National Toxicology Program

Technical Reports Peer Review Panel Meeting

October 29, 2013

National Institute of Environmental Health Sciences

Research Triangle Park, NC

Summary Minutes

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Table of Contents

I. Attendees

Peer-Review Panel:
Norman Barlow
Russell Cattley
Deborah Cory-Slechta
John Cullen (Panel Chair)
Terry Gordon
Joel Parker
Karen Regan
Timothy Zacharewski

NTP Board of Scientific Counselors Representative:
Richard Miller

Other Federal Agency Staff:
Frederick Beland, Food and Drug Administration/National Center for Toxicological Research (FDA/NCTR)
Paul Howard, FDA/NCTR
April Lake, Environmental Protection Agency (EPA)
Greg Olson, FDA/NCTR
Christy Powers, EPA
Kan Shao, EPA

National Institute of Environmental Health Sciences (NIEHS) Staff:

Charles Alden
Danica Andrews
Chad Blystone
John Bucher
Rajendra Chhabra
Michelle Cora
Helen Cunny
Michael DeVito
June Dunnick
Susan Elmore
Sue Fenton
Gordon Flake
Paul Foster
Dori Germolec
Robbin Guy
Ronald Herbert
Mark Hoenerhoff
Michelle Hooth
Essie Jones
Angela King-Herbert
Gabriel Knudsen
Robin Mackar
David Malarkey
Barry McIntyre
Tanasa Osborne
Erin Quist
Cynthia Rider
Kristen Ryan
Brian Sayers
Keith Shockley
Robert Sills
Stephanie Smith-Roe
Inok Surh
Gregory Travlos
Molly Vallant
Suramya Waidyanatha
Nigel Walker
Lori White
Kristine Witt
Mary Wolfe
Michael Wyde
Yun Xie
Haruhiro Yamashita

Contract Staff to NIEHS
Mamta Behl, Kelly Government Solutions
Amy Brix, Experimental Pathology Labs, Inc.
Nancy Bordelon, Battelle
Schantel Hayes, Charles River Laboratories, Pathology Associates International
Milton Hejtmancik, Battelle
Jessica Hoane, Charles River Laboratories, Pathology Associates International
Kyathanahalli Janardhan, Integrated Laboratory Systems
Rodney Miller, Experimental Pathology Laboratories, Inc.
James Morrison, Charles River Laboratories, Pathology Associates International
Arun Pandiri, Experimental Pathology Labs, Inc.
Barton Sparrow, Battelle

Public Attendees
Steve Anderson, Albemarle Corporation
Nicholas Ball, Dow Chemical Company (by telephone)
Susan Borghoff, ToxStrategies
Thomas Brock, Duke University
Ruth Danzeisen, Cobalt Development Institute (by telephone)
Marvin Friedman, SNF
Marcia Hardy, Albemarle Corporation (by telephone)
Ernie Hood, Bridport Services
W. Caffey Norman, Patton Boggs LLP (by telephone)
Steve Risotto, American Chemistry Council
Thomas Shaw, Sandvik
Glenn Simon, Solvay SA
Erik Van Miert, Solvay SA (by telephone)
Daniele Wikoff, ToxStrategies
Kimberly Wise, American Chemistry Council

II. Welcome and Introductions

The National Toxicology Program (NTP) Technical Reports Peer-Review Panel Meeting convened on October 29, 2013 in Rodbell Auditorium, National Institute of Environmental Health Sciences (NIEHS), Research Triangle Park, North Carolina. Dr. John Cullen served as chair. The other panel members present were Drs. Norman Barlow, Russell Cattley, Deborah Cory-Slechta, Terry Gordon, Joel Parker, Karen Regan, and Timothy Zacharewski. Dr. Richard Miller attended as the NTP Board of Scientific Counselors liaison. Dr. Paul Howard attended representing the FDA. Representing the NTP were Associate Director Dr. John Bucher, Dr. David Malarkey (group leader of NTP Pathology Group), Dr. Chad Blystone (toxicologist in Developmental and Reproductive Toxicology Group) and Dr. Nigel Walker (Deputy Division Director for Science).

Dr. Cullen welcomed everyone to the meeting and asked all attendees to introduce themselves. Dr. Bucher welcomed participants, thanked the panel members and staff for their work, and thanked Dr. Cullen for agreeing to chair the meeting. Designated Federal Officer Dr. Lori White read the conflict of interest policy statement. She noted that Dr. Zacharewski notified the NTP of a potential conflict of interest regarding tetrabromobisphenol A; thus, he would not participate in the discussion or vote for that draft technical report.

III. Peer Review of Draft NTP Technical Reports

Dr. Blystone briefly reviewed the NTP Technical Reports process for the panel, including the Levels of Evidence of Carcinogenic Activity categories used by the NTP to describe the draft conclusions. He also went over the panel's charge.

IV. Overview of the NTP Rat Models

The NTP Laboratory Animal Management Group leader Dr. Angela King-Herbert reviewed recent changes in the rat strains used by the NTP. She described advantages and concerns regarding the F344/N rat, which was used for over 30 years at the NTP. The NTP conducted a workshop in 2005 to consider rat model stocks and strains. A workshop recommendation was discontinuation of the F344/N strain. Soon after, the NTP discontinued use of the F344/N strain and temporarily started using the F344/NTac rat model, a substrain of the F344/N rat. Studies at NCTR have used the F344/NNctr model, which is another substrain of the F344/N rat and was bred at NCTR. In 2007, the NTP selected the Wistar Han rat as the default strain. Each of the strains mentioned was used in one or more of the NTP studies under peer-review consideration at this meeting.

Dr. Cullen asked Dr. King-Herbert to relate some idea of the robustness of the historical data for the different rat strains. She said there is some historical data for the Wistar Han and F344/NTac rat strains, but the database is still fairly small because the NTP only used the strains for a short period of time.

Dr. Gordon remarked that it is important to consider whether the rat strain would be sensitive to the majority of chemicals that might be tested by the NTP. Dr. Bucher referred to the 2005 workshop Dr. King-Herbert had mentioned. Those at the workshop recognized that all of the various strains had a variety of different background tumors, and there was not a database comparing the sensitivity across strains. Each strain was used in a variety of different cancer bioassays.

Dr. Cullen briefly reviewed the format for the peer review.

V. Draft NTP Technical Report TR-582 on Vinylidene Chloride

NTP Study Scientist Dr. Michael Wyde introduced the studies on vinylidene chloride (VDC). VDC is a high production volume chemical used to make common household products, artificial turf, pipes, lacquer resins and latex, and flame-resistant carpet backing. It was nominated for NTP study by the Agency for Toxic Substances and Disease Registry, primarily due to occupational exposure. Two-week, 3-month, and 2-year inhalation studies were conducted in F344/N rats and B6C3F1/N mice.

In genetic toxicity tests, VDC was negative in the bacterial mutagenicity tests, drosophila sex-linked recessive lethals tests, and erythrocyte micronucleus tests. In the mouse lymphoma cell mutagenicity tests, VDC was positive in the presence of exogenous metabolic activation provided by induced male rat liver S9 mix and equivocal without S9.

The draft report's proposed conclusions on VDC were:

> Under the conditions of this 2-year inhalation study, there was *clear evidence of carcinogenic activity* of vinylidene chloride in male F344/N rats based on increased incidences of malignant mesothelioma. Increased incidences of renal tubule carcinoma and respiratory epithelium adenoma in the nose of male rats were also considered to be related to vinylidene chloride exposure. There was *some evidence of carcinogenic activity* of vinylidene chloride in female 344/N rats based on increased incidences of C-cell adenoma or carcinoma in the thyroid gland and systemic mononuclear leukemia. Occurrences of malignant mesothelioma may have been related to vinylidene chloride exposure. There was *clear evidence of carcinogenic activity* of vinylidene chloride in male B6C3F1/N mice based on increased incidences of renal tubule adenoma and carcinoma. Increased incidences of hepatocholangiocarcinoma may have been related to vinylidene chloride exposure. There was *clear evidence of carcinogenic activity* of vinylidene chloride in female B6C3F1/N mice based on increased incidences of systemic hemangioma or hemangiosarcoma (combined). Hepatocholangiocarcinoma and hepatocellular adenoma and carcinoma (combined) in the liver of female mice were also considered to be related to vinylidene chloride exposure. Increased incidences of alveolar/bronchiolar carcinoma in the lungs and carcinoma of the small intestine may have been related to treatment.
>
> Exposure to vinylidene chloride caused increases in the incidences of nonneoplastic lesions in the nose of rats and mice, the liver of rats, the lung of male rats, and the kidney of male mice.

NTP Study Pathologist Dr. Mark Hoenerhoff described the molecular pathology studies of mesothelioma in VDC-exposed F344/N rats. He first provided background information about the use of molecular pathology studies in NTP Technical Reports. Molecular pathology studies generate supplementary and supportive data for the NTP on molecular characterization of chemically induced rodent tumors. The gene mutation or expression data help discriminate spontaneous tumors from those of chemical-exposed groups, but are not used for levels-of-evidence conclusions.

In the 2-year study, exposure to VDC caused a significant increase in malignant mesothelioma in male F344/N rats. The goal of the molecular analysis was to characterize the global gene expression changes that occurred in mesotheliomas from VDC-exposed F344/N rats, by comparing eight mesotheliomas from exposed animals with five spontaneous mesotheliomas from the frozen sample archives and a control mesothelial cell line called Fred-PE. Genomic profiling differentiated mesotheliomas from VDC-exposed rats and vehicle control rats. Mesotheliomas from exposed animals showed an overrepresentation of pro-inflammatory pathways and immune dysregulation.

Dr. Cullen noted receipt and distribution to the panel of written comments from Mr. W. Caffey Norman from Patton Boggs LLP on behalf of VDC producers. Dr. Cullen opened the floor for oral public comments.

The first commenter, Mr. W. Caffey Norman of Patton Boggs LLP, spoke by telephone on behalf of VDC producers. Mr. Norman suggested that the VDC bioassays in the draft report did not meet the NTP criteria for *clear evidence of carcinogenic activity*. He noted that in the literature there was a total of 18 cancer bioassays of VDC using multiple strains of rats, mice, and hamsters. The totality of those data did not show consistent evidence of carcinogenicity. Thus, he noted that the NTP cancer bioassay represents the first that demonstrates an apparent increase in tumors in both sexes of two species. He said the NTP had used dose levels that exceeded the maximum tolerated dose (MTD) in both rats and mice, noting that NTP and EPA guidance point to the need for caution when viewing cancer bioassays that exceed the MTD. He suggested that the observed tumors might have arisen as a consequence of stress placed on the animals by dosing at levels above the MTD. Based on that factor, he said the studies should not be considered adequate for the assessment of carcinogenicity, particularly as the results were so different from the previous studies.

The second commenter Dr. Erik Van Miert of Solvay spoke by telephone and focused on the VDC genotoxicity assessment. He proposed that the following statement from the draft technical report is not in line with genotoxicity data in the report and public domain: “The results from a variety of genetic toxicology studies…indicate that vinylidene chloride has mutagenic, clastogenic, and aneugenic properties.” He cited

several studies referenced in the draft report that indicated negative results with VDC. He also cited Concise International Chemical Assessment Document 51 from the World Health Organization, a report from the Scientific Committee on Occupational Exposure Limits of the European Commission, and a 2009 REACH [Registration, Evaluation, Authorisation and Restriction of Chemicals] dossier of VDC to suggest no evidence of genotoxicity with VDC. He called for more research on the mode of action of VDC.

The third commenter was Dr. Nicholas Ball, a toxicologist from the Dow Chemical Company, speaking on behalf of the VDC producers by telephone. He proposed that the study's experimental design and conduct did not support the conclusions regarding *clear evidence of carcinogenic activity* in male rats and male and female mice and *some evidence* in female rats. He provided two key reasons: (1) both mice and rat studies exceeded MTD according to NTP and EPA guidance and (2) inadequate dose spacing and lack of a dose providing a no-observed-adverse-effect level (NOAEL). He discussed his concerns in more detail and concluded that the peer-review panel should consider the study as inadequate to assess carcinogenicity to humans.

Dr. Cattley, the first primary reviewer, recommended that the methods and results concerning genetic toxicology testing be revised to account for different methodologies of bacterial mutagenesis assays. He urged the NTP to add findings from the molecular pathology appendix to the results section. He suggested resolution of an apparent discrepancy between the discussion and introduction sections concerning how data from the 1982 NTP Technical Report on VDC (TR #228) were referenced. He asked if a statement regarding "increased incidences of systemic neoplasms" referred only to malignant mesotheliomas or to other tumor types. He noted that hemangioma is often considered a benign end-stage lesion and asked that discussion of the progression between hemangioma and hemangiosarcoma be added. He recommended that the discussion concerning the mechanism of action of VDC account for the lack of *in vivo* genotoxicity. He said the report should not characterize VDC as a "weak initiator of tumorigenesis" without supporting context in the final sentence of the discussion.

Regarding the molecular pathology appendix, Dr. Cattley noted that isolated RNA from malignant mesotheliomas induced by VDC was compared to the cultured rat mesothelial cell (Fred-PE cell) RNA. He asked that the NTP discuss the potential for bias arising from different RNA isolation parameters for Fred-PE cells. He questioned why the results for the spontaneous mesothelioma in that study were not presented. He recommended adding a figure from Dr. Hoenerhoff's presentation to Appendix L to clarify confusion from Figure L2. He suggested including discussion and explanation of the relationship between inflammation and risk of mesothelioma as cited in the literature. He suggested that NTP discuss the predicted, if not actual gene expression results, of incubating Fred-PE cells with VDC, VDC metabolites, or VDC plus a

metabolic activation system. He found the conclusions in the draft report acceptable and agreed with the levels of evidence.

Dr. Gordon, the second primary reviewer, found the VDC study well conducted and the draft report well written. He suggested that the "time to first incidence" data deserved mention in the results section. He noted that the addition of the evaluation of global gene changes for the spontaneous vs. induced mesotheliomas is a major step in the right direction. However, the gene expression and pathway analyses should go beyond stating that a pro-inflammatory environment was associated with mesotheliomas, given that most cancers are thought to be associated with inflammation. He questioned the conclusion that pro-inflammatory and immune pathway genes were different for spontaneous vs. induced tumors because the gene expression changes presented in Table L3 appear similar for those pathways. He suggested that Table L2 needed more definitions. While he would have preferred the study to include some lower doses, he agreed with the conclusions in the draft report.

Dr. Parker, the third primary reviewer, focused his comments on the molecular pathology appendix. He noted that the analysis was done across all genes, so there was strong evidence for the segregation. However, the report should discuss possible factors that could constitute potential sources of bias (e.g., site of the tissue, RNA insolation differences) and include a statement that technical factors were not associated with the gene expression. He noted that there was some segregation in the plot for VDC in the Principal Component Analysis (PCA). While that is secondary to the segregation between the VDC and spontaneous mesothelioma plots, the segregation in the VDC plot itself may be important and could potentially be explained by known biological processes. He noted additional methods to illustrate segregation including cross-validation and machine learning techniques. Regarding the oncogenic signatures and inflammatory signatures, he said it was clear that there was significant overlap or enrichment of the genes of interest with these known pathways. However, he asked whether the direction of change supported overexpression or underexpression of those pathways. He suggested that producing a hypothesis or model system about the pathways involved and their direction of change would make the results much stronger. He showed interest for a direct comparison between the VDC-exposed and spontaneous mesothelioma microarray datasets; the control dataset may be inhibiting the detection of other significant pathways. Generally, he agreed with the results of the study.

Dr. Zacharewski, the fourth primary reviewer, also focused on the molecular pathology appendix. He proposed that the global gene profiling study was a valuable, complementary study and could be used to differentiate between a spontaneous and treatment-related tumor. However, he suggested the study is not the most appropriate

method to use to determine mechanism of action. He did not find the data overly compelling for indicating significant differences between the spontaneous and VDC-exposed tumors. He noted that a PCA is not necessarily a statistical analysis, but more of a classification method. Although PCA did show some separation, he suggested the separation might have been due to varying treatment of samples (e.g., the RNA was extracted in different ways, tumors were stored for different lengths of time). He suggested there would have been tremendous value in follow-up studies, such as qRT-PCR on individual genes, to demonstrate there were significant differences among the microarrays. He said microarrays are "last century's technology" and RNASeq should be employed in the future.

Dr. Cattley added that the discussion regarding the dose selection rationale for the 2-year study should be expanded, specifically to address the reduction in body weights in the male mice and the incidence and severity of nonneoplastic lesions.

Addressing the issue of the decrease in body weight, Dr. Zacharewski asked for clarification about the NTP's definition of an "inadequate study." He also asked whether MTD was defined strictly on body weight and survival without looking at any other endpoints. Dr. Walker replied that "inadequate" is defined as having major flaws in the design and conduct of a study. He added that for issues of MTD and dose selection, all available information is considered (e.g., body weight, historic experience). Dr. Walker noted that there are no hard and fast guidelines. Dr. Zacharewski asked whether NTP is obliged to follow EPA guidelines. Dr. Walker said those guidelines are considered in decision-making, but the NTP is not obliged to follow them.

Dr. Barlow remarked that based on the results of the 3-month study, a dose between 100 ppm and 200 ppm, perhaps 150 ppm, should have been used in the 2-year study. Upon reviewing the final data, however, he said it was clear that the doses used were appropriate and the study was adequately designed. He noted some ambivalence regarding if the data for C-cell tumors in female rats support the call of *clear evidence of carcinogenicity*, given that there was not a clear dose response, and he suggested a possible change to *some evidence*. He noted the gene expression information was useful and interesting, but he questioned how the data would be used and whether those studies should be conducted and reported outside of the technical reports. He asked for NTP's response on a public comment regarding genotoxicity statements in the discussion section of the draft report: "…a variety of genetic toxicology studies…indicate that vinylidene chloride has mutagenic, clastogenic, and aneugenic properties." He recommended correction to the statement "fixation quality of the rat testes was poor." He called for more discussion about the additional carcinogenicity studies in the literature that were not considered adequate.

Dr. Cullen mentioned that the NTP had not combined the hepatocholangiocellular carcinomas with the primary liver tumors. However, in later draft technical reports, hepatoblastomas were combined with hepatocellular tumors. He asked for the NTP's rationale regarding what is grouped together and what is not.

Dr. Wyde responded to Dr. Cattley's comments. Dr. Wyde would address clarifying the methodologies for previous studies in the Introduction and Discussion. He would address Dr. Cattley's suggestion to expand the discussion of the dose-selection rationale, and he acknowledged other editorial suggestions. He acknowledged Dr. Cattley's and Dr. Barlow's recommendations to amend the genetic toxicity discussion paragraph.

Dr. Hoenerhoff responded to comments from Dr. Cattley on gene profiling. He acknowledged Dr. Cattley's concern about potential bias from different RNA isolation parameters, but these parameters did not appear to have an impact on altering gene expression profiles. He would address the issue of different methods of isolation between the cell lines and tumors in the Discussion section. Regarding the spontaneous mesotheliomas, he said they were too small to trigger collection during necropsy, as were the female mesotheliomas. He noted that there was not a significant inflammatory component histologically in the study, and there was not a significant difference in inflammation between the spontaneous and treatment group mesotheliomas. However, the gene expression data suggested that there is a pro-inflammatory component in VDC mesotheliomas and those issues would be discussed further in the report. Regarding the potential gene expression results of incubating Fred-PE cells with VDC or VDC metabolites, he noted it would be valuable for follow-up *in vitro* validation experiments or more focused functional experiments.

Dr. Wyde responded to Dr. Gordon's comments. Dr. Hoenerhoff would address the time-to-tumor incidence data in the Results section. He agreed with Dr. Gordon about the global gene expression analysis being the first step in developing more focused, hypothesis-driven experiments to address specific questions. The gene expression experiment results suggest that there is an increased pro-inflammatory or immune dysfunction signature, and the Discussion would be updated to add more information on how that may influence tumorigenesis in the study. He noted that an additional figure, as seen in his presentation, would be added to Appendix L. He would address expanding Table L3 to include more of the differentially expressed genes and genes that are exclusively expressed in VDC mesotheliomas compared to spontaneous mesotheliomas.

Addressing Dr. Parker's comments, Dr. Hoenerhoff said the PCA plot included all of the genes on the array. For the Discussion section, he would address particular factors influencing gene expression that are technical or biologically related to tumor site, dose,

or method of RNA extraction. Regarding the variation within the VDC treatment group in principal component 3, he said there is some variation in those samples. Additional analysis solely on the VDC group could elucidate how those segregate and if it has any relationship to any other gene expression that might identify a subset of tumors or some kind of biological difference. For future studies, he would consider Dr. Parker's suggestion about additional studies for validation, cross-validation, or machine learning. He said discussion would be added regarding the directionality of the genes associated with the pro-inflammatory signature. For the comment about a direct comparison of VDC mesotheliomas with spontaneous mesotheliomas, he noted that the results from a direct comparison are difficult to interpret without the context of the normal tissue. The NTP could consider a direct comparison in future studies to see if additional information can be gained.

Dr. Hoenerhoff addressed Dr. Zacharewski's comments. Dr. Hoenerhoff said Tables L2 and L3 would be amended to include additional genes from a figure in his presentation. This figure would also be added to demonstrate those over-represented pathways and the genes within those pathways. He said the NTP agrees with the value of RNASeq, and that those assays are being implemented in studies.

Dr. Zacharewski asked whether the microarray data sets were submitted to public repositories such as GEO (Gene Expression Omnibus, NCBI). Dr. Hoenerhoff said they would be submitted to GEO, and that this particular data set is available in CEBS (Chemical Effects in Biological Systems, NIEHS). He added that once the final report is public, the data sets would be deposited into a public section of CEBS and would be available in GEO.

Dr. Wyde addressed Dr. Barlow's comments. Regarding the dosing issue, he said there was 100% mortality at 200 ppm, so there was hesitation about using any higher doses. There were liver and nasal lesions at the 100 ppm dose, so the dosing was appropriate. He said the call regarding C-cell tumors was primarily driven by the benign adenomas in the thyroid gland, and the significant increase in carcinomas was seen only at the low dose. Thus, this was supportive of *some evidence*, not *clear evidence*, of carcinogenicity. He agreed that the report would benefit from an expanded discussion of previous carcinogenicity studies.

Dr. Malarkey addressed Dr. Cattley's and Dr. Cullen's comments about combining hemangiomas and hemangiosarcomas. He noted that recent data suggest that hemangiomas can progress to hemangiosarcomas, providing evidence to support combining them for analysis. He said each of the tumor types was considered individually, along with which might be appropriate to combine. Anything that is of the same histogenesis was considered appropriate to combine. Dr. Malarkey acknowledged Dr. Cattley's suggestion to include the rationale in the report.

Dr. Foster addressed Dr. Barlow's comment regarding fixation of the testes. He said the NTP no longer fixes testes in formalin, which has improved histological profiles. Dr. Barlow noted that the necropsies were performed six years earlier. He suggested that the information should be released to the public more quickly, and it was important to keep up with current technology.

Dr. Cullen called for a motion on the conclusions for VDC. Dr. Cattley moved to accept the conclusions as written. Dr. Cory-Slechta seconded the motion. The panel voted unanimously (7 in favor, 0 opposed, 0 abstentions) to accept the conclusions as written.

VI. Draft NTP Technical Report TR-581 on Cobalt Metal

NTP contract toxicologist and Study Scientist Dr. Mamta Behl of Kelly Government Solutions introduced the studies on cobalt metal. The United Auto Workers and the Cobalt Development Institute nominated cobalt metal for toxicology and carcinogenesis studies, with support from OSHA and NIOSH. The nomination was based on widespread occupational exposure and occurrence of hard metal disease associated with exposure to cobalt and its compounds. Two-week and 3-month inhalation studies were conducted in F344/N rats. The 2-year studies were conducted in F344/NTac rats and B6C3F1/N mice. Genetic toxicology studies were conducted in *Salmonella typhimurium*, *Escherichia coli*, and mouse peripheral blood erythrocytes. Two-week, 3-month, and 2-year tissue-burden studies were conducted in additional groups of rats and mice. Molecular analysis was conducted on lungs of F344/NTac rats and B6C3F1/N mice from the 2-year studies. Detailed pathological evaluations were conducted on all studies.

Genetic toxicology results were positive in the TA98 strain (without S9) in the *Salmonella* assay and negative in the micronucleus assay (male and female mice).

The draft report's proposed conclusions on cobalt metal were:

> Under the conditions of these 2-year inhalation studies, there was *clear evidence of carcinogenic activity* of cobalt metal in male F344/NTac rats based on increased incidences of alveolar/bronchiolar adenoma and carcinoma in the lung, including multiples, and on increased incidences of benign and malignant pheochromocytoma of the adrenal medulla, including bilateral neoplasms. The increased incidences of pancreatic islet adenoma or carcinoma (combined) were considered related to exposure. The occurrences of cystic keratinizing epithelioma of the lung and of renal tubule adenoma or carcinoma (combined) may have been related to exposure. There was *clear evidence of carcinogenic activity* of cobalt metal in female F344/NTac rats based on increased incidences of alveolar/bronchiolar adenoma and carcinoma in the lung, including multiples,

and on increased incidences of benign and malignant pheochromocytoma of the adrenal medulla, including bilateral neoplasms. The occurrences of squamous cell neoplasms of the lung (predominantly cystic keratinizing epithelioma), and of mononuclear cell leukemia were considered related to exposure. The occurrences of pancreatic islet carcinoma may have been related to exposure. There was *clear evidence of carcinogenic activity* of cobalt metal in male and female B6C3F1/N mice based on increased incidences of alveolar/bronchiolar neoplasms of the lung (predominantly carcinoma), including multiple carcinoma.

Exposure to cobalt metal resulted in increased incidences of nonneoplastic lesions of the lung and nose in male and female rats, the testes in the male rats and mice, the adrenal medulla in female rats, and the lung, nose, larynx, and trachea in male and female mice.

NTP contract pathologist Dr. Arun Pandiri of Experimental Pathology Laboratories, Inc. described the molecular analysis of *Kras*, *Egfr*, and *Tp53* mutations in rat and mouse alveolar/bronchiolar carcinomas resulting from chronic inhalation exposure to cobalt metal. He presented data demonstrating that mutations within *Kras* were significantly higher than within *Egfr* and *Tp53* genes in both rat and mice alveolar/bronchiolar carcinomas resulting from chronic inhalation exposure to cobalt metal.

Dr. Cullen noted receipt and distribution to the panel of written comments from Dr. Steven Verberckmoes of Umicore S.A. and Dr. Ruth Danzeisen of the Cobalt Development Institute. Dr. Cullen opened the floor for oral public comments.

Dr. Ruth Danzeisen, a toxicologist at the Cobalt Development Institute, commented on the draft technical report by telephone. Dr. Danzeisen noted that the Cobalt Development Institute nominated cobalt metal for NTP testing. She anticipated that NTP studies on cobalt metal would lead to an industry self-classification of cobalt metal by the inhalation route, pending the outcome of the peer-review panel's deliberations. She felt that the study was well designed and conducted, but the particle size used was very small compared to typical human exposure scenarios. She said the high dose used was relatively high as reflected in the early reductions in body weights. There was no NOAEL, which made it more difficult to extrapolate the findings for risk assessment. Her group agrees with the NTP conclusion that there was *clear evidence* of carcinogenicity, but suggested limiting the conclusions to indicate that the evidence was by inhalation exposure and in the respiratory tract. The NTP findings were in line with her group's thinking and evidence from past human epidemiologic studies that cobalt causes cancer in the lung by causing local inflammation leading to reparative mechanisms. She noted that the systemic cancers were seen only in rats and not in mice, and are not relevant for humans and human risk assessment. She remarked that the systemic cobalt levels were highest in the liver, at times even exceeding lung levels, but the liver had no

neoplasms. The cobalt levels achieved in the tissues seemed disconnected with adverse effects, particularly neoplasms. She found this supported her group's hypothesis that there is local inflammation leading to reparative mechanisms, hyperplasia, and subsequently cancer. She stressed that the Cobalt Development Institute has strong evidence that cobalt is not a mutagen and agrees with the NTP review that oxidative stress causes interaction with the DNA.

Dr. Gordon, the first primary reviewer, indicated that the NTP's cobalt metal particle inhalation study was very well designed and conducted, and yielded important information regarding the carcinogenicity of a less soluble form of cobalt that complements the previous NTP study with the soluble form. He said considerable data suggest that the soluble and insoluble forms can have long-term toxicity or potency differences; thus, he commended the testing of cobalt metal in the NTP bioassay program. He found the choices of exposure concentrations to be generally acceptable, but an additional low concentration would have been appropriate for both the rat and mouse 2-year studies. On the basis of some of the 3-month endpoints (e.g., larynx), there may have been sufficient data to justify using a lower exposure concentration, which would enhance the relevance of the exposure concentrations. The multiple alveolar/bronchiolar carcinomas with dose response in rats and mice, and the statistical significance of the tumors, provide additional evidence of the carcinogenicity of the particles in the lung. He indicated that if the concentration was lower, a clearer dose response might have been seen. He suggested particle size be addressed earlier in the report. He noted that a stainless steel jet mill was used to break up the cobalt metal into respirable particle sizes; the report should explain the resulting chromium contamination of the bulk chemical and note contamination was minimal. The potential for cobalt metal to be carcinogenic is strengthened by the similar lung tumors seen with the soluble form of cobalt. He inquired about a potential miscalculation in the normalization of the exposure concentration of cobalt sulfate heptahydrate to elemental cobalt. He noted that the cobalt metal particles might have been more potent than the soluble form, which would support the mode of action suggested by the public commenter. He agreed with the conclusion of *clear evidence* in the lung. He noted that the other conclusions, except for the cystic keratinizing epitheliomas, may not warrant the higher ratings and should perhaps be lowered.

Dr. Cory-Slechta, the second primary reviewer, noted that the study was very well designed and conducted. Given that cobalt can be taken up by the nasal mucosa and into the brain, she proposed that there should have been analysis of brain tissue.

Dr. Regan, the third primary reviewer, said the study was well designed and had no interpretation differences at the *clear evidence* of carcinogenicity level. Regarding the *equivocal evidence of carcinogenicity* in the renal tubular adenomas and carcinomas,

she asked about the lack of preneoplastic lesions in the kidneys. She asked what triggered the extended evaluation of the kidneys. She asked whether there was any evidence of the amphophilic-vacuolar carcinomas that have been found to be spontaneous; if so, then that should be taken into account. Regarding the pancreatic islet tumors, she said there was an increase in incidence compared to the historical control data, but the historical control data were not appropriate for this particular study because of the strain used. Thus, she proposed that the pancreatic islet tumors were ranked too high, but agreed with the conclusions for all of the other tumor types.

Dr. Zacharewski, the fourth primary reviewer, asked whether the *Kras*, *Egfr*, and *Tp53* mutations mapped to any specific consequences in terms of the activity of those proteins subsequent to the mutation. He also inquired whether any additional studies could have been done to demonstrate that the mutation actually had functional significance to the protein itself. He asked whether there was any correlation between a mutation and a tumor outcome in terms of aggressiveness, metastatic ability, etc.

Dr. Barlow asked why no mutations were found in the concurrent controls, despite a robust response in the historical animals. He also noted that there was a well-known and direct mechanism for the development of adrenal medullary tumors, and asked for comment from the study pathologist.

Dr. Parker endorsed more large-scale sequencing efforts to allow for more accurate identification of genetic mutations and assessment of other types of mutations, such as indels, as opposed to just point mutations.

Dr. Cullen asked if cardiomyopathy was observed in the study. Dr. Behl replied that there was no evidence of cardiomyopathy in these studies. She said there was some evidence of cardiomyopathy in the cobalt sulfate subchronic studies.

Responding to Dr. Gordon's review, Dr. Behl agreed with his comments about non-neoplastic lesions in the larynx. She explained that when the NTP has different exposure concentrations in two species in studies involving inhalation chambers, it has elected to go with one less concentration rather than adding an additional group. Hence, a lower concentration was not used. She noted that the particle size used was consistent with the rat respirable range, and she would add more details on that topic in the report as well as information about the jet-milled cobalt metal and resulting minimal chromium contamination. Regarding the dosing calculation Dr. Gordon had questioned, she explained that the exposure concentration in the cobalt sulfate heptahydrate study was based on the mass percentage of cobalt in anhydrous cobalt sulfate. Dr. Gordon asked for better justification for the conclusion related to cystic keratinizing epitheliomas, suggesting perhaps that it should have been *equivocal*. Dr. Behl explained the basis for the *some evidence* call in the females. Because cystic

keratinizing epitheliomas are rare and are part of a continuum of lung lesions, their occurrence was included as a chemical-related effect in the conclusions. Dr. Herbert added that in the non-neoplastic lesions, there was some evidence of squamous cell hyperplasia within the lung and evidence of a progression from nonneoplastic lesions to benign lesions to carcinomas, leading to the *some evidence* conclusion.

Responding to Dr. Cory-Slechta's comment, Dr. Behl said the brain tissue was examined, and there was no evidence of neoplasms. Dr. Cory-Slechta said neoplasms might not have been expected, although there were likely non-neoplastic lesions, such as white matter injury. Dr. Herbert said there was no evidence of non-neoplastic or neoplastic lesions. Dr. Malarkey said the NTP is very interested in improving evaluation of the brain, having recently invoked a new method for its analysis.

Dr. Herbert responded first to Dr. Regan's question about what triggers an extended review in the kidney. He said the renal tubule adenomas are usually small tumors, and an extended review is triggered if there is an indication from the data that there could be an effect. Dr. Regan asked if there was a specific level used. Dr. Herbert said there was not. Dr. Barlow asked how often the extended review yields additional results that affect the conclusions. Dr. Herbert did not have data on that issue at the time. Dr. Regan asked whether any amphophilic-vacuolated renal tubular neoplasms were observed in the male rat study. Dr. Herbert said none were seen in this study and indicated that the NTP has not traditionally made a distinction between the amphophilic-vacuolated type and other types of renal tubular neoplasms in studies. He noted that one publication indicates such tumors are spontaneous, but the toxicologic pathology community does not generally accept this distinction. Dr. Regan mentioned that there are other publications on the topic. She asked how the NTP could know that such a tumor type did not occur, if the NTP does not distinguish that tumor type. Dr. Herbert indicated he had looked at all of the tumors, and that no amphophilic-vacuolated renal tubular neoplasms occurred in the study. Dr. Behl responded to Dr. Regan's comments about pancreatic islet tumors in the females and said the call *equivocal evidence* of carcinogenicity was primarily based on the increase in malignant neoplasms at the 5 mg/m^3 dose, as well as supporting evidence from the males (e.g., significant trend and pairwise comparisons in top two exposure concentrations).

Dr. Pandiri responded to Dr. Zacharewski's comments. Dr. Pandiri said the selection of the "hotspot" exons in all three genes was based on extensive literature review of human lung cancer as well as rodent models of chemical induced pulmonary carcinogenesis. Dr. Pandiri indicated that immunohistochemistry could be used to demonstrate the alterations in protein expression within the molecular pathways associated with the mutated genes.

Regarding Dr. Barlow's question about why no *Kras* mutations were observed in the spontaneous alveolar/bronchiolar carcinomas from the concurrent chamber controls, Dr. Pandiri speculated that there were in fact mutations present, but perhaps not in the exons examined.

In response to Dr. Parker's question about the primary focus being on point mutations, Dr. Pandiri said point mutations account for the majority of genetic changes seen in some of the well-known carcinogenesis studies in the literature. Dr. Pandiri agreed that massive parallel sequencing of the cancer genes in tumor tissue is a more powerful tool for detecting mutations and differentiating chemical induced tumors from spontaneous tumors. He also informed the committee that the NTP is currently running a pilot project using exome sequencing and RNA-seq technologies for evaluating chemically induced and spontaneous hepatocellular carcinomas from previous NTP chronic bioassays.

Dr. Malarkey responded to Dr. Gordon's comments regarding the cystic keratinizing epitheliomas and indicated that they are very rare in most species, so it is a significant finding when present. Even though they are benign, this tumor type would be considered in the levels of evidence of carcinogenic activity, especially because it can progress to a malignant tumor. Dr. Malarkey also responded to Dr. Barlow's question concerning how extended reviews of the kidneys are triggered and how often the extended reviews yielded additional results that affect the conclusions. He noted that when the response is weak, follow-up serial sections might confirm a finding.

Dr. Cullen called for a motion to accept the conclusions in the draft report as written. Dr. Cory-Slechta so moved, and Dr. Gordon seconded. The peer-review panel voted unanimously (7 in favor, 0 opposed, 0 abstentions) to accept the conclusions on cobalt metal as written in the draft report.

VII. Draft NTP Technical Report TR-588 on Glycidamide

Study Scientist Dr. Frederick A. Beland from the FDA's National Center for Toxicological Research introduced the studies on glycidamide in drinking water. He provided background information on acrylamide, of which glycidamide is a metabolite. Glycidamide is present in certain baked goods and fried, starchy foods, as well as cigarette smoke. The FDA Center for Food Safety and Applied Nutrition nominated glycidamide for NTP studies. The NTP performed parallel studies to determine and compare the long-term effects of acrylamide and glycidamide in male and female F344/N Nctr rats and B6C3F1/Nctr mice. Two-week, 3-month, and 2-year glycidamide drinking water studies were conducted in male and female F344/N Nctr rats and B6C3F1/Nctr mice.

The draft report's proposed conclusions on glycidamide were:

Under the conditions of this 2-year drinking water study, there was *clear evidence of carcinogenic activity* of glycidamide in male F344/N Nctr rats based upon increased incidences of malignant mesothelioma of the epididymis and testis tunica, malignant schwannoma of the heart, follicular cell adenoma or carcinoma of the thyroid gland, and oral cavity (oral mucosa or tongue) papillomas or carcinomas. An increased incidence of mononuclear cell leukemia may have been related to acrylamide exposure. There was *clear evidence of carcinogenic activity* of glycidamide in female F344/N Nctr rats based upon increased incidences of fibroadenomas of the mammary gland, oral cavity (oral mucosa or tongue) squamous cell papillomas or carcinomas, follicular cell adenoma or carcinoma of the thyroid gland, and carcinomas of the clitoral gland. Increased incidences of squamous cell papillomas of the forestomach and mononuclear cell leukemia were also considered to be related to glycidamide exposure.

There was *clear evidence of carcinogenic activity* of glycidamide in male B6C3F1/Nctr mice based upon increased incidences of adenoma of the Harderian gland, alveolar/bronchiolar neoplasms (primarily adenoma) of the lung, squamous cell neoplasms (primarily papilloma) of the skin, and squamous cell neoplasms (primarily adenoma) of the forestomach. There was *clear evidence of carcinogenic activity* of glycidamide in female B6C3F1/Nctr mice based upon increased incidences of adenoma of the Harderian gland, alveolar/bronchiolar neoplasms (primarily adenoma) of the lung, adenoacanthoma and adenocarcinoma of the mammary gland, squamous cell papilloma of the forestomach, and malignant mesenchymal neoplasms of the skin. The occurrence of benign granulosa cell tumor of the ovary may have been related to glycidamide exposure.

Exposure to glycidamide was associated with increased incidence in male and female rats of fibrosis of the spleen; increased incidences of exfoliated germ cells within the epididymis (males), hepatocyte degeneration (males), liver necrosis (males), increased incidences of bone marrow hyperplasia (females), mesenteric lymph node cellular infiltration (females), pituitary gland (pars distalis) hyperplasia (females), axonal degeneration of the lumbar spinal cord (females), and uterine endometrial hyperplasia (females).

In male and female mice, increased incidences of cataracts, corneal inflammation, forestomach squamous cell hyperplasia, hematopoietic cell proliferation of the spleen, preputial gland lesions (degeneration, ductal dilatation, inflammation) (males), ovarian cysts (females), hepatic angiectasis and necrosis

(females), and axonal degeneration of the cervical spinal cord (females) were associated with exposure to glycidamide.

Dr. Cullen opened the floor for oral public comments, and there were none. He proceeded to primary reviewers' comments.

Dr. Cory-Slechta, the first primary reviewer, remarked that the studies were well done. She agreed with the study's conclusions.

Dr. Cattley, the second primary reviewer, noted that the presentation had clarified the dose selection rationale for glycidamide, but the issue should be clarified in the report. He suggested addition of historical control data for incidence of alveolar/bronchiolar carcinomas in female mice to Table 13 on page 107 and Appendix Table D3a on page 204. He noted that in the table on pages 11-12, "gliosis" should be moved from neoplastic to nonneoplastic lesions. He agreed in principle with the conclusions of the report; however, he suggested limiting *clear evidence* in rats to oral cavity squamous papillomas, because the incidence of oral cavity squamous papilloma or carcinoma is almost entirely derived from the papilloma and not the carcinoma incidence. Similarly, for mice, he suggested limiting the conclusion *clear evidence* to alveolar/bronchiolar adenomas, because the incidence of alveolar/bronchiolar neoplasms is almost entirely derived from the adenoma and not the carcinoma incidence.

Dr. Barlow, the third primary reviewer, agreed that Dr. Beland's presentation had cleared up the issue of the dose rationale, and suggested the explanation should be added to the report. He noted that the report states there was decreased survival compared to controls due to tumors, but some of the tumors listed were not actually treatment-related and suggested clarification in the report. Noting that only 2 males and females in the high-dose group survived to study termination, he asked whether the study should have been terminated earlier. He thought that oral cavity papillomas or carcinomas merited only *some evidence* of carcinogenicity, rather than *clear evidence*. He suggested deleting squamous cell papillomas as increased in the results text for female rats and changing the conclusion regarding squamous cell papillomas to "may have been related". He stated that axonal degeneration should not be as significantly highlighted as it is currently in the report. He asked whether the non-neoplastic findings listed in the conclusions are truly increased related to treatment. He had similar comments for mesenteric lymph node cellular infiltrate and pituitary gland hyperplasia. He also questioned the conclusion regarding Zymbal's gland carcinoma, because only a few animals were examined. He suggested that the increase in alveolar/bronchiolar neoplasms in female mice should be listed as "considered related to," given a lack of clear dose response and only a mild increase at the high dose and that the benign granulosa cell tumors should be combined with several malignant granulosa cell tumors to strengthen the statement, perhaps to the level of *some evidence*.

Dr. Cory-Slechta noted that Dr. Barlow seemed to discount the axonal degeneration because it appears in different places in the two sexes. She said axonal degeneration is probably one of the best-documented effects of acrylamide in the neurotoxicology literature. She questioned why Dr. Barlow was discounting it. Dr. Barlow replied that he based his position on the doses that were used and the lack of a robust dose response.

Dr. Beland responded to Dr. Cattley's comments. He would address explaining the dose selection more thoroughly, adding references to lung carcinomas, and correcting the erroneous reference to gliosis in the summary table. Regarding the oral cavity tumors, he said squamous cell carcinoma is very rare in the control animals, so it was important to mention. Regarding the lung neoplasms in the mice, he said the conclusion states they were primarily adenomas, and he agreed to modify the text to describe that more clearly.

Regarding Dr. Barlow's comment, he agreed to better explain that the two studies were conducted simultaneously. Addressing the comment about survival, he clarified that the animals were not removed due to overt toxicity or weight loss, noting that the veterinary staff monitored the animals very closely. Animals were removed because of spontaneous or treatment-related tumors. Given the need for direct comparison with acrylamide-treated animals, he proposed it was permissible to keep animals on the study until tumor development dictated their removal.

Regarding the suggestion that the call be changed for oral cavity tumors in rats, he stated that the response was robust and monotonic. He believed that the conclusions regarding the clitoral gland and forestomach tumors were correct. He noted that there was much interest in neurotoxicity of glycidamide and acrylamide, and the axonal degeneration was included to demonstrate that a careful examination for potential neurotoxicity in the animals was conducted. He acknowledged that the material on page 126 mentioned by Dr. Barlow should have been deleted. Regarding Zymbal's gland tumors, he said if a lesion were observed during necropsy, the histopathology would be conducted. The statistics were compiled according to how many animals have such a tumor versus the entire cohort.

Regarding the suggestion to change the call on the lung neoplasms in female mice, he noted that the two highest doses exceeded the historical control by two- to three-fold, so *clear evidence* was proposed. Statistical analysis combining the benign granulose cell tumor with other malignant granulosa cell tumors indicated no significant effect.

Dr. Cullen asked how Dr. Beland would explain the lack of esophageal problems, given the mechanism of action. He also asked whether it was correct to assume that fibroadenomas do not have a high risk of converting into mammary carcinomas. Dr. Cullen understood the requirement for *clear evidence* of benign tumors is that it be a

benign tumor with a high risk of conversion into a malignant form. He felt the call of *clear evidence* on the fibroadenomas might be overreaching.

Referring to the lack of esophageal problems, Dr. Beland said he could not explain why cancer did not occur. He recalled discussion in the acrylamide study about fibroadenomas and the possibility of progressing to a malignant tumor. Dr. Bucher commented that the definition of *clear evidence* includes the presence of a marked increase in benign tumors. Dr. Beland was hesitant to change the *clear evidence* call because several regulatory agencies are using fibroadenomas in developing risk estimates for acrylamide. Editorial changes will also be made to the conclusions.

Dr. Malarkey noted that axonal degeneration is a common background lesion in mice and rats, so what is being sought is exacerbation beyond background levels, which did not occur in this study.

Dr. Cullen called for a motion to accept the conclusions in the draft report as written. Dr. Cory-Slechta so moved, and Dr. Gordon seconded. The peer-review panel voted (6 in favor, 1 opposed, 0 abstentions) to accept the conclusions on glycidamide as written in the draft report. Dr. Cattley explained that his negative vote was based on the combination of papilloma and carcinoma for oral cavity lesions in the rats, and the combination of adenomas and carcinomas in alveolar/bronchiolar lung tumors. He proposed that both of those responses for *clear evidence* are based on the benign neoplasm, not the malignant neoplasm.

VIII. Draft NTP Technical Report TR-587 on Tetrabromobisphenol A

NTP Study Scientist Dr. June Dunnick introduced the studies on tetrabromobisphenol A (TBBPA). Nominated by NIEHS, TBBPA is a high-production-volume flame retardant widely used in plastics, paper, electronics, textiles, and adhesives. It is present in a variety of household products such as computers, televisions, and mobile phones. Three-month oral gavage toxicology studies were conducted in F344/NTac rats and B6C3F1/N mice. Two-year oral gavage toxicology and carcinogenesis studies were conducted in Wistar Han rats and B6C3F1/N mice. There was an interim subhchronic study in Wistar Han rats, for comparison to the subchronic F344/NTac rat study. Genetic toxicity studies were negative.

The draft report's proposed conclusions on TBBPA were:

> Under the conditions of these 2-year gavage studies, there was *equivocal evidence of carcinogenic activity* of tetrabromobisphenol A in male Wistar Han rats based on the occurrence of testicular adenoma. There was *clear evidence of carcinogenic activity* of tetrabromobisphenol A in female Wistar Han rats based

on increased incidences of uterine epithelial tumors (predominantly uterine adenocarcinoma). There was *some evidence of carcinogenic activity* of tetrabromobisphenol A in male B6C3F1/N mice based on increased incidences of hepatoblastoma. The increased incidences of large intestine neoplasms and hemangiosarcoma (all organs) may have been related to chemical administration. There was *no evidence of carcinogenic activity* of tetrabromobisphenol A in female B6C3F1/N mice administered 250 or 500 mg/kg.

Administration of tetrabromobisphenol A resulted in increased incidences of nonneoplastic lesions of the uterus and ovary in female rats, the liver and kidney in male mice, and the forestomach in male and female mice.

NTP Study Pathologist Dr. Susan Elmore described the pathology review of TBBPA. She reported that the residual longitudinal tissue review of the remaining formalin fixed cervix, vagina, and uterine remnants sectioned longitudinally revealed additional adenomas and adenocarcinomas, which supported the original *clear evidence* call. Atypical hyperplastic lesions were also found that were not present in the original slides. This review was the first report of Malignant Mixed Müllerian Tumors (MMMTs) in an NTP study. They are very rare tumors considered more aggressive than adenocarcinomas. They were found in the original transverse sections due to their large size. They were combined with adenomas and adenocarcinomas because the current histogenesis theory and epithelial metastases indicate that the epithelial component is the driving force in their production. Atypical hyperplasia, a rare and potentially preneoplastic lesion seen in the uteri of the rats, was treatment-related. It was not found in the original transverse sections due to small lesion size. Renal tubule cytoplasmic alteration in the kidneys of male mice was considered to be treatment-related. It was found in subchronic and chronic studies, and may be associated with altered hormonal status.

Dr. Cullen noted receipt and distribution to the panel of written comments from Dr. James Popp from Stratoxon LLC on behalf of the American Chemistry Council's North American Flame Retardant Alliance. Dr. Dr. Cullen opened the floor for oral public comments.

The first public commenter was Dr. Marcia Hardy of the Albemarle Corporation, who spoke by telephone. She provided background information about TBBPA and its regulatory history. She noted the draft report relies on the peer-reviewed literature, with underrepresentation of unpublished data from guideline/GLP-compliant studies. Thus, she proposed the draft report does not present a clear and comprehensive overview of TBBPA toxicology. She listed several relevant unpublished data. She noted that TBBPA kinetics and metabolism are critically important in evaluating and interpreting the results

of NTP's work and listed several kinetic and metabolic elements. She made several comments related to use of the Wistar Han rat in the TBBPA 2-year study. She called for more information in the report on dose selection for the 2-year study, selection of gavage as the route of administration, the change to Wistar Han rats, the discontinuance of Wister Han rats, NTP's historical control data in the Wistar Han model, and the possible association of rat strain and the observed uterine adenocarcinomas. She suggested the Introduction section of the draft report also needed revision.

The second public commenter, Dr. Daniele Wikoff of ToxStrategies, Inc., spoke on behalf of the American Chemistry Council's North American Flame Retardants Alliance, reflecting her own and Dr. James Popp's written comments. She reported that Dr. Popp reviewed the hepatoblastomas in male mice and suggested the level of carcinogenic activity should be *equivocal evidence*, not *some evidence*. Dr. Wikoff presented some of Dr. Popp's key findings related to hepatoblastomas. Citing shortcomings in the comparison of the uterine tumor incidence to historical controls, she asked for clarifications related to historical control data and for all historical control data in the report to be made available. She also described limitations in the analysis and interpretation of the *Tp53* mutation data. She noted the limited relevance and unclear impact of NTP study dose levels. She remarked that even the lowest doses tested were substantially higher than human exposure, making it difficult to accurately extrapolate the study findings to humans. She asked that these issues be addressed in the Discussion section.

Dr. Cory-Slechta asked Dr. Wikoff about the issue of human-relevant dosing and why one would use human-relevant doses when testing in a mouse or a rat. She noted that such extrapolations between species are commonly done in terms of therapeutic compounds. Dr. Wikoff replied that use of human-relevant doses would help to better characterize responses in humans. Dr. Hardy, the previous public commenter, added by telephone that it was her understanding that for most pharmaceuticals, toxicology tests are run at multiple, potentially effective doses. In toxicology, dose levels are set very differently from pharmaceuticals.

Dr. Barlow, the first primary reviewer, suggested that the rats in the study were not dosed high enough to potentially drive a carcinogenic effect. In the highest dose, there was no effect on mortality, no body weight changes, and no histological changes in the 3-month study; yet, the highest dose for the 2-year study stayed at 1000 mg/kg. Also, the half-life is noted as less than 5 hours, and there was low bioavailability and no accumulation. He said there should be more elaboration on the statement in the Materials and Methods that formulation limitations precluded doses higher than 1000 mg/kg. He suggested the dose could have been pushed higher. He agreed with the

conclusion of *clear evidence* of uterine epithelial tumors in female rats, and questioned the combination of MMMTs with adenomas and adenocarcinomas. He proposed that MMMTs should be considered as potentially separate neoplasms that may have been related to exposure. He called for a better explanation in the report of how the uterine findings were handled. In general, he agreed with the calls as listed, except that the MMMTs should be separated out and characterized as "may have been related to exposure."

Dr. Regan, the second primary reviewer, suggested that the cervix and vagina deserved added attention as important structures in the female reproductive tract. She asked if there might have been a location bias regarding the atypical hyperplasias. She asked for clarification about the metastasis from the uterine adenocarcinomas and MMMTs in the treated and control animals. Also, she noted there should be a clearer distinction between metastases and local invasions. She was not surprised to see that carcinogenicity was found in the 2-year study despite the fact that there was none detected in the 3-month study. She supported the conclusions.

Dr. Parker, the third primary reviewer, said he understood the reasoning behind looking at the mutations from the coding regions in lieu of considering "silent" mutations, with respect to *Tp53* mutation data. He proposed that use of the term "hot spot" was an exaggeration, at least with respect to the human data available. He said it would be useful in the future to sequence the length of a gene for tumor suppressors such as *Tp53*, or at least all of the exons. He proposed that the number of *Tp53* mutations in the study was severely underestimated, which may have hurt the study by limiting power, rendering P values marginal.

Dr. Dunnick responded to Dr. Barlow's comments. Regarding his questions about the highest dose used, she said 1000 mg/kg was the maximum dose that could be used in the study due to solubility and gavagability. The five-day-per-week regimen was employed to mimic worker exposure. Dr. Elmore responded to Dr. Barlow's question regarding a separate call for the MMMTs. She reiterated that, based on NTP knowledge of the histogenesis of MMMTs, the epithelial component is considered to be the primary component in the MMMTs and the mesenchymal component is derived from the carcinoma. In this study, all the metastases were carcinomas, which supports this hypothesis. For this reason, the MMMTs were combined with the epithelial tumors.

Dr. Dunnick said the historical data are limited in the Wistar Han rats because few studies using this strain have been conducted. She said the cervix and vagina were studied in the longitudinal evaluation, and she would provide more data in the report.

Regarding points raised by Dr. Parker on the mutation analysis, Dr. Hoenerhoff said *Tp53* was screened because it is one of the most commonly deleted or mutated tumor

suppressor genes in human and rodent cancers. He agreed that a more comprehensive evaluation of the entire sequence of the gene would be beneficial. He also agreed with Dr. Parker's point regarding silent vs. coding mutations. He said the number of *Tp53* mutations might have been underestimated, and agreed that additional exome sequencing or a broader analysis could address that issue.

Dr. Cattley asked whether the NTP has a defined practice for when to combine hepatoblastomas with other hepatocellular neoplasms. Dr. Malarkey cited two publications that have served as guidance (by Drs. Amy Brix and Eugene McConnell). He said it is acceptable to combine them, but not required, as there is some evidence that they are individual types of tumors genetically. Dr. Cullen noted that there is flexibility on the issue, but asked for some discussion in the report about the decision to combine and the consequences of not combining. Dr. Elmore would address adding clarification in the report.

Dr. Cullen called for a motion to accept the conclusions in the draft report as written. Dr. Regan so moved, and Dr. Gordon seconded. The peer-review panel voted (4 in favor, 1 opposed, 0 abstentions) to accept the conclusions on TBBPA as written in the draft report. Dr. Barlow explained his negative vote as being based on his opinion that the uterine epithelial tumors and the MMMTs should not have been combined. Dr. Cory-Slechta left the meeting before the vote. Dr. Timothy Zacharewski was recused from the review of TBBPA.

Dr. Cullen thanked the panel for its participation. Dr. Bucher thanked the panel members for their hard work and service on the panel, and appreciated their comments on the molecular archeology of tumors and how to best use that information.

Dr. Cullen adjourned the proceedings at 4:12 PM, October 29, 2013.

These summary minutes have been read and approved by the Chair of the October 29, 2013, National Toxicology Program Technical Reports Peer Review Panel.

[Redacted]

Dr. John Cullen

Chair, NTP Technical Reports Peer Review Panel

Date: _1/20/2014_____