

**NATIONAL TOXICOLOGY PROGRAM
BOARD OF SCIENTIFIC COUNSELORS**

Summary Minutes

**Peer Review of Draft Technical Reports of
Toxicology and Carcinogenesis Studies
by the Technical Reports Review Subcommittee**

February 25, 2009

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Members in Attendance:

Tracie Bunton, Eicarte LLC
Russell Cattley, Amgen
David Eastmond, University of California
Mitzi Nagarkatti, University of South Carolina School of Medicine
Raymond Novak (Chair), Children's Hospital of Michigan
Michael Pino, Sanofi-Aventis
Kenneth Portier, American Cancer Society
Jim Riviere, North Carolina State University
James Sherley, Boston Biomedical Research Institute

Pending Members in Attendance:

Stephen Looney, Medical College of Georgia
Justin Teeguarden, Pacific Northwest National Laboratory

National Institute of Environmental Health Sciences (NIEHS) Staff:

Charles Alden	Ronald Melnick
Linda Birnbaum	Retha Newbold
John Bucher	Michael Sanders
Rajendra Chhabra	Barbara Shane
June Dunnick	Michael Shelby
Susan Elmore	Michael Snell
Gordon Flake	Robert Sills
Paul Foster	Cynthia Smith
Veronica Godfrey	William Stokes
Ronald Herbert	Raymond Tice
Angela King-Herbert	Gregory Travlos
Mark Hoenerhoff	Jacquelyn Tubbs
Michelle Hooth	Molly Vallant
Richard Irwin	Suramya Waidyanatha
Grace Kissling	Nigel Walker
Ruth Lunn	Kristine Witt
David Malarkey	Mary Wolfe
Scott Masten	Michael Wyde

Contractors to NIEHS

Abraham Nyska, Integrated Laboratory Systems
John Peckham, Experimental Pathology Laboratories, Inc.

Other Federal Agency Staff:

Paul Howard, Food and Drug Administration (FDA)/National Center for Toxicological Research (NCTR)
Mark Toraason, Centers for Disease Control and Protection (CDC)/National Institute of Occupational Safety and Health (NIOSH)
Sally S. White, Environmental Protection Agency

Public Attendees:

Steven Buecher, Dynamic Corporation
Milton Hejtmancik, Battelle
Peter Little, Charles River Laboratories Pathology Associates
Michael McGuffin, American Herbal Products Association

The meeting began at 8:30 a.m. on February 25, 2009 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. Materials from this meeting are available on the NTP website (<http://ntp.niehs.nih.gov/go/1584>).

Dr. John Bucher, NIEHS welcomed the subcommittee, thanked the members for the time they had spent in preparing their reviews, and acknowledged their valuable contribution to the NTP. Dr. Linda Birnbaum also welcomed the members and echoed Dr. Bucher's thanks. She said the NTP appreciated the input the subcommittee provides to the NTP regarding the reviews and noted that she was looking forward to hearing their deliberations.

Goldenseal Root Powder

Dr. June Dunnick, NIEHS, introduced the studies of goldenseal root powder by reviewing its use as a natural herbal product, the nomination of goldenseal root powder for study by the NTP, and the major plant alkaloids found in goldenseal and by describing the design of the short- and long-term studies and the effects of goldenseal root powder on survival, body weight, and liver lesions in rats and mice. The proposed conclusions were:

Under the conditions of these 2-year feed studies, there was *clear evidence of carcinogenic activity* of goldenseal root powder in male F344/N rats based on the increased incidences of hepatocellular adenomas and hepatocellular adenomas or carcinomas (combined). There was *clear evidence of carcinogenic activity* of goldenseal root powder in female F344/N rats based on the increased incidence of hepatocellular adenoma. There was *some evidence of carcinogenic activity* of goldenseal root powder in male B6C3F1 mice based on the increased incidences of hepatoblastoma and multiple hepatocellular adenoma. There was *no evidence of carcinogenic activity* of goldenseal root powder in female B6C3F1 mice exposed to 3,000, 9,000, or 25,000 ppm goldenseal root powder in feed for 2 years.

Administration of goldenseal root powder resulted in increased incidences of nonneoplastic lesions in the liver of male and female rats and male mice.

Dr. Raymond Novak noted that two written comments were submitted on behalf of the American Herbal Products Association. Mr. Michael McGuffin, representing the

American Herbal Products Association, spoke to clarify that goldenseal was not likely to be consumed for long periods of time by humans and that the highest daily dose used in the study was 1.5 times higher than would be consumed by humans daily.

Dr. Tracie Bunton, the first primary reviewer, said the report clearly described the progression of the development of the liver lesions over time, and agreed with the conclusions.

Dr. Justin Teegarden, the second primary reviewer, said the report was written clearly and the dose selection appeared appropriate. He asked if the conclusions could present comparison with expected human dosages and also if trends for decreases in tumor incidence would be noted.

Dr. James Sherley, the third primary reviewer, also agreed with the conclusions.

Dr. Dunnick noted that the actual human intake levels are largely unknown, and also that this NTP report is a hazard identification rather than a risk assessment document. Dr. John Bucher, NIEHS, explained that in general, the NTP avoids including dose levels in conclusions where carcinogenic effects were seen, as often the total dose range that elicits a carcinogenic response is not known. In cases where there is no evidence of carcinogenicity, the doses are included in the conclusion statements as this is important information.

Dr. Michael Pino asked for clarification on combining the incidences of adenoma and carcinoma in the conclusion statement, when only one carcinoma was observed. Dr. David Malarkey, NIEHS, said that adenomas and carcinomas were considered part of a continuum of progressive lesions and that the number of adenomas was driving the call. Dr. Michelle Hooth, NIEHS, noted that in six concurrent studies no liver carcinomas had been seen in 300 control male rats.

Dr. Kenneth Portier moved and Dr. David Eastmond seconded that the conclusion be accepted as written. The motion was approved unanimously with 8 yes votes, 0 no votes, and 0 abstentions.

Androstenedione

Dr. Chad Blystone, NIEHS, introduced the studies on androstenedione by describing its former use as a dietary supplement, the study design and dose selection for the rodent studies, the results of genetic toxicity assays, the effects of the chemical on body weight and reproductive tissues, and the incidence of lesions in the three-month and two-year studies. The proposed conclusions were:

Under the conditions of these 2-year gavage studies, there was *equivocal evidence of carcinogenic activity* of androstenedione in male F344/N rats based on increased incidences of alveolar/bronchiolar adenoma and alveolar/bronchiolar adenoma or carcinoma (combined). There was *equivocal evidence of carcinogenic activity* of androstenedione in female

F344/N rats based on increased incidences of mononuclear cell leukemia. There was *clear evidence of carcinogenic activity* in male B6C3F1 mice based on increased incidences of liver neoplasms. There was *clear evidence of carcinogenic activity* of androstenedione in female B6C3F1 mice based on increased incidences of hepatocellular adenoma and hepatocellular carcinoma. Increased incidences of pancreatic islet adenoma in male and female mice were also considered chemical related.

Androstenedione administration caused increased incidences in nonneoplastic lesions of the liver in male rats and male and female mice; pancreatic islets and exocrine pancreas of female rats; and clitoral gland, kidney and submandibular salivary gland of female mice.

Decreases in the incidences of testicular interstitial cell adenoma and mononuclear cell leukemia in male rats, mammary gland fibroadenoma, cysts, and hyperplasia in female rats, and malignant lymphoma in female mice were considered related to androstenedione administration.

Dr. Bunton, the first primary reviewer, felt the studies were limited by not having achieved a maximum tolerated dose level. She agreed with the proposed conclusions.

Dr. Eastmond, the second primary reviewer, raised questions about interpreting the statistical significance of tumors with high variability and high background rates. In particular, regarding liver tumors in male mice, he inquired if a rate of 48/50 of treated animals with tumors compared with 41/50 in controls could be deemed clear evidence of an effect. Dr. Grace Kissling, NIEHS, replied that in addition to statistical significance, knowledge of historical background rates and biological plausibility were a factor in study interpretation. Dr. Blystone noted that in addition to overall incidence, the increases in tumor multiplicity and the incidences of malignant carcinomas and hepatoblastomas added to the strength of clear evidence.

Dr. Teeguarden, the third primary reviewer, also agreed with the proposed conclusions.

Dr. Eastmond suggested that the conclusion for liver tumors in male mice specify the tumor types and multiplicity for liver neoplasms. The revised sentence for male mice was “There was *clear evidence of carcinogenic activity* of androstenedione in male B6C3F1 mice based on increased incidences of liver neoplasms, particularly multiple adenomas and carcinomas, and hepatoblastomas.” Dr. Eastmond moved and Dr. Mitzi Nagarkatti seconded that the conclusions be accepted with the suggested revision. The motion was approved unanimously with 8 yes votes, 0 no votes, and 0 abstentions.

2,3',4,4',5-Pentachlorobiphenyl (PCB 118)

Dr. Nigel Walker, NIEHS, introduced the technical report on PCB 118 by giving an overview of the series of NTP studies on dioxin-like compounds evaluating the concept of the toxic equivalency factor (TEF). He then described the study design and dose

selection for the long-term study on PCB 118, and the body weight, tissue disposition, and histopathologic findings in the study. The proposed conclusions were:

Under the condition of this 2-year gavage study, there was *clear evidence of carcinogenic activity* of PCB 118 in female Harlan Sprague-Dawley rats based on increased incidences of neoplasms of the liver (cholangiocarcinoma, hepatocholangioma, and hepatocellular adenoma) and cystic keratinizing epithelioma of the lung. Occurrences of carcinoma in the uterus were considered to be related to the administration of PCB 118. Occurrences of squamous cell carcinoma of the uterus and acinar neoplasms of the pancreas may have been related to administration of PCB 118.

Administration of PCB 118 caused increased incidences of nonneoplastic lesions in the liver, lung, adrenal cortex, pancreas, thyroid gland, nose, and kidney.

Dr. Jim Riviere, the first primary reviewer, felt that portions of the discussion were speculative, but he agreed with the overall conclusions.

Dr. Pino, the second primary reviewer, felt the occurrence of uterine carcinomas was a strong response and should be included in the category of *clear evidence*. He also inquired whether the four hepatocholangiomas should be considered *clear evidence of carcinogenicity*.

Dr. Portier, the third primary reviewer, had no additional comments.

Dr. Walker explained that the liver hepatocholangiomas were considered with the other liver neoplasms to give an overall conclusion of *clear evidence* at that site. Regarding the uterine carcinomas, Dr. Walker explained that the question of whether variations in body weight may have modulated the tumor response led to the lower level of evidence of carcinogenic activity of the chemical at that site.

Dr. Riviere moved and Dr. Eastmond seconded that the conclusions be accepted as written. The motion was approved with 7 yes votes, 1 no vote (Dr. Pino), and 0 abstentions. Dr. Pino voted no as he thought that the uterine carcinomas should be included in the category of *clear evidence of carcinogenicity*.

3,3',4,4'-Tetrachloroazobenzene (TCAB)

Dr. Michelle Hooth, NIEHS, introduced the toxicology and carcinogenesis studies of TCAB by describing its occurrence as a byproduct of herbicide production, its structural similarity to dioxins, the design of the short- and long-term studies, the toxic endpoints noted in the 3-month studies, and the body weight, survival, and neoplastic and nonneoplastic lesions observed in the 2-year studies. The proposed conclusions were:

Under the conditions of these 2-year gavage studies, there was *clear evidence of carcinogenic activity* of TCAB in male Harlan Sprague-

Dawley rats based on increased incidences of cystic keratinizing epithelioma of the lung, cholangiocarcinoma of the liver, and gingival squamous cell carcinoma of the oral mucosa. The increased incidences of follicular cell adenoma of the thyroid gland were also considered to be related to TCAB administration. The marginally increased incidence of malignant schwannoma may have been related to TCAB administration. There was *clear evidence of carcinogenic activity* of TCAB in female Harlan Sprague-Dawley rats based on increased incidences of cystic keratinizing epithelioma of the lung and gingival squamous cell carcinoma of the oral mucosa. The increased incidences of cholangiocarcinoma of the liver and squamous cell papilloma or squamous cell carcinoma (combined) of the forestomach were also considered to be related to TCAB administration. The marginally increased incidences of adenoma of the adrenal cortex may have been related to TCAB administration. There was *clear evidence of carcinogenic activity* of TCAB in male B6C3F1 mice based on increased incidences of transitional epithelial gland carcinoma of the urethra and alveolar/bronchiolar neoplasms of the lung. The increased incidences of squamous cell carcinoma of the forestomach were also considered to be related to TCAB administration. There was *clear evidence of carcinogenic activity* of TCAB in female B6C3F1 mice based on increased incidences of fibrosarcoma or malignant schwannoma (combined) of the skin. The increased incidences of transitional epithelial gland carcinoma of the urethra, alveolar/bronchiolar neoplasms and cystic keratinizing epithelioma of the lung, and squamous cell carcinoma of the forestomach were also considered to be related to TCAB administration. The marginally increased incidences of malignant lymphoma may have been related to TCAB administration.

TCAB administration caused increased incidences of nonneoplastic lesions of the lung, liver, oral mucosa, forestomach, adrenal cortex, pancreas, blood vessel, spleen, and mesenteric lymph node in male and female rats; the thyroid gland and testis in male rats; the nose in female rats; the urinary bladder, forestomach, glandular stomach, skin, spleen, thymus, liver, and heart in male and female mice; the urethra, ureter, and blood vessel in male mice; and the lung, clitoral gland, ovary, and bone marrow in female mice.

Dr. Pino, the first primary reviewer, inquired why fibrosarcomas and malignant schwannomas of the skin in female mice were combined for analysis. He felt that fibrosarcomas alone might constitute clear evidence. Dr. Hooth agreed that the incidence of fibrosarcomas was sufficient for clear evidence and explained that the incidences were combined because both are cutaneous tumors of mesenchymal origin.

Dr. Nagarkatti, the second primary reviewer, felt the study was well designed and agreed with the conclusions. She felt it was worth noting in the discussion that TCAB had a lower potency than TCDD because it degraded more rapidly and thus did not

bioaccumulate to the same extent. She offered suggestions for the possible design of future studies. She inquired about the possible interpretation of the lower incidence of pancreatic tumors in dosed animals compared to controls. Dr. Hooth replied that the difference in pancreatic tumor incidence may reflect the higher mortality in the high dosed males, and thus more control animals may have been at risk for these late-developing tumors. She added that frozen tissues were saved to study gene expression and immunological studies are ongoing.

Dr. Eastmond, the third primary reviewer, felt the study was well conducted and presented. He suggested clarifying the distinction between lesions contributing to clear evidence versus some evidence in the same study and providing the rationale for each call. He also inquired about the historical incidence of the rare urethral tumors. Dr. Kissling replied that in a review of not only the current 5-year study window of historical controls but also of a larger examination of NTP studies, no such tumors had been observed in approximately 10,000 control mice.

Dr. Pino suggested that the clear evidence conclusion for male mice should be based on “fibrosarcoma and fibrosarcoma or malignant schwannoma (combined) of the skin.” Dr. Eastmond moved and Dr. Nagarkatti seconded that the conclusions be accepted with the proposed amendment. The motion was accepted unanimously with 8 yes votes, 0 no votes, and 0 abstentions.

β-Myrcene

Dr. Rajendra Chhabra, NIEHS, representing NTP study scientist and lead author Dr. Po Chan, NIEHS, introduced the toxicology and carcinogenesis studies of β-myrcene by describing the natural occurrence of this plant product, its commercial uses, its structural similarity to the male kidney carcinogen *d*-limonene, the design of the short- and long-term studies, and the observed toxicity, mortality, and lesions observed in these studies. Dr. Mark Cesta, NIEHS, presented a more detailed histopathologic description of the spectrum of nonneoplastic kidney lesions observed in the study. The proposed conclusions were:

Under the conditions of these 2-year gavage studies, there was *clear evidence of carcinogenic activity* of β-myrcene in male F344/N rats based on increased incidences of renal tubule neoplasms. There was *equivocal evidence of carcinogenic activity* of β-myrcene in female F344/N rats based on increased incidences of renal tubule adenoma. There was *clear evidence of carcinogenic activity* of β-myrcene in male B6C3F1 mice based on increased incidences of liver neoplasms. There was *equivocal evidence of carcinogenic activity* of β-myrcene in female B6C3F1 mice based on marginally increased incidences of hepatocellular neoplasms.

Administration of β-myrcene induced nonneoplastic lesions in the kidney of male and female rats, nose of male rats, and liver of male and female mice.

Dr. Sherley inquired about the assessment of tumor types occurring with high spontaneous background rates, particularly whether the chemical might be thought to potentially promote spontaneously occurring tumors. Dr. Chhabra replied that the mechanism of action was not a criterion for study interpretation, and the comparison of incidences with the concurrent control group was a primary consideration. He added that the background and chemical-induced tumors are generally morphologically indistinguishable. Dr. Robert Sills, NIEHS, added that on occasion molecular biology techniques are used to try and distinguish between spontaneous and chemical-induced tumors, and in some cases different mutations have been found in the same gene in the spontaneous and chemical-induced tumors. Dr. Bucher, NIEHS, said most often in rodent studies the increases in tumors occur in tissues or organs that have measurable spontaneous background rates.

Dr. Cattley, the first primary reviewer, discussed the dose selection issues and said the absence of clinical pathology responses at 90 days may not be a sensitive indicator of toxicity. In discussing the possible association of α 2u-globulin with the various renal lesions, he inquired if it were known whether β -myrcene bound to that protein. He said it would be difficult in the discussion to postulate mechanisms for equivocal findings, such as the female rat kidney neoplasms. Regarding the conclusions, while agreeing with the overall calls, he felt it would be useful to specify the types of the liver neoplasms in male and female mice.

Dr. Chhabra replied that this was one of the very few studies in the history of the NTP where dose selection for the two-year study was so poor. He said no information is available about the possible binding of β -myrcene to α 2u-globulin. Dr. Chhabra proposed a statement indicating that the presence of renal neoplasms in female rats suggested a mechanism distinct from the accumulation of α 2u-globulin.

Dr. Stephen Looney, the second primary reviewer, discussed some issues of presentation of statistical details, including information about the number of outliers that were eliminated, and suggested that median survival might be a more informative measure than mean survival. Dr. Kissling replied that outlier information could be supplied, and noted that in studies where the overall survival was greater than 50% the median survival would be the same in all groups, hence the mean survival time has been included.

Dr. Riviere, the third primary reviewer, agreed with the conclusions and felt the discussion of the renal toxicity could be expanded.

Dr. Pino inquired about the practice of performing additional step sections in the kidney but not in other organs. Dr. Malarkey, NIEHS, replied that kidney tumors are small and additional sections would help confirm whether low incidences of tumors seen in the initial analyses were indeed chemical-related. Dr. Ronald Herbert, NIEHS, added that similar analyses were not performed on other smaller tissues such as the thyroid because

there was not sufficient tissue available for multiple slices and any tumors present in the gland usually were visible.

Dr. Cattley suggested that in the conclusions the liver neoplasms for male mice be specified as hepatocellular adenomas, hepatocellular carcinomas, and hepatoblastomas and that those for female mice be specified as hepatocellular adenomas and carcinomas. Dr. Cattley moved and Dr. Riviere seconded that the conclusions be accepted as amended. The motion was approved unanimously with 8 yes votes, 0 no votes, and 0 abstentions.

Tetralin

Dr. Po Chan, NIEHS, introduced the toxicology and carcinogenesis studies of tetralin by describing the uses, structure, and metabolism of the chemical, the design of the short and long-term studies, the body weights, clinical signs, and nonneoplastic lesions in the short-term studies, and neoplasms and nonneoplastic lesions in the two-year study. The proposed conclusions were:

Under the conditions of these 2-year inhalation studies, there was *some evidence of carcinogenic activity* of tetralin in male F344/N rats based on the increased incidence of cortical renal tubule adenoma. The increased incidence of testicular interstitial cell adenoma may have been related to tetralin exposure. There was *some evidence of carcinogenic activity* of tetralin in female F344/N rats based on the increased incidences of hepatocellular neoplasms and uterine stromal polyp. There was *no evidence of carcinogenic activity* of tetralin in male B6C3F1 mice exposed to 30, 60, or 120 ppm. There was *equivocal evidence of carcinogenic activity* of tetralin in female B6C3F1 mice based on the increased incidence of splenic hemangiosarcoma.

Exposure to tetralin resulted in nonneoplastic lesions of the nose in male and female rats and mice, kidney and testis in male rats, uterus in female rats, and urinary bladder in male and female mice.

Dr. Nagarkatti asked if the elevation in aspartate transaminase activity indicated liver toxicity rather than kidney toxicity. Dr. Gregory Travlos, NIEHS, replied that the aspartate transaminase measurements in this study were from urine, not serum.

Dr. Portier, the first primary reviewer, asked if the somewhat lower survival in the control female mice reduced the power of the study and impacted the ability to formulate a conclusion. He also inquired about the justification for the statement that testicular interstitial cell adenomas in male rats may have been related to tetralin administration. Dr. Chan explained that the statement 'may have been related' corresponded to an equivocal finding. Dr. Portier felt the incidences for this lesion represented background variation, as the concurrent control value was extremely low. Dr. Kissling said the statistical significance for the testicular interstitial cell adenomas would have remained even if all the control animals had survived to the end of the study.

Dr. Sherley, the second primary reviewer, inquired if the presence of decalin contamination in the tetralin test material could have contributed to the observed neoplasms. He suggested that more discussion be provided regarding the concentration of decalin in the exposure chamber. He also noted that for male rats, there were significant trends for increases in some skin lesions, although none were significant by pairwise comparison, and suggested these might constitute *equivocal evidence of carcinogenic activity*. He suggested this finding be added to the results section and addressed in the discussion section.

Dr. Chan replied that further analysis indicated less than 0.1% of the test material in the 2-year studies of tetralin was decalin, and in the NTP studies of decalin there were no neoplastic responses from exposure to 25 ppm. Regarding the skin neoplasms in male rats, Dr. Chan noted that when the squamous cell neoplasms and the basal cell neoplasms were combined, the statistical significance of the trend disappeared.

Dr. Cattley, the third primary reviewer, inquired why the extended evaluation of the kidney was performed in male rats but not in females. He noted that the stromal polyps were benign, with no evidence of progression to stromal sarcoma. Dr. Herbert explained that step sections normally are performed when there is some hint of lesions in the initial examination. Although there was little indication of kidney lesions in the female rats, step sections of the female kidneys were also performed more recently and confirmed there was no effect. Those data would be added to the final report.

Dr. Portier suggested that the statement about testicular tumors in male rats be removed from the conclusions. Dr. Paul Foster, NIEHS, explained that the observed atrophy of the seminal tubules could be linked to the occurrence of the testicular tumors. Dr. Portier agreed and withdrew his suggestion. Dr. Sherley again raised the question of whether the skin lesions might be considered *equivocal evidence*. Dr. Kissling provided the historical background rates for the skin lesions and said the papillomas fell into the historical range for inhalation studies. After further discussion it was agreed that the skin lesions would be mentioned in the results text but not in the conclusions. Dr. Cattley suggested that the liver neoplasms in female rats be identified as adenomas and adenomas or carcinomas combined. Dr. Eastmond moved and Dr. Pino seconded that the conclusions be accepted with the proposed revision. The motion was approved with 7 yes votes, 1 no vote (Dr. Sherley), and 0 abstentions. Dr. Sherley voted no because he thought that squamous cell papillomas might be related to tetralin exposure and this statement should be added to the conclusions.