NATIONAL TOXICOLOGY PROGRAM
BOARD OF SCIENTIFIC COUNSELORS
Technical Reports Review Subcommittee

May 16-17, 2007

NIEHS, Research Triangle Park, NC

Summary Minutes

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Attendees

Members:

Christopher Bradfield, University of Wisconsin
Kenny Crump, Environ Corporation
Prescott Deininger, Tulane University
Nancy Kerkvliet, (chair), Oregon State University
Jon Mirsalis, SRI International
Harish Sikka, State University of New York at Buffalo
Keith Soper, Merck Research Laboratories
Vernon Walker, Lovelace Respiratory Institute

Ad hoc Members:

Russell Cattley, Amgen
Raymond Novak, Wayne State University
Michael Pino, Sanofi-Aventis
Tammy Stoker, US EPA

NIEHS Attendees:

Charles Alden
Eddie Ball
Jack Bishop
John Bucher
Tom Burka
Po Chan
Rajendra Chhabra
Brad Collins
Allen Dearry
June Dunnick
Susan Elmore
Gordon Flake
Paul Foster
Jonathan Freedman
John (Jef) French
Sanford Garner
Angela King-Herbert
Ronald Herbert
Michelle Hooth
William Iverson
William Jameson
Marcia Johnston
Grace Kissling
Ruth Lunn
Robin Mackar
David Malarkey
Ronald Melnick
Retha Newbold
Denise Orzech (retired)
Joseph Roycroft
Michael Sanders
Barbara Shane
Michael Shelby
Robert Sills
Cynthia Smith
Matthew Stout
Gregory Travlos
Jacquelyn Tubbs
Nigel Walker
Samuel Wilson
Kristine Witt
Mary Wolfe

Agency Attendees:

Barry Delclos, FDA
Paul Howard, FDA
Mark Toraason, NIOSH

Public Attendees:
Susan Borghoff, ILS
John Butala, American Chemistry Council
Enrique Castro, Tierra Solutions, Inc.
Patrick Crockett, Constella Group
George Cruzan, ToxWorks
Reshan Fernando, RTI
Heather Burleigh-Flayer, PPG Industries
Milton Hejtmancik, Battelle
William Iverson, EPL
Christopher Koivisto, EPL
Keith Levine, RTI international
Thomas McKee, Interfaith Community Organization
Paul Mellick, Toxicological Associates at NCTR
Russell Morgan, Elementis Chromium
John Peckham, Experimental Pathology Laboratories
Deborah Proctor, Exponent
Roland Rossbacher, BASF
Ann Tveit, ISP
Galnelle Willson, GPL

Peer Review Meeting

The meeting began at 8:30 a.m. on May 16, 2007 in the Rodbell Conference Center of the David P. Rall Building, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina. For further information, contact Dr. Barbara Shane, Executive Secretary, at 919-541-4253 or shane@niehs.nih.gov.

Sodium Dichromate Dihydrate
Dr. Michelle Hooth, NIEHS, introduced the toxicology and carcinogenesis studies of sodium dichromate dihydrate by reviewing the uses of chromium, its occurrence in drinking water, the nomination of chromium VI for study by the NTP, and the effects of sodium dichromate in previous short-term studies. She then described the design of the long-term studies and the effects of sodium dichromate on the oral cavity, lymph nodes, and small intestine. Dr. Ronald Herbert, NIEHS, described the histopathologic changes in several of the observed lesions. The proposed conclusions were:

> Under the conditions of these 2-year drinking water studies there was *clear evidence of carcinogenic activity* of sodium dichromate dihydrate in male and female F344/N rats based on increased incidences of squamous cell neoplasms of the oral cavity. There was *clear evidence of carcinogenic activity* of sodium dichromate dihydrate in male and female B6C3F1 mice based on increased incidences of neoplasms of the small intestine (duodenum, jejunum, or ileum).

Exposure to sodium dichromate dihydrate resulted in histiocytic cellular infiltration in the liver, small intestine, and pancreatic and mesenteric lymph nodes of rats and mice, and diffuse epithelial hyperplasia in the small intestine of male and female mice.

Subcommittee discussion

Dr. Walker, the first principal reviewer, agreed with the overall conclusions of the report. He differed with one of the public comments to the report that suggested there should be more discussion of sites with decreased tumor incidence, noting that the primary purpose of the study was to detect carcinogenic responses, and not mechanisms underlying a reduction in cancer incidence. He felt the discussion of the epidemiology studies, some with conflicting results, was balanced. He said it was noteworthy that although chromium was detected at other tissue sites, the main carcinogenic response in mice was in the small intestine, primarily in the duodenum with decreasing incidences farther down the intestinal tract.

Dr. Novak, the second principal reviewer, also agreed with the overall conclusions. He inquired whether any clinical chemistry panel had been performed to detect changes in hormone levels. He asked if the observed histiocytic infiltration could be associated with inflammation.

Dr. Sikka, the third principal reviewer, agreed with the overall conclusions and inquired if there was any explanation for the difference in the organ-specific susceptibility of the two rodent species to the carcinogenic effects of sodium dichromate.

Dr. Hooth noted that a couple of tumors were seen in the ileum of male mice, and Dr. Walker replied that still it was interesting that the numbers of tumors decreased progressively down the intestinal tract. In response to Dr. Novak, Dr. Hooth replied that because this was a study of hexavalent chromium, examination of the effects of trivalent chromium on lipid or carbohydrate metabolism was not performed. Regarding the histiocytic infiltration, Dr. Herbert said there were no granulomas but accumulations of histiocytes. With granulomas, one usually finds a causative agent such as a bacterium, fungus, or necrosis, but in this study no additional inflammatory cells were seen in association with the infiltration. In response to Dr. Sikka, Dr. Hooth replied that in a survey of 21 NTP studies that caused neoplasms of the oral cavity in rats, none of the chemicals in these studies caused similar neoplasms in male mice and only one chemical caused similar neoplasms in female mice. Dr. Hooth added that further studies were underway to characterize the absorption of chromium in the stomach and intestine.

Dr. Mirsalis asked if the palatability of the chemical might have affected water consumption or body weights of the animals. Dr. Cattley asked about the sampling procedures for oral cavity lesions and if all the carcinomas were detected by gross examination. Dr. Herbert replied that a reexamination of all the tissue samples provided confidence that all potential lesions were detected.

Public comments
Dr. Kerkvliet said written comments had been submitted by six five organizations: The Sapphire Group; the Department of Health Sciences of the University of Genoa, Italy; the Interfaith Community Organization; Exponent, Inc.; Consulting Scientists; and the Environmental Working Group. Written comments were also received from two scientists affiliated with Health Science Resource Integration and ChemRisk.

Mr. Thomas McKee, representing the Interfaith Community Organization of New Jersey, said the NTP had produced a well-designed and meticulously executed study and an insightful and illuminating report. He urged that the final report be completed expeditiously in light of the need for the study results by regulatory agencies.

Ms. Deborah Proctor, representing Tierra Solutions, Inc., noted that the chromium concentrations in the NTP study were 50 to 100 times higher than the existing drinking water standards. She indicated that no human epidemiology studies in the literature indicated increases in oral cavity or intestinal tumors as a result of exposure to chromium. She also claimed that the body weights and water consumption of the study animals were significantly lower than for the control groups and suggested that lower palatability and dehydration may have affected the physiology of the exposed animals. She speculated that dehydration, which could lead to lower production of saliva, might be a factor in the ability of animals to reduce chromium VI in the oral mucosa.

Subcommittee discussion continued
Dr. Walker inquired if the water consumption was indeed altered significantly in this study. Dr. Hooth discussed the criteria for the diagnosis of dehydration, which included clinical observations of circulatory hypovolemia such as loss of skin turgor, dry mucous membranes, retraction of eyes, and even shock, and increases in clinical chemistry parameters such as albumin, hematocrit, blood urea nitrogen, or urinary specific gravity. None of these indicators was observed in the present study. Dr. Hooth also presented graphs showing that the body weights of only one dosed group of male rats varied by more than 10% from controls late in the study and that water consumption on a body weight basis was nearly identical for exposed and control rats.

Dr. Walker moved and Dr. Soper seconded that the conclusions be accepted as written. The motion was approved unanimously with 6 yes, 0 no votes, and 0 abstentions.

Formamide
Dr. Richard Irwin, NIEHS, introduced the toxicology and carcinogenesis studies of formamide by describing the study design, the nonneoplastic lesions observed in the 3-month studies, and the nonneoplastic and neoplastic lesions seen in the 2-year studies. The proposed conclusions were:

> Under the conditions of these 2-year gavage studies, there was *no evidence of carcinogenic activity* of formamide in male or female F344/N rats administered 20, 40, or 80 mg/kg. There was *clear evidence of*

carcinogenic activity of formamide in male B6C3F1 mice based on increased incidences of hemangiosarcomas of the liver. There was *equivocal evidence of carcinogenic activity* of formamide in female B6C3F1 mice based on increased incidences of hepatocellular adenoma or carcinoma (combined).

Mineralization of the testicular arteries and tunic and hematopoietic cell proliferation of the spleen in male mice were also associated with exposure to formamide.

Subcommittee discussion

Dr. Deininger, the first principal reviewer, noted that the results for formamide were different from those for dimethylformamide. He inquired if there was any insight into the cause of the erythrocyte damage. Dr. Irwin replied that there was little knowledge of the metabolites of formamide. He noted some suggestion in the literature of possible damage to membranes. The slight changes in the erythron and hematopoiesis were the only indications of red cell involvement. Dr. Kerkvliet inquired if there could also be a connection with the bone marrow hyperplasia. Dr. Elmore, NIEHS, stated that one cause of the erythocyte damage could be physical shearing as the cells passed through hemangiosarcomas, and thus the hyperplasia was secondary to the tumors and not vice-versa. Dr. Walker said that the most appropriate statement would be that causes of hematopoiesis were unknown. He also noted that studies involving immunohistologic staining for p53 stabilization might help provide information on a mechanism for this and other studies in which hemangiosarcomas were observed.

Dr. Mirsalis, the second principal reviewer, agreed in general with the conclusions. He inquired further of the possible causes of erythron changes. He asked for some comparison with a previous inhalation study that did show a liver tumor response. Dr. Irwin noted that the inhalation studies were on dimethylformamide.

Dr. Sikka, the third principal reviewer, agreed with the conclusions. He inquired if discussion could be added regarding the possible mechanism of carcinogenicity of this non-genotoxic chemical and also about the difference in response between rats and mice. Dr. Irwin replied that mechanistic speculation would be difficult given the lack of knowledge of the metabolites; he said that more information would be included about the effects of dimethylformamide and N-methylformamide.

Dr. Pino suggested that hyperplasia of the bone marrow in male rats be included in the conclusion statement. Dr. Mirsalis moved and Dr. Deininger seconded that with that addition, the conclusions be accepted as written. The motion was approved unanimously with 6 yes, 0 no votes and 0 abstentions.

Ethinyl Estradiol - Multigenerational Studies

Dr. Barry Delclos, NCTR, introduced the reports on ethinyl estradiol by describing the NIEHS/NTP-FDA/NCTR series of studies on endocrine disruptors, which include both

multigenerational studies to test for accumulative or persistent effects as well as carcinogenesis bioassays. Dr. Delclos discussed the uses, occurrence, and metabolism of the synthetic estrogen ethinyl estradiol and the results of the dose range finding study described in NTP Toxicity Report 79. He then described the dosing regimen for the various portions of the multigenerational study, the effect on body weight, the reproductive system endpoints for the different generations, and the occurrence of nonneoplastic lesions in the male rats. The proposed summary statement was:

> Ethinyl estradiol administered at exposure concentrations of 2, 10, or 50 ppb in a low phytoestrogen diet to NCTR CD (Sprague-Dawley) rats showed clear biological activity and potentially adverse effects. Ethinyl estradiol suppressed both preweaning and postweaning body weights of males and females during periods of direct exposure to dosed feed. Ethinyl estradiol accelerated the attainment of puberty of females under continuous exposure conditions (F_1 and F_2) and of animals where dosing was terminated at weaning (F_3). Perturbation of the estrous cycle (prolonged cycles, aberrant cycles, increased time in estrus) in young females after vaginal opening and prior to mating was observed in the F_1 and F_2 generations. In males, statistically significant inductions of male mammary gland hyperplasia (F_0 through F_3 generations) and mild mineralization of renal tubules (F_1 and F_2 generations) were observed. Treatment-related effects may have carried over into the unexposed F_4 generation since there was a marginal increase in the incidences of alveolar hyperplasia in the male mammary gland in that generation. The majority of these effects were observed at 50 ppb, but significant effects (body weight reduction, prolonged estrous cycle time, and male mammary gland hyperplasia) were observed at the lowest exposure concentration (2 ppb). With the possible exception of a 1.5-day delay of preputial separation in the F_2 males, effects of ethinyl estradiol did not appear to be magnified across exposed generations.

Subcommittee discussion

Dr. Walker, the first principal reviewer, felt the study was well reported. He expressed familiarity with the study design and did not have any major scientific criticisms.

Dr. Crump, the second principal reviewer, noted the large volume of data and questioned whether in some cases the analytic techniques indicated significant statistical differences when the values seemed nearly identical. He also queried whether it was appropriate to refer to reduced body weight as an adverse effect. He felt that the difference in estrous cycle length might not merit mention in the summary statement and that the final sentence of the conclusion might not be necessary.

Dr. Delclos replied that the analysis for the one table with similar values had been noticed and corrected and agreed that while body weight is a measure of chemical response, it might not be called an adverse effect.

Dr. Cattley, the third principal reviewer, inquired whether it was possible to detect the difference between spontaneously occurring and chemical induced hyperplasia in the male mammary gland. Dr. Paul Mellick, NCTR, replied that the feminization of the males by the chemical produced discernible differences in the mammary gland. Dr. Cattley asked for a statement about the consensus of the adequacy of dose selection.

Dr. Tammy Stoker, an *ad hoc* reviewer for this report, questioned whether there was indeed any direct or transplacental transfer of ethinyl estradiol to the fetuses or neonates from the gestational exposures, as ethinyl estradiol was not measured in the fetal blood or milk to substantiate a transfer. She suggested that the delay in preputial separation in males might have been a real effect, correlating well with a decrease in testosterone level and body weight. She queried the measurement procedure of taking smears to gauge estrous cycles three days after vaginal opening. Dr. Delclos replied that the timing was a consequence of the several other requirements of the study protocol.

In discussing the proposed summary, several statements were debated. Regarding the statement about mammary gland hyperplasia in the unexposed F_4 males, the statistical significance of the incidences and the possible distinction between feminized males were discussed. Dr. Kerkvliet called the question, and the panel voted unanimously with six votes to remove the sentence. Regarding body weight changes, discussion centered on whether the statements of effects implied they were adverse. In the second sentence, the decrease in body weight was to be noted, but not referred to as being "suppressed." That amendment was approved unanimously. Dr. Soper suggested that the mention of prolonged estrous cycle time be deleted from the penultimate sentence. The motion to remove that phrase and not include the other two effects as a parenthetical expression was approved unanimously. Next, Dr. Crump suggested removing mention of the preputial separation from the final sentence. Dr. Walker also thought that it was a singular point and questioned the use of the term "magnified." Dr. Delclos pointed out that this effect was seen in the F_2 generation but not in the F_1 generation that received a similar exposure. Dr. Kerkvliet noted that one of the major purposes of the study was to determine if there was any intergenerational accumulation of effects. Dr. Mirsalis felt the sentence was accurate and clear as written. Dr. Kerkvliet called the question, and the subcommittee approved retaining the final sentence as written by a vote of 4 yes to 2 no votes. Dr. Sikka suggested changing "and" to "including" in the first sentence, and the motion was approved unanimously. Dr. Cattley suggested deleting the word "increased" from the phrase "time in estrus" in the sentence about the perturbation of the estrous cycle; that motion carried unanimously. Finally Dr. Cattley moved, and Dr. Walker seconded, that the full summary be accepted as modified. The motion was approved unanimously with 6 yes votes, 0 no votes, and 0 abstentions. The approved summary statement was:

> Ethinyl estradiol administered at exposure concentrations of 2, 10, or 50 ppb in a low phytoestrogen diet to NCTR CD (Sprague-Dawley) rats showed clear biological activity including potentially adverse effects. Both preweaning and postweaning body weights of males and females

were decreased during periods of direct exposure to dosed feed. Ethinyl estradiol accelerated the attainment of puberty of females under continuous exposure conditions (F_1 and F_2) and of animals where dosing was terminated at weaning (F_3). Perturbation of the estrous cycle (prolonged cycles, aberrant cycles, time in estrus) in young females after vaginal opening and prior to mating was observed in the F_1 and F_2 generations. In males, statistically significant inductions of male mammary gland hyperplasia (F_0 through F_3 generations) and mild mineralization of renal tubules (F_1 and F_2 generations) were observed. The majority of these effects were observed at 50 ppb, but significant effects on body weight reduction and male mammary gland hyperplasia were observed at the lowest exposure concentration (2 ppb). With the possible exception of a 1.5-day delay of preputial separation in the F_2 males, effects of ethinyl estradiol did not appear to be magnified across exposed generations.

Ethinyl Estradiol - 2-year studies

Dr. Barry Delclos, NCTR, introduced the toxicology and carcinogenesis studies of ethinyl estradiol by noting that the chemical was already a known carcinogen. The purpose of the present study was to evaluate effects from chronic exposure to lower doses, reversibility of effects, and potential generational carryover effects. Dr. Delclos described the three exposure arms of the study, the body weights, survival, reproductive system effects, and nonneoplastic and neoplastic lesions observed in exposed rats. The proposed conclusions were:

Under the conditions of this 2-year feed study with continuous exposure to the test compound from conception through termination (F_1C), there was *no evidence of carcinogenic activity* of ethinyl estradiol in male or female Sprague-Dawley rats exposed to 2, 10, or 50 ppb. Nonneoplastic lesions were observed in the mammary gland and liver of males and in the uterus and liver of females.

Under the conditions of this 2-year feed study with exposure to the test compound from conception through 20 weeks followed by control feed until termination (F_1T140), there was *no evidence of carcinogenic activity* of ethinyl estradiol in male Sprague-Dawley rats exposed to 2, 10, or 50 ppb. There was *equivocal evidence of carcinogenic activity* of ethinyl estradiol in female Sprague-Dawley rats based on marginally increased incidences of uterine stromal polyps. Nonneoplastic lesions were observed in the mammary gland and liver of males and in the liver and clitoral gland of females.

Under the conditions of this study where offspring of two prior generations of animals exposed to ethinyl estradiol in feed were exposed from conception through weaning (PND 21), followed by control feed

through termination (F_3T21), there was *some evidence of carcinogenic activity* of ethinyl estradiol in male Sprague-Dawley rats based on increased incidences of preputial gland epithelial neoplasms. There was also a marginal increased incidence of mammary gland adenoma or adenocarcinoma (combined). A significantly increased incidence of male mammary gland alveolar hyperplasia was also observed. There was *equivocal evidence of carcinogenic activity* of ethinyl estradiol in female Sprague-Dawley rats based on marginally increased incidences of uterine stromal polyps. Increased incidences of squamous metaplasia and atypical hyperplasia in the uterus and hyperplasia in the clitoral gland were also observed.

Subcommittee discussion

Dr. Soper, the first principal reviewer, felt the complicated study was well performed and reported. He asked if there should be a statement in the conclusion that addressed whether or not there was some magnification of effect.

Dr. Bradfield, the second principal reviewer, inquired if differences in the serum levels of the chemical was known. Dr. Delclos replied that serum levels of the chemical were not measurable by a sensitive liquid chromatography-mass spectrometry method. The sensitivity of this method is adequate for measurement of ethinyl estradiol levels in humans, but rats metabolize the compound to a greater extent and blood levels are lower. Measurement of such low levels would have required greater volumes of blood than could be concentrated, as is done to measure low levels of ethinyl estradiol in wastewater samples. Dr. Bradfield also inquired about the ultimate use of these studies. Dr. Delclos replied that a comparative overview of the three environmental estrogen studies would be performed to look for commonalities, once the third one was completed. Dr. Bradfield also asked what might be learned about the mechanism of action of these compounds. Dr. Bucher replied that at the time these studies were designed, less was known about the possible mechanisms and the focus was on detecting common effects at low doses.

Dr. Pino, the third principal reviewer, suggested that the conclusion regarding the preputial gland tumors might better be classified as *equivocal evidence* rather than *some evidence*.

Dr. Walker also supported classifying the preputial gland tumors as *equivocal evidence*. Dr. Crump concurred. By a unanimous vote, the conclusion for the preputial gland tumors in male rats in the F_3 generation was changed from *some* to *equivocal evidence*. Subsequently, it was noticed that the mammary gland adenomas in these male rats, designated as "may also have been related," fit the same category, and the panel unanimously approved combining the two lesions under the category of *equivocal evidence*. The overall conclusions were then approved as amended with 6 yes votes, 0 no votes, and 0 abstentions. The final paragraph of the conclusions was:

> Under the conditions of this study where offspring of two prior generations of animals exposed to ethinyl estradiol in feed were exposed from conception through weaning (PND 21), followed by control feed through termination (F_3T21), there was *equivocal evidence of carcinogenic activity* of ethinyl estradiol in male Sprague-Dawley rats based on increased incidences of preputial gland epithelial neoplasms and a marginal increased incidence of mammary gland adenoma or adenocarcinoma (combined). A significantly increased incidence of male mammary gland alveolar hyperplasia was also observed. There was *equivocal evidence of carcinogenic activity* of ethinyl estradiol in female Sprague-Dawley rats based on marginally increased incidences of uterine stromal polyps. Increased incidences of squamous metaplasia and atypical hyperplasia in the uterus and hyperplasia in the clitoral gland were also observed.

Cumene

Dr. Po Chan, NIEHS, introduced the toxicology and carcinogenesis studies of cumene by describing the uses and potential human exposure to the chemical, the genetic toxicity profile, the study design, and the survival, body weights, and nonneoplastic and neoplastic lesions observed in the 3-month and 2-year studies. Dr. David Malarkey, NIEHS, described the histopathologic details of the lesions seen in the respiratory tract and some of the supplemental molecular pathology findings. The proposed conclusions were:

> Under the conditions of these 2-year inhalation studies, there was *clear evidence of carcinogenic activity* of cumene in male F344/N rats based on increased incidences of respiratory epithelial adenoma in the nose and renal tubule adenoma or carcinoma (combined). There was *some evidence of carcinogenic activity* of cumene in female F344/N rats based on the incidences of respiratory epithelium adenoma in the nose. There was *clear evidence of carcinogenic activity* of cumene in male B6C3F1 mice based on increased incidences of alveolar/bronchiolar neoplasms. There was *clear evidence of carcinogenic activity* of cumene in female B6C3F1 mice based on increased incidences of alveolar/bronchiolar neoplasms. Increased incidences of hepatocellular adenoma or carcinoma (combined) in female mice were also considered to be related to exposure to cumene.
>
> Exposure to cumene resulted in nonneoplastic lesions in the nose and kidney of male rats; the nose of female rats; the lung, nose, liver, and forestomach of male mice; and the lung and nose of female mice.

<u>Subcommittee discussion</u>

Dr. Mirsalis, the first principal reviewer, had no major scientific criticisms. He felt unconvinced that the rat micronucleus data showed a truly positive response.

Dr. Walker, the second principal reviewer, agreed that the micronucleus response was at best equivocal. He felt the study was well performed and especially liked the inclusion of

K-ras and p53 mutation analyses for the lung neoplasms.

Dr. Pino, the third principal reviewer, inquired about a statistically significant increase in interstitial cell adenomas of the testes in male rats that was not included in the results section of the report. He asked about the reason for the distinction between *clear* and *some evidence* for the nasal adenomas in male and female rats.

Dr. Crump noted two other sites in male mice for which significant occurrences of tumors were not included in the results or conclusion summary: hemangiosarcomas of the spleen and follicular cell adenomas of the thyroid gland. He also asked whether the kidney tumors in male rats merited a classification of *clear evidence*.

Public comments
Dr. Kerkvliet said that the American Chemistry Council had submitted written comments.

Dr. George Cruzan, representing the American Chemistry Council, noted that in the introduction to the report, cumene was incorrectly identified as being used in styrene synthesis. He suggested that the abstract of the report include a statement about the relevance of the findings to human disease and include genotoxicity results from the literature.

Subcommittee discussion continued
In considering the conclusion statements, Dr. Walker suggested that a statement be added indicating the association of the nonneoplastic kidney lesions in male rats with α2u-globulin. Dr. Crump suggested mentioning the three additional lesions: male rat testicular adenoma, hemangioma of the spleen and thyroid gland, and follicular cell adenomas in male mice. Dr. Bucher said if these tumors were considered equivalent to *equivocal evidence*, they would be termed "may have been related" to chemical administration. The panel voted unanimously with 6 yes votes, 0 no votes, and 0 abstentions to approve the inclusion of these three lesions as "may have been related" plus a statement concerning the α2u-globulin accumulation. The final conclusion was:

> Under the conditions of these 2-year inhalation studies, there was *clear evidence of carcinogenic activity* of cumene in male F344/N rats based on increased incidences of respiratory epithelial adenoma in the nose and renal tubule adenoma or carcinoma (combined). Increased incidences of interstitial cell adenomas of the testes may have been related to exposure to cumene. There was *some evidence of carcinogenic activity* of cumene in female F344/N rats based on the incidences of respiratory epithelium adenoma in the nose. There was *clear evidence of carcinogenic activity* of cumene in male B6C3F1 mice based on increased incidences of

alveolar/bronchiolar neoplasms. The increased incidences of hemangiosarcoma of the spleen and thyroid follicular cell adenoma may have been related to cumene exposure in male mice. There was *clear evidence of carcinogenic activity* of cumene in female B6C3F1 mice based on increased incidences of alveolar/bronchiolar neoplasms. Increased incidences of hepatocellular adenoma or carcinoma (combined) in female mice were also considered to be related to exposure to cumene.

Exposure of male rats to cumene resulted in nonneoplastic lesions of the kidney characteristic of α2u-globulin accumulation. Exposure to cumene resulted in nonneoplastic lesions in the nose of male and female rats; the lung, nose, liver, and forestomach of male mice; and the lung and nose of female mice.

At this point the meeting adjourned on the afternoon of May 16. The meeting resumed at 8:30 a.m. on May 17, 2007. Dr. Mirsalis was unable to attend the second day.

Cresols
Dr. Michael Sanders, NIEHS, introduced the toxicology and carcinogenesis studies of cresols by discussing the uses and human exposure for the chemical, the genetic toxicity of the various isomers, the results of short-term studies previously reported in NTP Toxicity Report 9, the design of the long-term studies in male rats and female mice, and the neoplastic and nonneoplastic lesions observed in the 2-year studies. The proposed conclusions were:

Under the conditions of these 2-year feed studies, there was *equivocal evidence of carcinogenic activity* of 60:40 *m*-/*p*-cresol in male F344/N rats based on the marginally increased incidence of renal tubule adenoma. There was *some evidence of carcinogenic activity* of 60:40 *m*-/*p*-cresol in female B6C3F1 mice based on the increased incidence of forestomach squamous cell papilloma.

Exposure to 60:40 *m*-/*p*-cresol resulted in increased incidences of nonneoplastic lesions in the kidney (hyperplasia), nose (inflammation, hyperplasia, and metaplasia), and liver (eosinophilic focus) of rats. Increased incidences of nonneoplastic lesions were observed in the respiratory tract (hyperplasia in the nose and lung), thyroid gland (follicular degeneration), and liver (eosinophilic focus) of mice exposed to *m*-/*p*-cresol.

Subcommittee discussion
Dr. Deininger, the first principal reviewer, inquired about the analyses on which the study protocol of just male rats and female mice was based. Dr. Sanders explained that in a 1990 survey of approximately 300 NTP studies, over 90% of the studies for which there

was a positive response had responses in at least one of those two sex/species groups. He noted also that in the present studies tumors were seen at the site of chemical contact, and he felt the study results validated the study design. Dr. Deininger also asked for an explanation of the rationale for the *some evidence* conclusion for the forestomach papillomas in the female B6C3F1 mice. Dr. Sanders replied that the conclusion was based on a significant increase in tumors that might be able to progress to malignancy. He added that the weight decrement in that group might be attributable to palatability of the dosed feed rather than to an excessive toxic effect.

Dr. Soper, the second principal reviewer, said that the kidney tumor response was well supported by a highly significant trend test. He agreed with the proposed conclusion of *equivocal evidence* in the male rats because that tumor type seldom progresses to malignancy.

Dr. Novak, the third principal reviewer, inquired if the thyroid gland tumors in male rats were worthy of mention. Dr. Walker noted that those tumors did not rise to any level of statistical significance.

Public comments
Dr. Kerkvliet said the American Chemistry Council submitted written comments.

Dr. John Butala, representing the Cresols Panel of the American Chemistry Council, mentioned that a series of reproductive and developmental toxicity studies of cresols had been performed, and he suggested that they might be referenced in the report. He also suggested that rodent forestomach tumors might not be relevant to human disease.

Subcommittee discussion continued
Dr. Soper moved and Dr. Deininger seconded that the conclusions be approved as written. The motion was approved unanimously with 6 yes votes, 0 no votes, and 0 abstentions.

Propargyl Alcohol
Dr. Michelle Hooth, NIEHS, described the toxicology and carcinogenesis studies of propargyl alcohol by describing the uses of the chemical, the survival and body weights in the short-term studies, and the neoplastic and nonneoplastic lesions observed in the 2-year studies. The proposed conclusions were:

> Under the conditions of these 2-year inhalation studies, there was *some evidence of carcinogenic activity* of propargyl alcohol in male F344/N rats based on increased incidences of nasal respiratory epithelial adenoma and mononuclear cell leukemia. There was *no evidence of carcinogenic activity* of propargyl alcohol in female F344/N rats exposed to 16, 32, or 64 ppm. There was *clear evidence of carcinogenic activity* of propargyl alcohol in male and female B6C3F1 mice based on increased incidences

of nasal respiratory epithelial adenoma. The increased incidences of Harderian gland adenoma in male B6C3F1 mice may have been related to exposure to propargyl alcohol.

Exposure to propargyl alcohol resulted in increased incidences of nonneoplastic nasal lesions in male and female rats and mice.

Subcommittee discussion

Dr. Bradfield, the first principal reviewer, inquired about the criteria used to formulate the levels of evidence in the conclusions and particularly about the interpretation of benign tumors such as adenomas. Dr. Malarkey, NIEHS, replied that findings from a couple of NTP studies suggested that nasal respiratory adenomas might have the potential to progress to malignancy.

Dr. Crump, the second principal reviewer, questioned whether the effects in the mouse nasal epithelium, though occurring in both sexes, rose to the level of *clear evidence* in the absence of any malignant tumors.

Dr. Cattley, the third principal reviewer, also questioned whether the appropriate conclusion for mice was *clear evidence* based on the given criteria. He felt the question was whether the ability of the adenomas to progress to malignancy was well established, and questioned what amount of increase was required to be considered "marked."

Dr. Bradfield asked if more could be said about the mechanism of tumor induction. Dr. Gordon Flake, NIEHS, replied that the cytochrome P450s are more concentrated in the olfactory epithelium and also localized in the transitional epithelium of the respiratory mucosa, the tissues in which adenomas developed. Dr. Novak added that the turnover of nasal epithelial cells was quite rapid.

In discussing the levels of evidence for the mouse studies, Dr. Walker noted that a dose related increase in nasal tumors occurred in both sexes. Dr. Malarkey said these were rare tumors, and thus the number of opportunities to observe progression to malignancy was small. He added for comparison that in dogs nasal adenomas, though considered benign, would continue to grow and act as malignant tissues, destroying the nasal tissue and invading the brain. Dr. Malarkey felt that for a tumor with a background incidence of 0.1%, the occurrence of several tumors in an exposed group could be considered a marked increase. Dr. Cattley said in this study there were nearly 100% incidences of hyperplasia of the respiratory epithelium and glandular respiratory epithelium, squamous metaplasia of the respiratory epithelium, and suppurative inflammation of the nasal passages, which would provide a severe test of the ability of these lesions to progress.

Dr. Pino asked for more explanation of why the mononuclear cell leukemia in male rats was considered *some evidence*. Dr. Walker explained that the effect was seen in just one exposed group, and for a commonly occurring tumor the trend test may be more relevant than one individual, pair wise comparison.

Dr. Cattley said no malignant nasal neoplasms were seen in mice with many nonneoplastic lesions, and Dr. Crump noted that pathologists seemed divided on whether there is evidence that a particular tumor type of adenoma can progress to malignancy. Dr. Flake cited the WHO International Classification of Tumors, which states that nasal carcinomas can arise from respiratory epithelium, mucosal glands, or from preexisting adenoma. Two literature sources indicated that these adenomas and carcinomas form a continuum with possible progression, but not with a high frequency. Although there is the possibility of these adenomas progressing to carcinomas, the Subcommittee felt that there was not sufficient evidence that this would occur. Dr. Soper moved, and Dr. Crump seconded, that the conclusion in male and female mice be changed from *clear evidence* to *some evidence*. The motion was carried by a vote of 4 yes votes, 2 no votes (Drs. Deininger and Walker) and 0 abstentions. Drs. Deininger and Walker disagreed with the change to the conclusions.