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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)

July 2011

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CONTENTS – TOXICOLOGICAL REVIEW OF VANADIUM PENTOXIDE
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LIST OF TABLES

LIST OF FIGURES

LIST OF ACRONYMS AND ABBREVIATIONS

BMC	benchmark concentration
BMCL	benchmark concentration (lower limit)
BMD	benchmark dose
BMDL	benchmark dose (lower limit)
BMDS	benchmark dose software
BMR	benchmark response
bw	body weight
cc	cubic centimeters
CD	caesarean delivered
CERCLA	Comprehensive Environmental Response, Compensation and Liability Act of 1980
CNS	central nervous system
cu.m	cubic meter
FIFRA	Federal Insecticide, Fungicide, and Rodenticide Act
g	grams
GI	gastrointestinal
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
m	meter
MCL	maximum contaminant level
MF	modifying factor
mg	milligram

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

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. 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, 1986a), *Guidelines for Mutagenicity Risk Assessment* (U.S. EPA, 1986b), *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, 2000a), *Benchmark Dose Technical Guidance Document* (U.S. EPA, 2000b), *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, 2006a), and *A Framework for Assessing Health Risks of Environmental Exposures to Children* (U.S. EPA, 2006b).

The literature search strategy employed for this compound was based on the Chemical Abstracts Service Registry Number (CASRN) and at least one common name. Any pertinent scientific information submitted by the public to the IRIS Submission Desk was also considered in the development of this document. The relevant literature was reviewed through May 2011.

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 (CASRN 7440-62-2) is a soft silver-grey 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 on vanadium pentoxide (CASRN 1314-62-1), but a short description of 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 a number of enzymes and is a potent inhibitor of the Na+K+-ATPase of plasma membranes (Harris et al. 1984; Patterson et al. 1986).

Figure 2-1. Vanadium pentoxide structure

Generally, V^{3+} and V^{4+} 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.

Vanadium compounds differ in their physicochemical properties which influence their solubility under different pH conditions and their accessibility and availability in biological systems [reviewed in (Assem and Levy 2009)]. An acidic pH favors tetravalent state (V^{+4}) keeping it as vanadyl, while an alkaline pH prefers pentavalent state (V^{+5}) as vanadate (Crans et al . 2004). In the case of oral ingestion, vanadium compounds are exposed to a range of pH solutions in the digestive tract starting from the stomach (pH typically between 1-3.5) followed by the small intestine (pH around 8). Bruyere et al. (1999) state that at pH between 1.3 and 3.3 the predominate 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. $VO_4(OH)_3$) and polymerized vanadium is predominant ($H_nV_{10}O_{29}^{(n-6)}$)(Bruyere 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 different valences as shown in Table 2-1 (HSDB 2009; IPCS 2001). The 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 vanadium compounds (V^{+5}), 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 solubility of 211 g/L, 100 g/L, 58 g/L, respectively (IPCS 2001). Furthermore, the rate (distinguished from the solubility – an equilibrium or thermodynamic parameter) of dissolution of various vanadium compounds may vary, resulting in different concentrations of specific forms of vanadium. It seems a reasonable hypothesis that these various forms of vanadium will be absorbed differently, which may result in different physiological effects. For example, $V(5^+)$ compounds can mimic phosphate and can 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 2009; IPCS 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 (IPCS 2001); 5.2 at 15°C (HSDB 2009)
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

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).

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 alkalies, 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, Non-combustible	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

Toxicokinetics of vanadium pentoxide have been investigated in limited studies described below and reviewed by Cooper (2007). Toxicokinetics of several other vanadium compounds have been evaluated in animal models and are reviewed elsewhere (Sabbioni et al. 1996; Barceloux 1999; Mukherjee et al. 2004; ATSDR 2009). Vanadium pentoxide is rapidly absorbed by both inhalation and oral exposures through lungs and the gastrointestinal tract, respectively, although the amount absorbed through the gastrointestinal tract is low. Laboratory animal studies show vanadium pentoxide is mainly distributed following inhalation and oral exposure to the bone, lungs, liver and kidney. Elimination of vanadium pentoxide has been studied only following inhalation exposure, and is mainly 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 (<7.44mg/m^3) of vanadium compounds, including vanadium pentoxide (Gylseth et al. 1979; Kiviluoto et al. 1981a; Lewis 1959; NIOSH 1983), with the majority excreted in urine within one day post 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 of 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 14 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 (NTP 2002; Dill et al. 2004); in the 2-year studies by NTP (2002), the increase in blood vanadium levels were concentration-related, although not statistically significant. Intratracheal studies suggest that vanadium pentoxide is

[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.

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, i.e., 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 abino rats (Edel and Sabbioni 1988). After 3 days, 90% of 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 components of a complex particulate matter mixture into its metal components and tracked the absorption of different metals in different tissues following a single intratracheal dose in rats. Healthy male Wistar rats were instilled with a single intratracheal dose of combustion particulate matter (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 lung 4-hrs post-instillation and 62.76 μg/rat was measured in lung 24-hrs post-instillation. In the plasma and lung, vanadium was significantly elevated 4-hr post-instillation (130,000 ng V/g lung tissue, and 350 ng V/g plasma) compared to 24-hrs (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 lung, plasma, heart and liver and was significantly increased compared to controls at both 4- and 24-hrs post-instillation compared to saline controls. This study permitted detectable changes in component metals of a complex mixture in various organs and provides evidence that metals dissociate from particulate matter 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.

The absorption of vanadium through the gastrointestinal tract of animals is low. Less than 0.1% of an intragastric dose was detectable in the blood of albino rats at 15 minutes post-exposure, 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). 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 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 that there is a higher absorption of vanadium in these 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 gastrointestinal tract.

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, although absorption by this route is generally considered to be very low (WHO 1988). Vanadium is a metal with low solubility, therefore 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 after other routes of exposure to vanadium pentoxide.

3.2 Distribution

Distribution was measured from autopsy cases with unknown routes of exposure. Vanadium has been detected in the lungs (in 52% of the cases) and intestines (in 16% of the cases) of humans with no known occupational exposure, collected from autopsy data and reviewed in Schroeder et al. (1963). In the gastrointestinal tract, it was primarily found in the ileum (37%), cecum (45.1%), sigmoid colon (15.9%), and rectum (26.2%). Most positive samples had 0.01μg 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 (Gylseth et al. 1979; Kiviluoto et al. 1981b). Analytical studies have shown low levels of vanadium in human kidneys and liver, with even less in brain,

heart, and 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 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 B6C3F1 mice exposed to 1.1 or 2.2 mg vanadium/m^3 as vanadium pentoxide, 6 hours/day, 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 (Oberg et al. 1978; Rhoads and Sanders 1985). 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 lungs, liver, kidneys, bone, testes and spleen with clearance at different timepoints post-exposure with little to no retention observed in stomach, intestines, heart or trachea (Edel and Sabbioni 1988). This is similar to the distribution seen following inhalation and oral exposure.

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 skeleton. 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 skeleton and blood of female Fischer rats.

Oral exposure for an intermediate duration produced the highest accumulation of vanadium in the kidney. In young male 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. There was an accumulation in muscle and fat, related to the growing mass of the tissues with age. The higher levels of vanadium in the young rat tissues may be due to the higher retention capacity of the undeveloped tissues, or a greater permeability of the intestinal wall. Adult rats exposed to 5 or 50 ppm vanadium in the 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 may have been due to phosphate displacement. All tissue levels plateaued at 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, retention of vanadium was 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 B6C3F1 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. Mainly, vanadium pentoxide is distributed 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 week 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 vs. 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, NADH) and oxygen, vanadium may be reduced, reoxidized, and/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 (Gylseth et al. 1979; Kiviluoto et al. 1981a; Lewis 1959; NIOSH 1983; Zenz et al. 1962). 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) (NIOSH 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 that 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 skeleton, and 2–7% was 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 (3h) but with some vanadium (2% original dose) remaining at 12 d post-exposure. All other tissues eliminated 98-99% of original dose by 3h post-exposure (Edel and Sabbioni 1988). Epidemiological studies and animal studies suggest that elimination of vanadium following inhalation exposure is primarily in the urine.

3.4.2 Oral Exposure

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

3.4.3 Dermal Exposure

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

3.5 Physiologically Based Pharmacokinetic (PBPK)/Pharmacodynamic (PD) Models

No PBPK models for vanadium pentoxide are available.

3.5.1 Animal-to-Human Extrapolations

There are no relevant data available to evaluate potential toxicokinetic differences

between humans and laboratory animals. Similar effects have been reported in humans and animals following inhalation or oral exposure to vanadium pentoxide; however, this conclusion is based on the limited human toxicity data. In absence of data to the contrary, rats or mice appear to be valid models for extrapolation to humans.

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 the vanadium levels in blood, but not hair is a sensitive or suitable indicator of environmental exposure.

4.1.2 Inhalation Exposure

Health effects of inhalation exposure to vanadium pentoxide and other vanadium compounds reported by case studies and epidemiological investigations 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 processing (Sjöberg, 1951; Sjöberg, 1956; Zenz et al., 1962; Kiviluoto et al., 1979; Kiviluoto, 1980; Kiviluoto et al., 1981; Musk and Tees, 1982; Irsigler et al., 1999) or to fuel-oil ash containing vanadium during cleaning and maintenence of oil-burning boilers (Williams, 1952; Sjöberg, 1955; Lees, 1980; Ross, 1983; Levy et al., 1984; Hauser et al., 1995a; Hauser et al., 1995b; Woodin et al., 1998; Woodin et al., 1999; Woodin et al., 2000; Hauser et al., 2001; Kim et al., 2004). Most of these studies did not quantify the inhalation exposure specifically to vanadium pentoxide, although it was the primary exposure in vanadium pentoxide production. Exposures to residual oil fly ash (ROFA) involves a mixture of pollutants including elemental vanadium, vanadium oxides, vanadium pentoxide, vanadium sulphates, particulate matter and other metal constituents (Hauser et al., 1995b). Vanadium pentoxide is a major

constituent of ROFA and the studies of health effects among boilermakers evaluated models in relation to vanadium content of respirable particulate matter. Therefore, these papers serve to inform the assessment of the nature and scope of the health response to vanadium pentoxide. More recently, epidemiology studies evaluated the metals content of ambient particulate matter (PM) and 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 (Lippmann et al., 2006; Dominici et al., 2007; Bell et al., 2009; Patel et al., 2009; Lagorio et al., 2006). 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 with several components that also may contribute to observed health effects.

4.1.2.1 Controlled Human Exposure Study

Zenz and Berg (1967) exposed nine volunteers to vanadium pentoxide dust to evaluate respiratory effects. 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 hrs; no control group was included in this study. Particle size analysis revealed that 98% of particles had a diameter <0.5 µm. Post-exposure assessments of chest x-ray, blood, urine, nasal smear samples and pulmonary function were compared with baseline values determined for each subject prior to exposure. All subjects were observed for clinical symptoms for 11-19 months after exposure. Subjects exposed to 1 mg/m^3 vanadium pentoxide developed sporadic cough after 5 hrs of exposure, which progressed to persistent cough during the last 3 hrs of exposure and continued for 8 days. No other signs of respiratory irritation were observed. Results of pulmonary function tests and chest x-ray 1, 2 and 3 weeks after exposure were similar to baseline (data not reported). Hematology and urinalysis parameters were not affected by exposure (data not reported). Nasal smears obtained 24 hrs, 72 hrs and 1 week after exposure were negative for eosinophilia. Three weeks after the initial exposure, two of the subjects were accidentally exposed to a "heavy cloud" of vanadium pentoxide dust (concentration not reported) for 5 minutes. Within 16 hrs of exposure, both subjects developed a "marked" productive cough with rales and expiratory wheeze, which continued for 1 week. However, pulmonary function test results were comparable to baseline (data not reported). Blood and nasal smear samples were negative for eosinophilia. Subjects in the 0.25 mg/m^3 exposure group developed a "loose" productive cough on the day after exposure, which lasted for 7-10 days. No additional

symptoms were observed and post-exposure pulmonary function and laboratory tests were comparable to baseline results (data not reported). Subjects exposed to 0.1 mg/m^3 developed "considerable" mucus formation within 24 hrs after exposure, lasting for 4 days. No other symptoms or positive findings for pulmonary function or laboratory tests were observed. No treatment-related symptoms or clinical findings were reported for any subject during the 11-19 months post-treatment period.

4.1.2.2 Occupational Exposure during Vanadium Pentoxide Mining and Processing

Respiratory and other symptoms have been documented among workers employed at facilities producing and processing vanadium pentoxide (Sjöberg, 1951; Sjöberg, 1956; Zenz et al., 1962; Kiviluoto et al., 1979; Kiviluoto, 1980; Kiviluoto et al., 1981a; Musk and Tees, 1982; Irsigler et al., 1999). 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 V_2O_5 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 to 7.5%. A significant proportion of the dust consisted of small, respirable particles (22% < 8µ, 39% < 12µ). Although vanadium pentoxide concentrations in air were not reported, vanadium was detected in the blood and urine of 23 and 27 individuals, respectively.

Sjöberg (1956) 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, a group of 703 workers from mines and sawmills in northern Sweden followed during the same time period and examined using the same methods. The authors assumed that the comparison population was exposed only to inert dust, however no sampling data was reported. The main symptoms of the upper respiratory tract were nasal irritation and/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. Acute changes in the lungs indicative of pneumonitis were noted in five workers. Spirometric measures were reported to be higher than those of the comparison group. A decrease in hemoglobin levels (7.8 ± 2.36% compared to 3.0 ± 2.07%) and red blood cell count was observed, 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 eosnophilia in blood samples. Six cases of dermatitis were observed, and one person had a positive response to a patch test with a sodium vanadate solution. Finally, weakness and fatigue were reported to be common symptoms after heavy exposure to dust, and symptoms of a neurasthenic character were noted in some cases.

Six workers with persistent symptoms were re-examined in December, 1953 to January, 1954 (Sjöberg, 1956). Reported symptoms included cough and wheezing (N=5), and dyspnea and fatigue (N=6). Clinical measurements and blood pressure were normal including sinuses, circulating eosinophils, erythrocyte sedimentation rate, and radiographic examination of the lungs and heart revealed no evidence of pneumoconiosis. Lung function measures had increased which the authors attributed to differences in technique. However, bronchoscopic examination and biopsy of bronchial mucosa showed evidence of chronic bronchitis (N=5).

Two additional published case summaries described symptoms that appeared 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). Symptoms similar to those reported by Sjöberg (1956) appeared by the end of the first day of exposure including eye and throat irritation and cough with sputum, and persisted 2 – 4 weeks after exposure ended. Lung function tests revealed reversible airflow obstruction in 3 of the 4 case histories reported by Musk and Tees, and bronchial reactivity to histamine was demonstrated in two of the four. One individual with a family history of asthma and positive skin prick tests to common allergens, continued to experience wheezing for 8 weeks. Zenz et al (1962) did not find reductions in airflow among the 18 men examined.

Kiviluoto et al. (1979; 1980; 1981a, b) 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. A total of 79 men employed at the vanadium factory ≥ 4 months were eligible and 63 men, aged 19-52 years, who were not on holiday or sick leave were enrolled (80% participation rate). A referent group of 63 men living in the same area were 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 5 cigarettes/day). Vanadium dust concentrations at various sites measured on 8 days over 2 shifts in March-May 1976, were 0.012 mg/m^3 ranging between 0.002 (LOD) and 0.043 mg/m^3. Vanadium concentrations in the breathing zone were 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). Air monitoring results were not reported for the referent population. Urinary vanadium concentrations among the exposed averaged 0.26 ± 0.17 umol/L (18 hour excretion), while concentrations among the referent group did not exceed the < 0.04 umol/L limit of detection (LOD).

Clinical assessments were conducted by health personnel with no knowledge of exposure status. The occurrence of wheeze differed by exposure. 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 was observed in nasal smears and these results are discussed in the section on immunological endpoints (Section 4.2.1). 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, there were no differences noted for serum cholesterol, serum triglyceride, or leukocyte differential.

Irsigler et al. (1999) evaluated the clinical histories of 40 men, who were employed at a vanadium pentoxide production plant in South Africa, and were referred by the plant's medical staff for more detailed medical assessment because of persistence of respiratory symptoms (cough, breathing difficulty, wheezing) between October 1995 – 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, who were referred and did not have bronchial hyperresponsiveness, matched by age and smoking. The authors concluded that the asthma symptoms and bronchial reactivity had occurred as a result of vanadium exposure because all were free of current symptoms or a

previous history of asthma when they began employment at the plant. Bronchial reactivity was determined by the repeated inhalation of histamine at successively doubled doses until a decrease of 20% or greater in FEV_1 was measured (Histamine PC20 FEV_1). A PC20 > 8 mg/ml was considered to be normal. Alternatively, an exercise challenge consisting of free tread mill running indoors for 6 minutes was used to assess bronchial reactivity. A positive test was defined as a decrease of 200 ml or a 15% or greater fall in FEV_1. 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. Concentrations of SO_2 and NH_3 were above their recommended occupational limits in the kiln 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 – 55 µg/g creatinine, with one person above 50 µg/g creatinine. Among 9 subjects who returned for a follow-up examination after 5 to 23 months with no vanadium pentoxide exposure, 8 still exhibited bronchial reactivity. Atopy was ruled out as a primary cause because 5 individuals with positive skin prick allergy tests were equally distributed in the reactive and nonreactive groups. In addition, no evidence of viral upper respiratory tract infection was found in the study subjects, and 10 cigarette smokers were also distributed equally in both groups. Work tasks did not appear to be different between the two groups. This small study was not informative regarding associations between vanadium pentoxide exposure and bronchial reactivity. Although the group with bronchial reactivity had two subjects with high urine vanadium levels, differences in biomarker levels or job site between groups were not analyzed statistically, and the sample size and study design were 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 sulphate and metals in ash that accumulate in oil- and coal-fired boilers, as well as other fuel types used in boilers. Several reports of case histories and epidemiology 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 (Williams, 1952; Sjöberg, 1955; Lees, 1980; Ross, 1983; Levy et al., 1984; Hauser et al., 1995a; Hauser et al., 1995b; Woodin et al., 1998; Woodin et al., 1999; Woodin et al., 2000; Hauser et al., 2001; Kim et al., 2004). Additional health parameters have

been investigated including pulmonary function (Lees, 1980; Levy et al., 1984; Hauser et al., 1995a; Woodin et al., 1999; , biomarkers of inflammation in nasal fluid (Hauser et al., 1995b; Woodin et al., 1998), and autonomic cardiac function (Magari et al., 2002). Studies have investigated acute effects occurring after jobs cleaning or overhauling boilers lasting 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 (Williams, 1952; Ross, 1983), Sweden (Sjöberg, 1955), and Canada (Lees, 1980). Two case series reports described exposure to dust during cleaning jobs of a few days to one week during 1946 – 1953. The onset of symptoms, including rhinorrhoea, sneezing, eye irritation, sore throat, and chest pain, began within 1 to 12 hours (Williams, 1952; Sjoberg, 1955). Symptoms with a delayed onset of 6 – 24 hours included a dry cough becoming paroxysmal and productive in some workers, wheezing, dyspnea upon exertion, and fatigue (Williams, 1952; Sjoberg, 1955). Bronchial irritation, bronchitis, and the development of rales in regions of the lung of some of the workers also were reported. Some workers also developed a greenish-black coating on the tongue. Symptoms were reported to persist for 3 days to one week after exposure was ended. Concentrations of vanadium pentoxide particles (10-20 μ in diameter) in the air inside the boilers during cleaning were 17 – 85 mg/m^3.

Lees (1980) reported on a clinical evaluation of 17 men occupationally exposed to bottom ash from cleaning the boiler of an oil-fired electricity generating station. Personal sampling in the breathing zone of four of the men indicated a mean time weighted average of 523 $\mu g/m^3$ (0.52 mg/m^3) for dust under 10μ. Vanadium content in the bottom ash and crusted deposits was 15.3% and 24.2 – 35%, respectively. The men wore cartridge filter type respirators during the cleaning operation, however subsequent testing documented that they leaked up to 9%. The air concentrations of vanadium pentoxide were not specifically reported. Symptoms and lung function after exposure were compared to health status assessed before exposure began. A medical history and clinical examination, performed before work began and the day after, indicated symptoms similar to those described in earlier reports, including cough with sputum, respiratory wheeze, and sore throat reported in 77%, 53%, and 41% of the men, respectively. Serial spirometry measurements at 24 hour intervals showed reductions with the lowest mean FVC and FEV_1 values at postexposure days 3 and 2, respectively, compared to baseline (percent of baseline: 88.6% and 86.6%, respectively; $p<0.05$). Reductions in forced mid-expiratory flow of 9-31% also were recorded. These measures had returned to preexposure levels after 4 weeks. No details were provided regarding the years the study was conducted and the characteristics of the men under study.

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 the course of approximately 6 weeks, October 15 – November 30, with most of the men working 10 hour days, six 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 fumes of copper and iron oxide 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, a questionnaire was distributed to all 100 workers through the union president and responses were received from 55 men over the next two months. All of the respondents, aged 23 – 60 years, reported symptoms, with over half describing 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%) 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 boilermakers a median of 10 years. Pulmonary function tests were performed on 35 individuals after they visited a physician. Median FVC was 87% of predicted, but was < 80% of predicted in 5 of 27 individuals. Median FEV_1 was 93% of predicted, but was < 80% of predicted in 8 of 27 men. Median FEV_1/FVC was 79% of predicted. Median $FEF_{25-75\%}$ among 24 workers tested was 57% of predicted with only 4 of 24 above 80% of predicted. $FEF_{25-75\%}$ was not correlated with smoking history among the 69% of workers for whom this information was obtained.

The symptoms and effects on lung function are consistent with previous reports of occupational exposures during the cleaning and maintenance of boilers that had burned oil contaminated with vanadium pentoxide. A marked deficit in pulmonary function, particularly in FEF, was observed in some of the workers. However, pulmonary function was assessed in only 60% of respondents by several different health providers. In addition, 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 before and after four weeks of work overhauling an oil-fired boiler did not observe an association with respirable vanadium dust concentrations (Hauser et al., 1995a). A total of 36 out of 80 eligible workers completed a baseline test, and 26 completed a postexposure test. The men averaged 42.5 years of age (27 – 60) and 16.9 years on the job (6 months – 35 years). Daily exposure estimates were developed for each subject based on work diaries detailing tasks and locations and personal sampling. Between 1 and 10 hour time weighted average 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. Lung function values were analyzed in relation to three exposure indices for the exposure period; average or peak concentrations, and concentrations on the day of the postexposure test. Average, peak and mean day-of vanadium concentrations in particulate matter < 10 μm (range) were estimated to be 12.2 ± 9.1 $\mu g/m^3$ (2.2 – 31.3), 20.2 ± 11.4 $\mu g/m^3$ (2.2-32.2) and 12.1 ± 10.9 (1.6-31.1), respectively. For the spirometric measures, the largest value from three acceptable curves was used in the analysis. Reductions in several lung function measures were observed over the average of 27 ±4.1 days between the baseline and postexposure tests that were statistically significant. FEV_1, FVC, and $FEF_{25-75\%}$ decreased by an average of 140 ± 160 ml (range: -390-420), 140 ± 200 ml (range: -580-320) and 270 ± 450 ml/s (range: -1170-870), respectively. Each lung function index was adjusted by dividing the change by the average of the pre- and post-exposure value. The adjusted value was analyzed for associations with exposure in multiple linear regression models adjusting for age and current smoking status. Peak PM_{10} was 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). 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 and the variation in the range of concentrations may not have been wide enough to detect a relation with lung function in this small sample. Alternatively, the deficits in lung function may have been caused by a different constituent in PM_{10}.

Woodin et al (1998, 1999, 2000) 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 six week period from mid-May, 1995 to late-June, 1995. The men had volunteered for the study and did not have allergic

symptoms two weeks prior to or during the overhaul. Data from one person who presented with cold or flu symptoms at one of the clinical assessments were excluded. All were white men aged 26 – 61 years and were employed a mean of 20.5 years (range: 3 – 39). Vanadium and PM_{10} exposure was calculated for each subject for each work day using information on task duration and location and use of personal protective equipment from job diaries, and PM_{10} or vanadium concentrations from personal exposure monitors in the breathing zone. Personal and stationary sampling of PM_{10} (< 10 μm) was conducted over 10 – 12 hour shifts in major work areas in and around the boiler. Environmental concentrations were compared before and during the boiler overhaul, and between the two occupational groups. Utility workers did not enter the boiler during the overhaul. Vanadium levels before the boiler work was comparable for boilermakers and utility workers (geometric mean (SD) $\mu g/m^3$: 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. Quartiles of lung vanadium dose (μg) were ≤ 0.90, > 0.90 - ≤ 5.30, > 5.30 - ≤ 22.30 and > 22.30. Quartiles of nasal vanadium dose (μg) were ≤ 2.50, > 2.50 - ≤ 23.20, > 23.20 - ≤ 68.30 and > 68.30.

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. 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. 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 dose 2, 3, and 4 compared to 1. Average lower airway frequency was increased by 0.19 ($p=0.02$), 0.39 ($p<0.01$) and 0.14 (0.07) in quartiles of lung vanadium dose 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). The highest value from three acceptable curves obtained during each test was used in repeated measures analysis of variance to test for differences over time. No changes in the four airflow

measures, FEF_{25}, FEF_{50}, FEF_{75}, and MMEF, were observed over the course of the study. Mean (SD) FEV_1 (l) values before, during and after the overhaul were 3.73 (0.61), 3.76 (0.54) and 3.65 (0.42), respectively. Mean (SD) FVC (l) values before, during and after the overhaul were 5.01 (0.67), 4.94 (0.61) and 4.92 (0.55), respectively. 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 considered the levels of other metals (cadmium, chromium, manganese, lead, arsenic, and nickel) to be low. All samples were 1-3 orders of magnitude below the 1996 TLV, and no significant changes were observed during the overhaul. Analyses of lung function and other pollutants were not reported.

Reductions in lung function over two years were investigated by Hauser et al. (2001) in a longitudinal study of boilermaker construction workers exposed to combustion particles from multiple sources including powerplants (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, a modified American Thoracic Society questionnaire on respiratory symptoms, and a work history questionnaire at baseline and two annual follow-up visits. The male cohort averaged 42.6 years of age (range: 20.5 – 56.5 years) and 97% were Caucasian. Technicians used standardized techniques and the same equipment to conduct spirometry testing for all workers during the study. Each worker, after some days off work, performed 3 – 7 forced vital capacity maneuvers to obtain at least three acceptable curves between 9:00 am and 2:00 pm on testing days. 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 equations adjusting for age, baseline FEV_1 and cigarette smoking status, the number of hours worked at gas-fired powerplants 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 gas-fired powerplants also showed FEV_1 reductions, however the associations were not statistically significant. Statistically significant reductions in annual FEV_1 also were observed among boilermakers who had ever worked at a coal, oil or gas-fired powerplant in models evaluating each fuel type separately. 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 (Magari et al., 2002). The group of apprentice and journeyman boilermakers was an average of 38 years old (18 – 59 years) and had worked an average of 13 years (0 – 40 years) in that occupation. Metals concentrations over an 8 – 10 hour work shift were determined from particle samples (< 2.5 μm) collected using personal monitors, and heart rate was monitored using a five-lead Holter monitor during the same period. Heart rate variability was estimated as the mean of 5 minute average SDNN (standard deviation of the normal-to-normal intervals). Vanadium concentrations (corrected for blank filter metal content) 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 48 personal samples were above the limit of detection 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) increase in SDNN index per $\mu g/m^3$ in mixed effects regression models with a random effect for each study subject and fixed covariates for smoking status, age, and mean heart rate. Lead also was associated with an increase in SDNN index (11.3 msec/$\mu g/m^3$, 95% CI: 2.88 – 19.73).[2] In contrast to earlier studies in this cohort that observed a decrease in SDNN measures, average $PM_{2.5}$ concentrations were not associated with SDNN index in this study (-0.77 msec/$\mu g/m^3$, 95% CI: -2.36 – 2.81). Vanadium concentrations were not correlated with either lead or $PM_{2.5}$ indicating that these pollutants were not likely to be confounders of the association of heart rate variability with vanadium. No associations with heart rate variability were observed for the other analyzed metals including nickel, chromium,

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

manganese, or copper. It is possible that the observation of an increase in heart rate variability with unit increases in vanadium and lead concentrations was the result of the temporal framework chosen for the analysis (e.g., averages over the work shift) rather than other time frames for exposure and SDNN averages. 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 night-time 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 authors did not report on the results of analyses for vanadium. 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). However, the effect on heart rate variability indicates that vanadium exposure may alter autonomic function. Alternatively, the observed association may have been due to chance.

In summary, case series, cross-sectional and longitudinal studies of occupational exposure to vanadium pentoxide or vanadium in residual oil fuel ash over a few days to several weeks reported symptoms of upper and lower respiratory tract irritation including headache, runny nose or sneezing, sore throat, cough with sputum, dyspnea on exertion, chest pain or discomfort, wheezing and tiredness (Sjöberg, 1951; Sjöberg, 1956; Zenz et al., 1962; Kiviluoto et al., 1979; Kiviluoto, 1980; Musk and Tees, 1982; Williams, 1952; Sjöberg, 1955; Lees, 1980; Ross, 1983; Levy et al., 1984; Woodin et al., 2000). Some symptoms began after a few hours while the onset of other symptoms occurred after one to several days. Vanadium pentoxide concentrations in production facilities where the studies were conducted varied by work location 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) and > 0.5 mg/m^3 at a pilot-plant operation (Zenz et al., 1962). 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).

The frequency and severity of symptoms were associated with vanadium among boilermakers exposed to relatively low ambient concentrations. Upper airway symptom severity scores (nasal congestion/irritation, throat irritation) increased across quartiles of estimated vanadium dose in the nose and the elevation was statistically significant in the third quartile compared to the first quartile in regression models adjusted for smoking (Woodin et al., 2000). Increases in lower airway symptom frequency and severity (chest tightness, wheeze, cough, and sputum production) were associated in a dose-related manner with estimates of lung vanadium dose. During the boiler overhaul, geometric mean respirable vanadium dust concentrations (SD)

were 8.9 (2.3) $\mu g/m^3$ (0.009 mg/m^3).

Vanadium concentrations in respirable particles (< 10 μm) inside the boiler during an overhaul (geometric mean (SD): 8.9 (2.3) $\mu g/m^3$) associated with increased upper and lower respiratory symptoms among boilermakers were not related to deficits in pulmonary function. Pulmonary function declines (FEV_1, FVC, $FEF_{25\%-75\%}$) over the course of a boiler overhaul were reported among boilermakers (Lees, 1980; Levy et al., 1984; Hauser et al., 1995a; Woodin et al., 1999). However, those studies with more systematic and detailed reporting of methods and results observed no association of FEV_1, FVC or flow measures with average vanadium concentrations in PM_{10} (Mean (SD): 12.2 (9.1) $\mu g/m^3$) or estimates of vanadium or PM_{10} dose in nasal passages or the lung (Hauser et al., 1995a; Woodin et al., 1999). Peak PM_{10} exposure (the highest concentration (1 – 10 hour TWA) reached on any day during the overhaul) (Mean (SD): 4.25 (1.58) mg/m^3) was inversely associated with ΔFEV_1, $\Delta FEV_{50\%}$, and ΔFVC, however peak vanadium concentrations (Mean (SD): 20.3 (11.4) $\mu g/m^3$) were not.

The authors reported that concentrations of respirable vanadium dust were relatively low and there may not have been enough variation in exposure to allow 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 may have prevented detection of associations between vanadium exposure and pulmonary function. The authors reported that they obtained fewer air samples inside the boiler where wearing the monitor was uncomfortable. Consequently, exposure and dose estimates for these locations may be more uncertain compared to other locations. The cohort had worked in this occupation an average of 20 years and may represent a healthy, less susceptible population. In addition to vanadium, boilermakers are exposed to increases in the levels of other metals during boiler overhauls including nickel, chromium and manganese (Liu et al., 2005). The observed pulmonary function declines amng boilermakers may be explained by exposure to other ROFA constituents.

4.1.2.4 Exposure to $PM_{2.5}$ Vanadium in Ambient Air

Vanadium is a constituent of ambient particulate generated by oil combustion. Recent studies of the short-term health effects of particulate matter 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 (Dominici et al., 2007; Bell et al., 2009).

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 on 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 the years 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 re-evaluated and extended by Dominici and colleagues (2007) using NMMAPS data for 90 communities from 1987 – 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 one-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 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, as well as the previous three days temperature and dew point temperature. 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} 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 $\mu g/m^3$ (range: 0.001-0.01) with an interquartile range of 0.001 $\mu g/m^3$. 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 particulate matter have a higher risk of mortality and hospital admissions related to daily change in $PM_{2.5}$ concentration is intriguing and 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. However, Bell et al (2009) 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, NO_2, copper, and iron, an interquartile range increase (0.003 $\mu g/m^3$) in three-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 ($p=0.08$). However, when the highest 5% of vanadium concentrations were excluded, 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 three 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 between 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, NO_2) during the cold/flu season or wheeze in other months (NO_2). $PM_{2.5}$ mass concentrations were not related to an increase in probability of symptoms.

The interpretation of the multi-pollutant 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 NO_2, a marker for traffic emissions, and the authors suggested that oil combustion for space heating may 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 $PM_{2.5}$ 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 COPD (N=11), asthma (N=11) or ischemic heart disease (N=7), 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 conduct repeated clinical examinations for two one-month periods. Pulmonary function testing was conducted according American Thoracic Society guidelines, and measures were expressed as the percentage of predicted based on subject-specific age, height and weight. An average of 15, 24 and 9 observations were obtained from each participant in the COPD, ischemic heart disease and asthma panels, respectively. Daily average $PM_{2.5}$ 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 $PM_{2.5}$ 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 asthma, ischemic heart disease, or COPD in generalized estimating equations models. Models adjusted for season (all), daily mean temperature (all), relative humidity (all), day of the week (COPD, IHD) 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 may not have been large enough to detect an association with the very low vanadium concentrations analyzed.

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 5 male Wistar rats were fed diets containing 0, 25, 50, 500 or 1000 ppm of vanadium incorporated in the form of pentoxide for 103 days (25 and 50 ppm groups; "low-exposure" groups) or 75 days (500 and 1000 ppm groups; "high-exposure" groups). After 35 days of treatment, dietary vanadium levels of the "low-exposure" groups were increased to 100 and 150 ppm, resulting in average daily doses of 0, 74.5, 116, 500, and 1000 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, though average weight gain in grams/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) 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 standard deviations were not reported for any result. Relative liver weight in the 69.6 mg/kg-day group

[3] Calculation: mg/kg-day = ppm(mg of compound per kg food) x mg food consumed/day x 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 – d of vanadium to amount vanadium pentoxide: [(mg/kg-d vanadium)(MW vanadium pentoxide, 181.9)]/(2xMW vanadium, 101.9).

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 4-1). A 20-30% decrease in erythrocyte count is considered biologically significant, however, no statistical analysis was reported by the study authors, and no measure of variance (SE or SD) was given for the means. Data were not reported for high dose groups. 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; though the researchers speculated that vanadium may 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. However, no statistical analysis was performed for the decrease in erythrocytes and body weight gain and no degree of variance was reported (precluding statistical analysis for this review), therefore, a NOAEL or LOAEL could not be determined from this study. However, compared to hematological data for Wistar rats (Wright et al., 2009; Charles River 2008), 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.

Table 4–1. Hematological results of oral vanadium pentoxide exposure in rats (Mountain et al. 1953).

	Control	10.5 mg/kg-day	16.4 mg/kg-day
Red Cell Count (M/mm^3)			
Start	8.0	7.8	8.0
Finish (103 days)	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 days)	15.0	14.5	13.7
Percent change between start and finish of expt (%)	3.9	4.6	10.5

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 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 were 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 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 derived as 17.9 ppm (1.41 mg/kg - day) vanadium pentoxide. However, the biological significance of decreased hair cystine is unclear 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

The 3-month exposure studies in F344/N rats were conducted to evaluate the cumulative toxic effects of subchronic inhalation exposure to vanadium pentoxide (NTP, 2002). Chemical identity and purity of vanadium pentoxide was evaluated prior to the beginning of and following the conclusion of all assays. 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 hrs per day, 5 days/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

[5] Converted to ppm vanadium pentoxide by [(ppm vanadium)(MW vanadium pentoxide, 181.9)]/(2xMW vanadium, 101.9). No information given on average rat weights so cannot be converted to mg/kg-day directly. Conversions based on data from subchronic study from same group (Moutain et al., 1953). Calculation: mg/kg-day =ppm(mg of compound per kg food) x mg food consumed/day x 1/kg body weight (usingreference 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])

[6] Complete histopathology was performed on 0, 8 (rats only), and 16 mg/m^3 rats and mice. In addition to gross

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. 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 4-2. 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.

Results of hematology assessments following 3-months of inhalation exposure are presented in Table 4-3. 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 red blood cell 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

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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 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. 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.

of altered iron metabolism and heme/hemoglobin production.

Table 4-2. Body Weight Gain and Lung Weights in Rats (F344/N) Exposed to Vanadium Pentoxide by Inhalation for 3 Months (Values are Means±Standard Error) (NTP, 2002)

Parameter	Exposure					
	Control	1 mg/m^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

Table 4-3. Selected Hematology Parameters in Rats (F344/N) Exposed to Vanadium Pentoxide by Inhalation for 3 Months (NTP, 2002)[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	0.2	0.22±0.03	0.19±0.02	0.23±0.03	0.25±0.02	0.8 ±0.08[b]

Table 4-3. Selected Hematology Parameters in Rats (F344/N) Exposed to Vanadium Pentoxide by Inhalation for 3 Months (NTP, 2002)[a]						
Parameter	**Exposure**					
	Control	**1 mg/m^3**	**2 mg/m^3**	**4 mg/m^3**	**8 mg/m^3**	**16 mg/m^3**
(10^6/µL)	±0.02					
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 ($p \leq 0.01$)

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

4-4. 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 diastolic, systolic 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. Pulmonary function results may indicate obstructive disease or may reflect the deteriorating condition of the 16 mg/m^3 rats, since histopathological finding in lungs of rats exposed to 8 and 16 mg/m^3 were similar. Taken together, results of pulmonary function tests 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 may have been present in rats exposed to 16 mg/m^3.

Table 4-4. Incidences of Selected Nonneoplastic Lesions of the Lung and Nose in Rats (F344/N) Exposed to Vanadium Pentoxide by Inhalation for 3 Months (NTP, 2002)

Lesion Location and Type	Numbers of Animals with Lesions (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]						

Table 4-4. Incidences of Selected Nonneoplastic Lesions of the Lung and Nose in Rats (F344/N) Exposed to Vanadium Pentoxide by Inhalation for 3 Months (NTP, 2002)						
Lesion Location and Type	**Numbers of Animals with Lesions (Avg Severity Score)**					
	Control	**1 mg/m^3**	**2 mg/m^3**	**4 mg/m^3**	**8 mg/m^3**	**16 mg/m^3**
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 ($p \leq 0.01$)

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, 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 2 mg/m^3 and greater. Results of pulmonary function tests 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 in male and female rats, the NOAEL and LOAEL values identified for 3-month inhalation exposure to vanadium pentoxide aerosols were 1 and 2 mg/m^3, respectively.

Three-month exposure studies in B6C3F$_1$ mice were conducted to evaluate the toxicity of subchronic inhalation exposure to vanadium pentoxide (NTP, 2002). Groups of 10 male and 10 female mice were exposed (whole-body exposure) to vanadium pentoxide aerosols at concentrations of 0, 1, 2, 4, 8 or 16 mg/m^3, 6 hrs per day, 5 days/week for 3 months. Particle size given in mass median aerodynamic diameter (MMAD) ± geometric standard deviation (GSD) for each dose groups 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 4-5. 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 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).

Table 4-5. Body Weight Gain and Lung Weights in Mice (B6C3F$_1$) Exposed to Vanadium Pentoxide by Inhalation for 3 Months (Values are Means±Standard Error) (NTP, 2002)

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 ($p \leq 0.01$)

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 4-6 (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 of the 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 inflammation 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.

Table 4-6. Incidences of Selected Nonneoplastic Lesions of the Lung in Mice (B6C3F$_1$) Exposed to Vanadium Pentoxide by Inhalation for 3 Months (NTP, 2002)

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, $p \leq 0.01$

In a study using cynomolgus monkeys, weekly provocation challenges (single 6-hr

exposures to 0.5 or 3.0 mg/m^3) with inhaled vanadium pentoxide aerosol for six weeks produced statistically significant pulmonary responses, prior to a subchronic exposure (6 hrs/day, 5 days/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 x time) with different exposure profile. 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, and 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 x 6/24 x 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. Pulmonary function tests, cytological and immunological analyses of blood and bronchiolar lavage fluid, and skin sensitivity tests were conducted before the pre- and post-exposure provocation challenges. Immunological analyses are described in Section 4.4.1.2. Pulmonary function tests and bronchiolar lavage fluid analyses were also performed one 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), forced vital capacity (FVC), residual volume (RV) and dynamic lung compliance (CL_{dyn}). Respiratory distress, characterized by audible wheezing and coughing, occurred in 3 out of 8 monkeys from the peak exposure group on peak exposure days during the first few weeks of the 26-week exposure; the responses developed within 3-4 hrs of exposure and occasionally required early removal of the affected monkeys from the exposure chamber. Impaired pulmonary function accompanied pre-exposure provocation challenges with V_2O_5 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 may 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. However, an apparent acute, but reversible, LOAEL of 1.38 mg/m^3 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 hr/day, 2 days/week for up to 12 weeks (Gonzalez-Villalva et al., 2006). A 0.02 M aqueous solution of vanadium pentoxide was aerosolized generating a reported average vanadium of 1436 µg/m^3 (1.44 mg/m^3 V), as measured by filters following the 12 weeks exposure. This study did not provide reliable exposure information, thus exposure concentrations in mg/m3 could not be determined. Groups of 8 exposed mice and 8 vehicle control mice (inhaling deionized water droplets) were evaluated after 24 hrs and weekly for 12 weeks. Evaluations consisted of a complete blood count and morphological examination of platelets. Platelet count was significantly increased in the exposed mice on weeks 3-12; counts increased from week 3 to a maximum at week 9 and subsequently declined, but still remained above controls (quantitative data inadequately reported). The morphology examinations showed the presence of giant platelets at unspecified longer exposure times. The study establishes an apparent LOAEL for increased platelet count and altered platelet morphology from short-term intermittent exposure to vanadium pentoxide at 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 6 hrs/day, 5 days/week for 104 weeks (Ress et al., 2003; NTP, 2002). 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)

[7] In addition to gross lesions and tissue masses, the following tissues were examined in the 2-yr 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.

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 52-58% in male rats and 30-40% in female rats. The percent survival of controls was 40% for male rats and 28% for female rats (Table 4-7). No infection was reported in this study that may account for these survival rates; however, these survival rates are comparable to historical rates of survival for male and female F344/N rats.

The incidences of nonneoplastic lesions of the respiratory tract in male and female rats are summarized in Table 4-7 (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 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.

Table 4-7. Selected Nonneoplastic Lesions of the Respiratory System in Rats Exposed to Particulate Aerosols of Vanadium Pentoxide for 2 Years (NTP, 2002)

Lesion Type and Location[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)

Table 4-7. Selected Nonneoplastic Lesions of the Respiratory System in Rats Exposed to Particulate Aerosols of Vanadium Pentoxide for 2 Years (NTP, 2002)

Lesion Type and Location[a]	Exposure Group			
	Control	0.5 mg/m^3	1 mg/m^3	2 mg/m^3
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)
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.

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. Compared to historical controls, alveolar/bronchiolar adenoma in 0.5 and 2 mg/m^3 males and 0.5 mg/m^3 females, alveolar/bronchiolar carcinoma in 0.5 and 2 mg/m^3 males, and combined alveolar/bronchiolar carcinoma in 0.5, 1 and 2 mg/m^3 males and in 0.5 mg/m^3 females were statistically significantly different (see Table 4-8 footnotes).

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 since the tumors were observed only at the lowest dose and no dose-response was evident.

Table 4-8. Incidences of Respiratory Tumors in Rats Exposed to Vanadium Pentoxide in a 2 Year Inhalation Study (NTP, 2002)[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 NIH-07 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 NIH-07 diet.

[e]Historical incidence of combined alveolar/bronchiolar adenoma or carcinoma male F344/N rats fed in inhalation chamber controls given NIH-07 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.

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 hrs per day, 5 days/week, for 104 weeks (Ress et al., 2003; NTP, 2002). Particle MMAD±GSD for each dose group was reported as follows: 1 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 non neoplastic 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 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 4-9).

The incidences of nonneoplastic lesions of the respiratory tract in male and female mice are summarized in Table 4-9 (Ress et al., 2003; NTP, 2002). In male mice, the incidences of nonneoplastic lesions of the lungs (hyperplasia of the alveolar and bronchiole epithelium, inflammation, alveolus histiocyte infiltration), larynx (squamous metaplasia of the epiglottis) and nose (olfactory and respiratory epithelium degeneration in males and olfactory epithelial degeneration and atrophy in females) were significantly increased compared to control in all 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.

Table 4-9. Selected Nonneoplastic Lesions of the Respiratory System in Mice Exposed to Vanadium Pentoxide in a 2 Year Inhalation Study (NTP, 2002)

Lesion Type and Location[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	10 (2.4)	36[c] (2.4)	45[c] (2.6)	49[c] (3.0)

infiltration				
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		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)
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 ($p \leq 0.01$)

The incidences of tumors of the respiratory tract in male and female mice exposed to vanadium pentoxide for 2 years are summarized in Table 4-10 (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 4-10. Incidences of Respiratory Tumors in Mice Exposed to Vanadium Pentoxide in the 2 Year Inhalation Study (NTP, 2002)					
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 ($p \leq 0.01$)

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 (10ug/g bw in corn oil; intraperitoneal injection) in week 1, followed by 5 weekly aspirations of either V2O5 (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 – 11). Differences were also observed between strains, with A/J mice showing increased tumorigenicity in response

to vanadium pentoxide. In the absence of MCA, V2O5 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 V2O5 (4 mg/kg bw) 4 times weekly, with BALF collected at 6hr, 1d, 3d, 6d, and 21d following the last aspiration. Cellular infiltrates and protein content was compared, and lungs were snap-frozen for histopathology. Increased pulmonary inflammation and hyperpermeability was increased in all exposed strains in a similar pattern as the tumor induction, with greater increases observed in A/J mice than in BALB/C. C57BL/6J showed the least amount of increase in all parameters compared to all mouse strains. All results returned to baseline at 21 days post-exposure. 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 -11. Lung tumor multiplicity in MCA-Treated mice exposed to V_2O_5 by pharyngeal aspiration (Rondini et al. 2010). [a,b]

	PBS Control	V_2O_5
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 parantheses.

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 hyplerplasia and pulmonary fibrosis. Vanadium pentoxide exposure for two years was associated with a wide spectrum of nonneoplastic pulmonary lesions in both rats and 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. There are currently no human epidemiology studies that examined carcinogenesis following exposure to vanadium pentoxide. However, there is clear evidence of carcinogenicity

in male and female mice exposed to vanadium pentoxide, and some evidence of 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 to 200 μmol/kg) for three days produced a significant increase in alkaline phosphatase activity and DNA content in the diaphysis of femoral bones, and suggests that vanadium pentoxide may 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 were exposed to 1.8 to 36.4 mg/kg (10 to 200 μmol/kg)[8] vanadium pentoxide orally 1 hr following an oral injection of 15.3 μmol Zn/100g) for three times at 24-h 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 as well blocked these deaths. All rats were killed 24h following the last vanadium administration, and blood immediately removed by cardiac puncture. Statistically significant increases were observed in serum from high-dose rats for calcium (27.3 – 36.4 mg/kg; $p < 0.05$) and decreased for phosphorus (36.4 mg/kg; $p < 0.01$). Administration of zinc completely prevented these serum changes. Femurs were also 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.

[8] Conversion factor: μmol = 0.1819 mg

Mravcova et. al (1993) assessed the extent of vanadium pentoxide accumulation in the bones of rats following 6 month exposure (full study description in 4.4.4.2). 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. However, no dose response data for these endpoints was reported.

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 (0.02M, apparently aqueous solution containing aerosolized vanadium pentoxide) for 1 hr two times 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 (Avila-Costa et. al., 2004). Thirty-six control animals inhaled only vehicle (deionized water). Groups of three exposed animals and three control animals were sacrificed per week for 12 weeks. Results indicated accumulation of vanadium pentoxide in testes (Mussali-Galante et al., 2005). Moreover, 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 may suggest changes in microtubule-involved function, such as cell division, which may impact 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 5-6 in vanadium-exposed animals. Spermatocytes exhibited cytoplasmic vacuolation, nuclear distortion and intercellular edema in response to vanadium pentoxide exposure. Spermatogonia (40% necrosis during weeks 6-7) were the most susceptible cell type, followed by spermatocytes and Sertoli cells. Moreover, vanadium pentoxide concentrations increased dramatically after one week of exposure and remained consistently elevated (avg 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, sperm motility), but it did 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 administration of vanadium pentoxide (Altamirano et al., 1991). Newborn male and female rats were injected with 0 or 12.5 mg/kg vanadium pentoxide in saline on every second day from birth to age 21 days; groups sizes were 5 (treated males) or 9 (male and female controls and treated females). Males were sacrificed at 55 days of age and the females were sacrificed on the day of first vaginal estrus. Other groups of females were injected with 0 or 12.5 mg/kg-day vanadium pentoxide (n = 10 and 6, respectively) from age 21 days to the day of first vaginal estrus, at which time they were sacrificed. Reported endpoints in the males consisted of absolute weights of 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; these 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 male CD-1 mice following intraperitoneal administration of vanadium pentoxide (Altamirano-Lozano et al., 1996). In the fertility assessment, groups of 20 and 15 male mice were injected with 0 and 8.5 mg/kg vanadium pentoxide in saline, respectively, every 3rd day for 60 days and mated 24 hrs 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 (avg = 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 5 evaluated after 10, 20, 30, 40, 50 or 60 days of treatment. Statistically significant effects included reduced sperm motility after ≥10 days, reduced sperm count and increased percentage of abnormal sperm after ≥20 days, decreased absolute testicular weight after ≥50 days (relative weight not reported), and decreased body weight after 60 days.

Developmental toxicity was evaluated in groups of 13 or 15 female CD-1 mice that were administered 0 or 8.5 mg/kg vanadium pentoxide in distilled water, respectively, by intraperitoneal injection on days 6-15 of gestation (Altamirano-Lozano et al., 1993). No maternal toxicity was reported (endpoints not specified). Developmental toxicity endpoints were assessed on gestation day 18. The endpoints included the 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), 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. (1991a) evaluated the developmental toxicity in NIH mice following intraperitoneal injection of 5 mg/kg-day vanadium pentoxide on days 1-5, 6-15, 7, 8, 9, 10, 11 or 14-17 of gestation. There were no adverse effects on preimplantation or implantation, 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. (1993a) evaluated developmental toxicity in Wistar rats following intraperitoneal injection of 0.33, 1.0 or 3.0 mg/kg-day on days 6-15 of gestation. Decreased placental weight and increases in embryo-fetus mortality and external or skeletal malformations (unspecified) occurred at 1.0 and 3.0 mg/kg-day. Maternal toxic symptoms (unspecified), decreased maternal weight gain during treatment and fetal growth retardation were observed at 3.0 mg/kg-day. In the third study, Zhang et al. (1993b) evaluated developmental toxicity in Wistar rats following intraperitoneal injection of vanadium pentoxide in doses of 3 mg/kg-day on days 6-15 of gestation or 5 mg/kg-day on days 9, 10, 11 or 9-12 of gestation. Effects in rats exposed on gestation days 6-15 and 9-12 included decreased maternal weight gain, increased fetal mortality, decreased fetal weight and

crown-rump length, delayed ossification of unspecified bones, and increased incidences of subcutaneous hemorrhage, wavy ribs, and dilation of lateral ventricles and renal pelvis. Effects in rats exposed on a single day of gestation included subcutaneous hemorrhage and unspecified visceral anomalies following exposure on days 9, 10 and 11, and increased fetal mortality and delayed ossification of unspecified bones following exposure on day 10. Additional study details were not available. Overall, the three studies identified an intraperitoneal 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 (1.1mg/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 (two days before parturition. Pre-implantation exposure had no effect on the fetuses as regards 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. However, the number of fetuses defined as having less mature skeletons[9] by the authors was significantly greater in mice given vanadium pentoxide on gestation day 8, but not gestation day 3 ($p < 0.001$, chi square test).

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

4.4 OTHER DURATION- OR ENDPOINT-SPECIFIC STUDIES

4.4.1 Acute and Short-Term Studies

4.4.1.1 Acute Studies

4.4.1.1.1 Oral

According to the Concise International Chemical Assessment Document 29 (WHO-IPCS, CICAD 29, 2001), rat oral LD_{50} values for vanadium pentoxide range from 86-137 mg/kg body weight (Yao et. al., 1986 as cited in WHO-IPCS, 2001). A rat oral LD_{50} value of 10 mg/kg body weight was reported in IARC Monographs, volume 86 (IARC, 2006) in a study by Lewis et al.

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

(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 was not reported (Yao et. al., 1986 as cited in WHO-IPCS, 2001). Histopathological analysis revealed liver necrosis and swelling of renal tubules. Other studies (WHO-IPCS, 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-IPCS, 2001). Signs of toxicity in rabbits mimicked those reported for rats.

4.4.1.1.2 Inhalation

A 1-hr inhalation exposure to vanadium pentoxide dust in rats led to an LC_{67} of 1.44 mg/L (1440 mg/m^3) (US EPA, 1992). Clinical signs of toxicity included respiratory difficulty, irritation of mucosa, and diarrhea (WHO-IPCS, 2001). Knecht et al. (1985) reported air flow restriction, as measured by pulmonary function tests, in sixteen adult male cynomolgus monkeys (*Macaca fasicularis*) exposed to vanadium pentoxide by whole-body inhalation at 5.0 mg/m^3 for 6 hrs 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 sixteen male cynomolgus monkeys were exposed to aerosols of 0.5 mg/m^3 vanadium pentoxide by whole-body inhalation for 6 hrs (Knecht et al., 1985). One week later, these monkeys were exposed to aerosols of 5 mg/m^3 vanadium pentoxide by whole-body inhalation for 6 hrs. Effects on airway function were evaluated in monkeys by comprehensive pulmonary function tests (PFTs) performed 24 hours post-exposure to 0.5 and 5 mg/m^3 and on pulmonary inflammation by analysis of bronchiolar lavage (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 following exposure to 5 mg/m^3 as follows: 16% increase in pulmonary resistance; 11% decrease in peak expiratory flow rate; 5-22% decreases in forced expiratory flow maneuvers; 33% increase in residual volume; and 24% increase in forced residual capacity. Results are consistent with air-flow limitation in both small peripheral and large central airways. 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 primarily due to a marked increase (approximately 425%; data presented graphically) in the number of polymorphonuclear leukocytes. Results suggest that pulmonary inflammation and release of bronchoconstrictive mediators from inflammatory cells may play a role in vanadium

pentoxide-induced air-flow restriction. An acute (single 6-hr 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 hr inhalation exposure to 0.02 M (2.5 mg/m^3 as Vanadium) vanadium pentoxide ($p < 0.05$). The dose-response relationship for these effects could not be evaluated, since only one dose was tested, and a NOAEL could not be established.

4.4.1.2. Short-term Studies

4.4.1.2.1 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 to5.3 mg/m^3 vanadium for 10 hr/day, 6 days/week, for 4 weeks (Levy et al., 1984). A LOAEL of 0.05 mg/m^3 was established. However, dose-response was not systematically measured, there were no controls, 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 relative lung weight in rodents (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 following 13-day or 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 13 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 13 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/group) and female mice (50/group) were also assessed for pulmonary inflammation (bronchiolar lavage 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, there was a localized inflammatory response in the lungs 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 bronchiolar lavage 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/m3 (LOAEL) for lung inflammation in female rats and mice, respectively, exposed to vanadium pentoxide for 13-days.

An additional 5 male and 5 female mice were exposed by inhalation to vanadium pentoxide for 6 hrs 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 $\geq$ 4 mg/m^3, and relative lung weight were significantly increased in males at $\geq$ 2 mg/m^3. Among females both absolute and relative lung weights increased in all exposure groups establishing the LOAEL of 2 mg/m3; no NOAEL was established.

Additional groups of 40-60 female mice were exposed to 0, 2, 4 or 8 mg/m^3 for 6 hrs per day, 5 days per week for 16 days (NTP, 2002). The nonneoplastic lung lesions noted on day 6 and 13 consisted of hyperplasia of the alvelolar 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, one hour inhalation exposure to 5.13 mg/m^3 vanadium pentoxide were suggestive of disruption of 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 pre-exposure to the virus exacerbated the vanadium pentoxide-induced lung inflammation and fibrosis. Animals were exposed intranasally to RSV (6 x 10^5 PFU in 100ul PBS) on day -1 and day 8, then exposed intranasally to V_2O_5 (4mg/kg in 50ul 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 mRNAs coding for pro-fibrogenic growth factors TGF-β-1, connective tissue growth factor (CTGF), platelet-derived growth factor-C (PDGF-C) and collagen Col1A2, anti-fibrogenic type I interferons-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. However, vanadium pentoxide-induced fibrotic response was less severe in the lungs of mice which received either pre- or post-RSV exposure, and no difference observed in pre- or post-RSV 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 pre-exposure, RSV pre-exposure 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 post-exposure to RSV led to a decrease in the severity of V2O5-induced fibrosis, and gene expression analysis showed decreases in several pro-fibrinogenic genes associated with innate immunity.

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

4.4.2 Immunological Endpoints

4.4.2.1 Human Studies

Some of the early case series observed dermatitis among affected workers employed at vanadium pentoxide processing facilities (Sjöberg, 1951; Zenz et al., 1962). Zenz et al. (1962) observed that respiratory symptoms, such as conjunctivitis, nasopharyngitis, hacking cough, fine rales, and wheezing recurred with greater severity when work resumed after three days of no exposure, even with the use of respirators, perhaps indicating immune system involvement.

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, 1980; Kiviluoto et al., 1979; Kiviluoto et al., 1981a). 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) who were employed at the magnetite ore mine and matched to the exposed by age and smoking habit (35% versus 7%, $p<0.001$, N=55 pairs). 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=57 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 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).

Motolese et. al. (1993) assessed whether exposure to vanadium pentoxide in the ceramics industry was associated with contact dermatitis or contact sensitization. One hundred and twenty-six enamellers and sixty-four decorators from five ceramics factories were tested for contact sensitization using skin patch testing after exposure to a variety of substances, including vanadium pentoxide. Among the 190 workers under study, twenty-two 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.

A pilot study using nasal lavage to evaluate an inflammatory response to fuel oil ash exposure, did not find an association of several exposure indices of vanadium or PM_{10} exposure with counts or percentages of polymorphonuclear cells, eosinophils, and epithelial cells (Hauser et al., 1995b). Thirty-six out of 50 volunteers 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. A total of 19 boilermakers involved in the overhaul of a large oil-fired boiler and 18 utility workers, full-time employees of the power company with lower exposure to fuel oil ash, were studied. 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 – 10 hour TWA) and a self-completed work diary completed by each participant listing tasks and job locations during the day. A total of 29 task/location exposure categories were identified, but only 3 or fewer samples were available to estimate concentrations at 23 of them. Environmental PM_{10} concentrations based on personal sampling were 50 to 4510 $\mu g/m^3$. Concentrations of respirable vanadium dust were 0.10 to 139.2 $\mu g/m^3$. Compared to baseline, the number of polymorphonuclear cells/ml recovered nasal fluid (adjusted by dividing the change by the mean of the baseline and postexposure value) increased by 40% (SD 100%, $p < 0.05$) with a range of -89% - 200%. The adjusted number of epithelial cells/ml recovered nasal fluid increased by 26.7% (SD 81.4%, $p > 0.05$) with a range of -83% - 200%.

Regression models controlled for age and smoking. 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 may 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 six week period from mid-May, 1995 to late-June, 1995. Biomarkers of upper airway inflammation in nasal lavage fluid were more prevalent among boilermakers during the overhaul compared to 11 utility workers (Woodin et al., 1998). Interleukin-8 (pg/ml) levels increased from a mean of 93.7 pg/ml (22.6-235.0) before the overhaul to 140.9 pg/ml (32.4-307.0) during the overhaul ($p < 0.05$), and decreased to levels comparable to those before the overhaul two weeks later (mean (range): 89.0 pg/ml (20.3-75.0)). Interleukin-8 levels among utility workers did not change substantially during the overhaul (mean (range): 69.2 (24.6-104.5), 58.5 (14.3-108.4) and 47.5 (12.8–74.7), respectively. Myeloperoxidase levels also increased among boilermakers during the overhaul, but not among utility workers. Among boilermakers, myeloperoxidase levels (ng/ml) were 22.7 (2.0-72.8), 33.9 (2.0-103.0) and 24.2 (3.9-58.1) before, during and after the overhaul, respectively (before versus during: $p < 0.05$). Among utility workers, myeloperoxidase levels were 25.6 (10.1-47.6), 27.2 (4.9-66.2) and 25.6 (4.9-51.7), respectively. 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 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) ($p < 0.001$)). However, vanadium concentrations in nasal lavage fluid were not associated with levels of either IL-8 or myeloperoxidase using Spearman's Rank Order Correlation Test.

Hauser et. al. (2002) reported on a prospective cohort study of 118 boilermaker construction workers (see Section 4.1.2). The participants provided information on their work at oil, gas and coal-fired powerplants in annual work history questionnaires, but exposure to specific components in the combustion particles were not quantified. Spirometry was used to measure lung function. Among the cohort, 6 workers reported having asthma diagnosed by a physician and 18 workers reported having symptoms of chronic bronchitis. Workers with asthma or chronic bronchitis experienced greater reductions in annualFEV_1 associated with the number of hours worked at gas or coal-fired powerplants during the year compared to the other workers. The generalized estimating equation models adjusted for age and smoking status, and interaction terms were statistically significant. In contrast, models adjusting for age, baseline FEV_1, and

cigarette smoking status did not find a similar effect of airway responsiveness, measured by methacholine challenge, on exposure-related reductions in FEV_1. The annual reduction in FEV_1 associated with the number of hours worked at gas, oil or coal-fired powerplants was similar between workers with and without reactive airways.

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 does not appear to be a common occurence. Although increases in the numbers of inflammatory cells in nasal smears or nasal lavage fluid have been observed in exposed workers, 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.

4.4.2.2 Animal Studies

Pinon-Zarate et. al. (2008) exposed 112 male CD1 mice to ~1.4 mg/m^3 vanadium pentoxide by inhalation for 1 hr/day, 2x a day, for 12 weeks as measured by filters following exposure. This study did not provide reliable exposure information, thus exposure concentrations in mg/m^3 could not be more specfically determined. Spleen weight and histology were determined. B-lymphocytes in the spleen were identified by immunohistochemical staining for CD19 (a cell surface marker which 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 and peaked at 9 weeks, and then decreased significantly. Splenic germinal centers were 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 may affect the avidity of antibodies – the ability of antibodies to bind effectively to substrates (affinity) and to engage multiple epitopes of the antigen at once.

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. Also, male and female ICR mice (groups of 10) were given vanadium pentoxide (0 or 6 mg/kg-day) by gavage 5x a 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 cells being converted 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 may 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 indicate that vanadium pentoxide may induce hypersensitivity responses at low doses.

Immunological endpoints were also analyzed as part of a pulmonary study using cynomolous monkeys weekly provocation challenges (single 6-hr exposures to 0.5 or 3.0 mg/m^3). Inhaled vanadium pentoxide aerosol for six weeks produced statistically significant pulmonary responses, prior to a subchronic exposure (6 hrs/day, 5 days/week for 26 weeks) (Knecht et al., 1992; study details in Sec 4.2.2.1). Immunological analyses of blood and bronchiolar lavage fluid, and skin sensitivity tests were conducted before the pre- and post-exposure provocation challenges. Bronchiolar lavage fluid analyses were also performed one day after the provocation challenges. Cytological endpoints included complete and differential blood cell counts and leukotriene C_4 levels. Immunological endpoints included total IgE, total IgG, albumin and total protein. The skin sensitivity tests assessed immediate and delayed responses to intradermal injections of vanadium pentoxide-monkey serum albumin conjugate. 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. The studies described here have not shown a statistically significant response in the endpoints tested, however, some effects were observed. These results are therefore inconclusive.

4.4.3 Neurological Endpoints

4.4.3.1 Human Studies

No studies on the neurological effects of vanadium pentoxide were reported in humans.

4.4.3.2 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. There was a statistically significant reduction in venous oxyhemoglobin in rats from the 0.027 mg/m^3. 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 CNS effects in albino rats with a NOAEL of 0.002 mg/m^3.

Three recent studies by Avila-Costa et al. (2004, 2005, 2006) found morphological changes in the central nervous system following inhalation exposure to vanadium pentoxide. Male CD-1 mice (n=48) were exposed to vanadium pentoxide by whole-body inhalation for 1 hr/day, 2 days/week for up to 8 weeks (Avila-Costa et al., 2004, 2005). Particle size was not reported in either study. The exposure concentration was reported as 0.02 M (Avila-Costa et al., 2004, 2005, 2006) or "2.5 mg/m^3 V" (Avila-Costa et al., 2005). The same group of investigators (Gonzalez-Villalva et al., 2006; Mussali-Galante et al., 2005) using the same exposure protocol reported that the 0.02 M solution generated an average chamber concentration of 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 one week and 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 hr/day, 2 days/week for up to 4 weeks. Groups of 6 exposed mice and 6 vehicle control mice (inhaling deionized water droplets) were evaluated after 24 hrs and weekly for 4 weeks. No clinical signs or body weight changes were observed. Spatial memory was tested using a modified Morris water maze task that was learned pre-exposure. Performance on this test, as assessed by latency (swimming time) to locate a hidden platform, was significantly impaired in the exposed mice at all time points in an increasing, time-related manner. Pyramidal neurons from the hippocampus CA1 region were evaluated for cytological and ultrastructural changes, because impairment in spatial memory is frequently seen 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; 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 the central nervous system 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-hr 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 hr two times a week for up to four weeks. Five mice were sacrificed from each group, per time point (24 h, 1, 2 and 4 weeks). The presence of matrix metalloproteinase (MMP) was determined by gel zymography. In the olfactory bulb, MMP-2 was not different between 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 elevanted 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 MMP's in different parts of CNS occur in association with dendritic spine loss as well as neuronal death, and could be related to blood-brain barrier disruption.

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

The preceding paragraphs have 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 (13 and 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.

4.5.1 Genotoxicity

The genotoxicity assays of vanadium pentoxide are summarized in Table 4-12.

4.5.1.1 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 non-exposed 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 measurement of serum and urine vanadium. 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 non-proliferating lymphocytes exhibited a significant increase in DNA migration in the alkaline comet assay compared to non-exposed cells only at the highest doses tested (250-500 μg/L). Cultured human fibroblasts, however, exhibited a dramatic dose-dependent increase in DNA migration after vanadate treatment at lower concentrations as well (as low as 0.5 μg/L) and suggest that fibroblasts are more sensitive to DNA damage in the presence of vanadate than are blood cells when exposed in vitro (Ivancsitis 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 non-exposed jail wardens (n=52) during October 2004 – May 2005. All subjects studied were male, and were exposed for their entire 8-hr shift while wearing protective masks. However, the duration of exposure and concentration of the inhaled vanadium was not determined. The median concentration (25^{th} – 75^{th} percentile) of vanadium in plasma was 7-fold 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. However, increases were observed 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 micronuclei formation, necrosis and apoptosis was determined in isolated lymphocytes using the CBMN Cyt assay. The number of micronuclei was 2.5 fold higher in 24 workers than 23 non-exposed 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 (7-fold and 3-fold) over controls. Numbers of necrotic and apoptotic cells were increased by 55% and 50% respectively in exposed workers. Together, these results suggest that occupational exposure to inhaled vanadium pentoxide may affect DNA stability by increasing

levels of oxidized bases and affecting DNA repair. Age, body mass index and smoking habit (cigarettes/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 were not due to vitamin deficits.

Kim and colleagues (2004) assessed the cross-shift change in urine levels of 8-hydroxy-2'-deoxyguanosine (8-OHdG), a marker of 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 $PM_{2.5}$ 8-hour TWA concentration, measured using personal exposure monitoring, was 0.44 mg/m^3 ($Q_{25\%}$ - $Q_{75\%}$: 0.29 – 0.76 mg/m^3). Total vanadium 8-hour TWA concentration, including vanadium oxides, was 1.23 μg/m^3 ($Q_{25\%}$ - $Q_{75\%}$: 0.47 – 3.53 μg/m^3). The workers were 18 – 59 years old (mean ± SD: 45.5 ± 12.0) and had been employed as boilermakers for 0.04 to 40 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 may not have been independent of the associations with the other metals. However, the finding of oxidative DNA injury and repair among healthy boilermakers 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 two days twice during one 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 may 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 (Rojas et al. 1996; Ramirez et al. 1997; Roldan 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 (0 – 0.1uM) lead 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 (0 – 6ug/ml) but no chromosomal aberrations were observed (Roldan and Altamirano 1990).

4.5.1.2 Laboratory in vivo and in vitro studies

Vanadium pentoxide produced gene mutations in two bacterial test systems (*Bacillus subtilus* 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). However, DNA damage and/or aneuploidy was observed in all in vitro studies performed in primary human cells (Ivancsits et al. 2002; Kleinsasser et al. 2003; Ramirez et al. 1997; Rojas et al. 1996; Roldan 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 (Roldan and Altamirano et. al., 1990). Negative results were reported for chromosomal aberrations (Roldan and Altamirano et. al., 1990). In Chinese hamster V79 lung fibroblast cells, positive results were observed for micronuclei 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 sister chromatid exchanges and gene mutations in vanadium pentoxide-treated Chinese hamster V79 firbroblast 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 seventeen 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 1hr. 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. However, DNA migration appeared to increase dose-dependently 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 and/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., 1993, 1996). Exposure caused no treatment-related effects on mitotic index, average generational time or sister chromatid exchanges in bone marrow cells (Altamirano-Lozano et al., 1993), although all doses induced DNA damage in testicular germ cells (Altamirano-Lozano et al., 1996). Altamirano-Lozano et al. (1999) assessed DNA damage in male CD-1 mice 24 hrs 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. There are few studies examining genotoxicity in humans in vivo, with equivocal results. Ivancsits et. al. (2002) reported no differences in DNA strand breaks, oxidative damage, or sister chromatid exchange 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 (Roldan 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.

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 and other chromosomal aberrations. DNA strand breaks (Rojas et al., 1996; Ivancsits et. al., 2002) and micronuclei formation (Zhong et. al., 1994) were indicated in some studies in cultured cells but were dependent 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. However, a number of studies by Altamirano-Lozano et al. (1993, 1996, 1999) 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.

Table 4-12. 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 non-exposure controls	_	avg 12.4y	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	Ivancsits et al. 2002
	–				DNA damage (8OHdG)	
	–				Sister chromatid exchange	
52 exposed workers; 52 non-exposed 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 MN induction, nucleoplasmic bridges and nuclear bud formation. Necrosis and apoptosis levels were also increased in exposed individuals.	Comet Assay	Ehrlich et al. 2008
	+				MN induction, oxidative nucleotides	
Laboratory Animals						
Male CD-1 mice (n = 4)	+	intraperitoneal injection, sacrificed 24h 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 24h 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 increases were observed in a dose-dependent manner ($p < 0.05$).	Comet assay	Altamirano-Lozano et al. 1996

Male CD-1 mice (n = 4)	-	intraperitoneal injection, sacrificed 24h post injection	0, 5.75, 11.5, 23 µg/g bw	Analysis of sister chromatid exchange demonstrated no effect of vanadium pentoxide exposure in this study.	cytogenetic assay	Altamirano-Lozano et al. 1993
B6C3F1 mice (M, F)	+	inhalation, 2yr	0, 1,2, or 4 mg/m^3	DNA was isolated from lung tumros 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
B6C3F1 mice (M, F)	+	inhalation, 2yr	0, 1,2, or 4 mg/m^3	Analysis of frequency of micronuclei in peripheral blood normochromatic erythrocytes demonstrated on effect of vanadium pentoxide exposure in this study.	Micronuclei assay	NTP 2002
In vitro						
primary human lymphocytes	+		25 – 500 µg/L	V2O5 exposure led to significant increase in DNA migration as measured by Comet assay at the highest doses tested (250 - 500ug/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 lead to an increase in aneuploidy with some interindividual variation observed between the four primary cell strains. Disruption of spindle formation may be due to interaction with microtubules.	FISH	Ramirez et al. 1997

primary human lymphocytes (n=4)	+	24h	0.3, 30, 3000 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 in 45 min post-exposure.	Comet Assay	Rojas et al. 1996
primary human lymphocytes (n = 1)	-	72h	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	
Syrian Hamster Embryo cells	+	0, 24h, 7d	0 to 0.875 µg/ml	Vanadium pentoxide exposed cells were positive at 7d exposure but not at 24h, similar to other tumor-promotion chemicals studied by this group.	SHE transformation assay	Kerckaert et al. 1996
Chinese hamster V79 cells	+	24h	0, 1, 3, 6, 9, 12 µg/ml	Vanadium pentoxide exposure led to increased MN induction ($p < 0.005$), apparently due to damage to the spindle apparatus but no significant increases in mutations or SCE.	MN 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	0 - 333 µg/plate	No increase in revertant colonies was observed following exposure.	gene mutation	NTP 2002

4.5.2 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 (Pierce et al. 1996; Bonner et al. 2002). These 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 bronchoalveolar lavage (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 one hr to ten days after exposure. Significant neutrophil influx was observed after 24 hrs exposure to vanadium pentoxide and peaked at 48-hr post-exposure with 20% neutrophils. MIP-2 mRNA expression levels were significantly elevated in vanadium pentoxide-treated rats compared to controls at early time points (1 hr to 48 hrs) suggesting pulmonary inflammation.

Pro-inflammatory prostaglandins such as PGE_2 produced by the enzymes COX-1 and COX-2, and PGE synthase mediate tissue homeostasis and/or 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 6-8 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 (wildtype) were instilled with 50 μL of saline (n = 3 to 4) or 1mg/kg vanadium pentoxide in saline (n = 5 to 6). Lungs were lavaged at 1, 3, 6, or 15 days post-instillation 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 wildtype 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 ~500 pg/mL in saline-instilled wildtype mice to ~1000 pg/mL in vanadium pentoxide-treated wildtype mice at 24 hrs

but not at other time points. The PGE2 level in saline-treated $COX1^{-/-}$ mice was ~10 pg/mL and about ~225 pg/mL after 24 hrs of vanadium pentoxide. PGE2 levels were ~200 pg/mL at 24 hrs 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 may 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 principle cell type that contributed to the observed fibrosis. Male Sprague-Dawley rats weighing ~200 g received intratreacheal instillation of sterile saline or 1 mg/kg vanadium pentoxide. Rats were additionally injected with BrdU (50 mg/kg, i.p.) one hr 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 post exposure and were 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 3.1-3.9-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 hrs 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 hr prior to intratracheal instillation of vanadium pentoxide

(1 mg/kg) and again two 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 five-fold increase in lung hydroxyproline content, an indicator of lung collagen and potentially fibrosis, 15 days after instillation. Prior and post-treatment with AG1296 reduced hydroxyproline content in vanadium pentoxide-treated animals to quantities similar to saline-instilled animals. Pre- and post-treatments with AG1478 reduced hydroxyproline content by ~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 post-treatment) and AP-1 (A/J and B6 mice; peaked at 6 hrs post-treatment) compared to PBS-controls, the activities in the order of A/J mice > B6 mice; 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 post-treatment 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 $\mu g/cm^2$ 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 hr. Vanadium pentoxide induced significant IFN-β expression after 18 and 24 hrs. 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 $\mu g/cm^2$ vanadium pentoxide or saline in vitro. RNA from cells was harvested 1, 4 8, 12, and 24 hrs post-treatment. 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 hrs of exposure. About 300 genes were found to be upregulated in response to vanadium pentoxide including inflammatory and immunomodulatory genes. Over 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 a number of 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 4-1).

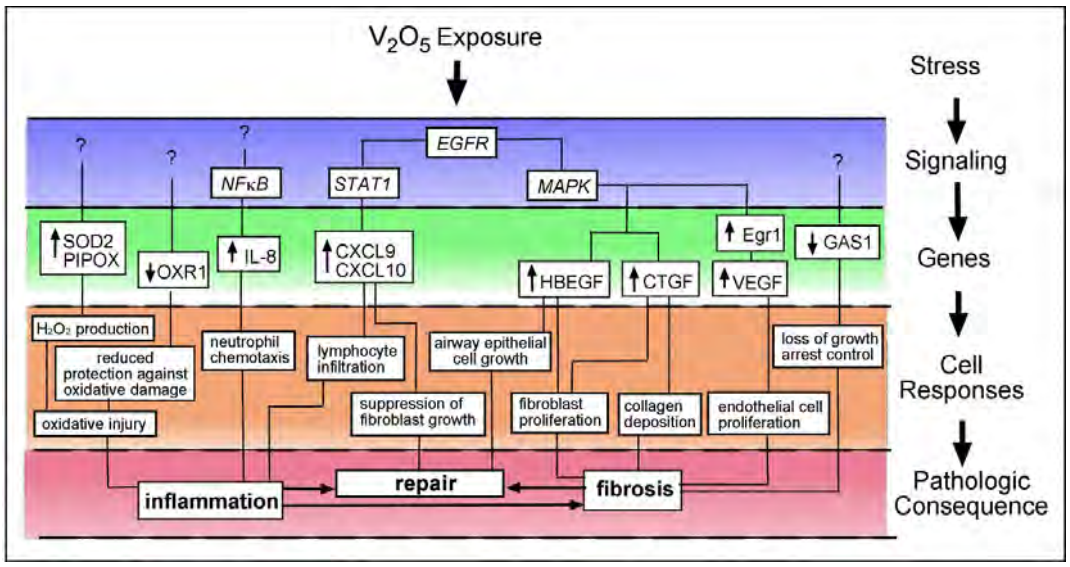

Figure 4-1: Genomics of V_2O_5-Induced Bronchitis (reprinted with permission from Ingram et al., (2007) Respir. Res. Apr 25;8(1):34)

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 may play key roles in this process. Moreover, fibroproliferative lesions contain collagen.

4.5.3 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 hr post exposure as well as 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 hrs. 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 that 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 hrs. 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 3hrs of exposure and persisted until 8 hrs 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 hrs. Quiescent cultured human lung fibroblasts were exposed for 3 hrs to 0, 10, 30 or 100 $\mu g/cm^2$ vanadium pentoxide. HB-EGF RNA was isolated and detected by Northern blot. 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 1hr post exposure. HB-EGF protein expression peaked at 6 hr post--exposure, as measured by Western blot. 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 hrs post exposure. 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 μg/cm^2 vanadium pentoxide for 15 min, 30 min, 1 hr, 3 hr, 6 hr or 24 hr. 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 MAPkinase and total p38. Peak phosphorylated ERK occurred at 30 min post exposure to H_2O_2. Vanadium-induced increases to p-ERK were biphasic, with one peak at 30 min post-exposure and the next at 24 hr post-exposure. Phosphorylated p38 was similarly maximally elevated at 30 min post-treatment with H_2O_2 and 24 hrs post treatment with vanadium pentoxide. Thus, this study suggests that HB-EGF expression occurs as a result of activation of the MAPKinase 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 there is some evidence of 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 mode of action (MOA) underlying cancer effects due to exposure of vanadium pentoxide is not as well characterized as mechanisms underlying non-cancer fibrotic effects. However, loss of heterozygosity (LOH) and DNA damage 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 2002 NTP study had mutations in *Kras2*. The *Kras2* 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, one spontaneous carcinoma, and two 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 with K-*ras* mutations but were not positive for LOH had elevated phosphorylated MAP kinase levels. However, these results should be interpreted cautiously 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 or 19 samples tested. However, the signaling events, and specifically the role of the MAPkinase pathway, associated with this oncogenic mutation 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 hr to 48 hrs).

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 may be dependent on inflammatory mediators such as IL-1B. HB-EGF expression is dependent on activation of the MAPKinase 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. It is not known whether lung neoplasms from male rats exhibit elevated activated MAP kinase or whether the rat tumors have increased K-*ras* mutation and LOH on chromosome 6.

4.6. SYNTHESIS OF MAJOR NONCANCER EFFECTS

4.6.1. Oral

Limited studies have been published examining the effects 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.

4.6.1.1 Acute

The only acute studies available report oral LD_{50} values in rats that range from ~10-137 mg/kg body weight and 64 mg/kg-body weight in mice depending on the source (Yao et. al., 1986b and CICAD 29, 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.

Table 4-13: Summary of Noncancer Results of Repeat-Dose Studies for Oral Exposure of Experimental Animals to Vanadium Pentoxide

Species (sex)	Avg Daily Dose (mg/kg-day)	Exposure Duration and Route	Response at LOAEL	NOAEL (mg/kg-day)	LOAEL (mg/kg/day)	Comments	Reference
Wistar Rat (male)	0, 10.5, 16.4, 69.6, 141.0 mg/kg-day (corresponds to 0, 74.5[a], 116.1[b], 500 and 1000 ppm)	103days in food	Decreased erythrocyte count	10.5 mg/kg-day	16.4 mg/kg-day	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-day (corresponds to 0, 74.5[a], 116.1[b], 500 and 1000 ppm)	103 days in food	Decreased relative liver weight	--	69.6 mg/kg-day		

Table 4-13: Summary of Noncancer Results of Repeat-Dose Studies for Oral Exposure of Experimental Animals to Vanadium Pentoxide							
	0, 10.5, 16.4, 69.6, 141.0 mg/kg-day (corresponds to 0, 74.5[a], 116.1[b], 500 and 1000 ppm)	103 days in food	Decreased Hair Cystine	10.5 mg/kg-day	16.4 mg/kg-day		
Rat (male)	1.41 and 14.1 mg/kg-day (corresponds to 17.9 and 179 ppm)	2.5 yrs in food	Decreased Hair Cystine	1.41 mg/kg-day	14.1 mg/kg-day	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.

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4.6.1.2 Subchronic

Data on toxicity following subchronic oral exposure to vanadium pentoxide are extremely limited. No comprehensive studies outlining toxic effects in response to vanadium pentoxide in humans have been reported. The primary noncancer health effects of subchronic oral exposure in animals include changes in relative liver weight, decreased erythrocyte count and hemoglobin counts, and decreased hair cystine content (Mountain et. al., 1953) in rats. Erythrocyte count and hemoglobin decreases are correlated and cannot be considered as separate effects. The hematological parameters were measured at low dose exposure levels. Relative liver weights were only measured for control and in the highest dose (69.6 mg/kg-day) group. Changes in hair cystine were measured in all dose groups but the biological significance of this effect is unknown; changes in hair cystine content may serve as a biomarker of exposure, rather than as an indication of an adverse effect. From these data, and using standard conversions for body weight and food consumption for Wistar rats, the NOAEL of 74.5 ppm was converted to 10.5 mg/kg-day for decreased erythrocyte count.

4.6.1.3 Chronic

No studies reporting chronic exposure to vanadium pentoxide in humans are available in the published literature. Chronic animal studies evaluating effects of oral exposure to vanadium pentoxide are limited. Previously, a study based on a 2.5 yr dietary exposure to vanadium (Stokinger et. al., 1953 reported in Patty's Industrial Hygiene and Toxicology, 3rd Ed., 1981.) utilized hair cystine as the critical effect, since hair cystine was significantly decreased (no details provided regarding the percent decrease or dose-response) in exposed rats and this change may reflect changes to enzymatic pathways. However, comprehensive toxicity endpoints were not evaluated, and the number and strain of rats used was not reported in this 1953 study. Decreased hair cystine may or may not be a biologically relevant effect. Decreased hair cystine may not be related to vanadium exposure, but rather an effect of poor nutritional status, as a result of an aversion to vanadium pentoxide in the feed. EPA has been unable to gain access to the raw data. No additional oral chronic exposure studies in animals were identified in the literature.

4.6.2. Inhalation

Table 4-20 presents a summary of the noncancer results for the acute subchronic and chronic studies of vanadium pentoxide toxicity in humans and experimental animals. 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 (Table 4-14). The most comprehensive animal studies evaluated toxicity following inhalation of vanadium pentoxide in F344/N rats and B6C3F$_1$ mice (NTP, 2002); these studies included acute, short-term, subchronic and chronic duration exposures and multiple toxicological endpoints. In those studies, the lung was identified as the most sensitive organ. The reported neurotoxic effects appear after as little as a single one hour exposure to inhaled vanadium pentoxide; although multiple effects were identified that increased in severity with increased duration of exposure, the studies were conducted using only one exposure level. Thus, a no-effect level for neurotoxicity could not be determined. Major data gaps also exist for studies on developmental toxicity, reproductive toxicity, and immunotoxicity following inhalation exposure to vanadium pentoxide, although effects have been seen in available studies (some of which used an exposure paradigm similar to that used for neurotoxicity studies).

4.6.2.1 Acute and Short-term

The primary identified noncancer health effect following acute inhalation exposure in humans is respiratory irritation, cough, and mucus formation. A human controlled exposure study (n = 100) performed by Zenz and Berg (1967) reported respiratory irritation, cough, and mucus formation in humans exposed to vanadium pentoxide at 0, 0.1, 0.5, and 1.0 mg/m^3, for 8 hrs. A NOAEL of 0.5 mg/m^3 was established. These effects are confirmed in male monkeys exposed to vanadium pentoxide at 0.5 or 5.0 mg/m^3 for 6 hrs (Knecht et. al., 1985). Air flow restriction, as measured by pulmonary function tests, was reported at 5.0 mg/m^3 (LOAEL); the NOAEL was 0.5 mg/m.^{3} Moreover, a WHO-IPCS (CICAD) document (2001) reports a 1-hr inhalation exposure to vanadium pentoxide dust in rats led to an LC_{67} of 1.44 mg/L (1440 mg/m^3). Clinical signs of toxicity included respiratory difficulty, increased respiratory tract mucus production, and irritation of the eyes, nose, and throat (WHO-IPCS, 2001, section 11.1.1). Neurotoxicity has also been observed in mice following a single inhalation exposure; Avila-Costa et al. (2006) noted significant changes in brain morphology and impairment in memory in

male CD-1 mice compared to controls following a single 1 hr exposure to 0.02 M (2.5 mg/m^3 V) inhaled vanadium pentoxide.

Respiratory irritation was observed following short-term inhalation exposure in humans. 100 workers were reportedly exposed to 0.05 to5.3 mg/m^3 vanadium for 10 hr/day, 6 days/week, for 4 weeks (Levy et al., 1984). A LOAEL of 0.05 mg/m^3 was established. However, dose-response was not systematically measured, there were no controls, and exposure 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 relative lung weight in rodents (NTP, 2002).

4.6.2.2 Subchronic

No comprehensive subchronic studies have evaluated inhalation effects in humans. The primary noncancer health effects of subchronic inhalation exposure in animals include nonneoplastic lung and nasal lesions (NTP, 2002), hematological parameter changes (NTP, 2002), decreased pulmonary function in male monkeys (Knecht et al., 1992), morphological changes to the CNS (Avila-Costa et al., 2004; 2005; 2006), increased platelet counts (Gonzalez-Villalva et al., 2006), and testicular malformation (Mussali-Galante et al., 2005; Fortoul et al., 2007). The NTP (2002) study evaluated pulmonary and nasal endpoints in both male and female rats and mice after 3 months 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 similar low doses in rats and mice. A LOAEL of 2.0 and NOAEL of 1.0 mg/m^3 were established. In addition, rats exhibited bronchiolar exudates, microcytic erythrocytosis, lung fibrosis, and nasal lesions. Associated LOAELs and NOAELs were established and are listed in Table 4-20. Body weight loss and increased absolute lung weight were reported in mice and resulted in a LOAEL of 8.0 mg/m^3 and NOAEL of 4.0 mg/m^3. An increased absolute and relative lung weight LOAEL was set at 4.0 mg/m^3 and 2.0 mg/m^3 for these lesions observed in mice. In summary, rats appear to be more sensitive to inhalation exposure to vanadium pentoxide than mice, based on the occurrence of a wider variety of nonneoplastic lesions throughout the respiratory tract.

Knecht et. al. (1992) observed that inhaled vanadium pentoxide leads to adverse lung effects by measuring 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 hrs/day, 5 days/week). A LOAEL of 0.5 mgm^3 and a NOAEL of 0.1 mg/m^3 was established. Pulmonary function parameters were reversible and did not repeat following subsequent challenge.

Selected hematology parameters (number of erythrocytes and reticulocytes and percent

hematocrit) were significantly altered in male and female rats at the highest dose level (NTP, 2002). A LOAEL of 16.0 mg/m^3 and a NOAEL of 8.0 mg/m^3 were established. Other effects such as increased platelet count following 12 weeks and altered testicular morphology following 20 weeks were tested at only one dose (5.13 mg/m^3).

4.6.2.3 Chronic

Limited information is available specific to the vanadium pentoxide exposure levels in human studies. Respiratory and other symptoms have been documented among workers employed at facilities producing and processing vanadium pentoxide (Sjoberg, 1951; Sjoberg, 1956; Zenz et al., 1962; Kiviluoto et al., 1979; Kiviluoto, 1980; Kiviluoto et al., 1981a; Musk and Tees, 1982; Irsigler et al., 1999). Similar effects were observed in occupational studies of boilermakers involved in the construction, cleaning and maintenance of oil-fired boilers (Williams, 1952; Sjoberg, 1955; Lees, 1980; Ross, 1983; Levy et al., 1984; Hauser et al., 1995a; 1995b; 2001; Woodin et al., 1998; 1999; 2000; Kim et al., 2004). Noncancer health effects of chronic inhalation exposure in animals included nonneoplastic pulmonary lesions in male and female rats and mice exposed to vanadium pentoxide for 2 yrs at 0, 0.5, 1.0 and 2.0 mg/m^3 and 0, 1.0, 2.0, and 4.0 mg/m^3, respectively (NTP, 2002). Increased incidence of 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 were reported. Based on these findings, the lowest dose for which a LOAEL could be established in rats was 0.5 mg/m^3. Additional findings observed in females included increased interstitial fibrosis. No NOAEL was established. In both male and female mice, increased incidences of pulmonary inflammation, and hyperplasia were reported. Increased incidences of nasal olfactory and/or respiratory epithelium degeneration, and epiglottis metaplasia were reported 1.0 mg/m^3 (LOAEL) in both male and female mice. The lung fibrosis and nasal inflammation were noted at $\geq$ 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. No treatment-related findings were observed in other tissues.

Table 4-14: Summary of Noncancer Results of Repeat-Dose Studies for Inhalation Exposure of Vanadium Pentoxide							
Species (sex)	**Avg Daily Dose (mg/m^3)**	**Exposure Duration**	**Response at LOAEL**	**NOAEL (mg/m^3)**	**LOAEL (mg/m^3)**	**Comments**	**Reference**
Acute Exposure							
Monkey (male)	0.5 or 5.0 mg/m^3	6 hrs (single exposure)	Air-flow restriction (measured by pulmonary function tests)	0.5[a]	5.0		Knecht et. al., 1985
Human (not reported)	0, 0.1, 0.5, 1.0 mg/m^3	8 hrs (single exposure)	Respiratory irritation, cough, mucus formation	0.5	1.0	Small sample size (n = 9)	Zenz and Berg, 1967
Mouse (male)	2.56 mg/m^3	1 hr (single exposure)	Impaired performance in spatial memory, decreased dendritic spines increased percentage of necrotic cells in hippocampus	---	2.56	Only one dose tested	Avila-Costa et. al., 2006
Short-term Exposure							
Rat and mice (female)	0, 1, 2, 4 mg/m^3	13 days, 6 hrs/day, 5 days/week	Histiocytic infiltrate and lung inflammation (rats); alveolar and bronchial hyperplasia (mice)	--	1.0 (rats) 2.0 (mice)	No study performed in male rats or mice	NTP, 2002
Rat (male)	0, 4, 8, 16, 32 mg/m^3	16 days, 6 hr/day, 5 day/week	BALFluid analysis: increased neutrophilia and decreased macrophage infiltration Significant increase in total cell count	8.0 4	16.0 8	Sample size = 22 rats. Rats exposed to 32 mg/m^3 were emaciated, 3 male rats died in the 32 mg/m^3 group,	
Mouse (male) Mouse (female)	0, 2, 4, 8, 16, 32 mg/m^3 16 days, , 6 hr/day, 5 day/week		Absolute lung weight Relative lung weight Decreased percentage of macrophages in BAL Fluid	2.0 -- 4.0	4.0 2.0 8.0	Sample size = 5 of each sex. All male mice died at 32 mg/m^3 Sample size 40-60.	

Table 4-14: Summary of Noncancer Results of Repeat-Dose Studies for Inhalation Exposure of Vanadium Pentoxide							
			Increased protein leakage into BAL Fluid and increased lymphocytes	- -	4.0	Females only.	
Mouse (male)	2.56 mg/m^3	30 days (1 hr, 2 times a week for up to 4 weeks)	Changes in matrix metalloproteinase levels in multiple regions of the brain	--	2.56	Signficant increases occurred after 1 month of exposure. Only one dose level was used.	Colin-Barenque et. al., 2008
Human (not reported)	Range of 0.05 to 5.3 mg/m^3	4 weeks, 10 hr/day, 6 days/week	Respiratory irritation	--	0.05[b]	Dose and response were not systematically measured, vanadium pentoxide exposure not accurate	Levy et. al., 1984
Subchronic Exposure							
Mouse (male)	2.56 mg/m^3	8 weeks, 1 hr, 2 times/week	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., 2004, 2005, 2006
Mouse (male)	2.56 mg/m^3	12 week,(1 hr/day, 2 days/weeks)	Increased platelet counts and altered platelet morphology	--	2.56		Gonzalez-Villalva et. al., 2006
Mouse (male)	2.56 mg/m^3	12 weeks (1 hr 2 times per week)	Decreased gamma globulin in testes, Increased cell death in spermatogonia	--	2.56	Only one dose used	Mussali-Galante et. al., 2005,

Table 4-14: Summary of Noncancer Results of Repeat-Dose Studies for Inhalation Exposure of Vanadium Pentoxide							
							Fortoul et. al., 2007
Monkey (male)	0.1, 0.5, or 1.1 mg/m^3	26 weeks (6 hrs per day, 5 days per week)	Impaired pulmonary function	0.1	0.5	Exposures occurred on alternate days for two sets of animals (some received 0.1 or 1.1 mg/m3 on alternate days, while a second group was exposed to a constant concentration (0.5 mg/m3) for all 5 days. Pulmonary function parameters were reversible and did not reappear following subsequent challenge. N= 26 animals total, n-= 8-9 per group.	Knecht et. al., 1992
Rat (both) and mouse (both)	0, 1, 2, 4, 8, 16 mg/m^3	3 months (6 hr/day, 5 days/week)	Lung hyperplasia	1.0	2.0		NTP, 2002
Rat (both)	0, 1, 2, 4, 8, 16 mg/m^3	3 months (6 hr/day, 5 days/week)	Lung fibrosis	2.0	4.0		
Rat (male)			Microcytic erythrocytosis, lung inflammation, and , lung epithelial hyperplasia,	1.0	2.0		
Mouse (male)	0, 1, 2, 4, 8, 16 mg/m^3	3 months (6 hr/day, 5 days/week)	Lung inflammation	2.0	4.0		
Mouse (female)				1.0	2.0		
Rat	0, 1, 2, 4,	3 months (6	Nasal hyperplasia and	4.0	8.0		NTP, 2002

Table 4-14: Summary of Noncancer Results of Repeat-Dose Studies for Inhalation Exposure of Vanadium Pentoxide							
(male)	8, 16 mg/m^3	hr/day, 5 days/week)	squamous metaplasia, body weight gain				
Rat (female)				2.0	4.0		
Rat and mouse (male) Rat and Mouse (female)	0, 1, 2, 4, 8, 16 mg/m^3	3 months (6 hr/day, 5 days/week)	Body weight loss	4.0 2.0	8.0 4.0		NTP, 2002
Rat and Mouse (male) Rat and Mouse (female)	0, 1, 2, 4, 8, 16 mg/m^3 0, 1, 2, 4, 8, 16 mg/m^3	3 months (6 hr/day, 5 days/week) 3 months (6 hr/day, 5 days/week)	Increased Relative lung weight Increased absolute lung weight	2.0 1.0 2.0	4.0 2.0 4.0		NTP, 2002
Rat (both)	0, 1, 2, 4, 8, 16 mg/m^3	3 months (6 hr/day, 5 days/week)	Increased levels of hematological parameters	8.0	16.0		NTP, 2002
Chronic Exposure							
Rat (male)	0, 0.5, 1, 2 mg/m^3	2 yrs (6 hr/day, 5 days/week)	Lung hyperplasia, histiocytic infiltration, epiglottis degeneration, , hyperplasia and squamous metaplasia, chronic inflammation of the larynx, , goblet cell hyperplasia	--	0.5	No other clinical findings or altered survival	NTP, 2002
Rat (female)			Interstititial fibrosis, and histiocytic infiltration, epiglottis degeneration, hyperplasia, and squamous	--	0.5		

Table 4-14: Summary of Noncancer Results of Repeat-Dose Studies for Inhalation Exposure of Vanadium Pentoxide							
			metaplasia, chronic inflammation of the larynx				
Rat (female)	0, 0.5, 1, 2 mg/m^3	2 yrs (6 hr/day, 5 days/week)	Lung inflammation	1.0	2.0	No other clinical findings or altered survival	NTP, 2002
Rat (female)	0, 0.5, 1, 2 mg/m^3	2 yrs (6 hr/day, 5 days/week)	Lung hyperplasia	0.5	1.0	No other clinical findings or altered survival	NTP, 2002
Mouse (both)	0, 1, 2, 4 mg/m^3	2 yrs (6 hr/day, 5 days/week)	Lung inflammation, and hyperplasia	--	1.0	Mice reported as thin, survival significantly decreased in male mice at 4 mg/m^3	NTP, 2002
Rat (female)	0, 0.5, 1, 2 mg/m^3	2 yrs (6 hr/day, 5 days/week)	Lung squamous metaplasia of alveolar and bronchiolar epithelium	1.0	2.0	No other clinical findings or altered survival	
Rat (male)	0, 0.5, 1, 2 mg/m^3	2 yrs (6 hr/day, 5 days/week)	Lung fibrosis	0.5	1.0	No other clinical findings or altered survival	NTP, 2002
Mouse (both)	0, 1, 2, 4 mg/m^3	2 yrs (6 hr/day, 5 days/week)	Lung fibrosis	1.0	2.0	Mice reported as thin, survival significantly decreased in male mice at 4 mg/m^3	NTP, 2002
Rat (male)	0, 0.5, 1, 2 mg/m^3	2 yrs (6 hr/day, 5 days/week)	Nasal olfactory degeneration and respiratory degeneration		0.5	No other clinical findings or altered survival	NTP, 2002
Mouse (both)	0, 1, 2, 4 mg/m^3	2 yrs (6 hr/day, 5 days/week)	Nasal inflammation	1.0	2.0		

Table 4-14: Summary of Noncancer Results of Repeat-Dose Studies for Inhalation Exposure of Vanadium Pentoxide							
Mouse (both)	0, 1, 2, 4 mg/m^3	2 yrs (6 hr/day, 5 days/week)	Nasal olfactory degeneration and respiratory degeneration	- -	1.0		NTP, 2002
Mouse (both)	0, 1, 2, 4 mg/m^3	2 yrs (6 hr/day, 5 days/week)	Epiglottis metaplasia	--	1.0		NTP, 2002

[a] Single exposures not adjusted for continuous exposure
[b] Not adjusted for continuous exposure because of the highly intermittent exposure protocol (1 hr/day, 2 days/week)

4.6.3 Mode of Action Information

There is currently insufficient evidence to establish the mode of action for vanadium pentoxide toxicity. However, the limited data to inform the mechanisms of various non-cancer health effects (pulmonary toxicity, neurotoxicity and reproductive toxicity) following inhalation exposure to vanadium pentoxide are summarized below.

4.6.3.1 Pulmonary Toxicity

The mechanism of action underlying the formation of pulmonary fibroproliferative lesions has been linked to inflammation, leading to regenerative hyperplastic responses, as evidenced by the presence of mitogens and observed smooth muscle thickening. Oxidative stress has also 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 ERK 1 or 2 and p38 kinase that lead to induction to 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. Oxidative stress has been implicated in the mechanism underlying vanadium pentoxide induced pulmonary injury. All species of vanadium may participate in redox cycling and can generate reactive oxygen species (Carter et. al.1997). Intracellular and extracellular H_2O_2 production increases significantly after 18 hr exposure to vanadium pentoxide compared to controls (Antao-Menezes et. al. 2008). Vanadium pentoxide induced significant IFN-β expression after 18 and 24 hrs but could be inhibited by catalase, an inhibitor of H_2O_2. Oxidative stress was also a suggested mechanism underlying STAT-1 activation, since 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). It is possible that hydrogen peroxide and vanadium pentoxide reacted to form peroxovanadium intermediates and/or ROS that led to the production of HB-EGF in fibroblasts. Oxidative stress could also be inducing ERK and p38 kinase that lead

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 may 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. 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 was observed beneath ciliated epithelial cells in vanadium pentoxide-exposed male Sprague-Dawley rats (Bonner et. al. 1998). Further, proliferating myofibroblasts were the principle 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, heparin-binding epidermal growth factor-like 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 MAPkinase) 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, and CTGF), chemokines (CXCL9, CXCL10), oxidative response genes (SOD2, PIPOX, OXRI) and DNA-binding proteins (GAS1, STAT1) (Ingram et. al., 2007).

Bonner et. al. (2003) has also reported that fibroproliferative lesions are resolved and repair 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 indicate the potential 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 (Figure 4-1). 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, as 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. It has been suggested that blood-brain barrier disruption may be related to brain region-specific changes in metalloproteinases (MMP-2 and MMP-9) that have been seen following vanadium pentoxide exposure (Colin-Barenque et. al. 2008), however more work is needed to fully characterize these findings.

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). Reproductive effects due to vanadium pentoxide inhalation exposure included morphological changes to spermatogonia, spermatocytes and Sertoli cells (Fortoul et. al., 2007). Estrous cycle length was increased in female rats but not mice following inhalation exposure at high doses, while decreased spermatozoal motility was observed in male mice but not male rats at high doses (NTP 2002). 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 may 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 mode of action 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 inhalation studies in male rats and male and female mice (NTP 2002). No studies evaluating the carcinogenic potential in humans exposed to inhaled vanadium pentoxide were identified. No studies suitable for evaluation of the oral carcinogenic potential for vanadium pentoxide were located in the published literature. There was clear evidence of carcinogenesis in both male and female mice based on the statistically significant and dose-related increased incidence of alveolar/bronchiolar tumors (NTP, 2002; Ress et al., 2003). There was some evidence of carcinogenic activity in male rats and equivocal evidence in female rats (NTP, 2002). 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 (Table 4-8). 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) 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* (U.S. EPA, 2005a) indicate for that for tumors occurring at a site other than the initial point of contact, the weight of evidence for carcinogenic potential may 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 V_2O_5 via the inhalation route is limited to examination of the respiratory tumors. Information on the carcinogenic effects of V_2O_5 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 a mode of action, this cancer descriptor applies only to the inhalation route of exposure. Therefore, V_2O_5 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, there are currently no epidemiology studies available in the published literature examining carcinogenicity.

Only two published laboratory animal studies provide evidence for the carcinogenic potential of vanadium pentoxide (NTP 2002; Rondini et al., 2010). Vanadium pentoxide has been shown to induce pulmonary tumors following inhalation exposure in a study performed by NTP (NTP 2002). F344 rats (50/sex/group) and B6C3F1 mice (50/sex/group) were exposed to vanadium pentoxide particles for six hours a day, five days per week for two 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 in male rats only (Table 4-15). 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 was not significantly different from controls. Both rats and mice showed other lesions of the respiratory tract, including inflammation, fibrosis and hyperplasia (Tables 4-7, 4-9). Decreased body weight gain was observed as early as 3 months post-exposure 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 V_2O_5 (4 mg/kg, 5 times weekly) (Table 4-11). A/J and BALB/C male mice showed increases in lung tumors following exposure to both MCA (initiator) and V_2O_5, but not V_2O_5 alone, suggesting V_2O_5 works as a tumor promoter.

4.7.3. Mode of Action Information

The U.S. EPA (2005a) *Guidelines for Carcinogen Risk Assessment* defines mode of action (MOA) as a sequence of key events and processes, starting with the interaction of an agent with a cell, proceeding through operational and anatomical changes, and resulting in cancer formation. Examples of possible modes of carcinogenic action include mutagenic, mitogenic, anti-apoptotic (inhibition of programmed cell death), and cytotoxic mechanisms with reparative cell proliferation and immunologic suppression. There is insufficient information to establish a carcinogenic mode of action 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 developings, however the 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 that there is a higher absorption of vanadium in these 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 gastrointestinal tract. Mravcova et. al (1993) assessed the extent of vanadium pentoxide accumulation in the bones of rats following 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. However, no dose response data for these endpoints was 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, and 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 and Ress, 2002). Thus, increased tumor incidence in rats is equivocal overall, but the lack of any increase in females may suggest a gender-related increase in susceptibility in males. It is unknown whether this observed difference is applicable to humans. There are no reported differences for response to vanadium pentoxide between genders for either animals or humans for any non-cancer endpoint.

4.8.3. Other Susceptible Populations

No data exists 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 may contribute to the severity of vanadium pentoxide-induced pulmonary inflammation and tumorigenicity in mice (Rondini et al., 2010). The NTP study (2002) used B6C3F1 mice in all of their exposure protocols for 16-day, 3 month, and 2 yr 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 pre-existing lung conditions would be differentially susceptible to inhaled vanadium pentoxide. Compared to exposed normal rats, Br rats exhibited delayed recovery from pre-existing 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 (full study description in Section 4.2.2.2). 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 – 11). Differences were also observed between strains, with A/J mice showing increased tumorigenicity in response to vanadium pentoxide. In the absence of MCA, V_2O_5 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), 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 post-treatment 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.

5. DOSE-RESPONSE ANALYSIS

5.1. ORAL REFERENCE DOSE (RfD)

Only two studies exist on human oral exposure to vanadium pentoxide, and neither examined health effects related to this exposure. Two studies 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, however, it is expected that these urinary levels of vanadium resulted from multiple exposure routes. Kucera et al. (1992) measured vanadium in the hair and blood of children and 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, and changes in hair cystine levels have not been correlated with adverse health effects. Additional studies of workers occupationally exposed to vanadium pentoxide exist, although it is presumed that these workers were exposed by multiple routes, inhalation was likely the primary route of exposure (Sjöberg, 1951; Sjöberg, 1956; Zenz et al., 1962; Kiviluoto et al., 1979; Kiviluoto, 1980; Kiviluoto et al., 1981b; Musk and Tees, 1982; Irsigler et al., 1999).

Mountain et al. (1953) is the only published, peer-reviewed study that evaluated the effects of subchronic oral vanadium pentoxide exposure in laboratory animals. Male Wistar rats (5/group; 200 – 350 gm bw) 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). However, these data were not accompanied by any explanation, statistical analysis, or measures of variability (SE or SD). 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. However, data to support a relationship between decreased hair cystine levels and adverse health outcomes are not available in the published literature. Thus, the biological significance of decreased hair cystine content is unclear.

Table 5–1. Hematological results of oral vanadium pentoxide exposure in rats (Mountain et al. 1953).

	Control	10.5 mg/kg-day	16.4 mg/kg-day
Red Cell Count (M/mm^3)			
Start	8.0	7.8	8.0
Finish (103 days)	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 days)	15.0	14.5	13.7
Percent change between start and finish of expt (%)	3.9	4.6	10.5

Relative liver weight increases and decreases in erythrocytes and hemoglobin levels were also reported by Mountain et al. (1953). 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 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). No statistical analysis was performed by the study authors on these data, and no measure of variance was reported, 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 were suggestive of 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 selected as the critical effect.

Although a dose-related decrease in RBC count was seen 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 is considered biologically significant only at 16.4 mg/kg-day, which

EPA thus identified as the LOAEL. The lowest dose of 10.5 mg/kg-day was then identified by EPA as a NOAEL.

5.1.1 Methods of Analysis

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.

The Agency endorses a hierarchy of approaches to derive human equivalent oral exposures from data from laboratory animal species, with the preferred approach being physiologically based toxicokinetic modeling (U.S. EPA, 2011). Other approaches may include using some chemical-specific information, without a complete physiologically based toxicokinetic model. In lieu of data to support either of these types of approaches, body weight scaling to the ¾ power (i.e., $BW^{3/4}$) is endorsed as a general default to extrapolate toxicologically equivalent doses of orally administered agents from all laboratory animals to humans for the purpose of deriving an oral Reference Dose (RfD). In general, the use of $BW^{3/4}$ scaling is considered appropriate for deriving an oral RfD when the observed effects are associated with the parent compound or a stable metabolite, not related to a portal-of-entry effect, and not related to developmental endpoints. Use of $BW^{3/4}$ scaling in combination with consideration of a reduced interspecies uncertainty factor, UF_A, is recommended as the Agency default approach.

No physiologically based toxicokinetic modeling information exists for vanadium pentoxide. The selected critical effect (decreased red blood cell 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:

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

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 a HED of 2.56 mg/kg-day as follows:

$$\begin{aligned} \text{HED} &= \text{Laboratory animal dose (mg/kg-day)} \times \text{DAF} \\ &= 10.5 \text{ mg/kg-day} \times 0.244 \\ &= 2.56 \text{ mg/kg-day} \end{aligned}$$

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

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

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

5.1.2 RfD Derivation – Including Application of Uncertainty Factors (UF)

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 application of uncertainty factors to the POD, converted to a HED, results in a composite uncertainty factor of 3,000 covering four areas of uncertainty: 3 for interspecies extrapolation from animals to humans (UF_A); 10 for human intraspecies variability (UF_H), 10 for extrapolation from a subchronic to a chronic study (UF_S), and 10 for database insufficiencies (UF_D). No UF is needed for extrapolation from a LOAEL to NOAEL because a NOAEL was used to identify the POD (US EPA, 2002).

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 (i.e., interspecies variability). This interspecies uncertainty factor is comprised of two separate but equal sources of uncertainty, toxicokinetic and toxicodynamic differences between animals and humans. For vanadium pentoxide, toxicokinetic uncertainty was accounted for by calculation of a human equivalent dose (HED) through application of a dosimetric adjustment factor (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 an UF_A of 3 is retained to account for this residual uncertainty.

An intraspecies uncertainty factor, UF_H, of 10 for intraspecies differences (human variability) was used to account for potentially susceptible individuals in the absence of quantitative information on the variability of response in humans.

An UF_S of 10 for extrapolation from a subchronic to chronic study is applied because a subchronic study was used for the POD.

An UF_L for LOAEL to NOAEL extrapolation was not used because a NOAEL was identified from the principal study and used as the POD.

An UF_D of 10 was used for database insufficiencies due to the lack of a developmental toxicity study and a multi-generation reproductive study for V_2O_5 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:

$$\text{RfD} = \text{NOAEL}_{\text{HED}} \div \text{UF}$$
$$= 2.56 \text{ mg/kg-day} \div 3000$$
$$= 0.000085 \text{ mg/kg-day or } 9\text{E-}04 \text{ mg/kg-day}$$

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

5.1.2. Previous RfD Assessment

U.S. EPA previously derived a chronic reference dose (RfD) of 9×10^{-3} mg/kg-day for vanadium pentoxide based on a 2.5-year dietary NOAEL of 0.89 mg/kg-day vanadium pentoxide for decreased hair cystine content that was entered on the IRIS database in 1987 (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 yrs) 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 of this study as described in Section 5.1.1, it 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 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., 1995; Levy et al., 1984; Musk and Tees, 1982; Kiviluoto, 1980; Lees, 1980; Kiviluoto et al., 1979; Zenz et al., 1962; Sjöberg, 1955; Vintinner et al., 1955; Williams, 1952, Lewis, 1959). 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 (Knecht et al., 1992; NTP, 2002). A 6 month cynomologus monkey study (Knecht et al., 1992) exposed monkeys (n=8) to vanadium pentoxide aerosol (0.5 or 3.0 mg/m^3) for 6 hrs/day, 5 days/week for 26 weeks. This study was not selected as the principal study due to limitations in the number of test animals, study duration and number of doses used.

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 a 3-month inhalation exposure to vanadium pentoxide. Pulmonary lesions were observed in F344/N rats (10 male and 10 female) and B6C3F1 mice (10 male and 10 female) exposed for 3 months, with NOAEL and LOAEL values of 1 and 2 mg/m^3, respectively, for minimal to mild epithelial hyperplasia (Tables 4-4 and 4-6). Other observed endpoints include increased lung bronchiolar exudate, lung fibrosis, altered nasal morphology, and increased nasal inflammation observed in rats. Significant exposure-related decreases in pulmonary function were also observed in male and female rats, with a LOAEL of 4 mg/m^3 (pulmonary function not assessed in mice) (NTP, 2002). Other effects identified from

the NTP (2002) 3 month inhalation study are the erythrocytosis , body weight and lung weight changes. 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 NOAELs for mild erythrocytosis were 1 mg/m^3 in male rats and 2 mg/m^3 in female rats. The erythrocytosis is considered to be a secondary effect arising from the primary lung lesions and was not considered as a critical effect.

Abnormal breathing, emaciation, and lethargy were observed in male and female rats exposed to concentrations of 8 mg/m^3 or higher (NOAEL 4 mg/m^3, LOAEL 8 mg/m^3) in response to vanadium pentoxide after 3 months of exposure. 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%). Thus, a NOAEL of 4 mg/m^3 and a LOAEL of 8 mg/m^3 suggested for body weight loss and associated behavioral changes in rats and male mice. Relative lung weights were significantly increased in male and female rats (4 mg/m^3) following 3-months inhalation exposure to vanadium pentoxide. Similarly, relative lung weights were significantly increased in male and female mice starting at 4 mg/m^3.

Other studies identified morphological changes to the central nervous system (CNS) in male mice exposed to vanadium pentoxide for up to 8 weeks (Avila-Costa et al., 2004, 2005). Avila-Costa et al. (2004, 2005) 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 mgV/m^3 for up to 8 weeks; effects on central nervous system function or other comprehensive endpoints were not reported. Using the same dose regimen (1.4 mgV/m^3 two times a week for up to one 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 may be related to the associated spatial memory loss. Morphological changes in the CNS reported by Avila-Costa et al. (2004; 2005; 2006) were not considered as the critical effect due to lack of information on the exposure-response relationship for morphological changes to the central nervous system, 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. Though 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 seen in humans, the NTP (2002) study is selected as the critical study. The 2-year exposure study in F344/N rats and B6C3F1 mice (50/sex/group) (NTP, 2002) examined the effects of chronic inhalation (0, 0.5, 1 or 2mg/m^3 in rats and 0, 1, 2, 4 mg/m^3 in mice for 6hrs/d, 5d/wk, 104 weeks) of vanadium pentoxide. The NTP (2002) observed nonneoplastic lesions of the respiratory tract in male and female rats and mice. Numerous lesions of the upper and lower respiratory tract were observed in male and female rats and mice at the lowest exposure concentrations tested. Specifically, epiglottis epithelial hyperplasia in male and female rats (LOAELs of 0.5 mg/m^3), and alveolar and bronchiolar hyperplasia was observed in male rats (LOAELs of 0.5 mg/m^3) and in male and female mice (1 mg/m^3); NOAELs were not identified (Tables 4-7 and 4-9). Other histological lesions observed at a LOAEL of 0.5 mg/m^3 include multiple lesions in the nose and larynx of male and female rats including epiglottis epithelial degeneration, hyperplasia, and squamous metaplasia in both sexes and nasal goblet cell hyperplasia in males. In male and female mice, histological lesions also occurred in the larynx and nasal tissues at 1 mg/m^3; it should be noted that 0.5 mg/m^3 was not used as a 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 LOAEL of 1 mg/m^3 (NOAEL 0.5 mg/m^3). The NTP (2002) chronic study is well-designed, well-controlled, and well-reported. Numerous toxicological endpoints were assessed. As with the subchronic exposure studies, results of this two-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 observed by NTP (2002) are among the most sensitive effects observed and were observed in both sexes of rats and mice (Table 5-3). 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 (Sjöberg, 1951; Sjöberg, 1956; Zenz et al., 1962; Kiviluoto et al., 1979; Kiviluoto, 1980; Kiviluoto et al., 1981a, b; Musk and Tees, 1982; Irsigler et al., 1999). Therefore, NTP (2002) was selected as the principal study, with effects on the upper and lower respiratory tract selected as the critical effects.

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 prior to any modeling (Table 5.4, 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 $POD_{[ADJ]}$ to a human equivalent concentration (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, 1994) using mean body weights for male and female rats and the average particle size MMAD±GSD of 1.24±1.89 for rats as reported by NTP (2002). Human equivalent concentration (HEC) conversions (in mg vanadium pentoxide/m^3) were calculated by multiplying $Conc_{[ADJ]}$ 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. Duration-adjusted exposure concentrations ($Conc_{[ADJ]}$) 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).

Table 5-3. Selected Nonneoplastic Lesions of the Respiratory System in Rats Exposed to Particulate Aerosols of Vanadium Pentoxide for 2 Years (NTP, 2002)

Lesion Type and Location[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)

Table 5-3. Selected Nonneoplastic Lesions of the Respiratory System in Rats Exposed to Particulate Aerosols of Vanadium Pentoxide for 2 Years (NTP, 2002)

Lesion Type and Location[a]	Exposure Group			
	Control (% incidence)	0.5 mg/m^3 (% incidence)	1 mg/m^3 (% incidence)	2 mg/m^3 (% incidence)
Interstitial, fibrosis	7 (14)	7 (14)	16[c] (33)	38[b] (76)
Alveolus, histiocyte 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, histiocyte 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)
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 lesion; numbers in parantheses indicate percent incidence compared to control.

[b]Significantly different from control by the Poly-3 test, p≤ 0.01
[c]Significantly different from control by the Poly-3 test, p≤ 0.05

Table 5-4: Human Equivalent Concentrations of Vanadium Pentoxide in the 2-Year Inhalation Studies in Rats (NTP, 2002)

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 and 5 days per week.
[c] Please refer to Appendix Table B-4.
[d] "Human Equivalent Concentration" = "Concentration as Reported" * "Continuous Exposure Adjustment Factor " * "RDDR"

To determine the POD for derivation of the RfC, benchmark dose 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 $BMCL_{[HEC]}$ 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. Thus, it was chosen as the POD for the basis of the RfC calculation.

Table 5-5: Candidate PODs for Vanadium Pentoxide Derived from NTP Studies (2002) through BMDS Modeling.

Endpoint	Selected	BM	HEC [b]

	Model [a]	R (Extra Risk)	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	LogLogistic	0.1	0.017	0.012
Respiratory Epithelium, Epiglottis, Hyperplasia	LogLogistic	0.1	0.008	0.006
Nose				
Goblet Cell, Respiratory Epithelium, Hyperplasia	LogLogistic	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	LogLogistic	0.1	0.005	0.003
Respiratory Epithelium, Epiglottis, Hyperplasia	LogLogistic	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 data set based on the draft Benchmark Dose Technical Guidance (2000).
[b] HEC=Human Equivalent Concentration.
[c] BMCL= the lower bound of 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, 2000b). Biological and statistical considerations were taken into account in the selection of a benchmark response (BMR) level for all data sets. In the absence of information indicating what magnitude of inflammatory changes in the larynx and epiglottis are

considered biologically significant, the benchmark response (BMR) of 10% increase in extra risk was used as the basis for the BMC (BMC_{10}), with the $BMCL_{10}$ representing by the 95% lower confidence limit on the BMC_{10} (US EPA 2000b). Following the model selection steps outlined in the draft Benchmark Dose Technical Guidance (US EPA, 2000b), the best-fitting model was selected. Biological and statistical considerations were taken into account in the selection of a benchmark response (BMR) level for this data set. 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, 2000b).

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 chi-square 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 (US EPA, 2000b), 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, NTP (2002)

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	**0.46**	**242.0**	**-0.88**	**-0.13**	Extra Risk 10%	**0.005**	**0.003**
LogProbit	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)						0.013	0.011
Weibull						0.014	0.011
Probit	0.03	247.5	1.99	1.99			
Logistic	0.03	247.6	1.97	1.97			

[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 3 stages were tested. Only the model with the lowest AIC and lowest stage was reported here.

Table 5-7: Benchmark Modeling Results for Incidence of Larynx Respiratory Epithelium, Epiglottis, Hyperplasia in Female Rats, NTP (2002)

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 3 stages were tested. Only the model with the lowest AIC and lowest stage was reported here.

5.2.3 RfC Derivation- Including Application of Uncertainty Factors (UFs)

Benchmark dose modeling of incidence data for chronic inflammation of the larynx in female rats yielded the $BMCL_{10}$ of 0.003 mg/m^3. A total UF of 300 was applied to the $BMCL_{10}$ of 0.003 mg/m^3: 3 for interspecies extrapolation from animals to humans (UF_A); 10 for human interspecies variability (UF_H), and 10 for database insufficiencies (UF_D). An uncertainty factor (UF_S) was not applied for extrapolation of subchronic to chronic study as data was used from a chronic 2-yr study.

An UF of 3 ($10^{1/2}$ = 3.16, rounded to 3) was applied for interspecies extrapolation (UF_A) to account for uncertainty in extrapolating from laboratory animals to humans (i.e., interspecies variability). This uncertainty factor is comprised of two separate and equal areas of uncertainty to account for differences in the toxicokinetics and toxicodynamics of animals and humans. In this assessment, toxicokinetic uncertainty was accounted for by the calculation of a human equivalent concentration by the application of a dosimetric adjustment factor as outlined in the RfC methodology (U.S. EPA, 1994). 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 UF_H of 10 for intraspecies differences (human variability) was used to account for potentially susceptible individuals in the absence of quantitative information or information on the variability of response in humans. Considering the pulmonary effects of V_2O_5, individuals with pre-existing respiratory disorders may be more susceptible to inhaled vanadium pentoxide.

An UF_L for LOAEL to NOAEL extrapolation was not used because the current approach is to address this factor as one of the considerations in selecting a BMR for BMD modeling (US EPA 2000b; 1994b). In this case, a BMR of a 10% increase in the incidence of chronic inflammation of the larynx and epithelial hyperplasia of the epiglottis was selected under the assumption that it represents a minimal, biologically significant change.

An UF_D of 10 was used for database insufficiencies due to the lack of a developmental toxicity study and a multi-generation reproductive study for V_2O_5 by the inhalation 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 pharmacokinetic models are available for conducting a route-to-route extrapolation.

The chronic RfC for vanadium pentoxide is calculated as follows:

$$\begin{aligned} \text{RfC} &= \text{BMCL}_{10} \div \text{UF} \\ &= 0.003 \text{ mg/m}^3 \div 300 \\ &= 0.00001 \text{ mg/m}^3 \text{ or } 1\text{E-}05 \text{ mg/m}^3 \end{aligned}$$

Note: Because vanadium exists in several different valence states, all of which are not equivalent toxicologically (WHO-IPCS, 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 V_2O_5 was not previously available on IRIS.

5.3 UNCERTAINTIES IN THE INHALATION REFERENCE CONCENTRATION (RfC)

The following discussion identifies uncertainties associated with the subchronic and chronic RfCs. As presented earlier in this section, EPA standard practices and RfC guidance (U.S. EPA 1994a,b, 1995, 2002b) were followed in applying an uncertainty factor approach to a

point of departure (POD). A $BMDL_{10}$ approach was used for derivation of the chronic RfC. Factors accounting for uncertainties associated with a number of steps in the analyses were adopted to account for extrapolating from an animal bioassay to human exposure, a diverse human population of varying susceptibilities, and to account for database insufficiencies. These extrapolations are carried out with standard approaches given the paucity of experimental and human data on vanadium pentoxide to inform individual steps.

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 genotoxity 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 for pulmonary fibrosis in response to vanadium pentoxide.

In addition to respiratory effects, immunological and neurological effects have been seen following vanadium pentoxide exposure in experimental animals. An inhalation study using vanadium pentoxide (12 weeks, 1.4 mg/m^3) in male mice noted altered immune status, including a temporary increase in spleen weight, and alterations in antibody avidity following exposure to vanadium pentoxide (Pinon-Zarate et. al. 2008). This in part supports the results of an earlier oral study (6 months, 0 – 14 mg/kg-day in drinking water) in rats that also observed increased spleen weight (Mravcova et al., 1993). Several recent studies document neurotoxic morphological and behavioral effects (decreased dendritic spine length and increased necrosis in neural cells) in animals following inhalation exposure to vanadium pentoxide (Avila-Costa et al., 2004; 2005; 2006; Colin-Barenque et al., 2008). Though these immune and neurological effects occurred at a low dose (1.4 mg/m^3), in most cases only one dose was tested and precludes formation of an adequate dose-response.

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

The selection of the benchmark dose model for the quantitation of the RfC does not lead to significant uncertainty in estimating the POD since benchmark effect levels were within the range of

experimental data for chronic inflammation of the larynx. However, the selected model, the log-logistic model, while the best fitting model, 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 point of departure in rodents are extrapolated to human response. Pharmacokinetic models are useful in examining species differences in pharmacokinetics; however, a PBPK model for vanadium pentoxide was not available. Therefore, toxicokinetic species differences were addressed by the determination of a HEC through inhalation dosimetry adjustments (as described in the RfC methodology, U.S. EPA, 1994b). A UF of 3 was applied to account for the remaining toxicokinetic uncertainties in the extrapolation from rats and humans.

Information was unavailable for quantitative assessment of toxicokinetic or toxicodynamic differences between animals and humans, so the three-fold uncertainty factor (UF) was used to account for uncertainty in extrapolating from laboratory animals to humans in the derivation of the subchronic and chronic RfC values.

Heterogeneity among humans is another uncertainty associated with the RfC. In the absence of vanadium pentoxide-specific data on human variation, an uncertainty factor of 10 was applied to account for uncertainty associated with variation in the human population in the derivation of the subchronic and chronic RfCs. The range of human response to vanadium pentoxide may be larger or smaller. Vanadium pentoxide-specific data to examine the potential magnitude of over- or underestimation are not available.

No NOAEL was identified in the NTP, 2002 study for either the subchronic or chronic RfCs. Effects in this study were found at the lowest exposure concentrations measured, therefore, the LOAEL determined for this study for both subchronic and chronic effects does not indicate where a threshold of effects would lie and the data provided in the study is not sufficient for a dose response analysis. The data were amenable to BMD analysis and a BMCL was used so the UF for LOAEL to NOAEL extrapolation is not applicable. The selected critical effect was observed in longer term studies (3-months and 2-yrs) at similar doses and appeared to be a persistent and sensitive effect.

Data gaps have been identified that are associated with uncertainties specific to the developmental and reproductive toxicities of vanadium pentoxide following inhalation exposure. Studies that used intraperitoneal injection to study pregnant rats exposed to vanadium pentoxide suggest that exposure to the developing fetus may have adverse health effects. However, no inhalation studies have assessed the risk to the developing fetus. The database also does not

include a comprehensive multigenerational reproductive study to help establish the full range of developmental endpoints and thus represents a gap in the database. To account for the lack of inhalation developmental studies and for the lack of multigenerational reproductive toxicity studies to establish the range of toxicities across development, a full uncertainty factor of 10 was applied to the chronic RfC derivations.

Overall confidence in the chronic RfC is medium. Confidence in the principal study (NTP, 2002) is high. It is a well-conducted study that used rats and mice, an adequate number of animals, and exposure at a range of doses. Multiple toxicity endpoints were assessed. However, confidence in the overall database is medium. The NTP study is the only chronic animal study available. Recent mechanistic studies have investigated vanadium pentoxide-induced pulmonary fibrosis and have contributed to a better understanding of a putative mode of action for pulmonary fibrosis in response to vanadium pentoxide. Health effects reported among workers exposed to vanadium pentoxide and other vanadium compounds in dust are consistent with effects observed in experimental studies. Reflecting high confidence in the principal study and medium confidence in the database, confidence in the RfC is medium.

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 and equivocal evidence of carcinogenic activity of vanadium pentoxide in female F344/N rats at the high dose. Male and female B6C3F1 mice had even greater incidences of these lesions, with a statistically significantly increased incidence of alveolar/bronchiolar adenomas or carcinomas in both male and female B6C3F1 mice following inhalation exposure to ≥ 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. There are no human or laboratory animal data to determine the carcinogenicity of vanadium pentoxide by the oral or dermal route.

Table 5-8. Incidences of Respiratory Tumors in Mice Exposed to Vanadium Pentoxide in the 2 Year Inhalation Study (NTP, 2002)

	Exposure Group

Tumor Type[a]	Historical Control (% 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 ($p \leq 0.01$)

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 for the derivation of an inhalation unit risk, 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 mean body weights for males and females reported by NTP (2002) and the average particle size MMAD±GSD of 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).

Table 5-9: Human Equivalent Concentrations of Vanadium Pentoxide in the 2-Year Inhalation Studies (NTP, 2002)

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 were exposed to vanadium pentoxide 6 hours per day and 5 days per week.
[c] Please refer to Appendix Table C-4.
[d] HEC=Human Equivalent Concentration = "Concentration as Reported" * "Continuous Exposure Adjustment Factor " * "RDDR".

According to the U.S. EPA *Guidelines for Carcinogen Risk Assessment* (US 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.[10] Since all non-control concentrations from the NTP carcinogenesis studies (2002) showed a plateau response, the data set 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. Therefore, a BMR based on the response at the control concentration and the first non-control concentration was calculated (Table C-6), and then used for estimation of POD (BMCL, extra risk of 0.71 for male mice, 0.67 for female mice).

Modeling was performed using the Benchmark Dose Modeling Software (BMDS; Version 2.1.2) developed by the National Center for Environmental Assessment (U.S. EPA, 2000b). The incidence of alveolar/bronchiolar adenomas and carcinomas in mice were combined; males and females were modeled separately (Table 5-10).Models were run using the

[10] 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. However, if the POD is far below all observed data points, it can introduce model uncertainty and parameter uncertainty that increase with the distance between the data and the POD. Use of a POD at the lowest level supported by the data seeks to balance these considerations.

default restrictions on parameters built into the BMD software. Goodness-of-fit was evaluated using the Chi-square statistic calculated by the BMDS program. Acceptable global goodness of fit was a p-value greater than or equal to 0.1. Each data set was first fitted with the dichotomous multi-stage 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.

Table 5- 10. BMDS Modeling Results for Combined Incidence of Alveolar/Bronchiolar Adenoma and Carcinoma in Male Mice, NTP (2002).

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							
LogLogistic	**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 data set based on the draft Benchmark Dose Technical Guidance (2000).
[b] HEC=Human Equivalent Concentration.
[c] BMCL= the lower bound of BMC at 95% confidence level.

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 multi-stage 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 point of departure (POD) for derivation of the inhalation unit risk as as this was the only model fit when including all doses for analysis of lung tumor formation following inhalation exposure to vanadium pentoxide.

5.4.4 Inhalation Unit Risk

Data to support a mode of action for the carcinogenicity of vanadium pentoxide are insufficient, although some data suggest that either a mutagenic or a cytotoxic and reparative proliferation mode of action is operative. In the absence of such data, extrapolation from the point of departure to lower doses was conducted by using a linear approach.

The inhalation unit risk 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 B6C3F1 mice exposed to vanadium pentoxide results in an inhalation unit risk of 3.4 $(mg/m^3)^{-1}$. This value was derived by linear extrapolation to the origin from the point of departure 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 has engendered some uncertainty in the results. Several types of uncertainty may be considered quantitatively, but other important uncertainties cannot be considered quantitatively. 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, where the increased incidence of neoplasms did not exceed what would be expected due to spontaneous tumor formation. Similarly, spontaneous tumors were observed in male rats

at control levels, though tumor incidence increased in male rats in response to exposure to vanadium pentoxide. The confidence in the database is low. Respiratory tract carcinogenicity was reported in two rodent species (i.e., mice and rats) in awell-conducted NTP study (2002) and supported by a study in mice (Rondini et al., 2010). Human variability in response to vanadium pentoxide 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 mode of action information is available. Overall confidence in the inhalation unit risk is low.

Choice of low-dose extrapolation approach. The Mode of Action (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 that supports 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 mostly from +3 to +5 valence state. The carcinogenic potential of other valence states of vanadium has not been established. It is not known whether vanadium pentoxide dissociates to other valence states with known carcinogenic potential or whether some other valence state or some combination of +5 and other valence states is responsible for the observed toxicity. 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 point of departure. Parameter uncertainty can be assessed through 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, there is a reasonably small degree of uncertainty at a 71% increase in tumor incidence (the point of departure for linear low-dose extrapolation).

Bioassay selection. The study by NTP (2002) was selected for development of an inhalation unit risk. This was a well-designed study, conducted in 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 an 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 inhalation unit risk for vanadium pentoxide was quantified using the tumor incidence data for male mice which was more amenable to BMD modeling than tumor incidence in female mice. In addition, lung neoplasm incidence was reported in male and

female rats, though 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 may have revealed more information about 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). While these incidence response rates were higher in male mice than those of the females at the comparable exposure level, suggesting greater sensitivity of the male mice, there is no information concerning 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 may 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 inhalation unit risk was derived from the combined tumor incidence of lung adenomas and carcinomas in male mice. The information investigating the mode of action of the lung tumors observed in the chronic animal bioassay, however, is limited. The genotoxicity studies provide inadequate evidence of a genotoxic mode of action, and there are inadequate data to support alternative mode-of-action hypotheses.

Human population variability. The extent of inter-individual 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

Consideration/ Approach	Impact on inhalation unit risk	Decision	Justification
Low-dose extrapolation procedure	Departure from EPA's *Guidelines for Carcinogen Risk Assessment* POD paradigm, if justified, could ↓ or ↑ slope factor 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 exists 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 if MLE used rather than lower bound on POD	LEC (method for calculating reasonable upper bound slope factor)	Lower bound is 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	There are no MOA data to inform extrapolation approach for any choice. It was assumed that humans are as sensitive as the most sensitive rodent gender/species tested; true correspondence is unknown. The carcinogenic response occurs across genders and species.

Consideration/ Approach	Impact on inhalation unit risk	Decision	Justification
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 may be carcinogenic through an unknown mode of action.
Human population variability in metabolism and response/ sensitive subpopulations	Low-dose risk ↑ to an unknown extent	Considered qualitatively	No data to support 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.

Toxicokinetics of orally administered vanadium pentoxide is 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 tied 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 B6C3F1 mice (Radike et al., 2002). In this study, vanadium was detected in small intestine, kidney and the femur. Similarly, there are a few studies that evaluated the toxicokinetics of inhaled vanadium, but there are no data in 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 toxicokinetics studies of inhaled or intratracheally administered vanadium pentoxide in rats show that vanadium pentoxide is absorbed from the lung, undergoes a wide distribution to liver, kidney, bone, blood, gastrointestinal tract, and ovary, and is eliminated primarily in the urine (NTP 2002; Dill et al., 2004; 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 RBC and hemoglobin (Mountain et. al., 1953), and a variety of reproductive and developmental effects following exposure to vanadium pentoxide (Altamirano et. al., 1993; Zhang et. al., 1993).

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). However, the chemical composition of the fuel-oil ash and vanadium dust or exposure measurements for vanadium pentoxide 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 μ 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 two weeks post-exposure, but would reappear upon re-exposure. Of 100 workers occupationally exposed to vanadium pentoxide fume (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 dose of vanadium was 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 of boilermakers and utility workers involved in the overhaul of a large, oil-fired boiler over a six week period (Woodin et al., 1998, 1999, 2000). Geometric mean concentrations of vanadium (9-10 hour shifts) measured in the breathing zone ranged from 1.1 – 8.9 μg/m^3. Reductions in pulmonary function were measured among boilermakers with exposure to fly ash for weeks or years, however an association with vanadium has not been established (Woodin et al., 1999; Hauser et al., 1995a; 2001).There are no reported cases of cancer in workers examined over 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 (NTP, 2002; Knecht et. al., 1985, 1992), there are some animal studies documenting changes to spermatogenesis and testicular ultrastructural changes (Mussali-Galante et. al., 2005) and CNS effects (Colin-Barenque et. al., 2007; Avila-Costa et. al., 2004, 2005, 2006) in response to inhaled vanadium pentoxide. Following repeated inhalation to vanadium pentoxide, the respiratory system is the most sensitive target for noncancer toxicity in rats and mice. Short term inhalation exposure (13-days) in F344 rats and B6C3F1 mice was associated with histiocytic infiltration at 1.0 mg/m^3, and short-term and subchronic inhalation exposure (3 month) in F344 rats and B6C3F1 mice was associated with increased lung epithelial hyperplasia and inflammation at 2.0 mg/m^3 (NTP, 2002). Long term exposure (2yr) in F344 rats and B6C3F1 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 LOAEL of 1 mg/m^3 (NOAEL 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 three days caused a significant increase in alkaline phosphatase activity and DNA content in the diaphysis of femoral bones suggesting that vanadium pentoxide may 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 (0.02M or 1.4 mg V/m^3). Gamma tubulin was significantly decreased in testes exposed to vanadium pentoxide and may suggest changes in microtubule function that may 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 (0.02M or 1.4 mg V/m^3).

A number of intraperitoneal 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 CD1 mice (Altamirano-Lozano et. al., 1996). In developmental studies, reduced ossification in the developing fore and hindlimbs of fetuses have been reported in response to intraperitoneal 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, b). Other reported effects range from reduced fetal weight and increased placenta weight.

The genotoxicity database for vanadium pentoxide is limited. The evidence for genotoxicity in humans is limited. There are few studies examining genotoxicity in humans in vivo, with equivocal results. Ivancsits et. al. (2002) reported no differences in DNA strand breaks, oxidative damage, or sister chromatid exchange 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 (Roldan 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 intraperitoneal injection caused no treatment related effects in mitotic index, average generational time, or sister chromatid exchange (Altamirano-Lozano et. al., 1993). However, DNA damage was detected in six organs from vanadium pentoxide-treated mice via intraperitoneal injection (Altamirano-Lorano 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 micronuclei induction but did not produce chromosomal aberrations or sister chromatid exchange in various cell lines (Ivancsits et al. 2002; Kleinsasser et al. 2003; Ramirez et al. 1997; Rojas et al. 1996; Roldan and Altamirano 1990; Zhong et al., 1994).

Most of the 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., 1998, Bonner et. al., 2000). 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 (Bonner et. al., 1998, Rice et. al., 1999, Ingram et. al., 2003). It is possible that pulmonary fibrosis could be a key event leading to eventual tumorigenesis.

Vanadium pentoxide is *"likely carcinogenic to humans"* by the inhalation route of exposure under the *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a). Existing cancer data comes 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. There is evidence of carcinogenicity in male rats exposed to vanadium pentoxide for 2 years, as respiratory tumor incidence exceeded that in historical controls. In female rats, the incidence of respiratory tumorigenesis in response to vanadium pentoxide did not exceed historical controls. Tumor incidence has not been documented in humans.

Information available on the carcinogenic effects of V_2O_5 via the inhalation route is limited to examination of the respiratory tumors. Information on the carcinogenic effects of V_2O_5 via the oral and dermal routes in humans or animals is absent. Based on the observance of only respiratory tumors following inhalation exposure, and in the absence of information to establish a mode of action, this cancer descriptor applies only to the inhalation route of exposure. Therefore, the database has *"inadequate information to assess carcinogenic potential"* of V_2O_5 via the oral or dermal route.

The mode of action underlying tumorigenicity in rats and mice has not been established. The genotoxicity database for vanadium pentoxide is equivocal, including both positive and negative studies for mutation, DNA damage and chromosomal aberrations. A nongenotoxic mode of action 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. However, the dose-response relationship is not robust and a clear relationship linking these effects to the tumor response has not been established. It is unknown whether cytotoxicity may be a required precursor event for vanadium pentoxide-induced cell proliferation. 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 was 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 between animals, therefore the continuous data was not amenable to BMD modeling. Derivation of an RfD was based on a NOAEL of 10.5 mg/kg-d for the critical effect decreased RBC counts and divided by a total UF of 3000, 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 subchronic to chronic study and 10 for database deficiencies to arrive at 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), limited endpoints and time points. Confidence in the critical effect is medium. Hematological effects have been documented in other rodent species in response to inhaled vanadium pentoxide (NTP, 2002). However, confidence in the overall database 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 RfC. Two laryngeal lesions were selected for modeling because they had the lowest Regional Deposited Dose Ratio (RDDR) value and corresponding $BMCL_{[HEC]}$. In accordance with U.S. EPA Benchmark Dose methodology (2000b), 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 Criteria (AIC) value was considered to provide a superior fit. Benchmark dose 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 to arrive at the chronic RfC of 1 x 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, a large sample size of two species of rodents, using both genders, numerous endpoints and time points. Confidence in the critical effect is high. Pulmonary effects have been documented in numerous species, including humans and primates, in response to inhaled vanadium pentoxide. Laryngeal lesions are a relevant proximal portal of entry target tissue for inhalation exposure and serves as an indicator of vanadium pentoxide-induced pulmonary injury. However, confidence in the overall database 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

There are no studies identifying cancer effects in 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 V_2O_5 via the inhalation route is limited to examination of the respiratory tumors. Information on the carcinogenic effects of V_2O_5 via the oral and dermal routes in humans or animals is absent. Based on the observance of only respiratory tumors following inhalation exposure, and in the absence of information to establish a mode of action, this cancer descriptor applies only to the inhalation route of exposure. Therefore, the database has *"inadequate information to assess carcinogenic potential"* of V_2O_5 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 for vanadium pentoxide. The calculated cancer inhalation unit risk for vanadium pentoxide is 3.4 ug/m^3 for the development of alveolar/bronchiolar adenomas or carcinomas in male B6C3F1 mice. This value was derived from BMCL10, the 95% lower bound on the dose associated with 71% extra cancer risk of

respiratory carcinoma in male B6C3F1 mice, by dividing the BMR (0.71) by the BMCL10, and represents the upper bound, continuous lifetime exposure estimate of cancer potency. The BMCL10, lower 95% bound on exposure at 71% risk, is 2.08 x 10^{-1} mg/m^3 and the slope of the linear extrapolation from the BMCL to the origin = 0.71/2.08 x 10^{-1} mg/m^3 = 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 supports any specific mode of carcinogenic action of vanadium pentoxide. Therefore, the derived inhalation unit risk 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; however, it is unknown how well this model or the linear low-dose extrapolation predicts low-dose risks for vanadium pentoxide. The selected model, while the best fitting model, is not the only model that adequately describes the data. Other models could conceivably be selected to yield different results consistent with the observed data, both higher and lower than those included in this assessment. The human equivalent inhalation unit risks 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 inhalation unit risk.

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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 RfC for vanadium pentoxide, inhalation toxicity effects observed in rats (NTP, 2002) were modeled to estimate the candidate PODs. Five endpoints in both male and female rats (Table B-2) were selected because of 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 data set was fitted with the dichotomous models available in EPA BMDS (version 2.1.2). Following the model selection steps outlined in the draft Benchmark Dose Technical Guidance (US EPA, 2000b), the best-fitting model for each data set was used to estimate the candidate POD, which was the BMCL at the selected BMR as 10% extra risk.

The highlights of the benchmark dose modeling results are:

- The POD value of 0.003 mg/m^3, from two endpoints in female rats, was the lowest POD derived and used for the calculation of 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.

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	LogLogistic	0.1	0.017	0.012
Respiratory Epithelium, Epiglottis, Hyperplasia	LogLogistic	0.1	0.008	0.006
Nose				
Goblet Cell, Respiratory Epithelium, Hyperplasia	LogLogistic	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	LogLogistic	0.1	0.005	0.003
Respiratory Epithelium, Epiglottis, Hyperplasia	LogLogistic	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 data set based on the draft Benchmark Dose Technical Guidance (2000).

[b] HEC=Human Equivalent Concentration.

[c] BMCL= the lower bound of BMC at 95% confidence level.

B.1. TREND TESTS ON THE INHALATION DATA SETS FOR BMDS MODELING

Ten inhalation toxicology data sets in rats were selected because of the lower concentrations of vanadium pentoxide administrated (as compared to mice), the significantly 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 data sets (Table B-2) before modeling.

Table B-2: Trend Tests on the Selected Data Sets from the 2-Year Inhalation Studies in Rats (NTP, 2002)

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
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 data set was calculated by combining the incidences of alveolar epithelium hyperplasia and bronchiole epithelium hyperplasia.

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 before any modeling and extrapolation.

First, the average life time animal body weights of rats were estimated based on the mean body weight at different weeks (Table B-3). Secondly, following the Methods for Derivation of

Inhalation Reference Concentrations and Application of Inhalation Dosimetry (US EPA, 1994b), RDDRs (regional deposited dose ratios) 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. Then, the human equivalent concentration 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 Life Time Animal Body Weight of Rats in the 2-Year Inhalation Studies of Vanadium Pentoxide (NTP, 2002)

Vanadium Pentoxide Concentration as Reported (mg/m^3)	Mean Animal Body Weight [a] (g)			Average Life Time Animal Body Weight [b] (g)
	1-13 weeks	14-52 weeks	53-104 weeks	
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 Life Time 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

Table B-4: RDDRs for Different Concentration/Sex Group in the 2-Year Inhalation Studies of Vanadium Pentoxide (NTP, 2002)

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 life time 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 GDSs reported for 2-year studies (NTP, 2002) .

[d] RDDR = regional deposited dose ratio; calculated with the RDDR program (V.2.3. US EPA);

Table B-5: Human Equivalent Concentrations of Vanadium Pentoxide in the 2-Year Inhalation Studies (NTP, 2002)

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 and 5 days per week.

[c] Please refer to Appendix Table B-4.

[d] "Human Equivalent Concentration" = "Concentration as Reported" * "Continuous Exposure Adjustment Factor " * "RDDR"

B.3. BMDS MODELING FOR INHALATION DATA SETS

Each selected inhalation data set was fitted 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 (US EPA, 2000b), 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.

Data Set 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 these 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 *Z* score as 8.83 and one-sided *p*-value as <0.0001.

Seven dichotomous models available in EPA BMDS (version 2.1.2.) were fitted to this data set. 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, 2000b), the Probit model was selected as the model for POD computation. The $BMCL_{10}$ of this model was 0.013 mg/m^3 and regarded as one of the candidate PODs.

Table B-6: Benchmark Modeling Results for Incidence of Lung Alveolar Epithelium Hyperplasia in Male Rats, NTP (2002)

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] (Stage3)	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
LogProbit	0.06	175.1	-1.31	0.67		0.026	0.016
LogLogistic	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.

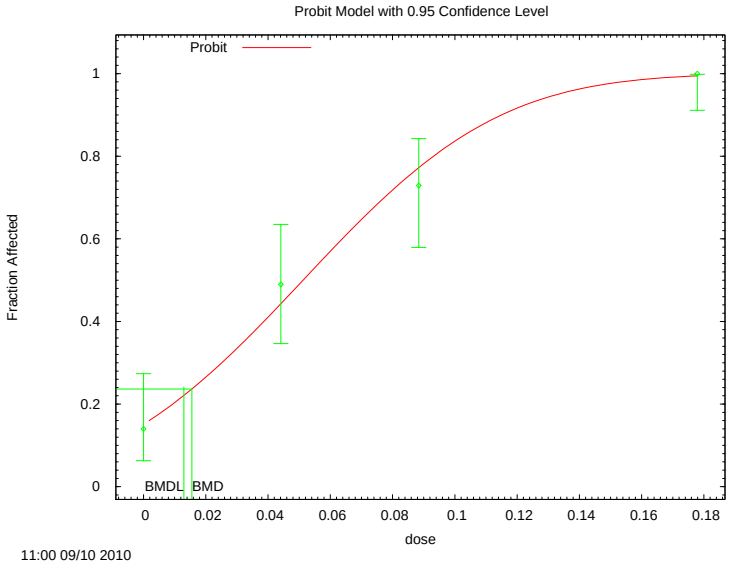
[c] HEC=Human Equivalent Concentration.

[d] BMCL= the lower bound of BMC at 95% confidence level.

[e] For Multistage model, up to 3 stages were tested. Only the model with the lowest AIC and lowest stage was reported here.

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
                                                        95.0% Wald Confidence Interval
      Variable         Estimate        Std. Err.     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
                                                                    Scaled
    Dose     Est._Prob.    Expected    Observed     Size       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
```


Probit Model with 0.95 Confidence Level
Probit
Fraction Affected
1
0.8
0.6
0.4
0.2
0
BMDL
BMD
0
0.02
0.04
0.06
0.08
0.1
0.12
0.14
0.16
0.18
dose
11:00 09/10 2010

Data Set 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 difference 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 *Z* score as 8.36 and one-sided *p*-value as <0.0001.

Seven dichotomous models available in EPA BMDS (version 2.1.2.) were fitted to this data set. 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 (US EPA, 2000b), the Logistic model was selected as the model for POD computation. The $BMCL_{10}$ of this model was 0.029 mg/m^3 and regarded as one of the candidate PODs.

Table B-7: Benchmark Modeling Results for Incidence of Lung Chronic Active Inflammation in Male Rats, NTP (2002)

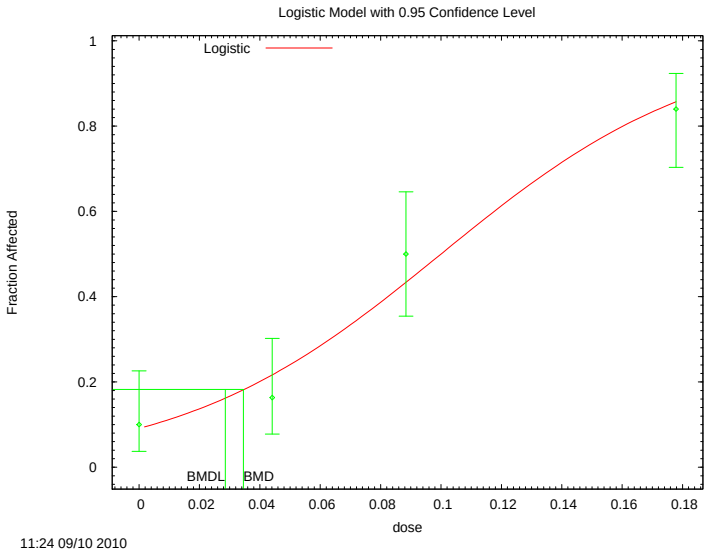
Model [a]	Goodness of Fit				BMR	HEC [c]	
	P-Value	AIC [b]	Largest Scaled Residual	Scaled Residual of Interest		BMC (mg/m^3)	BMCL [d] (mg/m^3)
Logistic	**0.40**	**192.5**	**-0.92**	**-0.92**	Extra Risk 10%	**0.035**	**0.029**
Probit	0.37	192.7	-1.00	-1.00		0.032	0.027
LogProbit	0.67	192.8	0.29	-0.26		0.046	0.032
LogLogistic	0.60	192.9	-0.34	-0.34		0.045	0.031
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] (Stage2)	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 Multistage model, up to 3 stages were tested. Only the model with the lowest AIC and lowest stage was reported here.

BMDS output file

==========

```
         Logistic Model. (Version: 2.13; Date: 10/28/2009)
         Input Data File:
C:\USEPA\BMDS212\Data\V2O5\NTP_2002NonCancerMaleRats\MaleRatsLungChronicActiveInflammation\NTP_20
02_Lung Chronic Active Inflammation_Logistic_0.1.(d)
         Gnuplot Plotting File:
C:\USEPA\BMDS212\Data\V2O5\NTP_2002NonCancerMaleRats\MaleRatsLungChronicActiveInflammation\NTP_20
02_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
```



Data Set 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 difference 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 *Z* score as 4.79 and one-sided *p*-value as <0.0001.

Seven dichotomous models available in EPA BMDS (version 2.1.2.) were fitted to this data set. 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 (US EPA, 2000b), the LogLogistic model was selected as the model for POD computation. The $BMCL_{10}$ of this model was 0.012 mg/m^3 and regarded as one of the candidate PODs.

Table B-8: Benchmark Modeling Results for Incidence of Larynx Chronic Inflammation in Male Rats, NTP (2002)

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	**0.12**	**229.1**	**1.63**	**-0.27**	Extra Risk 10%	**0.017**	**0.012**
LogProbit	0.09	229.8	-1.37	-0.01		0.005	0.000
Multistage [e] (Stage1)	0.05	230.7	2.14	-0.65		0.023	0.017

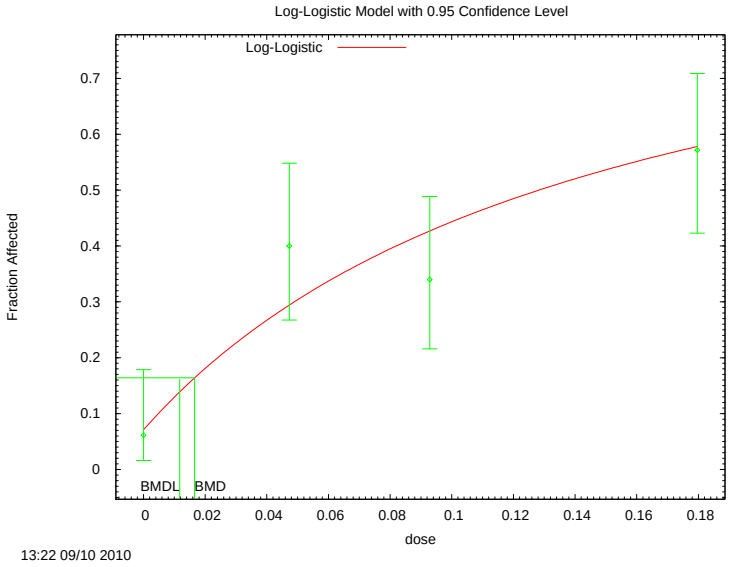
Gamma							
Weibull							
Probit	0.01	234.8	2.45	2.45		0.043	0.036
Logistic	0.01	235.2	2.43	2.43		0.046	0.038

[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 Multistage model, up to 3 stages were tested. Only the model with the lowest AIC and lowest stage was reported here.

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_L
arynx Chronic Inflammation_LogLogistic_0.1.(d)
         Gnuplot Plotting File:
C:\USEPA\BMDS212\Data\V2O5\NTP_2002NonCancerMaleRats\MaleRatsLarynxChronicInflammation\NTP_2002_L
arynx 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
```



Data Set 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 difference 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 *Z* score as -6.56 and one-sided *p*-value as <0.0001.

Seven dichotomous models available in EPA BMDS (version 2.1.2.) were fitted to this data set. 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 LogLogistic and LogProbit models demonstrated adequate goodness of fit *p*-value ≥ 0.1 and good visual fit, but the BMC/BMCL ratio in LogProbit was infinite, which was not acceptable. Based on the draft Benchmark Dose Technical Guidance (US EPA, 2000b), the LogLogistic model was selected as the model for POD computation. The $BMCL_{10}$ of this model was 0.006 mg/m^3 and regarded as one of the candidate PODs.

Table B-9: Benchmark Modeling Results for Incidence of Larynx Respiratory Epithelium, Epiglottis, Hyperplasia in Male Rats, NTP (2002)

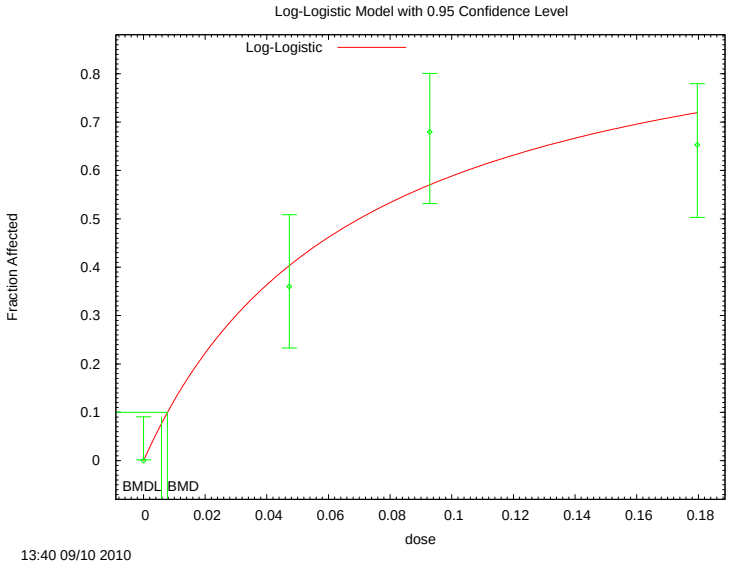
Model [a]	Goodness of Fit				BMR	HEC [c]	
	P-Value	AIC [b]	Largest Scaled Residual	Scaled Residual of Interest		BMC (mg/m^3)	BMCL [d] (mg/m^3)
LogLogistic	**0.27**	**197.3**	**1.56**	**0.000**	Extra Risk 10%	**0.008**	**0.006**
LogProbit	0.14	199.3	1.60	0.000		0.007	0.000
Gamma	0.04	201.5	-2.12	0.000		0.013	0.010
Multistage [e] (Stage1)							
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

[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 3 stages were tested. Only the model with the lowest AIC and lowest stage was reported here.

BMDS output file

```
 ================================================================
         Logistic Model. (Version: 2.13; Date: 10/28/2009)
         Input Data File:
C:\USEPA\BMDS212\Data\V2O5\NTP_2002NonCancerMaleRats\MaleRatsLarynxEpiglottisHyperplasia\NTP_2002
_Larynx Epiglottis Hyperplasia_LogLogistic_0.1.(d)
         Gnuplot Plotting File:
C:\USEPA\BMDS212\Data\V2O5\NTP_2002NonCancerMaleRats\MaleRatsLarynxEpiglottisHyperplasia\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
```



Data Set 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 difference from the control group as reported. The severity increase slightly as reported when the

concentration of vanadium pentoxide increased. The Cochran-Armitage test confirmed the statistically significant trend with *Z* score as 2.68 and one-sided *p*-value as 0.0037.

Seven dichotomous models available in EPA BMDS (version 2.1.2.) were fitted to this data set. 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 LogProbit model failed to compute a reasonable BMCL. Based on the draft Benchmark Dose Technical Guidance (U.S. EPA, 2000), the LogLogistic model was selected as the model for POD computation. The $BMCL_{10}$ of this model was 0.026 mg/m^3 and regarded as the one of the candidate PODs.

Table B-10: Benchmark Modeling Results for Incidence of Nose Goblet Cell, Respiratory Epithelium, Hyperplasia in Male Rats, NTP (2002)

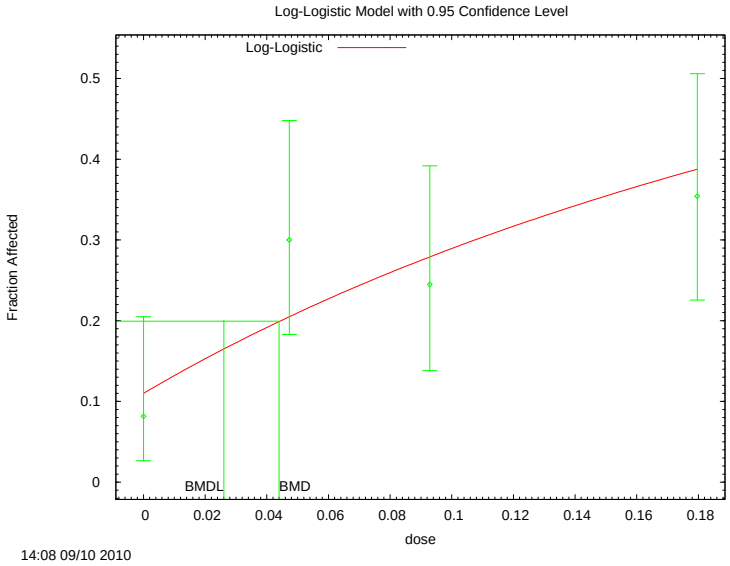
Model [a]	Goodness of Fit				BMR	HEC [c]	
	P-Value	AIC [b]	Largest Scaled Residual	Scaled Residual of Interest		BMC (mg/m^3)	BMCL [d] (mg/m^3)
LogLogistic	**0.16**	**213.2**	**1.66**	**1.656**	Extra Risk 10%	**0.044**	**0.026**
LogProbit	0.31	212.8	-0.83	-0.003		0.002	failed
Gamma	0.12	213.7	1.75	1.75		0.052	0.033
Multistage [e] (Stage1)							
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 Multistage model, up to 3 stages were tested. Only the model with the lowest AIC and lowest stage was reported here.

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_La
rynx Goblet Hyperplasia _LogLogistic_0.1.(d)
         Gnuplot Plotting File:
C:\USEPA\BMDS212\Data\V2O5\NTP_2002NonCancerMaleRats\MaleRatsNoseGobletEpiHyperplasia\NTP_2002_La
rynx 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
```



Data Set 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 difference 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 *Z* score as 9.53 and one-sided *p*-value as <0.0001.

Seven dichotomous models available in EPA BMDS (version 2.1.2.) were fitted to this data set. 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 LogLogistic model did not reelect in the observed data. Based on the draft Benchmark Dose Technical Guidance (US EPA, 2000b), the Gamma model was selected as the model for POD computation. The $BMCL_{10}$ of this model was 0.063 mg/m^3 and regarded as one of the candidate PODs.

Table B-11: Benchmark Modeling Results for Incidence of Lung Alveolar Epithelium Hyperplasia in Female Rats, NTP (2002)

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**
LogLogistic	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
LogProbit	0.78	157.9	0.20	0.00		0.085	0.068
Multistage (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 Multistage model, up to 3 stages were tested. Only the model with the lowest AIC and lowest stage was reported here.

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_L
ung Alveolar Bronchiole Combined Epithelium Hyperplasia_Gamma_0.1.(d)
         Gnuplot Plotting File:
C:\USEPA\BMDS212\Data\V2O5\NTP_2002NonCancerFemaleRats\FemaleRatsLungAlBrEpHyperplasia\NTP_2002_L
ung 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
```

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval 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

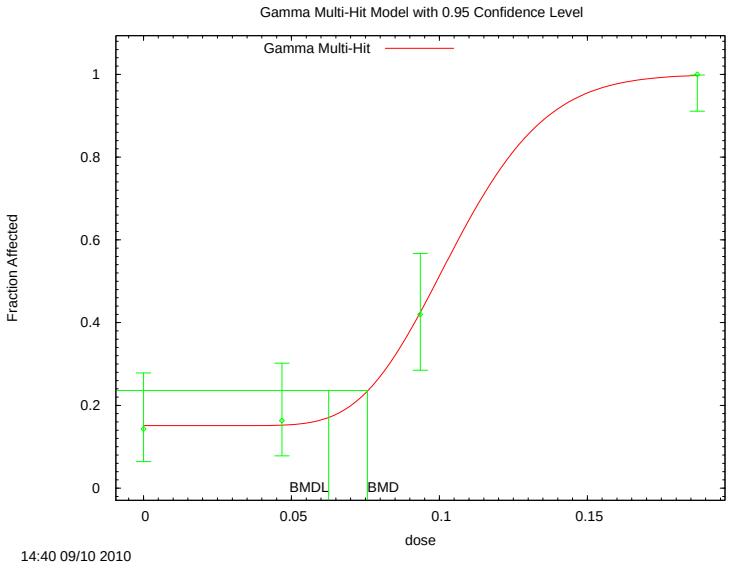
AIC: 156.194

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled 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



Data Set 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 *Z* score as 6.71 and one-sided *p*-value as <0.0001.

Seven dichotomous models available in EPA BMDS (version 2.1.2.) were fitted to this data set. 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 (US EPA, 2000b), the Multistage (Stage3) model was selected as the model for POD computation. The $BMCL_{10}$ of this model was 0.048 mg/m^3 and regarded as one of the candidate PODs.

Table B-12: Benchmark Modeling Results for Incidence of Lung Chronic Active Inflammation in Female Rats, NTP (2002)

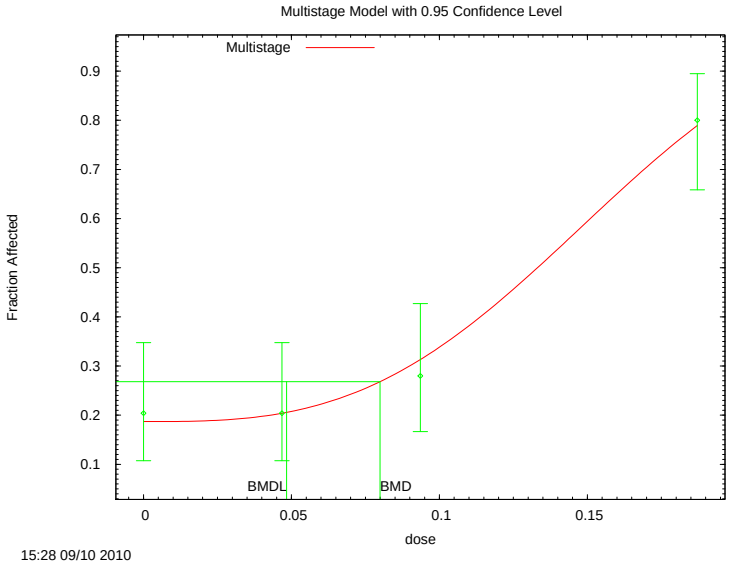
Model [a]	Goodness of Fit				BMR	HEC [c]	
	P-Value	AIC [b]	Largest Scaled Residual	Scaled Residual of Interest		BMC (mg/m^3)	BMCL [d] (mg/m^3)
Multistage [e] (Stage3)	**0.82**	**212.9**	**-0.51**	**-0.51**	Extra Risk 10%	**0.080**	**0.048**
LogProbit	1.00	214.5	0.00	0.00		0.094	0.068
Gamma	0.99	214.5	-0.01	0.00		0.094	0.063
LogLogistic	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.
[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 3 stages were tested. Only the model with the lowest AIC and lowest stage was reported here.

BMDS output file

```
 ================================================================
         Multistage Model. (Version: 3.2;  Date: 05/26/2010)
         Input Data File:
C:\USEPA\BMDS212\Data\V2O5\NTP_2002NonCancerFemaleRats\FemaleRatsLungChronicActiveInflammation\NT
P_2002_Lung Chronic Active Inflammation_Multi3_0.1.(d)
         Gnuplot Plotting File:
C:\USEPA\BMDS212\Data\V2O5\NTP_2002NonCancerFemaleRats\FemaleRatsLungChronicActiveInflammation\NT
P_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
                                                        95.0% Wald Confidence Interval
      Variable         Estimate        Std. Err.     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
                                                                    Scaled
     Dose     Est._Prob.    Expected    Observed     Size       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
```



Data Set 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 *Z* score as 5.38 and one-sided *p*-value as <0.0001.

Seven dichotomous models available in EPA BMDS (version 2.1.2.) were fitted to this data set. 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 (US EPA, 2000b), the LogLogistic model was selected as the model for POD computation. The $BMCL_{10}$ of this model was 0.003 mg/m^3 and regarded as one of the candidate PODs.

Table B-13: Benchmark Modeling Results for Incidence of Larynx Chronic Inflammation in Female Rats, NTP (2002)

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	**0.46**	**242.0**	**-0.88**	**-0.13**	Extra Risk 10%	**0.005**	**0.003**
LogProbit	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)							

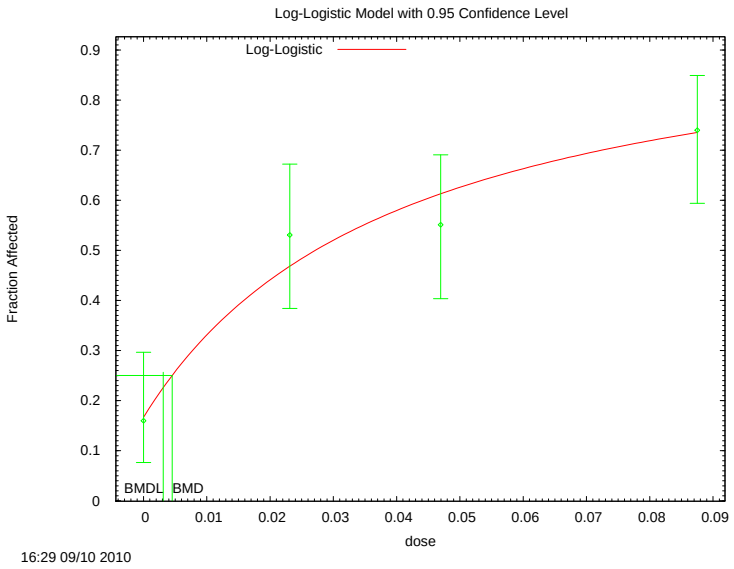
Weibull							
Probit	0.03	247.5	1.99	1.99		0.013	0.011
Logistic	0.03	247.6	1.97	1.97		0.014	0.011

[a] Selected (best-fitting) model shown in first row, in boldface type.
[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 3 stages were tested. Only the model with the lowest AIC and lowest stage was reported here.

BMDS output file

```
 ================================================================
         Logistic Model. (Version: 2.13; Date: 10/28/2009)
         Input Data File:
C:\USEPA\BMDS212\Data\V2O5\NTP_2002NonCancerFemaleRats\FemaleRatsLarynxChronicInflammation\NTP_20
02_Larynx Chronice Inflammation_LogLogistic_0.1.(d)
         Gnuplot Plotting File:
C:\USEPA\BMDS212\Data\V2O5\NTP_2002NonCancerFemaleRats\FemaleRatsLarynxChronicInflammation\NTP_20
02_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
```



Data Set 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 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 *Z* score as -5.98 and one-sided *p*-value as <0.0001.

Seven dichotomous models available in EPA BMDS (version 2.1.2.) were fitted to this data set. 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 LogLogistic and LogProbit models demonstrated adequate goodness of fit *p*-value ≥ 0.1 and good visual fit, but LogProbit model failed to compute a reasonable BMCL. Based on the draft Benchmark Dose Technical Guidance (U.S. EPA, 2000), the LogLogistic model was selected as the model for POD computation. The $BMCL_{10}$ of this model was 0.003 mg/m^3 and regarded as one of the candidate PODs.

Table B-14: Benchmark Modeling Results for Incidence of Larynx Respiratory Epithelium, Epiglottis, Hyperplasia in Female Rats, NTP (2002)

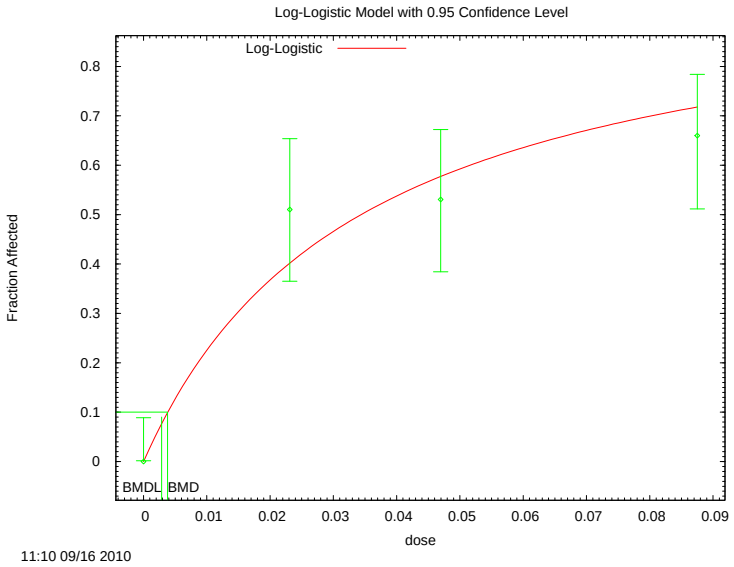
Model [a]	Goodness of Fit				BMR	HEC [c]	
	P-Value	AIC [b]	Largest Scaled Residual	Scaled Residual of Interest		BMC (mg/m^3)	BMCL [d] (mg/m^3)
LogLogistic	**0.30**	**205.3**	**1.53**	**0.000**	Extra Risk 10%	**0.004**	**0.003**
LogProbit	0.78	204.2	-0.57	0.000		0.000	failed
Gamma	0.01	212.3	2.85	0.000		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 3 stages were tested. Only the model with the lowest AIC and lowest stage was reported here.

BMDS output file

```
================================================================
        Logistic Model. (Version: 2.13; Date: 10/28/2009)
        Input Data File:
C:\USEPA\BMDS212\Data\V2O5\NTP_2002NonCancerFemaleRats\FemaleRatsLarynxEpiglottisHyperplasia\NTP_
2002_Larynx Epiglottis Hyperplasia_LogLogistic_0.1.(d)
        Gnuplot Plotting File:
C:\USEPA\BMDS212\Data\V2O5\NTP_2002NonCancerFemaleRats\FemaleRatsLarynxEpiglottisHyperplasia\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
                                                                      Scaled
     Dose      Est._Prob.     Expected     Observed      Size       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
```



Data Set 10: Incidence of Nose Goblet Cell, Respiratory Epithelium, Hyperplasia in Female Rats, NTP (2002)

Summary

One concentration groups (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 *Z* score as 3.46 and one-sided *p*-value as 0.0003.

Seven dichotomous models available in EPA BMDS (version 2.1.2.) were fitted to this data set. 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 (US EPA, 2000b), the Multistage (Stage2) model was selected as the model for POD computation. The $BMCL_{10}$ of this model was 0.014 mg/m^3 and regarded as one of the candidate PODs.

Table B-15: Benchmark Modeling Results for Incidence of Larynx Respiratory Epithelium, Epiglottis, Hyperplasia in Female Rats, NTP (2002)

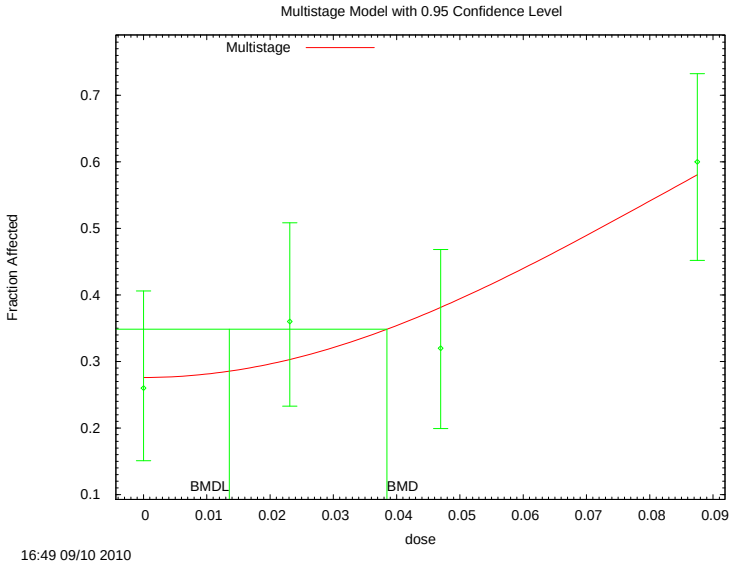
Model [a]	Goodness of Fit				BMR	HEC [c]	
	P-Value	AIC [b]	Largest Scaled Residual	Scaled Residual of Interest		BMC (mg/m^3)	BMCL [d] (mg/m^3)
Multistage (Stage2)	**0.43**	**258.3**	**-0.90**	**-0.896**	Extra Risk 10%	**0.038**	**0.014**
Logistic	0.33	258.9	-1.252	0.586		0.024	0.018
Probit	0.32	258.9	-1.273	0.564		0.023	0.018
Weibull (Quantal Linear)	0.22	259.7	-1.45	0.337		0.019	0.012
LogLogistic	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
LogProbit	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 Multistage model, up to 3 stages were tested. Only the model with the lowest AIC and lowest stage was reported here.

BMDS output file

```
 ================================================================
         Multistage Model. (Version: 3.2;  Date: 05/26/2010)
         Input Data File:
C:\USEPA\BMDS212\Data\V2O5\NTP_2002NonCancerFemaleRats\FemaleRatsNoseGobletEpiHyperplasia\NTP_200
2_Nose Goblet Hyperplasia_Multi2_0.1.(d)
         Gnuplot Plotting File:
C:\USEPA\BMDS212\Data\V2O5\NTP_2002NonCancerFemaleRats\FemaleRatsNoseGobletEpiHyperplasia\NTP_200
2_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
```


Multistage Model with 0.95 Confidence Level
Multistage
Fraction Affected
0.7
0.6
0.5
0.4
0.3
0.2
0.1
BMDL
BMD
0
0.01
0.02
0.03
0.04
0.05
0.06
0.07
0.08
0.09
dose
16:49 09/10 2010

APPENDIX C - BENCHMARK CONCENTRATION MODELING OF COMBINED LUNG ALVEOLAR AND BRONCHIOLAR TUMOR DATA SETS FROM NTP STUDIES (2002)

To derive the cancer slope factor of vanadium pentoxide, combined alveolar/bronchiolar adenoma and carcinoma data sets in B6C3F1 mice (Table C-2) by NTP (2002) were selected because of their biological and statistical significance.

The highlights of the benchmark concentration modeling results are:

- Cancer slope factor:

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

- BMDS modeling:

Each data set was firstly fitted with the recommended dichotomous model, multistage-cancer model, through EPA BMDS (version 2.1.2); if the goodness of fit *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 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 data set to estimate the candidate POD, which was the BMCL at the pre-determined BMR.

- BMR:

Since all the non-control concentrations showed essentially a plateau response, the tumor data sets provided limited information about the concentration-response relationship. So, BMR for each data set was calculated based on the response at the control and the first non-control concentration groups.

- Multistage Weibull (MSW) time-to-tumor modeling:

Since the survival curves reported by NTP (2002) showed a high percentage of deaths (22-46%) at the end of 2-year studies across the different concentrations groups, MSW time-to-tumor model was also used with these data sets. However, no adequate fit was noticed for either male or female mice data set.

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$_1$ Mice	0.71	0.360	0.208	3.4
Female B6C3F$_1$ Mice	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.

C.1. TREND TESTS BEFORE MODELING

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

Table C-2: Trend Tests on the Inhalation Carcinogenesis Data Sets in Mice from the 2-Year Inhalation Studies (NTP, 2002)

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 and/or carcinoma)/(animal sample size). Only the animals alive at 52 weeks or when the first tumor appeared, whichever was earlier, was counted toward the sample size.

C.2. DOSE CONVERSION

In the carcinogenesis studies reported by NTP (2002), mice (B6C3F$_1$) were exposed to

vanadium pentoxide through inhalation. To analyze the concentration response effect, the reported concentrations of vanadium pentoxide were converted to human equivalent concentrations before any modeling and extrapolation.

Firstly, the average life time animal body weights of rats were estimated based on the mean body weight at different weeks (Table C-3). Secondly, following the US EPA inhalation methods (1994), RDDRs (regional deposited dose ratio) were calculated with the RDDR program designed by US EPA (1994) and summarized in Table C-4. Then, the human equivalent concentration for each concentration/sex group was calculated from the reported concentration in mice by multiply the continuous exposure adjustment factor and the pulmonary RDDR (Table C-5).

Table C-3: Average Life Time Animal Body Weight of Mice for 2-Year Inhalation Studies of Vanadium Pentoxide (NTP, 2002)

Vanadium Pentoxide Concentration as Reported (mg/m^3)	Mean Animal Body Weight [a] (g)			Average Life Time 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 Life Time 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

Table C-4: RDDRs for Different Concentration/Sex Group of Mice in the 2-Year Inhalation Studies of Vanadium Pentoxide (NTP, 2002)

Vanadium Pentoxide Concentration as Reported (mg/m^3)	Average Life Time 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 life time 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 GDSs reported for 2-year studies (NTP, 2002) .
[d] RDDR = regional deposited dose ratio; calculated with the RDDR program from USA EPA (1994);
[e] Since the average life time animal body weight was out of the mice weight range (17-46g) accepted by RDDR program (V.2.3, US EPA), 46g was used to calculate the corresponding RDDRs.

Table C-5: Human Equivalent Concentrations of Vanadium Pentoxide in the 2-Year Inhalation Studies (NTP, 2002)

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 were exposed to vanadium pentoxide 6 hours per day and 5 days per week.
[c] Please refer to Appendix Table C-4.

[d] HEC=Human Equivalent Concentration = "Concentration as Reported" * "Continuous Exposure Adjustment Factor " * "RDDR"

C.3. BMR CALCULATION

Since all non-control concentrations from the NTP carcinogenesis studies (2002) showed essentially a plateau response, the data set 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, thus the BMD may be just below the first dose or orders of magnitudes lower. So, a BMR estimated based on the response at the control concentration and the first non-control concentration was calculated (Table C-6), and then used for estimation of BMCL.

Table C-6: BMR Estimation for Male and Female Mice Data Sets.

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 1st non-control concentration group.
[c] Calculated BMR = [P(1st Non-control Concentration) - P(Control)]/[1- P(Control)].

C.4. BMDS MODELING FOR INHALATION CARCINOGENESIS DATA SETS

Each data set was firstly fitted with dichotomous multistage-cancer model provided in EPA BMDS (version 2.1.2); if the goodness of fit *p*-value < 0.05, other dichotomous models available in EPA BMDS (version 2.1.2) were fitted; 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 data set to estimate the candidate POD, which was the BMCL at the calculated BMR for each data set.

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

Lung Tumor Data Set 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 *Z* score as 4.14 and one-sided *p*-value as <0.0001.

Because all the non-control 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 C-8.

Since 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 fitted to this data set. Two models demonstrated adequate goodness of fit *p*-value ≥ 0.05 and good visual fit, but LogProbit model failed to compute a reasonable BMCL. Based on the draft Benchmark Dose Technical Guidance (U.S. EPA, 2000), the LogLogistic model was selected and $BMCL_{71}$ of this model was 0.208 mg/m^3.

Further BMD modeling was performed for comparison purposes. Since the primary cancer model (Multistage-cancer) did not show adequate fit with all doses, the highest concentration was dropped for further modeling. After dropping the high dose, the primary cancer model (Multistage-cancer) 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, NTP (2002).

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							
LogLogistic	**0.19**	**200.632**	**-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 data set based on the draft Benchmark Dose Technical Guidance (2000).
[b] HEC=Human Equivalent Concentration.
[c] BMCL= the lower bound of BMC at 95% confidence level.

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_MaleMiceTum
       or4DosesAllModels_LogLogistic_0.71.(d)
        Gnuplot Plotting File:
       C:\USEPA\BMDS212\Data\V2O5\NTP_2002Cancer\MaleMiceTumor4DosesAllModel\NTP_2002_MaleMiceTum
       or4DosesAllModels_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
```

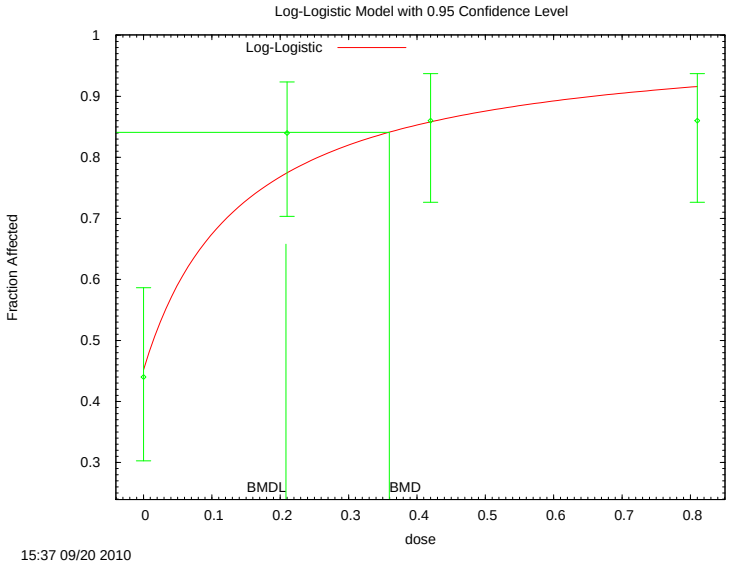
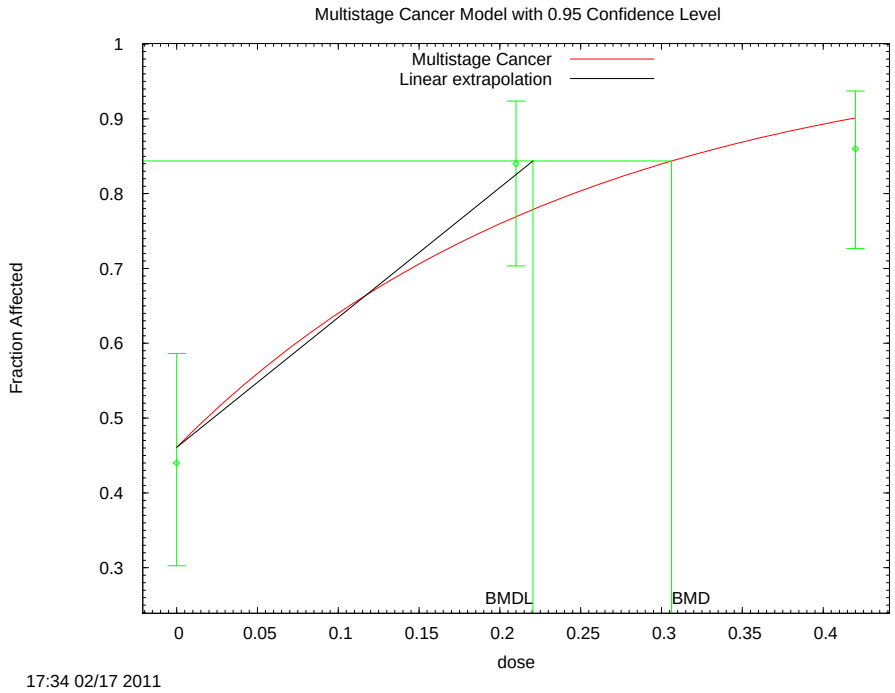


Table C- 8. BMDS Modeling Results for Combined Incidence of Alveolar/Bronchiolar Adenoma and Carcinoma in Male Mice after Dropping the Highest Concentration, NTP (2002).

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							
LogLogistic	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
LogProbit	NA	159.1		0.00		0.190	failed

[a] Selected model is the best-fitting model for the data set based on the draft Benchmark Dose Technical Guidance (2000).
[b] HEC=Human Equivalent Concentration.
[c] BMCL= the lower bound of BMC at 95% confidence level.

```
================================================================
         Multistage Cancer Model. (Version: 1.9;  Date: 05/26/2010)
         Input Data File:
C:\USEPA\BMDS212\Data\V2O5\NTP_2002Cancer\MaleMiceTumor3DosesAllModel\NTP_2002_MaleMiceTumor3Dose
sAllModels_MultiCanc1_0.71.(d)
         Gnuplot Plotting File:
C:\USEPA\BMDS212\Data\V2O5\NTP_2002Cancer\MaleMiceTumor3DosesAllModel\NTP_2002_MaleMiceTumor3Dose
sAllModels_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
```



Lung Tumor Data Set 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 *Z* score as 5.19 and one-sided *p*-value as <0.0001.

Because all the non-control 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 C-10.

Since 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 fitted to this data set. Only one model demonstrated adequate goodness of fit *p*-value ≥ 0.05, however, it failed to compute BMC and BMCL. So, the highest concentration was dropped for further modeling.

After dropping the highest dose, the primary cancer model (Multistage-cancer) still did not show an adequate fit. Then six other dichotomous models available in EPA BMDS (version 2.1.2.) were tried. Only the LogLogistc 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 LogLogistic model was selected and $BMCL_{67}$ of this model 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, NTP (2002).

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	0.00	218.8	-3.90	1.33	Extra Risk 67%	0.435	0.359
Other Dichotomous Models							
LogProbit	0.72	192.6	0.62	-999.00	Extra Risk 67%	Failed [d]	Failed [d]
LogLogistic	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 data set based on the draft Benchmark Dose Technical Guidance (2000).
[b] HEC=Human Equivalent Concentration.
[c] BMCL= the lower bound of BMC at 95% confidence level.

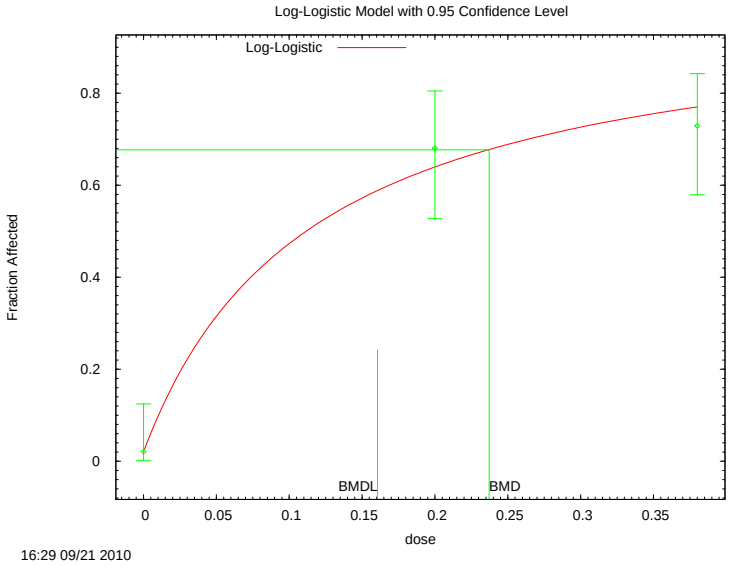
Table C- 10. BMDS Modeling Results for Combined Incidence of Alveolar/Bronchiolar Adenoma and Carcinoma in Female Mice after Dropping the Highest Concentration, NTP (2002).

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	0.06	132.2	1.42	1.42	Extra Risk 67%	0.264	0.213
Other Dichotomous Models							
LogLogistic	**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
LogProbit	NA	130.7	0.00	0.00		0.190	failed

[a] Selected model is the best-fitting model for the data set based on the draft Benchmark Dose Technical Guidance (2000).
[b] HEC=Human Equivalent Concentration.
[c] BMCL= the lower bound of BMC at 95% confidence level.

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_FemaleMiceTumor
3DosesAllModels_LogLogistic_0.67.(d)
         Gnuplot Plotting File:
C:\USEPA\BMDS212\Data\V2O5\NTP_2002Cancer\FemaleMiceTumor3DosesAllModels\NTP_2002_FemaleMiceTumor
3DosesAllModels_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.5. 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 POD. The inhalation cancer slope factor was the upper-bound estimation of risk and calculated as BMR/BMCL. It was used to estimate the lifetime lung tumor risk in human for vanadium pentoxide exposure through inhalation. Data are summarized in Table C-1.

C.6. MSW TIME-TO-TUMOR MODELING OF THE INHALATION CARCINOGENESIS DATA SETS

Multistage Weibull Time-to-tumor modeling was also used to model these data sets 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 concentrations groups. The individual animal data was obtained through the NTP website and summarized according to concentration and week of death (Table C-11).

Based on the results, MSW time-to-tumor model was not recommended to derive cancer slope factor for these data sets because of two reasons:

- The visual fit near the 1st non-control 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 non-convergence.

.

Lung Tumor Data Set 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 (NTP, 2002).

Human Equivalent Concentration (mg/m^3)	Week of Death	Response Category for Alveolar/Bronchiolar Adenoma and/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

Table C- 11. Grouped Data for MSW Time-to-tumor Modeling; $B6C3F_1$ Male Mice Exposed to Vanadium Pentoxide by Inhalation for 2 Years (NTP, 2002). (Continued)

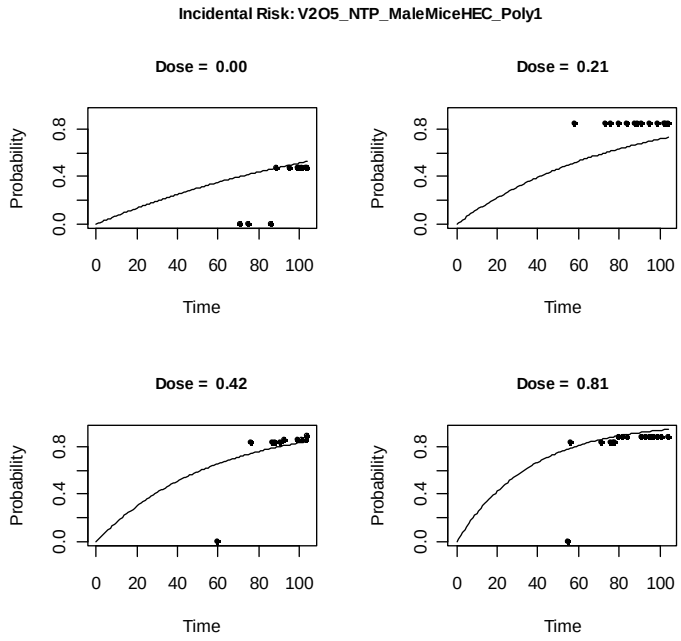
Human Equivalent Concentration (mg/m^3)	Week of Death	Response Category for Alveolar/Bronchiolar Adenoma and/or Carcinoma[b]	Number of Animals
0.42	36	C	1
	39	C	1
	40	I	1
	45	I	1
	50	C	1
	59	C	1
	70	C	1
	72	I	1
	76	I	1
	82	C	1
	83	I	1
	91	I	1
	92	I	1
	95	I	1
	95	C	1
	99	I	1
	101	I	2
	101	C	1
	104	I	7
	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] Concentration was 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 and/or adenoma were detected when the subjected was removed from the study due to scheduled sacrifice or unscheduled death.

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
```



Lung Tumor Data Set 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 (NTP, 2002).

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

Table C-12 . Grouped Data for MSW Time-to-tumor Modeling; B6C3F$_1$ Female Mice Exposed to Vanadium Pentoxide by Inhalation for 2 Years (NTP, 2002). (Continued)

Human Equivalent Concentration (mg/m^3)	Week of Death	Response Category for Alveolar/Bronchiolar Adenoma and/or Carcinoma [b]	Number of Animals
0.38	36	C	1
	39	C	1
	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
0.73	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 and/or adenoma were detected when the subjected was removed from the study due to scheduled sacrifice or unscheduled death.

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
                        CONTEXT
              C      F      I      U  Total  Expected Response
    DOSE
        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
```