

National Toxicology Program
Technical Reports Peer Review Panel Meeting

January 26, 2011

National Institute of Environmental Health Sciences

Research Triangle Park, NC

Summary Minutes

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I. Attendees

Members in Attendance:

Norman Barlow, Sanofi-Aventis
Diane Birt, Iowa State University
Russell Cattley, Amgen
David Dorman, North Carolina State University
James Klaunig, Indiana University
Mark Miller, Wake Forest University School of Medicine
Raymond Novak, Shriners Hospital for Children International (Chair)
Jerry Rice, Georgetown University Medical Center
Arlin Rogers, University of North Carolina at Chapel Hill (UNC)
Robert Smart, North Carolina State University
Dennis Wilson, University of California

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
Robert Paul Felton, FDA
Jonathan Gorin, US Environmental Protection Agency (US EPA)
Paul Howard, FDA
Marian Olsen, US EPA
Greg Olson, FDA
Mark Toraason, NIOSH

National Institute of Environmental Health Sciences (NIEHS) Staff:

| | | | |
|--------------------|-------------------|---------------------|------------------|
| Charles Alden | Robbin Guy | Cynthia Rider | Christopher Weis |
| Danica Andrews | Ronald Herbert | Joseph Roycroft | Lori White |
| Mamta Behl | Mark Hoenerhoff | William Schrader | Kristine Witt |
| Chad Blystone | Grace Kissling | Michael Shelby | Mary Wolfe |
| Amy Brix | Steven Kleeberger | Robert Sills | |
| John Bucher | Ruth Lunn | Cynthia Smith | |
| Po Chan | Robin Mackar | Matthew Stout | |
| Rajendra Chhabra | David Malarkey | Raymond Tice | |
| Bradley Collins | Scott Masten | Eric Tocar | |
| Michael Cunningham | Barry McIntyre | Molly Vallant | |
| Susan Elmore | Abraham Nyska | Michael Waalkes | |
| Paul Foster | Arun Pandiri | Suramya Waidyanatha | |
| John French | Deepa Rao | Nigel Walker | |

Public Attendees

John Bailey, Personal Care Products Council
Mary Draves, The Dow Chemical Company
Jerald Fagliano, New Jersey Department of Health and Senior Services
Dan Fagin, New York University
Robert Fensterheim, RegNet Environmental Services
Paul Forbes, Toxarus, Inc.
Linda Gillick (by phone), Citizens Action Committee on Childhood Cancer Cluster
Michael Ginevan, Mr. Ginevan & Associates
Joe Haseman, JK Haseman Consulting
Crystal Johnson, Charles River Laboratories
David Kistner (by phone), URS Corporation
Olga Naidenko, Environmental Working Group
Gerry O'Sullivan, University of Minnesota
Stephen Ross (by phone), Ross Public Affairs Group
Craig Rowlands, The Dow Chemical Company
James Swenberg, University of North Carolina at Chapel Hill
Chengguo Xing, University of Minnesota

II. Introductions and Welcome

The National Toxicology Program (NTP) Technical Reports Peer Review Panel Meeting convened on January 26, 2011, in Rodbell Auditorium, National Institute of Environmental Health Sciences (NIEHS), Research Triangle Park, North Carolina. Dr. Raymond Novak served as chair, welcomed everyone to the meeting, and asked all attendees to introduce themselves. The other Peer Review Panel members present were Drs. Norman Barlow, Diane Birt, Russell Cattley, David Dorman, James Klaunig, Mark Miller, Jerry Rice, Arlin Rogers, Robert Smart, and Dennis Wilson. Dr. Mitzi Nagarkatti attended the meeting as a liaison to the NTP Board of Scientific Counselors. Dr. Linda Birnbaum, Director of the NIEHS and NTP, and NTP Associate Director Dr. John Bucher also welcomed attendees. Dr. Lori White, Designated Federal Officer for the meeting, read the conflict of interest policy statement and stated that Drs. Klaunig and Novak would not participate in the discussion or voting on the styrene-acrylonitrile trimer report due to conflicts of interest.

III. Toxicology and Carcinogenesis Studies of Kava Kava Extract (TR 571)

Dr. Rajendra Chhabra, NIEHS/NTP, briefed the panel on the toxicology and carcinogenicity studies of kava kava extract, a leading dietary supplement with rapidly growing use in the United States market. Kava kava extract was nominated by the National Cancer Institute, based on widespread exposure, reports of hepatotoxicity in humans, increasing concern about its use by the US Food and Drug Administration and

the World Health Organization, and a lack of toxicity and carcinogenicity data. Two-week, three-month, and two-year gavage studies were conducted in male and female F344/N rats and B6C3F1 mice. The proposed conclusions of the studies were:

Under the conditions of these 2-year gavage studies, there was *equivocal evidence of carcinogenic activity* of kava kava extract in male F344/N rats based on marginal increases in the incidences of testicular adenoma. There was *no evidence of carcinogenic activity* of kava kava extract in female F344/N rats administered 0.1, 0.3, or 1.0 g/kg. There was *clear evidence of carcinogenic activity* of kava kava extract in male B6C3F1 mice based on increased incidences of hepatoblastoma and hepatocellular carcinoma or hepatoblastoma (combined). There was *some evidence of carcinogenic activity* of kava kava extract in female B6C3F1 mice based on increased incidences of hepatocellular adenoma or carcinoma (combined).

Kava kava extract administration was associated with the occurrence of nonneoplastic lesions in the liver, forestomach, kidney, eye, and pancreas of male and female rats, liver of male and female mice, and forestomach of female mice.

Dr. Birt, first primary reviewer, said she found the report to be well done and appreciated the opportunity to learn more about botanicals. She found the design of the experiments to be fully appropriate. She suggested several minor clarifications regarding statistical significance in the survival data; the relationship between the kavalactone component of dried kava kava and the lipid-soluble resin; the concentration of kava kava used in the studies; and inclusion of the genus, species, variety and accession information in the draft Technical Report, which Dr. Chhabra agreed to do.

Dr. Miller, second primary reviewer, inquired about the dosages used in the studies and their proximity to actual typical exposures. In addition, the percentage of the population that was exposed to kava kava extract and exhibited liver damage as a result should be reported, as well as any potential threshold dose at which liver damage could be anticipated. He noted the mention in the report about sedative effects during the early phases of the study that resolved upon extended use, and wondered if that might be expanded upon. Regarding the tables using a grading system for lesions, he suggested using figures to provide more transparency for the data. He provided other minor editorial suggestions.

Dr. Rice, third primary reviewer, concurred with prior comments that this was a standard bioassay that had been well conducted. He asked for clarification about how close to the actual “article of commerce” the studied extract was in terms of concentration of kavalactones, the pharmacologically active ingredient.

Dr. Chhabra said in toxicity and carcinogenicity studies, it is typical to use doses several times the normal human dosage in order to engender toxic or carcinogenic effects, if

any, while in safety studies it is more typical to dose at realistic exposure levels. He said there is very little information about human exposures to herbal products, given the fact that there has been little regulation. Dr. Howard, FDA, said that prior to 2004 or 2005, the agency had limited authority over dietary supplements. He added that FDA does take authority over a product and take action if clinical evidence of toxicity is found. Responding to Dr. Miller, Dr. Chhabra said there would be elaboration in the report about the sedative effects. He added that the NTP would look at a good way to provide more data on the grading system used in this and other studies. Dr. Walker said that individual animal severity data is typically not reported, but could be added on the website, and that would be considered for this and other reports. Per Dr. Rice's comments, Dr. Chhabra said the kava kava extract used in the current studies was comparable in its contents of kavalactones available to that from three to four different vendors.

Dr. Klaunig asked about the designation of *clear evidence* in the male mice versus *some evidence* in the females—whether it was the presence of hepatoblastomas in the males. Dr. Chhabra confirmed that conclusion.

Dr. Nagarkatti echoed an earlier comment by Dr. Birt that it would be important in any report on an extract to provide detailed information about the when and where the plant was harvested. Dr. Chhabra said the NTP tries to get as much of that type of information as possible. Dr. Walker added that such characterization information is not always easy to get, particularly with commercial materials. Dr. Cattley asked about prolactin levels. Dr. Chhabra explained that, after every Technical Report, staff members hold a meeting to discuss the potential needs for follow-up studies, and that the prolactin question may be appropriate for further characterization.

Dr. Barlow said that due to the earlier onset dates in the evidence of interstitial cell/Leydig cell adenomas in male rats, a change from *equivocal evidence* to *some evidence of carcinogenic activity* should be considered. Dr. Chhabra disagreed with that suggestion. Dr. Barlow also questioned the use of “combined” terminology in the report, and Dr. Chhabra replied that that question could be addressed when the study conclusions were considered.

Dr. Birt moved to accept the conclusions as written. Dr. Miller seconded the motion.

Dr. Cattley disagreed with the language in the conclusion regarding *clear evidence* based on increased incidence of carcinoma or hepatoblastoma, because that number was largely driven by the hepatoblastoma data. He recommended removing the language about the combined incidence and moved to amend the wording of the conclusion to state:

There was *clear evidence of carcinogenic activity* of kava kava extract in male B6C3F1 mice based on increased incidences of hepatoblastoma.

Dr. Klaunig seconded the motion and the panel voted unanimously (10 yes, 0 no, 0 abstentions) in favor of the motion to accept the revised conclusion for male mice and accept the other conclusions as written.

IV. Photocarcinogenesis Study of Retinoic Acid and Retinyl Palmitate (TR 568)

Dr. Mary Boudreau, FDA National Center for Toxicological Research (NCTR)/NTP, briefed the panel on the photocarcinogenicity study of retinoic acid (RA) and retinyl palmitate (RP). RP was nominated to the NTP for phototoxicity and photocarcinogenicity testing by the Center for Food Safety and Applied Nutrition (CFSAN) within the FDA, based upon widespread use of the compound in cosmetic retail products applied to sun-exposed skin and an association between topical application of retinoids and enhanced photocarcinogenesis. The objective of the 1-year photocarcinogenesis study was to determine whether the topical application of creams containing RA or RP would alter the process of photocarcinogenesis in SKH-1 mice exposed to simulated solar light (SSL), UVA, or UVB. The proposed conclusions were:

Control cream

Under the conditions of these studies, the topical treatment of SKH-1 mice with the control cream resulted in earlier onsets of in-life skin lesions and higher incidences and multiplicities of in-life skin lesions in the absence and presence of SSL or UVA, and higher incidences and multiplicities of squamous cell neoplasms when compared to untreated controls in the absence and presence of SSL.

Retinoic acid

Compared to the control cream, retinoic acid enhanced the photocarcinogenic activity of SSL and UVB in SKH-1 mice based upon earlier onsets and increased multiplicities of in-life skin lesions.

Retinyl palmitate

Compared to the control cream, retinyl palmitate enhanced the photocarcinogenicity activity of SSL and UVB in SKH-1 mice based upon earlier onsets and increased multiplicities of in-life skin lesions and increased incidences and multiplicities of squamous cell neoplasms.

Oral public comments were provided from the Environmental Working Group (EWG) and the Personal Care Products Council (PCPC).

Dr. Olga Naidenko, Senior Scientist at EWG, said the EWG strongly supported this “meticulous study,” which represents the culmination of a 10-year research program on RP begun at NCTR. Her comments covered three major points: the experimental protocol for the study was appropriately chosen, the lines of evidence all point to the

photocarcinogenic effect of RP in combination with sunlight, and the findings of the NTP study are in agreement with the research database on the phototoxicity and photocarcinogenicity of retinoid compounds. She concluded by stating that EWG considered the study, despite its limitations, both “clean, and very informative for public health.”

Dr. John Bailey, Executive Vice President for Science at PCPC, said PCPC was concerned about the use of this NTP study for risk management and risk assessment. Dr. Michael Ginevan, an independent consultant hired by PCPC to analyze the study and its results, said the group was pleased by the well-defined charge to the NTP panel. He expressed concern about the long lag time between the nomination and the report (11 years), and about the reasons listed for removing animals from the study, in that they may have skewed the results, leading to incorrect statistical analysis of outcomes. Another major concern was that there was no way to estimate the effects of RP independent from those of the control cream, which was in itself “a potent carcinogen.” He delineated several other concerns regarding the study’s methodology. Ultimately, he concluded, it was an “inadequate study of carcinogenic activity.”

Dr. Rice, first primary reviewer, noted that this study was obviously different from the “classic NTP bioassays.” He felt that ideally the control cream should have had no effect on the latency, incidence, and multiplicity of skin lesions. He was concerned about the effects of animals scratching themselves as a result of irritation from high doses of retinoids, which he noted by itself could be a co-carcinogenic stimulus. He added several editorial comments. He was concerned about the conclusion dealing with RA, in that the conclusion of photocarcinogenicity was not sufficiently supported by the data.

The other reviewers, Drs. Cattley, Klaunig, and Smart, all concurred with Dr. Rice’s statements. Each added specific editorial comments as well.

Responding to the reviewers’ comments, Dr. Boudreau noted that in studies such as these, the control cream must be customized to the compound being tested. Each control cream is formulated specifically to blend with the test article. She said the ingredients of the control cream are “quite generic.” Diisopropyl adipate, a common ingredient in cosmetics, was used as a carrier for the RA and RP in order to incorporate them into the control cream. She added that in most photocarcinogenesis protocols, there are three experimental groups: an untreated control group exposed to SSL only, a group exposed to the control cream and SSL, and the treated group. The control cream is compared to the untreated group at the same level of SSL to determine the effect of the control cream relative to SSL alone, and the treatment groups are compared to the control cream at the same level of SSL to determine the effect of treatment above that of the control cream. This protocol design allows for parsing out the specific effects of

the test articles. She said that the control cream was not irritating, and that no episodes of scratching were seen in the control cream-only group; scratching was seen only with the higher doses of RA and RP. Animals were removed according to specific guidelines regarding skin lesions and skin condition. Similar photocarcinogenesis protocols are used by industry; however histopathology is not typically conducted. That was the practice used to determine the effects of RA, because it would not be possible to discern whether the lesions were due to effects of radiation or because the skin was compromised.

Dr. Birt expressed concern that the report should be clearer that the cream being used is relevant to skin care products and enhances skin cancer, perhaps by adding language addressing that issue to the title of the report. Dr. Walker pointed out that such language was in the conclusions. Dr. Miller agreed with Dr. Birt that the effects of the control cream should be more prominently featured, perhaps in the report's introduction. He asked if there had been any similar previous studies in which a control cream had been used that had no effect. Dr. Boudreau acknowledged that a study of aloe vera used a control cream with no independent effect, and that there were plans to conduct follow-up studies with that type of cream and the retinoids. Dr. Howard said the FDA had reviewed the report and asked NCTR to conduct follow-up studies to clarify the role of the vehicle, seeking to clarify some of the issues that had also been raised by the panel.

The panel reviewed the draft conclusions and Dr. Novak suggested that the conclusions be rewritten to address the concerns raised in the discussion. The NTP rewrote the conclusions for consideration by the panel. The revised conclusions were:

These experiments investigated the effect of topical applications of creams containing RA or RP on the photocarcinogenic activity of SSL in male and female SKH-1 hairless mice. Skin lesions were assessed during the in-life phase and/or by histopathologic evaluation at necropsy.

Control Cream

Under the conditions of these studies, the topical treatment of SKH-1 mice with the control cream resulted in earlier onsets of in-life skin lesions and higher incidences and multiplicities of in-life skin lesions, when compared to untreated controls, in the absence and presence of SSL.

The topical treatment of SKH-1 mice with control cream resulted in higher incidences and multiplicities of squamous cell neoplasms of the skin when compared to untreated controls in the absence and presence of SSL.

Retinoic Acid

Compared to the control cream, RA further enhanced the effects of SSL in SKH-1 mice based upon earlier onsets and increased multiplicities of in-life skin lesions.

Retinyl Palmitate

Compared to the control cream, RP further enhanced the effects of SSL in SKH-1 mice based upon earlier onsets and increased multiplicities of in-life skin lesions.

Compared to the control cream, RP further enhanced the photocarcinogenic activity of SSL in SKH-1 mice based upon increased incidences and multiplicities of squamous cell neoplasms of the skin.

Dr. Rice moved that the conclusions be accepted as modified. Dr. Klaunig seconded the motion, which passed unanimously (10 yes, 0 no, 0 abstentions).

V. Toxicology and Carcinogenesis Studies of Methyl *trans*-Styryl Ketone (TR 572)

Dr. Michael Cunningham, NIEHS/NTP, briefed the panel on the toxicology and carcinogenesis feed and dermal studies of methyl *trans*-styryl ketone (MSK) in F344/N rats and B6C3F1 mice. MSK was nominated by the National Cancer Institute as a member of the structural class of α,β -unsaturated ketones, with human exposure occurring as a result of its use as synthetic flavoring and fragrance agents. Ninety-day feed and dermal studies were conducted in male and female rats and mice; two-year dermal studies were conducted in male and female rats and mice. The draft conclusions were:

Under the conditions of these 2-year dermal studies, there was *no evidence of carcinogenic activity* of methyl *trans*-styryl ketone in male or female F344/N rats or in male or female B6C3F1 mice administered 10, 30, or 90 mg/kg.

Administration of methyl *trans*-styryl ketone resulted in nonneoplastic lesions of the skin at the site of application in male and female rats and mice.

Dr. Smart, first primary reviewer, said that the report was well written and that the narrative accurately described the data presented. He made some minor editorial suggestions. He wondered whether ulceration should have been included as a clinical finding.

Dr. Wilson, second primary reviewer, agreed that it was “a nicely-done” study, and particularly liked the inclusion of the toxicokinetic study. He also had minor suggestions, including clarification of the dermal application process and additional discussion of nasal lesions.

Dr. Rogers, third primary reviewer, also felt that the report was well presented and that the conclusions were straightforward. He suggested more detail regarding references to non-neoplastic lesions in this and other NTP reports. He also expressed concern about the high background incidence of liver tumors in the B6C3F1 mice, with apparent increases recently in the strain.

Dr. Cunningham said that the materials and methods section of the report would be improved to better describe the technical aspects of how the dermal studies were conducted. Study pathologist Dr. Mark Cesta explained that the fungal infections in the nose were considered to be secondary to the nasal epithelial damage. Dr. Bucher mentioned that the program was aware of the liver tumor incidence in the mouse strain. Dr. Rice moved to accept the conclusions as written. Dr. Smart seconded the motion, which passed unanimously (10 yes, 0 no, 0 abstentions).

VI. Toxicology and Carcinogenesis Study of Styrene-Acrylonitrile Trimer (TR 573)

Dr. Birt chaired this portion of the meeting and Drs. Klaunig and Novak did not participate in this discussion or vote. Dr. Smart departed for the rest of the meeting.

Dr. Chhabra, Study Co-Scientist, briefed the panel on the perinatal and postnatal feed studies examining toxicology and carcinogenesis of styrene-acrylonitrile trimer (SAN Trimer) in F344/N rats. SAN Trimer is a by-product of the production of acrylonitrile styrene plastics, and is created in specific manufacturing processes for polymers of acrylonitrile and styrene. It was nominated for study by an Interagency SAN Trimer Workgroup established by the EPA due to reports by the New Jersey Department of Health and Senior Services that childhood cancer incidence was greater than expected between 1979 and 1995 in the vicinity of two Superfund sites in Toms River, NJ, where SAN Trimer was eventually identified to be a contaminant in the sites' groundwater plumes. NIEHS/NTP became a member of the Workgroup and worked with it during the course of the studies. The Workgroup led the preparation of the introduction section of the draft report, but the NTP was responsible for the conduct, interpretation and conclusions of the studies. The draft study conclusions were:

Under the conditions of this 2-year feed study preceded by perinatal exposure, there was *equivocal evidence of carcinogenic activity* of SAN Trimer in male F344/N rats based on the occurrence of astrocytomas and granular cell tumors in the brain and spinal cord. There was *no evidence of carcinogenic activity* of SAN Trimer in female F344/N rats given feed containing 400, 800, or 1,600 ppm SAN Trimer preceded by perinatal exposure.

Peripheral nerve degeneration and nonneoplastic lesions of the bone marrow and liver in male and female F344/N rats and urinary bladder lesions in female F344/N rats were attributed to exposure to SAN Trimer.

The incidences of pituitary gland adenoma and mononuclear cell leukemia in male and female F344/N rats and mammary gland fibroadenoma in female F344/N rats were decreased.

Oral public comments were provided by five attendees.

On the telephone, Dr. David Kistner, a project manager at URS Corporation, worked as a consultant to Union Carbide Corporation since 2002, at the Reich Farm, NJ Superfund site in conjunction with the US EPA. He questioned the NTP report's study of acrylonitrile, citing water-sampling data from the Reich Farm site from 1991 to 2001 showing no recorded detections of acrylonitrile which exceeded the technical quantitation limit of 2 milligrams per liter or 2 parts per billion. He requested reducing the amount of discussion on acrylonitrile in the draft report because he did not consider it relevant to the results of the study.

Ms. Linda Gilick, chair of the Citizens Action Committee on Childhood Cancer Cluster, provided comments by telephone. She expressed concern about the use of Batch 3 SAN Trimer (as provided by Union Carbide Corporation) in the tests, as opposed to Batch 1 or 2. She was concerned that true answers would not emerge from the study, and wondered whether further testing would occur, perhaps on other materials such as the dimer form of the compound.

Dr. James Swenberg, University of North Carolina at Chapel Hill, said he had been asked by the SAN Trimer Association (SANTA) to conduct a peer review of the brain and spinal cord and sciatic nerve lesions at the EPA, which he had done ten days previous with the assistance of staff at the NTP Archives, who prepared the appropriate slides for his review. He shared his blinded results with the panel, beginning with his evaluation of spinal cord and sciatic nerve degeneration. He agreed with the NTP discussion about the increase of incidence and severity of those degenerative lesions. In terms of his review of the data for brain and spinal cord neoplasms, Dr. Swenberg felt the conclusion for male rats should be *no evidence* instead of *equivocal evidence*. He also presented several specific editorial suggestions. He clarified that he was not speaking on behalf of SANTA, but had been asked by SANTA to review the data.

Dr. J. Craig Rowlands, Dow Chemical Company, said Dow felt the NTP cancer bioassay provided no evidence of carcinogenicity for SAN Trimer based on (1) no statistically significant increase in CNS tumors, (2) the incidence of CNS tumors was consistent with the background incidence in rats, and (3) increased incidence in the mid- and high-dose groups may have been due to increased survival. He recommended the conclusion should be that there is *no evidence of carcinogenetic activity*. He noted that past research had shown that SAN Trimer is not genotoxic or mutagenic. After discussing several other aspects of the study, he reiterated his opinion that SAN Trimer is not a carcinogen.

Dr. Joseph Haseman, representing the SAN Trimer Association, asked the panel two questions: "Should the reported brain/spinal cord tumors from the SAN Trimer study be judged differently than similar incidence patterns seen in previous NTP studies which concluded "no evidence of carcinogenic activity"? If SAN Trimer is responsible for a

marginal increase in brain/spinal cord tumors, then wouldn't one expect the extended histopathology review that was conducted in this case to find additional tumors in the mid or high dose groups?" Based upon his review of past similar NTP studies and the "clean" extended histopathology review in this case, he suggested that the conclusion should be *no evidence of carcinogenic activity* in male rats.

Dr. Cattley, first primary reviewer, said he found the study itself to be straightforward, but that some interpretation still needed additional consideration, particularly the use of severity grades in interpreting nerve degradation. He was concerned about the fact that the lesions described occur commonly in aging rats, and that there was no inclusion of historical control data on incidence of brain and spinal cord tumors, which would be key to determining whether the incidence of tumors in this study is or is not related to exposure to SAN Trimer. He added several editorial and methodological comments.

Dr. Barlow, second primary reviewer, said he felt that the severity scale used to assess the non-neoplastic lesions should be clarified further. Regarding the brain and spinal cord neoplasias, overall he felt that *equivocal evidence* was "too strong a call" for those neoplasms. He felt that the animal numbers in the study should have been increased, thus increasing statistical power, by not culling pups, as was done.

Dr. Miller, third primary reviewer, agreed that the brain tumor data presented was difficult to interpret. He concurred with Dr. Barlow's comment about a need for more animal numbers, and wondered why a second animal model had not been considered. He suggested transgenic models that would render the rats more sensitive to brain tumors, orthotopic tumor models in which the chemical could demonstrate increases in the growth or malignant characteristics of tumors, or murine models that would be able to detect potential effects of chemicals on blood cells. Dr. Miller suggested using figures to provide more transparency for the data and agreed with Dr. Cattley's call for more data regarding historical control rats.

Dr. Dorman, the fourth primary reviewer, shared Dr. Barlow's concern about the culling of pups. He wondered why the animals' diet had been changed in the middle of the study. He echoed previous remarks about the lack of historical control data, especially given the non-robust response. He called for the NTP to include more information about non-cancerous endpoints in its description, as many of these documents become critical for toxicologists, since they represent studies on materials that are unlikely to be repeated.

Dr. Deepa Rao, NIEHS/NTP pathologist, presented the findings of her blinded pathology review for peripheral nerve degeneration to the panel. She described her method of review, which was slightly different from the one employed by Dr. Swenberg

in his blinded review. She reported that statistical analysis had shown an increase in severity in the high-dose treated group compared to controls.

Panel members discussed Dr. Rao's review and several details contained in the draft Technical Report. Regarding the issue of changing diets in the study from NIH-07 to NTP-2000, Dr. Chhabra explained that the NIH-07 diet, which is higher in protein, was used for the pregnant and lactating animals in the study. The NTP-2000 diet was used for maintenance of the animals; the rationale for switching diets would be added to the report.

Dr. Rice said he had found the arguments that the proper call on brain tumors should be *no evidence*, rather than *equivocal evidence*, to be compelling.

Drs. Chhabra, Kissling, Walker, and Malarkey expressed the NTP's rationale for considering the glial and granular cell tumor responses as *equivocal evidence*, including evidence that each type was rare and very few occurred spontaneously or with treatment in NTP studies (>25) conducted in the past 5 years. Also, the occurrence is consistent with other NTP studies considered to have *equivocal evidence*.

Dr. Miller suggested that, given the concerns regarding the animal numbers, a conclusion of *inadequate* study could be considered. Dr. Walker said that finding would imply that the study was flawed; the panel agreed that the study was not flawed. After some discussion of this issue among panel members and NTP scientists, Dr. Rice moved for a straw poll to assess the panel's position. The poll showed 1 member in favor of retaining the conclusion of *equivocal evidence*, with 6 in favor of changing it to *no evidence*.

The panel amended the conclusion language to state:

Under the conditions of this 2-year feed study preceded by perinatal exposure, there was *no evidence of carcinogenic activity* of SAN Trimer in male and female F344/N rats given feed containing 400, 800, or 1,600 ppm SAN Trimer.

Nonneoplastic lesions of the peripheral nerve, bone marrow and liver in male and female F344/N rats and urinary bladder in female F344/N rats were more prevalent in the groups exposed to SAN Trimer.

The incidences of pituitary gland adenoma and mononuclear cell leukemia in male and female F344/N rats and mammary gland fibroadenoma in female F344/N rats were decreased.

Dr. Rice moved to approve the conclusions as amended. Dr. Barlow seconded the motion. The vote to approve the amended conclusions was 6 yes, 1 no, 0 abstentions. Dr. Miller voted no, stating he felt that the *equivocal* conclusion was more appropriate, given the evidence.

VII. Toxicology and Carcinogenesis Studies of α,β -Thujone (NTP TR 570)

Dr. Novak resumed chairing the meeting.

Dr. Chad Blystone, NIEHS/NTP, presented the details of the studies on α,β -thujone to the panel. It is a monoterpene found in several plant species. It exists in nature as a mixture of α and β stereoisomeric forms, and is used in herbal medicines, food and beverage flavorings, cosmetic products, and repellents. α -Thujone is the principal component of absinthe and has been identified as a γ -aminobutyric acid receptor antagonist. Thujone was nominated by the National Cancer Institute based on concerns of widespread exposure and lack of toxicity and carcinogenicity data. Two-week, three-month, and two-year toxicity and carcinogenicity studies were conducted in F344/N male and female rats and B6C3F1 male and female mice, as well as single-dose toxicokinetic studies in both genders of both species and genetic toxicology studies. An α,β -thujone mixture was selected for subchronic and chronic testing since it represents a common human exposure. The draft conclusions in the report were:

Under the conditions of these 2-year gavage studies, there was *some evidence of carcinogenic activity* of α,β -thujone in male F344/N rats based on increased incidences of preputial gland neoplasms; increased incidences of benign pheochromocytoma of the adrenal medulla may have been related to administration of α,β -thujone in male F344/N rats administered 12.5 or 25 mg/kg. There was *no evidence of carcinogenic activity* of α,β -thujone in female F344/N rats administered 12.5 or 25 mg/kg. There was *no evidence of carcinogenic activity* of α,β -thujone in male or female B6C3F1 mice administered 3, 6, or 12 mg/kg.

Administration of α,β -thujone for 2 years resulted in increased incidences of seizures in F344/N rats and B6C3F1 mice and increased incidences of nonneoplastic lesions in the brain and spleen of male and female F344/N rats, the kidney of male F344/N rats, and the pituitary gland of female F344/N rats.

Dr. Dorman, first primary reviewer, wondered about the cells of origin of the preputial tumors. He asked about urinary excretion, as related to the possibility that there may be a grooming effect in the animals, resulting in an atypical preputial exposure to the compound. He was concerned about the high level of contamination of the study compound with another compound with unknown toxicological characteristics, and felt that NTP should address that concern in its discussion in the report. He also expressed concern about lack of attention to the difference between nominal exposures and actual exposures in the study, in that the results could be skewed significantly as a result. He felt that there should have been more detail in the report regarding seizures, with a grading system and more information about clinical signs.

Dr. Birt, second primary reviewer, felt the report was clearly presented and easy to read, and that the study had been well designed and carefully conducted. She had no

scientific criticisms, but several suggestions about information that could be added to the report including the nature of the source (synthesized or isolated) of the compounds; data supporting the comments on alterations in diestrus; and inclusion of time on the study with different groups so that the reduced exposure time is reflected with the lesion data.

Dr. Wilson, third primary reviewer, had no arguments with the report's conclusions regarding carcinogenicity. He suggested further discussion about the potential mechanistic connection to 5-HT activity, which was mentioned briefly in the report.

Dr. Malarkey explained that the preputial gland is a modified sebaceous gland with squamous cells lining the ducts and the cell of origin for preputial gland neoplasms is likely a glandular epithelial, squamous, or stem cell. This will be added to the discussion. Regarding Dr. Dorman's question about contamination, Dr. Blystone replied that the bulk of the chemical came from cedar wood, and that in such natural products other chemicals are often present. There was further discussion of Dr. Dorman's question regarding nominal vs. actual dosing, as he recommended that reference to that issue be brought forward into the report's abstract.

Following subsequent discussion about several details concerning the studies' methodologies, the panel considered the draft conclusions. Dr. Dorman moved to accept the conclusions as written. Dr. Birt seconded the motion, which passed with a vote of 7 yes, 1 no, 0 abstentions. Dr. Barlow voted against the motion, suggesting that the passage regarding pheochromocytoma should have been related to the *some evidence* language rather than being characterized as *may have been related*.

Drs. Birnbaum and Bucher thanked the panel for their efforts in the review. Dr. Bucher announced that review of the draft NTP Technical Report on senna would be postponed until the next peer review panel meeting on April 5, 2011.

Dr. Novak adjourned the meeting.