

National Toxicology Program

Technical Reports Peer Review Panel Meeting

April 5, 2011

National Institute of Environmental Health Sciences

Research Triangle Park, NC

Summary Minutes

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I. Attendees

Members in Attendance:

Lucy Anderson, Private Consultant
Norman Barlow, Sanofi-Aventis
Diane Birt, Iowa State University
John Cullen, North Carolina State University (NCSU)
Wendy Heiger-Bernays, Boston University School of Public Health
James Klaunig, Indiana University
Mark Miller, Wake Forest University School of Medicine
Arlin Rogers, University of North Carolina at Chapel Hill (UNC)

NTP Board of Scientific Counselors Representative:

Mitzi Nagarkatti, University of South Carolina School of Medicine

Other Federal Agency Staff:

Frederick Beland, Retired – Food and Drug Administration (FDA)
Mary Boudreau, FDA
D. Gayle DeBord, NIOSH
Robert Paul Felton, FDA
Paul Howard, FDA
Paul Mellick, FDA
Maria Mendoza, FDA
Greg Olson, FDA
Brett Thorn, FDA

National Institute of Environmental Health Sciences (NIEHS) Staff:

Charles Alden	Angela King-Herbert	William Schrader
Danica Andrews	Ronald Herbert	Michael Shelby
Mamta Behl	Michelle Hooth	Robert Sills
Abee Briggs	Mark Hoenerhoff	Cynthia Smith
Amy Brix	Ed Kang	In Ok Suhr
John Bucher	Grace Kissling	Raymond Tice
Rajendra Chhabra	Robin Mackar	Molly Vallant
Michael Cunningham	David Malarkey	Suramya Waidyanatha
Michael DeVito	Scott Masten	Nigel Walker
June Dunnick	Barry McIntyre	Kristine Witt
Paul Foster	Arun Pandiri	Mary Wolfe
John French	Cynthia Rider	Richard Woychik
Robbin Guy	Michael Sanders	

Public Attendees

Ronald Amen, Tech Enterprises
Ezra Bejar, Herbalife International
Susan Collinge, JR Simplot Company
James Coughlin, Coughlin & Associates
Howard Feldman, Aloe Industry
Robert Fensterheim, RegNet Environmental Services
Marvin Friedman, SNF-SAS
Kenneth Jones, International Aloe Science Council (IASC)
Dennis Marroni, SNF-SAS
Ben McMakin, Aloe Industry
Clifton McClellan, NSF International
Devon Powell, IASC
Michael Scott, Aloe Industry
Jennifer Tallett, Global Prairie
Wally Winters, Aloe Industry

II. Introductions and Welcome

The National Toxicology Program (NTP) Technical Reports Peer Review Panel Meeting convened on April 5, 2011 in Rodbell Auditorium, National Institute of Environmental Health Sciences (NIEHS), Research Triangle Park, North Carolina. Dr. John Cullen served as chair. The other Peer Review Panel members present were Drs. Lucy Anderson, Norman Barlow, Diane Birt, Wendy Heiger-Bernays, James Klaunig (by telephone), Mark Miller, and Arlin Rogers. Dr. Mitzi Nagarkatti attended as the NTP Board of Scientific Counselors liaison. Dr. Paul Howard attended representing the FDA. Dr. Gayle DeBord attended representing NIOSH. Representing the NTP was Dr. John Bucher (Associate Director), Dr. Nigel Walker (Deputy Program Director for Science) and Dr. Dave Malarkey (Group leader for Pathology). Dr. Rick Woychik, Deputy Director of NIEHS, was present to represent the Office of the NIEHS Director in Dr. Linda Birnbaum's absence.

Dr. Cullen welcomed everyone to the meeting and asked all attendees to introduce themselves. Dr. Bucher also welcomed attendees, and mentioned that the meeting would highlight the interagency agreement between NTP and the National Center for Toxicological Research (NCTR), in that three of the four studies to be reviewed were actually performed at NCTR. Designated Federal Officer Danica Andrews read the conflict of interest policy statement. It was noted that Dr. Cullen had a conflict of interest with the AIDS Therapeutics report and that Dr. Birt would chair that review.

Dr. Klaunig only participated in peer review of the draft NTP Technical Report on Senna. Dr. Birt was not present for peer review of the draft NTP Technical Report on Senna.

CLARIFICATION: Clarification is needed to the NTP Technical Reports Peer Review Panel Meeting, April 5, 2011, Summary Minutes. On page 4, text is added to the minutes that Dr. Klaunig only participated in peer review of the draft NTP Technical Report on Senna and Dr. Birt was not present for peer review of the draft NTP Technical Report on Senna. This information was not present originally in the minutes. This clarification has been added to the PDF version of the minutes. [July 19, 2012]

III. Peer Review of Draft NTP Technical Reports

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

IV. Draft NTP Technical Report on the Toxicology Study of Senna in C57BL/6NTAC Mice and Toxicology and Carcinogenesis Study of Senna in Genetically Modified C3B6.129F1-Trp53^{tm1Brd} N12 Haploinsufficient Mice (NTP GMM 15)

NTP Study Scientist Dr. Inok Surh briefed the panel on the draft NTP Technical Report on senna. Senna is derived from the leaf and pod of *Senna alexandrina* P. Mill, and is marketed as a stimulant laxative and as a flavoring agent. Senna was nominated by the FDA due to its wide use, the positive genotoxicity *in vitro* of its components/metabolites, and its unknown carcinogenic potential. At the request of authorities at the FDA Center for Drug Evaluation and Research (CDER) it was decided to pursue senna studies in a p53 heterozygous mouse model, as a rat study was already being conducted by the manufacturer. Dr. Surh outlined the components of senna—dianthrones, anthraquinones, naphthalenes, and flavonoids. She also illustrated the metabolism of sennosides A and B, the main ingredients for laxative action in senna. They are metabolized by the bacteria in the large intestine into rhein anthrone, which is then rapidly oxidized into rhein following absorption.

NTP conducted *in vitro* and *in vivo* genetic toxicity studies, a range-finding study in mice, and a 40-week study in male and female C3B6.129F1-*Trp53*^{tm1Brd} (+/-) haploinsufficient mice. Illustrating the data with micrographs of the affected areas, Dr. Surh reported that there was evidence of dose-dependent epithelial hyperplasia in the colon and cecum of the test animals.

The draft report's conclusions on senna were presented to the panel for its consideration:

- Under the conditions of this 40-week feed study, there was *no evidence of carcinogenic activity* of senna in male or female C3B6.129F1-*Trp53*^{tm1Brd} N12 haploinsufficient mice exposed to 100, 300, 1,000, 3,000, or 10,000 ppm.
- Senna induced epithelial hyperplasia of the large intestine (colon and cecum) in male and female mice.

The first primary reviewer of the senna study was Dr. Klaunig. He had no criticisms of the scientific performance of the study, and felt that the report was well-written. He was particularly pleased with the discussion of the rationale for the construction of the test mice. He would have liked a clarification of the rationale for the 5-week study. He noted the mention of a reduction in heart weight in the 5-week study, but not in the chronic study, and wished to see some comment on that issue. He also wondered why the 5-week study had been conducted on wild-type mice and not the transgenics. He agreed with the report's conclusions.

Dr. Rogers, the second primary reviewer, agreed with Dr. Klaunig about the quality of the study and draft report, but felt that the choice of the p53 haploinsufficient mouse model may have been incorrect. He recommended that in the future if a substance is to be tested that presents with a lower bowel phenotype, models with a pro-inflammatory phenotype should be considered; models that would be prone to developing inflammatory bowel disease or bowel cancer. He said the p53 haploinsufficiency in the chosen model might have actually had a protective effect.

Dr. Barlow, the third primary reviewer, questioned whether the doses were set high enough in the studies to truly test the compound's carcinogenic potential. He also noted the mention in the report of reduction heart weight in the 5-week study, but felt that since it was biologically meaningless, it should be removed from the report, rather than discussed further.

Dr. Miller expressed "a great deal of concern about the choice of the p53 model," in that it is known to be unsusceptible to lower GI tumors, and there are many other choices of models that would have been more susceptible to such tumors. Thus, he said, a mouse was selected for a study of colon cancer that was unlikely to get colon cancer, constituting what he characterized as a "fatal flaw" in terms of valid conclusions. Dr. Bucher replied that the p53 model is one of the most evaluated of mouse models, and is accepted by the FDA for genotoxic and carcinogenic studies. He cited studies involving another laxative using that model as a precedent for the choice of models in the senna studies. Also, he said, the p53 model was not chosen based on a pre-assumption of colon cancer. Dr. Miller asked if any positive controls with known carcinogens had been

employed. Dr. Jef French of the NTP/NIEHS responded that studies in p53 haploinsufficient mouse models had shown induction of colorectal tumors by azoxymethane, which has been described in publication. Other panel members expressed similar concerns about the p53 model and lack of positive controls.

Dr. Surh said the 5-week study had been conducted to set doses for the 40-week study. She added that it had been felt that the 10,000 ppm dose was a sufficient challenge in the mouse model used.

Following additional comments from panel members about the mouse model, the panel proceeded to consider the draft conclusions regarding senna. Dr. Klaunig moved that the conclusions be accepted as written. Dr. Barlow seconded. The panel voted 4 yes, 2 no (0 abstentions) in favor of the motion. Dr. Miller voted against the motion, citing his concerns about the choice of mouse model. Dr. Heiger-Bernays also voted no, concurring with Dr. Miller's concern about the inappropriateness of the model, as well as feeling that the study should have lasted longer.

Following the vote, Dr. Bucher added that NTP had had similar discussions about the mouse model, and had been fairly concerned about the epithelial hyperplasia and the appearance of the colon in the test animals. He asked for the panel's impression of whether further studies of senna would be in the best interest of the NTP. Dr. Rogers endorsed further studies with animals with a pro-inflammatory-bowel phenotype. Dr. Klaunig mentioned that there had been a 2-year rat study of senna, and asked if anyone was familiar with the results. Dr. Surh replied that the rat study had shown no increase in tumors. Further discussion of potential animal models ensued. Dr. Bucher thanked the panel members for their comments.

V. Draft NTP Technical Report on the Toxicology and Carcinogenesis Studies of a Nondecolorized Whole Leaf Extract of *Aloe barbadensis* Miller (Aloe Vera) in F344/N Rats and B6C3F1 Mice (NTP TR 577)

Study Scientist Dr. Mary Boudreau of NCTR briefed the panel on the toxicology and carcinogenesis of drinking water studies on a nondecolorized whole leaf extract of *Aloe barbadensis* Miller in F344/N rats and B6C3F1 mice. She noted that Aloe vera is a frequently used synonym for the plant. Aloe vera was nominated by the National Cancer Institute (NCI) as a widely used cosmetic, dietary supplement, and herbal remedy with potential for widespread dermal and oral human exposure. It is suspected that components in Aloe vera may possess tumor-promoting activities, and there is a lack of toxicity information about the substance. Dermal exposure studies were previously conducted by NTP (NTP TR-553), so the current study examined exposure to Aloe vera via the oral route—it is widely promoted to alleviate constipation, promote digestive health, and reduce the risks of illness.

In the 14-day range-finding and metabolism studies in rats and mice, animals were administered Aloe vera gel extract, a nondecolorized whole leaf extract, and a decolorized whole leaf extract. There were no gross or microscopic treatment-related lesions from the 14-day study. In the 13-week and 2-year studies, animals received a variety of doses of the nondecolorized whole leaf extract. The nondecolorized whole leaf extract was selected for study because it contains all of the Aloe vera whole plant constituents. The nondecolorized whole leaf extract, which is commercially sold to consumers, contains higher concentrations of aloin than decolorized whole leaf extracts where much of the aloin is removed by activated carbon filtration. Decolorized whole leaf extracts are used in many Aloe vera-containing beverages. Aloin is a component suspected to contribute to tumor formation in rats.

Study conclusions included:

- Under the conditions of these 2-year studies, there was *clear evidence of carcinogenic activity* of a nondecolorized whole leaf extract of Aloe vera in male and female F344/N rats based upon increased incidences of adenomas and carcinomas of the large intestine.
- There was *no evidence of carcinogenic activity* in male and female B6C3F₁ mice exposed to 1%, 2%, or 3% (wt/wt) of a nondecolorized whole leaf extract of Aloe vera in drinking water.
- Exposure to a nondecolorized whole leaf extract of Aloe vera resulted in increased incidences of non-neoplastic lesions of the large intestine in male and female rats and mice, the small intestine of male and female rats, the stomach in male and female rats and female mice, the mesenteric lymph nodes in male and female rats and male mice, and the nose in male mice.

Panel members asked several clarification questions. Dr. Anderson asked Dr. Boudreau to describe how the animals' intestines were examined grossly for tumors. Study Pathologist Dr. Paul Mellick described the procedure. Dr. Anderson asked about the issue of multiplicity. Dr. Mellick replied that it was determined and quantified through microscopic examinations. NTP Pathologist Dr. David Malarkey added that different tumor types and hyperplasia in the same animal were quantified.

- A. Special Presentation: : Activation of MAPK, Wnt, and TGF signaling pathways in large intestinal tumors of F344/N rats chronically exposed to Aloe vera non-decolorized whole leaf extract

Dr. Malarkey introduced a special presentation by Dr. Arun Pandiri, who briefed the panel on alterations of the MAPK, Wnt, and TGF signaling pathways in large intestinal tumors of rats in the Aloe vera study. He described several histological similarities between the rat tumors and human sporadic colon cancer, which is the 4th most

commonly diagnosed cancer in the United States, but the 2nd leading cause of cancer-related death. He hypothesized that the large intestinal tumors observed in F344 rats exposed to Aloe vera nondecolorized whole leaf extract have similar genetic alterations as in human sporadic colon cancer. He presented data on mutation analysis of genes, as well as on molecular pathways important in human colon cancer using data from quantitative RT-PCR arrays. The results demonstrated that:

- Aloe vera nondecolorized whole leaf extract-induced colon tumors in F344 rats-
 - Contained point mutations in *Kras* or *Cttnb 1*
 - Appeared not to have *Tp53* mutations
 - Had alterations within Wnt, MAPK, and TGF- β signaling pathways
 - Shared morphological and molecular features with human colon cancer

Dr. Pandiri recommended that future research encompassed by the collaboration between NTP and FDA/NCTR include comparison of the genetic, epigenetic and protein changes in tumors and histologically normal colon tissue adjacent to tumors, as well as subchronic studies of Aloe vera and senna.

Following the morning break, the meeting resumed with Dr. Cullen asking for any oral public comments. The only public commenter was Dr. Wally Winters of ST&T Consultants. Dr. Winters took the opportunity to raise several questions regarding the draft Technical Report. He asked why data on Aloe vera products other than nondecolorized whole leaf extract were included in the report but not reflected in the title. He inquired about the rationale for the decisions regarding the various dosing regimens used in the studies. He wondered how the toxicity seen in the 2-year study related to human use of the product. He asked why dosage information for aloin had not been included in the 2-year study. He was concerned about the lack of discussion in the draft report about the other Aloe vera products in terms of their safety relative to the nondecolorized whole leaf extract. He mentioned that the Aloe vera gel and latex are discussed as components in the 2-year study results that may propagate the toxicity of nondecolorized whole leaf extract, but that the past literature indicates that there is negligible toxicity in doses comparable to human use. He expressed curiosity about plans for future research, as it might cover some of the limitations of the current study, such as how the study answered the NCI concerns about Aloe vera products. Lastly, he wondered if NTP had been sensitive to the Aloe Industry's concern that the negative report about the nondecolorized whole leaf product would adversely affect consumers who are unaware of the difference between that product and the Aloe vera gel and decolorized products available on the market.

Dr. Birt was the first primary reviewer. She felt the report was very well-developed, and had no scientific criticisms. She took some issue with the rationale for some aspects of the study. She said the compounds used should be clearly presented in the report's

abstract, as should the rationale for monitoring the Aloe vera components malic acid and aloin-A. She felt that dermal exposures should have been discussed more in the report, or, if it was mentioned, all of the exposures should be together. She asked what proportion of the animals had their entire intestinal tract collected. She felt that it would have been useful to include in the discussion some speculation about why lesions were seen in rats but not mice, as well as some attention to the industry concerns about the types of formulations used. She said she agreed with the conclusions included in the report.

The second primary reviewer, Dr. Rogers, also agreed with the conclusions, and had three primary points to discuss. He felt that it was entirely appropriate that NTP had looked at whole leaf nondecolorized Aloe vera extract, since that is presumably the starting material in many of the commercial products, and with no regulatory oversight it cannot be assumed that a label claiming a product was made from a certain part of the plant contains only that part. He also felt that while it was appropriate for the public to challenge the scientific rigor of the toxicity claims, it is also appropriate for the scientific community to challenge the scientific rigor of efficacy and benefit claims. He objected to the use of the term “goblet cell hyperplasia,” preferring “mucosal hyperplasia.” He noted that the data showed a non-dose-dependent decrease in liver cancer in the rats, but it was not commented on in the report.

Dr. Miller, the third primary reviewer, offered several specific editorial suggestions. He asked that the report include more information about how the Poly-3 test works. He noted that there was no mention of histopathology of the intestinal tract in the 14-day study, and wondered why it was left out or not looked at. He agreed with the report’s conclusions, and felt that it was very well-written.

Dr. Boudreau responded to several of the reviewers’ specific comments. She noted that the active ingredient in aloe is undefined, and the reason it gives health benefits is unknown. With more than 75 bioactive components in the plant, malic acid and aloin were chosen to monitor dose verification. She explained that the entire GI tract was collected from all of the animals, but that once the dose relationship in the tumors and adenomas was discovered, the pathologists began collecting frozen sections for special studies. She said in the 14-day study, there was no gross indication of any lesions in the intestinal tract, and so there was no further examination. She agreed to include a more detailed description of the Poly-3 test in the report. Dr. Boudreau, Dr. Miller, and Dr. Walker discussed the issue of developing a transparent, easily understandable severity scale to be used in Technical Reports.

The rest of the panel weighed in with several questions and discussion points. Dr. Anderson pointed out that it would have been useful to have more discussion in the report about the various Aloe vera products available on the market, dosages,

formulations, and for what conditions. Dr. Boudreau cautioned that including too much of that information might get into efficacy, which is inappropriate content for a Technical Report. Dr. Howard agreed that such information would be beyond the bounds of a toxicology study, linking into the risk assessment area.

Dr. Heiger-Bernays stressed that the Aloe vera is actually a complex mixture, and that its potency could be affected by diet or other external factors. She wondered whether the gamma irradiation of the test materials could modify their structures. In terms of the laxative effects of Aloe vera and other herbal remedies, she wondered whether specific effects of the agents based on their chemistry and potency were being examined, or non-specific effects. She recommended using a positive control in future studies. She agreed with previous comments that in future studies of this type, model animals known to be susceptible to the tumors in question should be used. Dr. Walker responded that NTP is quite aware of the issues raised by Dr. Heiger-Bernays regarding herbal remedies, botanicals, and mixtures, and chooses what to track in such studies very carefully.

Dr. Rogers moved to accept the study conclusions (see above) as written. Dr. Birt seconded the motion. The panel voted unanimously (6 yes, 0 no, 0 abstentions) in favor of the motion.

VI. Draft NTP Technical Report on the Toxicology and Carcinogenesis Studies of Acrylamide in F344/N Rats and B6C3F1 Mice (NTP TR 575)

Study Scientist Dr. Frederick A. Beland of the NCTR briefed the panel on the toxicology and carcinogenesis drinking water studies of acrylamide in F344/N rats and B6C3F₁ mice. Acrylamide is a high-production chemical used in industrial processes, and is a by-product in cigarette smoke and certain baked and fried starchy foods. Acrylamide was nominated by the FDA's Center for Food Safety and Applied Nutrition (CFSAN) for an in-depth toxicological evaluation by the NTP. Acrylamide carcinogenicity had been studied before in two 2-year drinking water bioassays in F344 rats, but the CFSAN felt that better dose-response data than those previously available are needed to develop risk estimates and inform regulatory decision-making. The mechanism of acrylamide carcinogenicity is unknown. Two-week, 3-month, and 2-year acrylamide studies were conducted in male and female rats and mice. The hypotheses for the studies were that (1) acrylamide is a genotoxic carcinogen as a result of metabolic conversion to glycidamide, which react with DNA, and (2) since the metabolic conversion of acrylamide to glycidamide occurs to a greater extent in mice as compared to rats, mice should be more sensitive than rats to the carcinogenic effects of acrylamide.

The following conclusions were included in the draft Technical Report:

- Male F344 Rats

- *Clear evidence of carcinogenic activity* based on increased incidences of:
 - Malignant mesothelioma of the epididymis and testis
 - Malignant schwannoma of the heart
 - Follicular cell neoplasms (adenoma or carcinoma) of the thyroid gland
- *Were also related to treatment:*
 - Neoplasms (primarily adenoma) of the pancreatic islets
- Female F344 Rats
 - *Clear evidence of carcinogenic activity* based on increased incidences of:
 - Fibroadenoma of the mammary gland
 - Squamous cell neoplasms (primarily papilloma) of the oral cavity (mucosa or tongue)
 - Mesenchymal neoplasms (fibroma, fibrosarcoma, or sarcoma) of the skin
 - Follicular cell neoplasms (adenoma or carcinoma) of the thyroid gland
 - *Were also related to treatment:*
 - Hepatocellular adenoma of the liver
 - Carcinoma of the clitoral gland
 - *May have been related to treatment:*
 - Malignant schwannoma of the heart
- Male B6C3F₁ Mice
 - *Clear evidence of carcinogenic activity* based on increased incidences of:
 - Neoplasms (primarily adenoma) of the harderian gland
 - Alveolar/bronchiolar neoplasms (primarily adenoma) of the lung
 - Squamous cell neoplasms (primarily papilloma) of the forestomach
- Female B6C3F₁ Mice
 - *Clear evidence of carcinogenic activity* based on increased incidences of:
 - Adenoma of the harderian gland
 - Alveolar/bronchiolar adenoma of the lung
 - Adenoacanthoma and adenocarcinoma of the mammary gland
 - Benign granulosa cell neoplasms of the ovary
 - Malignant mesenchymal neoplasms of the skin
 - *Were also related to treatment:*
 - Squamous cell papilloma of the forestomach
- Exposure to acrylamide was associated with increased incidences of:
 - Degeneration of the retina and sciatic nerve in male and female rats

- Forestomach epithelial hyperplasia and cataracts of the eye in male and female mice
- Hematopoietic cell proliferation of the spleen in female rats and male and female mice
- Epithelial hyperplasia of the lung in male mice
- Ovarian cysts in female mice

Dr. Marvin Friedman, a toxicology consultant to SNF, was the first public commenter. He said acrylamide is “very litigious...so being careful with the wording, being careful with the decisions, is enormously important.” He felt that the results from morphological studies by the Pathology Working Group should have been included in the report, particularly the brain cancers. He felt that the historical controls cited in the report were inappropriate. He also took issue with: the absence of a protocol, dietary vitamin E deficiency, the need for statistics on the 3-month staggered start, the need for analysis of the cage bottles, and lack of discussion of the results in the sentinel animals. Ultimately, he said, the report lacked sufficient detail to validate the findings. He also felt that the study should be combined with the ongoing NTP glycidamide study.

Dr. James Coughlin of Coughlin and Associates, a consultant in food and nutritional toxicology in Orange County, CA, was the second public commenter. His comments were offered on behalf of the Grocery Manufacturers Association in Washington, DC. He discussed three main issues. First, he pointed out that the FDA had nominated acrylamide and glycidamide together in 2002, and wondered why the acrylamide report was being released separately. He said it would be difficult to evaluate and peer review the report on one of the compounds without simultaneously doing so on the other, and appealed to the panel to not finalize its findings on acrylamide until the two could be considered together. Regarding the survival rates and early death data, he wondered whether the maximum tolerated dose had been exceeded in the studies. He asked how carefully cause of death analyses had been conducted to lead to the conclusion that tumors were the primary cause of death in both species, and whether it was simply an estimate as opposed to a rigorous analysis. Regarding the report’s conclusion on rat mammary gland fibroadenomas, he questioned the appropriateness of the historical controls used for comparison, and recommended that the more recent database published by NTP be used instead.

The first primary reviewer for acrylamide was Dr. Barlow. He said he thought the report was thorough and that the doses selected were appropriate. He felt that some of the data gaps had not been discussed adequately, leaving out essential information to understand why the study was conducted. He expressed concern that the existence of the previous bioassays on acrylamide may have introduced bias in the current study. He wondered whether the slight increases in mesothelioma in male rats seen at the

highest doses were enough to derive a “clear evidence” call. He noted that the squamous cell papillomas in the male and female mice had been rated differently, although the incidences were similar, and wondered if the call should be changed on one or the other. He asked for a more detailed discussion of historical control data to support the assertion that although it was older data, it was still valid for use. He also asked for more explanation of the staggered start of the study. He questioned the correlation in the report of Leydig cell tumors and mesothelioma, saying he was unaware of such a correlation.

Responding to the Dr. Barlow’s points, Dr. Beland said the mesothelioma response was similar to the past studies, and was “a real response.” He cited the large number of animals involved as the reason for the staggered start. He said he would add information to the rationale for the study in the report.

Dr. Heiger-Bernays was the second primary reviewer. She felt the report needed a more robust explanation of the confidence in the estimations of daily doses. She speculated that more attention may need to be paid in the report to the non-neoplastic lesions that occurred in the studies, to the results from the Neuropathology Working Group, and to the involvement of the hematopoietic system. She said the report should mention the actual absorbed dose, along with the administered dose, since food is one of the major sources of exposure to acrylamide in humans. She pointed out that epigenetic mechanisms may play a role, and recommended that more attention be paid to that question in the glycidamide study.

Dr. Beland replied that water consumption was measured weekly to help determine dose, and that estimations of dose were as good as could be done in a chronic bioassay. Addressing the differences between the mice and the rats, he said that the numbers regarding induction of cancer were very similar across the two species. He explained that less attention was paid to the non-neoplastic lesions due to typical NTP treatment, and the fact that there was so much cancer occurring in the studies. He agreed that epigenetic mechanisms were well worth looking at, and pointed out that NCTR has a large group devoted to epigenetic research.

Dr. Birt was the third primary reviewer. She commended Dr. Beland for his responses to the issues that had been raised. She asked that the inadequacy of the previous data stated in the study rationale be elaborated upon and clarified. She also asked for some discussion in the exposure section of occupational exposures to acrylamide. She encouraged inclusion in the discussion section of a table comparing results from these studies with the prior studies.

Further discussion ensued among the panel members regarding some of the details of the report, with comments from Dr. Beland. The panel then considered the draft

report's conclusions. An addition noting that in male rats "squamous cell neoplasms of the oral cavity (mucosa or tongue) may have been related to acrylamide exposure" was proposed and discussed, and Dr. Barlow moved for its adoption. The panel voted 3 in favor and 3 opposed to the motion; as tie-breaker, Dr. Cullen voted against it, and the motion was defeated.

Dr. Rogers moved that the conclusion regarding male rats be accepted as written. Dr. Heiger-Bernays seconded. The motion carried unanimously (6 yes, 0 no, 0 abstentions).

Dr. Barlow moved that the conclusion regarding female rats be accepted as written. Dr. Birt seconded. The motion carried unanimously (6 yes, 0 no, 0 abstentions).

Dr. Rogers moved that the conclusion regarding male mice be accepted as written. Dr. Heiger-Bernays seconded. The motion carried unanimously (6 yes, 0 no, 0 abstentions).

Dr. Heiger-Bernays moved that the conclusion regarding female mice be accepted as written. Dr. Birt seconded. The motion carried unanimously (6 yes, 0 no, 0 abstentions).

Dr. Rogers moved that the conclusion regarding non-neoplastic phenomena be accepted as written. Dr. Birt seconded. The motion carried unanimously (6 yes, 0 no, 0 abstentions).

VII. Draft NTP Technical Report on the Toxicology and Carcinogenesis Studies of Mixtures of 3'-Azido-3'-deoxythymidine (AZT), Lamivudine (3TC), Nevirapine (NVP), and Nelfinavir Mesylate (NFV) in B6C3F1 Mice (Transplacental Exposure Studies) (NTP TR 569)

Dr. Cullen stepped down as chair for this review; Dr. Birt assumed the chair.

Dr. Beland briefed the panel on the transplacental exposure studies of AIDS therapeutics. He noted that 40 million adults are infected with HIV worldwide, and 50% of them are women of childbearing age. In the absence of medical intervention, 25% of children born to HIV-positive women will become infected with the virus. Each year, 600,000 babies are infected with HIV due to mother-to-child transmission. Increasingly, multidrug antiretroviral regimens are being used by HIV-positive pregnant women, and although AZT is a known transplacental carcinogen in mice, there are limited data regarding the safety during pregnancy of other antiretroviral drugs or combinations. Thus, determining the long-term consequences of antiretroviral agents in non-infected children is important.

The goal of the studies was to determine the carcinogenicity of combinations of antiretroviral drugs in male and female B6C3F₁ mouse pups exposed transplacentally and monitored for two years.

The draft study conclusions were:

AZT

Under the conditions of this transplacental exposure study, there was *no evidence of carcinogenic activity* of AZT in male B6C3F₁ mice whose mothers were exposed to 80, 160, or 240 mg/kg by gavage. There was *equivocal evidence of carcinogenic activity* of AZT in female B6C3F₁ mice based on increased incidences of thyroidal gland neoplasms (primarily adenoma) and subcutaneous skin fibrosarcoma or sarcoma.

AZT and 3TC

Under the conditions of this transplacental exposure study, there was *no evidence of carcinogenic activity* of mixtures of AZT and 3TC in male B6C3F₁ mice whose mothers were exposed to 80/40, 160/80, or 240/120 mg/kg by gavage. There was *equivocal evidence of carcinogenic activity* of mixtures of AZT and 3TC in female mice based on increased incidences of lung alveolar/bronchiolar adenomas.

AZT, 3TC, and NVP

Under the conditions of this transplacental exposure study, there was *some evidence of carcinogenic activity* of mixtures of AZT, 3TC, and NVP in male B6C3F₁ mice whose mothers were exposed to these chemicals by gavage based on increased incidences of subcutaneous skin neoplasms (fibroma, fibrous histiocytoma, or fibrosarcoma). There was *equivocal evidence of carcinogenic activity* of mixtures of AZT, 3TC, and NVP in female B6C3F₁ mice based on an increased incidence of subcutaneous skin fibrosarcoma.

AZT, 3TC, and NFV

Under the conditions of this transplacental exposure study, there was *no evidence of carcinogenic activity* of mixtures of AZT, 3TC, and NFV in male or female B6C3F₁ mice whose mothers were exposed to 80/40/336, 160/80/672, or 240/120/1,008 mg/kg by gavage.

Dr. Miller was the first primary reviewer of the study. He said he found the report to be very well-written, but asked for pictures to accompany the pathology information. He had several specific editorial comments and questions for Dr. Beland. His first comment was that on page 79, the report stated that “With the exception of female mice treated with the high-dose combination of AZT/3TC/NVP, all changes in body weight were considered to have little biological importance”. He stated it was not clear what criteria were used to discriminate findings that were considered biologically relevant, as both males and females treated with the combination of AZT/3TC/NVP had decreases in body weight of 18% in the high dose groups.

He asked that a primer on statistical methods used be included in the report, particularly the Poly-3 analysis and in terms of how various elements were weighted. He noted that in the AZT and 3TC regimens, there were some tumors not seen in the triple combinations, implying that there was some level of tumor suppression occurring, and noted that there should have been some elaboration on that element, potentially from the literature. Dr. Beland responded to some of Dr. Miller’s specific suggestions, including that the page 79 statement was an error, as both male and female high dose body weight changes were significant and that will be corrected in the report. He also noted, for example, that the Poly-3 analysis is in fact survival-adjusted and corrects for animals that die early. Dr. Bucher provided more details about the Poly-3 test. Based on the evidence of increased incidence of Harderian gland neoplasms in the male mice, Dr. Miller speculated that it may be appropriate to change the call from *no evidence* to *equivocal evidence*.

Dr. Barlow was the second primary reviewer. He agreed that further discussion was called for regarding the Harderian gland data. He said he felt that the study did not mimic what was happening in the real world, where exposures continue after birth, and was concerned that effects may have been missed by not dosing the pups long enough. He was also concerned about the lack of clear evidence of carcinogenesis shown for AZT, as had been previously established in other studies—in this study, it was listed as *equivocal*. With that in mind, with AZT as basically a “quasi-positive control,” he questioned whether the study was valid at all, or whether at least it would have been more appropriate to compare results to the control group itself exclusively. Dr. Beland said there is a study in progress carrying the exposures out to 8 days after birth. He said the positive AZT studies had been conducted in CD1 mice, which he felt were more responsive than the B6C3F₁ model.

The third primary reviewer, Dr. Anderson, said she was looking forward to seeing the results of the neo-natal mouse studies, and felt that the CD1 mouse was probably a better model to use in this type of bioassay. She expressed concern that the “some”

call in the draft report on the AZT+3TC+NVP combination may need to be upgraded to “clear” evidence, because there was clear dose response, as well as several other reasons. Dr. Beland and Dr. Walker responded, elaborating on the rationale for the “some” call. Dr. Anderson agreed that there was “enough fuzziness here to stay with ‘some.’”

Dr. Rogers felt that the impact of body size on tumor risk should be addressed in the report. He also cautioned against drawing too much comparison with previous studies in CD1 mice, in that the ADME was different in those animals, as was the genotype of the pups. Dr. Anderson felt that the B6C3F₁ model was not sensitive enough, and recommended that NTP consider switching to another genetic model. Dr. Beland acknowledged that there probably would have been a better response if the study had used CD1 mice, but stopped short of recommending a switch. Dr. Bucher said NTP had had meetings to discuss the strains used in its bioassays, and that despite its drawbacks the B6C3F₁ model was still considered to be “the mouse of choice.” The panel further debated the issue of which mouse model was most appropriate.

Turning to the draft report’s conclusions:

Dr. Miller moved that the conclusions on AZT be accepted as written. Dr. Rogers seconded. The panel voted unanimously in favor of the motion (5 yes, 0 no, 0 abstentions).

Dr. Miller moved that the conclusions on AZT and 3TC be accepted as written. Dr. Anderson seconded. The panel voted unanimously in favor of the motion (5 yes, 0 no, 0 abstentions).

Dr. Rogers moved that in all of the conclusions, the word “mothers” be replaced with the word “dams.” The motion was adopted by consensus.

Dr. Miller moved that the conclusions on AZT, 3TC and NVP be accepted as written. Dr. Barlow seconded. The panel voted unanimously in favor of the motion (5 yes, 0 no, 0 abstentions).

Regarding the conclusions on AZT, 3TC and NFV, Dr. Miller moved that the call be changed to “equivocal” in the male mice. Thus the overall call would change from “no evidence” to “equivocal evidence” under the proposed change. Dr. Walker pointed out that the change would actually be split according to the genders, as in the AZT conclusions. He also elaborated on why that call had been made for the combination including NFV. Dr. Birt called for a second of Dr. Miller’s motion, which Dr. Barlow provided. Dr. Rogers suggested voting first on the amended language. The vote was taken, and there were two panel members in favor and two opposed to the motion. Dr. Birt as chair broke the tie, voting against the motion, which as a result failed.

Dr. Rogers moved to accept the language as written. Dr. Anderson seconded. There were two votes in favor, two opposed, and Dr. Birt as chair voted in favor. Thus the motion carried. Dr. Heiger-Bernays abstained from both votes, explaining that she did not feel qualified to weigh in on those particular issues.

Drs. Walker, Bucher, and Woychik closed the meeting with brief remarks and their thanks to the panel members for their hard work.

Summary Minutes – April 5, 2011
NTP Technical Reports Peer Review Panel Meeting

These summary minutes have been read and approved by the Chair(s) of the April 5, 2011, National Toxicology Program Technical Reports Peer Review Panel.

Dr. John Cullen
Chair, NTP Technical Reports Peer Review Panel, Sections I – VI

Date: _____

Dr. Diane Birt
Chair, NTP Technical Reports Peer Review Panel, Section VII

Date: _____