FINAL

REVIEWER COMMENTS

External Peer Review on the *Toxicological Review of Vanadium Pentoxide (V_2O_5)* (CASRN 1314-62-1)

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I. INTRODUCTION

EPA is seeking an external peer review of the draft "Toxicological Review of Vanadium Pentoxide (V_2O_5)" that will appear on the Agency's online database, the Integrated Risk Information System (IRIS). IRIS is an EPA database, prepared and maintained by the EPA's National Center for Environmental Assessment (NCEA) within the Office of Research and Development (ORD), which contains potential adverse human health effects that may result from chronic (or lifetime) exposure, or in select cases less-than-lifetime exposures, to chemicals in the environment. IRIS currently provides health effects information on over 500 chemical substances.

The IRIS program has developed a "Toxicological Review of Vanadium Pentoxide (V_2O_5)," which updates an assessment that was posted to the IRIS database in 1987, which contained an oral reference dose (RfD). The draft document has undergone EPA review for scientific accuracy and compliance with EPA risk assessment guidelines and procedures; the next step in the process is an external peer review. The external review draft "Toxicological Review of Vanadium Pentoxide (V_2O_5)" includes an RfD, an inhalation reference concentration (RfC), and a qualitative cancer descriptor and quantitative cancer risk value for the inhalation route of exposure (inhalation unit risk).

Peer Reviewers:

Mitchell D. Cohen, Ph.D.

Dr. Cohen, who served as Chair for this Peer Review Meeting, is Associate Professor in the Department of Environmental Medicine at the NYU School of Medicine (NYUSOM). He received his Ph.D. in Toxicology from the University of Florida (UF; Department of Nutrition) in 1988, before that, earning an M.S. in Toxicology (Department of Nutrition) at UF and a B.S. in Chemistry/Physics from SUNY Albany. Prior to coming to NYUSOM as a Postdoctoral Fellow, Dr. Cohen was a USDA Research Fellow at UF. At NYUSOM, Dr. Cohen's research has focused on the: immunotoxic potentials of inhaled metals in the lungs; effects of World Trade Center dusts on pulmonary biology/immunology, and potential mechanisms that have given rise to the increased incidence of airway hyper-responsivity and/or sarcoid-like granulomatous pulmonary disease in First Responders; effects on particulate matter on pulmonary resistance; in situ effects of ambient non-carcinogen oxidants on epigenetic effects of co-inhaled metal carcinogens; and effects from inhaled gases, metals, and mixtures on lung macrophage-cytokine interactions and resulting inducible cellular activities. Dr. Cohen currently serves as a Co-Chair of the Organizing Committee for the upcoming 8th International Vanadium Symposium (V8). Dr. Cohen also serves on the Editorial Board of several journals, including Inhalation Toxicology, Journal of Toxicology and Environmental Health, and Toxicology and Applied Pharmacology, and is the Editor-in-Chief (and Founder) of the Journal of Immunotoxicology. He has also acted as referee for > 30 other journals. Dr. Cohen's current professional memberships include the Society of Leukocyte Biology, the Society of Toxicology (Inhalation Toxicology and Immunotoxicology Specialty Sections), and the American Chemical Society.

Max Costa, Ph.D.

Dr. Costa currently serves as a Professor and Chairman at New York University School of Medicine's Department of Environmental Medicine. He is also the Director of the Nelson Institute of Environmental Medicine, the Program Leader for Environmental and Molecular Carcinogenesis NYU Cancer Center, the Director of the Center of Excellence in Environmental Health Sciences from NIEHS, and the Director of the NYU Superfund Program Project Grant funded by NIEHS. Dr. Costa received his Doctorate in Pharmacology from the University of Arizona, School of Medicine in 1976 and conducted postgraduate training as a Research Associate in the school's Division of Radiation Oncology. His research at NYU focuses on the molecular mechanisms of metal carcinogenesis. Dr. Costa has been an invited speaker at over 100 conferences and has authored over 340 papers and book chapters. Recently, he was the recipient of the Lifetime Achievement award from the Metals Specialty Section of the Society for Toxicology. Dr. Costa is currently a Member of the IUPAC Subcommittee on Environmental and Occupational Toxicology of Nickel, the ICOH Scientific Committee on the Toxicology of Metals, and the Organizing Committee for the International Association of Environmental and Analytical Chemistry. He serves on the Board of Associate Editors for *Toxicology and Applied Pharmacology* and on the Editorial Advisory Board of *Chemistry Central Journal* and serves as a Reviewer for the Agency for Toxic Substances and Disease Registry's "Toxicology Profiles." Dr. Costa is a member of the American Association for the Advancement of Science, the American Society for Pharmacology and Experimental Therapeutics, the Society for Toxicology, and the American Association for Cancer Research.

Ralph L. Kodell, Ph.D.

Dr. Kodell is a Professor in the Department of Biostatistics at the University of Arkansas for Medical Sciences. He received his Doctorate in Statistics from Texas A&M University in 1974, before that, receiving an M.S. and B.S. in Mathematics from Stephen F. Austin State University and University of the Ozarks, respectively. Before coming to the University of Arkansas for Medical Sciences as a Professor, Dr. Kodell served as Mathematical Statistician and Director of the Division of Biometry and Risk Assessment at the National Center for Toxicological Research. He also previously served as a Visiting Research Associate at Harvard School of Public Health's Department of Biostatistics. In the course of his career, Dr. Kodell has published over 150 articles in peer reviewed journals; the focus of most of his recent research and publications has been quantitative risk assessment and biostatistics modeling methods. Dr. Kodell previously served as the Chair of the Dose-Response Specialty Group, Society for Risk Analysis and has acted as the Associate Editor for several journals, including the *Journal of the American Statistical Association, Environmental and Ecological Statistics, the Journal of Agricultural, Biological and Environmental Statistics*, and others. Dr. Kodell's current professional memberships include the American Statistical Association (fellow), the International Biometric Society, the Society for Risk Analysis, and the Academy of Toxicological Sciences (elected).

Craig C. McLauchlan, Ph.D.

Dr. McLauchlan is currently an Associate Professor in the Department of Chemistry at Illinois State University. Dr. McLauchlan received his Doctorate in Inorganic Chemistry in 2000 from Northwestern University and served as a Postdoctoral Research Fellow at Harvard University from 2000 until 2002. He began at Illinois State as an Assistant Professor in 2002 and was promoted to Associate Professor in 2008. As part of a sabbatical leave in 2009, Dr. McLauchlan served as a Visiting Scholar at the University of Delaware, in its Department of Chemistry and Biochemistry. His current funded research focuses on applications of vanadium coordination chemistry, while his other research interests include inorganic and organometallic synthesis, chalcogenides, bio-mimetic model complexes, coordination chemistry, chemical education, X-ray crystallography, and air-sensitive manipulations. To date he has over twenty-five peer-reviewed publications on his research with his student co-workers. Dr. McLauchlan currently serves as a Co-Chair of the Organizing Committee for the upcoming 8th International Vanadium Symposium (V8), in addition to serving as the Treasurer and Webmaster for the American Chemical Society's Illinois Heartland Local Section. Dr. McLauchlan served as an External Program Reviewer for Northeastern Illinois University's Chemistry Program in 2010 and has also reviewed over a dozen chemistry textbooks and grant proposals for, inter alia, the National Science Foundation and the American Chemical Society. Dr. McLauchlan's professional memberships include the American Chemical Society, the American Association for the Advancement of Science, Sigma Xi, the National Science Teachers Association, the American Crystallographic Association, and others.

Xianglin Shi, Ph.D.

Dr. Shi is currently a Professor and the William A. Marquard Chair in Cancer Research at the University of Kentucky. Dr. Shi also serves as the Associate Director for Cancer Prevention and Environmental Toxicology at the University's Markey Cancer Center, as well as an Expert Consultant for the Pathology and Physiology Research Branch of NIOSH. Dr. Shi received his doctorate in Chemistry from West Virginia University, before then earning a M.Sc. in Nuclear Physics/Chemistry and a BS in Chemistry from the Chinese Academy of Sciences and Zhumadian Teachers College, respectively. Prior to joining the University of Kentucky as a Professor, Dr. Shi served as a Team Leader, Oxidative stress and Inflammation Team, the Pathology and Physiology Research Branch at NIOSH, the Director of the Institute for Nutritional Sciences, Chinese Academy of Sciences, as an Adjunct Associate Professor at the University of West Virginia, and as an NIH Intramural Research Fellow at the National Cancer Institute. Dr. Shi has over 370 publications, and his currently funded research includes the following focuses: reactive oxygen species in Ni(II) carcinogenesis; UV-induced carcinogenesis, prevention/protection by cyaniding-3-glucoside and quercitrin; GSK3beta and ethanol neurotoxicity; HIF-1alpha in Cr(IV) carcinogenesis; mechanisms of MnSOD expression (in normal and tumor cells); mechanism of arsenic-induced cell transformation; and cell survival and arsenic carcinogenesis. Dr. Shi has served as the Organizer for the Fourth, Fifth, and Sixth Conference on Molecular Mechanisms of Metal Toxicity and Carcinogenicity and he currently serves as a Member of the Science Advisory Board for West Virginia University's Cancer Center and Toxicology Excellence for Risk Assessment (TERA), as well as the Federal Food Quality Protection Act (FQPA) Science Review Board. Dr. Shi serves as an Associate Editor for the *Journal of Environmental Pathology* and has served as a Guest Editor for several other

scientific journals, including *Molecular and Cellular Biochemistry* and *Toxicology and Oncology*.

Qunwei Zhang, MD, MPH, PhD

Dr. Zhang is currently an Associate Professor in the Department of Environmental and Occupational Health Sciences at the University of Louisville's School of Public Health and Information Science. Dr. Zhang also serves as an Associate Scientist in the James Graham Brown Cancer Center at the University of Louisville. He holds a Medical Degree from Fujian Medical University and an MPH from Zhejiang University. Dr. Zhang also earned a Ph.D. in Medical Science from Japan's University of Fukui, School of Medicine. Prior to coming to the University of Louisville, Dr. Zhang served as a Research Associate at the NYU School of Medicine and the Institute for Environmental Medicine at the University of Pennsylvania. Dr. Zhang's major scientific interests include nanotoxicology; pulmonary toxicology; metal toxicology, genotoxicology, and carcinogenesis; lung ischemia and ion channels; free radicals and pulmonary disease; and gene expression and function. His funded research focuses on the health effects of nanomaterials and the activation of endothelial cells and gene expression in lungs following exposure to ultrafine particles. Dr. Zhang has served as a reviewer for over a dozen journals, including *Toxicology, Toxicology and Applied Pharmacology, Nanotoxicology, Environmental Health Perspectives,* and others. His current professional memberships include the Society for Toxicology, the American Thoracic Society, and the Kentucky Public Health Association.

Yiliang Zhu, Ph.D.

Dr. Zhu is currently Professor in the Department of Epidemiology and Biostatistics, College of Public Health and Department of Internal Medicine, Morsani College of Medicine at the University of South Florida, where he also serves as Director of the Center for Collaborative Research and Biostatistics Ph.D. program. Dr. Zhu received his Doctorate in Statistics from the University of Toronto in 1992, and conducted postdoctoral research in environmental health at Health Canada's Environmental Health Center. Dr. Zhu's current research ranges from quantitative methods for health risk assessment (e.g. PBPK/PD modeling, dose-response assessment, and uncertainty analysis), to spatiotemporal modeling of ambient air PM25 speciation, healthcare outcome evaluation, and global health. Dr. Zhu has developed statistical software, one of which (NEUROBMD) is currently being implemented as a module of the U.S. EPA's BMDS software. Dr. Zhu has been a member of various national Academies committees, including currently the NRC Committee *Science for EPA's Future,* IOM/NRC committee on Shipboard Hazard and Defense, as well as NAS/NRC committees on dioxin and formaldehyde. He also served on numerous independent panels to evaluate health risk assessment documents for IRIS, NIOSH/CDC and the Federal Consumer Product Safety Commission. Dr. Zhu is currently on the Editorial Board of *International Urogynecology Journal* and serves regularly as a reviewer for over a dozen other scientific journals including *Biometrics, Risk Analysis, Regulatory Toxicology and Pharmacology,* and *Environmental Health Perspectives.* He is a member of the Society of Risk Analysis, the Society of Advanced Disease Surveillance, the Institute of Mathematical Statistics, the American Statistical Association, the International Chinese Statistical Association, and the International Biometrics Society.

II. CHARGE TO REVIEWERS

Below is a set of charge questions that address scientific issues in the draft "Toxicological Review of Vanadium Pentoxide (V_2O_5)." Please provide detailed explanations for responses to the charge questions. EPA will also consider reviewer comments on other major scientific issues specific to the hazard identification and dose-response assessment of vanadium pentoxide. Please identify and provide the rationale for approaches to resolve these issues where possible. Please consider the accuracy, objectivity, and transparency of EPA's analyses and conclusions in your review.

General Charge Questions:

1. Is the Toxicological Review logical, clear and concise? Has EPA clearly presented and synthesized the scientific evidence for noncancer and cancer health effects of vanadium pentoxide?

2. Please identify any additional peer-reviewed studies from the primary literature that should be considered in the assessment of noncancer and cancer health effects of vanadium pentoxide.

Chemical-Specific Charge Questions:

(A) Oral reference dose (RfD) for vanadium pentoxide

1. A subchronic oral dietary study in Wistar rats (Mountain et al., 1953) was selected as the basis for the derivation of the RfD. Please comment on whether the selection of this study is scientifically supported and clearly described. If a different study is recommended as the basis for the RfD, please identify this study and provide scientific support for this choice.

2. A decrease in red blood cell count in male Wistar rats was concluded by EPA to be an adverse effect and was selected as the critical effect for the RfD. Please comment on whether the selection of this critical effect and its characterization is scientifically supported and clearly described. If a different endpoint is recommended as the critical effect for deriving the RfD, please identify this effect and provide scientific support for this choice.

3. The NOAEL/LOAEL approach was used in conjunction with dosimetric adjustments for calculating the human equivalent dose (HED) to identify the POD for derivation of the RfD. Please comment on whether this approach is scientifically supported and clearly described.

4. Please comment on the rationale for the selection of the uncertainty factors (UFs) applied to the POD for the derivation of the RfD. Are the UFs appropriate based on the recommendations described in *A Review of the Reference Dose and Reference Concentration Processes* (U.S. EPA, 2002; Section 4.4.5) and clearly described? If changes to the selected UFs are proposed, please identify and provide scientific support for the proposed changes.

(B) Inhalation reference concentration (RfC) for vanadium pentoxide

1. A two-year inhalation bioassay of vanadium pentoxide in F344/N rats (NTP, 2002) was selected as the basis for the derivation of the RfC. Please comment on whether the selection of this study is scientifically supported and clearly described. If a different study is recommended as the basis for the RfC, please identify this study and provide scientific support for this choice.

2. An increase in laryngeal inflammation in female F344/N rats was concluded by EPA to be an adverse effect and was selected as the critical effect for the RfC. Please comment on whether the selection of this critical effect and its characterization is scientifically supported and clearly described. If a different endpoint is recommended as the critical effect for deriving the RfC, please identify this effect and provide scientific support for this choice.

3. Benchmark dose (BMD) modeling was conducted using the incidence of laryngeal inflammation in female F344/N rats in conjunction with dosimetric adjustments for calculating the human equivalent concentration (HEC) to estimate the point of departure (POD) for derivation of the RfC. Has the modeling been appropriately conducted and clearly described based on EPA's draft *Benchmark Dose Technical Guidance Document* (U.S. EPA, 2000)? Is the choice of the benchmark response (BMR) for use in deriving the POD (i.e., a BMR of 10% extra risk of the incidence of laryngeal inflammation) supported and clearly described?

4. Please comment on the rationale for the selection of the UFs applied to the POD for the derivation of the RfC. Are the UFs appropriate based on the recommendations described in *A Review of the Reference Dose and Reference Concentration Processes* (U.S. EPA, 2002; Section 4.4.5) and clearly described? If changes to the selected UFs are proposed, please identify and provide scientific support for the proposed changes.

(C) Carcinogenicity of vanadium pentoxide

1. Under the EPA's *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005; www.epa.gov/iris/backgrd.html), the draft "Toxicological Review of Vanadium Pentoxide (V_2O_5)" characterizes vanadium pentoxide as "likely to be carcinogenic to humans" by the inhalation route of exposure. Please comment on whether this characterization of the human cancer potential of vanadium pentoxide is scientifically supported and clearly described.

2. The draft "Toxicological Review of Vanadium Pentoxide (V_2O_5)" concludes that there is insufficient information to identify the mode(s) of carcinogenic action. Please comment on whether this determination is appropriate and clearly described. If it is judged that a mode of action can be established for vanadium pentoxide, please identify the mode of action and its scientific support (i.e., studies that support the key events, and specific data available to inform the shape of the exposure-response curve at low doses).

Oral Slope Factor (OSF)

3. The draft "Toxicological Review of Vanadium Pentoxide (V_2O_5)" did not derive an OSF due to lack of available studies. Are there available data to support the derivation of an OSF for vanadium pentoxide? If so, please identify these data.

Inhalation Unit Risk (IUR)

4. A two-year inhalation bioassay of vanadium pentoxide in B6C3F1 mice (NTP, 2002) was selected as the basis for the derivation of the inhalation unit risk (IUR). Please comment on whether the selection of this study is scientifically supported and clearly described. If a different study is recommended as the basis for the IUR, please identify this study and provide scientific support for this choice.

5. The incidence of alveolar/bronchiolar adenomas or carcinomas in B6C3F1 male mice was selected to serve as the basis for the quantitative inhalation cancer assessment. Please comment on whether this selection is scientifically supported and clearly described. If a different cancer endpoint is recommended for deriving the IUR, please identify this endpoint and provide scientific support for this choice.

6. Benchmark dose (BMD) modeling was conducted using the incidence of alveolar/bronchiolar adenomas or carcinomas in male B6C3F1 mice in conjunction with dosimetric adjustments for calculating the human equivalent concentration (HEC) to estimate the point of departure (POD). A linear low-dose extrapolation from this POD was performed to derive the IUR. Has the modeling been appropriately conducted and clearly described based on EPA's draft *Benchmark Dose Technical Guidance Document* (U.S. EPA, 2000)? Has the choice of the benchmark response (BMR) for use in deriving the POD (i.e., a BMR of 71% extra risk of the incidence of alveolar/bronchiolar adenomas or carcinomas in male mice) been supported and clearly described?

III. GENERAL IMPRESSIONS

Mitchell D. Cohen

The *Toxicological Review of Vanadium Pentoxide (V_2O_5)* is logical, clear, and for the most part, concise. The document provides Readers with an up-to-date overview of what is known about: chemical and physical characteristics of V_2O_5; the paucity of information regarding toxicokinetics of how V_2O_5 is handled (i.e., absorption, distribution, metabolism, elimination) following human (occupational exposure scenarios) or animal model exposure; critical hazard identification information based on case reports, epidemiology, and clinical studies in humans as well as acute, subchronic, and chronic exposure(s) of animal models; both cancer and non-cancer effects from exposure(s) to V_2O_5; dose-response assessments (where available); and, potential mechanisms of action (MOA) as they pertain to specific portal-of-entry effects. Overall, the EPA has clearly, objectively represented and synthesized the limited scientific evidence for the non-cancer/cancer hazards from exposure to V_2O_5. For the most part, the majority of conclusions reported in the Profile appear sound. However, some conclusions need to be discussed further. There are also a few questions as to the 'appropriateness' of inclusion of a lot of information (primarily toward the earlier part of the document) about various other non-V_2O_5 pentavalent vanadium/vanadium-bearing agents (e.g., vanadates, PM, ROFA) as these obscure the focus of the report and have a potential to confuse Readers due to their complex mixture natures and chemistries. Once these points have been resolved by the 2012 Peer Review and any recommended changes made to the document, it is certain that a stronger Profile will be ready for release.

Max Costa

There was quite a bit of redundancy in the writing of this document. The same study was discussed numerous times and it is quite boring for the reader to have to look at what is essentially the same information from the same study over and over again. Pentavalent Vanadate was the only compound considered in the study since most experiments were conducted with this compound. There were essentially no human epidemiological studies examining health effects of V_20_5 by ingestion and inhalation; but, mouse studies, and to a smaller extent, rat inhalation studies, demonstrate that it is carcinogenic by the inhalation route of exposure. Reference doses for humans were calculated for both inhalation exposure and ingestion and they seem to be reliable numbers. However it is hard to understand how one can extrapolate from the NTP data since it goes from about 10% incidence to 72% at the first dose. This type of plateau data is difficult to work with. I agree with most of the conclusions of the authors of this document, except with the use of the NTP data to extrapolate to lower levels. How can this be done when all the doses give the same cancer incidence? There is a need to conduct more studies on V_20_5, especially epidemiological studies and human cell culture work.

Ralph L. Kodell

I believe that the information has been presented accurately for the most part, but I think the presentation needs to be clearer in certain places, and I question some of the interpretations and conclusions. I provide details of my concerns in my responses to the charge questions.

Craig C. McLauchlan

The Toxicological Review was easy to read, albeit repetitive, and appears accurate in its presentation of the material. As a non-toxicologist, this was appreciated. The document is clear and all conclusions include support for those conclusions and references to the studies and data that inform the decisions. As a chemist, the largest issue throughout is that the review may not actually be a toxicological review of V_2O_5 specifically, but of vanadium and vanadium oxides in general. For example, in pages 7-11, there are many references to "vanadium" and it is not clear if/that we are discussing V_2O_5 anymore, but vanadium and any of its compounds/complexes in general. That lack of clarity and several seemingly contradictory statements about the scope of the review undermine the document. The document needs to be clearer about what is being reviewed – if it is V_2O_5 only, much of the content becomes less relevant; if it is vanadium and vanadium oxides in general, more attention to the vast vanadium oxide literature must be given.

Xianglin Shi

Overall, the Toxicological Review is logical, clear, and concise. The Review covers a broad spectrum of literature. Key scientific issues were nicely summarized. The toxic effects of vanadium pentoxide are clearly presented and are very well supported by the scientific evidence. The carcinogenicity classification of vanadium pentoxide as "likely to be carcinogenic to humans" is adequately discussed and supported by the available animal data. The major supporting data are those from the chronic two-year study by NTP. This is the only major chronic carcinogenicity study. It is appropriate to derive the classification of vanadium pentoxide as "likely to be carcinogenic to humans" based on this study.

The following issues should be taken into consideration when evaluating the possible human carcinogenicity of vanadium pentoxide:

(a) It should be noted that Cr(VI) and arsenic are human carcinogens. For example, human exposure to these metals causes cancer of the lung. However, it is very difficult for these metals to induce lung cancer in mice or rats. Thus, whether mice or rats are a good model to evaluate human carcinogenesis of vanadium pentoxide needs to be considered.
(b) Oxidative stress is important in all stages of carcinogenesis. Similar to carcinogenic metals, Cr(VI) and arsenic, a vanadium(5+)-containing compound is able to generate reactive oxygen species (ROS) and cause oxidative stress. There is a fairly large amount of information indicating that ROS generated by vanadium(5+)-containing species play an important role in the health effects of vanadium(5+)-containing species. These species may cause oxidative stress, activation of oncogenic transformation factors, and certain oncogenic signaling proteins. These species increase the secretion of inflammation related proteins, such as TNF-α and Cox-2. The consequence of these ROS-responses will be the creation of an inflammatory tumor microenvironment, leading to cell neoplastic transformation and tumorigenesis. This aspect should be adequately discussed.
(c) Vanadium(V)-containing compounds are phosphatase inhibitors. These compounds inhibit various phosphatases, leading to alternations of cellular signaling transduction pathways and cell functions.
(d) Once entering the body, vanadium pentoxide will be changed into a different chemical structure and will be reduced to various oxidation states.

Qunwei Zhang

The toxicological review of vanadium pentoxide is logical, clear and concise. The EPA has clearly presented and synthesized the scientific evidence for both non-cancer and cancer of vanadium pentoxide. It is a high-quality evidence-based report.

Yiliang Zhu

The EPA's Toxicological Review of Vanadium Pentoxide (the Review) is a well-structured report, containing a comprehensive literature review that is up to date. The information provided in the Review is, for the most part, accurate and clear. The principle studies, endpoints, and methods used for deriving the RfD and RfC are reasonable, although there are issues that warrant further investigation and analysis, especially regarding the unit cancer risk estimate. Some of the issues arising from this Review also reflect IRIS risk assessment in general.

The first issue pertains to a systematic review of the literature. (See also comments in response to General Charge Questions 1 and 2). Systematic Review has become a standard in medicine and health, and is increasingly applied in other fields when it comes to synthesis of a large body of knowledge and evidence across different fields and across heterogeneous databases. Synthesis of scientific evidence is at the core of health risk assessment. EPA can benefit greatly by using systematic review to reduce the chance of unintentional omissions and to avoid "cherry-picking" which can be perceived otherwise. Systematic Review also enhances the transparency of EPA's risk assessment process.

The second issue is analysis of uncertainty. The Review, in fact, devotes Section 5.3 (for RfD and RfC) and Section 5.4.6 (for unit cancer risk) to discuss uncertainties. This discussion is very helpful, despite the fact it remains largely qualitative, compartmentalized with respect to the components of risk assessment, and somewhat superficial. To EPA's credit, this Review explores multiple endpoints in conjunction with multiple concentration-response models to demonstrate a range of the point of departure (POD) based on the BMD approach to RfC. Adopting the same approach to uncertainty quantification in other parts of the risk assessment would have strengthened this Review to a greater degree. For example, EPA could specify *minimum* and *preferred* criteria for study/endpoint inclusion, and advance *all* studies and endpoints that meet the inclusion criteria for concentration-response analysis and estimation of RfC, RfD or unit cancer risk. Such an approach would allow for the presentation of a range of variation in the final risk estimates; this range, in turn, serves as a basis for determining a sensible point of departure for RfC, RfD or unit risk. This approach can effectively separate risk management decision making from risk assessment itself. Although the current IRIS guideline has not explicitly dictated such an approach (involving quantification of uncertainty), EPA has increasingly emphasized the integration of uncertainty analysis in IRIS risk assessment. EPA should take an anticipatory and proactive position in adopting and practicing uncertainty quantification in its current IRIS documents, when feasible. A good case in point is the derivation of RfC of neurotoxicity of tetrachloroethylene (NRC, 2009). Additional comments are in my responses to the specific Charge Questions.

IV. RESPONSE TO CHARGE QUESTIONS

General Charge Questions

Question 1. Is the Toxicological Review logical, clear and concise? Has EPA clearly presented and synthesized the scientific evidence for noncancer and cancer health effects of vanadium pentoxide?

Mitchell D. Cohen

The Review is logical, clear and, for the most part, concise. The latter at times comes into question, as there are repetitions of sentences/paragraphs/points in differing sub-sections of the document. This Reviewer is certain that whether this is a (legal) requirement of this type of document or editorial oversight will be made clear to the Reviewers. In addition, there is an open question as to the 'appropriateness' of inclusion of much information (primarily in the earlier part of the document) about various other non-V_2O_5 pentavalent vanadium/vanadium-bearing agents (e.g., vanadates, PM, ROFA) as these tend to obscure the focus of the report and have a potential to confuse potential Readers.

Overall, EPA has clearly and objectively represented and synthesized the scientific evidence for non-cancer/cancer hazards from exposure to V_2O_5. The degree of accuracy will be left to the Reviewers to finalize based on comments of each Reviewer.

Max Costa

Except for being highly repetitious in discussing the very few animal studies that exist on this compound, the review presents and synthesizes scientific evidence for non-cancer and cancer health effects. It is problematic to use the NTP data for extrapolation since it the same incidence at all the doses.

Ralph L. Kodell

I don't think the Toxicological Review is entirely logical or entirely clear. It does not seem logical to use the Mountain et al. (1953) study to derive an oral RfD. I have some questions regarding the BMC modeling for deriving the inhalation RfC and the inhalation IUR. I don't follow the logic of the claim of a lung-tumor effect in male rats in the NTP (2002) study. I think the characterization of vanadium pentoxide as "likely to be carcinogenic to humans" is too strong.

Craig C. McLauchlan

The Toxicological Review is very clearly presented and the rationale for decisions and data informing such decisions are clearly presented. The authors have done a nice job of making studies comparable, when studies allowed it, by putting all studies on the same scale and doing all the appropriate conversions to make comparisons easier to perform. However clear the presentation, there remains the issue of the compound being reviewed. As stated earlier, as a chemist, the largest issue throughout is that the review may not actually be a toxicological

review of V_2O_5 specifically, but of vanadium and vanadium oxides in general. The document needs to be clearer about what is being reviewed – if it is V_2O_5 only, much of the content becomes less relevant; if it is vanadium and vanadium oxides in general, more attention to the vast vanadium oxide literature and the effects of those complexes must be given. The speciation of V_2O_5 in water, let alone in biological systems, is very complex. Upon "dissolution," one quickly obtains a variety of vanadium species, none of which are V_2O_5. This issue is briefly addressed in the first few pages of the review, but ought to be reinforced throughout. Ultimately, that change in speciation and lack of confining the review to only V_2O_5, despite claims to the contrary, undermines the power of the whole document.

Xianglin Shi

Overall, the Toxicological Review is logical, clear, and concise. The Review covers a broad spectrum of literature and the key scientific issues were nicely summarized. The toxic effects of vanadium pentoxide are clearly presented and are very well supported by the scientific evidence. The carcinogenicity classification of vanadium pentoxide as "likely to be carcinogenic to humans" is adequately discussed and supported by the available animal data. The scientific evidence for both non-cancer and cancer effects of vanadium pentoxide are clearly presented and synthesized.

Qunwei Zhang

The organization of the document is excellent and the format is comprehensive, clear and concise. It is a well written document. The reviewers have logically and clearly presented and synthesized the scientific evidence for both non-cancer and cancer effects of vanadium pentoxide.

Yiliang Zhu

This assessment is supported by a comprehensive review the published literature. It would be very helpful to briefly indicate the public health significance of vanadium pentoxide regarding the scope and size of exposed population. While some review criteria are stated clearly, such as the electronic databases within which the search was conducted and the search time frame (i.e., up to 2011), information gaps exist regarding the inclusion/exclusion criteria used, which resulted in a detailed review of some published studies but the exclusion of others. For example, it is unclear if co-exposure to other forms of vanadium was an exclusion criterion, or only studies published in the English language were considered for inclusion. A quick search on Google Scholar found studies that may have involved co-exposure to other forms of vanadium or other chemical compounds (e.g. PM) which were not reviewed. The Google search also revealed additional animal studies that were not included in the EPA's Review (Zychlinski et al., 1991; Zychlinski and Byczkowski, 1990; Toya et al., 2001). There are a considerable number of studies on possible mechanisms of vanadium pentoxide that were not included in the Review (see references and discussion in response to General Charge Question 2). The lack of clearly spelled-out inclusion/exclusion criteria is a source of confusion; it can compromise the transparency – or even the quality – of the literature review of this Review.

It has been advocated repeatedly in the past (e.g., NRC, 2006; 2010) that EPA's IRIS risk assessment process adopts a Systematic Review approach. The principle of a systematic review is fairly simple: develop and apply a set of inclusion/exclusion criteria uniformly, and on the basis of these criteria, determine if a study/publication will advance to the next level of analysis. IRIS risk assessment consists of multiple stages: inclusion for literature review, inclusion as candidate (principal) study, inclusion as candidate (critical) effects, inclusion for dose-response analysis, etc. Criteria in tiered sets can be developed and applied to advance studies through the stages.

General Charge Questions

Question 2. Please identify any additional peer-reviewed studies from the primary literature that should be considered in the assessment of noncancer and cancer health effects of vanadium pentoxide.

Mitchell D. Cohen

There are no additional published studies to examine - though other papers in the literature dealing with effects on pulmonary/systemic immunocompetence seem to have not been included in the document. These should be in place to let the greatest breadth of information be available to a Reader. One could certainly propose additional (new) studies designed to support development of the various guideline values (RfD, RfC, etc.) with a level of even greater confidence.

Max Costa

This may be after the cut-off date, but at this year's SOT, my lab has presented data on Pentavalent Vanadium induced cell transformation of human Bronchial Epithelial Cells (SOT 2012, Passantino et al. poster).

Ralph L. Kodell

I don't know of any such studies.

Craig C. McLauchlan

I am not as familiar with the toxicology literature as my toxicology colleagues will be, but I was struck by the lack of many recent references in deciding on health effects. I am aware of studies with other vanadium compounds, but none specifically for V_2O_5. Given the lack of clarity on whether the discussion relates to V_2O_5 or all vanadium compounds, I will suggest several of those studies here. Certainly Dr. Cohen can comment on the differences in effects of varying forms of vanadates, given his years of work in the area. Speciation studies of vanadates have been investigated for years in a variety of media; I'd suggest Lage Petterson's work and Debbie Crans' work there. Sakurai also published some speciation studies in rats in 1994. Katherine Thompson and John McNeill have done a lot of work in the 1990s and 2000s regarding vanadium complexes, including taking one species to clinical trials for the treatment of diabetes. Those data should be useful if you are considering beyond V_2O_5. The orthovanadate ion has been known since the 1890's to have medicinal value, so chronic studies ought to be available there. Japan has known areas of high vanadium (V) concentration in its water, so long term studies have effectively been run there for many years, though I do not know of a specific peer-reviewed study on that. The public comments seem to indicate that the NTP has already done some studies on oxovanadium ions, including orthovanadate and metavanadate. Much was made during the panel discussion about reduction of V(V) to V(IV) in biological media, but I know that Crans (Inorg. Chem. '10) and Hsu (JACS '11) have shown some V(V)-thiol complexes as well.

Xianglin Shi

Although the Review focuses on vanadium pentoxide, other low oxidation states of vanadium should also be mentioned and also briefly discussed. A significant amount of vanadium pentoxide will be reduced to V(4+)-containing species once it enters the body. Once entering the body, vanadium pentoxide will be changed into a different chemical structure and will be reduced to various oxidation states. A large number of cellular reductants can cause the reduction. Some of these examples are glutathione, glutathione reductase, and cysteine. During the one electron reduction process, molecular oxygen is reduced to generate superoxide radical and hydrogen peroxide. V(4+)-containing species will further react with hydrogen to produce hydroxyl radical (Reference: Flavoenzymes reduce vanadium(V) and molecular oxygen and generate hydroxyl radical. *Archives of Biochemistry and Biophysics* 289, 355-361, 1991; Shi, X., and Dalal, N.S. Glutathione reductase functions as vanadate (V) reductase. *Archives of Biochemistry and Biophysics* 278, 288-290, 1990). The reactive oxygen species (ROS) (superoxide radical, hydrogen, and hydroxyl radical) produced by cellular vanadium pentoxide reduction will cause oxidative stress and activate various carcinogenic proteins (Huang, C., Ding, M., Li, J., Leonard, S.S., Rojanasakul, Y., Castranova, V., Vallyathan, V., Ju, G., and Shi, X. Vanadium-induced NAFT activation through hydrogen peroxide. *Journal of Biological Chemistry* 276, 22397-22403, 2001; and Zhang, Z., Huang, C., Li, J., Leonard, S.S., Lanciotti, R., Butterworth, L., and Shi, X. Vanadate-induced cell growth regulation and the role of reactive oxygen species. *Archives of Biochemistry and Biophysics.* 392, 311-320, 2001). It may be noted that Cr(VI), Ni, Cd, and arsenic produce reactive oxygen species and are human carcinogens.

The peer-reviewed studies on oxidative stress induced by vanadium(V)-containing species should be provided.

Qunwei Zhang

Based on my knowledge, there are not any additional published studies to examine.

Yiliang Zhu

See also the response to General Charge Question 1.

EPA is capable of conducting a systematic review of the published literature, and is in a legitimate position to decide, using unequivocally stated criteria, whether or not to advance a study for an in-depth review, then for further assessment for cancer and non-cancer effects. Presenting all relevant studies that meet the selection criteria is important to ensure thoroughness and transparency of the review. For example, three animal studies are identified from a Google Scholar search (see comments to General Charge Question 1), which EPA did not review. This makes one wonder: did EPA miss it or did EPA exclude these studies? If excluded, what were EPA's criteria?

A similar issue is seen in the review of MOA. EPA concludes in the Review that mechanistic data on a feasible MOA are limited. However, there are a number of studies on MOA, which were not reviewed by EPA (see the references below). Without explanation or an independent

review (which is not required for this external review panel), this omission or exclusion creates an information gap that can only negate the transparency of the Review. Some of the animal studies are listed below:

Wang YZ, Bonner JC (2000) Mechanism of extracellular signal-regulated kinase (ERK)-1 and ERK-2 activation by vanadium pentoxide in rat pulmonary myofibroblasts. American Journal of Respiratory Cell and Molecular Biology **22**(5):590-6.

B.-Z. Zhong, Z.-W. Gu, W.E. Wallace, W.-Z. Whong, T. Ong (1994) Genotoxicity of vanadium pentoxide in Chinese hamster V79 cells. Mutation Research/Genetic Toxicology 321(1-2): 35–42.

K. Bhattacharya, H. Cramer, C. Albrecht, R. Schins, Q. Rahman, U. Zimmermann & E. Dopp. (2008) Vanadium Pentoxide-Coated Ultrafine Titanium Dioxide Particles Induce Cellular Damage and Micronucleus Formation in V79 Cells. *Journal of Toxicology and Environmental Health, Part A*: Current Issues, Issue (13-14): pp 976-980.

Wumin Dong, Petia P. Simeonova, Randle Gallucci,Joanna Matheson,Lori Flood,Shiyi Wang, Ann Hubbs, Michael I. Luster (1998)Toxic Metals Stimulate Inflammatory Cytokines in Hepatocytes through Oxidative Stress Mechanisms. Toxicology and Applied Pharmacology 151(2):359–366.

Chemical-Specific Charge Questions:

(A) Oral reference dose (RfD) for vanadium pentoxide

Question 1. A subchronic oral dietary study in Wistar rats (Mountain et al., 1953) was selected as the basis for the derivation of the RfD. Please comment on whether the selection of this study is scientifically supported and clearly described. If a different study is recommended as the basis for the RfD, please identify this study and provide scientific support for this choice.

Mitchell D. Cohen

Given that there was only one other study reported here (chronic one by Stokinger et al. [1953] that did not indicate rat strain), it would seem that this is the only study with any control over the actual dosing levels of V_2O_5 used. Nevertheless, there are several severe limitations acknowledged in the Profile – and some that were not acknowledged (i.e., whether the dosings were adjusted accordingly in light of any changes in host body weight), which cast strong doubt upon the reliability/utility of the Mountain et al. study for deriving a valid RfD.

Post-Meeting Update: It appeared to be a common view among the panel members that more efforts should be made to undertake a study (as soon as possible) that could better be used to define the RfD for V_2O_5. The panel also felt that, in the absence of a new study, other endpoints (see below) might be better selected (if actually available) from the Mountain et al. study to use in defining the RfD value.

Max Costa

There are few studies on this compound and the one used for derivation of the oral RfD is the only one available. However, it is problematic, not conducted very well, and many details are left out so it is questionable whether a reliable RfD can be obtained. Perhaps an RfD based on multiple endpoints needs to be used.

Ralph L. Kodell

I do not believe that the selection of the study of Mountain et al. (1953) is scientifically supported. The study suffered from many deficiencies. Only male rats were studied; females were not included in the study. The sample sizes were quite small, only five rats per dose group. Except for one isolated statistical comparison involving relative liver weights, no statistical analyses were reported for any of the endpoints. Standard deviations were not even reported. Different dose groups were observed for different lengths of time. Data on the endpoint chosen as the critical effect (see question 2 below) were reported for only the two lowest doses, but these doses were increased substantially during the study (one was quadrupled and one tripled). Even though average daily doses in mg/kg-day can be calculated for the two lowest dose groups to reflect the dose change, the fact that the doses were increased dramatically and that no results were reported for higher doses causes concern, in light of all the other inadequacies of the study.

I do not know of a suitable study to recommend as an alternative to the Mountain et al. (1953) study, but I do not think the Mountain study is adequate for deriving an oral RfD. The data simply cannot be trusted. I believe that it is better not to set an oral RfD than to set one based on faulty data that defy objective analysis and interpretation. Application of uncertainty factors cannot rescue data that are fundamentally flawed. It may be necessary to find a sponsor for a new study to provide data for setting an oral RfD.

Craig C. McLauchlan

The choice of this study and this criterion seem reasonable to me. The study may be quite old and have limitations, but perhaps it is the best choice for continuous exposure.

Xianglin Shi

The approach is scientifically supported and is properly described in the Review.

Qunwei Zhang

The subchronic oral dietary study in Wistar rats (Mountain et al., 1953) is the only available source for the derivation of the RfD. In this study, vanadium pentoxide was given orally. The results showed a biologically significant reduction in RBC count (21%) at an average dose of 10.1 mg/kg-day in male Wistar rats. Although there are some limitations in experimental design and the methods used, including lack of evaluation of a comprehensive number of toxicological endpoints, small sample size, and not clearly statistical analysis, this dose-related decrease in RBC count in this oral study is strongly supported by an inhalation study. Decrease in RBC count was considered an adverse effect of vanadium pentoxide and thus selected as a critical effect for the derivation of the RfD. To select Mountain's study as basis for the derivation of the RfD is scientifically supported and clearly described.

Yiliang Zhu

The study of Mountain et al. (1953) appears to be the only oral exposure study available. It generated dose-response data at 4 dose levels, in addition to a control. Data were only reported for the two lower dose levels, and information such as standard error for each group was not even given. No statistical analysis was reported in the paper. The Review reproduced the data of this study as reported in in the paper (Mountain et al., 1953), but does not discuss the limitation, uncertainty, and potential bias in adopting only a part of this study. Without knowing the data at the two higher dose levels or a valid statistical analysis, we do not know if the dose-response trend is significant, if it extends to the two higher dose levels, or if other endpoints are equally valid. The aging aspect of the study makes these unknowns even more unsettling. One would suspect if the Mountain et al. study would meet today's data quality standard for publication in a reputable journal. The confidence on this study is very low.

(A) Oral reference dose (RfD) for vanadium pentoxide

Question 2. A decrease in red blood cell count in male Wistar rats was concluded by EPA to be an adverse effect and was selected as the critical effect for the RfD. Please comment on whether the selection of this critical effect and its characterization is scientifically supported and clearly described. If a different endpoint is recommended as the critical effect for deriving the RfD, please identify this effect and provide scientific support for this choice.

Mitchell D. Cohen

Despite the caveats noted in item 1 above, among all the endpoints reported in Mountain et al., it appears that the change in RBC count is the only utilizable endpoint for possible designation as a Critical Effect to derive a point of departure (POD), NOAEL, LOAEL, etc. It should be noted that there are great doubts about the designations of the NOAEL and LOAEL here since the study Authors did not provide/seemingly perform statistical comparisons that would let one clearly state if the effects at the 10.5 and 16.4 mg/kg-d actually differed.

Max Costa

Since V_20_5 is widely distributed throughout the body and is even able to enter the CNS and the testes, a systemic endpoint is appropriate. Reduction of hair cystine, increased alkaline phosphatase (this is probably due to the fact that V_20_5 is a phosphatase inhibitor, however, this was not discussed very much as the MOA, but rather, the authors' focus was on oxidative stress, which may also be a MOA) and a number of other endpoints could have been used. Reduction of red blood cell count appears to be a critical effect, but it might have been interesting to have derived the oral RfD using a number of different endpoints to compare how the number varies across several toxic effects of V_20_5. Again, the study used is problematic.

Ralph L. Kodell

I do not think that the selection of this critical effect and its characterization is scientifically supported. As mentioned in response to Charge Question (A)1 above, the study of Mountain et al. (1953) from which the critical effect was selected, has many scientific deficiencies. In addition, although it is stated on page 74, lines 19-22, that the effects on RBC count and hemoglobin concentration observed in the Mountain study are consistent with the hematological effects observed in the 3-month inhalation study of vanadium pentoxide in rats (NTP, 2002), I do not think this is so. Although the decrease in hemoglobin levels might be consistent between the two studies depending on which NTP measure is used (Table 4-4), the effect on RBC (erythrocyte) count is reversed between the two studies. According to Table 4-2, RBC counts *decreased* with increasing dose in the Mountain study, but according to Table 4-4, erythrocyte counts *increased* with increasing dose in the NTP study. Even if the different routes of exposure are taken into account, I cannot see how these opposite dose-response effects between the two studies can be viewed as consistent, and therefore supportive of using the Mountain data. I do not believe that a decrease in red blood cell count reported in the Mountain study is a reliable endpoint for setting an oral RfD.

I am not able to recommend a different endpoint as the critical effect, as I do not think any of the data from the Mountain study can be trusted.

Craig C. McLauchlan

For many of these questions, I will defer to experts trained in the area. The effects listed seemed adverse to me. The choice was supported clearly in the document. It may be beneficial to use multiple endpoints, as suggested by my co-panelists.

Xianglin Shi

For endpoint selection, red blood count is a reasonably good choice to determine the adverse effect level.

Qunwei Zhang

The decrease in red blood cell count and hemoglobin is the most sensitive endpoint following oral exposure to vanadium pentoxide. This was first reported by Mountain and was further confirmed by other investigators. Therefore, to select it as the critical effect for deriving the RfD is scientifically supported and clearly described.

Yiliang Zhu

Selection of RBC decrease as a critical effect is acceptable. However, omission of data at the two higher dose groups and the absence of statistical analysis to confirm the significance of an exposure-related RBC decrease dampen the confidence of using this effect. The inconsistent description of this study in the Review further clouds this choice. On page 36, lines 30-32, it states:

> "A 20-30% decrease in erythrocyte count is considered biologically significant; no statistical analysis was reported by the study authors, however; and no measure of variance (standard error or SD) was given for the mean. Data were not reported for high dose groups."

On page 74, lines 18-19 it states:

> "Study authors performed no statistical analysis on these data and reported no measure of variance, precluding independent statistical analysis."

Yet, on the same page, lines 36-37 it states:

> "The study authors reported dose dependent, statistically significant decrease in RBC counts following inhalation (should be "oral" – noted by this reviewer) exposure to vanadium pentoxide …"

EPA explains the reason for choosing RBC count change as the critical effect (page 74, line 24-26): "Based on the dose-related decreases in RBC count in the oral study supported by the hematological effects observed in the inhalation study, decreased RBC count was considered adverse and selected as the critical effect." If what EPA stated is a strong enough a criterion, other effects, such as hemoglobin, can also be used as a critical effect. This is another case in

point that EPA appears to have a spectrum of ad hoc criteria – not well defined, but flexibly applied - in designating principle studies and choosing critical effects.

Regardless, confidence about this oral exposure study (Mountain et al., 1953) remains very low.

(A) Oral reference dose (RfD) for vanadium pentoxide

Question 3. The NOAEL/LOAEL approach was used in conjunction with dosimetric adjustments for calculating the human equivalent dose (HED) to identify the POD for derivation of the RfD. Please comment on whether this approach is scientifically supported and clearly described.

Mitchell D. Cohen

There is no issue with the dosimetric adjustments applied to arrive at the HED values used to derive the RfD. Based upon the stipulated caveats about rat age, portal-of-entry effects, etc., the use of the EPA Guidance is appropriate.

Max Costa

The U.S. EPA has established methods for calculating all of these numbers and appropriate logic and uncertainty factors were applied in arriving at the RfD, as well as the HED and POD. The study used is problematic.

Ralph L. Kodell

The characterization of the lowest dose of 10.5 mg/kg-d as a NOAEL for decreased RBC count is not justified. The control mean was 3.8 and the lowest-dose mean was 12.8, more than a three-fold difference. Even with only 5 rats per group, unless the standard deviation is extremely large, it seems more likely than not that significance would be achieved in a statistical comparison. Although I do not think the RBC data are adequate for deriving an RfD, if they are so used, then I believe that 10.5 mg/kg-d would more appropriately be characterized as a LOAEL than a NOAEL.

Dosimetric adjustments for calculating the human equivalent dose (HED) seem scientifically justified, provided a scientifically defensible NOAEL can be identified (or dose-response data are available for calculating a representative BMD).

Craig C. McLauchlan

I will defer to experts trained in the area. The effects listed seemed adverse to me. The choice was supported clearly in the document.

Xianglin Shi

The approach is scientifically supported and is adequately discussed.

Qunwei Zhang

Based on the experimental design and the results obtained in Mountain's study, using the NOAEL/LOAEL approach to identify the POD for derivation of the RfD is acceptable and reasonable.

Yiliang Zhu

The Review clearly described the HED computation process. This conversion is acceptable based on the available data and following EPA's own guidelines.

(A) Oral reference dose (RfD) for vanadium pentoxide

Question 4. Please comment on the rationale for the selection of the uncertainty factors (UFs) applied to the POD for the derivation of the RfD. Are the UFs appropriate based on the recommendations described in A Review of the Reference Dose and Reference Concentration Processes (U.S. EPA, 2002; Section 4.4.5) and clearly described? If changes to the selected UFs are proposed, please identify and provide scientific support for the proposed changes.

Mitchell D. Cohen

There is no issue with the various UF values applied. However, in light of Comment 3 above, this Reviewer would suggest that the UF_L might need to be re-visited.

NOTE: There is an error in the value provided on Page 77, line 34. There is one too many zeros (and so the value would read as $\approx 9 \times 10^{-5}$).

Max Costa

All UFs were appropriately based on the recommendations from *A Review of the Reference Dose and Reference Concentration Processes* (U.S. EPA, 2002; Section 4.4.5) and are clearly described. The study used is problematic, as discussed in the meeting.

Ralph L. Kodell

Assuming that an oral RfD can justifiably be derived from the selected critical effect, of which I am doubtful, then UF_A=3 seems adequate to account for toxicodynamics, as toxicokinetics has already been taken into account in the HED calculation. Applying UF_H = 10 and UF_S = 10 both seem appropriate, in the absence of intraspecies information or chronic-study information. I think UF_L = 1 is inadequate, as I think the identified NOAEL is more properly characterized as a LOAEL. A value of UF_L = 3 or 10 would be more appropriate. However, this may only serve to drive the POD lower, without actually improving the uncertainty characterization, because the "LOAEL" is so poorly defined. I agree that if the Mountain study is used, then UF_D = 10 is justified. However, the lack of other types of studies is not the only source of database uncertainty. In my opinion, a suitable subchronic study doesn't even exist. Instead of applying a database uncertainty factor, I think it would be more scientifically sound to simply not set an oral RfD for vanadium pentoxide. I don't see how there can be any confidence in the presently derived RfD.

Craig C. McLauchlan

I will defer to experts trained in the area. The use of uncertainty factors always relies on some level of assumptions and the ones chosen and the magnitude seem supported clearly in the document. The EPA seems to follow its own guidelines.

Xianglin Shi

My expertise makes me uncomfortable to provide my comments for this section.

Qunwei Zhang

There is an error in the numerical representation of the RfD on page 77. It is 0.00085 mg/kg-day, but not 0.000085 mg/kg-day. The overall UFs (UF_A, UF_H, UF_S, and UF_D) were carefully considered and calculated based on the recommendations described in "A Review of the Reference Dose and Reference Concentration Processes" (U.S. EPA, 2002; Section 4.4.5) and are also clearly described.

Yiliang Zhu

EPA followed its own guidelines regarding the use of UFs. However, the poor reporting and omission of the data from the original study (no higher dose groups, no variation measure, and no statistical analysis) remain significant concerns, of which the resulting uncertainty is not specifically accounted for.

(B) Inhalation reference concentration (RfC) for vanadium pentoxide

Question 1. A two-year inhalation bioassay of vanadium pentoxide in F344/N rats (NTP, 2002) was selected as the basis for the derivation of the RfC. Please comment on whether the selection of this study is scientifically supported and clearly described. If a different study is recommended as the basis for the RfC, please identify this study and provide scientific support for this choice.

Mitchell D. Cohen

There is no issue with the selection of the NTP 2002 study to derive the RfC. However, there are concerns that are not addressed in this Profile. For example, though the text indicates (Page 38) that "chemical identity and purity ….was evaluated," there is no specific citing of the purity anywhere in the Profile. This is a great concern, as there is significant potential that vanadate(s) might be present on the V_2O_5 and thus influenced some of the measured outcomes. In addition, there is no information provided as to the means of exposure (i.e., aerosols that were nebulized, dusts from Wright-Dust feeder [or similar system]??). These are key factors in determining the 'purity' of the actual material that the rats (as well as mice) were exposed to in these studies.

As these were extensive inclusive studies, there are no other studies to potentially recommend at this point for use in deriving an RfC. However, if the above-noted items lead to serious doubts about the NTP results, this issue should be re-visited and, potentially, another study should be chosen as the principal study.

Post-Meeting Update: It appeared to be a common view among the panel members that, based on comments of the various outside attendees, there might be several substantive issues not included in the Profile that could impact the defining of the RfC for V_2O_5. Many of these comments reinforced some original concerns about the NTP 2002 studies that had been raised by panel members (see above for some from this Reviewer). The panel appreciated the fact that the EPA had a timeline for generating the Profile and that this precluded inclusion of many of these pieces of information/publications (i.e., Duffus [?] et al., 2007). Thus, it would be best if an Addendum were placed into the final document that could include many of these items/issues/findings. Such an approach would be in the spirit of full openness to Readers of the Profile.

Max Costa

The cancer incidence in female rats was unequivocal, but there was a higher incidence of cancer in male rats in this study. However, the mouse study found a very high incidence of lung cancers in both sexes. It seems that mice were more responsive to V_20_5 than rats. A mouse toxic response may have been a better study to use than the rat study. However, the cancer incidence data in the mouse are the same for all the doses so extrapolation with these data is problematic.

Ralph L. Kodell

The NTP (2002) inhalation chronic study in rats was selected as the critical study on which to base the derivation of the RfC because of the dose-response and temporal relationship of effects

observed throughout the course of the 2-year study, and the concordance with effects observed in humans. The study is certainly a good-quality study and worthy of RfC derivation. However, it is not clear why the NTP (2002) chronic study in mice was not also considered in the assessment of potential endpoints upon which to base the inhalation RfC. The mouse study is just as scientifically sound as the rat study.

Craig C. McLauchlan

I will defer to experts trained in the area. The study choice seems to the best choice, albeit with some possible purity issues in the study. The effects listed seemed adverse to me. The choice was supported clearly in the document.

Xianglin Shi

The selection of this two-year study as the basis for the derivation of RfC is clearly described and is scientifically well supported.

Qunwei Zhang

The inhalation reference concentration (RfC) was calculated in the report (NTP, 2002) based on a 2-year inhalation bioassay of vanadium pentoxide in F344/N rats. In this study, chronic inflammation of the larynx in female rats was selected as the critical effect to calculate a chronic RfC. This study was well conducted in a state-of-the-art scientific facility and using state-of-the-art methodology. It is the most appropriate and reliable study that is available.

Yiliang Zhu

The NTP's two-year study is arguably the best designed study to date. It involved two species and both sexes; it evaluated a wide spectrum of health outcomes. Advancing this study to the final analysis of RfC is well justified. It can be argued and is well accepted that, instead of using a single study (or a single endpoint) as the basis for RfC derivation, multiple studies or endpoints can collectively serve as the basis for an RfC, as long as reasonable selection criteria are in place that are. The advantage of using multiple, quality studies or endpoints is clear: we will be able to describe the range of variation in RfC, which, in turn, captures the variability and uncertainty associated with the studies and endpoints. This, in essence, allows us to quantify the overarching uncertainty attributable to various aspects of a study and effects.

To this end, defensible criteria should be developed and applied to determine if a study will advance to the next step of analysis or the final RfC calculation. Factors to be considered include, for example, the number of dose levels, the amount and degree of dose-response information, or the minimum sample size per dose group. Biological feasibility and MOA information are also important to consider. The same selection criteria should be applied to all candidate studies, including the rat arm of the NTP's two-year study (the principle study), the mouse arm of the same study, as well as the subchronic inhalation study of mice and rats (NTP, 2002). From the outset, it's not clear why the mouse arm of the two-year NTP study was excluded. The subchronic study also seems to provide sufficient dose-response data on

hematological and histopathological endpoints (page 62, 1st paragraph). EPA's explanation for including or excluding a study appears casual and ad hoc.

(B) Inhalation reference concentration (RfC) for vanadium pentoxide

Question 2. An increase in laryngeal inflammation in female F344/N rats was concluded by EPA to be an adverse effect and was selected as the critical effect for the RfC. Please comment on whether the selection of this critical effect and its characterization is scientifically supported and clearly described. If a different endpoint is recommended as the critical effect for deriving the RfC, please identify this effect and provide scientific support for this choice.

Mitchell D. Cohen

There is no problem with the selection of this particular endpoint as the critical effect. Still, it remains a bit unclear if the fact that females tended to lower survival rates was a factor in this selection, since several of the reported endpoints (see Table 5-3) in male rats often appeared more severely impacted than in their female counterparts. This issue should be discussed at the Panel Meeting.

Post-Meeting Update: Upon hearing the comments of some of the outside attendees, it would seem that the potential use of the laryngeal inflammation in the female rats might not have been the optimal choice for defining a point-of-departure needed to generate the RfC value. However, it appears that selection of other endpoints (i.e., male lung hyperplasia) in these host animals might not have impacted the POD that would instead be used. Thus, it would be best if the final revision took this matter into account and modified the text to reflect the comments of the outside speaker(s) on this matter and note that even if a different endpoint had been selected, the POD (and, hence, the RfC) would not have been affected.

Max Costa

Some of the effects seen in mice include effects observed in the lungs (alveolar and bronchiolar epithelial hyperplasia, inflammation, alveolus histiocyte infiltration in both males and females), larynx (squamous metaplasia of the epiglottis in both males and females), and nose (olfactory and respiratory epithelium degeneration in males and olfactory epithelial degeneration and atrophy in females). These could easily have been chosen as well since many mice developed cancers. Why not calculate an RfC using both the rat and mouse study to compare what numbers are obtained? Again, since the incidence of cancer in rats was very low, it is somewhat problematic to use these data.

Ralph L. Kodell

Laryngeal inflammation was one of the most sensitive effects observed in both sexes of F344/N rats. Of all the endpoints for which benchmark dose modeling was done (see response to Charge Question (B)3, below), laryngeal inflammation in female F344/N rats gave the lowest $BMCL_{10}$ as a POD for deriving an RfC. EPA characterized this lowest $BMCL_{10}$ as indicative that the larynx was the most sensitive target for chronic inhalation exposure to vanadium pentoxide, and thus chose the $BMCL_{10}$ as the POD for RfC calculation (page 81, lines 31-36). This may be reasonable. However, calculation of the estimated BMC_{10} and its associated $BMCL_{10}$ for laryngeal inflammation required extrapolating substantially below the lowest experimental

concentration, 0.5mg/m^3, which was an approximate BMC_{37}. So, there was no experimental data near the estimated BMC_{10} to inform the concentration-response. Perhaps, it could be argued that a different endpoint than laryngeal inflammation, one that had concentration-response information in the neighborhood of the predicted BMC_{10}, would be an equally valid candidate for deriving a POD. I believe that more discussion of the substantial extrapolation involved in choosing laryngeal inflammation as the critical effect is warranted, and that more justification should be provided in the document for choosing this effect over endpoints that had better concentration-response information relevant to calculating a concentration corresponding to a BMR of 10%.

On the other hand, it is not clear why significant respiratory effects in mice of both sexes, which were also observed in the NTP (2002) study, were not also considered for RfC derivation (Table 4-10, pp. 51-52). It is stated in Appendix B (p. B-1), where the BMC modeling is described, that five endpoints in both male and female rats were selected for two reasons, one being that the study in rats was designed with lower concentrations of vanadium pentoxide. Presumably, this means lower concentrations relative to mice (0, 0.5, 1, 2 mg/m^3 for rats versus 0, 1, 2, 4 mg/m^3 for mice). I can see the logic of that, but I'm not sure that it necessarily implies that one couldn't get a lower $BMCL_{10}$ for any mouse endpoint. At the very least, if the lower concentrations in rats compared to mice is the reason for not considering mouse endpoints for deriving PODs (and thereby choose a critical effect), that needs to be stated in the main body of the document, not merely in Appendix B. If it's there, I missed it. If there is an alternative justification, such as that certain effects are not considered adverse, that needs to be stated.

Craig C. McLauchlan

I will defer to experts trained in the area. The typographical errors in the tables need to be fixed to allow sound conclusions to be drawn.

Xianglin Shi

The selection of this critical effect and its characterization is scientifically supported and clearly described.

Qunwei Zhang

Previous studies showed that exposure to vanadium pentoxide in fuel-oil ash and vanadium dust resulted in the irritation of the upper and lower respiratory tract. The NTP study (2002) also confirmed that exposure of rats and mice to vanadium pentoxide caused upper and lower respiratory tract inflammation. Although the lung is the most sensitive target for noncancer toxicity in rodents with chronic inhalation exposure to vanadium pentoxide, the nasal and laryngeal lesions were among the most sensitive effects observed and were observed in both sexes of rats and mice and the most proximal to route of exposure (inhalation). Therefore, selection of laryngeal inflammation as an adverse effect and the critical effect for the derivation of RfC of vanadium pentoxide exposure is appropriate and scientifically supported.

Yiliang Zhu

In fact, EPA advanced multiple endpoints in both male and female F344/N rats to concentration-response modeling and BMC (BMD) computation. EPA also applied multiple concentration-response models to these selected endpoints. This effort is highly commendable, reflecting a new approach that EPA is committed to adopt. Unfortunately, this effort is not concisely captured in the Review, nor was it applied in a consistently and transparent fashion.

The Review reported data on 10 endpoints (Table 5-3) out of the 12 endpoints listed in Table 4-8 for both male and female rats. This selection was not explained, except for the following statements:

> "[T]he nasal and laryngeal lesions reported in NTP (2002) are among the most sensitive effects observed and were observed in both sexes of rats and mice (Table 5-3)" (page 80, lines 34-36).
>
> "Therefore, NTP 2 (2002) was selected as the principal study, with inflammation of the larynx in female rats 3 considered adverse and selected as the critical effect" (page 81, lines 2-4).

Then, from the 10 selected endpoints, the Review advanced five for Benchmark analysis: two in the lung, two in the larynx, and one in the nose. The reason for not selecting the two endpoints in the larynx was given as follows:

> "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" (page 84, lines 1-5).

For the endpoint of "degeneration of the epiglottis epithelium," the Review seemed to refer to the incidence at the middle concentration level being lower than that at the lower concentration; but, non-monotone incidences with exposure are not unusual in toxicological experiments and do not necessarily mean a lack of dose-response. For the endpoint of "epithelial squamous metaplasia," EPA's reason for exclusion does not hold because instead of looking the statistical significance in the incidence difference between a dose group and the control, it is the statistical significance of dose-response over all dose groups that matters. The Review gave no explanation for excluding the other three endpoints.

In addition to the five endpoints reported in Table 5-5, all other endpoints (see Tables 4-7, 4-8, 4-10) of both species and both sexes may warrant further analysis in the absence of exclusion criteria on toxicological and concentration-response grounds. The shape of concentration-response in these plausible endpoints varies; some are moderate in dose-response gradation (e.g., bronchiole epithelium hyperplasia, interstitial fibrosis, and olfactory epithelium degeneration in both male and female mice, squamous metaplasia of epiglottis epithelium, alveolar epithelium hyperplasia in the lung in both male and female rats, interstitial fibrosis, bronchiole epithelium hyperplasia, chronic active inflammation in the lung of male rats) and others are of sizably and

somewhat uniformly elevated response at all concentration levels, somewhat similar to the case of larynx inflammation in female rats.

A uniformly elevated response is viewed by some as "lack of dose-response," and the shape of concentration-response at lower concentration levels is subject to significant uncertainty. It often leads to smaller value for an RfC because the "threshold" phenomenon may imply greater risk at lower concentration levels. It is therefore not surprising that the BMC is the smallest for larynx inflammation in female rats, among those reported in Table 5-4, because of abrupt elevation in the incidence from 16% in the control to 53%, 55%, and 76%. It seems that the Review identified larynx inflammation in female rats as *the* critical effect after the fact that it produced the smallest BMC!

It should be pointed out that the purpose of considering multiple studies, multiple endpoints and multiple models is not always looking for the smallest POD, but to see the range in variation. The final choice of POD could be the decision incorporating risk management decision.

(B) Inhalation reference concentration (RfC) for vanadium pentoxide

Question 3. Benchmark dose (BMD) modeling was conducted using the incidence of laryngeal inflammation in female F344/N rats in conjunction with dosimetric adjustments for calculating the human equivalent concentration (HEC) to estimate the point of departure (POD) for derivation of the RfC. Has the modeling been appropriately conducted and clearly described based on EPA's draft Benchmark Dose Technical Guidance Document (U.S. EPA, 2000)? Is the choice of the benchmark response (BMR) for use in deriving the POD (i.e., a BMR of 10% extra risk of the incidence of laryngeal inflammation) supported and clearly described?

Mitchell D. Cohen

There are no issues with the use of the application of the RDDR(s) to arrive at the HEC values. The subsequent modeling and choices of the BMR(s), POD(s), and the use of the $BMCL_{10}$ to maximize an implicit safety factor for the RfC generated was appropriate and adequately described in the Profile.

Max Costa

Again, considering that the mice got cancer from V_20_5, a mouse response should also have been included, and it would have been of interest to compare the mouse and the rat. One could have chosen bronchiolar inflammation, hyperplasie, or any of those listed above. Using the rat response, all the appropriate care in deriving the HEC and POD were followed. However, both the rat and mouse data suffer from a lack of dose response.

Ralph L. Kodell

The choice of the BMR of 10% extra risk of the incidence of laryngeal inflammation is supported by the statement that 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 concentration group (page 85, lines 5-7). This is true for dichotomous endpoints like laryngeal inflammation. However, as I mentioned in response to Charge Question B2 above, estimation of the BMC_{10} and its associated $BMCL_{10}$ for laryngeal inflammation required a substantial extrapolation below the lowest experimental concentration using the fitted concentration-response model. I believe that such an extrapolation is unusual for determining a POD. I checked the Benchmark Dose Technical Guidance Document and found the following paragraph in the Executive Summary:

> *"Determination of appropriate studies and endpoints on which to base BMD calculations.* Following the hazard characterization and selection of appropriate endpoints to use for the dose response assessment, the studies appropriate for modeling and BMD analysis can be evaluated. All studies that show a graded monotonic response with dose likely will be useful for BMD analysis, and the minimum data set for calculating a BMD should at least show a significant dose-related trend in the selected endpoint(s). It is preferable to have studies with one or more doses near the level of the BMR to give a better estimate of the BMD, and thus, a shorter confidence interval. Studies in which all the dose levels show

> changes compared with control values (i.e., there is no NOAEL) are readily useable in BMD analyses, unless the lowest response level is much higher than the BMR."

I was unable to find elaboration on this issue, but the above passage seems to imply that there is a concern about doing BMD analyses when the lowest response level is much higher than the BMR, as is the case here.

I did find it surprising that the log-probit model sometimes failed to produce a BMCL, and that the BMCLs for laryngeal inflammation for both male and female rats were given as 0.000. I wonder why the log-probit model, which was the second-best fitting model (in terms of AIC and p-value) compared to the log-logistic model for both males and females, was so problematic and so different from the other models. It would be nice if some insight could be added to Appendix B.

As I stated in response to Charge Question (B)2 above, I believe that either mouse respiratory effects ought to be included in the BMC modeling, or a clear justification for not doing so should be stated in Section 5.2 of the document.

Craig C. McLauchlan

I will defer to experts trained in the area. The effects listed seemed adverse to me. The choice was supported clearly in the document.

Xianglin Shi

The modeling has been appropriately conducted and clearly described based on EPA's draft *Benchmark Dose Technical Guidance Document* (U.S. EPA, 2000). The choice of the benchmark response (BMR) for use in deriving the POD (i.e., a BMR of 10% extra risk of the incidence of laryngeal inflammation) is supported and clearly described.

Qunwei Zhang

The critical effects after exposure to vanadium pentoxide (incidence of laryngeal inflammation in female F344/N rats) were amenable to BMD modeling. BMD modeling can interpolate POD significantly below the lowest exposure level in the principal study. As described in the document, modeling was performed using the Benchmark Dose Modeling Software (BMDA). Biological and statistical considerations were taken into account in the selection of a benchmark response (BMR) level of all datasets. In the document, it was clearly described why laryngeal inflammation was selected for BMD modeling, but degeneration of the epiglottis epithelium and epithelial squamous metaplasia was not chosen 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 was not significantly different from control at low-and mid-dose group. Therefore, it is reasonable to choose the benchmark response (BMR) for deriving the POD (i.e., a BMR of 10% extra risk of the incidence of laryngeal inflammation).

Yiliang Zhu

To its credit, EPA considered multiple concentration-response models for the endpoints considered in rats of both sexes, and determined the "best fit" model (Table 5-5) using a single criterion, the Akaike-information criterion. It should be noted that statistical model fitting criteria only assess the agreement between the data and the model, but tells nothing about the shape of the model where there are no data. This implies that there are often multiple models that are admissible, but they can be quite different in the exposure range where there is no data but within which BMR is designated. Subsequently, considering all admissible models helps better understand and quantify uncertainty arising from the model choice.

The choice of benchmark response (BMR) level of 10% excessive risk is consistent with EPA's Benchmark Dose Guidance Document. However, depending on the endpoint and the associated concentration-response data, the BMR level, in conjunction with the shape of dose-response model there, may produce quite a different BMC. Take laryngeal inflammation, for example; the excess risk at the lowest exposure level is 44%, more than four times that of 10% BMR. Thus, uncertainty in the model shape around BMR=10% resulted in substantial uncertainty of the BMC. In contrast, the excessive risk is about 6.6% at the lowest concentration for chronic active inflammation in the lung of male rats. In this case, using BMR=5% or BMR=10% is well supported by the data. The Review should consider various choices of BMR by taking consideration of the support that the data can afford.

(B) Inhalation reference concentration (RfC) for vanadium pentoxide

Question 4. Please comment on the rationale for the selection of the UFs applied to the POD for the derivation of the RfC. Are the UFs appropriate based on the recommendations described in A Review of the Reference Dose and Reference Concentration Processes (U.S. EPA, 2002; Section 4.4.5) and clearly described? If changes to the selected UFs are proposed, please identify and provide scientific support for the proposed changes.

Mitchell D. Cohen

There are no problems with the selection of UFs and the values assigned to each.

Max Costa

No comment.

Ralph L. Kodell

Assuming that the $BMCL_{10}$ for laryngeal inflammation is an appropriate POD, then a value of UF_A=3 seems adequate to account for toxicodynamics, as toxicokinetics has already been taken into account in the HEC calculation. A value of UF_H = 10 seems appropriate in light of the potential for pre-existing respiratory disorders in some individuals, with no data to suggest a lower number. Regarding the UF_L factor, the reasoning for choosing a value of 1 is probably in line with conventional thinking. However, I consider a BMC_{10} to be more reflective of a LOAEL than a NOAEL. Even though it is assumed that a 10% increase in the incidence of chronic inflammation of the larynx represents a "minimal, biologically significant change" (page 87, lines 3-5), it's still biologically significant and near the minimal statistical detection limit. I suggest that UF_L = 3 makes more sense. UF_S = 1 is appropriate, as the critical effect was observed in a chronic study. I do not like database uncertainty factors. If one must be applied, I suggest UF_D = 3. I don't see how studies using alternate routes of exposure showing adverse reproductive and developmental effects should cause major uncertainty in deriving an inhalation RfC.

Craig C. McLauchlan

I will defer to experts trained in the area. The use of uncertainty factors always relies on some level of assumptions and the ones chosen and their magnitude seem supported clearly in the document.

Xianglin Shi

My expertise makes me uncomfortable to provide my comments for this section.

Qunwei Zhang

BMD modeling of incidence data from the chronic inflammation of the larynx in female rats yielded a $BMCL_{10}$ of 0.003 mg/m^3. The uncertainty factors, which include interspecies UF

(UF_A) that is about 2, potentially susceptible individuals in the absence of data evaluating (UF_H) that is 10, a LOAEL-to NOAEL UF_L of 1 was applied because the current approach is to address this factor as one of the considerations in selecting a BMR for BMD modeling; and a subchronic to chronic UF_S of 1, and a database UF_D of 10 for lack of a developmental toxicity study and a multi-generation reproductive study for vanadium pentoxide by the inhalation route. Therefore, the UFs selection was carefully considered and clearly described in the document.

Yiliang Zhu

The use of UFs is consistent with EPA's guidance document. However, the traditional uncertainty factors may not directly take into consideration uncertainties related to the choice of endpoint, model, BMR level, etc. EPA can take advantage of admitting multiple studies, multiple effects, multiple models and various BMR levels to better understand the magnitude of associated uncertainties and make additional adjustment in the final RfC estimate.

(C) Carcinogenicity of vanadium pentoxide

Question 1. Under the EPA's Guidelines for Carcinogen Risk Assessment (U.S. EPA, 2005; www.epa.gov/iris/backgrd.html), the draft Toxicological Review of Vanadium Pentoxide characterizes vanadium pentoxide as "likely to be carcinogenic to humans" by the inhalation route of exposure. Please comment on whether this characterization of the human cancer potential of vanadium pentoxide is scientifically supported and clearly described.

Mitchell D. Cohen

According to IARC, an agent is 'carcinogenic' if it is capable of increasing the incidence of malignant neoplasms, reducing their latency, or increasing severity or multiplicity. Induction of benign neoplasms may in some circumstances contribute to a judgment that an agent is carcinogenic. The same agency also states "a cancer hazard is an agent capable of causing cancer under some circumstances, while a cancer risk is an estimate of the carcinogenic effects expected from exposure to a cancer hazard." Based on the first definition here, the data from the NTP study in mice (shown in Table 5-8) would support the designation that V_2O_5 is carcinogenic in this particular animal model.

However, this Reviewer is hesitant to make the jump to deeming V_2O_5 "likely to be carcinogenic to humans" by the inhalation route of exposure. Firstly, the lack of evidence that V_2O_5 acts by many/any of the commonly accepted means of inducing cell transformation. Though there are indications of clastogenicity for V_2O_5 reported in the Profile, as noted, the data is still somewhat incomplete. It is equally plausible that the V_2O_5 is causing significant immunomodulation *in situ* that could allow background cancer (cells) to proliferate and give rise to an appearance of increased adenomas and/or carcinomas in the lungs of the exposed hosts - albeit that the V might not have caused/triggered their initial transformations *per se*.

In light of these and other doubts (that should be discussed by the Panel), the decision by the draft *Toxicological Review of Vanadium Pentoxide* to characterizes V_2O_5 as "likely to be carcinogenic to humans" by the inhalation route of exposure is NOT scientifically supported.

Post-Meeting Update: The panel members appreciated receiving hard copies of the definitions used by the EPA to define test agents as "likely," "suggestive," "inadequate," etc. carcinogens. It was clear there was no common view among the panel as to which categorization best applied to V_2O_5. Based on multiple re-readings of each of the definitions and characteristics used to classify agents as carcinogens, it is the opinion of this Reviewer that V_2O_5 should be categorized as having either only "Suggestive Evidence of Carcinogenic Potential" [by the inhalation route only] or "Inadequate Information to Assess Carcinogenic Potential." The former can only be used if all the caveats raised by the Panel can be overcome and issues about the NTP 2002 study were fully resolved. In the absence of this clarity about the NTP study, the latter categorization should apply. Again, this is solely the opinion of this one Reviewer and clearly not that of all Panel members.

Max Costa

Inhalation of V_20_5 was clearly carcinogenic to both male and female mice; it exhibited tumor promotion activity in the mouse lung adenoma assay and it was carcinogenic by inhalation to male rats, but not females. These results clearly warrant its classification as likely to be carcinogenic to humans. However, because of the lack of human epidemiological studies, it cannot as yet be classified as a known human carcinogen. The lack of dose response in the mouse data is probably due to the fact that the dose range chosen was very narrow.

Ralph L. Kodell

From the discussion in section 4.7.1, I believe the characterization "likely to be carcinogenic in humans" was used because it was concluded that vanadium pentoxide caused alveolar/bronchial tumors in two species, mice and rats. Although I agree that the alveolar/bronchial tumors in both sexes of mice were associated with vanadium pentoxide exposure by the inhalation route (NTP, 2002), I am not convinced that a similar finding has been demonstrated in rats.

Compared to concurrent controls, tumor incidences in male and female rats were not significantly elevated. Although 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 (see lines 3-5, page 50), I am skeptical of the claim of a lung-tumor effect in male rats. The historical control percentages in Table 4-9, if correct, are actually higher than the concurrent control percentages, and more in line with the percentages in the exposed groups. So, the reason for using historical controls does not appear to be because concurrent control rates were higher than usual. They were actually on the low side of historical rates. That being the case, maybe it's primarily the large sample size in historical controls that induces statistical significance.

During the panel meeting, EPA's Guidelines for Carcinogen Risk Assessment were discussed. It seemed that the designation "likely to be carcinogenic to humans" could be applied if an agent were shown to be carcinogenic in both sexes of a single species. If this is so, then the characterization of vanadium pentoxide as "likely to be carcinogenic to humans" is consistent with the guidelines, even without carcinogenicity in rats having been demonstrated. However, it also seemed that the guidelines could be interpreted to allow the characterization of "suggestive" instead of "likely" if carcinogenicity were demonstrated in only one species. If there is room for interpretation, then I believe the characterization "likely to be carcinogenic to humans" is too strong, and that the data are "suggestive" that vanadium pentoxide is carcinogenic to humans. However, I do agree with the qualifier "by the inhalation route of exposure" because the lung tumors occurred at the initial point of contact with vanadium pentoxide.

Craig C. McLauchlan

There does not appear to be direct evidence for vanadium pentoxide as a carcinogen and such a statement is included in the document. However, amidst the other data and secondary evidence, it is probably reasonable to assign some cancer risk. A description as "suggestive evidence of carcinogenic potential" seems more reasonable to me.

Xianglin Shi

The characterization of human cancer potential of vanadium pentoxide is scientifically supported and clearly described.

Qunwei Zhang

The NTP (2002) studies showed that chronic vanadium pentoxide inhalation caused lung tumors in both sexes of mice and in male rats. Since the available information on the carcinogenic effects of vanadium pentoxide via the inhalation route is limited to examination of respiratory tumors, no data regarding human or laboratory animal carcinogenicity of vanadium pentoxide by oral or dermal route are available. Therefore, under the guidelines for Carcinogen Risk Assessment (EPA, 2005), the draft *Toxicological Review of Vanadium Pentoxide (V_2O_5)* characterizing vanadium pentoxide as "likely to be carcinogenic to humans" by the inhalation route of exposure is scientifically supported and clearly described.

Yiliang Zhu

The descriptor of "likely to be carcinogenic to human" is consistent with EPA's 2005 Cancer guideline. NTP's two-year experiment with vanadium pentoxide showed elevated respiratory track adenoma/carcinoma in mice of both sexes, and somewhat elevated tumor incidence of in male rats.

(C) Carcinogenicity of vanadium pentoxide

Question 2. The draft Toxicological Review of Vanadium Pentoxide concludes that there is insufficient information to identify the mode(s) of carcinogenic action. Please comment on whether this determination is appropriate and clearly described. If it is judged that a mode of action can be established for vanadium pentoxide, please identify the mode of action and its scientific support (i.e., studies that support the key events, and specific data available to inform the shape of the exposure-response curve at low doses).

Mitchell D. Cohen

The choice NOT to declare/define a mode(s) of carcinogenic action here is sound. This is even more the case in light of potential questions about the "carcinogenicity" of V_2O_5 in the traditional sense for classic carcinogens (see above). Nevertheless, if and when more relevant information becomes available, this issue needs to be revisited.

Post-Meeting Update: Unlike some of the Panel members, who believed that one or two sets of effects on select endpoints (i.e., enzyme functions) should be used to define the mode of carcinogenic action for V_2O_5, this Reviewer still believes that the choice NOT to declare/define a mode(s) of carcinogenic action in this Profile is sound. Furthermore, the attempts by some to simply state that because other metal carcinogens do "X," this must also apply to V_2O_5 as well, is foolish in the absence of further *in vivo/in vitro* scientific evidence of mode(s) of action for V_2O_5.

Max Costa

V_20_5 is a phosphatase inhibitor and it is used widely in the lab for this purpose. This MOA was not described very much in this review; rather, some mention of mutagenicity and oxidative stress was discussed in the review. More work needs to be done on the MOA of V_20_5. Data from my lab suggest that it has epigenetic effects and can alter histone modifications, but we have not as yet published these data. I think the document needs to indicate that V_20_5 in contact with biological fluids turns into a phosphatase inhibitor.

Ralph L. Kodell

The conclusion that there is insufficient information to identify the mode(s) of carcinogenic action appears to be appropriate. An extensive discussion is provided in Appendix D, but no firm conclusions can be drawn from the available data. It was suggested during the panel discussion that more information on the mode of action of vanadium pentoxide is available in the literature and ought to be included in the document.

Craig C. McLauchlan

The determination is clearly described. I am not aware of any mode of action.

Xianglin Shi

The determination that there is insufficient information to identify the mode(s) of carcinogenic action is appropriate and clearly described. Please note that there is a fairly large amount of information indicating that reactive oxygen species (ROS) by vanadium(5+)-containing species play an important role in the health effects vanadium(5+)-containing species. These species may cause oxidative stress, activation of oncogenic transformation factors, and certain oncogenic signaling proteins. These species increase the secretion of inflammation related proteins, such as TNF-α and Cox-2. The consequence of these ROS-responses will be the creation of inflammatory tumor microenvironment, leading to cell neoplastic transformation and tumorigenesis. These carcinogenic actions are very similar to those of other carcinogenic metals, such as Cr(VI) and arsenic. At present, there is insufficient information to identify the mode(s) of carcinogenic action.

Qunwei Zhang

Human data on the carcinogenic potential of inhalation exposure to vanadium pentoxide are not available, and data on the potential carcinogenic effects of vanadium pentoxide on human or laboratory animals by oral or dermal route are not available. The 2-year NTP inhalation cancer bioassay study is well-designed and well-conducted. Those tumor types are also considered relevant to humans. The IRU was derived from the combined tumor incidences of lung adenomas and carcinomas in male mice. The information is limited regarding the MOA of the lung tumors observed in the chronic animal bioassay. The genotoxicity study also did not provide adequate evidence of a genotoxic MOA, and the data that supported the alternative MOA hypotheses are inadequate. Therefore, the draft *Toxicological Review of Vanadium Pentoxide (V_2O_5)*'s conclusion that there is insufficient information to identify the mode(s) of carcinogenic action is appropriate and clearly described.

Yiliang Zhu

Information presented in the Review supports the assertion that the MOA for respiratory track cancers is unclear.

(C) Carcinogenicity of vanadium pentoxide - Oral Slope Factor (OSF)

Question 3. The draft Toxicological Review of Vanadium Pentoxide did not derive an OSF due to lack of available studies. Are there available data to support the derivation of an OSF for vanadium pentoxide? If so, please identify these data.

Mitchell D. Cohen

The lack of data available in the literature makes the choice NOT to develop a value here prudent. If and when relevant information becomes available to the EPA and the rest of the scientific community, this issue of a cancer OSF can and should be revisited.

Max Costa

There are no studies showing it is carcinogenic by the oral route.

Ralph L. Kodell

To my knowledge, there are no available data to support the derivation of an OSF for vanadium pentoxide.

Craig C. McLauchlan

I am not aware of any such studies.

Xianglin Shi

Due to a lack of available studies, it is correct that the draft *Toxicological Review of Vanadium Pentoxide* did not derive an OSF.

Qunwei Zhang

To my knowledge, there is no such kind of data available to support the derivation of an OSF for vanadium pentoxide.

Yiliang Zhu

I am not aware of additional studies or datasets that can support the calculation of an oral slope factor.

(C) Carcinogenicity of vanadium pentoxide - Inhalation Unit Risk (IUR)

Question 4. A two-year inhalation bioassay of vanadium pentoxide in B6C3F1 mice (NTP, 2002) was selected as the basis for the derivation of the inhalation unit risk (IUR). Please comment on whether the selection of this study is scientifically supported and clearly described. If a different study is recommended as the basis for the IUR, please identify this study and provide scientific support for this choice.

Mitchell D. Cohen

Please see response to Charge Question (C)1 with regard to general support for defining any V_2O_5 IUR at this time. In the abstract, selection of the NTP study for generation of an IUR could be scientifically supported.

Max Costa

This is the best study and it is clearly described. Lack of dose response in the study is problematic

Ralph L. Kodell

The selection of the NTP mouse study is scientifically supported by the statistically significantly elevated lung tumor incidences in male and female mice in the vanadium pentoxide exposure groups. In my opinion, the argument that there was also a significantly increased incidence of lung tumors in male rats in the NTP rat study is not compelling. However, if that argument is made, then the NTP rat study should be considered in the dose-response modeling for deriving the IUR because there are significant challenges in modeling the mouse data.

Craig C. McLauchlan

The study chosen seems to be the best study based on the arguments outlined. The choice is clearly explained. I am not aware of a better study.

Xianglin Shi

A two-year inhalation bioassay of vanadium pentoxide in B6C3F1 mice (NTP, 2002) was selected as the basis for the derivation of the inhalation unit risk (IUR). The selection of this study is scientifically supported and clearly described.

Qunwei Zhang

The NTP (2002) study was well designed and conducted on both sexes of two species with adequate number of animals per dose group. Exposure of male and female B6C3F1 mice to over 1 mg/m^3 of vanadium pentoxide caused a statistically significant increase in the incidence of alveolar/bronchiolar adenomas or carcinomas. These tumor types are relevant to humans. To my knowledge, the two-year inhalation bioassay is the only chronic carcinogenicity study currently

available. Therefore, its selection by EPA as the principal study for cancer assessment and to derive an IUR is scientifically supported and clearly described.

Yiliang Zhu

The NTP two-year study of vanadium pentoxide is, in fact, the only animal experiment that generated dose-response tumor incidence data. There is no published human study of vanadium pentoxide carcinogenicity. The Rondini et al. (2010) study investigated vanadium pentoxide as a tumor promoter, but did not provide dose-response data required for deriving a POD off the dose-response curve. The NTP experiment used both B6C3F1 mice and F334/N rats. The reason for not including male rat data was not clearly described. In fact, the crude incidence data of male rats show a dose-response similar in shape to those of the mice.

(C) Carcinogenicity of vanadium pentoxide - Inhalation Unit Risk (IUR)

Question 5. The incidence of alveolar/bronchiolar adenomas or carcinomas in B6C3F1 male mice was selected to serve as the basis for the quantitative inhalation cancer assessment. Please comment on whether this selection is scientifically supported and clearly described. If a different cancer endpoint is recommended for deriving the IUR, please identify this endpoint and provide scientific support for this choice.

Mitchell D. Cohen

Again, please see response to item Charge Question (C)1 concerning defining any V_2O_5 IUR at this time. As before, in the abstract, selection of the these particular endpoints in male (rather than female) mice could be scientifically supported, especially in light of the information provided on Page 91 of the Profile.

Max Costa

Yes, this is the best study and males had a better response than females. Lack of dose response in both sexes is problematic for any extrapolation.

Ralph L. Kodell

Unfortunately, it appears that alveolar/bronchiolar tumors (whether adenomas, carcinomas or adenomas and carcinomas combined) in mice represent the only tumor data in mice from which to derive the IUR. These tumor data are problematic for doing BMC modeling because the tumor incidences are very high in all three exposure groups, compared to controls. There is no information on the concentration-response relationship at concentrations below 50% extra risk. Nevertheless, a log-logistic concentration-response model was able to fit the data adequately in males with all concentration levels included. Although the incidence of alveolar/bronchial adenomas or carcinomas is far from ideal, I do not know of a different tumor endpoint in mice that could be used. On the other hand, even though I am not convinced that a carcinogenic effect in male rats has been demonstrated, such an effect has been claimed in the document. If that claim is carried forward, it seems that EPA ought to try to perform BMC modeling with the male rat tumor data to see if those data give better concentration-response information for BMC modeling than the mouse tumor data, which may provide a better basis for deriving an IUR.

Craig C. McLauchlan

The endpoint choice is well-explained. I am not aware of a better study. Interestingly, there seems to be a higher survival rate among the test subjects than the controls as shown, but this is apparently a typographical error that should be fixed.

Xianglin Shi

The incidence of alveolar/bronchiolar adenomas or carcinomas in B6C3F1 male mice was selected to serve as the basis for the quantitative inhalation cancer assessment. This selection is scientifically supported and clearly described.

Qunwei Zhang

It is very clear that inhalation exposure to vanadium pentoxide aerosol significantly increased the incidence of alveolar/bronchiolar adenomas and carcinomas in male and female B6C3F1 mice. Alveolar/bronchiolar adenomas and carcinomas are relevant to human lung cancer. The selection of the incidence of alveolar/bronchiolar adenomas or carcinomas in B6C3F1 male mice to be the basis for the quantitative inhalation cancer assessment is appropriate and scientifically supported.

Yiliang Zhu

The Review used the incidences of alveolar/bronchiolar adenomas or carcinomas in male and female mice for cancer dose-response assessment. The result of modeling for male mice was reported in Table 5-10, but there were troubles in fitting a model to female mouse data unless the highest dose group was removed. Criteria for choosing male rats were not clearly described. Rather, the text seems to imply that the choice was made largely based on the ability to fit a dose-response model. If model fitting indeed was the inclusion criterion reason, one wonders if additional efforts should have been made to fit models to both the female mouse and male rat data. BMD software is known for accepting built-in options to achieve model convergence.

(C) Carcinogenicity of vanadium pentoxide - Inhalation Unit Risk (IUR)

Question 6. Benchmark dose (BMD) modeling was conducted using the incidence of alveolar/bronchiolar adenomas or carcinomas in male B6C3F1 mice in conjunction with dosimetric adjustments for calculating the human equivalent concentration (HEC) to estimate the point of departure (POD). A linear low-dose extrapolation from this POD was performed to derive the IUR. Has the modeling been appropriately conducted and clearly described based on EPA's draft Benchmark Dose Technical Guidance Document (U.S. EPA, 2000)? Has the choice of the benchmark response (BMR) for use in deriving the POD (i.e., a BMR of 71% extra risk of the incidence of alveolar/bronchiolar adenomas or carcinomas in male mice) been supported and clearly described?

Mitchell D. Cohen

Please see response to item Charge Question (C)1 with regard to support for defining any V_2O_5 IUR at this point. In general, BMD modeling for the incidence of adenomas\carcinomas in conjunction with dosimetric adjustments for calculating the HEC to estimate a POD (followed by dose extrapolation from POD to derive IUR) is a proper, sound approach. Such modeling seemed to be appropriately conducted and clearly described in the Profile.

There is no clear reason for using the "71%" value. Based on the equation on Page 92, use of a 10% risk would have resulted in a far lower (safer?) IUR. The only clue to why '71%' was adopted for use seems to be provided on Page 94 (lines 8-11). It is hoped that someone with greater expertise in modeling/stats will be at the Peer Review Meeting to provide clarity where it is now lacking. In the interim, whether the choice of the BMR for use in deriving the POD (i.e., a BMR of 71% extra risk of incidence of adenomas or carcinomas in male mice) does NOT have clear support and was not clearly described**.**

Max Costa

All methodology was conducted properly. However, the lack of dose response in cancer incidence data is problematic for any extrapolation.

Ralph L. Kodell

The modeling has been described in Appendix C. Although it is not entirely clear, it appears that the approach taken was to choose the lowest experimental concentration as the BMC and then fix the BMR at the log-logistic model's fitted prediction of 71% extra risk in male mice at that concentration. That is, the predicted extra risk of 71% at the lowest concentration was selected as the BMR for getting the lower limit on the BMC; then, the lower 95% confidence on that concentration, i.e., the $BMCL_{71}$, was determined with the profile likelihood method and the log-logistic model. The reason given on page C-1 for the approach taken was that because of the plateau of the lung-tumor incidences at three nonzero concentration levels, there was limited information about the concentration-response relationship. While this is certainly true, it may not justify the choice of the BMR of 71% extra risk.

I understand not wanting to extrapolate below the experimental concentration range with a fitted model, but it's hard to have confidence that the IUR derived from the $BMCL_{71}$ really represents a "cancer slope factor" that is relevant at very low concentrations. In contrast to the present approach for cancer (IUR), the approach for nonneoplastic effects (RfC) was to extrapolate the fitted model below the lowest experimental concentration down to a 10% BMR to derive a POD. While I have commented that it might be better to select a nonneoplastic endpoint that would not require such extrapolation to establish a POD, nevertheless, I suggest that EPA consider doing the same thing for cancer that was done for nonneoplastic effects, using the conventional default BMR of 10% as an alternative approach to the BMR of 71% to see how the IUR derived from the $BMCL_{10}$ compares to the value derived from the $BMCL_{71}$. A large discrepancy might cause concern.

As I noted above in response to Charge Question (B)3, EPA expresses some concern in the BMD Guidance Document about BMD analyses when the lowest response level is much higher than the BMR. I was unable to find elaboration on that point. More discussion of why it was appropriate to perform BMC analysis on these data and why the BMR of 71% was chosen needs to be added to the document. In particular, it needs to be shown how this choice complies with the BMD Guidance Document. As was discussed at the panel meeting, it would be very helpful if EPA can cite instances when a similar approach has been used to derive an IUR.

In the interest of full disclosure, I want to mention that in 1982 I was a co-author of a paper that recommended extrapolating linearly below either the dose corresponding to 1% added risk for cancer or the lowest experimental dose, whichever is larger (Farmer et al., Risk Analysis, 1982). My recollection is that we were aiming to avoid using a fitted model to predict risk in the low-dose region. Although I doubt that we envisioned the lowest dose having a predicted extra risk as high as 71%, and even though I am somewhat uncomfortable with EPA's use of a BMC_{71} to essentially characterize the low-dose slope, technically, that approach is in line with my own previous recommendation.

Alternatively, even though I am not convinced of the claim of carcinogenicity in male rats in the NTP (2002) study, if that claim is made, then I think that EPA ought to consider modeling the male rat tumor data for deriving an IUR. Whatever is done, more discussion and justification is needed, and it may be helpful to have additional BMC experts in EPA to comment and offer advice.

I found it puzzling that the log-probit model failed to produce a $BMCL_{71}$ (see Table C-7), even though it fit better than the log-logistic model.

Craig C. McLauchlan

The choice is clearly described and justification is attempted. Based on the discussion with the remainder of the panel, more expertly trained in the area, it seems that the $BMCL_{71}$ value may be a bit contrived, i.e. the extrapolation is a bit more than the data allow.

Xianglin Shi

The modeling has been appropriately conducted and clearly described based on EPA's draft *Benchmark Dose Technical Guidance Document* (U.S. EPA, 2000). The choice of the benchmark response (BMR) for use in deriving the POD (i.e., a BMR of 71% extra risk of the incidence of alveolar/bronchiolar adenomas or carcinomas in male mice) is supported and clearly described.

Qunwei Zhang

Based on the data from the NTP (2002) chronic inhalation study for lung tumors in male and female B6CF1 mice, BMC and BMCL were estimated by using BMDS modeling. According to the U.S. EPA Guidelines for Carcinogen Risk Assessment, for each tumor response, a POD from the observed data should be estimated to mark the beginning of extrapolation to lower dose (U.S. EPA. 2005a). A BMR based on the response at the control concentration and the first non-control concentration was calculated and used to estimate a POD (BMCL, extra risk of 0.71 for male mice, 0.67 for female mice). Therefore, BMCL (HEC) was calculated as 0.208 mg/m^3 for male and 0.161 mg/m^3 for female. The BMCL71 of 0.208 mg/m^3 for male mice was selected as the POD for derivation of the IUR as this was the only model fit. The modeling was conducted appropriately and the BMR for use in the POD is also well supported and clearly described.

Yiliang Zhu

Because of the high tumor incidence data in male mice at the lowest exposure level, EPA chose a BMR level at the extra risk level observed at the lowest exposure level, rather than the conventional 5~10% level. For male mice, the BMR level = (.84-.28)/(1-.28) = .78 (from data given in Tables 4-11 and 5-8, where the control incidence is 0.28). The Review reported a BMD=0.71, based on the control incidence of 0.44 (Table C-6). This difference needs to be resolved.

Regardless, the use of such a high BMR level observed at the first non-control dose requires caution and warrants a thorough discussion. It is clear that with the crude incidence of 0.84 at the lowest exposure level and leveling responses of 0.86 at the next two higher exposure levels, the male mice adenoma/carcinoma data provided little information about the shape of concentration-response below the lowest non-control level. Thus, using the conventional BMR=0.10 amounts to an over-extrapolation of a POD over the data range. On the other hand, using BMR=0.71 observed at the lowest exposure level amounts to an over-extrapolation from the POD (which should be very close to the first non-control level if the model fits the data) to 0 excess risk. The BMD approach reported in this Review, based on the male mice tumor data, is not well supported according to EPA's Benchmark Dose Technical Guidance Document. A fundamental limitation is the lack of gradation in the dose-response data. As a result, the confidence in the dose-response model and the subsequent POD is extremely low, and the unit risk estimate (IUR=BMR/BMCL=0.71/0.208=3.4 per 1000 population for inhaling each additional 1 mg/m^3 of vanadium pentoxide) is extremely uncertain.

To see the degree of uncertainty, I would recommend EPA do a comparison by using the incidence of alveolar/bronchiolar carcinoma ion male mice. The gradation of concentration-

response curve is mild with the BMR level of 45% at the first non-control exposure level. Considering the use of male rat tumor data can serve the same purpose for better understanding uncertainty because the rat data are moderate in the elevation of tumor incidence.

Alternatively, one can attempt more sophisticated statistical modeling to allow for flexible dose-response shape, within a plausible range. Such a range may be determined in conjunction with an a priori dose-response shape, supported by external evidence. This approach still will not uncover the true dose-response shape. It will cast the range of uncertainty to support a risk management decision in choosing a POD.

Finally, a caution on the tumor incidence data: due to lower percentage of animal survival (40~58%), animals that died before the end of two years were examined earlier for neoplastic lesions. This could bias the tumor incidence data, especially if the latent time for tumor development is long. The Review should discuss this potential bias.

V. SPECIFIC OBSERVATIONS

Mitchell D. Cohen

Page	Paragraph or Line #	Comment or Question
77	33	Apparently too many zeros to get the number reported.
39	1-11/Table	The values for % decrease in weight gain do not seem to jive with the values reported in the table itself. An explanation would suffice for this Reviewer if my interpretation is incorrect.

Max Costa

None.

Ralph L. Kodell

Page	Paragraph or Line #	Comment or Question
41	7-10	Does this mean that only lung and nasal tissue was examined in groups 1, 2, and 4, and that those tissues were selected after observing treatment-related effects in those tissues in the 8 and 16 groups after complete histopathology? I think this needs clarification.
47	19-23	It needs to be clear that the ranges of 52-58% and 30-40% were for treated animals. In line 22, I think it would help to add the word "control," i.e., "…these control survival rates…"
48	Table 4-8	The survival percentages for females are incorrect.
49-50	18-21, 1-2	The text mentions several comparisons to historical controls that were statistically significant. However, footnote C of Table 4-9 "statistical comparison between NTP (2002) data and historical control data not conducted."
50	Table 4-9	The historical control numbers don't seem to make sense. The counts are too low to generate the corresponding percentages.
53	Table 4-11	Some historical control percentages for females are negative.
61	Table 4-13	Why aren't decreased hemoglobin levels in male Wistar rats included in the table?
78	26-27	I presume "this study" refers to Knecht et al. (1992), but it needs to be clearer.
80	36	Both rats and mice are mentioned in conjunction with Table 5-3, but mice are not in that table.
82	Table 5-3	The survival percentages for females are incorrect.
85	Table 5-6	It's not clear what 0.000 means for the log-probit model under the BMCL column. I guess it means < 0.001, but not actually 0.
91	15-17	This is not a sentence.

Page	Paragraph or Line #	Comment or Question
B-16	Table B-10	Exactly what does "failed" mean under the BMCL column for the log-probit model? It is actually the best-fitting model in the table, even better than the log-logistic. It's surprising that a BMCL couldn't be found. I think a footnote needs to be added to explain what "failed" means. Perhaps the BMDS developers can help.
B-24	Table B-13	Same issue as Table B-10.
C-1	25	I recommend inserting "predicted" or "fitted," i.e., "…based on the predicted response at the…"
C-5	21-22	Wasn't the Poly-3 test used, or was the Cochran-Armitage test actually used? With the survival differences shown in Table 4-10, I would think the Poly-3 test would be used. Clearly, it isn't needed when the effect is so pronounced, but what about other endpoints? On page 49, line 18 of the text, it states that the Poly-3 test was used for the rat tumor data.
C-6	Table C-7	The log-probit failed again to produce a BMCL, even though it was the best-fitting model. This is puzzling.
C-10	4-5	Same question as C-5, 21-22.
C-9	Table C-9	Same question as Tables B-10, C-7.
C-13 – C-14	7-16, 1-2	An alternative to the MSW would be to calculate the Poly-3 adjusted incidences and fit the quantal concentration-response models to those.

Craig C. McLauchlan

Page	Paragraph or Line #	Comment or Question
1	12/28	One of these unit sets is probably wrong.
3	11	There are many more recent studies in this area if we care to cite them. See my comments to General Charge Question 2.
3	17	What are the authors trying to say about covalent bonding? This is confusing to me. Most would consider solid-state V_2O_5 as being an extended solid, and that view is reinforced by its melting point and band gap. Yes, V_2O_5 is shown to have covalent bonding, but so would other, more molecular vanadates. The layered V_2O_5 structure is probably better described as an extended solid than a covalent structure, which might incorrectly imply that it is molecular in nature.
3	26, 27	I have now seen Bruyere's work; it IS the long known $V_{10}O_{28}$, not O_{29}. The decavanadate ion, as it is known, is not stable at high pH, only low pH. At high pH, you can have a complex set of equilibria between polyoxovanadates, but decavanadate is not usually part of the mix.
3	30	Try to stick with oxidation state instead of "valence" throughout the document.
4	9+	Figure 2-1: Yes, people think of V_2O_5 this way sometimes, but in the solid state it is composed of extended planes of vanadium in a square pyramidal/distorted octahedral environment, not a trigonal planar environment, as shown. Maybe I am being a picky solid-state chemist.

Page	Paragraph or Line #	Comment or Question
6	9-10	Here, we are discussing V_2O_5 as if it is present in the body; this is not likely at all. The species present should be other species, as indicated in the vast vanadium literature in the area.
7	4-8	The presence of metals other than vanadium (and no mention of V_2O_5) limits the value of this study to this document.
7	22	The lack of studies with V_2O_5 is surprising given the data with other vanadium compounds. A quick literature search for articles that cite Roshchin shows at least one study claiming to study V_2O_5 exposure - Kawai et al. 1989 -- PMID:2722251.
8	1	Most studies I am aware of state that vanadium is transported bound in transferrin, with some in albumin and some "free" in the plasma.
9	12	The lack of studies with V_2O_5 is surprising given the data with other vanadium compounds. A quick literature search for articles that cite Roshchin shows at least one study claiming to study V2O5 exposure… Kawai et al. 1989 -- PMID:2722251.
10	21-22	The metabolism of vanadium seems odd to be discussed if we are discussing V_2O_5. That aside, the vanadium species present in circulating blood is a complex equilibrium - yes, but not necessarily as free "polyvanadates." See my earlier comments about binding.
11	20-21	If we are expanding beyond V_2O_5, you may wish to examine the Japanese studies on patients undergoing dialysis exposed to vanadium-rich Japanese water (late '80s/early '90s).
55	15	Effects of multiple metals being confounding is a common theme in this report (e.g. D2).
77	35-37	This statement really limits the impact of the report. It is certainly a true statement, though. (same with page 87, lines 24-26)
82		V_2O_5 exposure appears to lead to higher survival rates. Apparently this is a typographical error. Sometimes percent survival is really a percentage and other times it is an actual value. Please correct.
83	Table 5-4	The superscripts do not match the notes.
96	10	See page 3, line 17 comment.

Xianglin Shi

None.

Qunwei Zhang

Page	Paragraph or Line #	Comment or Question
77	34	"0.000085 mg/kg-day" should be "0.00085 mg/kg-day."
83	Table 5-4 footnote	"c" should be "b" and "d" should be "c."

Yiliang Zhu

The following is a list of editorial observations.

Page	Paragraph or Line #	Comment or Question
14	31-32	"Workers who reported wheezing were twice as likely to work in the vanadium factory" should be "people working in the vanadium factory were twice as likely to report wheezing."
19	16-17	"The number of years worked as a boilermaker was a statistically significant predictor of annual FEV1 decrease": average decrease in FEV1 among (aging) adults is about 30 ml per year. The statement could be false if the workers are of mature age.
21	10	Strike out "1981?"
22	Table 4-1	Column labels are incorrect after the 1st page.
47	1	Typo: 312
48	Table 4-8	Instead of pairwise comparison of each dose group against the control, trend test should be considered to see the overall significance of dose-response.
53	Table 4-11	Historical controls cannot have negative incidences!
53	17	Spell out the full name of PBS.
67	3	Typo at the beginning?
74	38	Should be oral instead of "inhalation."
78	27	Change "This study" to "The study of Knecht et al (1992)."
84	8-9	BMR=10% was chosen uniformly. So it is unclear what biological and statistical considerations were taken in the selection of BMR.
85	4-5	Same as page 84, line 8-9.
87	1st paragraph	BMCL on average behaves like a NOAEL, thus UF=1. BMR=10% represents risk increase in the population, not "minimal, biologically significant change". Every case of larynx inflammation is biologically significant change.
88-89	37(88)-1(89)	The uncertainty must be considered under both the model and BMR level. The 10% BMR level is necessarily supported within the data range.
89	27	Should be Table 5-8.
90	Table 5-8	Subscript c is misplaced.
91	33	Delete the extra "as."
91	15-16	Check this sentence.

Additional References:

Lech Zychlinski, Janusz Z. Byczkowski and Arun P. Kulkarni. (1991) Toxic effects of long term intratracheal administration of vanadium pentoxide in rats. ARCHIVES OF ENVIRONMENTAL CONTAMINATION AND TOXICOLOGY 20(3), 295-298.

Lech Zychlinski and Janusz Z. Byczkowski (1990) Inhibitory effects of vanadium pentoxide on respiration of rat liver mitochondria. ARCHIVES OF ENVIRONMENTAL CONTAMINATION AND TOXICOLOGY. 19(1): 138-142.

Tadao TOYA, Kazuo FUKUDA, Mitsutoshi TAKAYA and Heihachiro ARITO (2001) Lung Lesions Induced by Intratracheal Instillation of Vanadium Pentoxide Powder in Rats Industrial Health 39, 8–15.