National Toxicology Program
Board of Scientific Counselors

Summary Minutes from Peer Review of Draft Technical Reports of Long-Term Toxicology and Carcinogenesis Studies by the Technical Reports Review Subcommittee

on

May 21, 1999

Research Triangle Park, NC

The meeting began at 9:00 a.m. on May 21, 1999 in the Conference Center, Building 101, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina. Members of the Subcommittee are: Drs. Gary Carlson (Chairperson), John Bailer, Steven Belinsky, James Bus, Linda Chatman, John Cullen, Harold Davis, Susan Fischer, Stephen Hecht, Michele Medinsky, and Jose Russo. Dr. Bus was not present. These minutes have been reviewed and approved by the Chairperson. They were written by Dr. Larry G. Hart.

When available, a final NTP Technical Report for the studies may be obtained through the Environmental Health Information Service (EHIS). Call 919-541-3841, Fax 919-541-0273, e-mail ehis@nih.gov, or subscribe on-line at ehis@niehs.nih.gov.

The next NTP Technical Reports Peer Review meeting will be held May 18, 2000 in Research Triangle Park, North Carolina. For information, contact Dr. Mary S. Wolfe, Executive Secretary, at 919-541-3971.

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NTP Technical Report Reviews

Gallium Arsenide Dr. J. H. Roycroft, NIEHS, introduced the toxicology and carcinogenesis studies of gallium arsenide by discussing the uses and rationale for study, describing the experimental design in mice and rats, reporting on survival and body weight effects, and commenting on compound-related neoplastic lesions in female rats and non-neoplastic lesions in male and female mice and rats. Additionally, lung burden studies were conducted in male rats from the 14-week and 2-year studies. The conclusions for carcinogenic activity for the two-year studies in rats and mice were that:

> Under the conditions of these 2-year inhalation studies, there was **no evidence of carcinogenic activity** of gallium arsenide in male F344/N rats exposed to 0.01, 0.1, or 1.0 mg/m^3. There was **clear evidence of carcinogenic activity** in female F344/N rats based on increased incidences of benign and malignant neoplasms in the lung. Increased incidences of benign neoplasms of the adrenal medulla and increased incidences of mononuclear cell leukemia in female rats were also considered to be exposure related. There was **no evidence of carcinogenic activity** in male or female B6C3F$_1$ mice exposed to 0.1, 0.5, or 1.0 mg/m^3.
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> Exposure to gallium arsenide caused a spectrum of non-neoplastic lesions in the lung of rats and mice, the larynx of male rats, and the tracheobronchial lymph node of mice.

Dr. Belinsky, a principal reviewer, agreed with the conclusions.

Dr. Davis, the second principal reviewer, agreed with the conclusions. He noted that the basis for selecting dose concentrations in the rat 2-year study were increased severity of lung lesions (proteinosis and inflammation) in the 14-week study, yet inflammation was not increased at 10 mg/m^3 over that at 1 mg/m^3, the high dose selected for the 2-year study. Dr. Roycroft responded that selection for exposure concentrations was based primarily on the proteinosis and the basis for exposure concentration selection would be clarified in the Report. Further, Dr. Davis wondered if tumors would have been seen in male rats had higher doses been used Dr. Roycroft agreed that a higher dose might have been tolerated, but based on lung weight increases of about 60% in the 14-week study at 1 mg/m^3 and the presence of an animal with fibrosis, the high dose chosen for the chronic study was sufficiently challenging. Dr. Davis stated that it would be helpful to have a reason for why lung burden was not assessed in female rats. Dr. Roycroft replied that there was considerable literature data in male rats on absorption of gallium and arsenic, as well as more experience with particulate studies.

Dr. Bailer, the third principal reviewer, agreed with the conclusions. He thought that in setting exposure levels it would have been useful to have linked them to typical human

occupational exposures. In this context, he was surprised to see human exposure estimates dated from 1981 and would have thought that more recent information would be available. Dr. Roycroft said the 1981 estimate was the best available, but was not specific to gallium arsenide. Dr. Mark Toraason, NIOSH, commented that NIOSH was embarking on a new effort to reinitiate the National Occupational Exposure Survey (NOES), so perhaps newer exposure data will be available in the future. Dr. Bailer stated that in plots of lung burdens in male rats for gallium and arsenic with increasing exposure concentrations over time (Figure 8), the superimposed lung deposition and clearance model did not fit the data for the high dose conditions for days 150 and beyond, most particularly in the high dose at 18 months. Dr. John Bucher, NIEHS, agreed that from a toxicological standpoint, the model clearly was inadequate to explain what is happening toward the end of the study. He said the interest in following lung burden throughout the study was to determine whether an overload situation was reached. Dr. Bailer observed that neither the data nor the model fit suggest an overload phenomenon.

In further discussion, Dr. Medinsky had a general philosophical question concerning balancing the need to obtain complete toxicokinetic information during a chronic study to aid in understanding mechanisms of toxicity while not delaying reporting out of primary toxicologic and carcinogenic information. Dr. George Lucier, NIEHS, agreed that the need to release toxicologic data may sometimes preclude reporting the complete toxicokinetic story. Dr. Russo asked whether the alveolar proteinosis appeared before hyperplasia. Dr. Ronald Herbert, NIEHS, responded that the proteinosis was most prominent in prechronic studies although there was some hyperplasia, while in the 2-year studies, these lesions were seen together in the same animals.

Dr. Belinsky moved that under the conditions of this study the Technical Report on gallium arsenide be accepted with revisions discussed and the conclusions as written for male rats and male and female mice, **no evidence of carcinogenic activity**, and for female rats, **clear evidence of carcinogenic activity**. Dr. Davis seconded the motion, which was accepted unanimously with nine yes votes.

Emodin Dr. Richard Irwin, NIEHS, introduced the toxicology and carcinogenesis studies of emodin by discussing the uses and rationale for study, describing the experimental design in mice and rats, reporting on survival and body weight effects, and commenting on possible compound-related neoplastic lesions in female rats and male mice and non-neoplastic lesions in male and female rats and mice. The conclusions for carcinogenic activity for the two-year studies in mice and rats were that:

> Under the conditions of these 2-year feed studies, there was **no evidence of carcinogenic activity** in male F344/N rats exposed to 280, 830, or 2,500 ppm of emodin. There was **equivocal evidence of carcinogenic activity** of emodin in female F344/N rats based on a marginal increase in the incidence of Zymbal's gland carcinoma. There was **equivocal evidence of carcinogenic activity** of emodin in male B6C3F_1 mice based on a low incidence of uncommon renal tubule

neoplasms. There was **no evidence of carcinogenic activity** of emodin in female B6C3F$_1$ mice exposed to 312,625, or 1,250 ppm.

Exposure of rats to emodin resulted in increased incidences of renal tubule hyaline droplets and pigmentation in males, increased incidences of renal tubule hyaline droplets in females, and increased severities of renal tubule pigmentation in males and females. Emodin exposure resulted in increased incidences of renal tubule pigmentation in male and female mice and increased incidences of nephropathy in female mice.

Decreased incidences of mononuclear cell leukemia occurred in male and female rats.

Dr. Hecht, a principal reviewer, agreed in principle with the conclusions. He did not agree with the statement that squamous cell carcinomas of the nose in rats could be considered unrelated to emodin exposure. Noting that the rat nose is known to be a rich source of cytochrome P450 enzymes, which could metabolically activate emodin, Dr. Hecht stated that the possibility that these tumors may be related to emodin exposure cannot be categorically ruled out. Dr. Irwin agreed that the potential for metabolic activation existed, but the fact that there were just two animals with neoplasms in the nose and no other indications of preneoplastic activity such as squamous metaplasia, spoke against an effect.

Dr. Chatman, the second principal reviewer, agreed with the conclusions. She wondered why emodin, being a cathartic, did not show laxative effects in rodents in the chronic study and inquired whether water consumption was monitored. Dr. Irwin said they were surprised not to see cathartic effects. Water consumption was not specifically monitored. Dr. Chatman asked whether in view of conflicting results on genotoxicity there were any additional studies planned. She noted the first pass effect and need for metabolic activation that suggested a metabolite as the genotoxic form. Dr. Irwin responded that further studies were not planned. A metabolite, 2-hydroxyemodin, is the genotoxin. Dr. Chatman commented on increased estrogenic activity of emodin reported in an early study and wondered whether there was potential for reproductive problems in women who abuse laxatives. Dr. Irwin said that was a good question and thought it might be worthwhile nominating emodin for endocrine disruptor screening.

Dr. Russo, the third principal reviewer, agreed with the conclusions. In response to a question from Dr. Russo about estrogenic effects of emodin, Dr. Irwin reported that there was a lengthening of the estrous cycle in treated rodents but no changes in morphology in the uterus.

There was considerable discussion among members and staff as to whether increased incidences of bone marrow hyperplasia and hematopoietic cell proliferation in rats were treatment-related effects or really secondary to decreased incidences of mononuclear cell leukemia (MCL) in male and female rats. Dr. Hailey and Dr. Abraham Nyska, NIEHS, concluded these increases were secondary to decreased MCL. They were asked to

discuss this interpretation in the report. Dr.Medinsky stated that there needed to be a richer discussion on the toxicokinetics, noting that low blood levels of emodin could be due to poor gastrointestinal absorption and/or an extensive first pass effect by the liver in which a majority of the parent compound is metabolized. Dr. Irwin agreed and noted that extensive studies were not done due to limited amounts of the test material and because of considerable information on metabolism and disposition in the literature. Dr. Cullen observed that emodin may not be a cathartic in rodents due to a different gut structure from humans and certainly different colonic function in terms of reabsorption capability. Dr. Bailer had questions about the lack of attention to several neoplasms where there were small, apparently dose-related increases, such as Harderian gland carcinomas. Dr. Hailey explained that NTP considers combined analysis of benign and malignant tumors to be the most important analysis in looking at tumorigenic effects, and in doing this with Harderian gland, there is neither a positive trend or pairwise differences. Dr. Chatman asked if possible anti-leukemic effects of emodin were being evaluated. Dr. Irwin replied that emodin was being evaluated for human use as an anticancer agent, as well as some derivatives, and he would add this to the report.

Dr. Hecht moved that under the conditions of this study the Technical Report on emodin be accepted with revisions discussed and with the conclusions as written for male rats and female mice, **no evidence of carcinogenic activity**, and for female rats and male mice, **equivocal evidence of carcinogenic activity**. Dr. Chatman seconded the motion, which was accepted unanimously with nine yes votes.

Anthraquinone Dr. Richard Irwin, NIEHS, introduced the toxicology and carcinogenesis studies of anthraquinone by discussing the uses and rationale for study, describing the experimental design in rats and mice, reporting on survival and body weight effects, and commenting on compound-related neoplastic and non-neoplastic lesions in male and female rats and mice. The conclusions for the 2-year studies in rats and mice were that:

> Under the conditions of these 2-year feed studies, there was **some evidence of carcinogenic activity** of anthraquinone in male F344/N rats based on increased incidences of renal tubule adenomas and of transitional epithelial papillomas of the kidney and urinary bladder. Hepatocellular neoplasms may have been related to exposure to anthraquinone. There was **clear evidence of carcinogenic activity** of anthraquinonein female in F344/N rats based on increased incidences of renal tubule neoplasms. Increases in the incidences of urinary bladder transitional epithelial papilloma or carcinoma (combined) and of hepatocellular adenomas in female rats were also related to anthraquinone exposure. There was **clear evidence of carcinogenic activity** in male and female $B6C3F_1$ mice based on increased incidences of liver neoplasms. Thyroid gland follicular cell neoplasms in male and female mice may have been related to anthraquinone exposure.
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> Exposure to anthraquinone for two years caused increases in the incidences of

nonneoplastic lesions of the kidney, liver, spleen, and bone marrow in male and female rats, and of the liver, urinary bladder, and spleen in male and female mice, and the thyroid gland and kidney in male mice.

Decreased incidences of mononuclear cell leukemia in male and female rats were attributed to exposure to anthraquinone.

Dr. Irwin reported that the metabolism of anthraquinone is extremely complicated and described short-term studies in rats that measured activities in the liver of two cytochrome P450 enzymes and levels of 8-hydroxy-2'-deoxyguanosine in liver, kidney and bladder. While there was only modest induction of the hepatic activity of ethoxyresorufin-O-dealkylase (P_{450} 1A1) activity, there was a strong induction of pentoxyresorufin-O-dealkylase (P_{450} 2B1) activity. This amounted to about an 80-fold increase over control in male rats and about a 40-fold increase over control in females. Dr. Irwin stated that cell proliferation was measured in liver, kidney and urinary bladder after administration of BrdU in drinking water. There was no increased proliferation in liver or kidney in rats but moderate increases in urinary bladder. Dr. Irwin compared neoplastic findings in anthraquinone, the parent compound, with findings in six substituted anthraquinone derivatives studied by the NTP. He concluded that the parent ring system confers carcinogenic potential while the various substituents play a major role in determining the target organs affected and strength of the carcinogenic response.

Dr. Christopher Portier, NIEHS, presented data on the toxicokinetics (TK) of anthraquinone, noting that standard TK protocols were run along with measurements of biliary levels after a single intravenous (IV) study in male rats. The model used was a standard physiologically based pharmacokinetic model (PBPK) for highly lipophilic compounds. Dr. Portier said that there did not appear to be a first pass effect in the liver but rather absorption was directly into venous blood, while distribution was through a restricted capillary: tissue transport mechanism. Metabolism was through inducible Michaelis-Menten kinetics in the liver. Elimination is through urinary and biliary excretion of parent and metabolites with some enterohepatic cycling. Dr. Portier presented graphical data comparing actual data points with points predicted by the model. He summarized conclusions drawn from the TK data. First, there is delayed absorption and very slow clearance. In the female rat, there are higher tissue concentrations due to slow clearance and slower metabolism of anthraquinone. Transport is diffusion limited in most tissues. There is markedly slower absorption from feed than from gavage dosing. Finally, chronic exposure induces metabolism of the parent compound.

Dr. Medinsky, a principal reviewer, agreed with the conclusions. She thought the pharmacokinetics supported the conclusions for carcinogenicity and provided an adjunct to our understanding, especially with regard to why there was **clear evidence** in female but not in male rats. Dr. Medinsky commented that it was difficult to adequately evaluate the model because of lack of explanatory text regarding assumptions underlying the model.

Dr. Cullen, the second principal reviewer, agreed with the conclusions. He asked for clarification on the relationship of the alpha2μ-globulin protein droplet renal injury in the 14-week studies in male rats and its relationship to risk of tumor development in the 2-year studies. Dr. Hailey responded that if we see a significant amount of alpha2μ-globulin in the kidney with angular crystals and an increase in renal tumors, then the mode of action seems fairly well described. However, this would not explain the increased incidence of renal tumors in female rats, who do not secrete much alpha2μ-globulin, and therefore, he didn't think we would be able to sort out what part of the kidney tumor effect in males might be related to alpha2μ nephropathy and what part might be related to a mechanism of action operative in females. Dr. Cullen asked for discussion on the rationale for setting higher chronic doses in mice than in rats and whether this may have impacted on the incidence of hepatocellular tumors in rats. Dr. Irwin commented that nephropathy is always a major consideration for setting doses in rats, and that along with increases in hepatocellular hypertrophy, were the major determinants for selecting doses in rats, while increases in liver weights in mice, as much as 30 % at the high dose in 14-week studies, were a major factor in setting doses in mice.

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

In further discussion about the dose setting, Dr. Davis asked if the lack of 14-day studies may have resulted in there not having been low enough doses set for the 14-week and 2-year studies, i.e., there was no dose in the latter where increased tumor incidence was not seen. He wondered what this meant with regard to human exposure. Dr. Russo observed that there was a clear dose-response. Dr. Davis agreed, but said it was still helpful to have a no effect level. Dr. Carlson suggested that a better explanation of how the doses were set is needed. Dr.Irwin said that there is always an attempt to reach a no effect level.

Dr. Medinsky moved that under the conditions of this study the Technical Report on anthraquinone be accepted with revisions discussed, with the inclusion that renal tubule neoplasms in male mice may have been related to anthraquinone exposure, and otherwise, with the conclusions as written for male rats, **some evidence of carcinogenic activity**, and for female rats and male and female mice, **clear evidence of carcinogenic activity**. Dr. Cullen seconded the motion, which was accepted unanimously with nine yes votes.

Fumonisin B_1 Dr. Bucher reported that the 2-year studies on fumonisin B_1 (FB1) were the first to come to the Subcommittee for peer review under an Interagency Agreement signed in 1992 between the Food and Drug Administration (FDA) and the National Institute of Environmental Health Sciences (NIEHS) to support the performance of studies evaluating the toxicology and carcinogenic activity of chemicals and agents that were primarily of interest to the FDA. Other agents being studied under the Agreement include chloral hydrate, malachite green, urethane and ethanol combinations, and most recently, endocrine disrupting compounds. Dr. Bucher added that the Agreement also has supported developmental toxicology, neurotoxicology and mechanistic studies on

FB1. Dr. Allaben pointed out that a driving force for the Agreement was the intent to give regulatory review scientists and researchers the kind of information needed to make quality sound regulatory decisions. He noted that a co-principal investigator with a National Center for Toxicological Research (NCTR) scientist is a scientist from the FDA Center that has regulatory responsibility for using the data at the conclusion of the studies. Dr. Paul Howard, NCTR/FDA, acknowledged the contributors to the studies at NCTR, Pathology Associates International (PAI), FDA Center for Food Safety and Applied Nutrition (CFSAN), and elsewhere, some of whom were present, including Dr. Ronald Lorentzen, CFSAN, and Dr. Ken Voss, United States Department of Agriculture (USDA) who were co-principal investigators with Dr. Howard.

Dr. Howard introduced the toxicology and carcinogenesis studies of fumonisin B_1 (FB1) by noting that FB1 is the most prevalent of a number of fungal metabolites of *Fusaria* species found primarily on corn in the U.S. and around the world and reporting on the principal mode of action of FB1 in interrupting sphingolipid synthesis. Dr. Howard characterized the toxicity of FB1 in various laboratory and domestic animal species. He said that to date there has not been a thorough study of human exposure using good analytical criteria. However, the association of human esophageal cancer with *Fusaria* contaminated corn in South Africa is suggested in correlative and epidemiological studies. These findings, along with findings from a South African study of FB1 in rats reporting a high incidence of hepatocellular neoplasms, led to nomination by CFSAN of FB1 to NTP for carcinogenicity testing. Dr. Howard then described the experimental design for 28-day and 2-year studies in rats and mice, including clinical chemistry indicators of hepatotoxicity, renal toxicity, sphingolipid metabolism, and measures of apoptotic activity. He reported on survival and organ and body weight effects, and commented on compound-related neoplastic lesions in male rats and female mice, and non-neoplastic lesions in male rats and male and female mice. The conclusions for carcinogenic activity for the 2-year studies in rats and mice were that:

> Under the conditions of these 2-year feed studies, there was **clear evidence of carcinogenic activity** of fumonisin B_1 in male F344/N rats based on the increased incidences of renal tubule neoplasms. There was **no evidence of carcinogenic activity** of fumonisin B_1 in female F344/N rats exposed to 5, 15, 50, or 100 ppm. There was **no evidence of carcinogenic activity** of fumonisin B_1 in male $B6C3F_1$ mice exposed to 5, 15, 80 or 150 ppm. There was **clear evidence of carcinogenic activity** of fumonisin B_1 in female $B6C3F_1$ mice based on the increased incidences of hepatocellular neoplasms.
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> The sphinganine/sphingosine ratios were increased in the urine and the kidney tissue of rats receiving diets containing fumonisin B_1. There was evidence of apoptosis and increased cell proliferation of the renal tubule epithelium in exposed rats, particularly in those groups of males that developed renal tubule neoplasms. Increased incidences of hyperplasia of the renal tubule epithelium also occurred in these groups of male rats.
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> In mice exposed to the higher concentrations of fumonisin B_1, males and females

had increased incidences of hepatocellular hypertrophy and females had increased incidences of hepatocellular apoptosis.

Dr. Fischer, a principal reviewer, agreed with the conclusions. She asked for better definition of the core study group and what the additional animals were, and which animals were being used for which part of the studies. Dr. Howard explained that the core animals were fed continously for 28 days while the other animals were fasted overnight at intervals for collection of urine or blood, and this distincion would be clarified. Dr. Fischer thought the Abstract would be more complete if information were added indicating that FB1 is an inhibitor of ceramide synthase and that this is responsible for the biological consequences of FB1 exposure. Dr. Howard agreed. Dr. Fischer noted that because there are such big species differences in response to FB1 with regard to the particular target tissue affected there should be more discussion about the relevance of all of these animal studies to the human situation. Dr. Howard commented that the mechanisms of action of these different target organ effects are not well understood, and further, there is no validated biomarker for human exposure so relevance of animal to human findings would be hard to discern at this point.

Dr. Bailer, the second principal reviewer, agreed with the conclusions although he thought the increases in alveolar/bronchiolar adenomas or carcinomas in female rats should be listed as an uncertain finding in the Abstract. Dr. Howard said that the 2% incidence in the high dose was not considered a significant enough increase, although this could be argued by some as fitting **equivocal evidence.** Dr. Bailer questioned not doing complete histopathology on some of the mid exposure groups, clearly decreasing the sensitivity of detecting tumor onset and trend with a loss of dose-response information. Dr. Howard responded that this was the protocol agreed on for the study. However, knowing that liver and kidney were likely target organs, these organs were examined from intermediate dose groups as well, and of course, animals dying before terminal sacrifice had complete histopathology, regardless of dose group. Dr. Bucher commented that where there is good interaction among pathologists, study directors, and the test laboratory, there is always the ability to go back and cut in tissues as needed. Dr. Carlson commented that in view of toxicity to heart, lung, and esophagus in other species, one could argue that there should have been complete histopathology on these organs. Dr. Bailer opined that incomplete histopathology may have led to some bizarre neoplasm patterns, e.g., the tumor burden for Harderian gland adenomas or carcinomas in male mice. Dr. Ralph Kodell, NCTR, said that his opinion was that ideally the data for the intermediate doses from animals dying before terminal sacrifice should not be used in a statistical analysis.

Dr. Davis, the third principal reviewer, agreed with the conclusions. He had some concerns about the dose selection noting the lack of tumor response in female rats and male mice and the statement that female rats could have tolerated a higher dose. Dr. Howard responded that doses for the 2-year study were selected based on multiple issues including the hepatotoxicity, nephrotoxicity, and literature information on mechanisms of target organ toxicity. Further, Dr. Davis criticized the lack of doing 90-day studies in conjunction with the 2-year bioassay while relying on 90-day studies done by Voss *et al.*

Dr. Howard stated that staff debated whether to conduct another 90-day study, but since the study by Voss *et al.* was conducted with test material provided by the NTP and was under NTP guidance, the study was considered adequate for moving ahead to a 2-year study. Dr. Davis also expressed concern about the underfeeding by 30% of the mice in the 2-year study. Dr.Howard explained that the doses in the feed were accurate, but the mice consumed 30 % less feed. As a partial explanation, it was realized about nine months into the study that the mice were lighter in weight than the average NTP mouse at this point. The decision was made to continue the study. Dr. Howard said that a factor in the decision not to stop and restart was the cost of the material, $40,000 a gram, and the lengthy time (years) required to purify it.

In other discussion, Dr. Russo asked what human doses from contaminated corn would be in relation to 100 ppm doses in animals. Dr. Howard reported that in South Africa where esophageal cancer is being seen and where corn is an everyday staple in the diet, estimates of human intake are around 0.2 mg/kg/day which is within a couple of orders of magnitude of the animal dose. Dr. Russo asked whether there were any pathologic changes in the esophagi of study animals. Dr. Howard responded that there were not, which is at variance with other reported rat studies in which hyperplasia was reported. Dr. Hecht stated that at least 50 nitrosamines can induce esophageal tumors in rats, which suggests that FB1 may not be the agent responsible for esophageal cancer in humans. Dr. Howard acknowledged the possible presence of nitrosamines in fungally contaminated corn. He concluded that a proper epidemiological study has not yet been done with fumonisin; rather the best studies available are correlative.

Dr. Fischer moved that under the conditions of this study the Technical Report on fumonisin B_1 be accepted with revisions discussed and the conclusions as written for male rats and female mice, **clear evidence of carcinogenic activity**, and for female rats and male mice, **no evidence of carcinogenic activity**. Dr. Davis seconded the motion, which was accepted unanimously with eight yes votes. (Dr. Bailer was not present.)

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