NATIONAL TOXICOLOGY PROGRAM
BOARD OF SCIENTIFIC COUNSELORS
Technical Reports Review Subcommittee

February 27-28, 2008

NIEHS, Research Triangle Park, NC

Summary Minutes

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ATTENDEES

Subcommittee Members:

Christopher Bradfield, University of Wisconsin
Tracie Bunton, Eicarte LLC
Russell Cattley, Amgen
Kenny Crump, Louisiana Tech University
Nancy Kerkvliet, Oregon State University (chairperson)
Jon Mirsalis, SRI International
Raymond Novak, Wayne State University
Michael Pino, sanofi-aventis
Keith Soper, Merck Research Laboratory

NIEHS Attendees:

Charles Alden
Jack Bishop
Douglas Bristol
John Bucher
Rajendra Chhabra
June Dunnick
Susan Elmore
Gordon Flake
Paul Foster
Veronica Godfrey
Ronald Herbert
Angela King-Herbert
Michelle Hooth
Richard Irwin
William Jameson
Grace Kissling
Ruth Lunn
David Malarkey
Ronald Melnick
Joseph Roycroft
Michael Sanders
Saija Savolainen
William Schrader
Barbara Shane
Robert Sills
Bhanu Singh
Cynthia Smith
Matthew Stout
Gregory Travlos
Jacquelyn Tubbs
Molly Vallant
Nigel Walker
Kristine Witt
Mary Wolfe

Agency Attendees:

Mary Boudreau, Food and Drug Administration (FDA)/ National Center for Toxicological Research (NCTR)
Fred Beland, FDA/NCTR
Paul Howard, FDA/NCTR
Ron Lorentzen, FDA/ Center for Food Safety and Applied Nutrition (CFSAN)
Paul Mellick, Toxicologic Associates
Brett Thorn, FDA/NCTR
Mark Toraason, National Institute for Occupational Safety and Health

Public Attendees:

Andrew Ballard, BNA, Inc.
Steven Buecher, Dynamic Corporation
Reshan Fernando, RTI International

Public Attendees:

Sanford Garner, Constella Group, LLC
Thomas Goodrich, AQUI-S New Zealand
Charles Hebert, Southern Research Institute
Milton Hejtmancik, Battelle
Georgette Hill, ILS, Inc.
Kristen Hobbie, ILS, Inc.
William Iverson, EPL, Inc.
Jessica Matthews, Constella Group, LLC
Susan Newbigging, ILS, Inc.
Abraham Nyska, ILS, Inc.
John Peckham, EPL, Inc.
Jacqueline Rams, Southern Research Institute
Mark Toneby, MIT miljolab AB

February 27, 2008

The meeting began at 8:30 a.m. on February 27, 2008, 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 shane1@niehs.nih.gov.

DIBROMOACETONITRILE

Dr. Ronald Melnick, NIEHS, introduced the toxicology and carcinogenesis studies of dibromoacetonitrile by reviewing the occurrence of dibromoacetonitrile as a drinking water disinfection by-product. He described the toxic effects of dibromoacetonitrile in short-term studies and the neoplastic and nonneoplastic lesions observed in the two-year studies. The proposed conclusions were:

Under the conditions of these 2-year drinking water studies there was *clear evidence of carcinogenic activity* of dibromoacetonitrile in male rats based on increased incidences of squamous cell papillomas or carcinomas of the oral cavity; adenomas in the glandular stomach of male rats were also considered to be exposure-related. There was *some evidence of carcinogenic activity* of dibromoacetonitrile in female rats based on an increased incidence of squamous cell papillomas of the oral cavity; increased incidences of basal cell or squamous cell neoplasms of the skin in female rats may have been related to dibromoacetonitrile exposure. There was *clear evidence of carcinogenic activity* of dibromoacetonitrile in male and female mice based on increased incidences of squamous cell papillomas or carcinomas of the forestomach. Increased incidences of neoplasms in the liver of male mice may have been related to dibromoacetonitrile exposure.

Exposure to dibromoacetonitrile for 2 years caused increased incidences of epithelial hyperkeratosis in the esophagus of male and female rats, ectasia of the glandular stomach and

squamous epithelial hyperplasia of the tongue in female rats, and squamous epithelial hyperplasia of the forestomach in male mice.

Subcommittee Discussion
Dr. Novak, the first principal reviewer, agreed with the conclusions. He felt the lack of a dose-response effect added to the complexity of the interpretation. He asked if comparisons could be made between the doses used in the NTP studies and human exposure levels.

Dr. Cattley, the second principal reviewer, suggested that the forestomach tumor response be considered separately for male and female mice, as no carcinomas were seen in females.

Dr. Soper, the third principal reviewer, agreed with the conclusions and had no major comments.

Dr. Melnick replied that higher concentrations were used in the 2-year rodent studies than occur in drinking water, because small groups of animals are serving to detect hazards for many millions of people exposed for longer time periods including sensitive subpopulations. He noted also that dibromoacetonitrile is one of several hundred drinking water disinfection by-products that occur in the water supply. Dr. Melnick said the discussion in the report would document the known progression of forestomach squamous cell papillomas to carcinomas. Dr. Flake, NIEHS, cited several comprehensive surveys in the pathology literature providing evidence of progression of squamous papillomas to carcinomas in the oral cavity and esophagus, in addition to the forestomach.

Dr. Pino noted that the testicular atrophy in the rats observed in the two-week studies did not occur in the longer-term studies, and suggested that the kidney nonneoplastic lesions in the two-year study be included in the summary. Dr. Melnick replied that there was a modest increase in the incidences of nephropathy with a high background rate, and said the kidney lesions would be added to the summary.

Dr. Mirsalis moved, and Dr. Novak seconded that the conclusions be revised to reflect that the conclusions in the female mouse are based on papillomas, while those in the male mouse are based on papillomas and carcinomas combined. The motion to approve the revised conclusions were approved with 7 yes votes and one no vote (Dr. Pino approved the original conclusions) and 0 abstentions.

The revised conclusions were:

Under the conditions of these 2-year drinking water studies, there was *clear evidence of carcinogenic activity* of dibromoacetonitrile in male rats based on increased incidences of squamous cell papillomas or carcinomas of the oral cavity; adenomas in the glandular stomach of male rats were also considered to be exposure-related. There was *some evidence of carcinogenic activity* of dibromoacetonitrile in female rats based on an increased incidence of

squamous cell papillomas of the oral cavity; increased incidences of basal cell or squamous cell neoplasms of the skin in female rats may have been related to dibromoacetonitrile exposure.

There was *clear evidence of carcinogenic activity* of dibromoacetonitrile in male mice based on increased incidences of squamous cell papillomas or carcinomas of the forestomach. There was *clear evidence of carcinogenic activity* of dibromoacetonitrile in female mice based on increased incidences of squamous cell papillomas of the forestomach. Increased incidences of neoplasms in the liver of male mice may have been related to dibromoacetonitrile exposure.

Exposure to dibromoacetonitrile for 2 years caused increased incidences of epithelial hyperkeratosis in the esophagus of male and female rats, ectasia of the glandular stomach and squamous epithelial hyperplasia of the tongue in female rats, and squamous epithelial hyperplasia of the forestomach in male mice.

BROMOCHLOROACETIC ACID

Dr. Melnick, NIEHS, introduced the toxicology and carcinogenesis studies of bromochloroacetic acid by reviewing its occurrence as a drinking water disinfection by-product. He described the toxic effects of bromochloroacetic acid in short-term studies and the neoplastic and nonneoplastic lesions observed in the two-year studies. The proposed conclusions were:

Under the conditions of these 2-year studies there was *clear evidence of carcinogenic activity* of bromochloroacetic acid in male F344/N rats based on increased incidences of malignant mesotheliomas and adenomas of the large intestine. There was *clear evidence of carcinogenic activity* of bromochloroacetic acid in female F344/N rats based on increased incidences of adenomas of the large intestine; increased incidences of multiple fibroadenomas of the mammary gland in female rats were also considered to be exposure related. Increased incidences of pancreatic islet adenomas in male rats and of hepatocellular adenomas in male and female rats may have been related to bromochloroacetic acid exposure. There was *clear evidence of carcinogenic activity* of bromochloroacetic acid in male and female B6C3F1 mice based on increased incidences of hepatocellular neoplasms and hepatoblastoma (males only).

Exposure to bromochloroacetic acid for 2 years resulted in increased incidences of nonneoplastic lesions in the liver of male rats, liver and lung of female rats, and liver of male and female mice.

Subcommittee Discussion

Dr. Soper, the first principal reviewer, felt the study was well done and agreed with the conclusions. He explored the occurrence of the hepatocellular adenomas in male and female rats and inquired about the rationale for classifying them as equivocal, rather than some evidence.

Dr. Bunton, the second principal reviewer, also felt the study was well done. She also inquired about the intent of the language for the conclusions for the hepatocellular lesions.

Dr. Novak, the third principal reviewer, inquired if any other clinical pathology measurements were made to assess the metabolic state of the animals.

Dr. Melnick explained that the phrase "may have been related" to chemical exposure was equivalent to *equivocal evidence* at that particular site, in a study where a higher level of evidence was seen in other tissues. He noted that while the hepatocellular adenomas were indeed uncommon tumors, occurring at a background rate of about 1%, the occurrence of just a few of these tumors best fit the interpretive category of equivocal evidence. He noted that the clinical pathology measurements were performed in the three-month studies, but not during the long-term studies.

Dr. Paul Howard, FDA, inquired about the rationale for classifying the malignant mesotheliomas and large intestine tumors in male rats as clear evidence because either the trend test or the pairwise comparison, but not both, were significant at each site. Dr. Melnick explained that large intestine neoplasms are extremely rare in control rats, and the occurrence of several such tumors in dosed animals was a strong indication of a chemical related effect. Moreover, for the intestinal tumors there was supporting evidence from an even stronger response in the female rats. Dr. Melnick also noted that the incidence of mesotheliomas exceeded the historical range in all dosed groups and that mesotheliomas were increased in a previous NTP study of a related chemical, dibromoacetic acid.

Dr. Crump asked if it was standard practice to combine the incidences of mesotheliomas from all sites in the analysis. Dr. Melnick said all of the mesotheliomas that occurred in the peritoneum were combined. Dr. Crump also suggested that the marginal increase in skin fibromas and fibrosarcomas be mentioned in the text.

Dr. Cattley noted that the conclusion for female rats was based largely on intestinal adenomas, and he suggested that more explanation of the knowledge from other studies about the possible progression of these tumors to malignancy be included in the discussion.

Dr. Soper moved, and Dr. Mirsalis seconded, that the conclusions be accepted as written. The motion was carried unanimously with 8 yes votes, 0 no votes, and 0 abstentions.

ALOE VERA

Dr. Mary Boudreau, NCTR, introduced the studies of photococarcinogenicity of Aloe vera by describing the use of aloe in cosmetic and therapeutic preparations, the various plant extracts used in the studies, the design and methodology of the photocarcinogenesis studies, and the survival, body weights, and skin lesions of the animals in the studies. She explained the distinction between normal in-life observations that note the presence of lesions and the subsequent histopathology diagnoses that identify the type of each lesion. The proposed conclusions were:

In male and female SKH-1 hairless mice treated topically with aloe gel creams and exposed to simulated solar light (SSL), the onset, incidence, and multiplicity of in-life observed skin lesions and the incidence of squamous cell neoplasms (papilloma, carcinoma *in situ*, and

carcinoma) did not differ from comparable measurements made in mice treated with control cream and exposed to SSL. The multiplicity of squamous cell neoplasms was significantly increased in female, but not male mice, treated topically with aloe gel creams and exposed to SSL, when compared to mice treated with control cream and exposed to the same dose of SSL.

In male and female SKH-1 hairless mice treated topically with aloe whole leaf creams and exposed to SSL, the onset, incidence, and multiplicity of in-life observed skin lesions and the incidence of squamous cell neoplasms did not differ from the comparable measurements made in mice treated with control cream and exposed to SSL. The multiplicity of squamous cell neoplasms was significantly increased in mice treated topically with aloe whole leaf creams and exposed to SSL, when compared to mice treated with control cream and exposed to the same dose of SSL.

In male and female SKH-1 hairless mice treated topically with decolorized aloe whole leaf creams and exposed to SSL, the onset, incidence, and multiplicity of in-life observed skin lesions and the incidence of squamous cell neoplasms did not differ from the comparable measurements made in mice treated with control cream and exposed to SSL. The multiplicity of squamous cell neoplasms was significantly increased in mice treated topically with decolorized aloe whole leaf creams and exposed to SSL, when compared to mice treated with control cream and exposed to the same dose of SSL.

In male and female SKH-1 hairless mice treated topically with aloe-emodin and exposed to SSL, the onset, incidence, and multiplicity of in-life observed skin lesions and the incidence of squamous cell neoplasms did not differ from the comparable measurements made in mice treated with control cream and exposed to SSL. The multiplicity of squamous cell neoplasms was significantly increased in female mice, but not male mice, treated topically with aloe-emodin creams and exposed to SSL, when compared to mice treated with control cream and exposed to the same dose of SSL.

Subcommittee Discussion

Dr. Crump, the first principal reviewer, noted the complexity of this type of study. He asked for more details on the source of the statistical analyses used and the definitions of the parameters and tests. He felt he was in general agreement with the conclusions but found them difficult to follow.

Dr. Cattley, the second principal reviewer, asked for clarification about the distinction between the presumptive positive control material and the other test articles and about the light used in the present studies and UV-B radiation. He asked for more clarification of the study rationale and of the neoplasm types that led to the conclusion statements.

Dr. Bunton, the third principal reviewer, felt the conclusions were more restatements of findings than interpretive conclusion statements. She also asked for clarification of the notes in the appendices regarding which tissues were examined for histopathology changes and which were not. (Editor's note: Sites examined, but for which no lesions are observed, are listed without

lines of zero incidences; however, those sites did receive histopathologic examination). Dr. Bunton also inquired about the 12-week period following exposure to irradiation before animal sacrifice.

Dr. Boudreau agreed to expand the description of the statistical tests used and the characterization of the aloe-emodin material. She explained that the final 12-week period was an observation period to monitor the progression of lesions following exposure. She noted that the light used in these studies, simulated solar light, and its distribution of wavelengths, was different from pure UV-B radiation in its tumorigenic efficiency.

Dr. P. Howard, NCTR, added that this study followed the standard exposure procedures for photocarcinogenesis experiments, with the only difference being an enhanced histopathologic examination of the animals.

In discussion of the proposed conclusions, Dr. Bunton inquired if the language could more than restate the findings, and Dr. Crump inquired if the standard levels of evidence were appropriate for this type of study. Dr. John Bucher, NIEHS, replied this was the second photococarcinogenesis study reviewed so far, and the program was still trying to develop language for studies that may show either cocarcinogenic or protective effects.

Dr. Howard said in previous studies the benchmark for conclusions were based on the ability of the test compound to alter the carcinogenic effects of the simulated solar light.

At this point an alternative conclusion drafted by the NTP was displayed:

These experiments investigated the potential of topical application of creams containing extracts of *Aloe barbadensis* Miller plant (aloe gel, whole leaf, decolorized whole leaf, or aloe-emodin) to alter the photocarcinogenic activity of filtered xenon arc simulated solar light (SSL) in male and female SKH-1 hairless mice.

Aloe gel or Aloe-emodin
Under the conditions of these studies, there was no effect of aloe gel or aloe-emodin on the photocarcinogenic activity of SSL in male SKH-1 mice. There was a weak enhancing effect of aloe gel or aloe-emodin on the photocarcinogenic activity of SSL in female SKH-1 mice based on an increase in the multiplicity of histopathologically determined squamous cell neoplasms. Neither aloe gel or aloe-emodin affected the SSL-induced onset, incidence, or multiplicity of in-life observed skin lesions or the SSL-induced incidence of histopathologically determined squamous cell neoplasms.

Aloe Whole leaf or Decolorized Whole leaf
Under the conditions of these studies, there was a weak enhancing effect of aloe whole leaf or decolorized whole leaf on the photocarcinogenic activity of SSL in both male and female SKH-1 mice based on an increase in the multiplicity of histopathologically determined squamous cell neoplasms. Neither aloe whole leaf or decolorized whole leaf affected the SSL-induced onset, incidence, or multiplicity of in-life observed skin lesions or the SSL-induced incidence of histopathologically determined squamous cell neoplasms.

Dr. Mirsalis said this set of conclusions was clearer. He felt that use of the traditional categories of levels of evidence might not be appropriate for this type of study.

Dr. Crump suggested that stating that incidences were not increased was less meaningful for studies where overall incidences approached 100% in all groups. Dr. Howard noted that in this type of study, time of onset was often more significant than the overall incidence of animals with skin lesions.

Drs. Crump and Mirsalis suggested that one sentence be added noting that no differences in lesions were detected in the in-life observations. Dr. Melnick suggested that the modifier "weak" was undefined by any formal criteria and might be difficult for subsequent readers to interpret.

Following a lunch recess, the panel reconvened to consider a revised version of the conclusions:

These experiments investigated the potential of topical application of creams containing extracts of *Aloe barbadensis* Miller plant (aloe gel, whole leaf, or decolorized whole leaf), or aloe-emodin to alter the photocarcinogenic activity of filtered xenon arc simulated solar light (SSL) in male and female SKH-1 hairless mice. Data on skin lesions were collected both on digital images during the in-life phase and by histopathologic evaluation at necropsy. No effects of creams upon SSL-induced skin lesions were identified from data collected during the in-life phase.

Aloe gel or Aloe-emodin
Under the conditions of these studies, there was a weak enhancing effect of aloe gel or aloe-emodin on the photocarcinogenic activity of SSL in female, but not in male SKH-1 mice based on an increase in the multiplicity of histopathologically determined squamous cell neoplasms.

Aloe Whole leaf or Decolorized Whole leaf
Under the conditions of these studies, there was a weak enhancing effect of aloe whole leaf or decolorized whole leaf on the photocarcinogenic activity of SSL in both male and female SKH-1 mice based on an increase in the multiplicity of histopathologically determined squamous cell neoplasms.

Following discussion, Dr. Mirsalis moved, and Dr. Novak seconded, that the conclusions be accepted as rewritten. The motion was approved unanimously with 8 yes votes, 0 no votes, and 0 abstentions.

CHROMIUM PICOLINATE

Dr. Matthew Stout, NIEHS, introduced the toxicology and carcinogenesis studies of chromium picolinate monohydrate by noting the trivalent form of chromium in this compound and the use

of chromium picolinate as a dietary supplement. He described the design of the short and long-term studies, the lack of in-life, clinical pathology, or histopathological effects in the short-term studies, the preputial gland lesions in male rats in the 2-year study, and the tissue concentration data from the 2-year study. The proposed conclusions were:

Under the conditions of these 2-year feed studies there was *equivocal evidence of carcinogenic activity* of chromium picolinate monohydrate in male F344/N rats based on an increase in the incidence of preputial gland adenoma. There was *no evidence of carcinogenic activity* of chromium picolinate monohydrate in female F344/N rats or in male or female B6C3F1 mice.

Subcommittee Discussion
Dr. Cattley, the first principal reviewer, felt the study was straightforward and adequate and he agreed with the conclusions.

Dr. Mirsalis, the second principal reviewer, agreed that overall the study was straightforward and well written. He drew attention to requirements for caging based on total body weight of animals and for documentation of feed analysis. He also questioned the characterization of the micronucleus response as equivocal in the females.

Dr. Novak, the third principal reviewer, also agreed with the conclusions and felt the study was well performed.

Dr. Cattley moved, and Dr. Mirsalis seconded, that the conclusions be accepted as written. The motion was passed unanimously with 7 yes votes, 0 no votes, and1 abstention. Dr. Pino abstained from voting due to a potential conflict of interest.

1,2-DIBROMO-2,4-DICYANOBUTANE

Dr. June Dunnick, NIEHS, introduced the toxicology and carcinogenesis studies of 1,2-dibromo-2,4-dicyanobutane. She described the uses of the chemical in cosmetics, the design of the short- and long-term studies, the skin toxicity observed in the studies, and the lack of a carcinogenic response. The proposed conclusions were:

Under the conditions of these 2-year dermal studies there was *no evidence of carcinogenic activity* of 1,2-dibromo-2,4-dicyanobutane in male or female F344/N rats administered 2, 6, or 18 mg/kg. There was *no evidence of carcinogenic activity* of 1,2-dibromo-2,4-dicyanobutane in male or female B6C3F1 mice administered 0.6, 2, or 6 mg/kg.

1,2-dibromo-2,4-dicyanobutane administration induced nonneoplastic lesions at the site of application in male and female rats and mice.

Subcommittee Discussion

Dr. Bradfield, the first principal reviewer, felt this was an appropriately conducted study and he had no major scientific criticisms. He suggested that transcriptional profiling would be a useful addition to the bioassay studies.

Dr. Mirsalis, the second principal reviewer, also felt the study was well conducted and agreed with the conclusions. He thought cell proliferation data would have been a useful addition.

Dr. Bunton, the third principal reviewer, also agreed with the conclusions. She inquired about the significance of some of the negative trends in tumor incidence. Dr. Dunnick replied that the decreased incidence highlighted in the mammary gland tumors in the high dose groups of female rats was not attributable to body weight differences, but may be related to chemical exposure although no mechanistic explanation for this decrease was evident. She added that there was a decrease in tumors in other tissues but these were all within the background tumor incidence, which was quite variable for a number of sites.

Dr. Mirsalis moved, and Dr. Bradfield seconded, that the conclusions be accepted as written. The motion was approved unanimously with 8 yes votes, 0 no votes, and 0 abstentions.

At this stage, the meeting was adjourned until the next day.

February 28, 2008

The meeting resumed at 8:30 a.m. on February 28, 2008 in the Rodbell Conference Center of the David P. Rall Building, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina.

ESTRAGOLE

Dr. Douglas Bristol, NIEHS, introduced the toxicity studies of estragole by describing the occurrence of the chemical in a variety of plants, its uses as a dietary chemical, and its structural characteristics. He described the toxicity of the allyl and iso-allyl phenylpropene analogues, the design of the three-month study of estragole, and the observation of a variety of lesions in rats and mice including neoplasms in the liver of male rats. Dr. David Malarkey, NIEHS, described the cholangiofibrosis and cholangiocarcinomas observed in this study. The proposed conclusions were:
Estragole is carcinogenic to male F344/N rats based on the occurrence of cholangiocarcinomas and a hepatocellular adenoma in the liver. Because rats and mice were exposed for only 3 months, these studies do not assess the full carcinogenic potential of estragole. The combined evidence from the present study and previously published studies of the genetic toxicity, carcinogenicity, and metabolism of estragole and structurally similar 2-phenylpropene analogs suggests that it is highly likely that estragole would be carcinogenic to additional sites in rats and mice after longer exposures.

Subcommittee Discussion

Dr. Pino, the first principal reviewer, inquired why an apparent difference in white blood cell count was discounted as an effect. He suggested that some nonneoplastic lesions that did not achieve statistical significance also merited mentioning, and that organ weight changes be identified as primary effects or secondary to other effects. He thought that periportal inflammation and histiocytic periportal infiltrates in the liver should be mentioned in the

discussion since it was not seen with methyleugenol while cholangiofibrosis was seen in both studies. He also suggested that the conclusion statement focus on the target organs.

Dr. Crump, the second principal reviewer, felt some qualifier was needed in the conclusion, rather than simply a statement that the chemical was carcinogenic (3 animals in the high dose group). He also questioned whether an extrapolation to other sites was warranted in the conclusion statement as the dose used in this study would not be used in a 2-year bioassay.

Dr. Bradfield, the third principal reviewer, suggested that transcriptional profiling would be useful, and asked for a clearer statement of the purpose of the study.

Dr. G. Travlos, NIEHS, described the manual method that he used to estimate numbers of leukocytes present in blood smear samples prepared from the estragole study. His reanalysis revealed that the total leukocyte counts obtained by automated analysis were inaccurately high. It was theorized that exposure to estragole increased the resistance of some erythrocytes to the lysing agent used in the automated analysis, resulting in their inclusion in the total leukocyte counts. Because erythrocytes ordinarily far outnumber leukocytes, a small increase in erythrocyte counts would easily account for the erroneously elevated, instrument-derived, total leukocyte counts.

Dr. Bristol agreed that the descriptions of organ weight changes and nonneoplastic lesions would be expanded. He said the conclusion statement was consistent with that used in other short-term studies in which neoplasms had been observed. He noted that observing three tumors in a group of only ten rats after short-term exposure was highly unusual, and that estragole had already been judged to be a carcinogen by the California Environmental Protection Agency (Cal/EPA).

Dr. Mirsalis said he thought the conclusion statement should be limited to the lesions observed and not predict results of longer-term studies. He also noted that the *Salmonella* and micronucleus genetic toxicity tests were not good predictors for liver carcinogens. Discussion ensued as to whether a 2-year bioassay for estragole was warranted to determine a dose response relationship for risk assessment. Dr. Michelle Hooth, NIEHS, replied that estragole is a known mouse carcinogen and thus it does not seem necessary to undertake a 2-year bioassay. Dr. Mirsalis suggested that a 2-year bioassay in the rat be considered at much lower doses than those used in the 3-month study.

Both Drs. Pino and Mirsalis suggested that the third sentence of the conclusions, regarding prediction of long-term effects, be deleted. Dr. Crump suggested that the first sentence specify the numbers of animals with tumors. He also suggested the second sentence indicate that the

doses used in the short-term study may have exceeded the maximum tolerated doses and were higher than might be used in a long-term study.

During a break, a revised discussion was formulated to incorporate the above suggestions. Dr. Mirsalis moved, and Dr. Soper seconded, that the statement be accepted. Dr. M. Hooth, NIEHS, and Dr. J. Bucher, NIEHS, said that the statements regarding maximum tolerated doses

were not applicable for short-term studies such as this. Dr. Mirsalis withdrew the motion. Dr. Pino moved, and Dr. Mirsalis seconded, that the conclusions be accepted with the deletion of the statement regarding maximum tolerated doses and the inclusion of the nonneoplastic lesions. The statement thus read:

Under the conditions of these three-month studies, estragole showed carcinogenic activity based on the occurrence of two cholangiocarcinomas and one hepatocellular adenoma in the liver of three of ten male F344/N rats in the high dose group. Because rats and mice were exposed for only three months, these studies do not assess the full carcinogenic potential of estragole.

Nonneoplastic effects were observed in the liver, glandular stomach, nose, kidney, and salivary gland of male and female rats and in the testes, epididymides, and pituitary gland of male rats. Nonneoplastic effects were also observed in the liver and nose of male and female mice and in the stomach of female mice.

The motion was passed unanimously with 8 yes votes, 0 no votes, and 0 abstentions.

ISOEUGENOL

Dr. Bristol, NIEHS, introduced the toxicology and carcinogenesis studies of isoeugenol by describing its natural occurrence in plants, its uses in fragrances and spices, and the structures of the related chemicals in the phenylpropenoid family. He described the design of the short- and long-term NTP studies, the survival, body weights, and nonneoplastic lesions observed in the studies, the occurrence of neoplasms in the long-term study, and an overall comparison of the results of the NTP studies of estragole, methyleugenol, and isoeugenol.
The proposed conclusions were:

Under the conditions of these 2-year gavage studies, there was *equivocal evidence of carcinogenic activity* of isoeugenol in male F344/N rats based on increased incidences of rarely occurring thymoma and mammary gland carcinoma. There was *no evidence of carcinogenic activity* of isoeugenol in female F344/N rats administered 75, 150, or 300 mg/kg. There was *clear evidence of carcinogenic activity* of isoeugenol in male B6C3F1 mice based on increased incidences of hepatocellular adenoma, hepatocellular carcinoma, and hepatocellular adenoma or carcinoma (combined). There was *equivocal evidence of carcinogenic activity* of isoeugenol in female B6C3F1 mice based on increased incidences of histiocytic sarcoma.

Exposure to isoeugenol resulted in nonneoplastic lesions of the nose in male and female rats and the nose, forestomach, and glandular stomach in male and female mice.

Subcommittee Discussion
Dr. Crump, the first principal reviewer, felt the study was well conducted and he agreed with the conclusions.

Dr. Pino, the second principal reviewer, suggested that body weight be included as part of the dose-setting rationale and asked for clarification of whether organ weight effects were primary

or secondary. He thought the nonneoplastic kidney effects were worth highlighting in the summary.

Dr. Bradfield, the third principal reviewer, agreed with the conclusions.

Dr. Mirsalis suggested that the statistical significance of the micronucleus tests were sometimes misleading given a low value for the control group compared with historical controls.

Public Comments
Dr. Thomas Goodrich, representing AQUI-S New Zealand Ltd., contrasted the gas chromatography procedures used in the NTP study and by the manufacturer, and suggested that the material used in the NTP study had exceeded its shelf life.

Dr. Mark Toneby, from Scan Aqua, the European representative of AQUI-S, presented results of an *in vivo* mouse micronucleus test showing no effect from isoeugenol. He suggested that considering the negative outcome of other *in vitro* genotoxic tests by the NTP as well, that isoeugenol is not genotoxic.

Subcommittee Discussion (continued)
Dr. Bristol replied that besides gas chromatography, Nuclear Magnetic Resonance and High Pressure Liquid Chromatography with UV detection were used to assess the purity of the test material and no significant polymerization or degradation of the test material was found. Some of Dr. Goodrich's information referred to material used in a much earlier NTP study.

Dr. Crump moved, and Dr. Soper seconded, to accept the conclusion as written, with the inclusion of mention of the kidney nonneoplastic lesions in the mouse. The motion was carried with 8 yes votes, 0 no vote, and 0 abstentions.

5-(HYDROXYMETHYL)-2-FURFURAL

Dr. Richard Irwin, NIEHS, introduced the toxicology and carcinogenesis studies of 5-(hydroxymethyl)-2-furfural by describing its ubiquitous occurrence in foods, reviewing the background literature relating to its metabolic activation to a potential carcinogen, and describing the design and results of the short- and long-term NTP studies. The proposed conclusions were:

Under the conditions of these 2-year gavage studies, there was *no evidence of carcinogenic activity* of 5-(hydroxymethyl)-2-furfural in male or female F344/N rats administered 188, 375, or 750 mg/kg. There was *no evidence of carcinogenic activity* of 5-(hydroxymethyl)-2-furfural

in male B6C3F1 mice administered 188 or 375 mg/kg. There was *some evidence of carcinogenic activity* of 5-(hydroxymethyl)-2-furfural in female B6C3F1 mice based on increased incidences of hepatocellular adenoma in the 188 and 375 mg/kg groups.

Administration of 5-(hydroxymethyl)-2-furfural was associated with increased incidences of lesions of the olfactory and respiratory epithelium of the nose in male and female rats and mice.

Subcommittee Discussion
Dr. Mirsalis, the first principal reviewer, thought the study was well performed and written. He inquired about the neurobehavioral effects and recognized that a follow-up study would be included in the final report. He inquired if an overall human exposure level could be estimated.

Dr. Soper, the second principal reviewer, also agreed with the conclusions. He suggested that a poly 3 trend test should not include a group that exceeded the maximum tolerated dose, as this group was not used for the consideration of carcinogenic response.

Dr. Pino, the third principal reviewer, inquired about the thyroid C-cell adenomas and clear cell liver foci that were mentioned in the results, but not in the summary. Dr Pino stated that the report should clearly indicate whether or not these changes are considered compound-related and why**.** Dr. Irwin replied that certain increases in lesion incidences in particular groups are mentioned for completeness even if they are not considered treatment related.

Dr. Howard said that inclusion of estimates of human consumption compared with doses administered would be quite difficult to calculate given the wide variety of diets. He added that inclusion of this information in the discussion would enter the domain of risk assessment.

Dr. Crump also thought the occurrence of the thyroid tumors seemed noteworthy. Dr. Irwin noted that the control incidence in this study was unusually low.

Dr. Mirsalis moved, and Dr. Soper seconded, that the conclusions be accepted as written. The motion was approved unanimously with 8 yes votes, 0 no votes, and 0 abstentions.