National Toxicology Program

Technical Reports Peer Review Panel Meeting

February 8-9, 2012

National Institute of Environmental Health Sciences

Research Triangle Park, NC

Summary Minutes

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I. Attendees

Members in Attendance:
Jane Alcorn, University of Saskatchewan
Lucy Anderson, Private Consultant
Hillary Carpenter III, Minnesota Department of Health
Russell Cattley, Auburn University
Michael Elwell, Covance Laboratories, Inc.
Jon Mirsalis, SRI International
Ofelia Olivero, National Cancer Institute (NCI)
Lisa Peterson, University of Minnesota
Michael Pino, Sanofi
Stephen Roberts, University of Florida (Panel Chair)
Keith Soper, Merck Research Laboratories

NTP Board of Scientific Counselors Liaison:
Richard Miller, GlaxoSmithKline

National Institute of Environmental Health Sciences (NIEHS) Staff:

Charles Alden	Ronald Herbert	Cynthia Rider
Danica Andrews	Mark Hoenerhoff	William Schrader
Linda Birnbaum	Michelle Hooth	Robert Sills
Mike Boyle	Amy Johnson	Cynthia Smith
John Bucher	Angela King-Herbert	Inok Surh
Mark Cesta	Grace Kissling	Sheetal Thakur
Po Chan	Robin Mackar	Raymond Tice
Rajendra Chhabra	David Malarkey	Gregory Travlos
Sheba Churchill	Scott Masten	Molly Vallant
Helen Cunny	Barry McIntyre	Suramya Waidyanatha
Michael DeVito	Minerva Mercado-Feliciano	Nigel Walker
June Dunnick	Alex Merrick	Lori White
Susan Elmore	Geoff Mueller	Kristine Witt
Paul Foster	Hiroaki Nagai	Mary Wolfe
John French	Arun Pandiri	Michael Wyde
Robbin Guy		

Contractor Staff to NIEHS
Mamta Behl, Kelly Services
Amy Brix, Experimental Pathology Labs., Inc.
Georgette Hill, Integrated Laboratory Systems, Inc. (ILS)
Abraham Nyska, ILS
Deepa Rao, ILS

Other Federal Agency Staff:
Paul Howard, Food and Drug Administration (FDA)
Julian Leakey, FDA
Greg Olson, Contractor to FDA

Mike Sanders, NCI
Brett Thorn, FDA
Elizabeth Whelan, National Institute for Occupational Safety and Health (NIOSH)

Public Attendees
Steven Dentali, American Herbal Products Association
Kimberly Ehman, Toxicology Regulatory Services
Lisa Fine, RadTech
Schantel Hayes, Charles River PAI
Jessica Hoane, Charles River PAI
Kyathanahalli Janardhan, Integrated Laboratory Systems
Karin Ke, Keller and Heckman LLP
Duffy MacKay, Council for Responsible Nutrition
Dave Moseley

February 8, 2012

II. Introductions and Welcome

The National Toxicology Program (NTP) Technical Reports Peer Review Panel Meeting convened on February 8 and 9, 2012, in Rodbell Auditorium, National Institute of Environmental Health Sciences (NIEHS), Research Triangle Park, North Carolina. Dr. Stephen Roberts served as chair. The other panel members present were Drs. Jane Alcorn, Lucy Anderson, Hillary Carpenter III, Russell Cattley, Michael Elwell, Jon Mirsalis, Ofelia Olivero, Lisa Peterson, Michael Pino, and Keith Soper. Dr. Richard Miller attended as the NTP Board of Scientific Counselors liaison. Dr. Paul Howard attended representing the FDA and Dr. Beth Whelan attended representing NIOSH. Representing the NTP were NIEHS/NTP Director Dr. Linda Birnbaum, Associate Director Dr. John Bucher, Dr. Dave Malarkey, and Dr. Michelle Hooth.

Dr. Roberts welcomed everyone to the meeting and asked all attendees to introduce themselves. Dr. Birnbaum thanked the panel members and staff for their work, as well as the FDA and NIOSH participants. She also recognized the work preparing for the meeting by Dr. Malarkey and Dr. Hooth. Dr. Bucher also welcomed attendees, and thanked Dr. Roberts for chairing the meeting. Designated Federal Officer Danica Andrews read the conflict of interest policy statement.

III. Peer Review of Draft NTP Technical Reports

Dr. Hooth briefly reviewed the NTP Technical Reports process for the panel, and went over the panel's charge. Dr. Roberts reviewed the agenda and format for the meeting.

IV. Draft NTP Technical Report TR-579 on *N,N*-Dimethyl-*p*-toluidine (DMPT)

Study Scientist Dr. June Dunnick introduced the draft Technical Report on *N,N*-dimethyl-*p*-toluidine (DMPT). DMPT is a high production chemical with potential for widespread human exposure due to its use in dental materials and bone cements. Dr. Dunnick noted the negative findings in genetic toxicity tests; the occurrence of hematologic toxicity and nonneoplastic lesions in the liver, nasal cavity, and hematopoietic system in short-term studies; and neoplastic and nonneoplastic lesions in the 2-year gavage studies. Decreased survival and body weight were observed in the high-dose (60 mg/kg) male and female rats in the 2-year studies.

The proposed conclusions on DMPT were:

> Under the conditions of these 2-year oral gavage studies, there was *clear evidence of carcinogenic activity* of *N,N*-dimethyl-*p*-toluidine in male F344/N rats based on increased incidences of hepatocellular carcinoma, and hepatocellular adenoma or carcinoma (combined), and increased incidences of nasal cavity neoplasms (primarily nasal cavity transitional epithelium adenoma). The increased incidences of thyroid gland follicular cell neoplasms may have been related to treatment. There was *clear evidence of carcinogenic activity* of *N,N*-dimethyl-*p*-toluidine in female F344/N rats based on increased incidences of hepatocellular carcinoma and hepatocellular adenoma or carcinoma (combined). The occurrence of nasal cavity transitional epithelium adenoma was considered to be related to treatment. There was *clear evidence of carcinogenic activity* of *N,N*-dimethyl-*p*-toluidine in male B6C3F1/N mice based on increased incidences of hepatocellular adenoma (multiple), hepatocellular carcinoma, and hepatoblastoma. There was *clear evidence of carcinogenic activity* of *N,N*-dimethyl-*p*-toluidine in female B6C3F1/N mice based on increased incidences of hepatocellular adenoma, hepatocellular carcinoma, and hepatoblastoma and increased incidences of alveolar/bronchiolar neoplasms (primarily adenoma). The increased incidences of forestomach squamous cell papilloma in female mice were considered to be related to treatment.
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> Administration of *N,N*-dimethyl-*p*-toluidine resulted in increased incidences of nonneoplastic lesions of the liver and nasal cavity in male and female rats and mice; the kidney in male and female rats; the spleen and bone marrow in male and female rats and female mice; the lung in male and female mice; the forestomach in male rats and female mice; the mesenteric lymph node in male rats and female mice; and the olfactory lobe in male and female mice.

N,N-Dimethyl-*p*-toluidine also caused hematologic toxicity and increases in methemoglobin levels in male and female rats and mice (as measured at 3 months).

Dr. Roberts invited comments from the public. There being none, he proceeded to the panel's peer review.

Dr. Pino, the first primary reviewer of the DMPT studies, felt that the studies were adequately conducted and that the dose selections for the 2-year studies were appropriate. He said that for the liver tumors in male rats, clear evidence of carcinogenicity should be based on carcinomas only, not combined with adenomas. He noted that the incidence of thyroid follicular adenomas in female rats was only slightly above the concurrent and historical ranges and asked whether those tumors were considered related to DMPT treatment or not. He noted that while the rat uterine stromal polyps and granulosa cell tumors and the tongue neoplasms were mentioned in the text, it was unclear if they were considered chemical-related effects. He suggested that the extended diestrus noted in female rats might be a secondary effect. Overall, he agreed with the conclusions, except for suggesting that the clear evidence in male rats was due to hepatocellular carcinomas, and should not be combined with adenomas.

Dr. Carpenter, the second primary reviewer, said he concurred with the calls that had been made by the staff, and that it was a very strong study. He noted that there is ample evidence for exposure to the general public, as well occupational exposure. He felt that the presence of rare tumors that were occurring was quite important and made the call much stronger.

Dr. Peterson was the third primary reviewer. She concurred with her colleagues, and supported the proposed conclusions.

Dr. Dunnick replied that the call on hepatocellular tumors was mainly related to the hepatocellular carcinomas, and the hepatocellular adenomas had been included because they are part of the same carcinogenic response. Regarding the thyroid tumors in the male rats, she said it was not a significant effect and was not considered to be a clear response to the chemical; its inclusion as "may have been related" in the conclusion statement was equivalent to a conclusion of *equivocal evidence*. The few tongue, uterine and ovarian tumors were noted in the results text for completeness but were not included in the overall conclusion because they were not considered compound related effects. Dr. Dunnick explained that after consulting with NTP reproductive toxicity experts, the staff felt that the extended diestrus indicated a potential for reproductive toxicity.

Regarding the hepatic carcinomas, Dr. Alcorn asked when NTP considers total tumor incidence in making their calls, when there are sometimes decreases. Dr. Dunnick said decreases in mononuclear cell leukemia are a phenomenon seen with other nitro-aromatic compounds, and that it was discussed as a finding typical with this class of chemical.

Dr. Cattley agreed with Dr. Pino that the hepatic carcinomas were the primary liver tumors in male rats, and suggested that the conclusion should reflect that point. Dr. Elwell asked about the standard protocol for when to examine the tongue. Study pathologist Dr. Brix said that occasionally wet tissue was examined when warranted by gross examination.

Dr. Alcorn suggested corn oil is a potential confounder in any study of a chemical's carcinogenic potential and asked whether NTP was planning to move away from corn oil as a delivery vehicle for lipophilic compounds. Dr. Bucher said there was no such plan in place presently. Dr. Anderson noted that questions have arisen about the nutritional role of the corn oil compared to the corn oil and other lipids in the animals' diets. Dr. King-Herbert explained that the NTP 2000 diet does include corn oil, and that there is a nutritional analysis of how much fat is in the diet. Dr. Anderson suggested that it would be useful for data to be provided regarding how much fat is in the diet, and how much is added in the gavage solution.

Dr. Roberts called for a motion regarding the conclusions for DMPT. Dr. Pino moved to modify the conclusion for male rats by striking reference to "and hepatocellular adenoma or carcinoma (combined)." Dr. Mirsalis seconded the motion. Dr. Malarkey mentioned that hepatocellular adenomas are less common in the rat compared to the mouse and are known to progress to carcinomas, which was the rationale for combining the tumor types. Dr. Carpenter added that it was his impression that this was a fairly standard way of referring to those tumors. Dr. Malarkey said that occasionally the reference is stated as "predominantly carcinomas." Dr. Carpenter said he would be more comfortable with that terminology.

Dr. Roberts called for a vote on Dr. Pino's motion to strike the line "and hepatocellular adenoma or carcinoma (combined)." The motion failed (3 yes, 7 no, 0 abstentions), with Drs. Alcorn, Anderson, Carpenter, Cattley, Elwell, Peterson, and Soper voting no as they agreed with the original language.

Dr. Carpenter suggested retaining the original language while adding "primarily carcinomas." Dr. Malarkey suggested "(primarily carcinoma)." Dr. Birnbaum and Dr. Sills noted that the original language was standard NTP language, and suggested it be retained as proposed.

Dr. Soper moved to accept the original language in the conclusion's first sentence. Drs. Elwell and Peterson seconded. Dr. Roberts called for a vote. The motion carried (8 yes, 2 no, 0 abstentions). Dr. Pino and Dr. Olivero voted no. Dr. Pino cited the reasons he had already stated, that the hepatic carcinomas were primarily responsible for the liver tumors in male rats and the conclusion should be reworded to state that. Dr. Olivero felt that the paragraph was not clear enough as it stood.

Dr. Carpenter moved to accept the full study conclusions as written. Dr. Peterson seconded. The panel voted in favor of the motion (8 yes, 2 no, 0 abstentions). Dr. Pino and Dr. Olivero voted no, for the same reasons they had stated for the prior motion.

V. Draft NTP Technical Report TR-578 on *Ginkgo biloba* Extract

Ms. Andrews announced that Drs. Pino and Elwell had conflicts of interest related to this report, and would not participate in the peer review.

Study Scientist Dr. Cynthia Rider introduced the draft NTP Technical Report on *Ginkgo biloba* extract noting that it was nominated by NIEHS for study based on widespread use as an herbal supplement, the known mutagenicity of the *Ginkgo biloba* extract constituent quercetin, and the lack of toxicity and carcinogenicity data. She provided information on the composition of *Ginkgo biloba* extract and its use as an herbal supplement both historically and currently, noting that in 2002 it was among the top five herbal supplements on the market. The test article was selected based on a wide distribution in commerce and the ratio of active ingredients were similar to marketed leaf extract EGb 761® (which was not itself available).

Dr. Rider presented results of mutagenicity studies and the nonneoplastic and neoplastic lesions observed in the 2-year gavage studies.

The proposed conclusions were:

> Under the conditions of these 2-year gavage studies, there was *some evidence of carcinogenic activity* of *Ginkgo biloba* extract in male F344/N rats based on increased incidences of thyroid gland follicular cell adenoma. The increased incidences of mononuclear cell leukemia and hepatocellular adenoma may have been related to *Ginkgo biloba* extract administration. There was *some evidence of carcinogenic activity* of *Ginkgo biloba* extract in female F344/N rats based on increased incidences of thyroid gland follicular cell neoplasms. Increased occurrence of respiratory epithelium adenomas in the nose may have been related to *Ginkgo biloba* extract administration. There was *clear evidence of carcinogenic activity* of *Ginkgo biloba* extract in male B6C3F1/N mice based on increased incidences of hepatocellular carcinoma and hepatoblastoma. The

increased incidences of thyroid gland follicular cell adenoma were also related to *Ginkgo biloba* extract administration. There was *clear evidence of carcinogenic activity* of *Ginkgo biloba* extract in female B6C3F1/N mice based on increased incidences of hepatocellular adenoma, hepatocellular carcinoma, and hepatoblastoma.

Administration of *Ginkgo biloba* extract resulted in increased incidences of nonneoplastic lesions in the liver, thyroid gland, and nose of male and female rats and mice and the forestomach of male and female mice. Increased severity of nephropathy in male rats was also due to administration of *Ginkgo biloba* extract. Increased severity of nephropathy in male rats was also due to administration of *Ginkgo biloba* extract.

Dr. Roberts opened the floor for oral public comments.

The first commenter, Dr. Stephen Dentali representing the American Herbal Products Association, said the unique *Ginkgo biloba* leaf extract discussed in the draft Technical Report is not representative of other *Ginkgo biloba* leaf extracts marketed in the United States and is almost certainly not sold in the US. He said that it is incorrect to represent it as similar to other *Ginkgo biloba* leaf extracts, based on the dissimilarity of its chemical composition to that of other commercially available products. He noted that the supplier had intended to make a unique extract for drug development, and that the extract contains (according to the company) “highly concentrated effective content” along with “further removal of inactive substances.” He presented data comparing the test article to other published analyses of *Ginkgo biloba* leaf extracts and to recognized standards and recommended that the report highlight these differences in composition. He also recommended that the report title be changed to delineate that the studies were done with a “specific” *Ginkgo biloba* leaf extract.

Dr. Ashley Roberts of Intertek Cantox presented public comments via telephone. He questioned the stability of the dosing formulation over the course of the study period, and the dose levels selected in the 2-year mouse study. He noted that the development of liver tumors was not surprising as they are known to occur spontaneously in the mouse population, and that the mouse strain used is highly susceptible to chemically induced liver tumors. He added that NTP should have worked to establish a “no observed adverse effect” level for risk assessment purposes. He asked the panel to consider downgrading the call in the 2-year rat study from *some evidence* to *equivocal evidence* based on the increased incidence of thyroid tumors. He said that the liver tumors in the mice and thyroid tumors in the rats may have little if any relevance in humans with the consumption of *Ginkgo biloba* leaf extract at much lower dose levels. He acknowledged that assessment of risk in humans is not the purpose of NTP studies,

but suggested NTP consider discussing the relevance of the findings to humans, given the widespread consumption of the herbal.

Dr. Howard noted for clarification that the panel is specifically not to enter into any risk assessment related to humans in its deliberations.

Dr. Anderson, the first primary reviewer, said the report was well done and that she agreed with the *clear evidence* calls in the male and female mice based on the liver tumors. For the thyroid adenomas in male rats, she noted the low incidence and no pair-wise significance, but said several other arguments suggested a chemical-related effect and she agreed with the call, as well as the *some evidence* call for female rats. For the mononuclear cell leukemia in male rats, she wondered whether further data such as age of death might support changing the call from *equivocal evidence* to *some evidence*. Regarding the liver tumors in the rats, she speculated that perhaps a *no evidence* call should be discussed but on balance agreed with the "may have been related" call. In the respiratory adenomas in the females, she wondered whether the call should be changed from *equivocal evidence* to *no evidence*. The call for the thyroid adenomas in the male mice was *some evidence*, which she felt was supported.

Dr. Mirsalis, the second primary reviewer, agreed with the proposed conclusions, although he agreed with Dr. Anderson that many of the calls seemed to fall into a "gray zone," where he didn't strongly agree or disagree. He made three general suggestions for the NTP Technical Reports: clearly state that all animals were specific pathogen-free (SPF), and what pathogens they are free of, specify the volume of the non-terminal blood collection and include a statement regarding FDA Part 11 Good Laboratory Practice (GLP) compliance in the quality assurance references. He said that his major issue with this study was the material used, in that it was different than the preparation most people are actually taking. He suggested including a table similar to the one shown by Dr. Dentali, comparing the components of the test article blend to commercially available compounds. He also felt that it would be good to point out how test exposures relate to human exposures, since the test exposures were many orders of magnitude higher.

The third primary reviewer Dr. Cattley agreed with Dr. Mirsalis' comments for the test material, and the usefulness of a chart for comparison. He agreed with the proposed call for the thyroid tumors in male rats; however, the ancillary evidence that supported the *some evidence* call in the males was lacking for the females; he wondered if the call in females should be *equivocal evidence*.

Dr. Rider responded to the reviewers' comments. She said that the *Ginkgo biloba* products available in the marketplace have a wide range of concentrations, and the test article's composition fell within the range of what is on the market. In response to

questions regarding nonneoplastic lesions, study pathologist Dr. Abraham Nyska noted that the findings were inconsistent across dose groups. Dr. Rider said that information about the SPF status is included in Appendix K of the report. She said that reporting the blood volumes relative to the animals' body weights would be difficult, and that all draws were within the Institutional Animal Care and Use Committee guidelines. She added that the NTP had not yet moved to fully electronic record keeping and thus has not triggered GLP Part 11. She agreed that several of the conclusions were complex and had been discussed thoroughly in staff reviews, where consistency across species and across sex was taken into account.

Dr. Anderson and Dr. Cattley suggested there was an over emphasis on genotoxicity in the report. Staff statistician Dr. Grace Kissling said that there was a slight correlation between age of death and dose, and agreed to analyze the available data as Dr. Anderson suggested, to determine average or mean ages of death. While age of death is a factor adjusted for in the statistical analysis of tumor incidence, it could also be addressed as a separate question.

Dr. Mirsalis recommended that NTP add mouse norovirus to its screening panel, and report the results. Dr. King-Herbert said that animals are not screened for norovirus when they are received by NTP, although such screening does take place frequently by the vendor. Dr. Mirsalis added that although he understood Dr. Rider's explanation regarding the test article's composition, NTP's rationale should be clear in the report.

In response to the public comment's suggestion that the NTP consider discussing the relevance of the *Ginkgo biloba* extract findings to humans, Dr. Howard offered caution given that these reports are not meant to evaluate risk for humans. Dr. Birnbaum noted such information would need proper context.

Dr. Roberts called for a motion on the conclusions for *Ginkgo biloba* extract. Dr. Mirsalis moved to accept the conclusions as written and Dr. Carpenter seconded the motion. Dr. Cattley felt that the call for the female rats' thyroid tumors should be changed from *some evidence* to *equivocal evidence*. Following further discussion of the issue, Dr. Roberts called for a vote on the motion to accept the conclusions as written. The panel voted in favor of the motion (7 yes, 1 no, 0 abstentions). Dr. Cattley voting no, re-stated his reason. Drs. Pino and Elwell recused themselves from the vote.

VI. Molecular Studies on *Ginkgo biloba* Extract (GBE) TR 578

Dr. Mark Hoenerhoff presented supplemental information on the molecular events seen in the *Ginkgo biloba* study. The study was designed to provide molecular and mechanistic context for the hepatocellular carcinomas (HCC) seen in the B6C3F1 mice

administered GBE compared to spontaneous HCC. Specifically, the study looked for relevant mutations, alterations in common HCC pathway expression, and differences in global gene expression profiling.

There was an increase in *β–catenin* mutations with increasing dose, with multiple mutations per tumor in several animals, as well as increased deletion mutations. There was a decreasing incidence of *H-ras* mutations with dose, in contrast to spontaneous tumors, in which *H-ras* mutations are common. Further protein analysis showed upregulation of the WNT/CTNNB1 pathway and alterations to the CTNNB1 protein not observed in spontaneous HCC.

Microarray analysis was conducted, and the gene expression profiles for the groups of vehicle control, spontaneous HCC, and GBE-treated HCC, each clustered distinctly upon Principal Component Analysis. "Although these tumors are extremely similar and often indistinguishable from one another at the cellular and morphological level, we see that in terms of their gene expression, they really are very different at the transcriptomic level," Dr. Hoenerhoff said. A heatmap representation also depicted the separation among the tumors with and without GBE treatment.

Dr. Hoenerhoff concluded that GBE hepatocarcinogenesis in B6C3F1 mice is a complex process involving multiple different pathways and genetic alterations, reflecting the complex nature of the compound. GBE-treated tumors exhibit genetic alterations and pathway dysregulation that are known to influence HCC development in both mice and humans.

Dr. Cattley asked about the increase seen in inflammatory pathways. Dr. Nyska replied that the increase was not considered significant. So, Dr. Cattley responded, the pathways were actually hepatocellular. Dr. Hoenerhoff said that it was common for there to be much overlap in cancer-related pathways.

Dr. Howard found the added information to be quite valuable, and asked whether at some point it would be added to the Technical Report. Dr. Bucher said that the decision had been made to publish the information separately, and although good for context, the information should not be taken to potentially change any of the report's calls. Dr. Howard felt including it in the report should be considered as the information was as valuable as the immunogenicity genetic toxicology data contained in the report. He asked if these slides would be posted on the NTP website. Dr. Bucher responded yes, with the other meeting information.

Dr. Howard asked about the sampling of the tumors, pointing out that tumor tissues are not typically homogeneous. Dr. Hoenerhoff said that tumor tissues are sectioned in the center of the tumor, avoiding necrosis or hemorrhage, and that there is always a

histopathologic slide made from the sample, which can be examined to see the constituents of the tumor.

The panel continued to discuss the inclusion of molecular information in the Technical Report, in this case or in general. Dr. Roberts summarized by saying that everyone agreed that it is important information, with the question being the best context for its presentation.

VII. Draft NTP Technical Report TR-580 on β-Picoline

Study Scientist Dr. Michael Wyde introduced the draft NTP Technical Report on the β-picoline by describing the chemical, which is structurally similar to pyridine, its uses, and its nomination based on environmental releases and potential for human exposure. Much of the initial knowledge used to design the studies was derived from data on pyridine. Dr. Wyde described the design and results of the short- and long-term studies in rats and mice, including nonneoplastic and neoplastic lesions in test animals. Decreases in body weight and water consumption and renal toxicity were observed in rats. The reduced water consumption was attributed to low palatability of the chemical at the higher concentrations. No significant treatment-related effects were observed in mice. In the 2-year studies, there were decreased body weights in male and female mice.

The proposed conclusions were:

> Under the conditions of these 2-year drinking water studies, there was *no evidence of carcinogenic activity* of β-picoline in male F344/N rats exposed to 156.25, 312.5, or 625 mg/L. There was *some evidence of carcinogenic activity* of β-picoline in female F344/N rats based on increased incidences of alveolar/bronchiolar adenoma and alveolar/bronchiolar adenoma or carcinoma (combined). There was *equivocal evidence of carcinogenic activity* of β-picoline in male B6C3F1/N mice based on increased incidences of alveolar/bronchiolar adenoma and alveolar/bronchiolar adenoma or carcinoma (combined). There was *clear evidence of carcinogenic activity* of β-picoline in female B6C3F1/N mice based on the increased incidences of alveolar/bronchiolar adenoma or carcinoma (combined) in the lung and of hepatocellular carcinoma and hepatoblastoma in the liver.
>
> Exposure to β-picoline caused increased incidences of nonneoplastic lesions of the lung in female mice and the nose in male and female mice.

Dr. Roberts invited oral comments from the public. There being none, he proceeded to the panel's peer review.

The first primary reviewer, Dr. Mirsalis felt that it was a good report and he agreed with its conclusions. He noted that the purity of the test article was 96%, and inquired about the other 4%. He requested more discussion on the compound's palatability. He noted the 78% "high" incidence of hepatocellular adenomas in the female mouse controls and questioned the significance of the increase in hepatocellular adenomas in the treated groups. Finally, he suggested that β-picoline might be a good candidate for reproductive toxicity tests in rats.

Dr. Pino, the second primary reviewer, recommended that the conclusion for male rats should have been *equivocal evidence* based on the alveolar/bronchiolar carcinomas, as the report indicated that while the combined incidences of alveolar/bronchiolar adenoma or carcinoma in males were similar between the control and treated groups, these observations (referring to the carcinomas in males) may suggest a treatment related progression from benign tumors to malignancy. He asked for more discussion in the text about whether or not hepatocellular adenomas in the female mice might be compound related, which was not mentioned in the conclusion. He asked whether the incidence of multiple alveolar/bronchiolar carcinomas in male mice was above the historical range, because if so, he felt that it should be included in the conclusion. He inquired why an increased severity of nephropathy was seen in the 3-month study in male rats, but not in the 2-year study at the same doses. He inquired whether the estrous data in rats were skewed by the fact that the estrous cycle was longer than 12 days or unclear in 4 of the 10 control rats, because if so, felt that information should be indicated in the discussion.

Dr. Alcorn, the third primary reviewer, questioned the doses selected for the studies, believing they may have been too high given changes in body weight and water consumption in the short-term studies. Based on those concerns, she endorsed changing the conclusion for female rats from *some evidence* to *equivocal evidence*. She also noted that the control mice lost significant weight in the last year of the study. She asked about why the CYP2B1 liver microsomes were assessed, as there was no indication of the importance in the report. She said she would like to see less reference to pyridine in the report.

Dr. Wyde said that most of the impurity in the test article was water, and that there were two impurities at 0.6% and 0.4%. Regarding the high incidence of liver tumors in the female mouse controls, he said the proposed conclusion was mainly based on the hepatoblastomas and the hepatocellular carcinomas rather than the adenomas. He said the NTP would consider conducting reproductive studies. He discussed the rationale for the *no evidence* call in the male rats. Dr. Elmore explained the approach

for evaluating the oral carcinomas. Dr. Mirsalis recommended including information about the compound's purity in the report.

Dr. Wyde said the intent of the dose selection had been to be sure the animals were challenged sufficiently. He said the information on pyridine was used as a starting point in the study design due to the lack of information on β-picoline. He said that the body weight loss in the mice in the second year of the study was a typical response. He also explained that the call of *some evidence* in the female rats was based on the occurrence of lung neoplasms in all three exposed groups, increased rates of hyperplasia in the exposed groups, and the potential for the adenomas to progress to carcinomas.

Dr. Roberts noted that there were at least two proposed changes to the conclusions. Dr. Pino said that perhaps the call for male rats should be *equivocal* based on the alveolar/bronchiolar carcinomas, or that the sentence regarding a possible compound-related progression to malignancy be deleted.

Dr. Anderson said there may have been some confusion as to nomenclature for reviewers who were not accustomed to reading the technical reports. Dr. Hooth explained that the NTP makes calls assigning one level of evidence for each sex and species based upon the highest call for each. Dr. Alcorn said she accepted Dr. Wyde's explanation about dosing, and, therefore, was comfortable with the conclusion regarding the female rats.

Dr. Roberts called for a motion on the conclusions for β-picoline. Dr. Mirsalis moved to accept the conclusions as written and Dr. Alcorn seconded the motion. The panel accepted unanimously (10 yes, 0 no, 0 abstentions) the conclusions as written.

VIII. Draft NTP Technical Report TR-574 on Pyrogallol

Study Scientist Dr. Minerva Mercado-Feliciano introduced the draft NTP Technical Report on pyrogallol describing its occurrence as a natural decomposition by-product of plant tannins, its use in a variety of manufacturing processes and consumer products, and its nomination for study based on its frequent occurrence as both a natural and manufactured product and lack of carcinogenicity data. She noted its positive response in genetic toxicity studies and short-term contact hypersensitivity tests identifying pyrogallol as a weak sensitizer and strong irritant and presented nonneoplastic and neoplastic lesions in the short- and long-term studies. Dermal studies were conducted because dermal occupational exposure is the most common human route. Similar nonneoplastic lesions were present at the site of application in both the short- and long-term studies.

The proposed conclusions were:

> Under the conditions of these 2-year dermal studies, there was *no evidence of carcinogenic activity* of pyrogallol in male or female F344/N rats administered 5, 20, or 75 mg/kg. There was *equivocal evidence of carcinogenic activity* of pyrogallol in male B6C3F1/N mice based on increased incidences of squamous cell papilloma of the skin at the site of application. There was *some evidence of carcinogenic activity* of pyrogallol in female B6C3F1/N mice based on increased incidences of squamous cell carcinoma of the skin at the site of application.
>
> Dermal administration of pyrogallol caused increased incidences of nonneoplastic lesions of the skin at the site of application in male and female rats and mice, skin adjacent to the site of application in male and female mice, and mammary gland in female mice.

Dr. Roberts opened the floor for oral public comments. There being none, he proceeded to the panel's reviews.

Dr. Cattley, the first primary reviewer, thought that the report justified the conclusions. He noted that discussion in the report had mentioned determination of a no observable adverse effect level (NOAEL), which he was unaccustomed to seeing in an NTP report. He felt that since the issue had been introduced, it should be addressed in the conclusions. He thought NTP had done a good job with dose selection, but wanted a bit more rationale about the top dose. He asked for more definition on how decisions were reached to remove animals from the study. In the 2-year study, he noted that all of the findings were at the site of application except the mammary gland hyperplasia, and asked what might have been the mechanism.

Dr. Alcorn, the second primary reviewer, asked for clarification as to why body weight was reduced in the female mice in the 2-year study. She asked if transference from the site of application might have taken place with regard to the squamous cell papillomas found on the dorsal nose of the rats, perhaps as a result of grooming behavior.

Dr. Soper, the third primary reviewer, thought that the study was well designed and the conclusions were well justified. He did not believe that the skin papillomas in the male rats rose to the level of *equivocal evidence*.

Regarding the endpoint being considered for the NOAEL, Dr. Mercado-Feliciano responded that several outcomes were considered. She said that 75 mg/kg had been chosen as the top dose because it appeared to be the minimum concentration that would give the maximum response, as had been seen in the 90-day study.

Study Pathologist Dr. Ron Herbert said that NTP specifications were followed for removal of animals, although the attending veterinarian is allowed latitude, particularly where the welfare of the animals is concerned. He added that in the pyrogallol study, the high number of moribund sacrifices in 75 mg/kg female mice was due to the presence of marked ulceration at the site of application. Regarding the question about mammary gland hyperplasia, Dr. Herbert was unsure what the mechanism might be, but added that there didn't seem to be any qualitative difference between the lesions that occurred in the control and treated animals. Regarding the decreasing weight in the female mice, Dr. Mercado-Feliciano said that feed consumption is not routinely monitored in dermal studies, so it was unclear what the reason might be. The squamous cell papillomas in the rats were not considered to be treatment-related, due to the low incidence and said that point would be clarified in the report.

Dr. Cattley said that instead of referring to NOAEL, perhaps it should be expressed as highest dose tolerated with no effect on survival. Dr. Roberts agreed. Dr. Alcorn suggested that monitoring of feed consumption be included as part of a study's humane intervention checklist. Dr. Soper spoke in support of the 75 mg/kg dose as the top dose, actually extrapolating from human to rodent, citing a human male who had dosed himself at approximately 143 mg/kg and died acutely.

Dr. Pino mentioned that the fact that the skin papillomas were not considered treatment-related should also appear in the abstract section of the report, or reference to the lesions should be removed from the abstract. Dr. Elwell felt that the female mice might have gotten an excessive dose, given their doubling in weight and resulting increase in dosing based on that higher weight. Dr. Mirsalis suggested that the micronucleus data be clarified.

Dr. Roberts called for a motion on the conclusions for pyrogallol. Dr. Soper moved to accept the draft's conclusions as written and Dr. Cattley seconded. The panel accepted unanimously (10 yes, 0 no, 0 abstentions) the conclusions as written.

IX. Draft NTP Technical Report TR-576 on Trimethylolpropane Triacrylate

Study Scientist Dr. Inok Surh introduced the draft NTP Technical Report on trimethylolpropane triacrylate (TMPTA) by noting its industrial applications, particularly in the production of ultraviolet curable dyes, and it nomination for study due to its high and increasing production and use, potential for human exposure, lack of adequate chronic toxicity and carcinogenicity data, and as a representative of the multifunctional acrylate class.

Studies conducted by NTP consisted of two phases. Dr. Surh reviewed Phase 1 of the TMPTA studies, which consisted of a genotoxicity study, a contact hypersensitivity study, an ADME study, 2-week and 3-month dermal studies in F344/N rats and B6C3F1/N mice, and 6-month dermal studies in FVB Tg.AC hemizygous mice that were reported in 2005 in the NTP GMM series (GMM 3). Phase 2, reported in TR-576, consisted of the current 2-year dermal studies of TMPTA in F344/N rats and B6C3F1/N mice. The genotoxicity assays were negative, and TMPTA was found to be an irritant, but not a contact sensitizer.

The proposed conclusions were:

> Under the conditions of these 2-year dermal studies, there was *some evidence of carcinogenic activity* of trimethylolpropane triacrylate in male F344/N rats based on increased incidences of malignant mesothelioma. There was *no evidence of carcinogenic activity* of trimethylolpropane triacrylate in female F344/N rats administered 0.3, 1.0, or 3.0 mg/kg. There was *no evidence of carcinogenic activity* of trimethylolpropane triacrylate in male B6C3F1/N mice administered 0.3, 1.0, or 3.0 mg/kg. There was *some evidence of carcinogenic activity* of trimethylolpropane triacrylate in female B6C3F1/N mice based on increased incidences of uncommon malignant hepatic neoplasms (hepatoblastoma and hepatocholangiocarcinoma) and stromal polyp or stromal sarcoma of the uterus.
>
> Dermal application of trimethylolpropane triacrylate for 2-years resulted in increased incidences of nonneoplastic lesions in the skin of male and female rats and mice.

Dr. Roberts opened the floor for oral public comments.

Dr. Kimberly Ehman of Toxicology Regulatory Services, speaking on behalf of the Specialty Acrylates and Methacrylates (SAM) Panel of the American Chemistry Council (ACC), said that it was very unlikely that consumers would be exposed to TMPTA, as its exposures mainly occur in the occupational setting. She questioned the relevance of the observed malignant mesothelioma in male rats, stating that they are very specific to F344 rats, and the extrapolation of stromal polyps and sarcoma in female mice to humans. She questioned the biological relevance of the hepatic neoplasms in the female mice because the incidence was not dose-dependent and TMPTA and numerous additional acrylates have been shown to be non-genotoxic *in vivo*. She asked the panel to consider revising the conclusion from *some evidence* to *equivocal evidence* of carcinogenic activity for both male rats and female mice, and to consider omitting reference to the NTP studies conducted with transgenic mouse strains.

In follow-up to Dr. Erhman's comments, Dr. Carpenter commented that malignant mesotheliomas actually occur in other strains of rats.

Dr. Karin Ke of Keller and Heckman LLP, speaking on behalf of RadTech International North America, cited studies suggesting the hepatic lesions in female mice were not as rare as reported. She requested that the panel consider changing the draft's conclusion in female B6C3F1/N mice to either "*no evidence of carcinogenic activity* or *equivocal evidence of carcinogenic activity*…based on a marginal increase of hepatic neoplasms that may be chemical related."

Dr. Carpenter, the first primary reviewer, said that he had no significant criticisms of the report, and agreed with the calls and the likelihood for exposures for consumers in addition to workers. He said he would like to have seen additional discussion of the tunica vaginalis mesothelioma tumors. He asked about the use of technical grade TMPTA and whether purer sources were available. He felt that a number of the public comments had indicated a lack of understanding that the NTP Technical Reports are not meant to provide comparisons of the findings with human toxicity and cancer.

Dr. Peterson, the second primary reviewer, said that the conclusions were well justified based on the data presented.

Dr. Elwell, the third primary reviewer, had no scientific criticisms and felt that the doses used in the studies had been chosen well. He asked for more discussion of the *some evidence* versus *equivocal evidence* call for the mesothelioma given the considerable variability of the lesion in historical controls. He also requested clarification in the discussion of the stromal polyps and stromal cell sarcomas in light of the uterine sarcoma observed in a control animal. He suggested that the results table for liver tumors include all the tumor types, rather than highlighting hepatoblastoma. Although a morphological description was provided for hepatocellular adenomas, there was no discussion of the incidence and relevance. Dr. Elwell requested more discussion of hepatocellular effects and of adrenal medullary hyperplasias. He questioned inclusion of the earlier Tg.AC study findings and noted their lack of concordance with the current findings.

In response to the reviewers' comments, Dr. Surh agreed to consider additional discussion of the rat mesothelioma tumors. Regarding the technical grade chemical used, Dr. Surh said TMPTA was only available commercially in technical grade. She explained the call regarding mesothelioma was based on a statistically significant trend and the incidence in the top dose group was significant and outside the historical control range. Study Pathologist Dr. Deepa Rao said that the origin of the uterine sarcoma tumor in the control animal was uncertain. Regarding the hepatocellular adenoma, Dr. Surh explained that only chemically related findings are included in the results section,

and since the hepatocellular adenoma was not considered to be a treatment effect, it was not included, but the information was available in the appendices. Regarding the hepatoblastoma and hepatocholangiocarcinoma, she noted that separate studies were conducted in male and female mice, and these uncommon tumors were more rare in female than male mice. She said that due to limitations of the Tg.AC transgenic mouse model, the NTP could not make a level of evidence determination for substances evaluated in this model. She said in the current study, the results in the male mice were negative.

Dr. Elwell commented that the liver tumor incidences seemed out of the norm. Dr. Malarkey agreed and said that that had been a consideration when the call was debated by NIEHS staff.

To help inform the discussion, Dr. Malarkey presented a short talk on hepatoblastomas, with morphological slides as examples. He said hepatoblastomas in the mouse are a primitive, poorly differentiated variant of a hepatocellular neoplasm that can arise from adenoma or carcinoma with relatively late onset in adult mice, and the males are much more affected than females. There is a metastatic rate of anywhere from 25% to 50% depending on the study. They have been reported in other mouse strains, but not in the rat. He added that the NTP has molecular studies in progress that are aimed at understanding the pathogenesis of hepatoblastoma and the relationship between hepatoblastoma and hepatocellular carcinoma.

Dr. Peterson asked if the same call would have been made if the same incidences had been seen in male mice. Dr. Malarkey replied probably not, given the higher background in male mice. Dr. Peterson felt that supported the *some evidence* call versus *equivocal evidence*. Dr. Anderson questioned citing the hepatoblastomas in the call, without including the precursor lesions and felt that the call should be *equivocal evidence* rather than *some evidence*. Dr. Robert Sills explained that some of the hepatoblastomas in controls arise independently of hepatocellular adenomas and carcinomas. Dr. Malarkey and Dr. Mark Cesta explained that when hepatoblastomas arise within hepatocarcinomas, only one diagnosis is recorded. With that explanation, Dr. Anderson rescinded her suggestion that the call be changed to *equivocal evidence*.

Dr. Roberts summarized the discussion to that point, noting that there had been reservations on the calls in the male rats and female mice.

Dr. Elwell reiterated that the incidence of mesotheliomas in male rats seemed to be sporadic in the limited sample cited in the report for other control groups from NTP studies, and questioned the *some evidence* call. Dr. Mirsalis felt that the call should be changed to *equivocal evidence*. Further discussion centered on the issue of incidences in historical versus concurrent controls, including some of the other NTP studies

considered during the day. Dr. Elwell said historical control data are generally considered to be secondary to concurrent controls, which was why he thought the NTP made the call. Dr. Carpenter noted that NTP does not make a differentiation histologically in different tissue types of mesothelioma. Dr. Malarkey concurred and added that mesotheliomas are generally of vaginal tunic origin and can be in the abdomen or thorax. Dr. Anderson said that given the statistically significant *p* value for trend, she supported the *some evidence* call.

Dr. Cattley moved to change the conclusion for female mice by not including hepatoblastoma as part of the *some evidence* category, noting it instead as "may have been related." Dr. Carpenter seconded the motion. The panel voted unanimously in favor of the motion (10 yes, 0 no, 0 abstentions). The call was changed to read:

> There was *some evidence of carcinogenic activity* of trimethylolpropane triacrylate in female B6C3F1/N mice based on increased incidences of an uncommon malignant hepatic neoplasm (hepatocholangiocarcinoma) and stromal polyp or stromal sarcoma of the uterus. The occurrence of hepatoblastoma may have been related to the chemical.

Dr. Mirsalis moved to change the call for male rats from *some evidence* to *equivocal evidence* and Dr. Soper seconded the motion. Prior to the vote, Dr. Mirsalis also suggested that the word "marginally" be inserted in the sentence prior to "increased incidences." The motion as amended was approved (8 yes, 2 no, 0 abstentions). Drs. Carpenter and Anderson voted no, explaining that they felt that the *some evidence* call was more appropriate. Thus, the conclusion was changed to read:

> Under the conditions of these 2-year dermal studies, there was *equivocal evidence of carcinogenic activity* of trimethylolpropane triacrylate in male F344/N rats based on marginally increased incidences of malignant mesothelioma.

Dr. Roberts then called for the panel to vote on the entire set of conclusions, as amended. Dr. Mirsalis moved to accept the entire set of conclusions and Dr. Soper seconded. The panel approved the motion (8 yes, 2 no, 0 abstentions), with Drs. Carpenter and Anderson voting no for the same reasons expressed regarding the call in the male rats.

Dr. Roberts adjourned the meeting for the day at 4:15 PM.

February 9, 2012

X. Introductions and Welcome, Day Two

Dr. Roberts convened the second day of the panel's proceedings. Attendees in the room introduced themselves and Ms. Andrews read the conflict of interest policy statement.

XI. Introductions to Studies on the Toxicology of AIDS Therapeutics

Dr. Howard presented an overview of NCTR's series of studies on the toxicology of AIDS therapeutics, briefly reviewing the bioassays that have been conducted.

He briefly defined AIDS and reviewed anti-retroviral therapy, adding that due to reports on the potential mutagenicity and/or carcinogenicity of nucleoside analogues, studies were designed to test those conditions in rodents as predictors of possible human disease outcomes. Initially, the manufacturer of 3'-Azido-3'-Deoxythymidine (AZT) published two 2-year bioassays in 1996 and 1997. NIH bioassays were performed in 1997, 1999, and 2007. Two NIEHS/NTP 2-year bioassays were conducted – Technical Reports (TR) 469 and 522. He noted that AZT is no longer administered alone but is always administered in combination with other therapeutic compounds. Thus, further studies have looked at whether or not the combinations of drugs have any impact on the known carcinogenicity of AZT. In TR-569 (reviewed at the April 5, 2011, Technical Reports peer review meeting), 2-year bioassays involving various combinations of the drugs were conducted. A 2-year bioassay of AZT, lamivudine (3TC), nevirapine (NVP), nelfinavir mesylate (NFV), and efavirenz (EFV) following transplacental/perinatal exposure will be reviewed in 2013. Because there was a desire to determine whether transgenic animals could be used to detect carcinogenicity and perhaps shed light on possible risk to humans, the studies being reviewed at the present meeting were transgenic mice of AZT alone and in combination with other drugs.

XII. Draft NTP Technical Reports 3'-Azido-3'-Deoxythymidine (GMM-14), and in Combination with Lamivudine and Nevirapine (GMM-16)

Study Scientist Dr. Julian Leakey introduced the Genetically Modified Model (GMM) Reports GMM-14 and GMM-16 in one presentation, and afterwards the peer review of each report was handled individually in terms of peer review comments, discussion, and vote on the proposed conclusions.

Dr. Leakey reviewed information on the toxicity and carcinogenicity of AZT in rodents and humans, including ADME issues and mechanisms of AZT-induced toxicity in eukaryotic cells. He presented information regarding AZT human toxicity, noting that long-term consequences of perinatal exposure to AZT are unknown. He provided background information regarding the development of the C3B6.129F1*Trp53*^{tm1Brd} *p53* haploinsufficient (+/-) mouse model, which is designed to develop tumors at an increased rate and thus shorten the duration of carcinogenicity studies.

GMM-14 was the first study to use the model with perinatal exposure. Dr. Leakey reviewed the experimental design for the main study and stop study, dosing AZT alone once per day from gestational day (GD) 12 to 9 months of age. He noted that the model was found to be sensitive enough to detect carcinogenesis, with a treatment-related tumor profile similar to that seen in the B6C3F1 mouse, mainly liver tumors and lymphomas. There was no evidence of clustering of lesions within litters.

The proposed conclusions for GMM-14 were:

> Under the conditions of these gavage studies, there was *clear evidence of carcinogenic activity** of AZT in male heterozygous F1 p53+/– mice based on the occurrence of hepatocellular neoplasms (predominantly adenomas) after 45 weeks of administration. The occurrence of malignant lymphoma may have been related to AZT administration for 30 weeks. There was *equivocal evidence of carcinogenic activity* of AZT in female heterozygous F1 p53+/– mice based on the occurrence of malignant lymphoma after 45 weeks of administration.

GMM-16 was designed to test AZT in combination with 3TC and NVP. Mice were dosed twice per day with AZT, 3TC, and NVP alone or in combination from GD12 until postnatal day (PND) 28. The study was designed to more closely mimic the clinical situation where infants are dosed with drug combinations twice daily, but only prenatally and in infancy. Dr. Leakey presented the results of the study, including a significant increase in liver tumors.

The proposed conclusions for GMM-16 were:

> Under the conditions of this gavage study, there was *clear evidence of carcinogenic activity* of AZT alone in male heterozygous F1 p53+/- mice based on increased incidences of hepatocellular adenoma. There was clear evidence of carcinogenic activity of AZT in combination with 3TC, and AZT in combination with 3TC and NVP in male heterozygous F1 p53+/- mice based on increased incidences of hepatocellular adenoma and hepatocellular adenoma or carcinoma (combined). The occurrence of malignant lymphoma may have been related to treatment with AZT alone and with AZT in combination with 3TC.

There was *no evidence of carcinogenic activity* of 3TC alone in male heterozygous F1 p53+/- mice administered 150 mg/kg. There was *no evidence of carcinogenic activity* of NVP alone in male heterozygous F1 p53+/- mice administered 168 mg/kg.

There was *equivocal evidence of carcinogenic activity* of NVP alone, AZT in combination with 3TC, and AZT in combination with 3TC and NVP in female heterozygous F1 p53+/- mice based on the occurrence of malignant lymphoma. There was *equivocal evidence of carcinogenic activity* of 3TC alone in female heterozygous F1 p53+/- mice based on the occurrence of mammary gland adenoacanthoma or adenocarcinoma (combined).

There was *no evidence of carcinogenic activity* of AZT alone in female heterozygous F1 p53+/- mice administered 240 mg/kg.

3'-Azido-3'-Deoxythymidine (GMM-14)

Dr. Roberts opened discussion of GMM-14 by asking whether there were any oral public comments. There being none, he proceeded to the panel's review.

Dr. Elwell, the first primary reviewer, noted that the presentations were very helpful for understanding these complex studies. He felt the studies were well designed and he had no scientific criticisms. He felt that it would be useful to have some discussion in the report of the differences, if any, between the perinatal exposure and the exposure for the full 45 weeks. He asked if the occurrence of lymphoma in the stop study should also be mentioned in "other findings." He suggested that "other findings" that were dismissed or not brought forward to the summary or conclusions should be clarified in the discussion section. Based on the comment in the report on group size and statistical significance, he asked if the sample size should have been increased in the stop study to improve the statistical ability of the study to discern small increases in tumor incidences. He said that there was indication of vagina examination only in the 30-week study tables, and asked whether that had also been examined in the 45-week study.

Dr. Soper, the second primary reviewer agreed that the studies were well designed and well-executed, and agreed with the proposed conclusions.

Dr. Olivero, the third primary reviewer, expressed concern about the limited historical control database.

Dr. Leakey replied that when this study was written and evaluated, it was the only one of its kind, and thus the historical controls consisted of only 103 animals from these two studies and one other. He said that nonetheless, they were confident in the tumor diagnoses. He felt that the haploinsufficiency was what was driving low tumor incidence, more than the actual dosing vehicle. He added that as the study series progresses, the historical control database would be built up. Regarding the stop study, he noted that body weight within the latter stage of dosing does affects liver tumor incidence. He said that he would add some discussion of the "other findings" such as bone and brain tumors to offer more explanation as to why certain neoplasms were in the conclusions while others were not.

Dr. Olivero asked about the increase in hemoglobin. Dr. Leakey said that while it was statistically significant, it was not outside the physiological range. He said the investigators were expecting to see more anemia, as seen in industry studies where dosing was twice per day.

Dr. Mirsalis inquired about the appearance of the malignant lymphomas compared to those seen in B6C3F1 mice. Study Pathologist Dr. Greg Olson replied that the lymphomas looked much like those normally seen. However, in the second study, there were several undifferentiated tumors that needed to be further characterized. Dr. Elwell asked how the fatal malignant lymphomas compare to those in B6C3F1 mice at 45 weeks. Dr. Olson said they were the same as those seen in normal chronic studies.

Drs. Anderson and Olivero mentioned similar studies in CD1 mice, which had yielded very different results. Dr. Leakey said he would include their discussion in the report.

Dr. Elwell moved that the conclusions be accepted as written and Dr. Mirsalis seconded the motion. The panel unanimously accepted (10 yes, 0 no, 0 abstentions) the conclusions as written.

3'-Azido-3'-Deoxythymidine in Combination with Lamivudine and Nevirapine (GMM-16)

Dr. Roberts asked if there were any public comments. There being none, he proceeded with the panel's reviews.

Dr. Olivero, the first primary reviewer, reiterated her concern that this mouse model may not be appropriate to evaluate the carcinogenic potential of the drugs. She acknowledged that the studies were very complex. She also found it concerning that the model did not produce anemia, since that has been a signature of other studies. She suggested that in the future the animals' micronuclei should be examined to determine whether they have intact chromosomes. She also suggested adding

protease inhibitors to future studies, since some evidence has shown that they have a protective carcinogenic effect.

Dr. Anderson, the second primary reviewer, called GMM-16 “a remarkable study.” She particularly appreciated the extra information on litter and paternal effects. She had no major scientific criticisms and agreed with the conclusions.

Dr. Elwell, the third primary reviewer, agreed with the previous reviewers and concurred with the calls as written. He suggested discussion in the report on the microscopic finding of centrilobular degeneration and inclusion of more information on the increased severity of vacuolization in the liver. He also noted inconsistency in the discussion of nonneoplastic findings.

Dr. Leakey replied that tests for anemia had not been conducted to avoid adding stress to the pups, which were already in a toxic environment. He agreed to add discussion of AZT lymphoma protection, and to clarify the discussion and treatment of nonneoplastic findings. Dr. Olson noted that the liver tumors were quite distinct. Dr. Leakey mentioned that in the GMM series the severity scores for graded nonneoplastic lesions were not in the report, but were available on the NTP website. Dr. Elwell asked whether the other liver findings had an impact on tumor response, and if there was something unusual about this study in that most of the animals, including controls, had liver degeneration. Dr. Olson agreed that it was not a typical response. After further discussion, it was recommended that the severity score information should be incorporated into the report.

Dr. Anderson moved to approve the conclusions as written and Dr. Olivero seconded. The panel voted unanimously to accept (10 yes, 0 no, 0 abstentions) the conclusions as written.

Dr. Bucher asked the panel for recommendations concerning future transgenic model studies. Dr. Olivero noted that in her research program, the models, dosing and other aspects were evolving, generating very different types of data than traditional studies. Dr. Cattley suggested formulating some type of guidance, because the studies are not full lifetime studies, the group sizes are smaller, and the genetic background is unusual. He noted what seemed to him to be a discrepancy between the intention to generate large changes in tumor incidence and the actual results, which had a more limited range. Dr. Anderson inquired whether the genetic models are in fact faster and cheaper, since they have their own special issues. Dr. Bucher said that the savings are not as great as had been anticipated, and said it remained an open question whether using the models would ultimately be cost-effective and rapid enough to actually influence clinical decision making in use of the therapeutics and evaluation of exposed

children. Dr. Anderson suggested that there are classical models that should be considered in this context.

Dr. Bucher thanked the panel and staff for their hard work on the meeting, and Dr. Roberts adjourned the proceedings at 10:25 AM, February 9, 2012.

These summary minutes have been read and approved by the Chair of the February 8-9, 2012, National Toxicology Program Technical Reports Peer Review Panel.

[Redacted]

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Dr. Stephen Roberts
Chair, NTP Technical Reports Peer Review Panel

Date: _____