**NATIONAL TOXICOLOGY PROGRAM**
**BOARD OF SCIENTIFIC COUNSELORS**

***Summary Minutes***

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

**November 19, 2009**

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**Members in Attendance**

Tracie Bunton, Eicarte LLC
Russell Cattley, Amgen
David Eastmond, University of California
Stephen Looney, Medical College of Georgia
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
Justin Teeguarden, Pacific Northwest National Laboratory

**National Institute of Environmental Health Sciences (NIEHS) Staff:**

Charles Alden
Scott Auerbach
Mamta Behl
Linda Birnbaum
Jack Bishop
Chad Blystone
John Bucher
Mark Cesta
Po-Chuen Chan
Rajendra Chhabra
Torrie Crabbs
Michael DeVito
June Dunnick
Susan Elmore
Gordon Flake
Paul Foster
John French
Dori Germolec
Ronald Herbert
Kembra Howdeshell
Michelle Hooth
Marsha Johnston
Angela King-Herbert
Grace Kissling
David Malarkey
Scott Masten
Debbie McCarley
Minerva Mercado-Feliciano
Daniel Morgan
Retha Newbold
Abraham Nyska
Andrew Rooney
Joe Roycroft
Michael Sanders
Michael Shelby
Robert Sills
Cynthia Smith
William Stokes
In Ok Surh
Kristina Thayer
Raymond Tice
Greg Travlos
Jacquelyn Tubbs
Molly Vallant
Suramya Waidyanatha
Nigel Walker
Lori White
Kristine Witt
Mary Wolfe
Coralie Zegre-Cannon

**Other Federal Agency Staff:**
Paul Howard, Food and Drug Administration (FDA)/National Center for Toxicological Research (NCTR)
Dennis Lynch, Centers for Disease Control and Prevention (CDC)/National Institute of Occupational Safety and Health (NIOSH)
Todd Stedeford, Environmental Protection Agency (EPA)

**Public Attendees:**
Dana Austin, North Carolina Central University (NCCU)
Antonio Baines, NCCU
Andrew Ballard, BNA, Inc.
Steven Brecher, Dynamac Corporation
Seema Chetti, NCCU
Stephanie Farmer, NCCU
Patience Hall, NCCU
Gaku Ichihara, Nagoya University, Japan
Kristin Lewis, NCCU
Balagopac Nacr, NCCU
John Peckham, EPL
Fabienne Serra, NCCU
LaShaya Smith, NCCU

The meeting began at 8:30 a.m. on November 19, 2009, in the Rodbell Conference Center of the David P. Rall Building, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina. There were no oral or written public comments. Dr. Justin Teeguarden had conflicts of interest with 1-bromopropane, diethylamine, and bis(2-chloroethoxy)methane. He did not participate in discussion or vote on the draft reports. Dr. Raymond Novak had a potential conflict of interest with bis(2-chloroethoxy)methane. Dr. Portier served as chair for this report. Information for this meeting is posted on the NTP Website (http://ntp.niehs.nih.gov/go/15849) or available by contacting Dr. Lori White, at 919-541-9834.

**1-Bromopropane**
Dr. Daniel Morgan, NIEHS, introduced the studies of 1-bromopropane by reviewing the chemical's extensive use as a solvent, the rationale for and design of the inhalation studies, the observed toxicity and body weight effects in the short-term studies, and the observed neoplasms and nonneoplastic lesions in the long-term studies. The proposed NTP conclusions were:

> Under the conditions of these 2-year inhalation studies, there was *some evidence of carcinogenic activity* of 1-bromopropane in male F344/N rats based on the occurrence of rare adenomas of the large intestine and increased incidences of neoplasms of the skin. Increased incidences of malignant mesothelioma and pancreatic islet adenoma may also have been related to 1-bromopropane

exposure. There was *clear evidence of carcinogenic activity* of 1-bromopropane in female F344/N rats based on increased incidences of adenoma of the large intestine. Increased incidences of neoplasms of the skin may also have been related to 1-bromopropane exposure. There was *no evidence of carcinogenic activity* of 1-bromopropane in male B6C3F1 mice exposed to concentrations of 62.5, 125, or 250 ppm 1-bromopropane. There was *clear evidence of carcinogenic activity* of 1-bromopropane in female B6C3F1 mice based on increased incidences of alveolar/bronchiolar neoplasms.

Exposure to 1-bromopropane resulted in increased incidences of nonneoplastic lesions in the nose of rats and mice, the larynx of rats and male mice, the trachea of female rats and male and female mice, and the lung of mice. Suppurative inflammatory lesions with Splendore-Hoeppli material were present primarily in the nose and skin of male and female rats exposed to 1-bromopropane.

Dr. Michael Pino, the first principal reviewer, had no scientific criticisms. He suggested that basal cell carcinomas as well as adenomas be listed in the results table for rats. He also suggested that pancreatic carcinomas as well as adenomas be added to the conclusion statement for male rats. He asked for clarification of the time and extent of the Splendore-Hoeppi inflammatory lesions and suggested specifying the types of skin tumors in rats. Finally, he questioned if some decreased tumor incidences should be discussed as in some other reports.

Dr. Morgan agreed to include mention of basal cell carcinomas in rat skin in the results section. He noted that the pancreatic carcinomas had not been included because they did not increase with dose and were not considered treatment related. Dr. Morgan said the decreased incidences of skin sarcomas and liver adenomas and carcinomas in female rats could be included under other findings in the results section.

Dr. David Eastmond, the second principal reviewer, felt the study was well conducted and the report well written. He felt the conclusion of *some evidence* for the intestinal tumors was fair even without statistical significance, given the rarity of those tumors. He suggested some caveats in reporting the results of the mutagenicity tests for this volatile chemical. He asked for clarification of the $T_{90}$ period at the start of the animal exposure period in the inhalation chambers and inquired if the pathology slides were coded during the initial diagnoses.

Dr. Morgan said more description would be provided about the mutagenicity test methodology and the chamber startup periods. Dr. Mark Cesta, NIEHS, said slides are not coded during the initial readings, though they are in subsequent reviews.

Dr. Kenneth Portier, the third principal reviewer, also agreed with the conclusions.

Dr. Russell Cattley expressed a preference for specifying the skin neoplasm types in the conclusion statement.

Dr. Mitzi Nagarkatti asked if any further studies were performed to determine if the chemical produced any sensitization leading to development of the Splendore-Hoeppli bodies. Dr. Morgan said no other studies were performed, but noted that a number of other brominated compounds were immunosuppressive.

Dr. Dennis Lynch, NIOSH, commented that the present data would be helpful in the current development of exposure guidelines.

Dr. Pino moved that the conclusion in male rats specify adenomas and carcinomas (combined) for pancreatic lesions, and that the skin tumors be identified as epithelial tumors. Dr. Cattley added that he thought the types of skin lesions should be specified, and Dr. Pino identified keratoacanthomas, squamous cell carcinoma, or basal cell neoplasms to be added parenthetically as the skin neoplasms for male rats.

Dr. Portier moved and Dr. Mitzi Nagarkatti seconded that the conclusions be accepted as modified. The motion was accepted with 9 yes votes, 0 no votes, and 0 abstentions. Dr. Justin Teeguarden was recused from the peer review and vote because of a conflict of interest.

**Ginseng**

Dr. Rajendra Chhabra, NIEHS and representing NTP study scientist and lead author Dr. Po-Chuen Chan, introduced the toxicology and carcinogenesis studies of ginseng by providing an overview of the NTP initiative on herbal products and dietary supplements. He described the uses of ginseng, the primary components of various ginseng products, and the design of the short- and long-term NTP studies. The proposed NTP conclusions from the long-term studies were:

> Under the conditions of these 2-year gavage studies, there was *no evidence of carcinogenic activity* of ginseng in male or female F344/N rats or B6C3F1 mice administered 1,250, 2,500, or 5,000 mg/kg.
>
> The incidence of mammary gland fibroadenoma was significantly decreased in 5,000 mg/kg female rats.

Dr. James Sherley, the first principal reviewer, noted a treatment-associated change in estrous cycling that was not discussed in the text of the report. He questioned a statement that the deaths of some of the treated animals were not

related to the chemical and suggested the statement be revised to reflect uncertainty about the cause of death. Otherwise, he agreed with the conclusions.

Dr. Chhabra replied that an error in reporting the denominators of affected animals led to the impression of altered cyclicity. With corrected data, no effect was noted. Regarding animal survival, Dr. Chhabra noted there was no statistical significance in survival rates and no histopathologic diagnoses indicating cause of death.

Dr. Stephen Looney, the second principal reviewer, raised some generic questions about the statistical methods used in the NTP studies, regarding power analyses, the use of Jonckheere's test, multiple comparisons, and the identification of outliers. He also suggested that median survival rather than mean survival might be a useful measure.

Dr. Grace Kissling, NIEHS, replied that the study design using 50 animals per dose group has been used from the outset of the program, and that an overall power analysis for a study with over 40 sites examined would be cumbersome. She noted that the Jonckheere's test was not an additional test, but rather was used as a decision-making test to determine which subsequent analysis was appropriate. Regarding analysis of various lesion types at a site, she replied that corrections for multiple testing were not applied. She noted that overall decisions for conclusions were not based solely on statistical criteria. Dr. Kissling said no outliers from the present study were included. She also noted that often, as in the present study, when survival is greater than 50% in a group the median survival would be identical to the full study length.

Dr. Teeguarden, the third principal reviewer, felt the study was well conducted and he agreed with the conclusions. He inquired about more detail concerning how the study material was extracted from the base plant material. Dr. Nagarkatti also asked if more detail could be provided about the source and preparation of the study material.

Dr. Chhabra replied that uncertainty about sources and strength of materials are commonplace with herbal products. In the present study, the several preparations of ginseng materials yielded consistent profiles of the component ginsenosides and thus were considered fairly standard.

Drs. Eastmond, Sherley, and Pino all mentioned that in various draft reports there was some inconsistency in how decreased incidences of tumors were presented in the conclusion statements. Dr. David Malarkey, NIEHS, noted that the studies were designed primarily to detect increases in tumor incidence and that level of evidence conclusions were not assigned to decreases. Dr. Michelle Hooth, NIEHS, added that factors such as historical background rates and concurrent decreases in body weight were also taken into account in considering

whether decreased incidences could be associated with treatment. Dr. Malarkey added that decreased tumor incidences in studies such as these should not be interpreted as evidence of protective effects.

Dr. Sherley moved, and Dr. Portier seconded, that the conclusions be accepted as written. The motion was approved with 6 yes votes, 4 no votes, and no abstentions. Drs. Eastmond, Teeguarden, Portier, and Pino voted no. They voted no because they felt the decrease in fibroadenoma should be clarified that it may or may not be related to ginseng administration.

**Pulegone**

Dr. Scott Auerbach, NIEHS, introduced the toxicology and carcingenesis studies of pulegone by describing the properties and uses of the essential oils constituting the herbal product, the genetic toxicity, absorption and metabolism studies of the chemical, and the design and results of the short- and long-term studies in rodents. Dr. Susan Elmore, NIEHS, followed with a presentation characterizing the hyaline glomerulopathy observed in rats and mice in these studies. The proposed NTP conclusions were:

> Under the conditions of these 2-year gavage studies, there was *no evidence of carcinogenic activity* of pulegone in male F344/N rats administered 18.75, 37.5, or 75 (stop-exposure) mg/kg. There was *some evidence of carcinogenic activity* of pulegone in female F344/N rats based on increased incidences of urinary bladder neoplasms. There was *clear evidence of carcinogenic activity* of pulegone in male and female B6C3F1 mice based on increased incidences of liver neoplasms. Osteomas and osteosarcomas in female B6C3F1 mice may have been related to pulegone administration.
>
> A unique renal lesion, hyaline glomerulopathy, was observed in all dosed groups of male and female mice and female rats and in 37.5 mg/kg and 75 mg/kg stop-exposure male rats. In rats, renal failure secondary to hyaline glomerulopathy and chronic progressive nephropathy, contributed to the decreased survival in the 75 mg/kg stop-exposure males and 150 mg/kg stop-exposure females.

Dr. Cattley, the first principal reviewer, asked whether the observed kidney nephropathy was associated with the glomerulopathy described and if circulating immunoglobulins were assayed. He requested that the tabular analyses of the liver neoplasms be expanded to include each tumor type and asked for more detail on the methodology of the analyses of the effects of intercurrent factors (survival and body weight) on expected tumor incidence. Regarding the conclusions, Dr. Cattley recommended specifying the tumor type rather than just the tissue of occurrence. He also asked for a discussion of the level of evidence assigned for the bladder neoplasms in rats.

Dr. Auerbach replied that it was unknown if there was any association between the nephropathy and the glomerulopathy, and no assays for immunoglobulins were performed. Dr. Elmore added that further immunohistochemical studies were being performed on the short-term studies to further characterize the lesions and the findings would be published separately. Dr. Auerbach explained that the conclusion for mice was framed to cover a variety of liver lesions, including adenomas, hepatoblastomas, and several preneoplastic lesions, rather than any one specific neoplasm type. Dr. Cattley suggested that at least specifying hepatocellular neoplasms might add clarity. Concerning the bladder tumors, Dr. Auerbach listed the various factors including rarity of the tumors, progression, early onset, and concomitant mortality due to toxicity in the urinary system that went into consideration of the proposed level of evidence.

Dr. Pino, the second principal reviewer, thought the conclusion for the female rat bladder tumors should be clear evidence. He questioned why certain decreases in tumor incidence were not included in the conclusion statement. He also questioned whether biliary tumors were included with liver tumors, as distinguished from hepatocellular lesions.

Dr. Auerbach replied that the conclusion regarding bladder tumors in female rats was the subject of considerable debate among the NTP staff as well. Regarding the seeming decrease in the incidence of pituitary adenomas and thyroid C-cell adenomas, he noted that the mortality in the high dose group and marginal statistical significance reduced confidence that it could truly be deemed a chemical-related effect.

Dr. Teeguarden, the third principal reviewer, agreed with the proposed conclusions and felt the report was complete. In light of the toxicity and extensive mortality in the high dose group, and the presence of toxicity in the short-term studies, he inquired about the dose-setting process in study design. He also suggested that language be included in the report clarifying that NTP Technical Reports are not risk assessment documents.

Dr. Auerbach replied that the dose selection for this study included consideration of a possible adaptive response to glutathione depletion, and the hyaline glomerulopathy was not fully diagnosed until a retrospective analysis of the short-term studies following the 2-year studies was completed. Dr. Bucher, NIEHS, noted that the foreword to the report indicates that risk assessment is beyond the purview of these studies.

Dr. Eastmond felt that because of the highly variable, spontaneous, background rate of liver neoplasms in male mice he would suggest a conclusion of some evidence rather than clear evidence in that study. Dr. Cattley questioned whether using a response in female mice as support for an effect in male mice is legitimate.

Dr. Auerbach agreed that male mouse liver adenomas are indeed variable and said the clear evidence conclusion for male liver neoplasms was based on a variety of factors, including a definite dose-response, preneoplastic lesions, the presence of hepatoblastomas, and an increased tumor incidence, even in the presence of a marked body weight reduction. He noted that while the studies are evaluated independently, one of the factors considered when determining the level of evidence is a corroborating response in the companion sex/species group.

Dr. Portier moved and Dr. Sherley seconded that the conclusions be accepted as written. The motion failed, with the consensus being that a modification of the descriptive language supporting the levels of evidence was needed.

Dr. Cattley recommended that the conclusion for mice be changed to clear evidence based on increased incidences of hepatocellular neoplasms (adenomas in both sexes and hepatoblastomas in males). A consensus vote of the panel indicated this change was satisfactory.

Dr. Pino suggested that the conclusion in female rats be changed from some evidence to clear evidence based on the presence of the rare urinary bladder tumors. Dr. Malarkey, NIEHS, said the competing considerations in drafting the conclusion were the presence of rare tumors but in the presence of toxicity at doses that exceeded the maximum tolerated dose.

Dr. Jim Riviere moved and Dr. Tracie Bunton seconded that the conclusions be accepted with the two changes proposed. The motion was approved with 6 yes votes, 4 no votes, and 0 abstentions. Drs. Sherley, Portier, Eastmond, and Teeguarden voted no. Dr. Sherley voted no because he agreed with the conclusion for mice as written, "*clear evidence*… based on increased incidences of liver neoplasms." He also did not agree with changing the conclusion for female rats to clear evidence. Dr. Portier agreed with conclusion for mice as clear evidence based on increased incidences of hepatocellular neoplasms (adenomas in both sexes and hepatoblastomas in males), but did not agree with the conclusion of clear evidence for female rats. Dr. Eastmond thought the conclusion should be some evidence rather than clear evidence in male mice. Dr. Teeguarden disagreed with clear evidence for female rats.

**Milk Thistle Extract**

Dr. June Dunnick, NIEHS, introduced the toxicology and carcinogenesis studies of milk thistle extract by describing the uses of the herbal product, the major alkaloids found in the plant extracts, the design of the short- and long-term studies, and the survival, body weights, and lesion incidences in the rodent studies. The NTP proposed conclusions were:

> Under the conditions of these 2-year feed studies, there was *no evidence of carcinogenic activity* of milk thistle extract in male or

female F344/N rats or B6C3F1 mice exposed to 12,500, 25,000, or 50,000 ppm.

Exposure to milk thistle extract resulted in increased incidences of clear cell and mixed cell foci in the liver of female rats and decreases in body weights of exposed groups of male and female mice.

Decreased incidences of mammary gland neoplasms occurred in exposed groups of female rats and decreased incidences of hepatocellular neoplasms occurred in exposed groups of male mice.

Dr. Bunton, the first principal reviewer, had no scientific criticisms and agreed with the conclusions. She noted the reduced incidences of a variety of lesions in exposed animal groups and inquired if NTP staff had any explanation for them. Dr. Dunnick replied that while the NTP studies were not designed to look for mechanisms of action for tumor decrease, there are reports in the literature about free radical scavenging properties.

Dr. Riviere, the second principal reviewer, had no scientific criticisms and agreed with the conclusions.

Dr. Looney, the third principal reviewer, indicated his critique focused on statistical issues that had been addressed in an earlier review.

Dr. Pino moved, and Dr. Teeguarden seconded, that the conclusions be approved as written. The motion was approved unanimously with 10 yes votes.

**Bis(2-chloroethoxy)methane**

Dr. Novak recused himself from the peer review of this draft report and it was chaired by Dr. Portier. Dr. Dunnick, NIEHS, introduced the toxicology and carcinogenesis studies of bis(2-chloroethoxy)methane by describing the uses of the solvent, the results of mutagenicity tests, background information on the absorption and metabolism of the chemical, and the design and results of the short- and long-term dermal exposure studies. The proposed NTP conclusions were:

Under the conditions of these 2-year dermal studies, there was *no evidence of carcinogenic activity* of bis(2-chloroethoxy)methane in male or female F344/N rats administered 75, 150, or 300 mg/kg. There was *no evidence of carcinogenic activity* of bis(2-chloroethoxy)methane in male B6C3F1 mice administered 150, 300, or 600 mg/kg or in female B6C3F1 mice administered 100, 200, or 400 mg/kg.

> Administration of bis(2-chloroethoxy)methane for 2 years resulted in increased incidences of nonneoplastic lesions in the nose of male and female rats, the forestomach of male rats, the heart of male and female mice, and the forestomach and skin of male mice.

Dr. Riviere, the first principal reviewer, felt the studies were well performed and reported. He asked that more details of the dermal exposure protocols be included.

Dr. Nagarkatti, the second principal reviewer, inquired if the thymic atrophy and bone marrow depletion warranted other studies on immunosupression. She inquired also if the dermal application could result in hypersensitivity. She thought the report explained well the different forms of cardiotoxicity and cardiomyopathy observed. Dr. Dunnick said the discussion would be expanded where appropriate.

Dr. Bunton, the third principal reviewer, inquired about the criteria for distinguishing the cardiotoxicity from cardiomyopathy, with the former sometimes masking the latter. Dr. Abraham Nyska, contractor to the NTP, explained that the damage associated with cardiotoxicity is more widespread, consisting of vacuolization, necrosis, and mononuclear cell infiltration, which mask the focal lesions characteristic of cardiomyopathy.

Dr. Riviere moved, and Dr. Nagarkatti seconded, that the conclusions be accepted as written. The motion was approved with 7 yes votes, 0 no votes, and 0 abstentions. Drs. Teeguarden and Novak were recused from participation in the peer review and vote because of potential conflicts of interest. Dr. Eastmond was absent for the vote.

**Diethylamine**

Dr. Novak resumed the role of chairperson. Dr. Morgan, NIEHS, introduced the toxicology and carcinogenesis studies of diethylamine by describing the chemical's uses as a chemical intermediate, the design of the inhalation studies, and the results of the short- and long-term studies. The proposed NTP conclusions were:

> Under the conditions of these 2-year inhalation studies, there was *no evidence of carcinogenic activity* of diethylamine in male or female F344/N rats exposed to 31, 62.5, or 125 ppm. There was *no evidence of carcinogenic activity* of diethylamine in male or female B6C3F1 mice exposed to 16, 31, or 62.5 ppm.
>
> Exposure to diethylamine resulted in increased incidences of nonneoplastic lesions of the nose in male and female rats and mice and of the pleura and lung in female rats.

Dr. Nagarkatti, the first principal reviewer, felt the studies answered all the important gaps in the database for this chemical and the report covered the background literature and study protocols adequately. She noted that exposure in inhalation chambers could result in exposure by other routes, e.g. dermal. She inquired about the occurrence of sporadic seizures in the control animals, about a possible link between thymic atrophy and immunosuppression, and whether other studies involving nitrosamine products were contemplated.

Dr. Morgan agreed that it was understood that whole body exposure would also entail some dermal and oral exposures in addition to inhalation. He noted that seizures had been noted in a number of studies involving singly housed Fischer rats. Extensive evaluation indicated no adverse effect of these seizures on the animals; nonetheless, the NTP has subsequently adopted a different strain for its studies. Dr. Gordon Flake, NIEHS, noted that while the weights of the thymus glands were decreased in rats and mice in the short-term studies, it might not be possible to discern between a stress-induced reaction and immunosuppression. Dr. Flake noted that the thymus is the most sensitive of the lymphoid organs to cortical hormones, but there was no histologic evidence of atrophy of the thymus or other lymphoid tissues in the two-year studies. Dr. Morgan added that a number of studies have attempted without great success to demonstrate nitrosamine formation from diethylamine.

Dr. Cattley, the second principal reviewer, suggested including mention of a short-term study of the related dimethylamine. He also noted an increased incidence of corneal lesions and suggested they be included in the conclusions.

Dr. Sherley, the third principal reviewer, questioned the rationale for discounting the decreased incidences of mammary gland carcinomas in female rats and female mice and thought they should be mentioned in the conclusions.

Dr. Morgan noted that generally conclusions concerning mammary gland are based on combined incidences of adenomas and carcinomas. In the present studies, no changes were seen in the incidences of mammary gland adenomas or fibroadenomas, and in contrast, the incidences of carcinomas were rather small, so little difference was seen in the overall combination.

Dr. Bucher, NIEHS, observed that in general, decreased tumor incidences receive less weight in consideration of study results, as the design of the studies is primarily to detect adverse effects with the goal of hazard identification. Only when tumor decreases are truly significant are they mentioned. He cautioned against misuse of the study results as evidence of protective or therapeutic effects of the chemicals studied.

Dr. Pino inquired if any conclusive association was being made concerning the occurrence of seizures in some study animals. Dr. Morgan replied that in an

overview examination of a number of studies, no direct chemical association and no histopathologic lesions,were detected in animals experiencing the seizures.

Dr. Cattley proposed the conclusions be accepted as written with the addition of lesions of the cornea in male rats. Dr. Sherley seconded the motion, which was approved with 8 yes votes, 0 no votes, and no abstentions. Drs. Teeguarden and Eastmond were absent for the peer review.

Dr. Bucher thanked the subcommittee for their advice and service to the NTP.

The meeting was adjourned at 3:30 pm.