effect, the uncertainties in applying this allometric scaling are minimal.

An adequate range of animal toxicology data is available for the inhalation hazard assessment of vanadium pentoxide, as described in Section 4. Included in these studies are short-term, subchronic, and chronic bioassays in rats, as well as a range of supporting genotocixity studies. Toxicity associated with inhalation exposure to vanadium pentoxide is observed in reproductive organs, the CNS, and particularly in the respiratory system, including a range of nasal and pulmonary nonneoplastic lesions, such as pulmonary inflammation, tissue morphology changes, and development of pulmonary fibrosis. Recent mechanistic studies have investigated vanadium pentoxide-induced pulmonary fibrosis and have contributed to some understanding of a putative mode of action (MOA) for pulmonary fibrosis in response to vanadium pentoxide.

For derivation of the chronic RfC for vanadium pentoxide, a 2-year inhalation study in rats and mice (NTP, 2002) was selected as the principal study and inflammation of the larynx was selected as the critical effect. Lung hyperplasia, nasal inflammation, and lung fibrosis were also sensitive effects. Inflammation of the larynx was selected as the critical effect because it is a sensitive indicator of vanadium pentoxide-induced respiratory toxicity and yielded the lowest POD.

The selection of the BMD model for quantifying the RfC does not lead to significant uncertainty in estimating the POD because benchmark effect levels were within the range of

experimental data for chronic inflammation of the larynx. Although the selected log-logistic model is the best fitting model, it is not the only model that adequately describes the data. Other models could be selected to yield more extreme results, both higher and lower than those included in this assessment.

Extrapolating from animals to humans entails further issues and uncertainties as the magnitude of the effect and the effect itself associated with the concentration at the POD in rodents are extrapolated to human response. Pharmacokinetic models are useful in examining species differences in pharmacokinetics; a PBPK model for vanadium pentoxide, however, was not available. Therefore, toxicokinetic species differences were addressed by determining an HEC through inhalation dosimetry adjustments as described in the RfC methodology (U.S. EPA, 1994b).

5.4. CANCER ASSESSMENT

5.4.1. Choice of Study/Data—with Rationale and Justification

The 2-year NTP (2002) inhalation cancer bioassay reported an increased incidence of alveolar/bronchiolar adenomas or carcinomas in male F344/N rats. Evidence for carcinogenic activity of vanadium pentoxide in female F344/N rats at the high dose was equivocal. Male and female $B6C3F_1$ mice had greater incidences of these lesions, with a statistically significantly increased incidence of alveolar/bronchiolar adenomas or carcinomas in both male and female $B6C3F_1$ mice following inhalation exposure to $\geq$1 mg/m^3 of vanadium pentoxide (NTP, 2002) (Table 5-8). These tumor types are considered relevant to humans. Human data on the carcinogenic potential of inhalation exposure to vanadium pentoxide are not available. No human or laboratory animal data to determine the carcinogenicity of vanadium pentoxide by the oral or dermal route are available.

5.4.2. Dose-Response Data

Data on the incidences of alveolar/bronchiolar adenomas or carcinomas in male and female mice from the NTP (2002) study were used for cancer dose-response assessment. These data are shown in Table 5-9.

5.4.3. Dose Adjustments and Extrapolation Methods

The NTP (2002) 2-year carcinogenicity study in mice was used to derive an inhalation unit risk (IUR), based on the dose-response relationship for alveolar/bronchiolar neoplasms (adenoma and carcinoma).

Using the RDDR computer program, as specified in the RfC guidelines (U.S. EPA, 1994b), HECs (in mg/m^3) were calculated at each exposure level for male and female mice using the mean body weights for males and females and the average particle size MMAD ± GSD of

Table 5-8. Incidences of respiratory tumors in B6C3F$_1$ mice exposed to vanadium pentoxide in the 2-year inhalation study

Tumor Type[a]	Exposure Group				
	Historical Control (% Historical Control)	Control	1 mg/m^3	2 mg/m^3	4 mg/m^3
Male mice					
Number of animals examined	1,071	50	50	50	50
Alveolar/bronchiolar adenoma[b]	201 (19%)	13 (26%)	16 (32%)	26c (53%)	15 (30%)
Alveolar/bronchiolar carcinoma	97(9%)	12 (24%)	29c (58%)	30c (60%)	35c (70%)
Alveolar/bronchiolar adenoma or carcinoma	285 (26.8%)	22 (28%)	42c (84%)	43c (86%)	43c (86)
Female mice					
Number of animals examined	1,075	50	50	50	50
Alveolar/bronchiolar adenoma	67 (6.3%)	1 (2%)	17[c] (34%)	23[c] (46%)	19[c] (38%)
Alveolar/bronchiolar carcinoma	43 (3.9%)	0 (0%)	23[c] (46%)	18[c] (36%)	22[c] (44%)
Alveolar/bronchiolar adenoma or carcinoma	109 (10.1%)	1 (2%)	32[c] (64%)	35[c] (70%)	32[c] (64%)

[a]Number of animals with tumor; numbers in parentheses indicate percent incidence; particle size mass mean aerodynamic diameter ± geometric standard deviation (MMAD ± GSD): 1 mg/m^3 = 1.3 ± 2.9; 2 mg/m^3 = 1.2 ± 2.9; 4 mg/m^3 = 1.2 ± 2.9.
[b]Historical incidence of alveolar/bronchiolar adenoma male B6C3F$_1$ mice fed in inhalation chamber controls given NIH-07 diet.
[c]Significantly different from control by the Poly-3 test ($p \leq 0.01$)
Source: NTP (2002).

Table 5-9. Human equivalent concentrations of vanadium pentoxide in the 2-year inhalation studies

Concentration as reported (mg/m^3)	Continuous exposure adjustment factor[a]	RDDR[b]	HEC[c] (mg/m^3)
		Pulmonary	Pulmonary
Male mice (B6C3F$_1$)			
0	0.179	1.168	0.00
1	0.179	1.168	0.21
2	0.179	1.168	0.42
4	0.179	1.134	0.81
Female mice (B6C3F$_1$)			
0	0.179	1.168	0.00
1	0.179	1.143	0.20
2	0.179	1.077	0.38
4	0.179	1.023	0.73

[a]Continuous exposure adjustment factor = (6/24) × (5/7); animals were exposed to vanadium pentoxide 6 hr/d, 5 d/wk.
[b]See Appendix Table C-4.
[c]HEC = human equivalent concentration = concentration as reported × continuous exposure adjustment factor × RDDR.
Source: NTP (2002).

1.26 ± 1.87 as reported by NTP (2002). HECs were calculated by multiplying $Conc_{[ADJ]}$ by the RDDR for male and female mice (Table 5-9).

According to the U.S. EPA *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a), for each tumor response, a POD from the observed data should be estimated to mark the beginning of extrapolation to lower doses. The POD is an estimated dose near the lower end of the observed range without significant extrapolation to lower doses.[11] All noncontrol concentrations from the NTP carcinogenesis studies (2002) showed a plateau response, and therefore the dataset provided limited information about the concentration-response relationship because the complete range of response from background to maximum must occur somewhere below the lowest dose. A BMR based on the response at the control concentration and the first noncontrol concentration was therefore calculated (Table C-6) and used to estimate a POD (BMCL, extra risk of 0.71 for male mice, 0.67 for female mice). Modeling was performed using BMDS (Version 2.1.2) developed by the National Center for Environmental Assessment (U.S. EPA, 2000a).

The incidences of alveolar/bronchiolar adenomas and carcinomas in mice were combined the incidence of tumor-bearing animals; males and females were modeled separately (Table 5-10). Models were run using the default restrictions on parameters built into the BMD software. Goodness-of-fit was evaluated using the χ^2 statistic calculated by the BMDS program. Each dataset was first fitted with the dichotomous multistage cancer model; if the goodness-of-fit *p*-value was < 0.05, other dichotomous models were fitted; if still no model showed adequate goodness of fit (*p*-value ≥ 0.05), the highest dose was dropped for further modeling. The BMDS modeling results for models meeting goodness-of-fit criteria are summarized in Appendix C. For incidence data in male mice, the log-logistic model was the only model that met goodness-of-fit criteria when all three dose groups were included, predicting BMC_{71} and $BMCL_{71}$ values of 0.360 and 0.208 mg/m^3, respectively (Appendix C). The multistage model fit the incidence data for male mice when the high dose was dropped, predicting BMC_{71} and $BMCL_{71}$ values of 0.306 and 0.220 mg/m^3, respectively.

None of the dichotomous models fit tumor incidence data for female mice when all three dose groups were included. One model (log-logistic) fit the incidence data for female mice when the high dose was dropped, predicting BMC_{67} and $BMCL_{67}$ values of 0.237 and 0.161 mg/m^3, respectively.

The $BMCL_{71}$ of 0.208 mg/m^3 for male mice was selected as the POD for derivation of the IUR as as this was the only model fit when including all doses for analysis of lung tumor formation following inhalation exposure to vanadium pentoxide.

[11]If the POD is above some data points, it can fail to reflect the shape of the concentration-response curve at the lowest doses and can introduce bias into subsequent extrapolations. If the POD is far below all observed data points, however, model and parameter uncertainty that increase with the distance between the data and the POD can be introduced Using a POD at the lowest level supported by the data tends to balance these considerations.

Table 5-10. BMDS modeling results for combined incidence of alveolar/bronchiolar adenoma and carcinoma in male mice

Model[a]	Goodness of fit				BMR	HEC	
	p-Value	AIC[b]	Largest scaled residual	Scaled residual of interest		BMC (mg/m^3)	BMCL[c] (mg/m^3)
Primary cancer models							
Multistage-cancer stage 1,2 and 3	0.01	207.0	2.05	0.72	Extra Risk 71%	0.532	0.379
Other dichotomous models							
Log-Logistic	**0.19**	**200.6**	**−1.42**	**0.04**	Extra Risk 71%	**0.360**	**0.208**
LogProbit	0.88	199.6	0.13	−0.06		0.146	failed
Gamma	0.01	207.0	2.05	0.72		0.532	0.379
Weibull							
Logistic	0.00	209.4	2.15	0.96		0.609	0.447
Probit	0.00	210.4	2.19	−1.54		0.654	0.495

[a]Selected model is the best-fitting model for the dataset based on the draft Benchmark Dose Technical Guidance (U.S. EPA, 2000a).
[b]HEC = human equivalent concentration.
[c]BMCL = the lower bound of BMC at 95% confidence level.
Source: NTP (2002).

5.4.4. Inhalation Unit Risk

The U.S. EPA *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a) recommend that the method used to characterize and quantify cancer risk from a chemical is determined by what is known about the mode of action of the carcinogen and the shape of the cancer dose-response curve. The linear approach is recommended if the mode of action of carcinogenicity is not understood (U.S. EPA, 2005a). In the case of vanadium pentoxide, data to support an MOA for the carcinogenicity are insufficient, although some data suggest that either a mutagenic or a cytotoxic and reparative proliferation MOA is operative. In the absence of such data, a linear approach was used to extrapolate from the POD to lower doses.

The IUR represents an upper-bound, continuous lifetime exposure risk estimate and is calculated as BMR/BMCL [0.71/(0.208 mg/m^3)]. The HEC $BMCL_{71}$ for extra risk of alveolar/bronchiolar adenomas or carcinomas in male $B6C3F_1$ mice exposed to vanadium pentoxide results in an IUR of 3.4 $(mg/m^3)^{-1}$. This value was derived by linear extrapolation to the origin from the POD of 0.208 mg/m^3 and represents an upper-bound estimate.

5.4.5. Oral Cancer Slope Factor

No human data or animal studies relevant to the carcinogenicity of vanadium pentoxide following oral exposure were located in the published literature. Therefore, an oral cancer slope factor is not derived.

5.4.6. Uncertainties in Cancer Risk Values

Extrapolation of study data to estimate potential risks to human populations from exposure to vanadium pentoxide introduces some uncertainty in the results. Several types of uncertainty can be considered quantitatively, but other important uncertainties cannot be. Section 5.4.5.1 and Table 5-11 summarize principal uncertainties.

Carcinogenicity due to chronic exposure of vanadium pentoxide was observed in two species (NTP, 2002), with the carcinogenicity more definitive in mice than in rats, particularly female rats. For female rats, the increased incidence of neoplasms was lower than what would be expected due to spontaneous tumor formation. Similarly, spontaneous tumors were observed in male rats at control levels, although tumor incidence increased in male rats in response to exposure to vanadium pentoxide. The confidence in the database is low. In the well-conducted NTP study (2002), respiratory tract carcinogenicity was reported in two rodent species (i.e., mice and rats), which was supported by a study in mice (Rondini et al., 2010). Human variability in response to vanadium pentoxide, on the other hand, is unknown, and humans occupationally exposed to vanadium pentoxide are often simultaneously exposed to other valence states of vanadium and to other inhaled environmental toxicants simultaneously. Genotoxicity is equivocal and no further MOA information is available. Overall confidence in the IUR is low.

Choice of low-dose extrapolation approach. The MOA is a key consideration in clarifying how risks should be estimated for low-dose exposure. A linear low-dose extrapolation approach was used to estimate human carcinogenic risk associated with vanadium pentoxide exposure due to the unavailability of data to support any specific mode of carcinogenic action of vanadium pentoxide.

Dose metric. Vanadium exists in the +5 valence state in vanadium pentoxide. Other valence states ranging from −1 to +5 exist. Frequently, vanadium exposures involve a mixture of vanadium compounds ranging primarily from +3 to +5 valence states. The carcinogenic potential of other valence states of vanadium has not been established. Whether vanadium pentoxide dissociates to other valence states with known carcinogenic potential is unknown. Similarly, whether some other valence state or some combination of +5 and other valence states is responsible for the observed toxicity is not known. If the actual carcinogenic moiety is proportional to administered exposure, then use of administered exposure as the dose metric is the preferred choice.

Statistical uncertainty at the POD. Parameter uncertainty can be assessed by examining confidence intervals. Each description of parameter uncertainty assumes that the underlying model and associated assumptions are valid. For the log-logistic cancer model applied to the male mice data, the degree of uncertainty at a 71% increase in tumor incidence (the POD for linear low-dose extrapolation) is reasonably small.

Bioassay selection. The NTP (2002) study was selected for development of an IUR. This study was well designed and conducted on both sexes in two species with an adequate

number of animals per dose group. The number of test animals allocated among the two dose levels and the untreated control group was adequate, with examination of appropriate toxicological endpoints in both sexes of rats and mice. Both genders of mice exhibited a statistically significant increased incidence of lung tumors.

Choice of species/gender. The IUR for vanadium pentoxide was quantified using the tumor incidence data for male mice, which were more amenable to BMD modeling than the tumor incidence in female mice. In addition, lung neoplasm incidence was reported in male and female rats, although neither gender of rat was as sensitive as mice. A 71% tumor incidence level was observed in female mice at the lowest exposure level (1 mg/m^3), suggesting that lower doses might have revealed more information about the low-dose region of the dose-response curve. Male mice demonstrated a high background rate of lung tumors, with spontaneous lung neoplasms observed in male mice at control levels (up to 28% of male mice). Tumor incidence increased significantly (84%) at the lowest dose level tested (1 mg/m^3). Although these incidence response rates were higher in male mice than in females at the comparable exposure level, suggesting greater sensitivity of the male mice, there is no information for the dose-response relationships at lower exposure levels. In other words, the behavior of vanadium pentoxide at 1.0 mg/m^3 in male mice might not inform the tumor response to vanadium pentoxide at lower exposures.

Relevance to humans. In the absence of direct human data, the most appropriate animal bioassays to use in the derivation of cancer risk values are chronic (i.e., lifetime) studies in two species of rodents. The IUR was derived from the combined tumor incidences of lung adenomas and carcinomas in male mice. Information investigating the MOA of the lung tumors observed in the chronic animal bioassay, however, is limited. Genotoxicity studies provide inadequate evidence of a genotoxic MOA, and data to support alternative MOA hypotheses are inadequate.

Human population variability. Interindividual variability in animals for vanadium pentoxide metabolism has not been characterized. Strain differences in the response to vanadium pentoxide-induced pulmonary fibrosis in rodents suggests a genetic component to susceptibility. Moreover, humans occupationally exposed to vanadium pentoxide are often simultaneously exposed to other valence states of vanadium and to other inhaled environmental toxicants simultaneously. This lack of understanding about potential differences in metabolism and susceptibility across exposed human populations thus represents a source of uncertainty.

Table 5-11. Summary of uncertainty in the vanadium pentoxide cancer risk assessment

Consideration/ Approach	Impact on inhalation unit risk	Decision	Justification
Low-dose extrapolation procedure	Departure from EPA's *Guidelines for Carcinogen Risk Assessment (2005a)* POD paradigm, if justified, could ↓ or ↑ slope factor by an unknown extent	Log-logistic cancer model to determine POD, linear low-dose extrapolation from POD	Available MOA data do not inform selection of dose-response model.
Dose metric	Alternatives could ↑ or ↓ slope factor by an unknown extent	Used administered exposure	Experimental evidence supports a role of other valence states of vanadium in the body on health effects, but no data exist to support carcinogenicity due to other forms of vanadium.
Cross-species scaling	Alternative methods could ↓ or ↑ inhalation unit risk [e.g., 3.5-fold ↓ (scaling by BW) or ↑ 2-fold (scaling by $BW^{2/3}$)]	RDDR	RDDR software was used to adjust for toxicokinetic differences in inhalation dosimetry.
Statistical uncertainty at POD	↓ slope factor by 1.7-fold if BMC used rather than the BMCL	BMCL (method for calculating reasonable upper-bound slope factor)	The lower bound is the 95% confidence interval on administered exposure.
Bioassay	Alternatives could ↑ or ↓ slope factor by an unknown extent	NTP study	Alternative bioassays were unavailable.
Species/gender combination	Human risk could ↓ or ↑, depending on relative sensitivity	Male mice lung cancer	The lung cancer response was concordant in male and female mice. There are no MOA data to inform the extrapolation approach for any choice. Humans were assumed to be as sensitive as the most sensitive rodent gender/species tested; true correspondence is unknown. The carcinogenic response occurs across genders and species.
Human relevance of mouse tumor data	Human relevance of mouse tumor data could ↓ slope factor	Lung tumors in mice are relevant to human exposure	Vanadium pentoxide could be carcinogenic through an unknown MOA.
Human population variability in metabolism and response/sensitive subpopulations	Low-dose risk ↑ to an unknown extent	Considered qualitatively	No data are available to support the range of human variability/sensitivity.

6. MAJOR CONCLUSIONS IN THE CHARACTERIZATION OF HAZARD AND DOSE RESPONSE

6.1. HUMAN HAZARD POTENTIAL

Vanadium is a metal commonly found in ores, tars, coals, and oils and is used as an alloy in steel. The toxicity of vanadium depends on its valence state, which can range from −1 to +5, depending on pH and other factors. Vanadium pentoxide (V_2O_5), sodium metavanadate ($NaVO_3$), and ammonium metavanadate (NH_4VO_3) all contain vanadium in the +5 oxidation state. This Toxicological Review focuses exclusively on vanadium pentoxide (V_2O_5), the most common form of vanadium used commercially. In addition, V_2O_5 is the only compound that is covalently bonded. Occupational exposure to vanadium pentoxide occurs primarily via inhalation of dust generated during vanadium processing and fuel-oil ash generation during cleaning of oil-burning boilers and furnaces.

The toxicokinetics of orally administered vanadium pentoxide are not available in humans. Kucera et al. (1992) attempted to measure vanadium in the hair of children who had been exposed to vanadium in drinking water, but exposure could not be linked to vanadium pentoxide specifically. Oral toxicokinetic studies in animals are limited. One study investigated the toxicokinetics of components of various metals, including vanadium, in female $B6C3F_1$ mice (Radike et al., 2002). In this study, vanadium was detected in the small intestine, kidney, and femur.

Similarly, a few studies have evaluated the toxicokinetics of inhaled vanadium, but there are no data for humans. Occupational studies of inhaled vanadium pentoxide indicate that vanadium is absorbed by humans, and levels in blood and urine rapidly declined following exposure (Kiviluoto et al., 1981b). Results of toxicokinetic studies of inhaled or intratracheally administered vanadium pentoxide in rats show that vanadium pentoxide is absorbed from the lung, undergoes a wide distribution to the liver, kidney, bone, blood, gastrointestinal (GI) tract, and ovary, and is eliminated primarily in the urine (Dill et al., 2004; NTP, 2002; Roshchin et al., 1980).

The animal oral toxicity database reported decreased hair cystine (Mountain et al., 1953; Stokinger et al., 1953); hematological effects, including a decrease in red blood cell (RBC) count and hemoglobin (Mountain et al., 1953); and a variety of reproductive and developmental effects following exposure to vanadium pentoxide (Altamirano-Lozano et al., 1993)(Zhang et al., 1993a; 1993b).

Studies of human exposure to inhaled vanadium pentoxide include primarily occupational reports and one controlled human exposure study. Several case study summaries reported upper and lower respiratory tract irritation and inflammation among workers with inhalation exposure to vanadium pentoxide and other vanadium compounds in dust during vanadium processing or to fuel-oil ash during cleaning and maintenence of oil-burning boilers (Section 4.1.2). The

chemical composition of the fuel-oil ash and vanadium dust or exposure measurements for vanadium pentoxide, however, generally were not reported. Thus, in many cases, specific relationships between vanadium pentoxide and adverse respiratory effects cannot be definitively determined. Sjöberg (1955) published seven case reports of vanadium-induced bronchitis in workers cleaning boilers where the concentrations of vanadium pentoxide particles (10–20 μm in diameter) in the air were 2–85 mg/m^3. Self-reported respiratory symptoms included cough, rhinitis, wheeze, sore throat, and conjunctivitis. All symptoms resolved by 2 weeks postexposure, but reappeared upon re-exposure. Of 100 workers occupationally exposed to vanadium pentoxide fumes (0.05–5.3 mg/m^3) via an oil-to-coal power-plant conversion, 74 reported severe respiratory tract irritation (Levy et al., 1984). Estimated daily nasal and lung doses of vanadium were associated with incidence and severity of upper airway symptoms (nasal congestion/irritation, throat irritation) and lower airway symptoms (chest tightness, wheeze, cough, and sputum production) in a dose-related manner in a prospective clinical study. This clinical study involved boilermakers and utility workers overhauling a large, oil-fired boiler over a 6-week period (Woodin et al., 2000; Woodin et al., 1999; Woodin et al., 1998). Geometric mean concentrations of vanadium (9–10 hour shifts) measured in the breathing zone ranged from 1.1 to 8.9 μg/m^3. Reductions in pulmonary function were measured among boilermakers with exposure to fly ash for weeks or years but an association with vanadium has not been established (Hauser et al., 2001; Woodin et al., 1999; Hauser et al., 1995b). No cases of cancer were reported in workers examined over a sufficient latency period as a result of exposure to vanadium pentoxide.

Respiratory effects in experimental animals are well documented and include lesions in the nasal compartment, larynx, and lung. In addition to respiratory effects observed in animals (Yu et al., 2011; Turpin et al., 2010; NTP, 2002; Knecht et al., 1992; Knecht et al., 1985), some animal studies document spermatogenesis changes and testicular ultrastructural changes (Mussali-Galante et al., 2005) and central nervous system (CNS) effects (Colín-Barenque et al., 2007; Avila-Costa et al., 2006; Avila-Costa et al., 2005; Avila-Costa et al., 2004) in response to inhaled vanadium pentoxide. Short-term inhalation exposure (16 days) in F344/N rats and B6C3F$_1$ mice was associated with histiocytic infiltration at 1.0 mg/m^3, and short-term and subchronic inhalation exposures (3 months) in F344/N rats and B6C3F$_1$ mice were associated with increased lung epithelial hyperplasia and inflammation at 2.0 mg/m^3 (NTP, 2002). Long-term exposure (2 years) in F344/n rats and B6C3F$_1$ mice similarly resulted in increased incidence of respiratory toxicity and carcinogenicity (NTP, 2002). Lung inflammation was observed at 1.0 mg/m^3 and lung hyperplasia was observed at 2.0 mg/m^3 in female rats. Lung inflammation and hyperplasia were observed at 1.0 mg/m^3 in both male and female mice. In male and female mice, nonneoplastic lesions also occurred in the larynx (squamous metaplasia of epiglottis epithelium) and nasal tissues (degeneration and atrophy of olfactory epithelium and degeneration of respiratory epithelium) at 1 mg/m^3; it should be noted that 1 mg/m^3 was the

lowest dose in mice. In mice exposed to 1 mg/m^3, lesions were observed in the nose, bronchioles, and lung of male mice and the nose, larynx, bronchioles, and lung of female mice. Chronic, active inflammation and interstitial fibrosis was observed in male rats at a lowest-observed-adverse-effect level (LOAEL) of 1 mg/m^3 (no-observed-adverse-effect level [NOAEL] was 0.5 mg/m^3). The nasal and laryngeal lesions appear to be among the most sensitive effects observed at the lowest dose tested. Moreover, multiple lesion types were observed in both sexes of rats and, thus, the LOAEL of 0.5 mg/m^3 for nasal and laryngeal lesions was selected as the critical effect.

Reproductive and developmental studies reveal various altered endpoints in response to vanadium pentoxide via oral and inhalation routes. Vanadium pentoxide delivered orally to weanling rats for 3 days caused a significant increase in alkaline phosphatase activity and DNA content in the diaphysis of femoral bones, suggesting that vanadium pentoxide might be linked to bone formation in the developing rat (Yamaguchi et al., 1989). Mussali-Galante et al., (2005) identified accumulation of vanadium pentoxide in the testes after 1 week of inhalation exposure in male CD-1 mice (0.02 M or 1.4 mg V/m^3). Gamma tubulin was significantly decreased in testes exposed to vanadium pentoxide and might suggest changes in microtubule function that could impact spermatogenesis. In a follow-up study, Fortoul et al. (2007) reported necrotic cell death in spermatogonia and increased nuclear distortion in spermatocytes in male CD-1 mice exposed by inhalation to vanadium pentoxide (0.02 M or 1.4 mg V/m^3).

Several intraperitoneal (i.p.) studies have evaluated reproductive and developmental endpoints. Common reproductive effects include increased reproductive organ weights in male rats (Altamirano et al., 1991), increased incidence of apoptotic spermatogonia in male mice (Aragon et al., 2005) and reduced sperm motility, reduced sperm count, and increased numbers of abnormal sperm in male CD-1 mice (Altamirano-Lozano et al., 1996). In developmental studies, reduced ossification in the developing forelimbs and hindlimbs of fetuses have been reported in response to i.p. exposure to vanadium pentoxide during gestation days 6–15 in pregnant CD-1 mice (Altamirano-Lozano et al., 1993) and Wistar rats (Zhang et al., 1993a; 1993b). Other reported effects range from reduced fetal weight and increased placental weight.

The genotoxicity database for vanadium pentoxide is limited. The evidence for genotoxicity following exposure to vanadium pentoxide in humans is limited. A few studies have examined genotoxicity in humans in vivo, with equivocal results. Ivancsits et al. (2002) reported no differences in DNA strand breaks, oxidative damage, or SCE frequency in leukocytes between control and vanadium pentoxide-exposed workers. Ehrlich et al., (2008) noted changes in DNA stability and DNA repair in leukocytes of occupationally exposed workers as compared to controls. Studies have demonstrated a genotoxic effect of vanadium pentoxide on human cells in vitro. Ivancsits et al. (2002) demonstrated significant increases in DNA damage as measured by the Comet assay in both leukocytes and fibroblasts but with different dose sensitivity, while Kleinsasser et al (2003) noted DNA migration differences

occurred dose dependently in peripheral blood lymphocytes but not in nasal mucosa cells. Earlier studies in human lymphocyte cultures also demonstrated increased aneuploidy (Ramirez et al., 1997; Rojas et al., 1996) and DNA damage (Roldán and Altamirano, 1990) following exposure to vanadium pentoxide. Thus, vanadium pentoxide-induced mutagenicity may occur at doses higher than those measured in these occupational exposures, may be tissue-specific, and may be associated with oxidative stress rather than direct DNA damage. Experimental data in animals provide evidence of some types of genotoxicity following in vivo exposure to vanadium pentoxide. Vanadium pentoxide administered by inhalation to mice or rats did not increase the frequency of micronucleated normochromatic erythrocytes in peripheral blood (NTP, 2002). Genotoxicity assessed in male CD-1 mice following i.p. injection caused no treatment-related effects in mitotic index, average generational time, or SCE (Altamirano-Lozano et al., 1993). DNA damage was detected, however, in six organs from vanadium pentoxide-treated mice via i.p. injection (Altamirano-Lozano et al., 1999). Vanadium pentoxide produced gene mutations in two bacterial test systems (Kada et al., 1980; Kanematsu et al., 1980) but negative results in the NTP (2002) study. Vanadium pentoxide produced DNA strand breaks, aneuploidy, and micronucleus induction but did not produce chromosomal aberrations or SCE in various cell lines (Kleinsasser et al., 2003; Ivancsits et al., 2002; Ramirez et al., 1997; Rojas et al., 1996; Zhong et al., 1994; Roldán and Altamirano, 1990).

Most effects of vanadium pentoxide are thought to be produced by the parent compound, primarily by inducing cell damage and pulmonary fibrosis (NTP, 2002; Bonner et al., 2000; Bonner et al., 1998). Pulmonary fibrosis is mediated either directly by vanadium pentoxide-induced changes to cell signaling molecules or via vanadium pentoxide-induced oxidative stress that induces cellular changes leading to fibrosis (Ingram et al., 2003; Rice et al., 1999; Bonner et al., 1998). Pulmonary fibrosis could be a key event leading to eventual tumorigenesis.

Vanadium pentoxide is "likely to be carcinogenic to humans" by the inhalation route of exposure under the *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a). Existing cancer data come from two animal species (mice, rats) reported in one well-characterized study (NTP, 2002). Increased incidence of pulmonary adenomas and carcinomas were observed in male and female mice exposed to vanadium pentoxide for 2 years. Carcinogenicity in male rats exposed to vanadium pentoxide for 2 years is evident, as respiratory tumor incidence exceeds that in historical controls. In female rats, the incidence of respiratory tumorigenesis in response to vanadium pentoxide does not exceed historical controls. Tumor incidence has not been documented in humans.

Information available on the carcinogenic effects of vanadium pentoxide via the inhalation route is limited to examination of respiratory tumors. Information on the carcinogenic effects of vanadium pentoxide via the oral and dermal routes in humans or animals is absent. Based on the observation of only respiratory tumors following inhalation exposure, and in the

absence of information to establish a mode of action (MOA), this cancer descriptor applies only to the inhalation route of exposure. Therefore, the database has "inadequate information to assess carcinogenic potential" of vanadium pentoxide via the oral or dermal route.

The MOA underlying tumorigenicity in rats and mice has not been established. The genotoxicity database for vanadium pentoxide is equivocal, including studies that report both positive and negative results for mutation, DNA damage, and chromosomal aberrations. A nongenotoxic MOA hypothesis involving hyperplasia and development of fibrotic pulmonary lesions is supported by the presence of hyperplastic lesions and pulmonary fibrosis at earlier time points and at lower doses. The dose-response relationship is not robust, however, and a clear relationship linking these effects to the tumor response has not been established. Whether cytotoxicity is a required precursor event for vanadium pentoxide-induced cell proliferation is unknown. Sufficient data regarding a plausible dose response and temporal progression from cytotoxicity to hyperplasia to fibrosis to tumorigenesis are not available.

6.2. DOSE RESPONSE

6.2.1. Noncancer

Limited studies are available examining the toxicity of vanadium pentoxide following oral exposure in humans or laboratory animals. Decreased RBC count and hemoglobin were observed following subchronic oral exposure to vanadium pentoxide in rats (Mountain et al., 1953). The authors reported the decrease of RBC as a mean with no measure of variability among animals and the continuous data therefore were not amenable to benchmark dose (BMD) modeling. Derivation of an oral reference dose (RfD) was based on a NOAEL of 10.5 mg/kg-d for the critical effect, decreased RBC counts, and divided by a total uncertainty factor (UF) of 3,000: 3 to represent interspecies toxicodynamic uncertainties, 10 for interhuman variability in the absence of quantitative information on the variability of response in humans, 10 for extrapolation from a subchronic to a chronic study, and 10 for database deficiencies. The result was the chronic **RfD of 9×10^{-4} mg/kg-day**.

Confidence in the principal study for derivation of the RfD (Mountain et al., 1953) is low. Mountain et al. (1953) is a well-conducted study with numerous doses, but is a subchronic study with a small sample size (n = 5) for one species of rodents (Wistar rat), using one gender (male), and limited endpoints and time points. Confidence in the overall database, however, is low. Mountain et al. (1953) is the single, relevant, peer-reviewed study for derivation of the chronic RfD. Thus, overall confidence in the chronic RfD is low.

Pulmonary effects have been documented in numerous species, including humans and primates, in response to inhaled vanadium pentoxide. Inflammation and histiocytic infiltrate are common hallmarks of initial vanadium-induced pulmonary injury. The database also includes occupational (inhalation) and laboratory animal (inhalation and oral) studies demonstrating possible immunotoxicity following exposure to vanadium pentoxide. In addition, the database

includes studies of neurotoxicity and reproductive and developmental toxicity studies following inhalation exposure to vanadium pentoxide in rodents. Overall, the lung is the most sensitive target for noncancer toxicity in rats and mice following chronic inhalation exposure to vanadium pentoxide. Nonneoplastic lung lesions (specifically laryngeal lesions) were selected as the most sensitive endpoint from a well-conducted chronic study (NTP, 2002) (2-year rodent bioassay). The chronic inflammation of the larynx and epithelial hyperplasia of the epiglottis in rats were chosen as critical effects because they are the most sensitive effects and the most proximal to route of exposure (inhalation). These effects were observed in both male and female rats and mice.

The dose-response pattern for laryngeal lesions (NTP, 2002) was amenable to BMD modeling and was used for derivation of the chronic inhalation reference concentration (RfC). Two laryngeal lesions were selected for modeling because they had the lowest regional deposited dose ratio (RDDR) and corresponding benchmark concentration lower limit (adjusted for dosimetric differences across species to humans; $BMCL_{[HEC]}$). In accordance with U.S. EPA BMD methodology (2000a), a benchmark response (BMR) of 10% increase in extra risk was selected to represent a minimally adverse level. All available dichotomous models were fit to the incidence data for chronic inflammation and for epithelial hyperplasia of the epiglottis. The model with the lowest Akaike's information criterion value was considered to provide a superior fit. BMD modeling of incidence data for chronic inflammation of the larynx and epithelial hyperplasia of the epiglottis in female rats yielded the same $BMCL_{10}$ of 0.003 mg/m^3. The shared $BMCL_{10}$ of 0.003 mg/m^3 for either chronic inflammation of the larynx or epithelial hyperplasia of the epiglottis was divided by a total UF of 300: 3 to represent interspecies toxicodynamic uncertainties, 10 for interhuman variability in the absence of quantitative information on the variability of response in humans, and 10 for database deficiencies. The result was the chronic **RfC of 1×10^{-5} mg/m^3**.

Confidence in the principal study for derivation of the RfC (NTP, 2002) is high. NTP (2002) is a well-conducted study with numerous doses, large sample sizes of two species of rodents using both genders, and numerous endpoints and time points. Confidence in the overall database, however, is medium. NTP (2002) remains the single relevant study for use in the derivation of the chronic RfC. Thus, overall confidence in the chronic RfC is medium.

6.2.2. Cancer

No studies have identified cancer effects following oral exposure to vanadium pentoxide in either humans or animals, or following inhalation exposure in humans.

Under the *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a), the database for vanadium pentoxide indicates that it is "likely to be carcinogenic to humans" via the inhalation route of exposure. This determination is based predominantly on the NTP (2002) study, which found positive evidence of lung tumors in both sexes of mice and male rats after

chronic vanadium pentoxide inhalation exposure. This weight-of-evidence conclusion takes into consideration the NTP (2002) cancer bioassay, the available human studies, and other laboratory animal studies. Information available on the carcinogenic effects of vanadium pentoxide via the inhalation route is limited to examination of the respiratory tumors. Information on the carcinogenic effects of vanadium pentoxide via the oral and dermal routes in humans or animals is absent. Based on the observation of only respiratory tumors following inhalation exposure, and in the absence of information to establish an MOA, this cancer descriptor applies only to the inhalation route of exposure. Therefore, the database has "inadequate information to assess carcinogenic potential" of vanadium pentoxide via the oral or dermal route.

The increased incidence of lung tumors in male mice observed in the NTP (2002) 104-week inhalation study was used to calculate the inhalation unit risk (IUR) for vanadium pentoxide. The calculated cancer IUR for vanadium pentoxide is 3.4 $\mu g/m^3$ for the development of alveolar/bronchiolar adenomas or carcinomas in male $B6C3F_1$ mice. This value was derived from $BMCL_{10}$, the 95% lower bound on the dose associated with 71% extra cancer risk of respiratory carcinoma in male $B6C3F_1$ mice, by dividing the BMR (0.71) by the $BMCL_{10}$ and represents the upper bound, continuous lifetime exposure estimate of cancer potency. The $BMCL_{10}$, lower 95% bound on exposure at 71% risk, is 2.08×10^{-1} mg/m^3 and the slope of the linear extrapolation from the BMCL to the origin, which is equal to $0.71/2.08 \times 10^{-1}$ mg/m^3, or 3.4 $(mg/m^3)^{-1}$. A linear low-dose extrapolation approach was used to estimate human carcinogenic risk associated with vanadium pentoxide exposure due to a lack of data that support any specific mode of carcinogenic action of vanadium pentoxide. Therefore, the derived **IUR to vanadium pentoxide is 3.4 per mg/m^3**.

Areas of uncertainty exist for this cancer assessment. The log-logistic model was selected to model lung tumor incidence in male mice; how well this model or the linear low-dose extrapolation predicts low-dose risks for vanadium pentoxide, however, is unknown. The selected model, although the best-fitting, is not the only model that adequately describes the data. Conceivably, other models could be selected that yield different results consistent with the observed data, both higher and lower than those included in this assessment. The human equivalent IURs estimated from the statistically significant increase in lung tumors ranged from 1.4 mg/m^3 in male mice to 4.2 mg/m^3 in female mice. These tumors are considered to be relevant to humans. As there is no information to inform which species or gender of animals would be most applicable to humans, the most sensitive group was selected for the basis of the IUR.

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APPENDIX A. SUMMARY OF EXTERNAL PEER REVIEW AND PUBLIC COMMENTS AND DISPOSITION

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APPENDIX B. BENCHMARK CONCENTRATION MODELING OF INHALATION STUDIES IN RATS FROM NTP, 2002

To derive the reference concentration (RfC) for vanadium pentoxide, inhalation toxicity effects observed in rats (NTP, 2002) were modeled to estimate the candidate points of departure (PODs). Five endpoints in both male and female rats (see Table B-2) were selected for two reasons:

- The study in rats was designed with lower concentrations of vanadium pentoxide.
- The results showed both biological significance and statistically significant trends.

Each dataset was fit with the dichotomous models available in EPA Benchmark Dose Software (BMDS; version 2.1.2). Following the model selection steps outlined in the draft *Benchmark Dose Technical Guidance* (U.S. EPA, 2000), the best-fitting model for each dataset was used to estimate the candidate POD, which was the benchmark concentration (BMC) lower limit (BMCL) at the selected benchmark response (BMR) as 10% extra risk.

The highlights of the benchmark dose (BMD) modeling results are:

- The POD value of 0.003 mg/m^3, from two endpoints in female rats, was the lowest POD derived and was used for calculating the RfC. The two endpoints were larynx chronic inflammation and larynx respiratory epithelium epiglottis hyperplasia.
- All candidate PODs and the selected models are summarized in Table B-1.

B.1. TREND TESTS ON THE INHALATION DATASETS FOR BMDS MODELING

Ten inhalation toxicology datasets in rats were selected because of the lower concentrations of vanadium pentoxide administrated (as compared to mice), the statistically significant biologically adverse effects observed, and the statistical significance reported by NTP (2002). A Cochran-Armitage test was conducted to confirm the significance of the statistical trend for the selected datasets (Table B-2) before modeling.

Table B-1. Candidate PODs for vanadium pentoxide derived from NTP studies (2002) through BMDS modeling

Endpoint	Selected model[a]	BMR (extra risk)	HEC[b]	
			BMC (mg/m^3)	BMCL[c] (mg/m^3) (candidate POD)
Male F344/N Rats				
Lung				
Alveolar epithelium hyperplasia	Probit	0.1	0.016	0.013
Chronic active inflammation	Logistic	0.1	0.035	0.029
Larynx				
Chronic inflammation	Log-Logistic	0.1	0.017	0.012
Respiratory epithelium, epiglottis, hyperplasia	Log-Logistic	0.1	0.008	0.006
Nose				
Goblet cell, respiratory epithelium, hyperplasia	Log-Logistic	0.1	0.044	0.026
Female F344/N Rats				
Lung				
Alveolar epithelium hyperplasia	Gamma	0.1	0.076	0.063
Chronic active inflammation	Multistage (Stage 3)	0.1	0.080	0.048
Larynx				
Chronic inflammation	Log-Logistic	0.1	0.005	0.003
Respiratory epithelium, epiglottis, hyperplasia	Log-Logistic	0.1	0.004	0.003
Nose				
Goblet cell, respiratory epithelium, hyperplasia	Multistage (Stage 2)	0.1	0.038	0.014

[a]Selected model is the best-fitting model for the dataset based on the draft *Benchmark Dose Technical Guidance* (U.S. EPA, 2000).
[b]HEC = human equivalent concentration.
[c]BMCL = the lower bound of BMC at 95% confidence level.

Table B-2. Trend tests on the selected datasets from the 2-year inhalation studies in rats

Endpoint	Concentration as reported (mg/m^3)[a]				Trend test[b]	
	0.0	0.5	1.0	2.0	*Z*-score	*p*-value
Incidence[c] in male F344/N rats						
Lung						
Alveolar epithelium hyperplasia[d]	7/50	24/49	35/48	50/50	8.83	<0.0001
Chronic active inflammation	5/50	8/49	24/48	42/50	8.36	<0.0001
Larynx						
Chronic inflammation	3/49	20/50	17/50	28/49	4.79	<0.0001
Respiratory epithelium, epiglottis hyperplasia	0/49	18/50	34/50	32/49	-6.56	<0.0001
Nose						
Goblet cell, respiratory epithelium, hyperplasia	4/49	15/50	12/49	17/48	2.68	0.0037

Table B-2. Trend tests on the selected datasets from the 2-year inhalation studies in rats

Endpoint	Concentration as reported (mg/m^3)[a]				Trend test[b]	
	0.0	0.5	1.0	2.0	*Z*-score	*p*-value
Incidence[c] in female F344/N rats						
Lung						
Alveolar epithelium hyperplasia[d]	7/49	8/49	21/50	50/50	9.53	<0.0001
Chronic active inflammation	10/49	10/49	14/50	40/50	6.71	<0.0001
Larynx						
Chronic inflammation	8/50	26/49	27/49	37/50	5.38	<0.0001
Respiratory epithelium, epiglottis hyperplasia	0/50	25/49	26/49	33/50	-5.98	<0.0001
Nose						
Goblet cell, respiratory epithelium, hyperplasia	13/50	19/50	16/50	30/50	3.46	0.0003

[a]Concentrations are as reported by NTP (2002).
[b]One-sided Cochran-Armitage trend test.
[c]Incidence = (number of animals affected)/(number of animals examined).
[d]This dataset was calculated by combining the incidences of alveolar epithelium hyperplasia and bronchiole epithelium hyperplasia.

Source: NTP (2002).

B.2. DOSE CONVERSION

In the toxicology and carcinogenesis studies reported by NTP (2002), rats (F344/N) were exposed to vanadium pentoxide through inhalation. To analyze the concentration-response effect of vanadium pentoxide, the reported concentrations of vanadium pentoxide were converted to human equivalent concentrations (HECs) before any modeling and extrapolation were completed.

First, the average lifetime animal body weights of rats were estimated based on the mean body weight at different weeks (Table B-3). Then, following the *Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry* (U.S. EPA, 1994), regional deposited dose ratios (RDDRs) were calculated with the RDDR program designed by US EPA (1994). Although six regional RDDRs were reported by the RDDR program for each concentration/sex group, only two regional RDDRs (i.e., extrathoracic and pulmonary) are relevant to the selected endpoints, which are summarized in Table B-4. Finally, the HEC for each concentration/sex group was calculated from the reported concentration in rats by multiplying the continuous exposure adjustment factor and RDDR (Table B-5). For endpoints in the lung, the pulmonary RDDR was applied; for endpoints in the larynx and nose, the extrathoracic RDDR was applied.

Table B-3. Average lifetime animal body weight of rats in the 2-year inhalation studies of vanadium pentoxide

Vanadium pentoxide concentration as reported (mg/m^3)	Mean animal body weight[a] (g)			Average lifetime animal body weight[b] (g)
	1–13 wk	14–52 wk	53–104 wk	
Male rats (F344/N)				
0	238	411	504	440
0.5	241	422	513	449
1	242	414	508	443
2	233	404	494	432
Female rats (F344/N)				
0	151	227	326	269
0.5	150	229	319	266
1	150	227	326	269
2	147	217	308	256

[a]As reported in Table 11-12 of the NTP report (2002).
[b]Average lifetime animal body weight = (mean body weight 1-13 weeks × 13 + mean body weight 14−52 weeks × 39 + mean body weight 53−104 weeks × 52)/104.
Source: NTP (2002).

Table B-4. RDDRs for various concentration/sex groups in the 2-year inhalation studies of vanadium pentoxide

Vanadium pentoxide concentration as reported (mg/m^3)	Average life time animal body weight[a] (g)	Average MMAD[b]	Average GSD[c]	RDDR[d]	
				Extrathoracic	Pulmonary
Male rats (F344/N)					
0	440	1.24	1.89	0.516	0.496
0.5	449			0.530	0.494
1	443			0.520	0.495
2	432			0.503	0.498
Female rats (F344/N)					
0	269	1.24	1.89	0.263	0.524
0.5	266			0.259	0.524
1	269			0.263	0.524
2	256			0.245	0.524

[a]All average lifetime animal body weights were calculated in Table B-3.
[b]MMAD = mass median aerodynamic diameter; calculated based on the MMADs reported for 2-year studies (NTP, 2002).
[c]GSD = geometric standard deviation; calculated based on the GSDs reported for 2-year studies (NTP, 2002).
[d]RDDR = regional deposited dose ratio; calculated with the RDDR program (V.2.3. US EPA).
Source: NTP (2002).

Table B-5. Human equivalent concentrations of vanadium pentoxide in the 2-year inhalation studies

Concentration as reported[a] (mg/m^3)	Continuous exposure adjustment factor[b]	RDDR[c]		Human equivalent concentration[d] (mg/m^3)	
		Extrathoracic	Pulmonary	Extrathoracic	Pulmonary
Male rats (F344/N)					
0	0.179	0.516	0.496	0.00	0.00
0.5	0.179	0.530	0.494	0.05	0.04
1	0.179	0.520	0.495	0.09	0.09
2	0.179	0.503	0.498	0.18	0.18
Female rats (F344/N)					
0	0.179	0.263	0.524	0.00	0.00
0.5	0.179	0.259	0.524	0.02	0.05
1	0.179	0.263	0.524	0.05	0.09
2	0.179	0.245	0.524	0.09	0.19

[a]*Toxicology and carcinogenesis studies of vanadium pentoxide in F344/N rats and B6C3F$_1$ mice*, NTP (2002).
[b]Continuous exposure adjustment factor = (6/24) × (5/7); animals were exposed to vanadium pentoxide 6 hours per day, 5 days per week.
[c]Refer to Appendix Table B-4.
[d]Human equivalent concentration = concentration as reported × continuous exposure adjustment factor × RDDR.
Source: NTP (2002).

B.3. BMDS MODELING FOR INHALATION DATASETS

Each selected inhalation dataset was fit with all the standard dichotomous models available in EPA BMDS (version 2.1.2.). Following the model selection steps outlined in the *Benchmark Dose Technical Guidance* (U.S. EPA, 2000), the best-fitting model was used to estimate the candidate POD for each endpoint, which was the BMCL at the selected BMR as 10% extra risk.

The selected models and candidate PODs for all endpoints are summarized in Table B-1.

B.3.1. Dataset 1: Incidence of Lung Alveolar Epithelium Hyperplasia in Male Rats, NTP (2002)

Summary

All three concentration groups (0.5, 1, and 2 mg/m^3) showed statistically significant differences from the control as reported. The severity of the nonneoplastic lesions increased from mild to moderate as reported when the concentration of vanadium pentoxide increased. The Cochran-Armitage test confirmed the statistically significant trend with a Z-score = 8.83 and one-sided p-value < 0.0001.

Seven dichotomous models available in EPA BMDS (version 2.1.2.) were fit to this dataset. A 10% extra risk of the combined incidence was used as the BMR to determine the POD. The goodness of fit, BMC, and BMCL for each model are summarized in Table B-6.

Five models demonstrated adequate goodness of fit (p-value ≥ 0.1) and good visual fit. Based on the draft *Benchmark Dose Technical Guidance* (U.S. EPA, 2000), the Probit model was selected as the best-fitting model for this dataset. Using this model, the $BMCL_{10}$ was 0.013 mg/m^3 and this value was regarded as a candidate for the POD.

Table B-6. Benchmark modeling results for incidence of lung alveolar epithelium hyperplasia in male rats

Model[a]	Goodness of Fit				BMR	HEC[c]	
	p-Value	AIC[b]	Largest scaled residual	Scaled residual of interest		BMC (mg/m^3)	BMCL[d] (mg/m^3)
Probit	**0.53**	**170.0**	**-0.70**	**-0.23**	Extra Risk 10%	**0.016**	**0.013**
Logistic	0.38	171.0	-0.87	-0.20		0.016	0.013
Multistage[e] (Stage 3)	0.43	171.3	-0.50	-0.08		0.011	0.007
Weibull	0.18	172.7	-0.90	-0.15		0.019	0.010
Gamma	0.13	173.6	-1.05	-0.10		0.021	0.009
Log-Probit	0.06	175.1	-1.31	0.67		0.026	0.016
Log-Logistic	0.04	176.4	-1.38	0.67		0.025	0.015

[a]Selected (best-fitting) model shown in first row, in boldface type.
[b]AIC = Akaike Information Criterion
[c]HEC = human equivalent concentration.
[d]BMCL = the lower bound of BMC at 95% confidence level.
[e]For the Multistage model, up to three stages tested; only the model with lowest AIC and lowest stage is reported here.
Source: NTP (2002).

BMDS output file

```
 ================================================================
        Probit Model. (Version: 3.2;  Date: 10/28/2009)
        Input Data File:
C:\USEPA\BMDS212\Data\V2O5\NTP_2002NonCancerMaleRats\MaleRatsLungAlBrEpHyperplasia\NTP
_2002_Lung Alveolar Bronchiole Combined Epithelium Hyperplasia_Probit_0.1.(d)
        Gnuplot Plotting File:
C:\USEPA\BMDS212\Data\V2O5\NTP_2002NonCancerMaleRats\MaleRatsLungAlBrEpHyperplasia\NTP
_2002_Lung Alveolar Bronchiole Combined Epithelium Hyperplasia_Probit_0.1.plt
================================================================
   The form of the probability function is:
   P[response] = CumNorm(Intercept+Slope*Dose),
   where CumNorm(.) is the cumulative normal distribution function

   Dependent variable = Response
   Independent variable = Dose
   Slope parameter is not restricted

   Total number of observations = 4
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008

                  Default Initial (and Specified) Parameter Values
                     background =            0   Specified
                      intercept =     -1.04337
                          slope =      20.1821
          Asymptotic Correlation Matrix of Parameter Estimates
          ( *** The model parameter(s)  -background
                have been estimated at a boundary point, or have been specified by
the user,
                and do not appear in the correlation matrix )
```

	intercept	slope
intercept	1	-0.78
slope	-0.78	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
intercept	-1.02981	0.180284	-1.38316	-0.676464
slope	20.0578	2.82623	14.5184	25.5971

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-82.2383	4			
Fitted model	-82.9994	2	1.52225	2	0.4671
Reduced model	-133.424	1	102.372	3	<.0001

AIC: 169.999

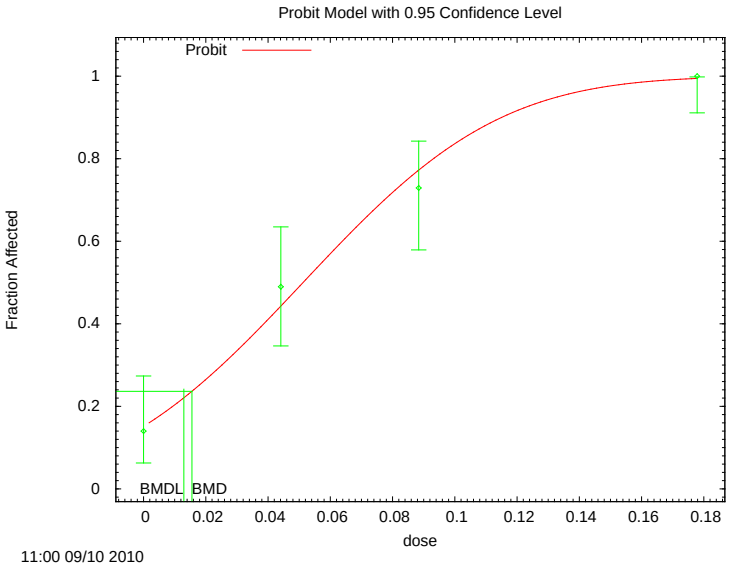
Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.1515	7.577	7.000	50	-0.228
0.0441	0.4423	21.673	24.000	49	0.669
0.0884	0.7713	37.023	35.000	48	-0.695
0.1779	0.9944	49.721	50.000	50	0.530

Chi^2 = 1.26 d.f. = 2 P-value = 0.5316

Benchmark Dose Computation

Specified effect = 0.1
Risk Type = Extra risk
Confidence level = 0.95
BMD = 0.0155483
BMDL = 0.0129496



B.3.2. Dataset 2: Incidence of Lung Chronic Active Inflammation in Male Rats, NTP (2002)

Summary

Two concentration groups (1 and 2 mg/m^3) showed statistically significant differences from the control group as reported. The severity increased from minimal to mild as reported when the concentration of vanadium pentoxide increased. The Cochran-Armitage test confirmed the statistically significant trend with a *Z*-score = 8.36 and a one-sided *p*-value < 0.0001.

Seven dichotomous models available in EPA BMDS (version 2.1.2.) were fit to this dataset. A 10% extra risk of the incidence was used as the BMR to determine the POD. The goodness of fit, BMC, and BMCL for each model are summarized in Table B-7.

All models demonstrated adequate goodness of fit (*p*-value ≥ 0.1) and good visual fit. Based on the draft *Benchmark Dose Technical Guidance* (U.S. EPA, 2000), the Logistic model was selected as the best-fitting model for this dataset. Using this model, the $BMCL_{10}$ was 0.029 mg/m^3 and this value was regarded as a candidate for the POD.

Table B-7. Benchmark modeling results for incidence of lung chronic active inflammation in male rats

Model[a]	Goodness of fit				BMR	HEC[c]	
	p-Value	AIC [b]	Largest scaled residual	Scaled residual of interest		BMC (mg/m^3)	BMCL[d] (mg/m^3)
Logistic	**0.40**	**192.5**	**-0.92**	**-0.92**	Extra Risk 10%	**0.035**	**0.029**
Probit	0.37	192.7	-1.00	-1.00		0.032	0.027
Log-Probit	0.67	192.8	0.29	-0.26		0.046	0.032
Log-Logistic	0.60	192.9	-0.34	-0.34		0.045	0.031

Table B-7. Benchmark modeling results for incidence of lung chronic active inflammation in male rats

Model[a]	Goodness of fit				BMR	HEC[c]	
	p-Value	AIC [b]	Largest scaled	Scaled residual of		BMC	BMCL[d]
Gamma	0.40	193.3	0.58	-0.52		0.042	0.027
Weibull	0.27	193.9	-0.75	-0.75		0.038	0.024
Multistage[e] (Stage 2)	0.24	194.0	0.88	-0.66		0.040	0.019

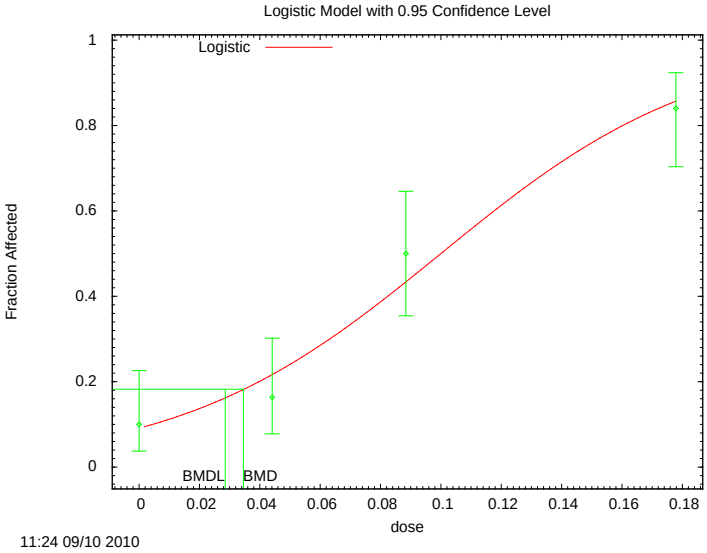
[a]Selected (best-fitting) model shown in first row, in boldface type.
[b]AIC = Akaike Information Criterion.
[c]HEC = human equivalent concentration.
[d]BMCL = the lower bound of BMC at 95% confidence level.
[e]For the Multistage model, up to three stages were tested. Only the model with the lowest AIC and lowest stage is reported here.

Source: NTP (2002).

1 *BMDS output file*

```
 ================================================================
        Logistic Model. (Version: 2.13; Date: 10/28/2009)
        Input Data File:
C:\USEPA\BMDS212\Data\V2O5\NTP_2002NonCancerMaleRats\MaleRatsLungChronicActiveInflamma
tion\NTP_2002_Lung Chronic Active Inflammation_Logistic_0.1.(d)
        Gnuplot Plotting File:
C:\USEPA\BMDS212\Data\V2O5\NTP_2002NonCancerMaleRats\MaleRatsLungChronicActiveInflamma
tion\NTP_2002_Lung Chronic Active Inflammation_Logistic_0.1.plt
================================================================
   The form of the probability function is:
   P[response] = 1/[1+EXP(-intercept-slope*dose)]

   Dependent variable = Response
   Independent variable = Dose
   Slope parameter is not restricted

   Total number of observations = 4
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008

                  Default Initial Parameter Values
                     background =            0   Specified
                      intercept =      -2.2183
                          slope =      21.8588
           Asymptotic Correlation Matrix of Parameter Estimates
           ( *** The model parameter(s)  -background
                 have been estimated at a boundary point, or have been specified by
the user,
                 and do not appear in the correlation matrix )

              intercept        slope
 intercept            1        -0.82
     slope        -0.82            1

                                Parameter Estimates
                                                        95.0% Wald Confidence
Interval
```

```
       Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf.
Limit
      intercept         -2.29582         0.320666           -2.92431            -
1.66732
          slope          23.0148          3.22048            16.7027
29.3268

                        Analysis of Deviance Table
       Model      Log(likelihood)  # Param's  Deviance  Test d.f.   P-value
     Full model        -93.3159         4
   Fitted model        -94.2613         2        1.8908      2          0.3885
  Reduced model        -132.664         1       78.6961      3         <.0001
           AIC:         192.523
                                Goodness of  Fit
                                                                      Scaled
     Dose     Est._Prob.    Expected    Observed     Size       Residual
  ---------------------------------------------------------------------------
    0.0000     0.0915         4.574     5.000          50        0.209
    0.0441     0.2174        10.654     8.000          49       -0.919
    0.0884     0.4350        20.880    24.000          48        0.908
    0.1779     0.8578        42.892    42.000          50       -0.361
 Chi^2 = 1.84      d.f. = 2        P-value = 0.3976

   Benchmark Dose Computation
Specified effect =            0.1
Risk Type        =      Extra risk
Confidence level =           0.95
             BMD =      0.0345486
            BMDL =      0.0285337
```



B.3.3. Dataset 3: Incidence of Larynx Chronic Inflammation in Male Rats, NTP (2002)

Summary

Three concentration groups (0.5, 1, and 2 mg/m^3) showed statistically significant differences from the control group as reported. The severity increased from minimal to about mild as reported when the concentration of vanadium pentoxide increased. The Cochran-Armitage test confirmed the statistically significant trend with a *Z*-score = 4.79 and a one-sided *p*-value < 0.0001.

Seven dichotomous models available in EPA BMDS (version 2.1.2.) were fit to this dataset. A 10% extra risk of the incidence was used as the BMR to determine the POD. The goodness of fit, BMC, and BMCL for each model are summarized in Table B-8.

Only one model demonstrated adequate goodness of fit (p-value ≥ 0.1) and good visual fit. Based on the draft *Benchmark Dose Technical Guidance* (U.S. EPA, 2000), the Log-Logistic model was selected as the best-fitting model for this dataset. Using this model, the $BMCL_{10}$ of was 0.012 mg/m^3 and this value was regarded as a candidate for the POD.

Table B-8. Benchmark modeling results for incidence of larynx chronic inflammation in male rats

	Goodness of Fit					HEC[c]	
Model[a]	***p*-Value**	**AIC[b]**	**Largest scaled residual**	**Scaled residual of interest**	**BMR**	**BMC (mg/m^3)**	**BMC[d] (mg/m^3)**
Log-Logistic	**0.12**	**229.1**	**1.63**	**-0.27**	Extra Risk 10%	**0.017**	**0.012**
Log-Probit	0.09	229.8	-1.37	-0.01		0.005	0.000
Multistage[e] (Stage 1)	0.05	230.7	2.14	-0.65		0.023	0.017
Gamma							
Weibull							
Probit	0.01	234.8	2.45	2.45		0.043	0.036
Logistic	0.01	235.2	2.43	2.43		0.046	0.038

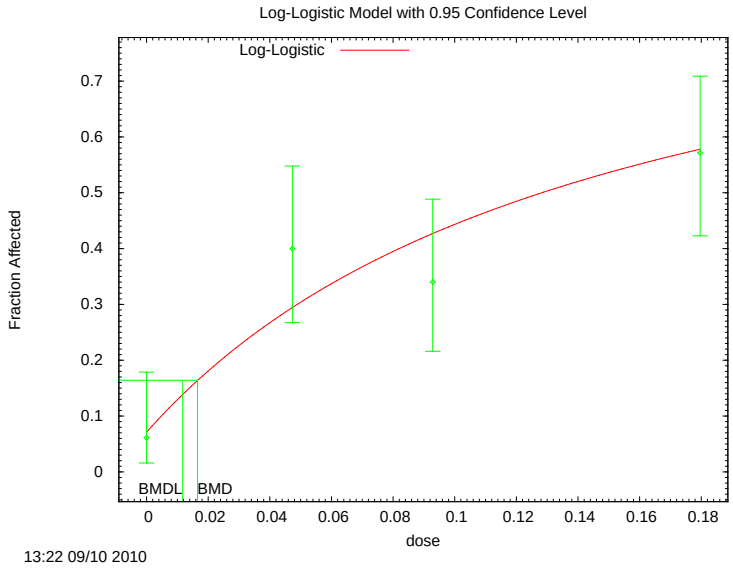
[a]Selected (best-fitting) model shown in first row, in boldface type.
[b]AIC = Akaike Information Criterion.
[c]HEC = human equivalent concentration.
[d]BMCL = the lower bound of BMC at 95% confidence level.
[e]For the Multistage model, up to three stages were tested. Only the model with the lowest AIC and lowest stage is reported here.

Source: NTP (2002).

BMDS output file

```
 ================================================================
        Logistic Model. (Version: 2.13; Date: 10/28/2009)
        Input Data File:
C:\USEPA\BMDS212\Data\V2O5\NTP_2002NonCancerMaleRats\MaleRatsLarynxChronicInflammation
\NTP_2002_Larynx Chronic Inflammation_LogLogistic_0.1.(d)
        Gnuplot Plotting File:
C:\USEPA\BMDS212\Data\V2O5\NTP_2002NonCancerMaleRats\MaleRatsLarynxChronicInflammation
\NTP_2002_Larynx Chronic Inflammation_LogLogistic_0.1.plt
 ================================================================
   The form of the probability function is:
   P[response] = background+(1-background)/[1+EXP(-intercept-slope*Log(dose))]

   Dependent variable = Response
   Independent variable = Dose
   Slope parameter is restricted as slope >= 1
```

```
   Total number of observations = 4
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008

   User has chosen the log transformed model
                 Default Initial Parameter Values
                    background =    0.0612245
                     intercept =       1.9744
                         slope =             1

          Asymptotic Correlation Matrix of Parameter Estimates

          ( *** The model parameter(s)  -slope
                have been estimated at a boundary point, or have been specified by
the user,
                and do not appear in the correlation matrix )

             background    intercept
background            1        -0.53
 intercept        -0.53            1

                                Parameter Estimates
                                                        95.0% Wald Confidence
Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf.
Limit
     background        0.0712314            *                *                   *
      intercept           1.9037            *                *                   *
          slope                1            *                *                   *
* - Indicates that this value is not calculated.

                       Analysis of Deviance Table
       Model      Log(likelihood)  # Param's  Deviance  Test d.f.   P-value
     Full model        -110.451         4
   Fitted model         -112.55         2       4.19924      2          0.1225
  Reduced model        -127.371         1       33.8403      3         <.0001

          AIC:         229.101

                                Goodness  of  Fit
                                                                    Scaled
     Dose     Est._Prob.    Expected    Observed     Size       Residual
  ---------------------------------------------------------------------
    0.0000     0.0712         3.490     3.000          49       -0.272
    0.0473     0.2951        14.754    20.000          50        1.627
    0.0929     0.4278        21.390    17.000          50       -1.255
    0.1796     0.5789        28.366    28.000          49       -0.106

 Chi^2 = 4.31      d.f. = 2        P-value = 0.1162

   Benchmark Dose Computation
Specified effect =            0.1
Risk Type        =      Extra risk
Confidence level =           0.95
            BMD =      0.0165573
           BMDL =      0.0117279
```



B.3.4. Dataset 4: Incidence of Larynx Respiratory Epithelium, Epiglottis, Hyperplasia in Male Rats, NTP (2002)

Summary

Three concentration groups (0.5, 1, and 2 mg/m^3) showed statistically significant differences from the control group as reported. The severity increased slightly when the concentration of vanadium pentoxide increased. The Cochran-Armitage test confirmed the statistically significant trend with a *Z*-score = -6.56 and a one-sided *p*-value < 0.0001.

Seven dichotomous models available in EPA BMDS (version 2.1.2.) were fit to this dataset. A 10% extra risk of the incidence was used as the BMR to determine the POD. The goodness of fit, BMC, and BMCL for each model are summarized in Table B-9.

Both Log-Logistic and Log-Probit models demonstrated adequate goodness of fit (*p*-value ≥ 0.1) and good visual fit but the BMC/BMCL ratio in Log-Probit was infinite, which is not acceptable. Based on the draft *Benchmark Dose Technical Guidance* (U.S. EPA, 2000), the Log-Logistic model was selected as the best-fitting model for this dataset. The $BMCL_{10}$ was 0.006 mg/m^3 and this value was regarded as a candidate for the POD.

Table B-9. Benchmark modeling results for incidence of larynx respiratory epithelium, epiglottis, hyperplasia in male rats

	Goodness of Fit					HEC[c]	
Model[a]	***p*-Value**	**AIC[b]**	**Largest scaled residual**	**Scaled residual of interest**	**BMR**	**BMC (mg/m^3)**	**BMCL[d] (mg/m^3)**
Log-Logistic	**0.27**	**197.3**	**1.56**	**0.000**	Extra Risk	**0.008**	**0.006**
Log-Probit	0.14	199.3	1.60	0.000		0.007	0.000

Table B-9. Benchmark modeling results for incidence of larynx respiratory epithelium, epiglottis, hyperplasia in male rats

Model[a]	Goodness of Fit				BMR	HEC[c]	
	p-Value	AIC[b]	Largest scaled	Scaled residual of		BMC	BMCL[d]
Gamma	0.04	201.5	-2.12	0.000	10%	0.013	0.010
Multistage[e] (Stage 1)							
Weibull							
Probit	0.00	226.4	3.16	1.180		0.031	0.027
Logistic	0.00	227.1	3.05	1.078		0.032	0.027

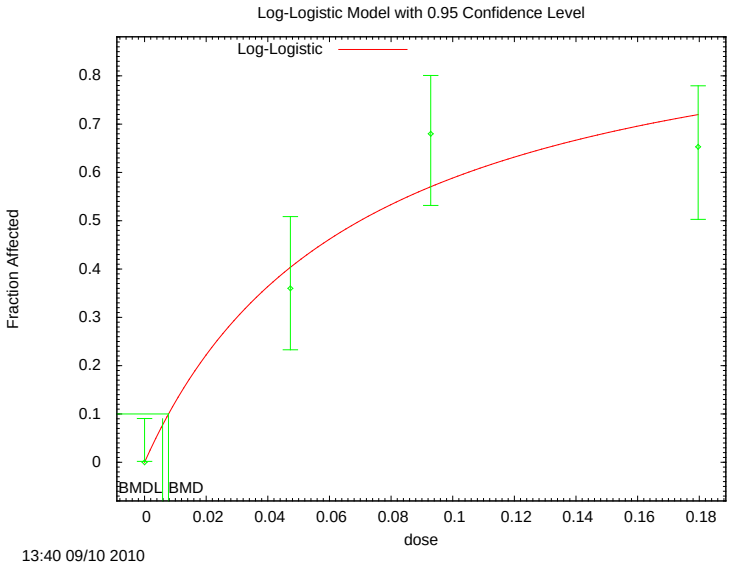
Selected (best-fitting) model shown in first row, in boldface type.
[b]AIC=Akaike Information Criterion.
[c]HEC = human equivalent concentration.
[d]BMCL = the lower bound of BMC at 95% confidence level.
[e]For the Multistage model, up to three stages were tested. Only the model with the lowest AIC and lowest stage is reported here.

Source: NTP (2002).

1 ***BMDS output file***

```
 ================================================================
        Logistic Model. (Version: 2.13; Date: 10/28/2009)
        Input Data File:
C:\USEPA\BMDS212\Data\V2O5\NTP_2002NonCancerMaleRats\MaleRatsLarynxEpiglottisHyperplas
ia\NTP_2002_Larynx Epiglottis Hyperplasia_LogLogistic_0.1.(d)
        Gnuplot Plotting File:
C:\USEPA\BMDS212\Data\V2O5\NTP_2002NonCancerMaleRats\MaleRatsLarynxEpiglottisHyperplas
ia\NTP_2002_Larynx Epiglottis Hyperplasia_LogLogistic_0.1.plt
================================================================
   The form of the probability function is:
   P[response] = background+(1-background)/[1+EXP(-intercept-slope*Log(dose))]

   Dependent variable = Response
   Independent variable = Dose
   Slope parameter is restricted as slope >= 1

   Total number of observations = 4
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008

   User has chosen the log transformed model
                 Default Initial Parameter Values
                    background =            0
                     intercept =      2.65432
                         slope =            1

          Asymptotic Correlation Matrix of Parameter Estimates

          ( *** The model parameter(s)  -background    -slope
                have been estimated at a boundary point, or have been specified by
the user,
                and do not appear in the correlation matrix )

             intercept
```

```
 intercept            1

                                 Parameter Estimates
                                                        95.0% Wald Confidence
Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf.
Limit
     background                0            *                *                   *
      intercept          2.66112            *                *                   *
          slope                1            *                *                   *

* - Indicates that this value is not calculated.

                        Analysis of Deviance Table
       Model      Log(likelihood)  # Param's  Deviance  Test d.f.   P-value
     Full model        -95.6454         4
    Fitted model       -97.6263         1       3.96181      3          0.2656
  Reduced model        -134.962         1       78.6325      3         <.0001

           AIC:         197.253

                                  Goodness  of  Fit
                                                                      Scaled
     Dose      Est._Prob.     Expected     Observed      Size       Residual
  ----------------------------------------------------------------------------
    0.0000      0.0000          0.000      0.000          49        0.000
    0.0473      0.4038         20.190     18.000          50       -0.631
    0.0929      0.5706         28.532     34.000          50        1.562
    0.1796      0.7200         35.279     32.000          49       -1.043

 Chi^2 = 3.93      d.f. = 3        P-value = 0.2694

   Benchmark Dose Computation
Specified effect =           0.1
Risk Type        =      Extra risk
Confidence level =          0.95
             BMD =     0.00776335
            BMDL =     0.00584847
```



B.3.5. Dataset 5: Incidence of Nose Goblet Cell, Respiratory Epithelium, Hyperplasia in Male Rats, NTP (2002)

Summary

Three concentration groups (0.5, 1, and 2 mg/m^3) showed statistically significant differences from the control group as reported. The severity as reported increased slightly when the concentration of vanadium pentoxide increased. The Cochran-Armitage test confirmed the statistically significant trend with a *Z*-score = 2.68 and a one-sided *p*-value = 0.0037.

Seven dichotomous models available in EPA BMDS (version 2.1.2.) were fit to this dataset. A 10% extra risk of the incidence was used as the BMR to determine the POD. The goodness of fit, BMC, and BMCL for each model are summarized in Table B-10.

Five models demonstrated adequate goodness of fit (*p*-value ≥ 0.1) and good visual fit but Log-Probit model failed to compute a reasonable BMCL. Based on the draft *Benchmark Dose Technical Guidance* (U.S. EPA, 2000), the Log-Logistic model was selected as the best-fitting model for this dataset. Using this model, the $BMCL_{10}$ was 0.026 mg/m^3 and this value was regarded as a candidate for the POD.

Table B-10. Benchmark modeling results for incidence of nose goblet cell, respiratory epithelium, hyperplasia in male rats

Model[a]	Goodness of Fit				BMR	HEC[c]	
	p-Value	AIC[b]	Largest scaled residual	Scaled residual of interest		BMC (mg/m^3)	BMCL[d] (mg/m^3)
Log-Logistic	**0.16**	**213.2**	**1.66**	**1.656**	Extra Risk 10%	**0.044**	**0.026**
Log-Probit	0.31	212.8	-0.83	-0.003		0.002	failed
Gamma	0.12	213.7	1.75	1.75		0.052	0.033
Multistage[e] (Stage 1)							
Weibull							
Probit	0.08	214.9	1.787	-0.155		0.077	0.056
Logistic	0.07	215.0	1.78	-0.128		0.081	0.060

[a]Selected (best-fitting) model shown in first row, in boldface type.
[b]AIC = Akaike Information Criterion.
[c]HEC = human equivalent concentration.
[d]BMCL = the lower bound of BMC at 95% confidence level.
[e]For the Multistage model, up to three stages were tested. Only the model with the lowest AIC and lowest stage is reported here.

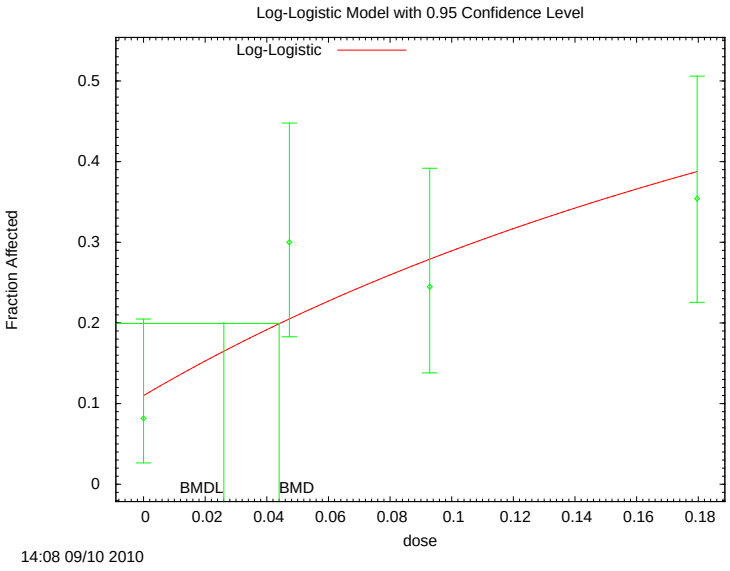
Source: NTP (2002).

BMDS output file

```
==============================================================
        Logistic Model. (Version: 2.13; Date: 10/28/2009)
        Input Data File:
C:\USEPA\BMDS212\Data\V2O5\NTP_2002NonCancerMaleRats\MaleRatsNoseGobletEpiHyperplasia\
NTP_2002_Larynx Goblet Hyperplasia _LogLogistic_0.1.(d)
```

```
        Gnuplot Plotting File:
C:\USEPA\BMDS212\Data\V2O5\NTP_2002NonCancerMaleRats\MaleRatsNoseGobletEpiHyperplasia\
NTP_2002_Larynx Goblet Hyperplasia _LogLogistic_0.1.plt

 ====================================================================
   The form of the probability function is:
   P[response] = background+(1-background)/[1+EXP(-intercept-slope*Log(dose))]

   Dependent variable = Response
   Independent variable = Dose
   Slope parameter is restricted as slope >= 1

   Total number of observations = 4
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008

   User has chosen the log transformed model
                 Default Initial Parameter Values
                    background =    0.0816327
                     intercept =      1.21717
                         slope =            1

          Asymptotic Correlation Matrix of Parameter Estimates

          ( *** The model parameter(s)  -slope
                have been estimated at a boundary point, or have been specified by
the user,
                and do not appear in the correlation matrix )

             background    intercept
background            1        -0.72
 intercept        -0.72            1

                                Parameter Estimates
                                                        95.0% Wald Confidence
Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf.
Limit
     background         0.110444            *                *                 *
      intercept         0.926382            *                *                 *
          slope                1            *                *                 *

* - Indicates that this value is not calculated.



                        Analysis of Deviance Table
      Model      Log(likelihood)  # Param's  Deviance  Test d.f.   P-value
     Full model        -102.873         4
   Fitted model        -104.611         2       3.47486      2           0.176
  Reduced model        -109.105         1       12.4644      3         0.00595

          AIC:         213.221

                                  Goodness of  Fit
                                                                    Scaled
    Dose     Est._Prob.    Expected    Observed     Size       Residual
  ---------------------------------------------------------------------
    0.0000     0.1104         5.412     4.000          49       -0.643
    0.0473     0.2054        10.270    15.000          50        1.656
    0.0929     0.2794        13.691    12.000          49       -0.539
    0.1796     0.3881        18.627    17.000          48       -0.482

 Chi^2 = 3.68      d.f. = 2        P-value = 0.1590
```

```
   Benchmark Dose Computation
Specified effect =            0.1
Risk Type        =      Extra risk
Confidence level =           0.95
             BMD =      0.0439982
            BMDL =       0.026051
```



B.3.6. Dataset 6: Incidence of Lung Alveolar Epithelium Hyperplasia in Female Rats, NTP (2002)

Summary

Two concentration groups (1 and 2 mg/m^3) showed statistically significant differences from the control as reported. The severity of these nonneoplastic lesions increased from minimal to moderate as reported when the concentration of vanadium pentoxide increased. The Cochran-Armitage test confirmed the statistically significant trend with a *Z*-score = 9.53 and a one-sided *p*-value < 0.0001.

Seven dichotomous models available in EPA BMDS (version 2.1.2.) were fit to this dataset. A 10% extra risk of the combined incidence was used as the BMR to determine the POD. The goodness of fit, BMC, and BMCL for each model are summarized in Table B-11.

Five models demonstrated adequate goodness of fit (*p*-value ≥ 0.1) but the extreme curvature of Log-Logistic model did not reelect in the observed data. Based on the draft *Benchmark Dose Technical Guidance* (U.S. EPA, 2000), the Gamma model was selected as the best-fitting model for this dataset. Using this model, the $BMCL_{10}$ was 0.063 mg/m^3 and this value was regarded as a candidate for the POD.

1

Table B-11. Benchmark modeling results for incidence of lung alveolar epithelium hyperplasia in female rats

Model [a]	Goodness of Fit				BMR	HEC [c]	
	P-Value	AIC [b]	Largest scaled residual	Scaled residual of interest		BMC (mg/m^3)	BMCL[d] (mg/m^3)
Gamma	**0.89**	**156.2**	**0.37**	**-0.12**	Extra Risk 10%	**0.076**	**0.063**
Log-Logistic	0.96	155.9	0.20	0.00		0.086	0.070
Weibull	0.91	157.9	0.08	-0.01		0.071	0.053
Log-Probit	0.78	157.9	0.20	0.00		0.085	0.068
Multistage[e] (Stage 3)	0.46	158.1	0.87	-0.24		0.055	0.041
Logistic	0.00	168.1	2.34	-0.78		0.035	0.028
Probit	0.00	168.4	2.32	-1.11		0.031	0.025

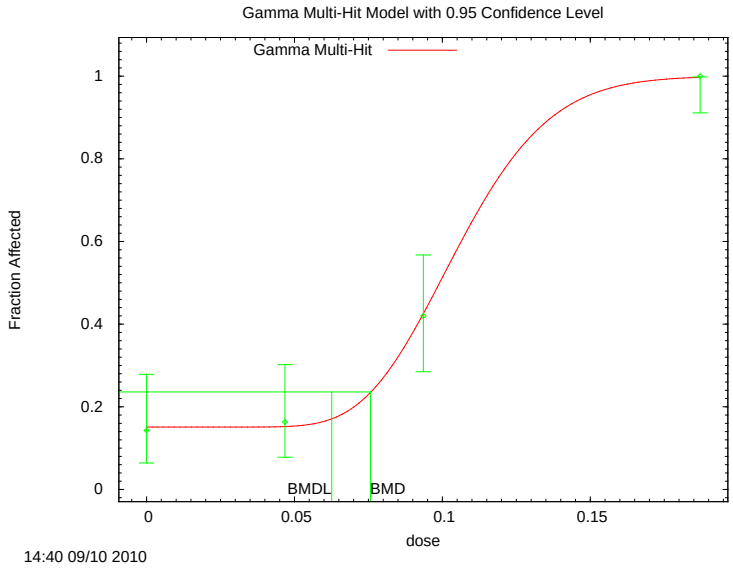
[a]Selected (best-fitting) model shown in first row, in boldface type.
[b]AIC = Akaike Information Criterion.
[c]HEC = human equivalent concentration.
[d]BMCL = the lower bound of BMC at 95% confidence level.
[e]For the Multistage model, up to three stages were tested. Only the model with the lowest AIC and lowest stage is reported here.

Source: NTP (2002).

2 ***BMDS output file***

```
 ================================================================
        Gamma Model. (Version: 2.15;  Date: 10/28/2009)
        Input Data File:
C:\USEPA\BMDS212\Data\V2O5\NTP_2002NonCancerFemaleRats\FemaleRatsLungAlBrEpHyperplasia
\NTP_2002_Lung Alveolar Bronchiole Combined Epithelium Hyperplasia_Gamma_0.1.(d)
        Gnuplot Plotting File:
C:\USEPA\BMDS212\Data\V2O5\NTP_2002NonCancerFemaleRats\FemaleRatsLungAlBrEpHyperplasia
\NTP_2002_Lung Alveolar Bronchiole Combined Epithelium Hyperplasia_Gamma_0.1.plt
================================================================
   The form of the probability function is:

   P[response]= background+(1-background)*CumGamma[slope*dose,power],
   where CumGamma(.) is the cummulative Gamma distribution function

   Dependent variable = Response
   Independent variable = Dose
   Power parameter is restricted as power >=1

   Total number of observations = 4
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008

                  Default Initial (and Specified) Parameter Values
                     Background =     0.156863
                          Slope =      139.011
                          Power =      16.8546


            Asymptotic Correlation Matrix of Parameter Estimates
```

```
          ( *** The model parameter(s)  -Power
                have been estimated at a boundary point, or have been specified by
the user,
                and do not appear in the correlation matrix )

             Background        Slope
Background            1        -0.32
     Slope        -0.32            1

                                Parameter Estimates
                                                          95.0% Wald Confidence
Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf.
Limit
     Background         0.151066          0.03605            0.080409
0.221723
          Slope          169.414          9.58875             150.621
188.208
          Power               18               NA

NA - Indicates that this parameter has hit a bound
     implied by some inequality constraint and thus
     has no standard error.

                        Analysis of Deviance Table

       Model      Log(likelihood)  # Param's  Deviance  Test d.f.   P-value
     Full model        -75.9175         4
   Fitted model        -76.0968         2      0.358619      2          0.8358
  Reduced model        -135.531         1       119.227      3         <.0001

           AIC:         156.194

                                  Goodness  of  Fit
                                                                      Scaled
     Dose     Est._Prob.    Expected    Observed     Size       Residual
  ---------------------------------------------------------------------------
    0.0000     0.1511         7.402     7.000          49       -0.160
    0.0468     0.1523         7.462     8.000          49        0.214
    0.0936     0.4286        21.431    21.000          50       -0.123
    0.1871     0.9973        49.864    50.000          50        0.369

 Chi^2 = 0.22      d.f. = 2        P-value = 0.8945

   Benchmark Dose Computation
Specified effect =            0.1
Risk Type        =      Extra risk
Confidence level =           0.95
             BMD =      0.0756821
            BMDL =     0.0626004
```



B.3.7. Dataset 7: Incidence of Lung Chronic Active Inflammation in Female Rats, NTP (2002)

Summary

One concentration group (2 mg/m^3) showed statistically significant difference from the control group as reported. The severity did not change much when the concentration of vanadium pentoxide increased. The Cochran-Armitage test confirmed the statistically significant trend with a *Z*-score = 6.71 and a one-sided *p*-value < 0.0001.

Seven dichotomous models available in EPA BMDS (version 2.1.2.) were fit to this dataset. A 10% extra risk of the incidence was used as the BMR to determine the POD. The goodness of fit, BMC, and BMCL for each model are summarized in Table B-12.

Five models demonstrated adequate goodness of fit (*p*-value ≥ 0.1) and good visual fit. Based on the draft *Benchmark Dose Technical Guidance* (U.S. EPA, 2000), the Multistage (Stage 3) model was selected as the best-fitting model for this dataset. The $BMCL_{10}$ was 0.048 mg/m^3 and this value was regarded as a candidate for the POD.

Table B-12. Benchmark modeling results for incidence of lung chronic active inflammation in female rats

	Goodness of fit					HEC[c]	
Model[a]	***p*-Value**	**AIC[b]**	**Largest scaled residual**	**Scaled residual of interest**	**BMR**	**BMC (mg/m^3)**	**BMCL[d] (mg/m^3)**
Multistage[e] (Stage 3)	**0.82**	**212.9**	**-0.51**	**-0.51**	Extra Risk 10%	**0.080**	**0.048**
Log-Probit	1.00	214.5	0.00	0.00		0.094	0.068
Gamma	0.99	214.5	-0.01	0.00		0.094	0.063

Table B-12. Benchmark modeling results for incidence of lung chronic active inflammation in female rats

Model[a]	Goodness of fit				BMR	HEC[c]	
	p-Value	AIC[b]	Largest scaled	Scaled residual		BMC	BMCL[d]
Log-Logistic	0.97	214.5	-0.03	0.00		0.094	0.065
Weibull	0.94	214.5	-0.06	0.01		0.094	0.059
Logistic	0.04	218.8	1.64	-0.48		0.040	0.033
Probit	0.03	219.4	-1.71	-0.62		0.037	0.031

[a]Selected (best-fitting) model shown in first row, in boldface type.
AIC = Akaike Information Criterion.
[c]HEC = human equivalent concentration.
[d]BMCL = the lower bound of BMC at 95% confidence level.
[e]For the Multistage model, up to three stages were tested. Only the model with the lowest AIC and lowest stage is reported here.

Source: NTP (2002).

BMDS output file

```
 ========================================================================
        Multistage Model. (Version: 3.2;  Date: 05/26/2010)
        Input Data File:
C:\USEPA\BMDS212\Data\V2O5\NTP_2002NonCancerFemaleRats\FemaleRatsLungChronicActiveInfl
ammation\NTP_2002_Lung Chronic Active Inflammation_Multi3_0.1.(d)
        Gnuplot Plotting File:
C:\USEPA\BMDS212\Data\V2O5\NTP_2002NonCancerFemaleRats\FemaleRatsLungChronicActiveInfl
ammation\NTP_2002_Lung Chronic Active Inflammation_Multi3_0.1.plt
========================================================================
   The form of the probability function is:
   P[response] = background + (1-background)*[1-EXP(
                 -beta1*dose^1-beta2*dose^2-beta3*dose^3)]

   The parameter betas are restricted to be positive
   Dependent variable = Response
   Independent variable = Dose

 Total number of observations = 4
 Total number of records with missing values = 0
 Total number of parameters in model = 4
 Total number of specified parameters = 0
 Degree of polynomial = 3

 Maximum number of iterations = 250
 Relative Function Convergence has been set to: 1e-008
 Parameter Convergence has been set to: 1e-008

                  Default Initial Parameter Values
                     Background =      0.17928
                        Beta(1) =            0
                        Beta(2) =            0
                        Beta(3) =      214.579

            Asymptotic Correlation Matrix of Parameter Estimates

            ( *** The model parameter(s)  -Beta(1)    -Beta(2)
                  have been estimated at a boundary point, or have been specified by
the user,
                  and do not appear in the correlation matrix )
```

	Background	Beta(3)
Background	1	-0.41
Beta(3)	-0.41	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
Background	0.186734	*	*	*
Beta(1)	0	*	*	*
Beta(2)	0	*	*	*
Beta(3)	206.262	*	*	*

* - Indicates that this value is not calculated.

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-104.257	4			
Fitted model	-104.451	2	0.389305	2	0.8231
Reduced model	-130.861	1	53.209	3	<.0001

AIC: 212.903

Goodness of Fit

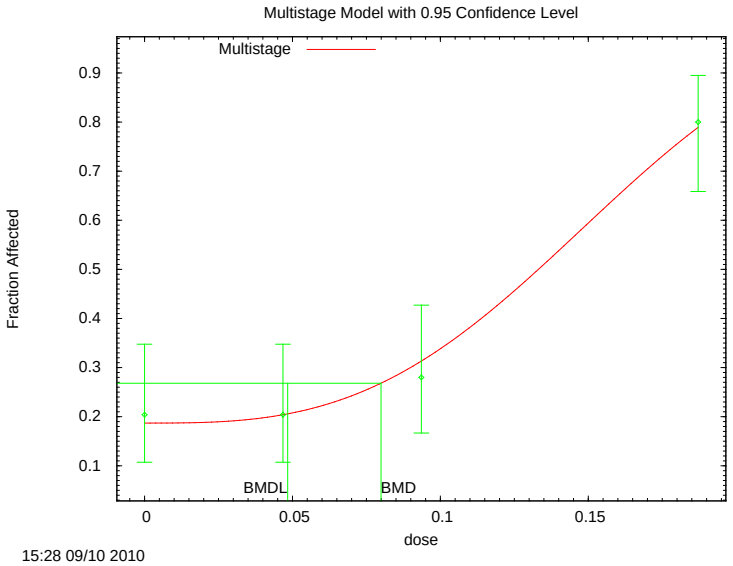
Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.1867	9.150	10.000	49	0.312
0.0468	0.2037	9.983	10.000	49	0.006
0.0936	0.3132	15.659	14.000	50	-0.506
0.1871	0.7896	39.478	40.000	50	0.181

Chi^2 = 0.39 d.f. = 2 P-value = 0.8246

Benchmark Dose Computation

Specified effect = 0.1
Risk Type = Extra risk
Confidence level = 0.95
BMD = 0.079938
BMDL = 0.0483842
BMDU = 0.0900697

Taken together, (0.0483842, 0.0900697) is a 90% two-sided confidence interval for the BMD



B.3.8. Dataset 8: Incidence of Larynx Chronic Inflammation in Female Rats, NTP (2002)

Summary

Three concentration groups (0.5, 1, and 2 mg/m^3) showed statistically significant difference from the control group as reported. The severity did not change much when the concentration of vanadium pentoxide increased. The Cochran-Armitage test confirmed the statistically significant trend with a *Z*-score = 5.38 and a one-sided *p*-value < 0.0001.

Seven dichotomous models available in EPA BMDS (version 2.1.2.) were fit to this dataset. A 10% extra risk of the incidence was used as the BMR to determine the POD. The goodness of fit, BMC and BMCL for each model are summarized in Table B-13.

Five models demonstrated adequate goodness of fit (*p*-value ≥ 0.1) and good visual fit. Based on the draft *Benchmark Dose Technical Guidance* (U.S. EPA, 2000), the Log-Logistic model was selected as the best-fitting model for this dataset. Using this model, the $BMCL_{10}$ was 0.003 mg/m^3 and this value was regarded as a candidate for the POD.

Table B-13. Benchmark modeling results for incidence of larynx chronic inflammation in female rats

	Goodness of fit					HEC[c]	
Model[a]	***p*-Value**	**AIC[b]**	**Largest scaled residual**	**Scaled residual of interest**	**BMR**	**BMC (mg/m^3)**	**BMCL[d] (mg/m^3)**
Log-Logistic	**0.46**	**242.0**	**-0.88**	**-0.13**	Extra Risk 10%	**0.005**	**0.003**
Log-Probit	0.27	243.6	-0.89	-0.02		0.003	0.000
Gamma	0.19	243.7	1.58	-0.57		0.007	0.006
Multistage[e] (Stage 1)							
Weibull							
Probit	0.03	247.5	1.99	1.99		0.013	0.011
Logistic	0.03	247.6	1.97	1.97		0.014	0.011

[a]Selected (best-fitting) model shown in first row, in boldface type.
[b]AIC = Akaike Information Criterion.
[c]HEC = human equivalent concentration.
[d]BMCL = the lower bound of BMC at 95% confidence level.
[e]For the Multistage model, up to three stages were tested. Only the model with the lowest AIC and lowest stage is reported here.

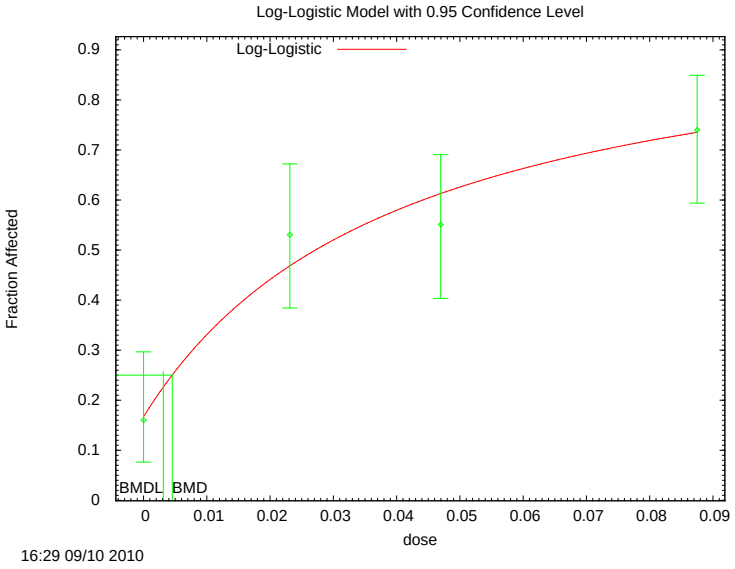
Source: NTP (2002).

BMDS output file

```
================================================================
        Logistic Model. (Version: 2.13; Date: 10/28/2009)
```

```
        Input Data File:
C:\USEPA\BMDS212\Data\V2O5\NTP_2002NonCancerFemaleRats\FemaleRatsLarynxChronicInflamma
tion\NTP_2002_Larynx Chronice Inflammation_LogLogistic_0.1.(d)
        Gnuplot Plotting File:
C:\USEPA\BMDS212\Data\V2O5\NTP_2002NonCancerFemaleRats\FemaleRatsLarynxChronicInflamma
tion\NTP_2002_Larynx Chronice Inflammation_LogLogistic_0.1.plt
================================================================
   The form of the probability function is:
   P[response] = background+(1-background)/[1+EXP(-intercept-slope*Log(dose))]

   Dependent variable = Response
   Independent variable = Dose
   Slope parameter is restricted as slope >= 1

   Total number of observations = 4
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008

   User has chosen the log transformed model
                Default Initial Parameter Values
                   background =         0.16
                    intercept =       3.2396
                        slope =            1

          Asymptotic Correlation Matrix of Parameter Estimates
          ( *** The model parameter(s)  -slope
                have been estimated at a boundary point, or have been specified by
the user,
                and do not appear in the correlation matrix )

             background    intercept
background            1        -0.54
 intercept        -0.54            1

                                Parameter Estimates
                                                        95.0% Wald Confidence
Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf.
Limit
     background         0.166778            *                *                 *
      intercept          3.19725            *                *                 *
          slope                1            *                *                 *

* - Indicates that this value is not calculated.

                        Analysis of Deviance Table

       Model      Log(likelihood)  # Param's  Deviance  Test d.f.   P-value
     Full model        -118.217         4
   Fitted model        -118.997         2       1.55893      2          0.4587
  Reduced model        -137.233         1       38.0314      3         <.0001

          AIC:         241.994

                                  Goodness  of  Fit
                                                                    Scaled
    Dose     Est._Prob.    Expected    Observed     Size       Residual
  ----------------------------------------------------------------------
   0.0000     0.1668         8.339     8.000          50       -0.129
   0.0231     0.4678        22.925    26.000          49        0.881
   0.0470     0.6123        30.001    27.000          49       -0.880
   0.0875     0.7347        36.735    37.000          50        0.085

 Chi^2 = 1.57      d.f. = 2        P-value = 0.4553
```

```
   Benchmark Dose Computation
Specified effect =            0.1
Risk Type        =      Extra risk
Confidence level =           0.95
             BMD =     0.00454158
            BMDL =     0.00312534
```



B.3.9. Dataset 9: Incidence of Larynx Respiratory Epithelium, Epiglottis, Hyperplasia in Female Rats, NTP (2002)

Summary

Three concentration groups (0.5, 1, and 2 mg/m^3) showed statistically significant differences from the control group as reported. The severity did not change much when the concentration of vanadium pentoxide increased. The Cochran-Armitage test confirmed the statistically significant trend with a *Z*-score = -5.98 and a one-sided *p*-value < 0.0001.

Seven dichotomous models available in EPA BMDS (version 2.1.2.) were fit to this dataset. A 10% extra risk of the incidence was used as the BMR to determine the POD. The goodness of fit, BMC, and BMCL for each model are summarized in Table B-14.

Both the Log-Logistic and Log-Probit models demonstrated adequate goodness of fit (*p*-value ≥ 0.1) and good visual fit but the Log-Probit model failed to compute a reasonable BMCL. Based on the draft *Benchmark Dose Technical Guidance* (U.S. EPA, 2000), the Log-Logistic model was selected as the best-fitting model for the dataset. Using this model, the $BMCL_{10}$ was 0.003 mg/m^3 and this value was regarded as a candidate for the POD.

Table B-14. Benchmark modeling results for incidence of larynx respiratory epithelium, epiglottis, hyperplasia in female rats

Model[a]	Goodness of fit	BMR	HEC[c]

	p-Value	AIC[b]	Largest scaled residual	Scaled residual of interest		BMC (mg/m^3)	BMCL[d] (mg/m^3)
Log-Logistic	**0.30**	**205.3**	**1.53**	**0.000**	Extra Risk 10%	**0.004**	**0.003**
Log-Probit	0.78	204.2	-0.57	0.000		0.000	failed
Gamma	0.01	212.3	2.85	0.000		0.006	0.005
Multistage[e] (Stage 1)							
Weibull							
Probit	0.00	234.6	3.258	3.258		0.016	0.013
Logistic	0.00	235.3	-3.295	3.174		0.016	0.014

[a]Selected (best-fitting) model shown in first row, in boldface type.
[b]AIC = Akaike Information Criterion.
[c]HEC = human equivalent concentration.
[d]BMCL = the lower bound of BMC at 95% confidence level.
[e]For the Multistage model, up to three stages were tested. Only the model with the lowest AIC and lowest stage is reported here.

Source: NTP (2002).

1 ***BMDS output file***

```
================================================================
        Logistic Model. (Version: 2.13; Date: 10/28/2009)
        Input Data File:
C:\USEPA\BMDS212\Data\V2O5\NTP_2002NonCancerFemaleRats\FemaleRatsLarynxEpiglottisHyper
plasia\NTP_2002_Larynx Epiglottis Hyperplasia_LogLogistic_0.1.(d)
        Gnuplot Plotting File:
C:\USEPA\BMDS212\Data\V2O5\NTP_2002NonCancerFemaleRats\FemaleRatsLarynxEpiglottisHyper
plasia\NTP_2002_Larynx Epiglottis Hyperplasia_LogLogistic_0.1.plt
================================================================
   The form of the probability function is:
   P[response] = background+(1-background)/[1+EXP(-intercept-slope*Log(dose))]

   Dependent variable = Response
   Independent variable = Dose
   Slope parameter is restricted as slope >= 1

   Total number of observations = 4
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008

   User has chosen the log transformed model
                 Default Initial Parameter Values
                    background =           0
                     intercept =     3.38546
                         slope =           1
          Asymptotic Correlation Matrix of Parameter Estimates
          ( *** The model parameter(s)  -background    -slope
                have been estimated at a boundary point, or have been specified by
the user,
                and do not appear in the correlation matrix )

              intercept
 intercept            1
                            Parameter Estimates
                                                    95.0% Wald Confidence
Interval
```

Variable	Estimate	Std. Err.	Lower Conf. Limit	Upper Conf. Limit
background	0	*	*	*
intercept	3.3735	*	*	*
slope	1	*	*	*

* - Indicates that this value is not calculated.

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-99.8781	4			
Fitted model	-101.66	1	3.56345	3	0.3126
Reduced model	-134.962	1	70.1671	3	<.0001

AIC: 205.32

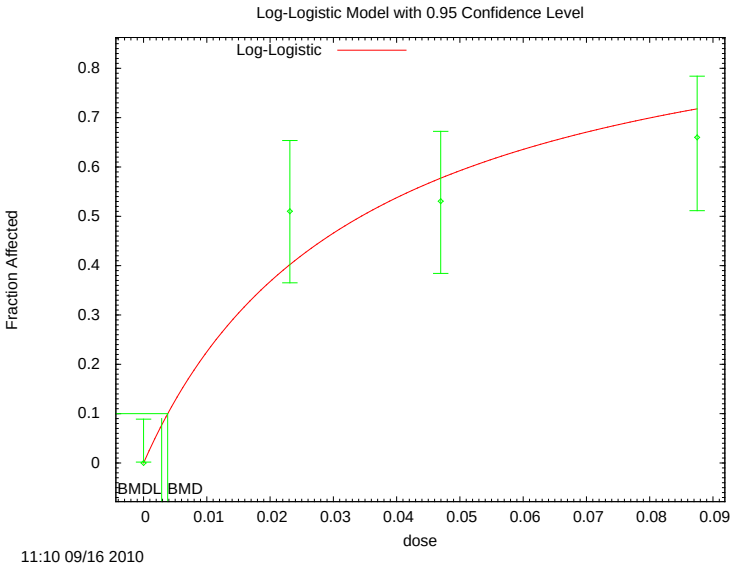
Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0000	0.000	0.000	50	0.000
0.0231	0.4029	19.743	25.000	49	1.531
0.0470	0.5781	28.329	26.000	49	-0.674
0.0875	0.7186	35.929	33.000	50	-0.921

Chi^2 = 3.65 d.f. = 3 P-value = 0.3022

Benchmark Dose Computation

Specified effect = 0.1
Risk Type = Extra risk
Confidence level = 0.95
BMD = 0.00380772
BMDL = 0.0028644



B.3.10. Dataset 10: Incidence of Nose Goblet Cell, Respiratory Epithelium, Hyperplasia in Female Rats, NTP (2002)

Summary

One concentration group (2 mg/m^3) showed statistically significant difference from the control group as reported. The severity did not change much when the concentration of

vanadium pentoxide increased. The Cochran-Armitage test confirmed the statistically significant trend with a *Z*-score = 3.46 and a one-sided *p*-value = 0.0003.

Seven dichotomous models available in EPA BMDS (version 2.1.2.) were fit to this dataset. A 10% extra risk of the incidence was used as the BMR to determine the POD. The goodness of fit, BMC, and BMCL for each model are summarized in Table B-15.

All models demonstrated adequate goodness of fit (*p*-value ≥ 0.1) and good visual fit. Based on the draft *Benchmark Dose Technical Guidance* (U.S. EPA, 2000), the Multistage (Stage 2) model was selected as the best-fitting model for the dataset. Using this model, the $BMCL_{10}$ was 0.014 mg/m^3 and this value was regarded as a candidate for the POD.

Table B-15. Benchmark modeling results for incidence of nose goblet cell, respiratory epithelium, hyperplasia in female rats

Model[a]	Goodness of fit				BMR	HEC[c]	
	P-Value	AIC[b]	Largest scaled residual	Scaled residual of interest		BMC (mg/m^3)	BMCL[d] (mg/m^3)
Multistage[e] (Stage 2)	**0.43**	**258.3**	**-0.90**	**-0.896**	Extra Risk 10%	**0.038**	**0.014**
Logistic	0.33	258.9	-1.252	0.586		0.024	0.018
Probit	0.32	258.9	-1.273	0.564		0.023	0.018
Weibull (Quantal Linear)	0.22	259.7	-1.45	0.337		0.019	0.012
Log-Logistic	0.28	259.8	0.78	-0.027		0.064	0.014
Gamma	0.28	259.8	0.77	-0.005		0.063	0.014
Log-Probit	0.28	259.8	0.765	-0.001		0.062	0.015

[a]Selected (best-fitting) model shown in first row, in boldface type.
[b]AIC = Akaike Information Criterion.
[c]HEC = human equivalent concentration.
[d]BMCL = the lower bound of BMC at 95% confidence level.
[e]For the Multistage model, up to three stages were tested. Only the model with the lowest AIC and lowest stage is reported here.

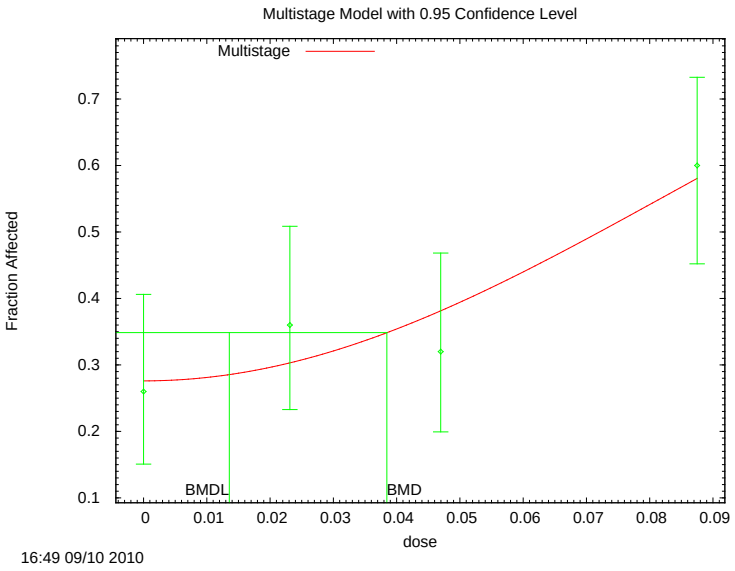
Source: NTP (2002).

BMDS output file

```
 ================================================================
        Multistage Model. (Version: 3.2;  Date: 05/26/2010)
        Input Data File:
C:\USEPA\BMDS212\Data\V2O5\NTP_2002NonCancerFemaleRats\FemaleRatsNoseGobletEpiHyperpla
sia\NTP_2002_Nose Goblet Hyperplasia_Multi2_0.1.(d)
        Gnuplot Plotting File:
C:\USEPA\BMDS212\Data\V2O5\NTP_2002NonCancerFemaleRats\FemaleRatsNoseGobletEpiHyperpla
sia\NTP_2002_Nose Goblet Hyperplasia_Multi2_0.1.plt
================================================================
   The form of the probability function is:
   P[response] = background + (1-background)*[1-EXP(
                -beta1*dose^1-beta2*dose^2)]
```

```
   The parameter betas are restricted to be positive

   Dependent variable = Response
   Independent variable = Dose

 Total number of observations = 4
 Total number of records with missing values = 0
 Total number of parameters in model = 3
 Total number of specified parameters = 0
 Degree of polynomial = 2

 Maximum number of iterations = 250
 Relative Function Convergence has been set to: 1e-008
 Parameter Convergence has been set to: 1e-008

                 Default Initial Parameter Values
                    Background =     0.270822
                       Beta(1) =            0
                       Beta(2) =      75.6009

           Asymptotic Correlation Matrix of Parameter Estimates

           ( *** The model parameter(s)  -Beta(1)
                 have been estimated at a boundary point, or have been specified by
the user,
                 and do not appear in the correlation matrix )

             Background      Beta(2)
Background            1        -0.59
   Beta(2)        -0.59            1


                                Parameter Estimates
                                                        95.0% Wald Confidence
Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf.
Limit
     Background         0.276279            *                *                  *
        Beta(1)                0            *                *                  *
        Beta(2)          71.2588            *                *                  *

* - Indicates that this value is not calculated.

                        Analysis of Deviance Table
       Model      Log(likelihood)  # Param's  Deviance  Test d.f.   P-value
     Full model        -126.318         4
   Fitted model        -127.169         2       1.70254      2          0.4269
  Reduced model        -133.292         1       13.9479      3        0.002977

           AIC:         258.338

                                Goodness  of  Fit
                                                                      Scaled
     Dose     Est._Prob.    Expected    Observed     Size       Residual
  ---------------------------------------------------------------------------
    0.0000     0.2763        13.814    13.000          50       -0.257
    0.0231     0.3033        15.167    18.000          50        0.872
    0.0470     0.3815        19.077    16.000          50       -0.896
    0.0875     0.5806        29.030    30.000          50        0.278

 Chi^2 = 1.71      d.f. = 2        P-value = 0.4262

   Benchmark Dose Computation
Specified effect =            0.1
Risk Type        =      Extra risk
Confidence level =           0.95
```

```
          BMD =      0.0384521
         BMDL =      0.0135524
         BMDU =      0.0544996

Taken together, (0.0135524, 0.0544996) is a 90% two-sided confidence
interval for the BMD
```



B.4. REFERENCES

NTP. (National Toxicology Program). (2002). NTP toxicology and carcinogensis studies of vanadium pentoxide (CAS No.;1314-62-1) in F344/N rats and B6C3F1 mice (inhalation). Washington, DC.

U.S. EPA. (U.S. Environmental Protection Agency). (1994). Methods for derivation of inhalation reference concentrations and application of inhalation dosimetry. (EPA/600/8-90/066F). Research Triangle Park, NC: U.S. Environmental Protection Agency, Office of Research and Development, Office of Health and Environmental Assessment, Environmental Criteria and Assessment Office. http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=71993.

U.S. EPA. (U.S. Environmental Protection Agency). (2000). Benchmark dose technical guidance document [external review draft]. (EPA/630/R-00/001). Washington, DC: U.S. Environmental Protection Agency, Risk Assessment Forum. http://www.epa.gov/raf/publications/benchmark-dose-doc-draft.htm.

APPENDIX C. BENCHMARK CONCENTRATION MODELING OF COMBINED LUNG ALVEOLAR AND BRONCHIOLAR TUMOR DATASETS FROM NTP STUDIES (2002)

To derive the cancer slope factor for vanadium pentoxide, combined alveolar/bronchiolar adenoma and carcinoma datasets in $B6C3F_1$ mice (Table C-2) from NTP (2002) were selected because of their biological and statistical significance.

The highlights of the benchmark concentration (BMC) modeling results are the cancer slope factor derivation and the benchmark dose (BMD) modeling results.

Cancer Slope Factor

The cancer slope factor was estimated as 3.4 per mg/m^3 after linear extrapolation. The selected model for each dataset, the candidate points of departure (PODs) and the calculation of cancer slope factor are summarized in Table C-1.

Benchmark Dose Software (BMDS) Modeling

Each dataset was first fit with the recommended dichotomous model, the multistage-cancer model, through EPA BMDS (version 2.1.2); if the goodness of fit test showed a p-value < 0.05, other dichotomous models available in EPA BMDS (version 2.1.2) were used; if still no model showed adequate goodness of fit (p-value ≥ 0.05), the highest dose was dropped from further modeling. Following the general model selection steps outlined in the draft *Benchmark Dose Technical Guidance* (U.S. EPA, 2000), the best-fitting model was selected for each dataset to estimate the candidate POD, which was the benchmark concentration lower limit (BMCL) at the predetermined benchmark response (BMR).

C.1. BMR

Because all noncontrol concentrations showed essentially a plateau response, the tumor datasets provided limited information about the concentration-response relationship. So, BMR for each dataset was calculated based on the response at the control and the first noncontrol concentration groups.

Multistage Weibull (MSW) Time-to-tumor Modeling

Because the survival curves reported by NTP (2002) showed a high percentage of deaths (22–46%) at the end of the 2-year studies across the different concentration groups, the MSW time-to-tumor model also was used with these datasets. No adequate fit was obtained, however, for either the male or the female mouse dataset.

Table C-1. Summary of candidate PODs and cancer slope factors

Animal	BMR (extra risk)[a]	HEC[b]		Cancer slope factor[d] (per mg/m^3)
		BMC (mg/m^3)	BMCL[c] (candidate POD, mg/m^3)	
Male B6C3F$_1$ mouse	0.71	0.360	0.208	3.4
Female B6C3F$_1$ mouse	0.67	0.237	0.161	4.2

[a]BMR was calculated based on the response at the control concentration and the first non-control concentration (Table C-6).
[b]HEC = human equivalent concentration.
[c]BMCL = the lower bound of BMC at 95% confidence level.
[d]Cancer Slope Factor = BMR/BMCL, as linear extrapolation was used.

Source: NTP (2002).

C.2. TREND TESTS BEFORE MODELING

Two inhalation carcinogenesis datasets were selected because of the biological and the statistical significance reported by NTP (2002). The Cochran-Armitage test was conducted to confirm the significance of the statistical trend for the selected datasets (Table C-2) before modeling.

Table C-2. Trend tests on the inhalation carcinogenesis datasets in mice from the 2-year inhalation studies

Endpoint	Concentration as reported (mg/m^3)[a]				Trend test[b]	
	0.0	1.0	2.0	4.0	*Z* -Score	*p*-value
Incidence[c] in male B6C3F$_1$ mice						
Lung alveolar/bronchiolar adenoma and carcinoma	22/50	42/50	43/50	43/50	4.14	<0.0001
Incidence[c] in female B6C3F$_1$ mice						
Lung alveolar/bronchiolar adenoma and carcinoma	1/48	32/47	35/48	32/49	5.19	<0.0001

[a]Concentrations are as reported by NTP (2002).
[b]One-sided Cochran-Armitage trend test.
[c]Incidence = (number of animals with alveolar/bronchiolar adenoma or carcinoma)/(animal sample size). Only the animals alive at 52 weeks or when the first tumor appeared, whichever was earlier, were counted toward the sample size.

Source: NTP (2002).

C.3. DOSE CONVERSION

In the carcinogenesis studies reported by NTP (2002), mice (B6C3F$_1$) were exposed to vanadium pentoxide by inhalation. To analyze the concentration-response effect, the reported

concentrations of vanadium pentoxide were converted to human equivalent concentrations (HECs) before any modeling and extrapolation were completed.

First, the average lifetime animal body weights of rats were estimated based on the mean body weight at different weeks (Table C-3). Then, following the EPA's *Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry* (U.S. EPA, 1994), regional deposited dose ratios (RDDRs) were calculated with the RDDR program designed by U.S. EPA (1994) and summarized in Table C-4. Finally, the HEC for each concentration/sex group was calculated from the reported concentration in mice by multiplying the continuous exposure adjustment factor and the pulmonary RDDR (Table C-5).

Table C-3. Average lifetime animal body weight of mice for 2-year inhalation studies of vanadium pentoxide

Vanadium pentoxide concentration as reported (mg/m^3)	Mean animal body weight[a] (g)			Average lifetime animal body weight[b] (g)
	1–13 weeks	14–52 weeks	53–104 weeks	
Male Mice ($B6C3F_1$)				
0	30.8	46.7	54.1	48.9
1	30.5	47.0	52.9	48.3
2	30.3	45.4	51.4	46.9
4	29.8	43.7	46.1	43.6
Female Mice ($B6C3F_1$)				
0	25.5	42.7	56.1	47.7
1	24.9	40.9	50.7	44.2
2	24.8	36.8	44.8	39.7
4	24.5	34.2	40.1	36.3

[a]As reported in Table 20-21of the NTP report (2002).
[b]Average lifetime animal body weight = (mean body weight 1-13 weeks × 13 + mean body weight 14–52 weeks × 39 + mean body weight 53–104 weeks × 52)/104

Source: NTP (2002).

Table C-4. RDDRs for different concentration/sex group of mice in the 2-year inhalation studies of vanadium pentoxide

Concentration as reported (mg/m^3)	Average lifetime animal body weight[a] (g)	Average MMAD[b]	Average GSD[c]	RDDR[d]
				Pulmonary
Male mice ($B6C3F_1$)				
0	48.9[e]	1.26	1.87	1.168[e]
1	48.3[e]			1.168[e]
2	46.9[e]			1.168[e]
4	43.6			1.134
Female mice ($B6C3F_1$)				
0	47.7[e]	1.26	1.87	1.168[e]
1	44.2			1.143
2	39.7			1.077
4	36.3			1.023

[a]Average lifetime animal body weight of each concentration/sex group was calculated in Table C-3.
[b]MMAD = mass median aerodynamic diameter; calculated based on the MMADs reported for 2-year studies (NTP, 2002).
[c]GSD = geometric standard deviation; calculated based on the GSDs reported for 2-year studies (NTP, 2002).
[d]RDDR = regional deposited dose ratio; calculated with the RDDR program from USA EPA (1994).
[e]Because the average lifetime animal body weight was out of the mouse weight range (17–46g) accepted by the RDDR program (V.2.3, US EPA), 46 g was used to calculate the corresponding RDDRs.
Source: NTP (2002).

Table C-5. Human equivalent concentrations of vanadium pentoxide in the 2-year inhalation studies

Concentration as reported[a] (mg/m^3)	Continuous exposure adjustment factor[b]	RDDR[c]	HEC[d] (mg/m^3)
		Pulmonary	Pulmonary
Male mice ($B6C3F_1$)			
0	0.179	1.168	0.00
1	0.179	1.168	0.21
2	0.179	1.168	0.42
4	0.179	1.134	0.81
Female mice ($B6C3F_1$)			
0	0.179	1.168	0.00
1	0.179	1.143	0.20
2	0.179	1.077	0.38
4	0.179	1.023	0.73

[a]*Toxicology and carcinogenesis studies of vanadium pentoxide in F344/N rats and B6C3F$_1$ mice*, NTP (2002).
[b]Continuous exposure adjustment factor = (6/24) × (5/7); animals exposed to vanadium pentoxide 6 h/d, 5 d/wk.
[c]Refer to Appendix Table C-4.
[d]HEC = human equivalent concentration = concentration as reported × continuous exposure adjustment factor × RDDR.
Source: NTP (2002).

C.4. BMR CALCULATION

Because all noncontrol concentrations from the NTP carcinogenesis studies (2002) showed essentially a plateau response, the dataset provided limited information about the concentration-response relationship, given that the complete range of response from background to maximum must occur somewhere below the lowest dose. Thus, the BMD could be just below the first dose or it could be orders of magnitude lower. A BMR was therefore estimated based on the response at the control concentration, and the first noncontrol concentration was calculated (Table C-6) and then used to estimate the BMCL.

Table C-6. BMR estimation for male and female mice datasets

Animal	P(Control)[a]	P(1st non-control concentration)[b]	Calculated BMR (extra risk)[c]
Male B6C3F$_1$ Mice	0.44	0.84	0.71
Female B6C3F$_1$ Mice	0.02	0.68	0.67

[a]The combined alveolar/bronchiolar adenoma and carcinoma incidence in the control group.
[b]The combined alveolar/bronchiolar adenoma and carcinoma incidence in the first noncontrol concentration group.
[c]Calculated BMR = [P(1st Noncontrol Concentration) − P(Control)]/[1 – P(Control)].

C.5. BMDS MODELING FOR INHALATION CARCINOGENESIS DATASETS

Each dataset was first fit with the dichotomous multistage-cancer model provided in EPA BMDS (version 2.1.2); if the goodness of fit test showed a p-value < 0.05, other dichotomous models available in EPA BMDS (version 2.1.2) were fit; if still no model showed adequate goodness of fit (p-value ≥ 0.05), the highest dose was dropped for further modeling.

Following the general model selection steps outlined in the draft *Benchmark Dose Technical Guidance* (U.S. EPA, 2000), the best-fitting model was selected for each dataset to estimate the candidate POD, which was the BMCL at the calculated BMR for each dataset.

The selected models and candidate PODs for all endpoints are summarized in Table C-1.

C.5.1. Lung Tumor Dataset 1: Combined Incidence of Alveolar/Bronchiolar Adenoma and Carcinoma in Male Mice, NTP (2002)

Summary

The Cochran-Armitage test confirmed the statistically significant trend with a Z-score = 4.14 and a one-sided a p-value < 0.0001.

Because all the noncontrol concentrations showed a similar effect, a BMR as 71% extra risk was calculated and used to estimate the POD. The goodness of fit, BMC, and BMCL for each model are summarized in Table C-7 and Table C-8.

Because the primary cancer model (Multistage cancer) did not show an adequate fit, six other dichotomous models available in EPA BMDS (version 2.1.2.) were fit to this dataset. Two models demonstrated adequate goodness of fit (p-value ≥ 0.05) and good visual fit, but

Log-Probit model failed to compute a reasonable BMCL. Based on the draft *Benchmark Dose Technical Guidance* (U.S. EPA, 2000), the Log-Logistic model was selected as the best-fitting model. Using this model, the $BMCL_{71}$ was 0.208 mg/m^3.

Further BMD modeling was performed for comparison purposes. Because the primary cancer model (Multistage cancer) did not show adequate fit with all doses, the highest concentration was dropped from further modeling. After dropping the high dose, the Multistage-cancer model demonstrated adequate goodness of fit (*p*- value ≥ 0.05) and good visual fit.

Table C-7. BMDS modeling results for combined incidence of alveolar/bronchiolar adenoma and carcinoma in male mice

Model[a]	Goodness of fit				BMR	HEC	
	p-Value	AIC[b]	Largest scaled residual	Scaled residual of interest		BMC (mg/m^3)	BMCL[c] (mg/m^3)
Primary cancer models							
Multistage cancer Stages 1,2, and 3	0.01	207.0	2.05	0.72	Extra risk 71%	0.532	0.379
Other dichotomous models							
Log-Logistic	**0.19**	**200.632**	**-1.42**	**0.04**	Extra Risk 71%	**0.360**	**0.208**
Log-Probit	0.88	199.6	0.13	-0.06		0.146	failed
Gamma	0.01	207.0	2.05	0.72		0.532	0.379
Weibull							
Logistic	0.00	209.4	2.15	0.96		0.609	0.447
Probit	0.00	210.4	2.19	-1.54		0.654	0.495

[a]Selected model is the best-fitting model for the dataset based on the draft *Benchmark Dose Technical Guidance* (2000).
[b]AIC = Akaike Information Criterion.
[c]BMCL = the lower bound of BMC at 95% confidence level.

Source: NTP (2002).

BMDS output file

```
================================================================
        Logistic Model. (Version: 2.13; Date: 10/28/2009)
        Input Data File:
C:\USEPA\BMDS212\Data\V2O5\NTP_2002Cancer\MaleMiceTumor4DosesAllModel\NTP_2002_MaleMic
eTumor4DosesAllModels_LogLogistic_0.71.(d)
        Gnuplot Plotting File:
C:\USEPA\BMDS212\Data\V2O5\NTP_2002Cancer\MaleMiceTumor4DosesAllModel\NTP_2002_MaleMic
eTumor4DosesAllModels_LogLogistic_0.71.plt
================================================================
   The form of the probability function is:
   P[response] = background+(1-background)/[1+EXP(-intercept-slope*Log(dose))]

   Dependent variable = Effect
   Independent variable = Dose
   Slope parameter is restricted as slope >= 1
```

```
   Total number of observations = 4
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008

 User has chosen the log transformed model

                 Default Initial Parameter Values
                    background =         0.44
                     intercept =       1.9384
                         slope =            1
           Asymptotic Correlation Matrix of Parameter Estimates
           ( *** The model parameter(s)  -slope
                 have been estimated at a boundary point, or have been specified by
the user,
                 and do not appear in the correlation matrix )

             background    intercept
background            1        -0.58
 intercept        -0.58            1

                                Parameter Estimates
                                                        95.0% Wald Confidence
Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf.
Limit
     background         0.451808            *                *                   *
      intercept          1.91813            *                *                   *
          slope                1            *                *                   *
* - Indicates that this value is not calculated.

                       Analysis of Deviance Table
       Model      Log(likelihood)  # Param's  Deviance  Test d.f.   P-value
     Full model        -96.7763         4
   Fitted model        -98.3159         2       3.07913      2          0.2145
  Reduced model        -112.467         1       31.3814      3         <.0001
           AIC:         200.632

                                Goodness  of  Fit
                                                                      Scaled
     Dose     Est._Prob.    Expected    Observed     Size       Residual
  ---------------------------------------------------------------------
    0.0000     0.4518        22.590    22.000          50       -0.168
    0.2100     0.7744        38.719    42.000          50        1.110
    0.4200     0.8580        42.898    43.000          50        0.041
    0.8100     0.9159        45.793    43.000          50       -1.423
 Chi^2 = 3.29      d.f. = 2        P-value = 0.1934

   Benchmark Dose Computation
Specified effect =          0.71
Risk Type        =      Extra risk
Confidence level =          0.95
            BMD =      0.359607
           BMDL =      0.208416
```

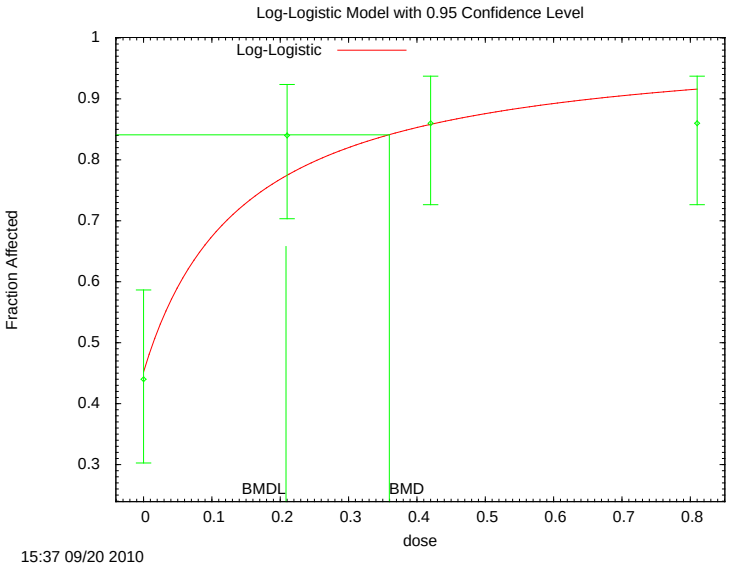


Table C-8. BMDS modeling results for combined incidence of alveolar/bronchiolar adenoma and carcinoma in male mice after dropping the highest concentration

Model[a]	Goodness of fit				BMR	HEC	
	p-Value	AIC[b]	Largest scaled residual	Scaled residual of interest		BMC (mg/m^3)	BMCL[c] (mg/m^3)
Primary Cancer Models							
Multistage cancer Stage 1,2, and 3	**0.12**	**159.5**		**1.19**	**Extra Risk 71%**	**0.310**	**0..220**
Other Dichotomous Models							
Log-Logistic	0.43	157.7		0.52	Extra Risk 71%	0.260	0.140
Gamma	0.12	159.5		1.19		0.310	0.220
Weibull							
Probit	0.04	161.5		-1.03		0.340	0.270
Logistic	0.05	160.1		-1.09		0.330	0.250
Log-Probit	NA	159.1		0.00		0.190	failed

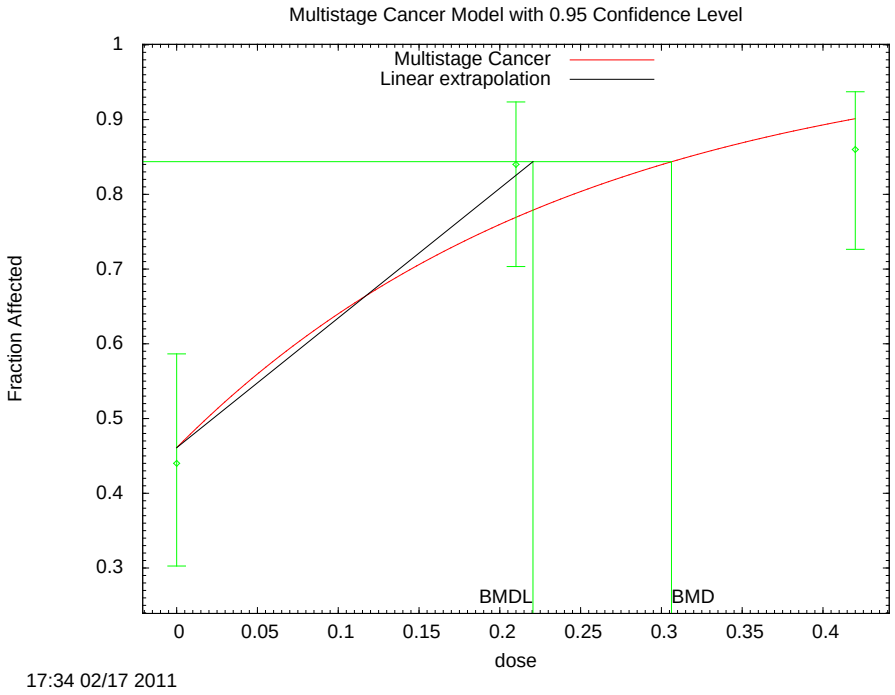
[a]Selected model is the best-fitting model for the dataset based on the draft *Benchmark Dose Technical Guidance* (2000).
[b]AIC = Akaike Information Criterion.
[c]BMCL = the lower bound of BMC at 95% confidence level.

Source: NTP (2002).

1 ***BMDS output file***

```
================================================================
        Multistage Cancer Model. (Version: 1.9;  Date: 05/26/2010)
        Input Data File:
C:\USEPA\BMDS212\Data\V2O5\NTP_2002Cancer\MaleMiceTumor3DosesAllModel\NTP_2002_MaleMic
eTumor3DosesAllModels_MultiCanc1_0.71.(d)
        Gnuplot Plotting File:
C:\USEPA\BMDS212\Data\V2O5\NTP_2002Cancer\MaleMiceTumor3DosesAllModel\NTP_2002_MaleMic
eTumor3DosesAllModels_MultiCanc1_0.71.plt
                                                Thu Feb 17 17:24:36 2011
================================================================
   The form of the probability function is:
P[response] = background + (1-background)*[1-EXP(
                -beta1*dose^1)]
   The parameter betas are restricted to be positive
   Dependent variable = Effect
   Independent variable = Dose
 Total number of observations = 3
 Total number of records with missing values = 0
 Total number of parameters in model = 2
 Total number of specified parameters = 0
 Degree of polynomial = 1

 Maximum number of iterations = 250
 Relative Function Convergence has been set to: 1e-008
 Parameter Convergence has been set to: 1e-008

                 Default Initial Parameter Values
                    Background =     0.535297
                       Beta(1) =       3.3007
          Asymptotic Correlation Matrix of Parameter Estimates
            Background      Beta(1)
Background            1         -0.6
   Beta(1)         -0.6            1

                                Parameter Estimates
                                                      95.0% Wald Confidence
Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf.
Limit
     Background         0.461149            *                *                  *
        Beta(1)          4.04221            *                *                  *
* - Indicates that this value is not calculated.

                        Analysis of Deviance Table
       Model      Log(likelihood)  # Param's  Deviance  Test d.f.   P-value
     Full model        -76.5282         3
   Fitted model        -77.7667         2       2.47711      1          0.1155
  Reduced model         -89.871         1       26.6857      2         <.0001
           AIC:         159.533

                                  Goodness  of  Fit
                                                                    Scaled
     Dose     Est._Prob.    Expected    Observed     Size       Residual
  ---------------------------------------------------------------------
    0.0000     0.4611        23.057    22.000          50       -0.300
    0.2100     0.7694        38.471    42.000          50        1.185
    0.4200     0.9013        45.067    43.000          50       -0.980
 Chi^2 = 2.45      d.f. = 1        P-value = 0.1172

   Benchmark Dose Computation
Specified effect =           0.71
Risk Type        =      Extra risk
Confidence level =           0.95
             BMD =       0.306237
```

```
            BMDL =       0.220508
            BMDU =       0.471328
Taken together, (0.220508, 0.471328) is a 90     % two-sided confidence
interval for the BMD
Multistage Cancer Slope Factor =       3.21985
```



C.5.2. Lung Tumor Dataset 2: Combined Incidence of Alveolar/Bronchiolar Adenoma and Carcinoma in Female Mice, NTP (2002)

Summary

The Cochran-Armitage test confirmed the statistically significant trend with a *Z*-score = 5.19 and a one-sided *p*-value < 0.0001.

Because all noncontrol concentrations showed a similar effect, a BMR as 67% extra risk was calculated and used to determine the POD. The goodness of fit, BMC, and BMCL for each model are summarized in Table C-9 and Table C-10.

Because the primary cancer model (Multistage cancer) did not show an adequate fit, six other dichotomous models available in EPA BMDS (version 2.1.2.) were fit to this dataset. Only one model demonstrated adequate goodness of fit (*p*-value ≥ 0.05); it failed, however, to compute BMC and BMCL. So, the highest concentration was dropped from further modeling.

After dropping the highest dose, the Multistage-cancer model still did not show an adequate fit. Six other dichotomous models available in EPA BMDS (version 2.1.2.) were tried. Only the Log-Logistc model demonstrated adequate goodness of fit (*p*-value ≥ 0.05) and good visual fit. Based on the draft *Benchmark Dose Technical Guidance* (U.S. EPA, 2000), the Log-Logistic model was selected as the best-fitting model. Using this model, the $BMCL_{67}$ was 0.161 mg/m^3.

Table C-9. BMDS modeling results for combined incidence of alveolar/bronchiolar adenomas and carcinomas with all four concentrations in female mice

Model [a]	Goodness of fit				BMR	HEC	
	p-Value	AIC [b]	Largest scaled residual	Scaled residual of interest		BMC (mg/m^3)	BMCL[c] (mg/m^3)
Primary cancer models							
Multistage cancer Stages 1,2, and 3	0.00	218.8	-3.90	1.33	Extra Risk 67%	0.435	0.359
Other dichotomous models							
Log-Probit	0.72	192.6	0.62	-999.00	Extra Risk 67%	Failed [d]	Failed [d]
Log-Logistic	0.00	202.7	-2.87	0.52		0.351	0.255
Gamma	0.00	218.8	-3.90	1.33		0.435	0.359
Weibull							
Logistic	0.00	239.4	-4.00	-2.62		0.657	0.532
Probit	0.00	239.6	-3.97	-2.48		0.670	0.553

[a]Selected model is the best-fitting model for the dataset based on draft *Benchmark Dose Technical Guidance* (2000).
[b]AIC = Akaike Information Criterion.
[c]BMCL = the lower bound of BMC at 95% confidence level.
Source: NTP (2002).

Table C-10. BMDS modeling results for combined incidence of alveolar/bronchiolar adenoma and carcinoma in female mice after dropping the highest concentration

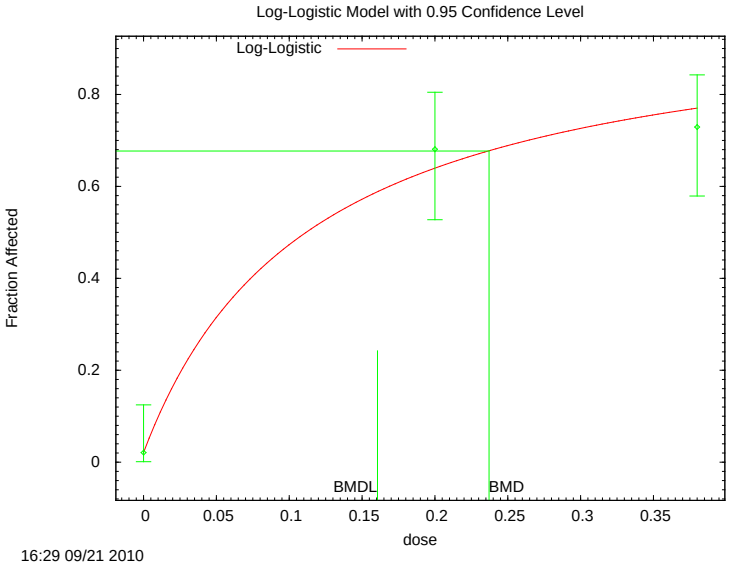
Model[a]	Goodness of fit				BMR	HEC	
	p-Value	AIC[b]	Largest scaled residual	Scaled residual of interest		BMC (mg/m^3)	BMCL[c] (mg/m^3)
Primary cancer models							
Multistage cancer Stages 1,2 and 3	0.06	132.2	1.42	1.42	Extra risk 67%	0.264	0.213
Other dichotomous models							
Log-Logistic	**0.37**	**129.453**	**-0.67**	**0.60**	Extra risk 67%	**0.237**	**0.161**
Gamma	0.06	132.2	1.42	1.42		0.264	0.213
Weibull							
Probit	0.00	145.9	3.00	-1.85		0.309	0.272
Logistic	0.00	146.5	2.84	-1.99		0.303	0.263
Log-Probit	NA	130.7	0.00	0.00		0.190	failed

[a]Selected model is the best-fitting model for the dataset based on draft *Benchmark Dose Technical Guidance* (2000).
[b]AIC = Akaike Information Criterion.
[c]BMCL = the lower bound of BMC at 95% confidence level.
Source: NTP (2002).

1 ***BMDS output file***

```
 ================================================================
        Logistic Model. (Version: 2.13; Date: 10/28/2009)
        Input Data File:
C:\USEPA\BMDS212\Data\V2O5\NTP_2002Cancer\FemaleMiceTumor3DosesAllModels\NTP_2002_Fema
leMiceTumor3DosesAllModels_LogLogistic_0.67.(d)
        Gnuplot Plotting File:
C:\USEPA\BMDS212\Data\V2O5\NTP_2002Cancer\FemaleMiceTumor3DosesAllModels\NTP_2002_Fema
leMiceTumor3DosesAllModels_LogLogistic_0.67.plt
      ================================================================
   The form of the probability function is:

   P[response] = background+(1-background)/[1+EXP(-intercept-slope*Log(dose))]

   Dependent variable = Effect
   Independent variable = Dose
   Slope parameter is restricted as slope >= 1

   Total number of observations = 3
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008

   User has chosen the log transformed model

                 Default Initial Parameter Values
                    background =    0.0208333
                     intercept =      2.36858
                         slope =            1

           Asymptotic Correlation Matrix of Parameter Estimates

           ( *** The model parameter(s)  -slope
                 have been estimated at a boundary point, or have been specified by
the user,
                 and do not appear in the correlation matrix )

             background    intercept
background            1        -0.14
 intercept        -0.14            1

                                Parameter Estimates
                                                        95.0% Wald Confidence
Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf.
Limit
     background        0.0210689            *                *                  *
      intercept          2.14725            *                *                  *
          slope                1            *                *                  *
* - Indicates that this value is not calculated.

                        Analysis of Deviance Table
       Model      Log(likelihood)  # Param's  Deviance  Test d.f.   P-value
     Full model        -62.3295         3
   Fitted model        -62.7264         2       0.79377      1           0.373
  Reduced model        -98.9486         1       73.2384      2         <.0001
           AIC:         129.453

                                  Goodness  of  Fit
                                                          Scaled
     Dose     Est._Prob.    Expected    Observed     Size       Residual
  -----------------------------------------------------------------------
    0.0000     0.0211         1.011     1.000          48       -0.011
```

```
    0.2000     0.6391        30.036    32.000          47        0.596
    0.3800     0.7698        36.952    35.000          48       -0.669
 Chi^2 = 0.80      d.f. = 1        P-value = 0.3699

   Benchmark Dose Computation
Specified effect =           0.67
Risk Type        =      Extra risk
Confidence level =           0.95
             BMD =        0.23715
            BMDL =       0.160575
```



C.6. EXTRAPOLATION METHOD AND INHALATION CANCER SLOPE FACTOR

As explained in Chapter 5, linear extrapolation was applied in this assessment and BMCL at the calculated BMR was regarded as the POD. The inhalation cancer slope factor was the upper-bound estimation of risk and was calculated as BMR/BMCL. The inhalation slope factor was used to estimate the lifetime lung tumor risk in humans for vanadium pentoxide exposure by inhalation. Data are summarized in Table C-1.

C.7. MSW TIME-TO-TUMOR MODELING OF THE INHALATION CARCINOGENESIS DATASETS

MSW time-to-tumor modeling also was used to model the datasets because the survival curves reported by NTP (2002) showed a high percentage (22–46%) of deaths at the end of 2-year studies across the different concentration groups. The individual animal data were obtained through the NTP website and summarized according to concentration and week of death (Table C-11).

Based on the results, the MSW time-to-tumor model was not recommended to derive cancer slope factors for these datasets for two reasons:

- The visual fit near the first noncontrol concentration was not adequate.

- The parameters, including c, beta_0, and beta_1, were very close to the initial values, which suggested the model was nonconvergent.

C.7.1. Lung Tumor Dataset 1: Combined Incidence of Alveolar/Bronchiolar Adenomas and Carcinomas in Male Mice, NTP (2002)

Table C-11. Grouped data for MSW time-to-tumor modeling; B6C3F$_1$ male mice exposed to vanadium pentoxide by inhalation for 2 years

Human equivalent concentration (mg/m^3)	Week of death	Response category for alveolar/bronchiolar adenoma or carcinoma [b]	Number of animals
0.0	2	C	1
	26	C	1
	72	C	1
	89	C	1
	92	C	1
	95	C	1
	97	C	1
	98	C	2
	99	C	1
	101	C	1
	102	C	1
	104	C	6
	104	I	1
	105	C	31
0.21	3	C	3
	64	C	1
	75	I	1
	76	I	1
	81	I	1
	83	I	1
	86	C	1
	87	I	1
	91	I	1
	93	I	1
	94	I	2
	95	C	1
	101	I	1
	103	I	1
	104	I	8
	104	C	1
	105	C	11
	105	I	13
0.42	36	C	1
	39	C	1

Table C-11. Grouped data for MSW time-to-tumor modeling; B6C3F$_1$ male mice exposed to vanadium pentoxide by inhalation for 2 years

Human equivalent concentration (mg/m^3)	Week of death	Response category for alveolar/bronchiolar adenoma or carcinoma [b]	Number of animals
	40	I	1
	45	I	1
	50	C	1
	59	C	1
	70	C	1
	72	I	1
	76	I	1
	82	C	1
	83	I	1
	91	I	1
	92	I	1
	95	I	1
	95	C	1
	99	I	1
	101	I	2
	101	C	1
	104	I	7
	105	I	17
	105	C	7
0.81	36	C	1
	68	I	1
	77	I	1
	79	I	1
	79	C	1
	81	C	1
	81	I	1
	87	C	2
	91	I	1
	93	I	1
	98	I	1
	99	C	1
	101	I	2
	104	I	7
	104	C	3
	105	I	16
	105	C	9

[a]Concentrations were the original doses reported in the publication from NTP (2002). No adjustment was applied.
[b]Categories of response: "C" = Neither carcinoma nor adenoma was detected when the subject was removed from the study due to scheduled sacrifice or unscheduled death; "I" = carcinoma or adenoma, or both, were detected when the subject was removed from the study due to scheduled sacrifice or unscheduled death.

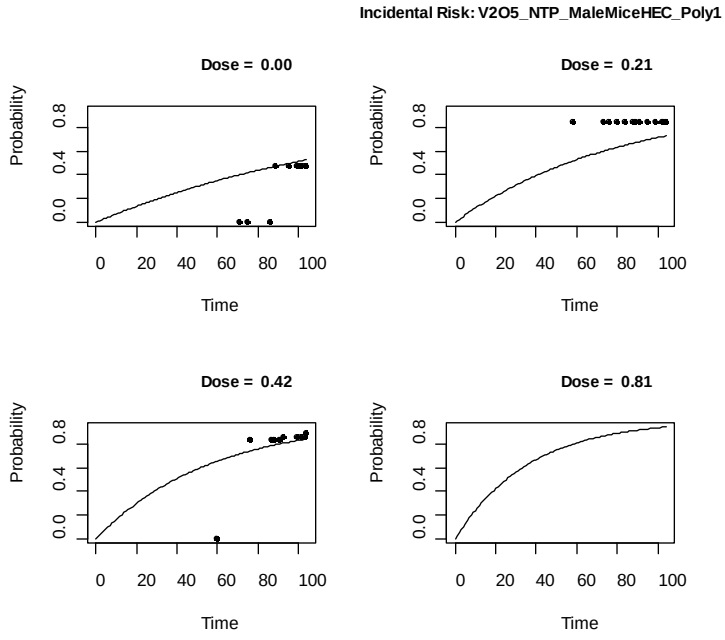
Table C-11. Grouped data for MSW time-to-tumor modeling; B6C3F$_1$ male mice exposed to vanadium pentoxide by inhalation for 2 years

Human equivalent concentration (mg/m^3)	Week of death	Response category for alveolar/bronchiolar adenoma or carcinoma [b]	Number of animals

1 ***MSW Time-to-tumor output***

```
 ========================================================================
        Multistage Weibull Model. (Version: 1.6.1;  Date: 11/24/2009)
        Solutions are obtained using donlp2-intv, (c) by P. Spellucci
        Input Data File: V2O5_NTP_MaleMiceHEC_Poly1.(d)
 ========================================================================
 V2O5_NTP_MaleMiceHEC
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
   The form of the probability function is:
   P[response] = 1-EXP{-(t - t_0)^c *
                 (beta_0+beta_1*dose^1)}

   The parameter betas are restricted to be positive

   Dependent variable = CONTEXT
   Independent variables = DOSE, TIME

 Total number of observations = 200
 Total number of records with missing values = 0
 Total number of parameters in model = 4
 Total number of specified parameters = 1
 Degree of polynomial = 1

   User specifies the following parameters:
          t_0    =          0

 Maximum number of iterations = 64
 Relative Function Convergence has been set to: 2.22045e-016
 Parameter Convergence has been set to: 1.49012e-008

                  Default Initial Parameter Values
                        c      =      1.05882
                        t_0    =            0   Specified
                        beta_0 =    0.0054829
                        beta_1 =    0.0193401

           Asymptotic Correlation Matrix of Parameter Estimates
           ( *** The model parameter(s)  -c         -t_0
                 have been estimated at a boundary point, or have been specified by
the user,
                 and do not appear in the correlation matrix )

                 beta_0       beta_1
    beta_0            1        -0.61
    beta_1        -0.61            1

                                Parameter Estimates
                                                        95.0% Wald Confidence
Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf.
Limit
         c                    1              NA
```

```
         beta_0       0.00720175       0.00201449          0.00325342
0.0111501
         beta_1        0.0252605       0.00794841          0.00968191
0.0408391

NA - Indicates that this parameter has hit a
     bound implied by some inequality constraint
     and thus has no standard error.

                Log(likelihood)   # Param            AIC
   Fitted Model        -101.116         3         208.233

                    Data Summary
                        CONTEXT
              C      F      I      U  Total  Expected Response
    DOSE
        0    28      0     22      0     50    25.86
     0.21     8      0     42      0     50    35.33
     0.42     7      0     43      0     50    41.53
     0.81     7      0     43      0     50    46.15


   Benchmark Dose Computation
Risk Response    =     Incidental
Risk Type        =          Extra
Specified effect =           0.71
Confidence level =            0.9
Time             =            104
             BMD =       0.471196
            BMDL =       0.390136
            BMDU =       0.716564
```



1

C.7.2. Lung Tumor Dataset 2: Combined Incidence of Alveolar/Bronchiolar Adenoma and Carcinoma in Female Mice, NTP (2002)

Table C-12. Grouped data for MSW time-to-tumor modeling; $B6C3F_1$ female mice exposed to vanadium pentoxide by inhalation for 2 years

Human equivalent concentration (mg/m^3)	Week of death	Response category for alveolar/bronchiolar adenoma and/or carcinoma [b]	Number of animals
0.00	2	C	1
	26	C	1
	72	C	1
	89	C	1
	92	C	1
	95	C	1
	97	C	1
	98	C	2
	99	C	1
	101	C	1
	102	C	1
	104	C	6
	104	I	1
	105	C	31
0.20	3	C	3
	64	C	1
	75	I	1
	76	I	1
	81	I	1
	83	I	1
	86	C	1
	87	I	1
	91	I	1
	93	I	1
	94	I	2
	95	C	1
	101	I	1
	103	I	1
	104	I	8
	104	C	1
	105	C	11
	105	I	13
0.38	36	C	1
	39	C	1
	40	I	1

Table C-12. Grouped data for MSW time-to-tumor modeling; B6C3F$_1$ female mice exposed to vanadium pentoxide by inhalation for 2 years

Human equivalent concentration (mg/m^3)	Week of death	Response category for alveolar/bronchiolar adenoma and/or carcinoma [b]	Number of animals
	45	I	1
	50	C	1
	59	C	1
	70	C	1
	72	I	1
	76	I	1
	82	C	1
	83	I	1
	91	I	1
	92	I	1
	95	I	1
	95	C	1
	99	I	1
	101	I	2
	101	C	1
	104	I	7
	105	I	17
	105	C	7
0.73	36	C	1
	68	I	1
	77	I	1
	79	I	1
	79	C	1
	81	C	1
	81	I	1
	87	C	2
	91	I	1
	93	I	1
	98	I	1
	99	C	1
	101	I	2
	104	I	7
	104	C	3
	105	I	16
	105	C	9

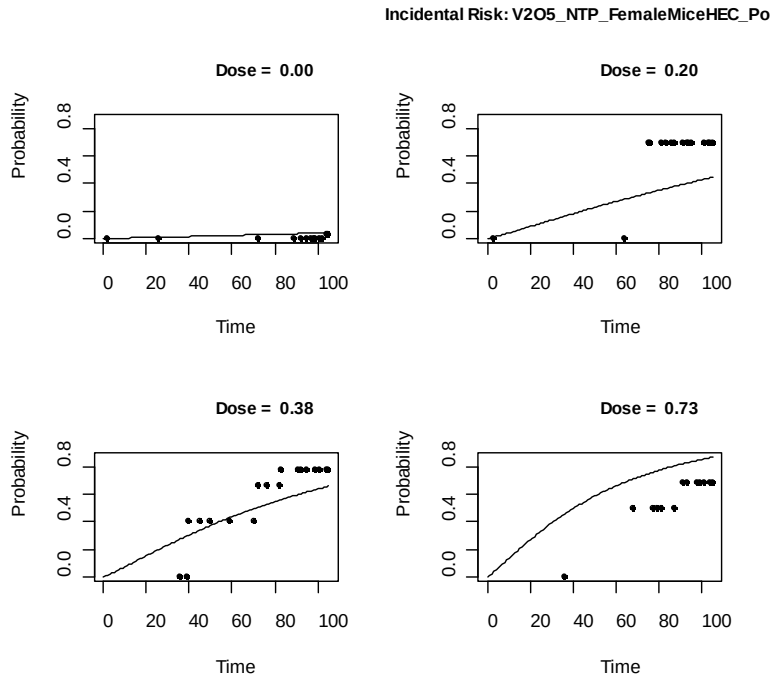
[a]Concentration was the original one reported in the publication from NTP (2002). No adjustment was applied.
[b]Categories of response: "C"=Neither carcinoma nor adenoma was detected when the subject was removed from the study due to scheduled sacrifice or unscheduled death; "I"= carcinoma or adenoma, or both, were detected when the subject was removed from the study due to scheduled sacrifice or unscheduled death.
Source: NTP (2002).

MSW Time-to-tumor output

```
=================================================================
        Multistage Weibull Model. (Version: 1.6.1;  Date: 11/24/2009)
        Solutions are obtained using donlp2-intv, (c) by P. Spellucci
        Input Data File: V2O5_NTP_FemaleMiceHEC_Poly1.(d)
=================================================================
 V2O5_NTP_FemaleMiceHEC
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
   The form of the probability function is:
   P[response] = 1-EXP{-(t - t_0)^c *
                 (beta_0+beta_1*dose^1)}

   The parameter betas are restricted to be positive

   Dependent variable = CONTEXT
   Independent variables = DOSE, TIME

 Total number of observations = 200
 Total number of records with missing values = 0
 Total number of parameters in model = 4
 Total number of specified parameters = 1
 Degree of polynomial = 1

   User specifies the following parameters:
          t_0    =          0

 Maximum number of iterations = 64
 Relative Function Convergence has been set to: 2.22045e-016
 Parameter Convergence has been set to: 1.49012e-008

                  Default Initial Parameter Values
                         c      =        1.125
                         t_0    =            0   Specified
                         beta_0 =  0.000224032
                         beta_1 =    0.0144984

           Asymptotic Correlation Matrix of Parameter Estimates
           ( *** The model parameter(s)  -t_0
                 have been estimated at a boundary point, or have been specified by
the user,
                 and do not appear in the correlation matrix )

                 c            beta_0       beta_1
    c               1        -0.93           -1
    beta_0       -0.93            1         0.93
    beta_1          -1         0.93            1


                                  Parameter Estimates
                                                        95.0% Wald Confidence
Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf.
Limit
         c              1.12496         0.770137           -0.384483
2.6344
         beta_0      0.000224043      0.000862599         -0.00146662
0.00191471
         beta_1        0.0144991        0.0511302          -0.0857143
0.114712

                Log(likelihood)   # Param            AIC
   Fitted Model        -109.298         3         224.597

                    Data Summary
```

DOSE	CONTEXT C	F	I	U	Total	Expected Response
0	49	0	1	0	50	1.94
0.2	18	0	32	0	50	19.95
0.38	15	0	35	0	50	30.09
0.73	18	0	32	0	50	42.08

```
   Benchmark Dose Computation
Risk Response    =     Incidental
Risk Type        =          Extra
Specified effect =           0.67
Confidence level =            0.9
Time             =            104
             BMD =       0.411511
            BMDL =       0.339589
            BMDU =       0.516352
```



1

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APPENDIX D. SUPPLEMENTAL INFORMATION

D.1. EXPOSURE TO $PM_{2.5}$ VANADIUM IN AMBIENT AIR

Vanadium is a constituent of ambient PM generated by oil combustion. Recent studies of the short-term health effects of PM and its constituents have found higher risks of mortality and hospitalization in locations with a higher fractional content of vanadium, nickel, and elemental carbon in PM (Bell et al., 2009; Dominici et al., 2007).

Lippmann et al. (2006) evaluated the impact of average concentrations of 16 $PM_{2.5}$ components across 60 U.S. communities on the association between the daily change in PM_{10} concentration and daily all-cause mortality risk in those communities. Community-specific mortality risk estimates per daily change in PM_{10} concentrations were obtained from the National Morbidity, Mortality, and Air Pollution Study (NMMAPS) database for 1987–1994. Annual average concentrations for PM constituents were obtained for 2000–2003 from the $PM_{2.5}$ speciation network. The authors used weighted linear regression to evaluate for each chemical constituent whether annual average concentration altered the association between PM_{10} concentration on the previous day and mortality risk. The constituents, nickel and vanadium, were found to increase PM_{10} mortality risk.

These results were reevaluated and extended by Dominici and colleagues (2007) using NMMAPS data for 90 communities from 1987 to 2000 and data on $PM_{2.5}$ composition for 187 U.S. counties for 2000–2005. A total of 69 U.S. communities in the NMMAPS database also had data on $PM_{2.5}$ composition and were included in this analysis. Using a Bayesian hierarchical regression model to estimate the association between the l-day lag PM_{10} mortality risk and average county-level PM constituent concentrations, counties with high average concentrations of nickel and vanadium had higher PM_{10} mortality risk with a 1-day lag. When the three counties that comprise the NMMAPS New York community were excluded from the analysis, the effect of nickel and vanadium was much weaker and lost statistical significance. The authors stated that the three New York counties had nickel and vanadium concentrations that were 8.9 and 3.4 times higher than the other counties in the analysis.

Bell et al. (2009) used a Bayesian hierarchical regression model to evaluate the effect of $PM_{2.5}$ chemical constituents as percent of $PM_{2.5}$ total mass on $PM_{2.5}$-associated cardiovascular and respiratory hospital admissions by county and season for 106 U.S. counties during 1999–2005. Counties with a population of 200,000 or greater for which data on PM and constituent concentrations were available were selected. Models were adjusted for day of the week, seasonality, long-term trends using a smoothing function, daily temperature and dew point temperature, and temperature and dew point temperature for the previous three days. County- and season-specific $PM_{2.5}$ relative risk for cardiovascular and respiratory hospital admissions were higher in counties and seasons with a nickel, vanadium, or elemental carbon fraction of total $PM_{2.5}$ in the 75^{th} percentile compared to the 25^{th} percentile. The effect of these PM

constituents was statistically significant. The average concentration of vanadium across the counties was 0.003 μg/m^3 (range: 0.001–0.01) with an interquartile range of 0.001 μg/m^3. The interquartile range as percent of $PM_{2.5}$ total mass was 0.01%. Each interquartile range increase in the fraction of $PM_{2.5}$ total mass for vanadium was associated with a 27.5% (95% posterior interval: 10.6–44.4) increase in $PM_{2.5}$-associated cardiovascular hospitalizations and a 392% (95% posterior interval: 46.3–738) increase in $PM_{2.5}$-associated respiratory hospitalizations. Associations also were observed for elemental carbon and nickel, and effect estimates were not always stable in multipollutant models.

The finding that communities with a higher fractional content of vanadium, nickel, and elemental carbon in ambient PM have a higher risk of mortality and hospital admissions related to daily change in $PM_{2.5}$ concentration is intriguing: It indicates the need for further research on the contribution of fuel-oil combustion to regional and local air pollution, and the contribution of specific metals, including possibly vanadium pentoxide, to elevated health risks. The time series study design used in these investigations evaluates exposure-disease associations at the county level and therefore, individual-level assessments of exposure and the impact of possible confounders is not possible. Bell et al. (2009), however, investigated whether county-level indicators of socioeconomic status, racial composition, and degree of urbanization could be alternative explanations for the observed effect modification by $PM_{2.5}$ constituents and concluded that this was not the case.

Ambient concentrations of $PM_{2.5}$ and $PM_{2.5}$ fractions of nickel, vanadium, zinc, and elemental carbon were evaluated in relation to respiratory symptoms among young Dominican and African American children, aged 3–24 months, followed as part of a birth cohort study in Northern Manhattan and the South Bronx in New York City between 1998 and 2007 (Patel et al., 2009). Among 653 24-month-old children with questionnaire data (90% of total enrolled), 3-month average ambient vanadium concentrations were associated with an increase in the presence of wheeze during the cold and flu season (September 1–March 31). After adjusting for elemental carbon, nitrogen dioxide, copper, and iron, an interquartile range increase (0.003 μg/m^3) in 3-month average vanadium concentrations was associated with a 31% increased probability of wheeze during the cold season ($p < 0.0003$). When not stratified by season, an IQR increase in vanadium concentration was associated with a 14% increased probability of wheeze ($p = 0.08$). When the highest 5% of vanadium concentrations were excluded, however, the association of wheeze with vanadium lost significance in the multipollutant model. Vanadium concentrations were not associated with cough. Twenty-four-hour average ambient concentrations of $PM_{2.5}$ and $PM_{2.5}$ fractions of nickel, vanadium, zinc, and elemental carbon, measured every third day at two stationary sites in the Bronx, were obtained from the New York State Department of Environmental Conservation. Exposure levels were assigned to each subject by calculating 3-month moving average concentrations of each pollutant based on each follow-up questionnaire date and the previous 3 months. Exposures were assigned

to each subject's address using inverse-distance-weighted concentrations from the two stationary monitors. Associations were evaluated using generalized additive mixed effects models and a first-order autoregressive correlation structure to account for correlation in the up to 8 repeated observations for each individual. The models also adjusted for sex, ethnicity, postnatal ETS exposure, and a smoothed term for calendar time using natural cubic splines. Other pollutants also were associated with increased probability of wheeze (nickel) or cough (elemental carbon, nitrogen dioxide) during the cold/flu season or wheeze in other months (nitrogen dioxide). $PM_{2.5}$ mass concentrations were not related to an increase in probability of symptoms.

The interpretation of the multipollutant models for vanadium is complicated because other PM constituents are correlated with vanadium concentrations, resulting in less stable risk estimates. Nickel was not evaluated in the same model with vanadium for this reason. The association with vanadium was independent of the association with nitrogen dioxide, a marker for traffic emissions, and the authors suggested that oil combustion for space heating might contribute to the observed respiratory symptoms in the very young children in this study. Although vanadium cannot be singled out as the responsible agent for the probability of wheeze observed in this study, the association is consistent with the respiratory symptoms observed among boilermakers with exposure to high levels of residual oil fuel ash for periods of days to weeks.

The effect of the metal content of ambient PM2.5 on lung function also was evaluated in a time-series panel study of 29 patients with chronic obstructive lung disease, asthma, or ischemic heart disease in Rome, Italy in the spring and winter of 1999 (Lagorio et al., 2006). Outpatients of the Pneumology and Cardiology Departments of the Catholic University Hospital in Rome who met eligibility requirements for chronic obstructive pulmonary disease (COPD) (n = 11), ischemic heart disease (n = 7), or asthma (n = 11), and who lived in census tracts less than 2 kilometers from one of six air monitoring stations were selected for the study. The subjects volunteered to participate in repeated clinical examinations for two 1-month periods. Pulmonary function testing was conducted according to American Thoracic Society guidelines, and measures were expressed as the percentage of predicted based on subject-specific age, height, and weight. Averages of 15, 24, and 9 observations were obtained from each participant in the COPD, ischemic heart disease, and asthma panels, respectively. Daily average PM2.5 concentrations were calculated based on measurements obtained at two fixed site monitors set up for the study. PM content of cadmium, chromium, iron, nickel, lead, platinum, vanadium, and zinc was calculated as the ratio of the metal content in each PM sample to the air volume collected during the sampling. The mean 24-hour PM2.5 concentrations during the spring and winter of 1999 were 18.2 ± 5.0 $\mu g/m^3$ and 36.7 ± 24.1 $\mu g/m^3$, respectively. The mean 24-hour vanadium concentrations during the spring and winter of 1999 were 2.4 ± 1.6 ng/m^3 and 1.1 ± 0.52 $\mu g/m^3$, respectively. Vanadium was not associated with daily change in percent predicted pulmonary function among subjects with COPD, ischemic heart disease, or asthma in

generalized estimating equation models. Models were adjusted for season (all), daily mean temperature (all), relative humidity (all), day of the week (COPD, ischemic heart disease), and ß-2 agonist use (asthma). Although the repeated measures design was a strength of the study, the number of subjects in each disease panel was small, and might have precluded detection of an association, given the very low vanadium concentrations analyzed.

D.2. LABORATORY ANIMAL AND IN VITRO STUDIES

D.2.1. Other Endpoint and Duration Studies

D.2.1.1. *Acute and Short-Term Studies*

D.2.1.1.1. *Acute studies*

D.2.1.1.1.1. *Oral*. According to the *Concise International Chemical Assessment Document* 29 (WHO, 2001), rat oral LD_{50} values for vanadium pentoxide range from 86 to 137 mg/kg body weight [Yao et al., 1986 as cited in WHO (2001)]. A rat oral LD_{50} value of 10 mg/kg body weight was reported in IARC Monographs, volume 86 (2006) in a study by Lewis et al. (2000). Lewis et al. (2000) also reported a mouse oral LD_{50} value at 23 mg/kg body weight. Clinical signs of acute toxicity included lethargy, excessive tearing (lacrimation), and diarrhea but dose-response data were not reported [Yao et al., 1986 as cited in WHO (2001)]. Histopathological analysis revealed liver necrosis and swelling of renal tubules. Other studies (WHO, 2001) have identified LD_{50}s of ~10 mg/kg body weight in rats and 23 mg/kg body weight in mice. An oral LD_{50} at 64 mg/kg body weight in rabbits was established (WHO, 2001). Signs of toxicity in rabbits mimicked those reported for rats.

D.2.1.1.1.2. *Inhalation*. A 1-hour inhalation exposure to vanadium pentoxide dust in rats led to an LC_{67} of 1.44 mg/L (1440 mg/m^3) [U.S. EPA, 1992 as cited in WHO (2001)]. Clinical signs of toxicity included respiratory difficulty, irritation of mucosa, and diarrhea (WHO, 2001). Knecht et al. (1985) reported air flow restriction, as measured by PFTs, in 16 adult male cynomolgus monkeys *(Macaca fasicularis)* exposed to vanadium pentoxide by whole-body inhalation at 5.0 mg/m^3 for 6 hours but not at the lower dose tested (0.5 mg/m^3). From this study, a LOAEL of 5.0 mg/m^3 and a NOAEL of 0.5 mg/m^3 were established. The lung was also identified as a target organ in response to acute inhalation exposure to vanadium pentoxide. Following a baseline measurement of pulmonary function, each of 16 male cynomolgus monkeys was exposed to aerosols of 0.5 mg/m^3 vanadium pentoxide by whole-body inhalation for 6 hours (Knecht et al., 1985). One week later, the monkeys were exposed to aerosols of 5 mg/m^3 vanadium pentoxide by whole-body inhalation for 6 hours. Effects on airway function were evaluated in monkeys by comprehensive PFTs performed 24 hours postexposure to 0.5 and 5 mg/m^3 and on pulmonary inflammation by analysis of BAL fluid in monkeys performed after exposure to 5 mg/m^3.

Significant changes in pulmonary function parameters compared to baseline values were observed only after exposure to 5 mg/m^3 as follows: 16% increase in pulmonary resistance; 11% decrease in peak expiratory flow rate; 5–22% decreases in FEF maneuvers; 33% increase in RV; and 24% increase in forced residual capacity. Results are consistent with air-flow limitation in both small peripheral and large central airways. An 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^3 vanadium pentoxide. The increase in BAL fluid total cell number was due primarily 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 could play a role in vanadium pentoxide-induced air-flow restriction. An acute (single 6-hour exposure) LOAEL for vanadium pentoxide of 5 mg/m^3 for pulmonary function in monkeys was established in this study, with a NOAEL of 0.5 mg/m^3.

A study in male CD-1 mice (n = 48) by Avila-Costa et al. (2006) noted significantly impaired performance on memory tasks, significantly decreased dendritic spine length, and significant increases in percentages of necrotic cells in the hippocampus compared to controls following a 1-hour inhalation exposure to 0.02 M vanadium pentoxide (2.5 mg/m^3 as vanadium) ($p < 0.05$). The dose-response relationship for these effects could not be evaluated because only one dose was tested, and a NOAEL could not be established.

D.2.1.1.2. *Short-term studies*

D.2.1.1.2.1. *Inhalation and aspiration*. The primary noncancer health effect of short-term inhalation exposure in humans is respiratory irritation where 100 workers were reportedly exposed to 0.05–5.3 mg/m^3 vanadium for 10 hours per day, 6 days per week, for 4 weeks (Levy et al., 1984). A LOAEL of 0.05 mg/m^3 was established. Dose-response was not systematically measured, however, no controls were used, and exposure due to vanadium pentoxide could not be directly correlated to effects. The primary noncancer health effects of short-term inhalation exposure in animals include increased pulmonary inflammation, and dose-related decreases in body weight and increases in relative lung weight in rodents (16-day exposure) (NTP, 2002).

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 during a 16-day 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 16 days, with a LOAEL of 1 mg/m^3; a NOAEL was not established. Similar results were observed for female mice (assessments not made in male mice) exposed for 16 days, with a LOAEL of 2 mg/m^3 for minimal to mild epithelial hyperplasia and inflammation; a NOAEL was not established.

Male rats (22 per group) and female mice (50 per group) were also assessed for pulmonary inflammation (BAL analysis) and systemic immunotoxicity (pulmonary bacteriocidal activity) following exposure to 0, 4, 8, and 16 mg/m^3 vanadium pentoxide for 16 days (NTP, 2002). Observed effects included significant alterations in the percentage of recoverable bronchial lavage cells (macrophages and neutrophils) (LOAEL of 8 mg/m^3 and NOAEL of 4 mg/m^3), and increased lung protein and lysozyme in male rats (LOAEL of 4 mg/m^3). No NOAEL was established. In female mice exposed to vanadium pentoxide for 16 days, a localized inflammatory response in the lungs occurred based on increase in lymphocytes, protein, and lysozymes at all concentrations; the NOAEL was not established. A significant decrease in the percentage of macrophages from BAL fluid led to a LOAEL of 8 mg/m^3 and a NOAEL of 4 mg/m^3. These responses, NOAEL, and LOAELs are commensurate with those observed in the 3-month study in rats and mice of both genders (NTP, 2002). Thus, the lowest concentrations at which adverse effects were observed were 1 and 2 mg/m^3 (LOAEL) for lung inflammation in female rats and mice, respectively, exposed to vanadium pentoxide for 16 days.

An additional five male and five female mice were exposed by inhalation to vanadium pentoxide for 6 hours per day, 5 days a week for 16 days at concentrations of 0, 2, 4, 8, 16, or 32 mg/m^3 (NTP, 2002). All male mice exposed to 32 mg/m^3 died before study completion. Body weight was significantly decreased in male and female mice at 16 and 32 mg/m^3, respectively. Absolute lung weights were significantly increased in a dose-dependent manner in males at ≥4 mg/m^3, and relative lung weight was significantly increased in males at ≥2 mg/m^3. Among females, both absolute and relative lung weights increased in all exposure groups establishing the LOAEL of 2 mg/m^3; no NOAEL was established.

Additional groups of 40-60 female mice were exposed to 0, 2, 4, or 8 mg/m^3 for 6 hours per day, 5 days per week for 16 days (NTP, 2002). The nonneoplastic lung lesions noted on days 6 and 13 consisted of hyperplasia of the alveolar and bronchiolar epithelium at all exposure levels. Increase in severity of lesions was correlated with increasing concentration and time. The LOAEL for nonneoplastic lung lesions was 2 mg/m^3. A NOAEL was not established.

A duration-dependent decrease in the number of immunoreactive TH+ neurons (Avila-Costa et al., 2004) after 4 weeks and increased quantities in metalloproteinase (MMP)-2 and MMP-9 in CNS after 8 weeks in male mice (Colin-Barenque et al., 2008) following twice weekly, 1-hour inhalation exposure to 5.13 mg/m^3 vanadium pentoxide. These effects are suggestive of disruption of the blood-brain barrier.

Turpin et al. (2010) exposed male AKR mice to vanadium pentoxide by intranasal aspiration following exposure to respiratory syncytial virus (RSV) to determine if preexposure to the virus exacerbated the vanadium pentoxide-induced lung inflammation and fibrosis. Animals were exposed intranasally to RSV (6×10^5 plaque-forming units [PFU] in 100 µL PBS) on day 1 and day 8, then exposed intranasally to vanadium pentoxide (4 mg/kg in 50 µL PBS) on day 0 and day 7. One hour before euthanasia, animals were given BrdU (50 mg/kg) by i.p. for

analyzing cell proliferation in bronchus-associated lymphoid tissue (BALT). Lungs were lavaged with PBS and BALF collected for analyzing differential cell counts (neutrophils, macrophages, and lymphocytes). Total RNA from lung was analyzed by real time RT-PCR for mRNA coding for profibrogenic growth factors TGF-β-1, connective tissue growth factor (CTGF), platelet-derived growth factor-C (PDGF-C) and collagen Col1A2, anti-fibrogenic type I interferon-alpha (IFN-α) and -beta (IFN-β), and IFN-inducible chemokines CXCL9 and CXCL10.

Lung sections stained with Masson's trichrome staining to show collagen had an inflammation score of two, representing mild fibrosis with vanadium pentoxide exposure alone. Vanadium pentoxide-induced fibrotic response, however, was less severe in the lungs of mice that received either pre- or post-RSV exposure, and no differences were observed in pre- or post-RSV exposure alone and the negative controls. In addition, BALF from mice exposed to vanadium pentoxide alone or with RSV-post exposure had significantly higher total cell count compared to RSV preexposure, RSV preexposure plus vanadium pentoxide, or controls. In particular, vanadium pentoxide alone caused a significant increase in the levels of neutrophils and lymphoctyes compared to controls. Both pre- and postexposure to RSV led to a decrease in the severity of vanadium pentoxide-induced fibrosis, and gene expression analysis showed decreases in several profibrinogenic genes associated with innate immunity (Turpin et al., 2010).

To analyze mucin production by the airway epithelium following exposure to vanadium pentoxide, Yu et al. (2011) exposed female AKR mice (n = 5) by laryngeal aspiration to vanadium pentoxide (4 mg/kg bw) at two time points (day 1 and 7) and sacrificed on day 8. Lung tissue analyzed by histopathology demonstrated an increase in the level of inflammation and mucin production following exposure to vanadium pentoxide. These results were supported by RT-PCR analysis demonstrating a statistically significant increase in the major airway mucin (Muc5ac) ($p < 0.05$).

The acute and short-term studies described here support those results described previously in the subchronic and chronic studies and suggest progressive lung effects from vanadium pentoxide exposure similar to those observed in respiratory disease observed in occupational studies. The studies further support that the lung is the most sensitive organ to vanadium pentoxide exposure.

D.2.1.2. *Immunological Endpoints*

D.2.1.2.1. ***Human studies.*** Study methods and results are described in Table 4-1. Some early case series observed dermatitis among affected workers employed at vanadium pentoxide processing facilities (Zenz et al., 1962; Sjoberg, 1951). Motolese et al. (1993) assessed whether exposure to vanadium pentoxide in the ceramics industry was associated with contact dermatitis or contact sensitization. Testing for contact sensitization using skin patch testing was conducted on 126 enamellers and 64 decorators from 5 ceramics factories after exposure to a variety of substances, including vanadium pentoxide. Among the 190 workers under study, 22 individuals were found to have dermatitis and 17 reported having had skin lesions in the past. One worker responded with a positive skin patch test indicating sensitization to a 10% solution of vanadium pentoxide.

Kiviluoto published a series of reports regarding an investigation in 1975 of respiratory symptoms and clinical findings among employees (process workers, repairmen, foremen, and a laboratory worker) at a factory making vanadium pentoxide from magnetite ore (Kiviluoto et al., 1981; Kiviluoto, 1980; Kiviluoto et al., 1979). A higher proportion of the exposed group (n = 63) had an elevated number of neutrophils in nasal smears compared to the referent group (n = 63) (35% versus 7%, $p < 0.001$, n = 55 pairs). The referent group comprised individuals who were employed at the magnetite ore mine and were matched to the exposed group members by age and smoking habit. In biopsies of the nasal mucosa, a higher proportion in the exposed groups had elevated plasma cells and round cells (26% versus 0%, $p < 0.05$, n = pairs and 48% versus 29%, $p < 0.05$, n = 56, respectively). The prevalence of elevated numbers of eosinophils did not vary by exposure, leading the authors to conclude that the cytological and histological response in the vanadium-exposed group was an irritant, not an allergic response. Vanadium concentrations in the breathing zone averaged 0.028 mg/m^3 (TWA) with a range of 0.002-0.42 mg/m^3. Higher concentrations were found where grinding and packing of smelt were conducted (TWA [range]: 2.3 mg/m^3 [one sample] and 0.13 mg/m^3 [0.02–0.37]).

A pilot study using nasal lavage to evaluate an inflammatory response to fuel-oil ash exposure did not find an association between several exposure indices of vanadium or PM_{10} exposure and counts or percentages of polymorphonuclear cells, eosinophils, and epithelial cells (Hauser et al., 1995). Thirty-six of fifty volunteers (boilermakers and utility workers) with no symptoms of cold or flu provided a nasal lavage sample both at baseline after at least 36 hours away from work and after 72 hours of exposure at a local electric company. Daily exposure estimates for PM_{10} (<10 μm) and vanadium (adjusted for filter extraction efficiency) were assigned to each individual using data from personal air sampling (1- to 10-hour TWA) and a self-completed work diary completed by each participant listing tasks and job locations during the day. Environmental PM_{10} concentrations based on personal sampling were 50–4,510 μg/m^3. Concentrations of respirable vanadium dust were 0.10–139.2 μg/m^3. Although boilermakers

(N=19) exhibited a larger increase in the number of polymorphonuclear cells per mL recovered from nasal fluid (adjusted by dividing the change by the mean of the baseline and postexposure value) and the adjusted number of epithelial cells/mL compared to utility workers, regression models, controlling for age and smoking, did not indicate an association with vanadium exposure. Models did not control for ozone levels or respirator use. The wide variation in the change in cell counts after exposure, especially among nonsmokers, and the small number of subjects could have precluded the detection of an association with vanadium or particulate exposure. Alternatively, the authors considered the levels of vanadium dust to be low, possibly not enough to cause inflammation in these workers.

Woodin et al. (1998) analyzed nasal lavage fluid from 18 boilermakers before, during, and after the overhaul of a large, oil-fired boiler over a 6-week period from mid-May 1995 to late June 1995. Biomarkers of upper airway inflammation in nasal lavage fluid, including interleukin-8 and myeloperoxidase, were more prevalent among boilermakers during the overhaul compared to 11 utility workers (Woodin et al., 1998). Mean IL-6 and eosinophilic cationic protein levels did not change during the overhaul work, suggesting that the inflammatory response was not due to an allergy or a respiratory infection. During the boiler work, vanadium levels rose to a geometric mean SD of 8.9 (2.3) $\mu g/m^3$ inside the boiler but did not change appreciably outside the boiler where the utility workers were located (geometric mean SD $\mu g/m^3$: 1.4 [1.6] [$p < 0.001$]). Vanadium concentrations in nasal lavage fluid, however, were not associated with levels of either IL-8 or myeloperoxidase using Spearman's Rank Order Correlation Test.

In conclusion, case studies of occupational exposure to vanadium pentoxide dust or ROFA have reported individuals with dermatitis, positive skin patch reactions, and bronchial reactivity, although this occurrence appears to be uncommon. Increases in the numbers of inflammatory cells in nasal smears or nasal lavage fluid have been observed in exposed workers but no associations were observed in relation to estimates of respirable vanadium dust or vanadium concentrations in nasal fluid. The authors did not report increases in eosinophils, suggesting that the inflammation was due to irritation and was not an allergic response.

D.2.1.2.2. ***Animal studies.*** Pinon-Zarate et al. (2008) exposed 112 male CD1 mice to ~1.4 mg/m^3 vanadium pentoxide by inhalation for 1 hour per day, 2 times per day, for 12 weeks, as measured by filters following exposure. Because this study did not provide reliable exposure information, exposure concentrations in mg/m^3 could not be more specifically determined. Spleen weight and histology were determined. B-lymphocytes in the spleen were identified by immunohistochemical staining for CD19 (a cell surface marker that acts as a co-receptor for other CD markers). In addition, eight control and eight vanadium pentoxide-treated mice were immunized with Hepatitis B surface antigen, a well-known T-cell-dependent antigen. Avidity to the resulting antibody was measured. Spleen weight of vanadium pentoxide-exposed animals increased significantly, peaking at 9 weeks, and then decreased significantly. Splenic germinal centers significantly increased in vanadium pentoxide-treated mice and contained a significantly increased number of CD19+ cells compared to controls. The authors suggest that vanadium pentoxide does not act as a direct antigen and does not induce a "host humoral response." These data suggest that vanadium pentoxide might affect the avidity of antibodies – the ability of antibodies to bind effectively to substrates (affinity) and to engage multiple epitopes of the antigen simultaneously.

Mravcova et al. (1993) conducted experiments to determine effects of subchronic exposure to low doses of vanadium pentoxide on the immune system. Weanling and adult male and female Wistar rats (n = 10 per group) were given vanadium pentoxide in drinking water (0, 1, 100 mg/L, or 0, 0.14, 14 mg/kg-day) for 6 months. In addition, male and female ICR mice (groups of 10) were given vanadium pentoxide (0 or 6 mg/kg-day) by gavage 5 times per week for 6 weeks. Immunotoxicity endpoints included spleen and thymus weight, spleen cellularity, number of peripheral white blood cells, phagocytosis and natural killer cell activity, the extent of plaque-forming cell conversion to T-dependent antigen, and several cell-mediated immunity endpoints (Concanavalin A [ConA], pokeweed mitogen responsiveness [PWM], and phytohemagglutinin responsiveness [PHA] assays). Of these endpoints, spleen weight was significantly elevated over controls at 14 mg/kg-day in rats. The ConA and PHA assays illustrated significant cell-mediated immune activation at 1 mg/L in rats over control (3 and 2.5 times higher than control values, respectively) but statistical significance was not indicated or reported. At the high dose (14 mg/kg-day vanadium), ConA and PHA assay results were close to control values. Thus, no dose-response pattern was detected. The low-dose response could be a transient or compensatory response. Results of other endpoints were not reported. No significant differences in these parameters were reported in mice. The authors suggested that the high Con A response of T suppressor cells indicates that vanadium pentoxide might induce hypersensitivity responses at low doses.

Immunological endpoints also were analyzed as part of a pulmonary study using weekly provocation challenges cynomolgus monkeys (single 6-hour exposures to 0.5 or 3.0 mg/m^3). Inhaled vanadium pentoxide aerosol for 6 weeks produced statistically significant pulmonary

responses, prior to a subchronic exposure (6 hours per day, 5 days per week, for 26 weeks) (Knecht et al., 1992); study details are presented in Section 4.2.2.1). Immunological analyses of blood and BAL fluid, and skin sensitivity tests were conducted before the pre- and postexposure provocation challenges. BAL fluid analyses were also performed 1 day after the provocation challenges. Cytological endpoints included complete and differential blood cell counts and leukotriene C_4 levels. Immunological endpoints included total immunoglobulin E, total immunoglobulin G, albumin, and total protein. The skin sensitivity tests assessed immediate and delayed responses to intradermal injections of vanadium pentoxide-monkey serum albumin conjugate. BAL fluid analysis showed a significant influx of inflammatory cells (polymorphonuclear leukocytes) into the lung. 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.

In summary, immunological effects of vanadium pentoxide exposure have not been comprehensively studied. Although the studies described here have not shown a statistically significant response in the endpoints tested, some effects were observed. These results are therefore inconclusive.

D. 2.1.3. *Neurological Endpoints*

D.2.1.3.1. ***Human studies.*** No studies on the neurological effects of vanadium pentoxide were reported in humans.

D.2.1.3.2. ***Animal studies.*** Pazynich (1966) exposed 33 male albino rats (species not specified) to 0.027 mg/m^3 or 0.002 mg/m^3 aerosolized vanadium pentoxide "round the clock" for 70 days. A third group of rats served as control. The animals were evaluated for general condition, body weight, motor chromaxy of antagonistic muscles, whole-blood cholinesterase activity, and oxyhemoglobin content. Motor chromaxy of extensor muscles decreased significantly ($p < 0.01$) while that of flexor muscles increased ($p < 0.001$) in animals exposed to 0.027 mg/m^3. Blood cholinesterase levels were statistically significantly reduced after exposure to 0.027 mg/m^3 and the reduction persisted throughout the 90-day recovery period. A reduction in venous oxyhemoglobin in rats at 0.027 mg/m^{3}was statistically significant. Recovery was observed after 20 days. No difference in these parameters was reported in the 0.002 mg/m^3 group compared to controls. These results suggest a LOAEL of 0.027 mg/m^3 for hematological and central nervous system (CNS) effects in albino rats with a NOAEL of 0.002 mg/m^3.

Three recent studies by Avila-Costa et al. (2006; 2005; 2004) found morphological changes in CNS following inhalation exposure to vanadium pentoxide. Male CD-1 mice ($n = 48$) were exposed to vanadium pentoxide by whole-body inhalation for 1 hour per day, 2 days per week for up to 8 weeks (Avila-Costa et al., 2005; Avila-Costa et al., 2004). Particle size was not reported in either study. The exposure concentration was reported as 0.02 M

(Avila-Costa et al., 2006; Avila-Costa et al., 2005; Avila-Costa et al., 2004) or "2.5 mg/m^3 V" (Avila-Costa et al., 2005). The same group of investigators (González-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 2.5 mg/m^3, as vanadium metal (MW = 50.94), corresponding to 2.57 mg/m^3 vanadium pentoxide (MW = 181.9). The number of immunoreactive-TH$^+$ neurons in the substantia nigra region of the basal ganglia in the mesencephalon (Avila-Costa et al., 2004) and the morphology of the blood-brain barrier (Avila-Costa et al., 2005) were assessed at the end of each week for up to 8 weeks of exposure. No clinical signs of toxicity were reported in either study. A duration-dependent decrease in the number of immunoreactive-TH$^+$ 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 also observed starting at week 1and increasing with duration 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 hippocampal neurons in male CD-1 mice that were exposed by whole-body inhalation for 1 hour per day, 2 days per week, for up to 4 weeks. Groups of six exposed mice and six 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 preexposure. 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 impairment in spatial memory is frequently observed following damage to 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, and the loss increased with time, in a manner that correlated with the memory impairments. The ultrastructural analysis showed a significantly increased percentage of necrotic hippocampal cells at all time points, with a maximum of 33% after 4 weeks of exposure; other findings included hyperdense postsynaptic terminals and edema in mitochrondria, dendrites, dendritic spines, and presynaptic terminals. These three studies establish a LOAEL for morphological changes to CNS accompanied by behavioral effects following acute and short-term intermittent exposure to vanadium pentoxide at concentrations of 2.56 mg/m^3 two times per week, for 1-hour duration/exposure.

Colin-Barenque et al. (2008) investigated whether the vanadium pentoxide-mediated disruption of the blood-brain barrier was associated with the activation of matrix metalloproteinases (MMPs), protein degrading enzymes that are involved in tissue remodeling. Male CD-1 mice (n = 20 per group) were exposed to 0.02 M (2.56 mg/m^3) aerosolized vanadium

pentoxide in deionized water or deionized water alone for 1 hour, two times a week, for up to 4 weeks. Five mice were sacrificed from each group, per time point (24 hours, 1, 2, and 4 weeks). The presence of MMP was determined by gel zymography. In the olfactory bulb, MMP-2 was the same in vanadium pentoxide-treated mice and controls, regardless of time point. MMP-9 was significantly elevated (300% and ~420%) in vanadium pentoxide-exposed mice compared to controls at 2 and 4 weeks, respectively. Both MMP-2 and MMP-9 were detected in the prefrontal cortex; MMP-2 was not different between controls and treated animals at any time point but MMP-9 was significantly elevated over control values at 1, 2, and 4 weeks of exposure (150%, ~175%, and 250% of control values, respectively). In the hippocampus, MMP-9 from vanadium pentoxide-treated mice was significantly elevated over controls after 1, 2, and 4 weeks of exposure (200%, 340%, and ~370%), while MMP-2 in exposed animals was significantly increased at 4 weeks (~150% over controls). In the striatum, MMP-9 from exposed mice was significantly elevated over that of controls after 4 weeks of exposure; MMP-2 levels from exposed mice were significantly elevated over control values after 2 (160%) and 4 weeks (~250%) of exposure, but were not documented at earlier time points. These findings suggest that vanadium-induced increases in MMPs in different parts of CNS occur in association with dendritic spine loss and with neuronal death, and could be related to blood-brain barrier disruption.

D.2.2. Mechanistic Data and Other Studies in Support of the Mode of Action for Pulmonary Fibrosis and Cancer

The preceding paragraphs highlighted the main noncancer and cancer health effects that result from exposure to vanadium pentoxide. Noncancer effects in the lung range from histiocytic infiltration and alveolar inflammation to hyperplasia of alveolar epithelium and pulmonary fibrosis. These endpoints exist in a plausible biological response continuum—from inflammation to reparative hyperplasia to fibrosis. These effects also display a temporal and dose-response continuum, ranging from inflammatory and hyperplastic responses that occur at earlier time points (16 days) and at lower doses (2 mg/m^3) to fibrosis that occurs at later time points (3 months) and at higher doses (4 mg/m^3). Inflammation and hyperplasia are biologically relevant as precursor events to pulmonary fibrosis. Several investigators have systematically investigated the molecular mechanisms underlying vanadium pentoxide-induced pulmonary inflammation and fibrosis. These studies are summarized below.

D.2.2.1. *Genotoxicity*

The genotoxicity assays of vanadium pentoxide are summarized in Table D-1.

D.2.2.1.1. ***Human studies.*** Two studies investigated mutagenic activity in humans exposed to vanadium pentoxide (Ehrlich et al., 2008; Ivancsits et al., 2002). 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 nonexposed controls (Ivancsits et al., 2002). The average exposure duration for workers was 12.4 years. Workers reported using protective masks at least occasionally. Measurements or estimates of worker exposure to vanadium pentoxide were not reported, although exposure to vanadium was confirmed through serum and urine analyses. No significant differences between vanadium-exposed and control workers were observed for DNA strand breaks (as assessed by alkaline comet assay), 8-hydroxy-2'deoxyguanosine (8-OHdG), an oxidized DNA base common indicative of oxidative stress, or the frequency of sister chromatid exchange (SCE) in leukocytes. When normal human leukocytes or human fibroblasts were cultured in vitro and exposed to vanadate (25-500 μg/L), both whole blood cells and isolated nonproliferating lymphocytes exhibited a significant increase in DNA migration in the alkaline comet assay compared to nonexposed cells only at the highest doses tested (250500 μg/L). Cultured human fibroblasts, however, exhibited a dramatic dose-dependent increase in DNA migration after vanadate treatment also at lower concentrations (as low as 0.5 μg/L) and suggest that fibroblasts are more sensitive to DNA damage in the presence of vanadate than blood cells when exposed in vitro (Ivancsits et al., 2002).

Ehrlich et al. (2008) investigated the impact of inhaled vanadium pentoxide on DNA stability in vanadium production workers (n = 52) compared to nonexposed jail wardens (n = 52) during October 2004–May 2005. All subjects studied were male and were exposed for their entire 8-hour shift while wearing protective masks. The duration of exposure and concentration of the inhaled vanadium, however, was not determined. The median concentration ($25^{th}75^{th}$ percentile) of vanadium in plasma was sevenfold higher in exposed workers compared to the unexposed reference group. Leukocytes were then assayed (Comet assay) for DNA damage, and endogenous levels of oxidized purines and pyrimidines were determined. No differences in DNA migration by exposure were noted in leukocytes under standard conditions, demonstrating that exposure is not associated with increases in single- and double-strand breaks. Increases were observed, however, in both oxidized purine (7% increase, $p = 0.02$) and pyrimidine (33% increase, $p = 0.002$) formation in exposed individuals. Moreover, DNA damage induced by bleomycin was 25% greater in leukocytes from the exposed workers ($p < 0.0001$), and DNA repair after bleomycin administration was less evident ($p < 0.0001$). The extent of micronucleus formation, necrosis, and apoptosis was determined in isolated lymphocytes using the cytokinesis-block micronucleus cytome (CBMN Cyt) assay. The number of micronuclei was 2.5-fold higher in 24 workers than in 23 nonexposed referents ($p= 0.01$). The frequency of nucleoplasmic bridges and nuclear buds (which indicate evidence of misrepaired DNA breaks and gene amplification, respectively) were significantly increased (sevenfold and threefold) over controls.

Numbers of necrotic and apoptotic cells were 55% and 50% higher, respectively, in exposed workers. Together, these results suggest that occupational exposure to inhaled vanadium pentoxide could affect DNA stability by increasing levels of oxidized bases and affecting DNA repair. Age, body mass index, and smoking habit (number of cigarettes per day) were similar between the two groups. Folate levels also were similar but vitamin B_6 and B_{12} levels were lower among the unexposed, indicating that the effects on DNA in the exposed group were not due to vitamin deficits.

Kim and colleagues (2004) assessed the cross-shift change in urine levels of 8-OHdG, a marker for DNA repair of oxidative DNA damage, over a 5-day period in 1999 among a group of 20 boilermakers involved in the overhaul of oil-fired boilers at a power plant (74% of eligible). Median total 8-hour TWA $PM_{2.5}$ concentration, measured using personal exposure monitoring, was 0.44 mg/m^3 ($Q_{25\%}Q_{75\%}$: 0.290.76 mg/m^3). Total vanadium 8-hour TWA concentration, including vanadium oxides, was 1.23 µg/m^3 ($Q_{25\%}Q_{75\%}$: 0.473.53 µg/m^3). The workers were 18-59 years old (mean ± SD: 45.5 ± 12.0) and had been employed as boilermakers for 0.0440 years (mean ± SD: 21.7 ± 12.9). The mean cross-shift change in creatinine adjusted 8-OHdG levels in urine was 1.88 µg/g creatinine (SD = 0.74). Pre-shift levels, measured an average of two days away from work, were significantly different from post-shift levels (p = 0.02). In linear mixed regression models, a 1 mg/m^3 increase in total $PM_{2.5}$ 8-hour TWA concentration was associated with an increase in urinary 8-OHdG concentrations of 1.67 µg/g creatinine (95% CI: 0.21, 3.14), adjusting for urinary cotinine, chronic bronchitis status, and age. A 1 µg/m^3 increase in $PM_{2.5}$ vanadium concentration was associated with an increase in urinary 8-OHdG concentrations of 0.23 µg/g creatinine (95% CI: 0.04, 0.42) in a model with the same covariates. $PM_{2.5}$ manganese, nickel and lead concentrations also were associated with 8-OHdG levels in urine when analyzed separately in similar models. The concentrations of the metals were correlated ($0.52 < r < 0.92$) and so the association with vanadium might not have been independent of the associations with the other metals. The finding of oxidative DNA injury and repair among healthy boilermakers, however, is consistent with similar reports among vanadium pentoxide workers.

Another marker of oxidative DNA damage, 7-hydro-8-oxo-2'-deoxyguanosine (8-oxodG), was assessed in relation to water-soluble transition metal content in ambient $PM_{2.5}$ among male and female nonsmoking students, 20–33 years of age, living in central Copenhagen (Sorensen et al., 2005). Personal samples of $PM_{2.5}$ were collected over 2 days twice in 1 year, once during summer and once during autumn. Median (interquartile range) concentrations of $PM_{2.5}$ were 20.1 µg/m^3 (13.1 - 27.7) in November and 12.6 µg/m^3 (9.4 - 24.3) in August. The median (interquartile range) concentration of vanadium in $PM_{2.5}$ was 3.0 (0.3 - 4.7) in November and 3.2 (1.4 - 5.7) in August. Of 66 participating students, 32 provided measurements for both seasons. Median (interquartile range) levels of 8-OxodG in lymphocytes (per 105 dG) were 0.55 (0.34 - 0.78) and 0.58 (0.47 - 0.70) in November and August, respectively. Vanadium and

chromium concentrations in aqueous suspensions of $PM_{2.5}$ were associated with the 8-oxodG concentration in lymphocytes in mixed regression models with subject as a random factor and adjusting for $PM_{2.5}$ mass and season. A 1-μg/L increase in either vanadium or chromium was associated with a 1.9% (95% CI: 0.6, 3.3) or 2.2% (95% CI: 0.8, 3.5) increase in 8-oxodG concentrations in lymphocytes, respectively. Platinum, nickel, copper, and iron were not associated with 8-oxodG concentration in lymphocytes, and no metals were associated with 8-oxodG concentration in urine. This study suggests that metal content of ambient fine particulate matter increases oxidative DNA damage in lymphocytes and that vanadium could be one of the responsible agents along with other metal constituents in particulate air pollution at levels common in urban settings.

Three other studies demonstrated a genotoxic effect of vanadium pentoxide on primary human lymphocytes in vitro (Ramirez et al., 1997; Rojas et al., 1996; Roldán and Altamirano, 1990). These studies examined chromosomal aberrations and aneuploidy by fluorescence in situ hybridization (FISH) and SCE assays, as well as DNA damage by the comet assay. Cells exposed to vanadium pentoxide had significantly increased DNA migration indicative of DNA damage at all doses tested in primary human leukocytes ($p < 0.05$) and in the high doses in three of four donor lymphocyte cell strains ($p< 0.05$). Vanadium pentoxide (00. μM) led to an increase in aneuploidy with some interindividual variation observed in four primary human lymphocyte cell strains (Ramirez et al., 1997). An increase in aneuploidy was observed in one primary human cell strain exposed to vanadium pentoxide (06μg/mL) but no chromosomal aberrations were observed (Roldán and Altamirano, 1990).

D.2.2.1.2. ***Laboratory in vivo and in vitro studies.*** Vanadium pentoxide produced gene mutations in two bacterial test systems (*Bacillus subtilis* and *Escherichia coli*) (Kada et al., 1980; Kanematsu et al., 1980); although negative results were reported by NTP (2002) in a reverse mutation assay in *Salmonella typhimurium* (TA97,TA98,TA100, TA102,TA1535 with or without metabolic activation). Negative results were also reported in a gene mutation assay in Chinese hamster V79 fibroblast cells (Zhong et al., 1994). DNA damage or aneuploidy, however, was observed in all in vitro studies performed in primary human cells (Kleinsasser et al., 2003; Ivancsits et al., 2002; Ramirez et al., 1997; Rojas et al., 1996; Roldán and Altamirano, 1990). Positive results were observed for DNA strand breaks in cultured human lymphocytes (Rojas et al., 1996) at high doses of vanadate (Ivancsits et al., 2002) and in cultured human fibroblasts at lower, more environmentally relevant doses of vanadate (0.5 μg/L) (Ivancsits et al., 2002). Positive results have been noted for aneuploidy (Ramirez et al., 1997) and polyploidy (Roldán and Altamirano, 1990). Negative results were reported for chromosomal aberrations (Roldán and Altamirano, 1990). In Chinese hamster V79 lung fibroblast cells, positive results were observed for micronucleus formation (Zhong et al., 1994), altered mitosis (Zhong et al., 1994), and cell transformation in Syrian hamster embryo cells (Kerckaert et al., 1996) at concentrations that were not cytotoxic. Negative results were reported for SCE and gene mutations in vanadium pentoxide-treated Chinese hamster V79 fibroblast cells (Zhong et al., 1994).

One study evaluated the genotoxicity of vanadium pentoxide in primary human cell cultures. Kleinsasser et al. (2003) took mucosal biopsies from inferior nasal turbinates and blood samples from 17 healthy volunteers. Isolated lymphocytes and mucosal cells were cultured and exposed to 0, 0.06, 0.12, 0.24, and 0.47 mM vanadium pentoxide in vitro for 1 hour. Mucosal cells and lymphocytes were assessed for DNA migration by the Comet assay. Extent of migration was measured qualitatively (image analysis) and quantitatively ("Olive Tail Moment" method). DNA migration was not significantly different in exposed human nasal mucosal cells compared to controls. DNA migration appeared to increase dose-dependently, however, in exposed human lymphocytes compared to controls ($p = 0.001$). Cytotoxicity was limited in both cell types at all doses as measured by trypan blue exclusion assay. These results suggest that human lymphocytes, but not nasal mucosal cells demonstrate genotoxic damage (single strand breaks or alkali-labile damage) in response to vanadium pentoxide.

Experimental data in animals provide evidence of some types 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^3) 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 Section 4.2.1.2). 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., 1996; Altamirano-

Lozano et al., 1993). Exposure caused no treatment-related effects on mitotic index, average generational time, or SCEs 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 CD1 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_{50}, $^1/_2$ LD_{50} and $^1/_4$ LD_{50}, 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.

In summary, the evidence for mutagenicity in humans is limited. Few studies examine genotoxicity in humans in vivo, with equivocal results. Ivancsits et al. (2002) reported no differences in DNA strand breaks, oxidative damage, or SCE frequency in leukocytes between control and vanadium pentoxide-exposed workers. Ehrlich et al. (2008) noted changes in DNA stability and DNA repair in leukocytes of occupationally exposed workers as compared to controls. Studies have demonstrated a genotoxic effect of vanadium pentoxide on human cells in vitro. Ivancsits et al. (2002) demonstrated significant increases in DNA damage as measured by the Comet assay in both leukocytes and fibroblasts but with different dose sensitivity, while Kleinsasser et al. (2003) noted DNA migration differences occurred dose dependently in peripheral blood lymphocytes but not in nasal mucosa. Earlier studies in human lymphocyte cultures also demonstrated increased aneuploidy (Ramirez et al., 1997; Rojas et al., 1996) and DNA damage (Roldán and Altamirano, 1990) following exposure to vanadium pentoxide. Thus, vanadium pentoxide-induced mutagenicity could occur at doses higher than those measured in these occupational exposures, could be tissue specific, and could be associated with oxidative stress rather than direct DNA damage.

In vitro tests in bacterial and yeast systems provide mixed evidence of vanadium pentoxide-induced mutagenicity. In general, classic gene mutation assays were negative, as were tests that assessed SCE and other chromosomal aberrations. DNA strand breaks (Ivancsits et al., 2002; Rojas et al., 1996) and micronucleus formation (Zhong et al., 1994) were indicated in some studies in cultured cells but depended on cell type. Fibroblasts appear to be more sensitive to vanadium exposure in vitro than are blood cells. Similarly, experimental data from animal studies is equivocal. NTP (2002) reported that the frequency of micronucleated normochromatic erythrocytes in peripheral blood was not increased in exposed compared to control mice. Several studies by Altamirano-Lozano et al. (1999; 1996; 1993), however, have noted DNA damage in specific target tissues in vanadium pentoxide-treated mice. It should be noted that Altamirano-Lozano et al. (1993) consistently used intraperitoneal injection as the route of exposure for these studies.

D.2.2.2. *Mechanisms of Inflammation and Fibrosis*

Increases in markers of pulmonary inflammation have been observed in the BAL fluid of rats and susceptible mice following intratracheal instillation exposure to vanadium pentoxide (Bonner et al., 2002; Pierce et al., 1996). These markers include macrophage inflammatory protein-2 (MIP-2), keratinocyte-derived chemokine (KC), interleukin-6 (IL-6), and IL-8. Further, the increased expression of prostaglandin-generating enzymes cyclooxygenases (COX) and prostaglandin E synthases have been associated with exposure to vanadium pentoxide (Bonner et al., 2002; Pierce et al., 1996), further suggesting increased inflammation.

Pierce et al. (1996) investigated the ability of several vanadium compounds to increase mRNA levels of cytokines in BAL fluid. Female CD rats received 42 or 420 μg of vanadium pentoxide or phosphate-buffered saline (PBS) by intratracheal instillation. BAL fluid was recovered 1 hour to 10 days after exposure. Significant neutrophil influx was observed after 24 hours of exposure to vanadium pentoxide and peaked at 48 hours postexposure with 20% neutrophils. MIP-2 mRNA expression levels were significantly elevated in vanadium pentoxide-treated rats compared to controls at early time points (1 hour to 48 hours), suggesting pulmonary inflammation.

Proinflammatory prostaglandins such as PGE_2 produced by the enzymes COX-1 and COX-2, and PGE synthase mediate tissue homeostasis or are known to be associated with various inflammatory diseases. Bonner et al. (2002) assessed the role of COX-1 and COX-2 enzymes in vanadium pentoxide-induced pulmonary inflammatory and fibrotic responses using 6- to 8-month-old male and female mice (of a hybrid C57BL/6J and 129/Ola genotype) that were deficient in either COX-1 ($COX1^{-/-}$) or COX-2 ($COX2^{-/-}$) enzyme. These COX-deficient mice and genotype-matched controls (wild type) were instilled with 50 μL of saline (n = 3 or 4) or 1 mg/kg vanadium pentoxide in saline (n = 5 or 6). Lungs were lavaged at 1, 3, 6, or 15 days postinstillation and BAL fluid was collected and analyzed for tumor necrosis factor-alpha (TNF-α) and prostanoids (e.g., PGE2) by ELISA. Lungs were removed and preserved for histopathology, hydroxyproline assay, or COX immunoblotting. Histopathology showed marked inflammation and increased injury in $COX2^{-/-}$ mice compared to wild-type and $COX1^{-/-}$ mice 3 days following vanadium pentoxide instillation. Hydroxyproline content was not different in wild-type or $COX1^{-/-}$ mice in response to vanadium pentoxide compared to saline-instilled controls. Hydroxyproline content in vanadium pentoxide-exposed $COX2^{-/-}$ mice was increased twofold compared to saline-instilled $COX2^{-/-}$, mice suggesting enhancement of lung fibrosis following vanadium pentoxide exposure. PGE2 levels increased from about 500 pg/mL in

Table D-1. Genotoxicity data following exposure to vanadium pentoxide

Test System/Species	Results	Exposure	Dose	Effects	Endpoint	Reference
In Vivo						
Human						
49 exposed male workers at vanadium pentoxide processing plant; 12 nonexposure controls	– – –	avg 12.4 y	Not reported - exposure confirmed through blood and urine measurements of vanadium	Genotoxicity measured in isolated lymphocytes and whole blood leukocytes. Study also examined in vitro exposure (below).	Comet Assay DNA damage (8-OHdG) Sister chromatid exchange	Ivancsits et al. (2002)
52 exposed workers; 52 nonexposed workers (jail wardens)	– +	inhalation	Not reported - 8hr shift, protective masks; exposure confirmed through blood levels of vanadium	Genotoxicity was measured in isolated leukocytes by Comet assay, with no increases in single- and double-DNA strand breaks observed. However, increases were observed in oxidized purines and pyrimidine formation in exposed workers. CBMN Cyt assay demonstrated increased micronucleus induction, nucleoplasmic bridges, and nuclear bud formation. Necrosis and apoptosis levels were also increased in exposed individuals.	Comet Assay micronucleus induction, oxidative nucleotides	Ehrlich et al. (2008)
Laboratory Animals						
Male CD-1 mice (n = 4)	+	intraperitoneal injection, sacrificed 24 h post injection	0, 5.75, 11.5, 23 µg/g bw	DNA damage was observed in all tissues examined except for bone marrow. This included liver, kidney, lung, spleen, and heart.	Comet assay	Altamirano-Lozano et al. (1999)
Male CD-1 mice (n = 2)	+	intraperitoneal injection, sacrificed 24 h post injection	0, 5.75, 11.5, 23 µg/g bw	As part of a larger study on reprotoxicity, DNA damage in sperm cells was analyzed. Significant ($p < 0.05$) dose-dependent increases were observed.	Comet assay	Altamirano-Lozano et al. (1996)
Male CD-1 mice (n = 4)	–	intraperitoneal injection, sacrificed 24 h post injection	0, 5.75, 11.5, 23 µg/g bw	Analysis of SCE demonstrated no effect of vanadium pentoxide exposure in this study.	Cytogenetic assay	Altamirano-Lozano et al. (1993)

Table D-1. Genotoxicity data following exposure to vanadium pentoxide

Test System/Species	Results	Exposure	Dose	Effects	Endpoint	Reference
$B6C3F_1$ mice (M, F)	+	inhalation, 2 yr	0, 1, 2, or 4 mg/m^3	DNA was isolated from lung tumors and normal tissue from exposed animals. Of the 20 tumors analyzed, 13 had either K-ras mutations or LOH at chromosome 6 or both.	Cytogenetic assay	Devereux et al. (2002)
$B6C3F_1$ mice (M, F)	+	inhalation, 2 yr	0, 1,2, or 4 mg/m^3	Analysis of frequency of micronuclei in peripheral blood normochromatic erythrocytes demonstrated an effect of vanadium pentoxide exposure.	Micronucleus assay	NTP (2002)
In Vitro						
Primary human lymphocytes	+		25–500 µg/L	Vanadium pentoxide exposure led to a significant increase in DNA migration as measured by Comet assay at the highest doses tested (250–500 µg/L) for whole-blood lymphocytes and leukocytes, and at all doses tested in cultured fibroblasts (*p*-values not given).	Comet assay	Ivancsits et al. (2002)
Primary human whole blood leukocytes	+					
Cultured human fibroblasts	+					
Primary human lymphocytes	+		0–47 mM	Exposure to vanadium pentoxide led to a dose-dependent increase in DNA migration in lymphocytes but not in mucosal cells.	Comet assay	Kleinsasser et al. (2003)
Primary human nasal mucosal cells	–					
Primary human lymphocytes	+		0–0.1 µM	Vanadium pentoxide led to an increase in aneuploidy with some interindividual variation observed between the four primary cell strains. Disruption of spindle formation might be due to interaction with microtubules.	FISH	Ramirez et al. (1997)
Primary human lymphocytes (n=4)	+	24 h	0.3, 30, 3,000 µM	Cells exposed to vanadium pentoxide had significantly increased DNA migration at all doses tested in the leukocytes ($p < 0.05$) and in the high doses in lymphocytes for three of the four donors ($p < 0.05$). DNA repair occurred generally within 45 min post exposure.	Comet assay	Rojas et al. (1996)
Primary human lymphocytes (n = 1)	–	72 h	0, 2, 4, 6 µg/mL	Vanadium pentoxide-exposed cells had an increase in aneuploidy and a decrease in mitotic index, with no changes in SCE or chromosomal aberrations.	SCE assay	Roldan and Altamirano (1990)
	+				Aneuploidy	

Table D-1. Genotoxicity data following exposure to vanadium pentoxide

Test System/Species	Results	Exposure	Dose	Effects	Endpoint	Reference
Syrian hamster embryo cells	+	0, 24 h, 7 d	0 to 0.875 µg/mL	Vanadium pentoxide-exposed cells were positive at 7 d of exposure but not at 24 h, similar to other tumor-promotion chemicals studied by this group.	SHE transformation assay	Kerckaert et al. (1996)
Chinese hamster V79 cells	+	24 h	0, 1, 3, 6, 9, 12 µg/mL	Vanadium pentoxide exposure led to increased micronucleus induction ($p < 0.005$), apparently due to damage to the spindle apparatus but no significant increases in mutations or SCE.	Micronucleus induction	Zhong et al. (1994)
	–				SCE assay	
	–				HGPRT mutation	
Bacterial Systems						
Bacillus subtilis	Positive with and without activation			Study details not available.	Recombination repair	Kada et al. (1980)
Escherichia coli	Positive without activation (not tested with activation)			Study details not available.	Gene mutation	Kanematsu et al. (1980)
Salmonella typhimurium						
Salmonella typhimurium (TA97,TA98,TA100, TA102,TA1535 with or without metabolic activation)	All strains negative with and without activation (both hamster and rat S9 fractions)	48 h	0333 µg/plate	No increase in revertant colonies was observed following exposure.	Gene mutation	NTP (2002)

saline-instilled wild-type mice to about 1,000 pg/mL in vanadium pentoxide-treated wildtype mice at 24 hours but not at other time points. The PGE2 level in saline-treated $COX1^{-/-}$ mice was about 10 pg/mL and about 225 pg/mL after 24 hrs of vanadium pentoxide. PGE2 levels were about 200 pg/mL at 24 hours in saline-treated $COX2^{-/-}$ mice and did not differ significantly from vanadium pentoxide-treated $COX2^{-/-}$ mice regardless of time point. This study suggests that vanadium pentoxide-induced inflammation could also be at least partially mediated by prostaglandins such as PGE2 generated by COX-2.

Myofibroblasts, the principal proliferating cells that produce collagen, are involved in fibrogenic response of lungs following exposure to pulmonary irritants. Bonner et al. (2000) observed that proliferating myofibroblasts were the principal cell type that contributed to the observed fibrosis. Male Sprague-Dawley rats weighing about 200 g received intratracheal instillation of sterile saline or 1 mg/kg vanadium pentoxide. Rats were additionally injected with BrdU (50 mg/kg, i.p.) 1 hour prior to sacrifice. Sacrifice occurred at 3, 6, and 15 days after vanadium pentoxide instillation. Excised lung tissue was assessed morphometrically and by immunohistochemistry for vimentin and desmin, two biomarkers for myofibroblasts and smooth muscle cells, respectively. Trichrome staining was used to assess collagen levels, an indicator of the extent of fibrosis. Vanadium pentoxide exposure induced thickening of the desmin-positive bronchiolar smooth muscle cell layer by day 6 postexposure, which was identified as myofibroblasts. A 2.3-fold increase in airway smooth muscle cell nuclear profile, suggesting increase in smooth muscle cell proliferation was due to hyperplasia. Serial sections of the peribronchiolar region stained positive for vimentin and desmin, and were mainly myofibroblasts. The thickness of the subepithelial trichrome-positive layer was 3.1- to 3.9-fold higher at day 15 in vanadium pentoxide vs. control samples. The peak appearance of peribronchiloar myofibroblasts occurred at day 6 and declined by day 15. A thickened collagen ring was apparent by day 15 in vanadium pentoxide-exposed samples compared to controls.

Rice et al. (1999) using both in vitro and in vivo models showed that myofibroblasts do proliferate in response to vanadium pentoxide, and are dependent on platelet-derived growth factor (PDGF) and epidermal growth factor (EGF). Rat lung myofibroblasts were isolated from exposed male Sprague-Dawley rats, as stated in Bonner (1998) above, and were grown to confluency. Cultures were incubated for 24 hours with increasing concentrations of one of two inhibitors of the PDGF-R (AG1296) and EGF-R (AG1478), respectively, at a concentration of 100 μmol/L. Autophosphorlyation of PDGF-R and EGF-R in vitro was specifically blocked by AG1296 and AG1478, respectively. Tritiated [^{3}H] thymidine uptake, a measure of mitogenesis, was blocked by selective inhibition of PDGF- and EGF-receptors. An in vivo study was carried out at the same time. Male Sprague-Dawley rats were treated with AG1296 or AG1478 (50 mg/kg) by intraperitoneal injection 1 hour prior to intratracheal instillation of vanadium pentoxide (1 mg/kg) and again 2 days after vanadium pentoxide was administered. Rats were sacrificed 3, 6, and 15 days after instillation and lungs were preserved for bromodeoxyuridine

(BrdU) immunohistochemistry and hydroxyproline assays to measure DNA replication and cell division, respectively. Quantitation of BrdU-labeled cells in the nuclei of rat lung tissue was significantly reduced in vanadium pentoxide-treated animals that had received injections of AG1296 and AG1478, compared to vanadium pentoxide-treated animals that were injected with vehicle alone. Vanadium pentoxide treatment induced a fivefold increase in lung hydroxyproline content, an indicator of lung collagen and potentially fibrosis, 15 days after instillation. Prior and posttreatment with AG1296 reduced hydroxyproline content in vanadium pentoxide-treated animals to quantities similar to saline-instilled animals. Pre- and posttreatments with AG1478 reduced hydroxyproline content by about 50%, but were still significantly higher than in saline-instilled controls.

Two recent studies examined the mechanism of inflammation in mice (Rondini et al., 2010; Turpin et al., 2010). Rondini et al. (2010) examined the effect of exposure to vanadium pentoxide in three mouse strains of varying susceptibility to lung cancer (A/J, BALB/C, and C57BL/6J) in an initiation/promotion model (full study description in Section 4.2.2.2). Significantly higher transcriptional activity was observed for NFkB (A/J mice; peaked at 1 day posttreatment) and AP-1 (A/J and B6 mice; peaked at 6 hours posttreatment) compared to PBS controls in vanadium pentoxide-treated mice. Overall, the differential inflammatory responses observed in the three strains of mice appear to positively correlate with increased levels of chemokines, such as keratinocyte-derived chemokine (KC) and monocyte chemotactic protein-1 (MCP-1), and increased binding of transcriptional factors NFκB and AP-1 (c-Fos), and sustained activation of MAP kinases (MAPKs) and extracellular signal-regulated kinases 1 and 2 (ERK 1/2), suggesting inflammation as a major response in mice. Turpin et al. (2010) examined pulmonary inflammation and fibrosis following intranasal aspiration exposure to vanadium pentoxide with and without respiratory syncytial virus (RSV) exposure (full study description in Section 4.4.1.2). In this study, vanadium pentoxide exposure also caused a significant increase in cell proliferation in the airways and lung parenchyma, lung mRNAs for TGF-β-1, CTGF, PDGF-C, Col1A2, and mRNAs for IFN-α and -β and IFN-inducible chemokines CXCL9 and CXCL10 compared to controls. Pre- or posttreatment with RSV caused a significant reduction in the all mRNAs. Together, results from this study showed that vanadium pentoxide induces inflammatory and fibrogenic response in mouse lung and these effects were suppressed by RSV infection.

To elucidate the potential cell signaling cascades associated with these endpoints, Antao-Menezes et al. (2008) investigated the role of the signaling molecule, signal transducer, and activator of transcription (STAT)-1 in vanadium pentoxide-induced pulmonary fibrosis. Their work identified another inflammatory molecule, interferon-beta (IFN-β), as a mediator of vanadium pentoxide-induced STAT-1 activation in normal human lung fibroblasts. Briefly, confluent, quiescent cultures of human lung fibroblasts were either placed in serum-free defined medium (SFDM) or SFDM supplemented with 10 μg/cm^2 vanadium pentoxide. Neutralizing

anti-IFN-α and anti-IFN-β antibodies were used to quench activity of IFN-α and IFN-β and the ratio of phosphor-STAT-1 to STAT-1 was then measured by quantitative RT-PCR or Western blot. Vanadium pentoxide-induced STAT-1 activation could be inhibited by a broad spectrum NADPH inhibitor at 24 hour. Vanadium pentoxide induced significant IFN-β expression after 18 and 24 hours. This effect was nearly completely abolished by addition of the NAPDH inhibitor. Activation of STAT-1 (as measured by a ratio of phosphor-STAT1 to total STAT-1) was significantly decreased by addition of neutralizing IFN-β antibodies and also by addition of a Janus Associated Kinase (JAK) inhibitor. In summary, STAT-1 was activated in response to vanadium pentoxide exposure, and was linked to IFN-β as its primary mediator. This study identifies a putative signaling pathway leading to expression of genes that control proliferation of myofibroblasts.

Ingram et al. (2007) performed gene array analysis to determine a list of candidate genes altered by exposure to vanadium pentoxide. Normal human lung fibroblasts were exposed to 10 μg/cm^2 vanadium pentoxide or saline in vitro. RNA from cells was harvested 1, 4 8, 12, and 24 hours posttreatment. Labeled cRNA hybridized to the Affymetrix Human Genome Array U133A 2.0 gene chip was used to assess gene expression at various time points up to 24 hours of exposure. About 300 genes were found to be upregulated in response to vanadium pentoxide including inflammatory and immunomodulatory genes. More than 1,000 genes were downregulated in response to vanadium pentoxide, including genes from the ubiquitin cycle and cell cycle genes. A dozen genes were confirmed by RT-PCR and included growth factors (heparin-binding EGF-like growth factor [HB-EGF], vascular endothelial cell growth factor [VEGF], and connective tissue growth factor [CTGF]); chemokines (IL-8, CXCL9, CXCL10); oxidative response genes (superoxide dismutase [SOD]2, pipecolic acid oxidase [PIPOX], oxidative stress response [OXR]1); and DNA-binding proteins (growth arrest specific [GAS]1, STAT1). The gene array analysis thus confirms that several mitogens, growth factors, chemokines, cytokines, oxidative response genes, and DNA-binding proteins are all critical to the formation of fibroproliferative lesions in response to vanadium pentoxide exposure in vitro (Figure D-1).

Together, these results indicate that proliferating myofibroblasts are the primary cell type associated with vanadium pentoxide-induced pulmonary fibrosis and that cellular proliferation depends on activated mitogens such as PDGF and EGF, in addition to HB-EGF. The STAT-1 and MAPKinase pathways might play key roles in this process. Moreover, fibroproliferative lesions contain collagen.

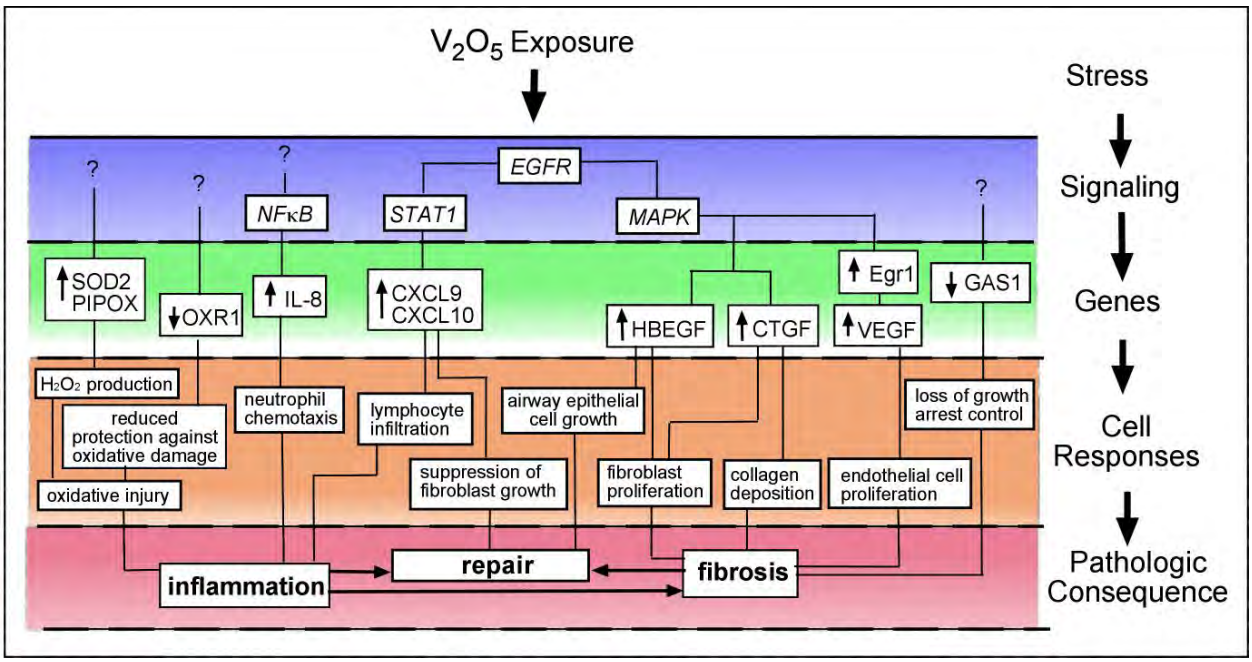

Figure D-1. Genomics of V_2O_5-induced bronchitis.

Note: Reprinted with permission from Ingram et al. (2007) Respir. Res. Apr 25; 8(1):34.

D.2.2.3. *Mechanisms of Hyperplasia and Carcinogenicity*

Molecular events underlying the mechanism of reparative hyperplasia and carcinogenesis have been documented. Bonner et al. (1998) exposed male Sprague-Dawley rats to vanadium pentoxide or sterile saline by intratracheal instillation at 1 mg/kg. Animals were sacrificed at 3, 6 and 15 days after instillation. Lungs were preserved and analyzed for PDGFR-α by immunohistochemical analysis and morphometry for fibroproliferative lesions. Smooth muscle thickening was observed beneath ciliated epithelial cells, as indicated by increased desmin localization, on day 6 in exposed samples. Trichrome staining revealed increased collagen deposition around bronchioles in vanadium-pentoxide-exposed samples. The thickness of the subepithelial layer increased by 3.1- to 3.9-fold at day 15 after instillation of vanadium pentoxide, determined by morphometric techniques.

Platelet-derived growth factor (PDGF) is a mitogen and chemoattractant for fibroblasts. Male Sprague-Dawley rats were intratracheally instilled with sterile saline or 2 mg/kg of vanadium pentoxide (Bonner et al., 1998). Tissues were harvested at 24, 48, and 72 hours postexposure and at 6 and 15 days. Pulmonary myofibroblasts and alveolar macrophages were isolated. Isolated total lung RNA was assessed for quantitation of PDGF by Northern blot analysis. PDGF-receptor alpha mRNA and protein expression were significantly elevated in vanadium pentoxide-exposed animals compared to controls at 24 and 48 hours. PDGF-receptor beta was not significantly elevated over controls at any time points. Confluent cultures of lung

myofibroblasts were stimulated with vanadium pentoxide. Similarly, cultures of lung macrophages were stimulated with vanadium pentoxide. Levels of PDGF in myofibroblasts were not affected by direct stimulation by vanadium pentoxide, but were affected by a factor released by vanadium pentoxide-stimulated macrophages, and were associated with interleukin (IL)-1B, an inflammatory cytokine. Together, these results suggest that hyperplasia occurs under the control of inflammatory mediatiors such as IL-1B that can recruit mitogens such as PDGF which can stimulate growth of myofibroblasts, the main cell type involved in the development of fibrotic lesions.

Zhang et al. (2001a) investigated the ability of vanadium pentoxide to induce heparin-binding epidermal growth factor-like growth factor (HB-EGF) (another mitogen) in vitro, using normal human bronchial epithelial cells (NHBECs). Mature cultures of NHBECs were incubated with vanadium pentoxide at 0, 1, 10 and 50 $\mu g/cm^3$ for 3 hours. Total RNA was then isolated and RT-PCR was used to quantitate HB-EGF. HB-EGF mRNA was significantly and dose-dependently increased in response to vanadium pentoxide compared to controls. In a second time course study (using only the high dose 50 $\mu g/cm^3$ of vanadium pentoxide), the peak of HB-EGF induction occurred after 3 hours of exposure and persisted until 8 hours of exposure.

In a follow-up study, Ingram et al. (2003) similarly showed a peak induction of HB-EGF in quiescent cultured human lung fibroblasts exposed to 10 $\mu g/cm^2$ vanadium pentoxide at 3 hours. Quiescent cultured human lung fibroblasts were exposed for 3 hours to 0, 10, 30 or 100 $\mu g/cm^2$ vanadium pentoxide. HB-EGF RNA was isolated and detected by Northern blot analysis. HB-EGF was significantly elevated in a dose-dependent manner in vanadium pentoxide-exposed fibroblasts compared to controls. Stimulating human lung fibroblasts with H_2O_2 (10 μM) similarly induced HB-EGF, with a peak mRNA expression at 1 hour postexposure. HB-EGF protein expression peaked at 6 hours postexposure, as measured by Western blot analysis. Quiescent human lung fibroblasts stimulated with 10 $\mu g/cm^2$ vanadium pentoxide were found to initially quench spontaneous H_2O_2 production (at early time points) and then boost H_2O_2 production at a peak of 12 hours postexposure. To assess the role of extracellular signal-regulated protein kinase (ERK), and the p38 subunit of mitogen-activated protein (MAP) kinase in vanadium pentoxide-induced HB-EGF production, Ingram et al. (2003) exposed quiescent confluent human lung fibroblast cells in culture to 500 μM H_2O_2 or 10 $\mu g/cm^2$ vanadium pentoxide for 15 minutes, 30 minutes, 1 hours, 3 hours, 6 hours, or 24 hours. Cell lysates were collected and Western blot was performed for the phosphorylated form of ERK or total ERK protein or for the phosphorylated p38 subunit of MAP kinase and total p38. Peak phosphorylated ERK occurred at 30 minutes postexposure to H_2O_2. Vanadium-induced increases to p-ERK were biphasic, with one peak at 30 minutes postexposure and the next at 24 hours postexposure. Phosphorylated p38 was similarly maximally elevated at 30 minutes posttreatment with H_2O_2 and 24 hours posttreatment with vanadium pentoxide. Thus, this study

suggests that HB-EGF expression occurs as a result of activation of the MAP kinase and ERK pathways.

NTP (2002) and Ress et al. (2003) concluded that exposure to vanadium pentoxide caused alveolar and bronchiolar adenomas and carcinomas in male and female mice and some evidence suggests carcinogenicity in male rats, based on observations of alveolar and bronchiolar neoplasms in groups exposed to vanadium pentoxide that exceeded historical controls. The body of evidence that has investigated the MOA underlying cancer effects due to exposure of vanadium pentoxide is not as well characterized as mechanisms underlying noncancer fibrotic effects. Loss of heterozygosity (LOH) and DNA damage, however, has been documented.

Using mouse lung tumor tissues from the NTP (2002) chronic inhalation study from mice exposed to 0, 1, 2 or 4 mg/m^3 vanadium pentoxide, Zhang et al. (2001b), observed LOH on chromosome 6 (in the region of the K-*ras* gene) in 17 of 19 vanadium pentoxide-induced mouse tumor samples. Moreover, 29 of 40 (73%) vanadium pentoxide-induced murine adenocarcinomas from the NTP (2002) study had mutations in K-*ras2*. The K-*ras2* mutations typically were the result of either a GA→AT transition or a GA→TA transversion in the second base of codon 12 (Zhang et al., 2001b). To determine the effect of the K-*ras* mutations and LOH on activated MAP kinase, Devereux et al. (2002) used tissues from the NTP (2002) 2-year carcinogenicity study in female and male B6C3F1 mice to isolate protein from 17 vanadium pentoxide-induced alveolar and bronchiolar carcinomas, 1 spontaneous carcinoma, and 2 normal (untreated) lung tissue samples. Levels of total MAP kinase and activated MAP kinase were assessed by probing isolated lung protein for total and phosphorylated MAP kinase with anti-phospho-MAP kinase antibody in all samples. Only qualitative analysis was reported. Total MAP kinase was not different between normal tissue and lung tumor tissue. Activated MAP kinsase was elevated in five of six tumors that had both LOH and K-*ras* mutations, and was barely detectable in all seven tumors examined where no K-*ras* mutations were detected. Four of five tumors that had K-*ras* mutations but were not positive for LOH had elevated phosphorylated MAP kinase levels. These results should be interpreted cautiously, however, as LOH was difficult to detect due to interference from infiltrating lymphocytes. In summary, mouse tumor tissue excised from mice used in the NTP (2002) study showed LOH in the region of the K-*ras* oncogene location in 17 of 19 samples tested. The signaling events, and specifically the role of the MAP kinase pathway, associated with this oncogenic mutation, however, remain unclear.

Pierce et al. (1996) identified proinflammatory cytokines associated with vanadium pentoxide-exposure. Pierce et al. (1996) reported increased mRNA expression of macrophage inflammatory protein (MIP-2) and keratinocyte-derived cytokine (KC) in bronchiolar lavage (BAL) fluid in vanadium pentoxide-treated female CD rats compared to controls at early time points (1 hour to 48 hours).

Montiel-Davalos et al. (In Press) examined the effect of vanadium pentoxide exposure on endothelial cells. Exposure to vanadium pentoxide to human umbilical vein endothelial cells

(HUVEC; 3.12 μg/cm^2) induced enhanced adhesion of U937 macrophage cell line to HUVECs. HUVECs exposed to vanadium pentoxide also demonstrated an increase in reactive oxygen species and nitric oxide production, as well as diminished proliferation. Yu et al. (2011) demonstrated in a primary cell strain and the NCI-H292 cell line an increase in genes related to mucin production, but not an increase in reactive oxygen species. The results demonstrated an increase in gene expression related to mucin expression following exposure to vanadium pentoxide that seemed unrelated to ROS production.

In summary, hyperplastic responses following exposure to vanadium pentoxide are associated with increased expression of various mitogens such as PDGF and HB-EGF. Moreover, PDGF activation could be dependent on inflammatory mediators such as IL-1B. HB-EGF expression is dependent on activation of the MAP kinase and ERK signaling pathways.

No information is available concerning the rat neoplasm data. Indeed, the marginal increase in lung neoplasms observed in female rats was not statistically significant. Whether lung neoplasms from male rats exhibit elevated activated MAP kinase or the rat tumors have increased K-*ras* mutation and LOH on chromosome 6 is unknown.

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