

National Toxicology Program

Technical Reports Peer Review Panel Meeting

May 22, 2014

National Institute of Environmental Health Sciences

Research Triangle Park, NC

Summary Minutes

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I. Attendees

Peer Review Panel Members:

Hillary Carpenter (Panel Chair)
Michael Conner
Michelle Fanucchi
Charles Mahrt (by telephone)
Jon Mirsalis
Gary Perdew
Karen Regan

NTP Board of Scientific Counselors Representative:

Sonya Sobrian, Howard University

National Institute of Environmental Health Sciences (NIEHS) Staff:

Charles Alden	Michelle Hooth	Robert Sills
Scott Auerbach	Angela King-Herbert	Stephanie Smith-Roe
Chad Blystone	Grace Kissling	Vicki Sutherland
John Bucher	Robin Mackar	Raymond Tice
Mark Cesta	David Malarkey	Gregory Travlos
Michael DeVito	Barry McIntyre	Molly Vallant
June Dunnick	Rebecca Moore	Suramya Waidyanatha
Susan Elmore	Daniel Morgan	Nigel Walker
Gordon Flake	Arun Pandiri	Lori White
Paul Foster	Cynthia Rider	Kristine Witt
Dori Germolec	Kristen Ryan	Michael Wyde
Robbin Guy	Brian Sayers	Yun Xie
Ronald Herbert		

Contract Staff to NIEHS

Mamta Behl, Kelly Government Solutions
Nancy Bordelon, Battelle
Amy Brix, Experimental Pathology Labs, Inc.
Pragati Coder, Battelle
Torrie Crabbs, Experimental Pathology Labs, Inc.
Milton Hejtmancik, Battelle
Kyathanahalli Janardhan, Integrated Laboratory Systems
Rodney Miller, Experimental Pathology Labs, Inc.
Rebecca Moore, Experimental Pathology Labs, Inc.
Tom Steinbach, Experimental Pathology Labs, Inc.
Gabrielle Willson, Experimental Pathology Labs, Inc.

Other Federal Agency Staff:

Paul Howard, Food and Drug Administration (FDA)

Public Attendees

Ann Ball, Independent Lubricant Manufacturers Association
Walden Dalbey, DalbeyTox, LLC
Ernie Hood, Bridport Services
John Howell, GHS Resources Inc.
Jeffrey Leiter, Independent Lubricant Manufacturers Association
Robert Maronpot, Maronpot Consulting LLC
Amanda Phelka, NSF International
Ria Scheuren, Houghton International
David Slinkman, Houghton International
Julie Thomas, Independent Lubricant Manufacturers Association

Webcast Registrants

Kristin Aillon, MRIGlobal
Joseph Algaier, MRIGlobal
Aouatef Bellamine, Lonza
Shannon Berg, Centers for Disease Control and Prevention (CDC)/National Institute for Occupational Safety and Health (NIOSH)
Karen Coker, Biotechnical Services, Inc.
Bradley Collins, NIEHS
Steven Dentali, Herbalife International of America
Carol Eisenmann, Personal Care Products Council
William Frez, The Lubrizol Group
Perry Gideon, Biotechnical Services, Inc.
Susan Gunnels, Biotechnical Services, Inc.
Xiaowen Guo, Kemin Industries, Inc.
Lynn Harper, Biotechnical Services, Inc.
Melody Harwood, Herbalife International of America
Kristina Hatlelid, Consumer Product Safety Commission
Jennifer Hsieh, California Environmental Protection Agency
Jiang Hu, Herbalife International of America
Trudy Kumpe, Biotechnical Services, Inc.
Kirstin Kosemund-Meynen, Procter & Gamble
Rachel Leven, Bloomberg BNA
Donna McMillan, Procter & Gamble
Kirstin Meynen, Procter & Gamble
Franklin Mirer, CUNY School of Public Health
Helen Phipps, Booz Allen Hamilton
Anne Pilaro, FDA, Center for Biologics Evaluation and Research (CBER)
Resha Putzrath, Navy and Marine Corps Public Health Center
Phyllis Rathman, Biotechnical Services, Inc.
Wilson Rumbeiha, Iowa State University
Del Serbus, Biotechnical Services, Inc.
Maged Sharaf, American Herbal Products Association
Glenn Simmons, Biotechnical Services, Inc.

Meng Sun, California Environmental Protection Agency
Loren Tapp, CDC/NIOSH
Sheetal Thakur, RTI International
Jessica Vaughn, Morgan, Lewis, and Bockius
Mary Wolfe, NIEHS
Andrea Wong, Council for Responsible Nutrition
Judy Woods, Biotechnical Services, Inc.
Jie Zhang, Procter & Gamble
Merle Zimmerman, American Herbal Products Association (telephone)

II. Welcome and Introductions

The National Toxicology Program (NTP) Technical Reports Peer Review Panel Meeting convened on May 22, 2014 in Rodbell Auditorium, National Institute of Environmental Health Sciences (NIEHS), Research Triangle Park, North Carolina. Dr. Hillary Carpenter served as chair. The other peer review panel members present were Drs. Michael Conner, Michelle Fanucchi, Charles Mahrt (by telephone), Jon Mirsalis, Gary Perdew, and Karen Regan. Dr. Sonya Sobrian attended as the NTP Board of Scientific Counselors liaison. Dr. Paul Howard attended representing the FDA. Representing the NTP were Associate Director Dr. John Bucher, Dr. David Malarkey (group leader of the NTP Pathology Group), Dr. Chad Blystone (toxicologist in the Developmental and Reproductive Toxicology Group) and Dr. Nigel Walker (Deputy Division Director for Science).

Dr. Carpenter welcomed everyone to the meeting and asked all attendees to introduce themselves. Dr. Bucher welcomed participants and thanked the board members and staff for their work, and thanked Dr. Carpenter for agreeing to chair the meeting. Designated Federal Officer Dr. Yun Xie read the conflict of interest policy statement.

III. Peer Review of Draft NTP Technical Reports: Charge

Dr. Blystone briefly reviewed the NTP Technical Reports process for the panel, including the Levels of Evidence of Carcinogenic Activity used in the draft conclusions. He also went over the committee's charge.

IV. Overview of the NTP Rat Models

NTP Laboratory Animal Management Group leader Dr. Angela King-Herbert provided an overview of the rat models used in the NTP studies to be presented in the current peer review meeting. She described advantages and concerns of using the F344/N rat, which was used for about 30 years at the NTP. One of the recommendations from the 2005 NTP workshop to consider rat stocks and strains was to discontinue use of the F344/N strain. Soon after, the NTP discontinued use of that strain and temporarily used

the F344/NTac rat model, a substrain of the F344/N rat strain. Dr. King-Herbert described programmatic changes and desired traits in selecting an NTP rat model. In 2007, the NTP selected the Wistar Han rat, but it was discontinued due to several issues. In 2009, the NTP selected the Harlan Sprague Dawley rat model and continues to use this model.

The F344/N, F344/NTac, Wistar Han, and Harlan Sprague Dawley rat strains were used in the studies to be discussed in the current meeting.

V. Residual Longitudinal Review for Identifying Uterine Proliferative Lesions

NTP Pathologist Dr. Susan Elmore briefed the panel on the uterine longitudinal sectioning protocol, which was used for Green Tea Extract, Indole-3-Carbinol, and CIMSTAR 3800. She discussed and illustrated the original transverse tissue review and the residual longitudinal tissue review. The residual longitudinal reviews revealed additional uterine tumors and nonneoplastic lesions in all groups and identified preneoplastic lesions in some groups. It also has helped to determine the primary site of invasive tumors and to avoid misinterpretation of gross lesion incidences. Dr. Elmore noted that residual longitudinal sectioning has now been incorporated as standard protocol for NTP subchronic and chronic studies.

VI. Draft NTP Technical Report on Green Tea Extract (TR-585)

Dr. Carpenter briefly reviewed the format for the peer review.

NTP Study Scientist Dr. Chad Blystone briefed the panel on the draft NTP Technical Report on green tea extract (GTE). The National Cancer Institute (NCI) nominated epigallocatechin gallate (EGCG), a component of green tea, for study by the NTP. The NTP elected to study GTE due to its wide human exposure. GTE is one of the most commonly used herbal supplements in the U.S. Gavage studies were conducted in F344/NTac rats (3-month), Wistar Han rats (2-year), and B6C3F1/N mice (3-month and 2-year). Genotoxicity tests were conducted *in vitro* and in the 3-month B6C3F1/N mice study.

GTE was mutagenic in the presence of S9 in *Salmonella typhimurium* TA98 and TA100 tests, but was not mutagenic without S9 up to 2.0 mg/plate. It was not mutagenic in the *E. coli* assay, nor in a 3-month *in vivo* B6C3F1/N mice study.

The draft NTP report's conclusions on GTE were:

Male and Female Wistar Han Rats

- **No evidence of carcinogenic activity** at 100, 300, or 1,000 mg/kg

Male B6C3F1/N mice

- **No evidence of carcinogenic activity** at 30, 100, or 300 mg/kg

Female B6C3F1/N mice

- **Equivocal evidence of carcinogenic activity:**
 - Occurrence of squamous cell neoplasms (squamous cell papilloma or squamous cell carcinoma) of the tongue

Administration of green tea extract resulted in increased incidences of nonneoplastic lesions of the liver, glandular stomach, small intestine (duodenum, ileum, and jejunum), nose, lung, heart, and spleen in male and female rats; bone marrow of female rats; the nose, mandibular lymph node, and bone marrow of male and female mice; and the liver of male mice.

Dr. Carpenter noted receipt and distribution to the panel of written comments from Scott J. Smith on behalf of Taiyo Kagaku Co. Ltd. Japan; Dr. Thomas L. Kurt on his own behalf; Dr. Andrea W. Wong, Dr. James C. Griffiths, and Haiuyen Nguyen on behalf of the Council for Responsible Nutrition; Michael McGuffin on behalf of the American Herbal Products Association; Mark Blumenthal, Dr. Stefan Gafner, and Amber Guevara on behalf of the American Botanical Council; and Stephen Paul Mahinka on behalf of non-U.S. parties. He then recognized an oral public commenter, Dr. Merle Zimmerman of the American Herbal Products Association (AHPA), who spoke to the panel by telephone.

Dr. Zimmerman said that the AHPA has concerns about the test article used in the GTE studies, including the identify of the part of the tea plant used as the starting material to manufacture the GTE and the solvent used in the production of the extract. He said there was insufficient information about the level of contaminants in the extract, which could contribute to the toxicological effects observed. He asked NTP researchers to clarify that the studies described in the draft report are relevant only to the specific test article used, not to the wide variety of GTEs in the U.S. marketplace. He cited a statement from the Senate Appropriations Committee related to that issue. He also provided comments from the Council for Responsible Nutrition (CRN). CRN stated there was a lack of information in the report on contaminant testing for aflatoxins and pesticides, as well as residual solvent, microbiological contaminants, or polycyclic

aromatic hydrocarbons. He noted the observed effects on reproductive parameters could be attributed to response to stress in the test animals. In the 2-year studies, he noted GTE administration resulted in statistically significant reductions in the incidence rate of tumors for several tissues and organs, as well as the overall rate of malignant neoplasms in the animals. While the data are presented in the appendices, they are neither noted nor discussed in the Results or Discussion sections.

Dr. Mirsalis, the first primary reviewer, noted the report accurately described and interpreted the findings of the subchronic and chronic studies of GTE in rats and mice. He recommended re-emphasizing in the report that as a result of the switch to Wistar Han rats, there are no historical data. He stated the 1,000 mg/kg dose was too high, which could have been predicted from the effects seen in the 3-month study. Many of the findings in the report were only seen at that high dose. He asked why bilirubin was not measured when the substance had previously demonstrated biliary toxicity and recommended the measurement should be included in the future. He speculated the accidental deaths in the 2-year rat study could be dose related and could be related to stress. He asked for clarification on the makeup of the GTE used in the studies, along with information on how it was prepared. He noted every batch of GTE would be slightly different, and the material selected appeared to be representative of commercially available GTE. He asked how common nasal lesions due to retrograde aspiration into the nasal cavity were, because he did not recall seeing these effects in other NTP studies. He recommended inclusion of a statement about when the studies were conducted, so the reviewer could assess the state of technology and science at the time when evaluating the studies. He suggested removing references to “censoring” data on accidental deaths, as that is a politically charged term. Overall, he agreed with the conclusions of the report.

Responding Dr. Mirsalis’ comments, Dr. Blystone said more context could be provided regarding the historical controls issue. He noted the 1,000 mg/kg dose was dose-limiting and affected survival. He could clearly state in the discussion when pathologies were observed and at what dose. Regarding the biliary toxicity, he said there were two markers of biliary toxicity that were assayed, and that was deemed sufficient. The accidental deaths were evaluated and deemed to be gavage accidents with fluid in the lungs. He could move the table in the discussion describing the constituents of green tea extract up to the methods section. He agreed that retrograde aspiration is an uncommon occurrence, and could include more discussion about it in the report. He noted that the dates of the studies had been provided in Table 1 on page 46. He said “censored” is actually a statistical term. Dr. Kissling noted the term does have a specific statistical meaning. Dr. Mirsalis said he would defer to the statistical term use.

Dr. Conner, the second primary reviewer, asked whether the lower survival rate had an impact on data interpretation, as well as the lack of stopping rules. Dr. Blystone said the low survival at the high dose could affect tumor formation response; however, no tumor

response was seen at lower doses, so this did not affect the evaluation. Dr. Conner said the lesions underlying the “*equivocal*” call were not sufficient and would recommend “*no evidence*.” He also said there is often some degree of retrograde aspiration with compounds known to be irritants, and as rats are nose breathers, both incidental and treatment-related mortality are common. Dr. Blystone responded that the rarity of the tongue tumors in female B6C3F1/N mice was the basis for the call of equivocal evidence. Dr. Malarkey added that a retrospective study is underway to assess irritancy and reflux in the lung and nasopharynx.

Dr. Perdew, the third primary reviewer, addressed the reflux issue. He proposed the GTE is quite viscous, and as the gavage needle is being withdrawn, some of the material may remain on the needle. He recommended adding details on how the extract is made to the report. If the company involved is not willing to share the information, NTP should re-think buying from commercial sources. He asked to see full analyses performed on the GTE, particularly heavy metal analyses, perhaps in an appendix. He asked to see more information on the rationale for the doses chosen, stating that citing literature is not an adequate rationale. He asked for an estimate on how many cups of green tea might be equivalent to the doses used. He said there should be more information in the report about the mutagenic potential of the components of GTE, which should be available in the literature.

Dr. Blystone said more details about the extract and its preparation could be added to the report; however, the information is limited. NTP did measure the components of GTE. For the initial dose justification, the information available in the literature was used as the starting point. NCI had provided some initial information. He agreed to emphasize that analyses were of the extract, not the beverage. He could provide some information about typical exposure to GTE, although that is beyond the purview of the report and enters into risk assessment. He agreed to review the information in the report regarding the mutagenicity of individual components. Referring to the Ames assay, Dr. Perdew asked what it would mean for the mutagenicity of GTE if individual components of GTE were not mutagenic. Dr. Walker noted the extract contained more than the identified components, and this is typical of botanical extracts. Regarding whether to make or purchase the extracts, he said there is criticism attached to both methods. Dr. Perdew suggested that in the future when a commercial acquisition is planned, a deal should be made with the company to provide full information about the extract.

Dr. Howard cautioned against making any statements of relevance to human consumption, because that would force a risk assessment decision with inadequate information within the study. Dr. Walker added that the testing was done on GTE extract, so it would be inappropriate to draw any comparisons with the green tea beverage. Dr. Perdew said perhaps he should have asked for inclusion of information

about how the data would relate to the usual dose of GTE in humans. Dr. Howard reiterated that great care should be taken about relating this type of data to human consumption. Dr. Walker said in past reports on botanicals, simple statements relating to human consumption had been included, so it could be discussed for inclusion.

Dr. Regan echoed Dr. Conner's remarks about reflux irritation in the nose. She asked if the study group had ever looked for inflammation in the animals' middle ears. Dr. Brix said they had not. Dr. Conner agreed that irritation is often seen in Eustachian tubes. Dr. Malarkey said if there was any inflammatory response at that location, it would have been noticed, but that site was not specifically examined. Dr. Regan said it was unlikely it would have been seen unless the area had been specifically examined. She also stated the incidence of two tumors of the tongue was not actual evidence of carcinogenicity. Dr. Mahrt agreed that the lesions of the nose may be related to retrograde aspiration.

Dr. Carpenter called for the draft conclusions to be projected.

Dr. Conner moved that the *equivocal evidence* conclusion be deleted for female mice, and the conclusion should be there is *no evidence of carcinogenic activity* at the various doses in both species and both sexes. Dr. Regan seconded the motion. Dr. Walker reminded the panel why the equivocal call had been made, in that the squamous cell carcinoma of the tongue or oral cavity had never been seen before in the mice in the historical controls. Dr. Mirsalis said he was more comfortable with the equivocal call, based on the rarity.

Dr. Carpenter called for a vote on the motion. The motion carried, with 5 in favor and 1 opposed. Dr. Mirsalis explained his vote was due to the rarity of the tumor seen in two animals.

Thus, the panel voted to accept the following amended conclusion for GTE:

Male and Female Wistar Han Rats

- ***No evidence of carcinogenic activity*** at 100, 300, or 1,000 mg/kg

Male and Female B6C3F1/N mice

- ***No evidence of carcinogenic activity*** at 30, 100, or 300 mg/kg

Administration of green tea extract resulted in increased incidences of nonneoplastic lesions of the liver, glandular stomach, small intestine (duodenum, ileum, and jejunum), nose, lung, heart, and

spleen in male and female rats; bone marrow of female rats; the nose, mandibular lymph node, and bone marrow of male and female mice; and the liver of male mice.

VII. Draft NTP Technical Report on Indole-3-Carbinol (TR-584)

NTP Study Scientist Dr. Michael Wyde briefed the panel on the draft NTP Technical Report on indole-3-carbinol (I3C). I3C, a commercially available dietary supplement, was nominated for NTP study by the NCI based on its occurrence in natural products and its potential use as a cancer chemopreventive agent. It is derived from cruciferous vegetables such as Brussels sprouts, broccoli, cauliflower, cabbage, kale, and turnips. It activates the Ah receptor, a mechanism often associated with toxicity and carcinogenicity. It has been found to be a tumor promoter in rodent and trout models, and has been seen to be chemoprotective in a number of animal models. Three-month gavage studies were conducted in F344/N rats and B6C3F1/N mice. Two-year gavage studies were conducted in Harlan Sprague Dawley Han rats and B6C3F1/N mice. Genotoxicity tests were also conducted.

The draft NTP report's conclusions on I3C were:

Male Harlan Sprague Dawley Rats

- **No evidence of carcinogenic activity** at 75, 150, or 300 mg/kg

Female Harlan Sprague Dawley Rats

- **Some evidence of carcinogenic activity:**
 - Increased incidences of malignant uterine neoplasms (primarily adenocarcinoma)
- May have been related (equivocal evidence):
 - Occurrences of fibroma and fibrosarcoma in the skin

Male B6C3F1/N mice

- **Clear evidence of carcinogenic activity:**
 - Increased incidences of liver neoplasms (hepatocellular adenoma, hepatocellular carcinoma, and hepatoblastoma)

Female B6C3F1/N mice

- **No evidence of carcinogenic activity** at 62.5, 125, or 250 mg/kg

Administration of indole-3-carbinol caused increased incidences of nonneoplastic lesions in the small intestine, mesenteric lymph node, and liver of male and female rats, the thyroid gland of male rats, the uterus of female rats, and the liver, glandular stomach, and nose of male and female mice.

Dr. Carpenter noted that there were no written or oral public comments for I3C.

Dr. Perdew, the first primary reviewer, noted the study was well done. He asked if high concentrations of I3C were insoluble in corn oil, and if the insolubility might affect the results. He asked whether any inflammatory markers were examined in tissues or serum. Dr. Wyde said all doses used in the studies were suspensions, and no inflammatory markers were looked at in tissue or serum. Dr. Perdew asked if it was standard practice to not look at inflammatory markers. Dr. Wyde said it was. Dr. Perdew questioned a statement on page 25 of the report regarding comparative affinity for the Ah receptor. Dr. Wyde said he could adjust the language accordingly. Dr. Perdew recommended wording more carefully the discussion on page 96 about I3C's mechanism of toxicity and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). Dr. Wyde said NTP staff had discussed how much to write about the Ah receptor and dioxins, versus just the mechanism associated with I3C.

Dr. Regan, the second primary reviewer, said the original and extended uterine examinations should be presented together, as that is the basis of the carcinogenicity conclusion. She suggested less emphasis on the findings from the original and extended examinations alone in the report, focusing discussion to positive trends and statistical significance seen when the two are combined. She noted there was no observable increase in atypical endometrial hyperplasia in the tumors. She suggested clarifying the data on cycling animals in Appendix H, Table H2. Regarding nose inflammation, she said the animals with foreign material present in the nasal cavity should be shown separately in the incidence table, as inflammation could be attributed to the foreign material. She asked if assays for cytochrome P450 (CYP) induction were conducted on the nose. Dr. Wyde responded the carcinogenicity call was based on the combined data, both the standard and extended examinations. Dr. Regan recommended limiting discussion in the text to the combined incidences. Dr. Bucher noted this was presented based on a historical precedent, with substantial historical control information available. Because there was no substantial historical database in this study, he understood Dr. Regan's suggestion. Dr. Wyde said he could clarify the data in Table H2. He added that NTP staff had not conducted assays for CYP induction in the nose. Dr. Herbert, the study pathologist, said foreign bodies are often diagnosed in the nose and often associated with inflammation. The NTP has diagnosed the foreign

body, not the inflammatory change, because the inflammatory change is considered secondary to the foreign body. He described how the issue had been approached in the I3C studies. He said there was only one diagnosis of foreign body in the mid-dose group with no evidence of inflammation and two diagnoses in the high dose group, both of which were in animals that had a treatment-related diagnosis of inflammation. Dr. Regan suggested adding more information in the report reflective of Dr. Herbert's statement.

Dr. Mirsalis, the third primary reviewer, said lower doses may not impact the overall conclusions, but it would be good to see if there was a threshold by using lower doses. He said the increases in liver weights were equally striking in rats and mice, and recommended bringing the table for mice into the main body of the text from the appendix. The report has an extended discussion about how I3C is an AhR agonist, and compares the response to those seen with dioxin. The increases in mouse liver tumors could be explained by induction of CYPs resulting in increased liver size, which has been shown to be a significant risk factor for mouse liver tumors. Therefore, he recommended removing the dioxin discussion. Dr. Perdew asked Dr. Mirsalis to clarify his point, because the CYP induction is through the Ah receptor in the liver. Dr. Mirsalis said the response in the mice is different than that seen with dioxin, and noted that in the report, after extensive discussion about dioxin, the conclusion was that the response was not similar to dioxin. Dr. Bucher noted the nomination of this substance was related to the question about how there could be an Ah receptor agonist that was unlike dioxin. He noted perhaps there was overemphasis in the discussion about dioxin. Dr. Wyde said it would be remiss not to mention the dioxin comparison at all. Dr. Fanucchi recommended adding language to the discussion related to Dr. Bucher's response.

Dr. Wyde agreed with Dr. Mirsalis that the information on organ weights in mice could be brought forward in the report.

Dr. Carpenter called for the draft conclusions to be projected.

Dr. Mirsalis moved to accept the draft conclusions as written. Dr. Perdew seconded the motion.

Dr. Regan recommended changing the reference to malignant uterine neoplasms in the female rats from *some evidence* to *equivocal evidence*. There was no dose-related increase in malignant neoplasms at the high dose, and without pre-neoplastic changes or a positive trend test, she could not support the *some evidence* call. Dr. Walker explained in the criteria, *some evidence* is for when there are treatment-related increases; in this case, there was a pair-wise significance against the concurrent control. Dr. Conner disagreed with mentioning an increase that did not reach statistical significance; thus, he agreed that the call should be *equivocal evidence* rather than

some evidence. Dr. Mirsalis said with the multiple adenocarcinomas involved, the *some evidence* call was appropriate. Dr. Kissling stated statistical significance is only one piece of evidence used to make calls, and that there are cases where a biological increase is seen without reaching statistical significance.

Dr. Malarkey said it could be difficult to interpret pre-neoplastic lesions. Dr. Regan said even if the pre-neoplastic lesions were added in this case, it would not change the numbers.

Dr. Carpenter called for a vote on the motion. The panel voted 4-2 in favor of the motion, so the conclusions were accepted as written. Dr. Regan and Dr. Connor cited their previous comments regarding the uterine neoplasm as their reasons for voting against the motion.

VIII. Draft NTP Technical Report TR-586 on CIMSTAR 3800

NTP Study Scientist Dr. Daniel Morgan briefed the panel on the draft NTP Technical Report on CIMSTAR 3800.

CIMSTAR 3800 is a semi-synthetic metalworking fluid (MWF). MWFs are complex mixtures that may contain a variety of additives depending on the particular use. In occupational setting, MWFs are typically re-used, which generates even more complex mixtures due to contaminants. NIOSH nominated the neat, unused MWFs for study, reasoning that a better understanding of the toxicity of unused MWFs is needed before evaluating the contaminated fluids. CIMSTAR 3800 is one of four MWFs selected for NTP studies. In genetic toxicity studies, CIMSTAR 3800 was classified as a weak mutagen. Inhalation studies were conducted in F344/NTac rats (3-month), Wistar Han rats (2-year), and B6C3F1/N mice (3-month and 2-year).

The draft NTP report's conclusions on CIMSTAR 3800 were:

Male Wistar Han Rats

- ***Equivocal evidence of carcinogenic activity:***
 - Incidences of prostate gland adenoma or carcinoma (combined)
 - Incidences of benign or malignant granular cell tumors (combined) of the brain

Female Wistar Han Rats

- ***Equivocal evidence of carcinogenic activity:***
 - Incidences of squamous cell papilloma and keratoacanthoma (combined) of the skin

- Incidences of adenocarcinoma or mixed malignant Mullerian tumor (combined) of the uterus

Male B6C3F1/N mice

- **No evidence of carcinogenic activity** at 10, 30, or 100 mg/m³

Female B6C3F1/N mice

- **Some evidence of carcinogenic activity:**
 - Incidences of follicular cell carcinoma of the thyroid gland
 - Incidences of alveolar/bronchiolar adenoma or carcinoma (combined) of the lung

Exposure to CIMSTAR 3800 resulted in increased incidences of nonneoplastic lesions of the nose, larynx, and lung in male and female rats and mice, lymph nodes in male and female rats, and thyroid gland in female mice.

Dr. Marsalis asked Dr. Morgan about the justification for using inhalation versus dermal exposure studies. Dr. Morgan replied that inhalation is likely the most common occupational exposure.

Dr. Howard asked if the target droplet size was based on industry standards for conducting inhalation toxicology studies, and not necessarily the droplet size to which workers could be exposed. Dr. Morgan said droplet size was based on NTP standards for inhalation studies in rodents.

Dr. Carpenter noted receipt and distribution to the panel of written comments from Jeffrey L. Leiter on behalf of the Independent Lubricant Manufacturers Association. He introduced one oral public comment by telephone, Dr. Franklin Mirer from the CUNY School of Public Health, who would speak on his own behalf.

Dr. Mirer described his background related to the subject of metalworking fluids. He related a history of the project, which dated back to a petition by the United Automobile Workers to the NTP for bioassay of respiratory effects and carcinogenicity of representative MWFs. He said respiratory effects were of as much public health concern as carcinogenicity. He recommended the report take note there were nearly 100% upper respiratory effects at the lowest dose tested for the 90-day study and the 2-year study in rats. He said the adjusted incidence should be calculated for non-tumor pathology so the results could be included in benchmark dose analysis, and only NTP

could do so. He argued the lung tumors in female mice should be seen as “clear evidence.” He asked for clearer discussion of composition in the report, and listed some of the details for clarification.

Dr. Carpenter introduced an oral public commenter present at the meeting, Dr. Walden Dalbey of DalbeyTox, LLC, speaking on behalf of the Independent Lubricant Manufacturers Association (ILMA). Dr. Dalbey questioned the characterization of exposures in the report, particularly whether a vapor phase was monitored during exposures. He questioned the method used for gravimetric measurement of the aerosol phase, and noted the composition of the aerosol was not apparent. He said the criteria for evidence of carcinogenicity was sometimes unclear in the report, referring to the calls related to prostate tumors and brain tumors in male rats and the lung tumors in mice. Similarly, he raised questions about the basis for the *equivocal evidence* call for skin tumors in female rats. He approved of calling CIMSTAR 3800 “weakly mutagenic.”

Dr. John Howell of GHS Resources, Inc., also presented oral public comments on behalf of ILMA. He provided general information about MWFs and the characterization of CIMSTAR 3800. He stressed MWFs are complex mixtures, with thousands of formulations commercially available. He suggested several editorial changes to the report, including addition of references to unintended substances in MWFs. He suggested addition of language describing occupational levels of aerosol exposure to MWF as opposed to experimental concentrations used in the study. He questioned the characterization of CIMSTAR 3800 in the report, and recommended adding a table describing the composition of CIMSTAR 3800. He asked for correction on the statement that no biocide was present, citing the updated MSDS.

Dr. Regan, first primary reviewer, said this was a difficult study from a pathology standpoint and was very well done. She said comments about the uterine tumors should be limited to the combined final numbers. She asked whether there were any animals that had both granulosa cell tumors of the ovary and adenocarcinomas or Mullerian tumors of the uterus. She asked whether the skin tumors in rats were associated with any of the chronic, active inflammation that was observed. She suggested adding a table for the components analyzed in the starting MWF, along with information about whether there was any indication of bacterial or fungal growth or bacterial endotoxin in the CIMSTAR 3800. She asked whether the historical control data presented also had residual uterine exams performed.

Dr. Morgan said he could add a table as suggested by Dr. Regan. He noted the information on bacterial and fungal counts was in Appendix H, and the information could be brought into the Materials and Methods section. He said endotoxin was not measured. Regarding the discussion about relative exposures, he noted it was addressed in the discussion section, but it could be expanded.

Study pathologist Dr. Mark Cesta said none of the animals had both granulosa cell tumors of the ovary and adenocarcinomas or Mullerian tumors of the uterus. He stated none of the skin tumors in rats were associated with chronic, active inflammation. Regarding the historical control data, he said it only included the original sections, not residual sections. Dr. Regan noted the overall incidence of adenocarcinomas in rats was at 7 in 150, and asked if that incidence was based solely on original sections, which Dr. Cesta confirmed.

Dr. Fanucchi, the second primary reviewer, agreed with Dr. Regan's comments. She said in Appendix H, it was shown that boron was originally used as a marker, which changed to a gravimetric method. She asked how the methods were correlated. She noted on page 28 of the report, a statement regarding epidemiological studies suggest that laryngeal cancer may be associated with occupational exposure to machining fluids, but the observed increases in metaplasia in the larynges of treated animals was not considered in the evidence of carcinogenicity. She asked about the presence of a biocide in CIMSTAR 3800. Two compounds tentatively identified in the chemical analysis may act as biocides. She noted the 2-year study began in 2008, and a 2008 MSDS did not list a biocide.

Dr. Morgan said a biocide had tentatively been identified, and the report could be changed to reflect its presence. He said the switch to the gravimetric method had been made four months into the 2-year study. It was done to eliminate drift in the relative amounts of compounds. Dr. Fanucchi said what was written was unclear as to whether boron was a bad marker, and how it would be known that the first four months matched with the rest of the study period. Dr. Morgan said chamber concentrations with and without animals present were measured. He said boron worked well in the 3-month study with not as many animals in the chamber. After four months of the 2-year study, with more animals in the chambers, drift was noted, and they switched to the gravimetric method. He said the issue could be clarified in the report.

Dr. Cesta said squamous cell metaplasia is not considered to be a pre-neoplastic lesion, and it does not lead to laryngeal neoplasia. Dr. Fanucchi asked the point to be clarified in the report. Dr. Conner said laryngeal changes are seen commonly in respiratory studies.

Dr. Mahrt, the third primary reviewer, asked whether the NTP considered looking at the literature or other sources for Wistar Han rats to add to the information on historical controls. Dr. Morgan said that had not been done, as such information would be very study-dependent and may not be relevant to the NTP study.

Dr. Mahrt asked for clarity in the report on the historical controls involved with the original uterine sectioning and if there were some historical controls with the residual

longitudinal uterine sectioning. Dr. Cesta said it could be clarified in the report which animals were included in the historical controls.

Dr. Carpenter called for the study conclusions to be projected for further discussion.

Dr. Regan moved that the conclusion regarding “incidences of benign or malignant granular cell tumors (combined) of the brain” in male Wistar Han rats be changed from *equivocal evidence* to *no evidence*. Dr. Mirsalis seconded the motion, noting the incidence of three tumors at the lowest dose was not strong. Dr. Regan added there was also the presence of such tumors in the controls of female rats. Dr. Mahrt agreed, noting there was limited historical control data.

Dr. Carpenter called for a vote on the motion. The panel voted 6-0 in favor of the motion. Thus, the panel voted to accept the following amended conclusions for CIMSTAR 3800:

Male Wistar Han Rats

- ***Equivocal evidence of carcinogenic activity:***
 - Incidences of prostate gland adenoma or carcinoma (combined)

Female Wistar Han Rats

- ***Equivocal evidence of carcinogenic activity:***
 - Incidences of squamous cell papilloma and keratoacanthoma (combined) of the skin
 - Incidences of adenocarcinoma or mixed malignant Mullerian tumor (combined) of the uterus

Male B6C3F1/N mice

- ***No evidence of carcinogenic activity at 10, 30, or 100 mg/m³***

Female B6C3F1/N mice

- ***Some evidence of carcinogenic activity:***
 - Incidences of follicular cell carcinoma of the thyroid gland
 - Incidences of alveolar/bronchiolar adenoma or carcinoma (combined) of the lung

Exposure to CIMSTAR 3800 resulted in increased incidences of nonneoplastic lesions of the nose, larynx, and lung in male and

female rats and mice, lymph nodes in male and female rats, and thyroid gland in female mice.

IX. Draft NTP Technical Report TR-583 on Bromodichloroacetic Acid

NTP Study Scientist Dr. Michael DeVito briefed the panel on the draft NTP Technical Report on bromodichloroacetic acid (BDCA). The U.S. Environmental Protection Agency and the American Water Works Association Research Foundation nominated BDCA for NTP study. The nomination was based on widespread exposure to BDCA through drinking water, its structural similarity to other haloacetic acids that are carcinogenic in rodents, and a lack of toxicity and carcinogenicity data. BDCA is a water disinfection by-product.

Genetic toxicity studies found that BDCA was mutagenic in *Salmonella typhimurium* strains TA97, TA98, and TA100; *E. coli* WP2 *uvrA* without S9 activation; and *E. coli* strain with S9 activation. Equivocal results were observed in *S. typhimurium* strains TA97, TA98, and TA100 with S9 activation. No increases in micronucleated erythrocytes were observed in mice following a 3-month exposure in drinking water. 2-week, 3-month, and 2-year drinking water toxicity and carcinogenicity studies were conducted in rats (2-week and 3-month in the F344/N rat; 2-year in the F344/NTac rat) and B6C3F1 mice.

The draft NTP report's conclusions on BDCA were:

Male F344/NTac Rats

- ***Clear evidence of carcinogenic activity:***
 - Increased incidences of malignant mesothelioma
 - Combined incidences of epithelial tumors of the skin (squamous cell papilloma, keratoacanthoma, sebaceous gland adenoma, basal cell adenoma, basal cell carcinoma, or squamous cell carcinoma)
- Related to exposure (some evidence):
 - Increased incidences of glioma or oligodendroglioma (combined) of the brain
 - Increased incidences of squamous cell papilloma or squamous cell carcinoma of the oral cavity (oral mucosa or tongue)
 - Increased incidences of subcutaneous fibromas
- May have been related to exposure (equivocal evidence):
 - Occurrences of adenoma of the large intestine

- Occurrences of fibroadenoma of the mammary gland

Female F344/NTac Rats

- **Clear evidence of carcinogenic activity:**
 - Increased incidences of fibroadenoma and carcinoma of the mammary gland
- Related to exposure (some evidence):
 - Increased incidences of glioma or oligodendroglioma (combined) of the brain

Male B6C3F1/N mice

- **Clear evidence of carcinogenic activity:**
 - Increased incidences of hepatocellular carcinoma and hepatoblastoma
 - Increased incidences of adenoma or carcinoma (combined) of the Harderian gland

Female B6C3F1/N mice

- **Clear evidence of carcinogenic activity:**
 - Increased incidences of hepatocellular adenoma, hepatocellular carcinoma, and hepatoblastoma

Exposure to bromodichloroacetic acid for 2 years resulted in increased incidences of nonneoplastic lesions in the bone marrow and liver of male and female rats, spleen of female rats, liver of male and female mice, and testis and epididymis of male mice.

Dr. Carpenter said there were no written or oral public comments for BDCA.

Dr. Conner, the first primary reviewer, agreed in general there was clear evidence of carcinogenicity of BDCA in the two species. He questioned the calls of carcinogenicity on certain tumor types in his written comments, but he did not want to change the overall conclusions. He asked if the low survival in the female rats in the high dose group had an impact on some of the assessments. He questioned whether the low survival in some groups could impede the interpretation of the study. For numerous places in the report, he questioned claims that biological differences were found that did not reach statistical significance. He also questioned comparisons of BDCA to other compounds in the chemical class, particularly a discussion about the reproductive

effects of halogenated acetic acids and related compounds. He recommended adding a table comparing and contrasting the effects of the various related compounds in each type of assay, because the discussion in the report was lengthy and hard to follow. He disagreed with the conclusion regarding intestinal adenomas in male rats, recommending the call be changed from *equivocal evidence* to *no evidence*, because of a lack of statistical significance in the data.

Dr. DeVito noted he could put the incidence numbers of the tumors in the report when there were mentions of biological difference that did not reach statistical significance, and he could indicate the differences were non-significant. He noted the challenge in this study of the low-incidence tumors, and confirmed they had been classified as *equivocal evidence*, which means it may or may not have been related to the exposure. Dr. Bucher addressed the issue of study length and survival. He said the survival-adjusted statistics used by NTP are designed to account for when survival is different among dose groups; a weighted statistical assessment is used. Dr. Kissling said the statistics are survival-adjusted, helping to equalize the risk period across dose groups. Dr. Conner asked whether that approach still worked well in extremes of survival. Dr. Kissling said that depends on when the survival starts falling off.

Dr. Mahrt, the second primary reviewer, agreed with most of the conclusions, but noted some of the conclusions labeled *some evidence* could easily be called *equivocal*.

Dr. Fanucchi, the third primary reviewer, asked for the addition of a table to Appendix J to compare the characterization of the different lots of BDCA for the multiple studies. Dr. DeVito said he could add the table.

Dr. Carpenter asked for a motion upon consideration of the draft conclusions as projected.

Dr. Mahrt moved to change the *some evidence* conclusion in male rats on gliomas (brain) and squamous cell papillomas (oral cavity) to *equivocal evidence*. Dr. Conner seconded.

For the extended evaluation conducted for brain tumors, Dr. Regan asked whether the historical controls had the same evaluation. Dr. Kissling said the historical controls were based only on the original sections.

Dr. Sills said it is important to remember that brain tumors are rare in rodents, and the gliomas seen in NTP studies are similar to human brain tumors. He noted the adult rat is generally insensitive to neurocarcinogens, resulting in a low incidence of tumors. He supported the *some evidence* conclusion.

The vote was taken on the motion, which was restricted to the conclusions on male rats. The vote was unanimous in favor of the motion, 6-0.

Dr. Conner moved that the conclusion regarding increased incidences of gliomas in female rats be downgraded from *some evidence* to *equivocal evidence*. Dr. Mirsalis seconded the motion. The vote was unanimous in favor of the motion, 6-0.

Dr. Mirsalis moved that the conclusions regarding the male and female mice be accepted as written. Dr. Conner seconded. The vote was unanimous in favor of the motion, 6-0.

Thus, the panel voted to accept the following amended conclusions for BDCA:

Male F344/NTac Rats

- ***Clear evidence of carcinogenic activity:***
 - Increased incidences of malignant mesothelioma
 - Combined incidences of epithelial tumors of the skin (squamous cell papilloma, keratoacanthoma, sebaceous gland adenoma, basal cell adenoma, basal cell carcinoma, or squamous cell carcinoma)
- Related to exposure (some evidence):
 - Increased incidences of subcutaneous fibromas
- May have been related to exposure (equivocal evidence):
 - Occurrences of adenoma of the large intestine
 - Occurrences of fibroadenoma of the mammary gland
 - Increased incidences of glioma or oligodendroglioma (combined) of the brain
 - Increased incidences of squamous cell papilloma or squamous cell carcinoma of the oral cavity (oral mucosa or tongue)

Female F344/NTac Rats

- ***Clear evidence of carcinogenic activity:***
 - Increased incidences of fibroadenoma and carcinoma of the mammary gland
- May have been related to exposure (equivocal evidence):
 - Increased incidences of glioma or oligodendroglioma (combined) of the brain

Male B6C3F1/N mice

- ***Clear evidence of carcinogenic activity:***
 - Increased incidences of hepatocellular carcinoma and hepatoblastoma
 - Increased incidences of adenoma or carcinoma (combined) of the Harderian gland

Female B6C3F1/N mice

- ***Clear evidence of carcinogenic activity:***
 - Increased incidences of hepatocellular adenoma, hepatocellular carcinoma, and hepatoblastoma

Exposure to bromodichloroacetic acid for 2 years resulted in increased incidences of nonneoplastic lesions in the bone marrow and liver of male and female rats, spleen of female rats, liver of male and female mice, and testis and epididymis of male mice.

Dr. Carpenter noted that the standard business of the peer review proceedings was concluded. He polled the panelists on whether they thought the bulleted information presented was a clearer format for the conclusions. Dr. Conner said he found it very useful and succinct, and recommended continuing it. Dr. Mirsalis and Dr. Mahrt agreed. None of the other panelists voiced disagreement.

Dr. Bucher thanked the panel members for their hard work on the meeting, and appreciated their thorough and thoughtful analyses. Dr. Carpenter adjourned the proceedings at 3:00 PM, May 22, 2014.

Summary Minutes – May 22, 2014
NTP Technical Reports Peer Review Panel Meeting

These summary minutes have been read and approved by the Chair of the May 22, 2014, National Toxicology Program Technical Reports Peer Review Panel.

[Redacted]

Hillary M. Carpenter III, Ph.D.

Chair, NTP Technical Reports Peer Review Panel

Date: 8/4/2014