# National Toxicology Program

# Technical Reports Peer Review Panel Meeting

# February 16, 2016

# National Institute of Environmental Health Sciences

# Research Triangle Park, NC

## *Peer Review Report*

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# I. Attendees

**Members in Attendance:**
William Brock
Michael Elwell
Jon Mirsalis (Panel Chair)
Kent Pinkerton
Michael Pino
Tara Sabo-Attwood
Madhuri Singal

**NTP Board of Scientific Counselors Representative:**
George Corcoran (via Webcast)

**National Institute of Environmental Health Sciences (NIEHS) Staff:**

Mamta Behl
Chad Blystone
John Bucher
Natasha Catlin
Vivian Chen
Sheba Churchill
Michael DeVito
June Dunnick
Gordon Flake
Paul Foster
Dori Germolec
Robbin Guy
Ron Herbert
Michelle Hooth
Angela King-Herbert
Kelly Lenox
David Malarkey
Scott Masten
Barry McIntyre
Rachel McIntosh-Kastrinski
Dan Morgan
Arun Pandiri
Cynthia Rider
Georgia Roberts
Veronica Robinson
Kristen Ryan
Kelly Shipkowski
Stephanie Smith-Roe
Matt Stout
Vicki Sutherland
Eric Tokar
Molly Vallant
Suramya Waidyanatha
Nigel Walker
Kristine Witt
Mary Wolfe
Yun Xie

**Contract Staff to NIEHS**
Charles Alden, Kelly Services
Amy Brix, Experimental Pathology Labs, Inc.
Steven Brecher, CSS-Dynamac
Sudha Iyer, CSS-Dynamac
Kyathanahalli Janardhan, Integrated Laboratory Systems
Amy Johnson, Charles River Pathology Associates
Ramesh Kovi, Experimental Pathology Labs, Inc.
Rachel McIntosh-Kastrinski, Kelly Services
Marjo Smith, Social and Scientific Systems, Inc.
Varghese Tharakan, CSS-Dynamac

**Public Attendees**
Ann Ball, Independent Lubrication Manufacturers Association
Patricia Beattie, Master Chemical Corporation
Craig Boreiko, CJB Risk Analysis, LLC
Daniel Bryant, Independent Lubrication Manufacturers Association
Walden Dalbey, DalbeyTox, LLC
Steve Florio, Master Chemical Corporation
Ernie Hood, Bridport Services, LLC
John Howell, GHS Resources, Inc.
Jeffrey Nelson, Master Chemical Corporation
Ria Scheuren, Houghton International
Julie Thomas, Master Chemical Corporation

## II. Welcome and Introductions

The National Toxicology Program (NTP) Technical Reports Peer Review Panel Meeting convened on February 16, 2016, in Rodbell Auditorium, National Institute of Environmental Health Sciences (NIEHS), Research Triangle Park, North Carolina. Dr. Jon Mirsalis served as chair. The other panelists in attendance were Drs. William Brock, Michael Elwell, Kent Pinkerton, Michael Pino, Tara Sabo-Attwood, and Madhuri Singal. Dr. George Corcoran attended by webcast as the NTP Board of Scientific Counselors liaison.

Dr. Mirsalis welcomed everyone to the meeting and asked all attendees to introduce themselves. Dr. Bucher welcomed participants and thanked the board members and staff for their work. Designated Federal Officer Dr. Yun Xie read the conflict of interest statement.

## III. Peer Review of Draft NTP Technical Reports: Charge

Dr. Chad Blystone, toxicologist in the Toxicology Branch of the Division of NTP (DNTP), briefly reviewed the Levels of Evidence of Carcinogenic Activity guidelines used to express the draft NTP conclusions. He also stated the panel's charge.

## IV. Draft NTP Technical Report on Antimony Trioxide (TR590)

### A. Presentation

NTP study scientist Dr. Matt Stout briefed the panel on the draft NTP technical report on antimony trioxide. Antimony trioxide is the primary and most commercially significant form of antimony. It is used as a flame retardant synergist and a catalyst in the production of polyethylene terephthalate plastics. The Consumer Product Safety Commission and NIEHS

nominated antimony trioxide to NTP for study because of substantial human exposure in occupational settings and a lack of adequate 2-year exposure carcinogenicity studies.

NTP conducted 2-week and 2-year whole body inhalation studies in male and female Wistar Han rats and B6C3F1/N mice. The 2-year studies were designed following review of the 2-week study data and subchronic and chronic studies in the literature. The highest exposure concentration in the 2-year studies was 30 mg/$m^3$.

Based on the 2-year studies, the draft NTP report's conclusions on antimony trioxide were:

Male Wistar Han rats

- ***Some evidence of carcinogenic activity***
    - Increased combined incidences of alveolar/bronchiolar adenoma or carcinoma in the lung
    - Increased incidences of benign pheochromocytoma of the adrenal medulla

Female Wistar Han rats

- ***Some evidence of carcinogenic activity***
    - Increased incidences of alveolar/bronchiolar adenoma in the lung
    - Increased combined incidences of benign or malignant pheochromocytoma of the adrenal medulla
- May have been related to exposure (equivocal evidence)
    - Combined occurrence of cystic keratinizing epithelioma and squamous cell carcinoma in the lung

Male B6C3F1/N mice

- ***Clear evidence of carcinogenic activity***
    - Increased incidences of alveolar/bronchiolar adenoma or carcinoma in the lung
- Related to exposure (some evidence)
    - Increases in the combined incidences of fibrous histiocytoma or fibrosarcoma in the skin

Female B6C3F1/N mice

- ***Clear evidence of carcinogenic activity*:**
    - Increases in the incidences of alveolar/bronchiolar adenoma and alveolar/bronchiolar carcinoma of the lung
    - Increased incidences of malignant lymphoma
- May have been related to exposure (equivocal evidence)
    - Occurrence of squamous cell carcinoma of the skin

Exposure to antimony trioxide resulted in increased incidences of nonneoplastic lesions of the lung, nose, larynx, trachea, bronchial and mediastinal lymph nodes, and bone marrow of male and female rats and mice; the adrenal medulla, arteries of multiple tissues (mesentery, pancreas, mediastinum, kidney, and lung), and the eye of male and female rats; the thymus and heart of male and female mice; the forestomach of male mice; and the spleen of female mice.

## B. Questions for Clarification

Dr. Pinkerton asked whether the skin reaction seen in the test animals was due to inhalation or dermal exposure from whole body exposures. Dr. Stout replied that there is no clear answer; however, the skin reaction could have been due to direct contact with the skin during whole body exposure. Dr. Pinkerton asked Dr. Stout about the plateau seen in lung deposition and clearance with the 3 mg/$m^3$ dose versus no plateau for the 30 mg/$m^3$ dose. He speculated the difference was due to the animals being unable to clear the particles at the higher dose. Dr. Stout agreed that lung burden increased over time due to decreased pulmonary clearance from particle overload at the 30 mg/$m^3$ dose. Dr. Pinkerton asked how lung burden was measured. Dr. Stout said that inductively coupled plasma-atomic emission spectrometry was used to measure antimony and this data was mathematically converted to antimony trioxide concentrations, which were multiplied by lung weights to calculate lung burden.

Dr. Brock asked whether there was a dose-related change in lung overload. Dr. Stout said there was and there was a graded response in both rats and mice. Dr. Brock noted that survival and body weight at the higher dose seemed to suggest that maximum tolerated dose had been exceeded. Dr. Stout noted that the exposure concentrations were selected following a review of the inhalation studies in the literature and the NTP 2-week studies. There were no data in any of these studies that would point to a decrease in survival at the exposure concentrations selected. In addition, exposure concentrations were selected to sufficiently challenge the animals, particularly given that these studies were conducted to determine if antimony trioxide was carcinogenic following inhalation exposure.

Dr. Singal asked about hyaline droplet formation in the kidney. Dr. Stout said the lesion might be due to an accumulation of surfactant. NTP study pathologist Dr. Gordon Flake noted it was very likely because there were increased levels of surfactant in the blood; however, the cause of the lesions is ultimately unknown, and the lesions did not seem to be a major issue in the kidney in terms of causing morbidity in the animals. Dr. Singal asked about thymic weight increases and cellular depletion in the mice. Dr. Flake replied that the thymic histology was complicated, with the weights increased in the first year and cellular depletion noted

histologically in the second year. Dr. Flake described several observations at the first and second year. In the second year, some of the animals developed lymphoma, while others did not develop lymphoma and had stress-related atrophy. He proposed adding a statement to the report's Discussion on this issue.

Dr. Brock asked Dr. Stout about clinical signs seen only in the second year, inquiring how soon in the second year they had occurred, noting that no temporal relationship had been addressed. Dr. Stout said the data were available on the NTP public website. In most cases, particularly in the higher doses, the clinical observations developed in the last few months of the study. Dr. Brock asked how clinical observations could be correlated with lung burden, and found discussion of that missing from the report. He asked about the B- and T-cell lymphomas, and whether there was proportionality associated with those responses. Dr. Flake said that he found a predominance of B-cell lymphomas in the lung and spleen, with the T-cells possibly reacting to the presence of the B-cell malignancy. He said such a process has been observed in humans.

Dr. Pinkerton asked about foreign bodies and whether antimony is always a solid particle, or if it can become soluble and reform into another particle. Dr. Stout replied that there were no data to address that question.

Dr. Mirsalis asked whether the observed pattern of neoplastic or nonneoplastic lesions in this study is similar to observations from other NTP inhalation studies of metals. Dr. Stout said that in general, the observations have similarities. He noted that skin reactions were not always seen.

## C. Public Comments

Dr. Mirsalis noted receipt and distribution to the panel of written comments from Dr. Craig Boreiko of CJB Risk Analysis, LLC on behalf of the International Antimony Association. He then recognized Dr. Boreiko for oral public comments.

Dr. Boreiko spoke on behalf of the International Antimony Association and noted he would be touching on highlights of the written comments he had previously submitted. He said there was no question that something was happening in the mouse; however, he proposed that the question of why remained largely unresolved in the report. He was surprised by the results in the rat. He speculated that deep alveolar loading was likely to be significantly greater in the rat studies than that seen in previous studies. He noted that particle overload had been discussed in the body of the report; however it was not in the abstract. He questioned interpretation of the Comet assay data, and asked whether the micronucleus data were actually biologically significant. He questioned the conclusion of clear evidence of lymphoma in female mice.

## D. Peer Reviewer Comments

Dr. Pino, the first reviewer, stated the studies in both rats and mice were well conducted, and the results overall supported the NTP's conclusions regarding the long-term toxicity and carcinogenicity of antimony trioxide. Referring to his preliminary written comments, he made several specific suggestions regarding information he thought should be added to or deleted from the report. For example, he suggested adding mention of foreign body in the summary findings in the abstract, providing solubility of antimony trioxide in water, clarifying the paragraph about animal source, and discussing whether or not cataracts observed in female mice were considered related to exposure. He also suggested giving the reasons for why the findings in the prostate gland and skin of rats and the adrenal medullary pheochromocytomas in mice were not considered exposure related. He disagreed that the incidence of mammary gland carcinoma in rats was meaningfully decreased. He also made several specific editorial corrections and comments. He suggested changes to the report's conclusions, including rewriting a sentence in the first paragraph to indicate that the incidence of fibrous histiocytoma alone is also increased and replacing "lesions of" with "findings in" in the second paragraph. He noted that there was no increase in reticulocytes in the micronucleus test in rats at 12 months to correlate with the bone marrow increase in erythroid precursors at two years. This was in contrast to observations in the mouse. He suggested hematology should have been performed to determine what was happening with circulating erythrocytes. He agreed with the discussion in the report that significance of increased micronuclei in mice was of questionable significance.

Dr. Stout responded that "foreign body" was deliberately not mentioned in the report's abstract to avoid repetition. Dr. Pino suggested that a brief sentence discussing foreign body observations would be helpful. Dr. Stout concurred. For Dr. Pino's other suggestions, Dr. Stout agreed with his comments and noted that sections of the report would be updated accordingly.

Dr. Pinkerton, the second reviewer, stated the study design, conduct, and findings were excellent. He noted that the conclusions made by NTP were scientifically sound and logical. While he did not recommend deleting any information from the report, he suggested that tables should be adjusted to provide a clear designation of the total number of animals examined. He suggested adding a definition of "thinness" for the animals. He asked for more information on inflammation of the pleura and appreciated the report's inclusion of the rationale for changing the rat model.

Dr. Stout said he would ensure that the number of animals is included in all tables. Regarding "thinness," he said it is a general body shape observation. Dr. Flake discussed pleural inflammation, stating that particles in or below the pleura appeared to cause the fibrosis that had been observed. Dr. Pinkerton asked if it was dose-related. Dr. Flake replied that it was.

Dr. Singal, the third reviewer, stated that overall the study design was appropriate and suggested improvements to enhance the report's thoroughness and of additional endpoints that could have been added to the studies. She asked for more information about how the material affected the system as a whole. She suggested that urinary excretion could have been added. She asked for further evaluation of the difference in thymus weight increases between rats and mice. She suggested lung lavage data would have been informative of the proteinaceous/cellular changes in the lungs. She asked for further evaluation of the observation regarding karyorrhectic debris, as well as evaluation of lymphocytic infiltrates in rats. She noted that skin lesions had been observed in mice, and suggested that future studies should include a more detailed evaluation of dermal changes due to the relevance for effects observed in humans. She suggested further investigation of hyaline droplet formation in the kidney. She suggested inclusion of Comet assay data, as it is mentioned in the text and not shown, to allow a basis for comparison of the differing DNA damage results in mice and rats. In reference to the discussion in the report about potential intrinsic toxicity of antimony trioxide, she noted that particle surface chemistry may contribute to free radical generation and asked for further investigation. Regarding microscopic image slides, she asked for more information about the collection and fixing process and the method of sectioning. She suggested adding proper labeling with magnification information. She recommended adding images of sections of the upper respiratory tract. She noted that there was reference to deposition and clearance modeling without data or methodology. She said her observations would enhance the report and not detract from the study's findings.

Dr. Stout stated that urinary excretion is not typically assessed in these types of studies and acknowledged that it might have been useful in this case. He said that lung lavage data are typically obtained in satellite studies. Dr. Flake addressed Dr. Singal's comment regarding arterial inflammation, noting that the cause was unknown and there are several possibilities. Regarding Dr. Singal's question about pulmonary edema, he noted that the associated histologic changes were not observed. He proposed the vascular changes were most likely related to increased blood volume. Regarding the karyorrhectic debris, he said it was related to the neutrophils and alveolar macrophages in the lung, which tend to form aggregates and ultimately break down to form the debris seen. Regarding the lymphocytic infiltrates in the rats, he said it was a less prominent reaction than in the mice. Dr. Stout concurred with Dr. Singal's comment about cytokine analysis being useful to include; however, he said the molecular analyses were retrospective; therefore, there was not an opportunity to collect specimens for cytokine analysis. He agreed that the skin effects warranted further investigation. He said Dr. Singal's suggestion regarding hyaline droplet accumulation was noteworthy. Regarding the DNA damage results, he noted that the genotoxicity data are publicly available on the website and that there was no observed explanations for the difference between the results in the rats and

the mice. He said that the issue of particle surface chemistry in the study of metal particles could be important to investigate in better understanding the toxicity. He said that the final magnification was not shown on the photos because it could be different when it is published. He stated that collecting and fixing sections of the lungs followed standard NTP practices. He agreed that it would be helpful to add more detail about the inflation fixing pressure and that images of the upper respiratory tract would be helpful. He noted that deposition and clearance information had been included in the report's appendix and would be checked for completeness.

Dr. Pinkerton noted that if scale bars were included with the images, the magnification would not change in printing.

## E. Panel Discussion and Vote

Dr. Mirsalis opened the discussion to general comments from the panel.

Dr. Brock asked whether the panelists' discussions and reviews would be captured and processed for inclusion in the final reports and future studies. Dr. Blystone replied that all comments are discussed and addressed, and suggestions are taken into account. Dr. Brock asked if there was a process for protocol review. Dr. Blystone said that there are multiple steps for protocol review and reviewer comments are considered. Dr. Brock said that he had found the dose level justification weak given the results of the study. He suggested enhancing the dose justification in the report.

Dr. Elwell asked whether the adenoma in the nose (listed in the summary tables in the appendix) of one of the high-dose male rats should be mentioned in the body of the report, given that it was at a target site. He asked about the incidence of adenomas in males at the highest dose in the lung; the table in the report indicated that there were eight, which did not seem to agree with the incidence in the summary table of the report. Dr. Stout noted that eight is the correct number.

Dr. Mirsalis stated that following standard regulatory guidelines, the genotoxicity data would be considered negative. Regarding the micronucleus data, he said that it is important to consider the historical control data for comparison. He emphasized that contrary to language in the report, there was a lack of genotoxic carcinogen response. He recommended toning down that section of the report. Ms. Kristine Witt, genetic toxicology group leader in the Biomolecular Screening Branch of DNTP, noted that the report reflected what had been observed. The level of response was low, and the historic control data were taken into account.

Dr. Mirsalis called for the conclusions to be projected. Dr. Brock moved to accept the conclusions as written, and Dr. Pinkerton seconded the motion. Dr. Pino suggested adding the

phrase “incidence of fibrous histiocytoma and the” to the second bullet point under the male mice. In the discussion of nonneoplastic issues, Dr. Pino suggested changing the word “lesions” to “findings,” and the addition of “the kidney and” between “and” and “eye” later in the sentence.

Given Dr. Pino’s suggestions, Dr. Brock withdrew his original motion. Dr. Pino moved to accept the conclusions as revised. Dr. Pinkerton seconded the motion. Dr. Mirsalis called for a vote on the motion. The panel voted unanimously to accept the motion.

Thus, the panel voted to accept the following amended conclusions for antimony trioxide:

Male Wistar Han rats

- ***Some evidence of carcinogenic activity***
    - Increased combined incidences of alveolar/bronchiolar adenoma or carcinoma in the lung
    - Increased incidences of benign pheochromocytoma of the adrenal medulla

Female Wistar Han rats

- ***Some evidence of carcinogenic activity***
    - Increased incidences of alveolar/bronchiolar adenoma in the lung
    - Increased combined incidences of benign or malignant pheochromocytoma of the adrenal medulla
- May have been related to exposure (equivocal evidence)
    - Combined occurrence of cystic keratinizing epithelioma and squamous cell carcinoma in the lung

Male B6C3F1/N mice

- ***Clear evidence of carcinogenic activity***
    - Increased incidences of alveolar/bronchiolar adenoma or carcinoma in the lung
- Related to exposure (some evidence)
    - Increases in the incidence of fibrous histiocytoma and the combined incidences of fibrous histiocytoma or fibrosarcoma in the skin

Female B6C3F1/N mice

- ***Clear evidence of carcinogenic activity*:**
    - Increases in the incidences of alveolar/bronchiolar adenoma and alveolar/bronchiolar carcinoma of the lung
    - Increased incidences of malignant lymphoma
- May have been related to exposure (equivocal evidence)
    - Occurrence of squamous cell carcinoma of the skin

Exposure to antimony trioxide resulted in increased incidences of nonneoplastic findings of the lung, nose, larynx, trachea, bronchial and mediastinal lymph nodes, and bone marrow of male and female rats and mice; the adrenal medulla, arteries of multiple tissues (mesentery, pancreas, mediastinum, kidney, and lung), and the kidney and eye of male and female rats; the thymus and heart of male and female mice; the forestomach of male mice; and the spleen of female mice.

## V. Draft NTP Technical Report on TRIM® VX (TR591)

### A. Presentation

NTP study scientist Dr. Kristen Ryan briefed the panel on the draft NTP technical report on TRIM® VX. TRIM® VX is a water-soluble metalworking fluid (MWF) in the class known as soluble oils. Due to their high production volume, the large number of occupationally exposed workers, and the lack of carcinogenicity and toxicology data, the National Institute for Occupational Safety and Health nominated MWFs for NTP study. TRIM® VX was one of four MWFs selected for study from the original slate of 30.

NTP conducted 3-month and 2-year whole body inhalation exposure studies in male and female Wistar Han rats and male and female B6C3F1/N mice.

The 3-month study showed that the major target of TRIM® VX exposure was the respiratory tract, with similar toxicity in both sexes and species. Lung fibrosis was also seen, which was a distinct finding in NTP comparison studies with MWFs. No effects were seen on overall survival, clinical observations, or body weights in both sexes and species.

Based on the 2-year studies, the draft NTP report's conclusions on TRIM® VX were:

Male Wistar Han rats

- ***Equivocal evidence of carcinogenic activity***
    - Combined occurrences of alveolar/bronchiolar adenoma or carcinoma of the lung

Female Wistar Han rats

- ***Equivocal evidence of carcinogenic activity***
    - Occurrences of alveolar/bronchiolar adenoma of the lung

Male B6C3F1/N mice

- ***Clear evidence of carcinogenic activity***
    - Increased combined incidences of alveolar/bronchiolar adenoma or carcinoma of the lung

Female B6C3F1/N mice

- ***Clear evidence of carcinogenic activity:***
    - Increased combined incidences of alveolar/bronchiolar adenoma or carcinoma (primarily carcinoma) of the lung

Exposure to TRIM® VX resulted in increased incidences of nonneoplastic lesions of the lung, nose, and larynx of male and female rats and mice; the bronchial lymph node in male and female rats and male mice, and the mediastinal lymph node in male and female rats.

## B. Questions for Clarification

Dr. Sabo-Attwood asked Dr. Ryan to comment on the bacterial fungal growth assays performed, and whether they also included an endotoxin assay. Dr. Ryan said they had not done an endotoxin assay. An assessment for bacterial and fungal growth was done, and no evidence of bacterial or fungal growth was detected throughout the studies.

Dr. Brock questioned the stability assessments performed on the test article. He asked for clarification on Dr. Ryan's presentation to "data compared to frozen reference sample upon receipt of the material." Dr. Ryan replied that when the test material was received, aliquots were taken out and frozen, so that current data could be compared to the reference samples at any point during the study, to see if there was any degradation over time. Dr. Brock noted that method assumed that the frozen samples would not also degrade. Dr. Ryan said no evidence of degradation was seen. Dr. Suramya Waidyanatha, chemistry group leader in the Program Operations Branch of DNTP, described the procedures that had been conducted to assess stability and clarified that the test article was stored at 5°C. Dr. Brock said he found the description in the report confusing.

## C. Public Comments

Dr. Mirsalis noted receipt and distribution to the panel of written comments from Ms. Holly Alfano of the Independent Lubricant Manufacturers Association (ILMA) and Dr. Steven Florio of Master Chemical Corporation. Dr. Mirsalis then recognized an oral public commenter, Dr. Franklin Mirer of the CUNY School of Public Health, who spoke on his own behalf by telephone.

Dr. Mirer described the history of the MWFs project, which began with a petition to NTP from the United Auto Workers. He noted that the respiratory effects of MWFs are as important to public health as carcinogenic effects. He noted the contrast between fresh fluids and in-use, contaminated fluids. He said that the respiratory effects of in-use MWFs of all types are generally accepted, with the dispute being whether they stem from microbial contamination or whether the fluids themselves have toxic potential. He cited several previous studies from the literature. He asked NTP to publish complete analyses of all nine test articles, especially the four

articles subjected to 90-day testing. He noted that Master Chemical Corporation has already discontinued TRIM® VX. He pointed out that sulfonate had not appeared as a component of TRIM® VX in NTP analysis. He stated that lung tumors in male rats should be "some evidence," not "equivocal evidence." He agreed with the "clear evidence" conclusion for lung tumors in mice of both genders.

Next, Dr. Mirsalis acknowledged a series of four public commenters. Dr. Walden Dalbey of DalbeyTox, LLC and Dr. John Howell of GHS Resources, Inc. spoke on behalf of ILMA. Dr. Patricia Beattie of SciVera, LLC and Dr. Steven Florio of Master Chemical Corporation (MCC) spoke on behalf of MCC.

Dr. Dalbey asked NTP to clarify the rationale for the selection of TRIM® VX. He questioned the handling of TRIM® VX samples and asked why the material had not been diluted with water, as is common in the workplace. He asked for the rationale and validation of the methods used to determine particle size and monitor total aerosol concentration. He noted a discrepancy of NTP's analysis of mineral oil content in TRIM® VX compared with the MSDS. He noted other issues with NTP's analyses of TRIM® VX, including the reported pH of the test article. He asked for a more explicit statement on the lack of systemic effects than what is currently in the draft report. He recommended NTP to consider a different mode of action than what is stated in the report.

Dr. Beattie provided details about TRIM® VX and the history of its toxicity testing. She noted that the tested TRIM® VX concentrate would become alkaline when mixed with the moisture in the respiratory tract, likely causing the irritation, inflammation, and tumors formed at the site of contact. She said that the negative results in genotoxicity tests, the lack of systemic toxicity or tumors, and tumors only seen at the site of contact at the highest dose, all suggest a non-genotoxic mechanism. She noted that she made a request to NTP for more detailed chemical characterization and stability analytical information, and NIEHS denied the release of the additional analytical information.

Dr. Florio provided further background information about TRIM® VX and listed several analytical concerns related to the NTP study, particularly the chemical degradation and analysis of the test article. He noted that the test article used in the study had aged outside of its recommended one-year shelf life, affecting its stability. He concluded that the two lots of material tested were not chemically equivalent to the TRIM® VX produced and marketed by Master Chemical Corporation.

Dr. Howell discussed the NTP selection process for MWFs, with which ILMA had cooperated. He asserted that NTP should find the TRIM® VX study inadequate because of significant issues regarding the characterization of the test article, such as the potential for bacterial and fungal

growth, variations in the characterization of the compound, and use of the product well beyond its stated shelf life. He noted that TRIM® VX is a unique formulation and that according to OSHA regulations, the study results cannot be extended to other MWFs.

## D. Peer Reviewer Comments

Dr. Elwell, the first reviewer, stated that overall the report was well written and the results supported the conclusions. Regarding the findings, he cited Table 9 in the report, and asked why some of the nonneoplastic results (nasal polyps) listed in the summary table in the appendix had not been discussed in the body of the report. He asked whether the cystic keratinizing epithelioma (CKE) seen in a high-dose female had been considered to be part of the equivocal evidence. He recommended adding discussion in the report about the occurrence of multiple adenomas in rats and mice. He asked for clarification of the sentence on page 62, "Alveolar/bronchiolar neoplasms were morphologically typical of those that occur spontaneously." He agreed in principal with the conclusions; however, he suggested considering CKE as part of the equivocal evidence and asked for further information about nasal tumors in rats for possible inclusion in the report.

Dr. Ryan said that the nasal polyps appeared to be more inflammatory than neoplastic, and were deemed to not warrant inclusion in the report's text. NTP study pathologist Dr. Ron Herbert agreed with Dr. Ryan's statement. Regarding the CKE, Dr. Ryan said that the corresponding text in the report could be clarified, and noted that since it was a single incidence, it was not considered part of the call. Regarding the multiple adenomas in the rats and mice, she said additional discussion would be added to report. Dr. Herbert explained that the bronchial adenoma had been listed as a single finding and was not included in the call. Dr. Ryan said further text changes in report would be considered for clarification.

Dr. Brock, the second reviewer, said that the report was generally well-written. He noted that because TRIM® VX is a mixture and the specific chemical composition has not been revealed, the results from the current TRIM® VX studies make it difficult to determine the potential hazard associated with other soluble metal working fluids. He suggested that the authors insert an appendix or other reference for the chemical composition of other soluble MWFs to allow subsequent use of the toxicological data in the report for comparison to other mixtures. He noted the discrepancy between NTP's stated chemical composition for TRIM® VX and the manufacturer's listing. He stated the high dose used in the 2-year studies was too high, and that an exposure concentration of 50 mg/m$^3$ would have been sufficient. Thus, he asked for more description of dose selection in the discussion section of the report. He proceeded to provide specific recommendations for each section of the report. Notably, he asked for clarification on the methods used to test the stability of the test article and validate aerosol chamber

concentrations. He asked for an explanation on what was unique about the occurrence of lung fibrosis.

Regarding Dr. Brock's comment on the selection of TRIM® VX, Dr. Ryan said that TRIM® VX was selected as an example of a soluble oil and not to reflect all soluble oils. She said this would be clarified in the report. As to the dose selection rationale, she said the aim was to design a study to allow cross-species comparison; thus, the concentration needed to challenge both rats and mice. A dose of 50 mg/$m^3$ would have been sufficient in rats; however, a higher dose was needed to challenge the mice. Thus, 100 mg/$m^3$ was chosen as a dose to not limit survival or have overt toxicity. There was also an aim to compare the TRIM® VX study to the prior CIMSTAR study, which had similar dose selection. Dr. Brock said that although he appreciated the complexity of 2-year bioassays, using the same dose in rats and mice because it is easier is not a good answer. He renewed his request for a more robust dose justification. Dr. Ryan responded to several of Dr. Brock's specific suggestions. Regarding test article stability, she said that it was evaluated with a qualified method described in the appendix, and that there was no evidence of degradation in the bulk test article. She stated that the analysis of the chamber concentration was based on the quantitative assessment of three TRIM® VX components as mentioned in the appendix. She said additional text could be added to the report to further describe the methods for test article stability and validating aerosol chamber concentrations. She noted that data regarding clinical signs of toxicity were available on a website, which would be added to the report. Regarding the lung fibrosis, it was not unique on its own; however, it was interesting that lung fibrosis was found in the TRIM® VX studies in both species and this fibrosis was not seen in NTP studies of other MWFs.

Dr. Sabo-Attwood, the third reviewer, said the report represented a scientifically sound study and was well written. She asked for more clarity about the fibrotic lesions. Like Dr. Brock, she thought TRIM® VX was representative of the larger class of water-soluble MWFs based on the text in the draft report. She recommended edits to the text to avoid that confusion. TRIM® VX is a complex mixture, so it is unclear how the component chemicals are distributed *in vivo*. Based on the description provided, she anticipated that the constituents would be highly soluble and would not be the particulates in the aerosols that would be consistent with fibrotic lesions associated with particulate exposure. She noted the fibrotic lesions are described in the report as those commonly seen with irritation. She asked for clarification on what the irritation was and whether it was a distinguishing lesion for this particular material, especially compared to other MWFs that were tested. She noted tissues were stained with Oil Red O to indicate the presence of TRIM® VX in immune cells, inflammatory lesions, and hyperplastic alveolar epithelium; however, the increase in staining could also represent phospholipid inclusions due

to disruption of lung surfactant or result from cell death, perhaps of alveolar cells.[1] She suggested additions to the report because there are limited data available regarding metabolism and clearance of TRIM® VX. She also asked for explanation for the use of Oil Red O stain in rats and not in mice. She asked about endotoxin testing, and whether the inflammatory responses that were seen were indicative of exposures to endotoxins or other pathogens.

Dr. Ryan would consider adding text to further describe the fibrotic lesions in the report; however, the studies were not designed to directly link particulates or components of TRIM® VX with the fibrotic lesions. The fibrotic lesions were not indicative of the pre-cancerous lesions. Dr. Herbert said that the fibrotic changes were not considered a part of the continuum from pre-neoplastic change to the tumors that were seen in the lung, which were mainly alveolar/bronchiolar carcinomas. He explained that Oil Red O staining had not been done in mice because the entire mouse lung tissue was embedded for preparation of histological slides; therefore, there was no residual wet lung tissue that could be used to perform Oil Red O staining. He said that the stain had been used because although TRIM® VX is soluble, the lesions were morphologically similar to those observed with inhalation exposure to a particulate. Thus, there was an interest in seeing if there was an appreciable amount of oil left in the lung that was causing a foreign body reaction. Residue from oil was found in the tissues. Dr. Sabo-Attwood asked if one could distinguish it from material identified as TRIM® VX and phospholipid damage, which would show up from staining. Dr. Herbert said he would add statements to the report to reflect Dr. Sabo-Attwood's comments. Dr. Brock asked if the tissue had been formalin-fixed, which would remove all lipids. Dr. Herbert said the lipid was not removed by formalin fixation of the lungs and would only be removed if the tissue were subjected to histological processing. Thus, the wet tissue would still have oil present in the lungs. Dr. Ryan noted that endotoxin was not measured in the study; however, there was assessment of bacterial and fungal growth at several study time points. There was no evidence of bacterial or fungal growth.

Dr. Sabo-Attwood asked if there was anything indicative in the inflammatory response that was relevant to an endotoxin-type exposure. Dr. Herbert explained that with so many inflammatory changes, it was not possible to delineate whether they were due to an endotoxin or exposure and direct contact with the chemicals.

## E. Panel Discussion and Vote

Dr. Singal asked about necrotic lesions that were attributed to increased exudative pressure, and inquired about other characteristics aside from the necrotic changes would suggest the potential for increased barotrauma. Dr. Herbert said there was an accumulation of exudate in

[1] Epping G, Van Baarlen J, Van Der Valk PD. 2011. Toxic alveolitis after inhalation of a water repellent. Int J Occup Med Environ Health 24(4): 409-413.

the ventral portions of the nasal cavity, which was severe in some cases and most likely resulted in pressure degeneration, necrosis, and ultimately rupture and perforation of the nasal septa.

Dr. Pinkerton asked if the lung fibrosis was thought to lead to greater potential for carcinogenesis. Dr. Pinkerton asked if there was any evidence that greater fibrosis was associated with greater propensity to see tumors. Dr. Herbert replied that could not be stated with any certainty. There are some inhalation studies with particulates where fibrosis is observed without this type of scar tumors.

Dr. Mirsalis commented on the selection of TRIM® VX. He suggested making the following points clear in the introduction: TRIM® VX is an example of a MWF, relatively small volume of it was in use, and it has since been discontinued. He noted that wider conclusions about soluble MWFs should not and could not be drawn based on this study, which stands on its own. Dr. Bucher confirmed Dr. Mirsalis' statement, adding that there should be an idea from the discussions about how difficult it was to sort through all of the different products on the market and determine their identifiable components versus trade secret components. It was difficult to select materials for 2-year study that would give some indication of whether some of the effects that were seen in the MWFs could be attributed to materials that were not contaminated with bacteria during the course of their use. Due to the complexity of the field, the materials chosen are not representative, but are individual materials.

Regarding the question of NTP's chemical analysis of the materials, Dr. Mirsalis said that the information should be included in the final report, as it should be readily available. Regarding the earlier comment that the study should be considered inadequate because the material was not actually tested, he said that the study is an adequate study. One may argue the relevance of the study; however, it meets the requirements for an adequate study.

With no further discussion and no requests for edits to the conclusions, Dr. Mirsalis called for a motion on the conclusions. Dr. Elwell moved that the conclusions be accepted as written. Dr. Pinkerton seconded.

Dr. Brock asked for further clarification on the issue of the fibrosis and the carcinomas seen in the study. Dr. Herbert elaborated that in terms of the neoplasms seen in the study, fibrosis was not seen as a prerequisite.

Dr. Mirsalis called for a vote on the motion. The panel voted (5 yes, 0 no, and 0 abstentions) to accept the conclusions as written. Dr. Pino was recused and not present during the peer review of the draft NTP technical report on TRIM® VX.

Dr. Bucher thanked the peer review panel for the depth and seriousness with which they reviewed the draft reports. He noted the peer review process always improves the reports.

Dr. Mirsalis thanked the panel members for their participation and adjourned the meeting at 4:00 p.m., February 16, 2016.

## VI. Approval of the Peer Review Report by the Chair of the Peer Review Panel

This peer review report has been read and approved by the Chair of the February 16, 2016, National Toxicology Program Technical Reports Peer Review Panel.

[Signature Redacted]

Jon C. Mirsalis, Ph.D., D.A.B.T.

Chair, NTP Technical Reports Peer Review Panel

Date: 4-28-2016