Board of Scientific Counselors
National Toxicology Program

Summary Minutes
from

Peer Reviews of Draft Technical Reports of Long-Term Toxicology and Carcinogenesis Studies by the Technical Reports Review Subcommittee and Panel of Experts
on

October 3 and 4, 1988
Research Triangle Park, North Carolina

The review meeting began at 9:00 a.m. on October 3 in the Conference Center, Building 101, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina. Members of the Subcommittee are: Drs. Robert Scala (Chairperson), Michael Gallo and Frederica Perera. Members of the Panel of Experts are: Drs. John Ashby, Robert Garman, Lois Gold, Curtis Klaassen William Lijinsky, Barbara McKnight, Franklin Mirer, Paul Newberne, and James Popp. Drs. Lijinsky and Scala were unable to attend this meeting. Dr. Perera served as Chairperson. These minutes have been reviewed and approved by all members of the Subcommittee and Panel present. They were written by Dr. Larry Hart, Executive Secretary.

When available, a limited number of final NTP Technical Reports for the studies may be obtained free of charge from the NTP Public Information Office, MD B2-04, P. O. Box 12233, Research Triangle Park, NC 27709. Telephone: (919) 541-3991; FTS: 629-3991. Subsequently, they may be purchased from the National Technical Information Service, U. S. Department of Commerce, 5285 Port Royal Road, Springfield, Virginia 22161, (703) 487-4650.

The next NTP technical reports peer review meeting will be held March 13, 1989, in Research Triangle Park, North Carolina. For information, contact Dr. Hart, (919) 541-3971; FTS: 629-3971.

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Bromoethane. Dr. J.H. Roycroft, NIEHS, NTP Chemical Manager, introduced the toxicology and carcinogenesis studies of bromoethane by reviewing the experimental design, results, and proposed conclusions:

> Under the conditions of these 2-year inhalation studies, there was equivocal evidence of carcinogenic activity of bromoethane for male F344/N rats, as indicated by marginally increased incidences of pheochromocytomas of the adrenal gland and neoplasms of the brain and lung. For female F344/N rats, there was equivocal evidence of carcinogenic activity, as indicated by marginally increased incidences of neoplasms of the brain and lung. For male B6C3F1 mice, there was equivocal evidence of carcinogenic activity, based on marginally increased incidences of neoplasms of the lung. There was clear evidence of carcinogenic activity for female B6C3F1 mice, as indicated by neoplasms of the uterus.

Dr. Mirer, a principal reviewer, agreed with the conclusions for female rats and male and female mice. He proposed that the conclusion for male rats be changed to some evidence of carcinogenic activity based on the increased incidence of pheochromocytomas. He thought there should be some discussion on significance of the non-malignant pheochromocytomas including whether there was evidence of progression in other studies. Dr. Roycroft commented that pheochromocytomas do progress; however, they are late appearing and not considered life-threatening, and in this study most of the tumors were small and not seen at necropsy. Dr. Mirer said it appeared that rats of both sexes and male mice could have been given higher doses.

Dr. Newberne, the second principal reviewer, agreed with the conclusions.

Dr. Gallo opined that the increased incidence in pheochromocytomas along with increased incidences of uncommon tumors of the lung and brain were supportive of some evidence in male rats. Dr. Perera noted the increased incidence in brain neoplasms in female rats and commented on similar increases in female rats in a companion study of chloroethane asking why the analogous findings would not lend support to a conclusion of some evidence in female rats. Dr. Roycroft responded that in both studies the increases were not statistically significant either from pairwise comparisons or from a trend test. Additionally, there were no supporting increases in hyperplasia. However, these are uncommonly occurring neoplasms.

Dr. Mirer moved that the conclusion for male rats be changed from equivocal evidence to some evidence of carcinogenic activity based on increased incidences of pheochromocytomas of the adrenal gland. Dr. Gallo seconded the motion, which was approved by six yes votes (Gallo, Gold, Klaassen, McKnight, Mirer, Newberne) to two no votes (Garman, Popp). Dr. Mirer moved that the conclusion for female rats be accepted as written, equivocal evidence of carcinogenic activity. Dr. Gold seconded the motion, which was approved unanimously with eight votes. Dr. Mirer moved that the conclusion for male mice be accepted as written, equivocal evidence of carcinogenic activity. Dr. Gallo seconded the motion, which was approved unanimously with eight votes. Dr. Mirer moved that the conclusion for female mice be accepted as written, clear evidence of carcinogenic activity. Dr. Gold seconded the motion, which was approved unanimously with eight votes.

Chloroethane. Dr. J.H. Roycroft, NIEHS, NTP Chemical Manager, introduced the toxicology and carcinogenesis studies of chloroethane by reviewing the experimental design, results, and proposed conclusions:

> Under the conditions of these 2-year inhalation studies, there was equivocal evidence of carcinogenic activity of chloroethane for male F344/N rats, as indicated by benign and malignant epithelial neoplasms of the skin. For female F344/N rats, there was equivocal evidence of carcinogenic activity, as indicated by three uncommon malignant astrocytomas of the brain in the exposed group. The study in male B6C3F1 mice was considered to be an inadequate study of carcinogenic activity because of reduced survival in the exposed group. However, there was an increased incidence of alveolar/bronchiolar neoplasms of the lung. There was clear evidence of carcinogenic activity for female B6C3F1 mice, as indicated by carcinomas of the uterus. A marginally increased incidence of hepatocellular neoplasms was observed in the exposed group.
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> Although no chemically related toxic effects were observed in the short-term studies, concerns about potential flammability and explosion led to selection of 0 and 15,000 ppm as the exposure concentrations for rats and mice for the 2-year studies.

Dr. Newberne, a principal reviewer, agreed with the conclusions for female rats and male and female mice. He thought the conclusion for male rats should be changed to no evidence of carcinogenic activity.

Dr. Mirer, a second principal reviewer, agreed with the conclusions in male and female rats and female mice although he thought the incidence of hepatocellular neoplasms in female mice should be considered part of the evidence also, and not be designated as a marginal effect. He argued that the increase in lung tumors in male mice was observed in spite of the high mortality and should be considered supportive of some evidence of carcinogenic activity. Dr. J. Haseman, NIEHS, said that if the NTP considered the increase in lung tumors to be a positive effect, the study would not have been called inadequate. Dr. J. Huff, NIEHS, commented that the early mortality also decreased the sensitivity of the studies for detecting tumors developing later in life. Dr. Mirer stated that the choice of a single dose compromised the ability of the experiments to observe any dose response given the overwhelming effect in female mice. Dr. Perera suggested adding a sentence in the Abstract explaining the use of a single dose. Dr. Roycroft said a single dose was chosen after no toxic effects were seen in 90-day studies at up to 19,000 ppm.

Dr. Newberne moved that the conclusion for male rats be changed to no evidence of carcinogenic activity. The motion was not seconded. Dr. Newberne then moved that the conclusion for male rats be accepted as written, equivocal evidence of carcinogenic activity. Dr. Gallo seconded the motion, which was approved unanimously with eight votes. Dr. Newberne moved that the conclusion for female rats be accepted as written, equivocal evidence of carcinogenic activity. Dr. Mirer seconded the motion, which was approved unanimously with eight votes. Dr. Newberne moved that the conclusion for male mice be accepted as written, inadequate study of carcinogenic activity. Dr. Gallo seconded the motion, which was approved by six yes votes (Gallo, Garman, Gold, Klaassen, Newberne, Popp) to two no votes (McKnight, Mirer). Dr. Newberne moved that the conclusion for

female mice be accepted as written, clear evidence of carcinogenic activity. Dr. Popp seconded the motion. There was discussion as to whether or not the word 'marginally' should be removed from the sentence: "A marginally increased incidence of hepatocellular neoplasms was observed in the exposed group". A request by Dr. Mirer to amend the motion was denied. The original motion was then approved by five yes votes (Gallo, Garman, Gold, Newberne, Popp) to three no votes (Klaassen, McKnight, Mirer).

Dimethoxane. Dr. K.M. Abdo, NIEHS, NTP Chemical Manager, introduced the toxicology and carcinogenesis studies of dimethoxane by reviewing the experimental design, results, and proposed conclusions:

> Under the conditions of these 2-year corn oil gavage studies, there was no evidence of carcinogenic activity of dimethoxane for male F344/N rats receiving 62.5 or 125 mg/kg or for female F344/N rats receiving 125 or 250 mg/kg per day. There was equivocal evidence of carcinogenic activity of dimethoxane for male B6C3F1 mice, as indicated by an increased incidence of forestomach neoplasms. There was no evidence of carcinogenic activity for female B6C3F1 mice receiving 250 or 500 mg/kg per day. Acanthosis and hyperkeratosis occurred at increased incidences in the forestomach of high dose rats. Inflammation, acanthosis with hyperkeratosis, and focal hyperplasia occurred at increased incidences in the forestomach of dosed mice.

Dr. Ashby, a principal reviewer, agreed with the conclusions although he asked for clarification as to why the conclusion in male mice was not some evidence of carcinogenic activity since the levels of squamous papillomas of the forestomach were similar to levels in a previous study (benzyl acetate) that was called some evidence. Dr. Abdo said the conclusion for male mice in the study with benzyl acetate (NTP TR No. 250) was based primarily on increased incidence of liver tumors with supporting evidence from the lesions of the forestomach. Dr. S. Eustis, NIEHS, added that with the exception of a carcinoma in a high dose mouse, the rest of the forestomach neoplasms were papillomas that only met the minimum pathology requirements for diagnosis of a papilloma. Dr. Ashby opined that the impurities (20%) might play a role in the toxicity of this chemical, and, more specifically, the genetic toxicity was probably due to two of the impurities, acetaldehyde and crotonaldehyde.

Dr. Garman, the second principal reviewer, agreed with the conclusions. He asked for a brief discussion concerning human exposure to the hydrolysis products of dimethoxane and suggested that a repeat of the earlier inadequate water gavage study be considered. Dr. Abdo said the literature would be searched, in particular for information on the toxicity and chemical disposition of the major contaminants and hydrolysis products and relevant data would be added to the report. Dr. Garman asked for some clarification of pathology descriptive terminology used, especially in distinguishing between acanthosis and hyperplasia of the forestomach.

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

Dr. M. Manowitz, Givaudan Corporation, said he believed that his company was the only manufacturer of dimethoxane, and under contract in the mid 1970s had conducted a skin painting study in CD-1 Swiss Webster albino mice. The study lasted 80 weeks and gave no indication of local or systemic oncogenic or other toxic effects. The study was not published but a report of the study was submitted to the National Cancer Institute. He also pointed out that the chemical is not used in cosmetic preparations nor in products that are ingested or directly applied to human skin. Dr. J. Huff, NIEHS, recommended that these studies be published.

Dr. Ashby moved that the Technical Report on dimethoxane be accepted with the revisions discussed and with the conclusions as written for male and female

rats and female mice, no evidence of carcinogenic activity, and for male mice, equivocal evidence of carcinogenic activity. Dr. Klaassen seconded the motion, which was approved unanimously with nine votes.

Diphenhydramine Hydrochloride. Dr. R.L. Melnick, NIEHS, NTP Chemical Manager, introduced the toxicology and carcinogenesis studies of diphenhydramine hydrochloride by reviewing the experimental design, results, and proposed conclusions:

> Under the conditions of these 2-year feed studies, there was equivocal evidence of carcinogenic activity of diphenhydramine hydrochloride for male F344/N rats, based on marginally increased incidences of uncommon brain neoplasms (astrocytomas or gliomas) and of alvelolar/bronchiolar neoplasms. There was equivocal evidence of carcinogenic activity for female F344/N rats, based on a marginal increase in the incidence of pituitary gland adenomas. There was no evidence of carcinogenic activity for male or female B6C3F1 mice fed diets containing 156 or 313 ppm diphenhydramine hydrochloride.

Dr. Garman, a principal reviewer, agreed with the conclusions. In view of the rather high frequency of glial tumors in male rats, he wondered if consideration had been given to evaluating spinal cords from these rats. Dr. S. Eustis, NIEHS, said pieces of spinal cord were saved but not the organ in entirety. He doubted they would locate any additional tumors.

Dr. McKnight, the second principal reviewer, agreed with the conclusions. Both Dr. Garman and Dr. McKnight asked that more specific and detailed information be included about the Pathology Working Group (PWG) process. Dr. Melnick described the PWG processes used for the tissues from these studies, and mentioned that additional sections of brain from control and exposed male and female rats were evaluated. Dr. Garman suggested that identification of the target organ(s) evaluated by PWGs should be included in the writeup.

Dr. Klaassen, the third principal reviewer, agreed with the conclusions although he voiced some reservation about the conclusion in male rats, noting that the increase in incidence of brain tumors in male rats was not statistically significant, was not observed in the other sex, and, there was not a dose response. Dr. Melnick said the term 'marginal' as used with equivocal meant to him a borderline effect with potential biological significance. There was a lengthy discussion about factors considered in interpreting a marginal increase, and, specifically about the difficulty in assigning the correct level of evidence based on the brain tumors.

Dr. Adrianne Rogers, Boston University, representing Parke Davis, stated that the conclusions for both male and female rats should be no evidence of carcinogenic activity as far as either lung or pituitary tumors are concerned. In male rats, the increase in lung tumors represents primarily adenomas, there is no increase in hyperplasia, and the trend test is not significant at the 5% level. In female rats, the incidences of pituitary gland tumors in all dose groups were within historical control ranges and, there were no associated increases in the incidence of hyperplasia. In response to Dr. Ashby, Dr. Rogers concurred with the conclusion for male rats based on the brain tumors.

Dr. Garman moved that the Technical Report on diphenhydramine hydrochloride be accepted with the revisions discussed and with the conclusions as written for male and female rats, equivocal evidence of carcinogenic activity, and for

male and female mice, no evidence of carcinogenic activity. Dr. Gallo seconded the motion, which was approved by eight yes votes with one abstention (Newberne).

Hexachloroethane. Dr. W.C. Eastin, NIEHS, NTP Chemical Manager, introduced the toxicology and carcinogenesis studies of hexachloroethane by reviewing the experimental design, results, and proposed conclusions:

> Under the conditions of these 2-year gavage studies, there was clear evidence of carcinogenic activity of hexachloroethane for male F344/N rats, based on the increased incidences of renal neoplasms. The marginally increased incidences of pheochromocytomas may have been related to hexachloroethane administration to male rats. There was no evidence of carcinogenic activity of hexachloroethane for female F344/N rats administered 80 or 160 mg/kg by gavage for 103 weeks.
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> An increased severity of nephropathy, linear mineralization of the renal papillae, and hyperplasia of the transitional epithelium of the renal pelvis were present in dosed male rats. An increased incidence of nephropathy was present in dosed female rats.

Dr. Popp, a principal reviewer, agreed with the conclusion in female rats but thought the conclusion in male rats should be reduced to some evidence of carcinogenic activity based on the incidence of renal neoplasms (1 in vehicle controls vs. 2 in the low dose group and 7 in the high dose group) and on the hyaline droplet nephropathy and its likely relationship to the renal tubular cell neoplasms. He opined that the high dose for male rats was too low, being below the lowest dose evaluated in the 13-week study. Dr. Eastin reported that the high dose for male rats reflected the fact that there was toxicity even at the lowest dose used in the 13-week studies. Dr. J. Huff, NIEHS, commented that if all dose groups have significant lesions in the 13-week studies, the Program considers doing another short-term study; for this chemical, the decision was made not to repeat the 13-week study. Dr. Popp stated that the discussion section dealing with hyaline droplet nephropathy could be better organized; further, he believed that the alpha-2u-globulin concept is more widely supported than is indicated in the discussion. Based on the revisions required, he recommended that a final vote on the report be deferred until revision is completed.

Dr. Mirer, the second principal reviewer, agreed with the conclusions and thought the increased incidences of pheochromocytomas in male rats to be more supportive than indicated. He opined that the treatment of the alpha-2u-globulin hypothesis in the Discussion section was appropriate but reference to the hypothesis should be deleted from the Abstract. He said the appearance of renal toxicity in female rats should receive more discussion, especially in that such toxicity was not seen in an earlier study with d-limonene.

Dr. McKnight, the third principal reviewer, agreed with the conclusions. She asked for more specific descriptions in the text of how certain pathology and statistical analyses were performed.

In the discussion, Dr. Garman and Dr. Gallo argued for leaving the statement associating renal nephropathy with alpha-2u-globulin in the Abstract. Since there seemed to be analogies with issues around the role of the protein as discussed in the review by the Panel of the Technical Report on d-limonene (NTP-TR No. 347), April 18, 1988, Dr. Perera read from relevant sections of the

minutes of that review. She noted that there was a consensus by the Panel that the statement about alpha-2u-globulin be retained in the Abstract but no mention made of the uniqueness to male rats of the alpha-2u-globulin-associated nephropathy. Dr. Perera felt the Panel had concluded that the relationship between the protein and nephropathy was fairly well established but that the correlation with tumorigenicity was not established. Dr. Ashby suggested that the discussion in the current report on hexachloroethane be completed by stating that the induction of hyaline droplets in the male rat kidney may or may not provide insight into a possible non-mutagenic mechanism.

In response to the proposal by Dr. Popp that the report be deferred to allow for major revision of the discussion on hyaline droplet nephropathy, Dr. Huff asked that action on the report not be delayed but suggested that a revised discussion be mailed to the Panel members for their comments and approval. Dr. Eastin said the revision would take into consideration the major points made by the Panel. This course of action was acceptable to Dr. Popp.

Dr. Mirer moved that the Technical Report on hexachloroethane be accepted with the revisions as discussed, with retention of the statement on alpha-2u-globulin in the Abstract, and with the conclusions as written for male rats, clear evidence of carcinogenic activity, and for female rats, no evidence of carcinogenic activity. Dr. McKnight seconded the motion, which was approved by five yes votes (Gallo, Garman, Klaassen, McKnight, Mirer) to two no votes (Newberne,Popp) with two abstentions (Ashby, Gold). Dr. Ashby's abstention was for reason of company affiliation. The revised discussion will be mailed to Panel members present for their review and approval.

Hydroquinone. Dr. F.W. Kari, NIEHS, NTP Chemical Manager, introduced the toxicology and carcinogenesis studies of hydroquinone by reviewing the experimental design, results, and proposed conclusions:

> Under the conditions of these 2-year gavage studies, there was clear evidence of carcinogenic activity of hydroquinone for male F344/N rats exposed to hydroquinone, as shown by marked increases in tubular cell adenomas of the kidney. There was some evidence of carcinogenic activity of hydroquinone for female F344/N rats, as shown by increases in mononuclear cell leukemia. There was no evidence of carcinogenic activity of hydroquinone for male B6C3F1 mice administered 50 or 100 mg/kg in water by gavage. There was some evidence of carcinogenic activity of hydroquinone for female B6C3F1 mice, as shown by increases in hepatocellular neoplasms, mainly adenomas.
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> Administration of hydroquinone was associated with thyroid follicular cell hyperplasia in both male and female mice, and anisokaryosis, multinucleated hepatocytes, and basophilic foci of the liver in male mice.

Dr. Popp, a principal reviewer, agreed with the conclusions although he thought the conclusion in male rats, clear evidence of carcinogenic activity, to be a borderline call between clear evidence and some evidence. He said a better rationale needed to be given as to why the oral instead of dermal route of administration was chosen. Dr. Kari said practical limitations of how much chemical could be applied dermally and lack of toxicity in the short-term studies justified the use of gavage for optimizing the potential for observing systemic toxicity and carcinogenicity. Dr. Popp stated that the relationship between nephropathy and renal carcinogenicity in male rats needed to be clarified in the discussion. He said that likelihood of finding hyaline droplets was dependent on the time that had elapsed between animal sacrifice and examination for droplets. For the short-term studies, Dr. Kari said there were 72 hours between cessation of exposure and necropsy; however, there were no other indices of hyaline droplet formation seen such as granular cast formation in the loop of Henle or mineralization in the renal papilla. Dr. J. Huff, NIEHS, pointed out that in the NTP studies on d-limonene, there were still clearly increased levels of hyaline droplets in kidneys of treated male rats after 72 hours. Nonetheless, the time factor was most important and should be highlighted in the report.

Dr. Gallo, the second principal reviewer, agreed with the conclusions in female rats and male and female mice but disagreed with the conclusion in male rats thinking it should be changed to some evidence of carcinogenic activity. He based this on the presence of nephropathy in nearly all male and most female rats of all dosed groups and vehicle controls, on the possibility of products of reduction/oxidation cycling in the kidney as a function of pH and high renal concentrations of hydroquinone, and on the activity of cysteine lyase in the kidney and the role of thiol adducts in acute nephrotic syndrome as a precursor to hyperplasia. Dr. Gallo questioned the use of the oral route of exposure considering the major route of human exposure appears to be dermal. He said a complete absorption, distribution, metabolism and excretion profile should have been developed before initiating two-year studies. He asked that the report be deferred until chemical disposition data could be incorporated. Dr. Kari agreed

that such data would be meaningful for interpretation but lack of it does not detract from the validity of the information obtained by the oral route. Further, there is no indication that the route of exposure would influence the overall outcome.

Dr. Mirer, the third principal reviewer, agreed with the conclusions in male rats and male mice. He argued for changing the conclusion in female mice to clear evidence of carcinogenic activity based on highly significant dose related increases in incidences of hepatocellular neoplasms for both low and high dose groups. Dr. Kari mentioned that there was not a clear dose response relation, the numbers were not overwhelming, and there was no supporting evidence in the other sex or other species. Dr. Mirer asked for more discussion about changing the conclusion in female rats to clear evidence. Dr. Ashby commented that the high and quite variable historical control incidence of mononuclear cell leukemia was not supportive of a higher level of evidence in female rats. Dr. Mirer noted that skin absorption had been observed in preliminary animal studies, a finding of importance for drawing public health conclusions.

Ms. Susan Murphy, Goodyear Tire and Rubber Company, and Chairperson, Toxicology Research Task Group of the Hydroquinone Program Panel, Chemical Manufacturers Association, made a presentation. She asked the Panel to consider inclusion of more discussion on the role of nephrotoxicity in tumor formation in the kidney while noting the high incidence of spontaneous nephropathy in all rat groups, and to consider changing the conclusion in female rats to equivocal evidence of carcinogenic activity based on the high and variable historical control rates for mononuclear cell leukemia. Dr. Caroline English, Eastman Kodak Company, also made a presentation in which she expressed concern that changes in feed consumption, water consumption, body weight, and the virological status of study animals may have contributed to nephrotoxic responses observed, and consequently was associated with the production of renal tumors in male rats. She also asked that results of recent hydroquinone metabolism studies be considered before finalizing the report, as metabolism in the F344 rat produces a cysteine conjugate which may be a nephrotoxin. Dr. Huff pointed out that these data have not been published, and that the NTP ordinarily does not cite unpublished studies.

Dr. Ashby thought that the discussion about hydroquinone playing a role in the carcinogenicity of benzene was overstated, citing the large difference between the chemicals in physico-chemical characteristics as making this unlikely. Dr. Mirer thought the metabolic connection between the two chemicals lent support to raising the call in female rats to clear evidence in that benzene is a potent leukemogen. Dr. J. Haseman, NIEHS, commented that for tumors with quite variable rates such as mononuclear cell leukemia the concurrent control rate is most appropriate.

There was considerable discussion among Panel members and staff regarding the degree of correlation between toxicity (nephropathy) and carcinogenicity (renal tubular adenomas) in male rats. Dr. Huff concluded there was not a significant correlation. Dr. Popp noted that the definition of clear evidence called for dose-related increases in malignant neoplasms, or a combination of malignant and benign neoplasms, or a marked increase in benign neoplasms. He questioned whether eight adenomas in the top dose group constituted a marked increase.

Dr. Gallo moved that the conclusion for male rats be reduced to some evidence of carcinogenic activity. Dr. Popp seconded the motion, which was approved by five yes (Gallo, Garman, Klaassen, Newberne, Popp) to four no votes (Ashby, Gold, McKnight, Mirer). Dr. Gallo moved that the conclusion be accepted as written for female rats, some evidence of carcinogenic activity. Dr.Popp seconded the motion, which was approved by seven yes to two no votes (McKnight,Mirer). Dr. Gallo moved that the conclusion be accepted as written for male mice, no evidence of carcinogenic activity. Dr. Popp seconded the motion, which was approved unanimously with nine votes. Dr. Gallo moved that the conclusion be accepted as written for female mice, some evidence of carcinogenic activity. Dr. Garman seconded the motion, which was approved by eight yes to one no vote (Mirer).

Iodinated Glycerol. Dr. J.E. French, NIEHS, NTP Chemical Manager, introduced the toxicology and carcinogenesis studies of iodinated glycerol by reviewing the experimental design, results, and proposed conclusions:

> Under the conditions of these 2-year gavage studies, there was some evidence of carcinogenic activity for male F344/N rats administered iodinated glycerol, as indicated by increased incidences of mononuclear cell leukemia and follicular cell carcinomas of the thyroid gland. Adenomas of the nasal cavity in two high dose male rats may have been related to the administration of iodinated glycerol. There was no evidence of carcinogenic activity for female F344/N rats administered 62 or 125 mg/kg iodinated glycerol by gavage for 103 weeks. There was no evidence of carcinogenic activity for male B6C3F1 mice administered 125 or 250 mg/kg iodinated glycerol by gavage for 103 weeks. There was some evidence of carcinogenic activity for female B6C3F1 mice administered iodinated glycerol, as indicated by increased incidences of adenomas of the anterior pituitary gland and neoplasms of the harderian gland. Squamous cell papillomas of the forestomach may have been related to the administration of iodinated glycerol.
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> Significant nonneoplastic lesions considered related to exposure to iodinated glycerol were squamous metaplasia and focal atrophy of the salivary glands in male and female rats. Dilatation of the thyroid gland follicle and follicular cell hyperplasia were observed in male and female mice.

Dr. Gallo, a principal reviewer, agreed with the conclusions for female rats and male mice. He thought the conclusion in male rats should be reduced to equivocal evidence of carcinogenic activity because the occurrence of mononuclear cell leukemias is variable and may have been affected by faulty environmental controls at the laboratory. For the thyroid, he stated that without knowledge of the functional status of the thyroid, association of follicular cell tumors with chemical cannot be fully assessed. Dr. French responded that because of the variability, the concurrent controls were more appropriate for comparison of the leukemia incidences. With regard to the thyroid glands, all of the tumors were carcinomas, an uncommon event. Dr. Gallo said the conclusion in female mice should be reduced to equivocal evidence of carcinogenic activity because due to lack of knowledge of thyroid functional status association of pituitary tumors with chemical exposure cannot be assessed. Dr. Gallo said the discussion should include more description of the role of free iodide on thyroid function and how it may impact on the thyroid glands in this study. He suggested a short-term study might be appropriate to evaluate the effect of iodinated glycerol on thyroid function. Dr. French said a 13-week study was in progress including thyroid function tests and results may be available in time to add to the discussion.

Dr. Gold, the second principal reviewer, agreed with the conclusions. She noted that the target sites in female mice, pituitary and harderian glands, have only infrequently been considered as target sites in NTP studies. She commented that it would be desirable to state the category of evidence separately for each target site instead of the current practice of adding a sentence without the evaluative category, in this case, adenomas of the nasal cavity in male rats.

Dr. Garman, the third principal reviewer, agreed with the conclusions. He echoed Dr. Gallo's concern about there being more information about the level of thyroid activity as well as a more detailed description of thyroid gland morphology including representative photomicrographs of thyroid glands from control and high dose animals. Dr. S. Eustis, NIEHS, commented that the lesions in the thyroid were not typical of a goitrogenic effect. He said a more detailed description would be provided including photomicrographs.

Dr. J. Haseman, NIEHS, observed that the low dose group should be the focus for evaluation in male rats because of the high mortality occurring late in the study in the high dose animals. Dr. Perera cautioned that judgment of the levels of evidence should be based on the actual results and not on proposed mechanisms. Dr. Gallo agreed but said consideration of mechanism was important to understanding how a chemical alters tissue physiology.

Dr. William H. Butler, British Industrial Biological Research Association, representing Carter-Wallace, Inc., discussed certain aspects of the pathology, noting that he had reviewed the microslides at the NTP Archives and participated in two NTP Pathology Working Groups (PWGs). He began by describing deficiencies in the conduct of the study. He commented that in male rats, the incidences of leukemias were within the historical control range with no shortening of latency, while thyroid tumors were elevated only in the low dose group and there was no evidence of goitrogenic effects; thus, the conclusion should be no evidence. In female mice, pituitary tumors are common and highly variable and there was no decrease in latency, while the increase in harderian gland tumors was marginal; thus, the conclusion should be, at best, equivocal evidence.

Dr. Gallo moved that the conclusion for male rats be accepted as written, some evidence of carcinogenic activity. Dr. Garman seconded the motion. Dr. Gallo noted that he primarily based his motion on the follicular cell carcinomas of the thyroid. The motion was approved by six yes votes to one no vote (Gold). Dr. Gallo moved that the conclusions for female rats and male mice be accepted as written, no evidence of carcinogenic activity. Dr. Gold seconded the motion, which was approved unanimously with seven votes. Dr. Gallo moved that the conclusion for female mice be accepted as written, some evidence of carcinogenic activity, but with squamous cell papillomas of the forestomach considered to be related rather than "may have been related". Thus, the tumors supporting the level of evidence would be in order of importance: adenomas of the anterior pituitary gland, neoplasms of the harderian gland, and papillomas of the forestomach. Dr. Mirer seconded the motion. In discussion, Dr. Gold disagreed and thought that each organ should be evaluated separately and noted that the increases in squamous cell papillomas of the forestomach were not statistically significant so the evaluation should not change. The motion was not accepted by six no votes to one yes vote (Gallo). Dr. Gallo then moved to accept the conclusion as written, some evidence of carcinogenic activity, with no changes. Dr. Garman seconded the motion, which was approved unanimously with seven votes.

N-Methylolacrylamide. Dr. J.R. Bucher, NIEHS, NTP Chemical Manager, introduced the toxicology and carcinogenesis studies of N-methylolacrylamide by reviewing the experimental design, results, and proposed conclusions:

> Under the conditions of these 2-year aqueous gavage studies, there was no evidence of carcinogenic activity of N-methylolacrylamide for male or female F344/N rats receiving doses of 6 or 12 mg/kg per day. There was clear evidence of carcinogenic activity of N-methylolacrylamide for male B6C3F1 mice, based on increased incidences of neoplasms of the harderian gland, liver, and lung. There was clear evidence of carcinogenic activity of N-methylolacrylamide for female B6C3F1 mice, based on increased incidences of neoplasms of the harderian gland, liver, lung, and ovary.
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> In rats, because no biologically important nonneoplastic lesions were attributed to N-methylolacrylamide administration, somewhat higher doses might have been used to increase the sensitivity of these studies for determining the presence or absence of a carcinogenic response; in female mice, ovarian atrophy was compound related.

Dr. Ashby, a principal reviewer, agreed with the conclusions. He said that since there was more than one tumor site supporting the level of evidence perhaps some indication could be given regarding the tumor incidence(s) from which the category of evidence was primarily derived. He asked whether the presence of Sendai virus might invalidate the findings for lung tumors in mice. Dr. Ashby noted that the chemical seemed to be a specific clastogen, much like acrylamide, so despite a negative Ames test N-methylolacrylamide should be considered to be genotoxic.

Dr. Klaassen, the second principal reviewer, agreed with the conclusions.

Dr. Popp, the third principal reviewer, agreed with the conclusions. He stated that the criteria used for dose selection for the two-year study in rats based on the shorter term study results were correct even though the end results indicated higher doses could have been used. Dr. Popp also asked for clarification of the impact of Sendai virus infection on the incidence of lung tumors in mice. Dr. Bucher said that recent analysis of a large number of studies indicated no difference in the incidence of lung tumors between Sendai positive and Sendai negative control groups. This analysis also did not indicate a cocarcinogenic effect of Sendai in the induction of lung tumors by chemicals; however, a cocarcinogenic effect with N-methylolacrylamide could not be ruled out.

Dr. Ashby moved that the Technical Report on N-methylolacrylamide be accepted with the revisions discussed and with the conclusions as written for male and female rats, no evidence of carcinogenic activity, and for male and female mice, clear evidence of carcinogenic activity. Dr. Klaassen seconded the motion, which was approved unanimously with nine votes.

Pentaerythritol Tetranitrate. Dr. J.R. Bucher, NIEHS, NTP Chemical Manager, introduced the toxicology and carcinogenesis studies of pentaerythritol tetranitrate by reviewing the experimental design, results, and proposed conclusions:

> Under the conditions of these 2-year feed studies, there was equivocal evidence of carcinogenic activity of pentaerythritol tetranitrate (PETN), NF, for male and female F344/N rats, based on a marginal increase in neoplasms of the Zymbal gland. Female rats might have tolerated a higher dose. There was no evidence of carcinogenic activity of PETN, NF, for male or female B6C3F1 mice fed diets containing 25,000 or 50,000 ppm for 2 years. No nonneoplastic lesions were attributed to PETN, NF, administration.

Dr. Newberne, a principal reviewer, agreed with the conclusions. He asked for an explanation of why the doses in the two-year studies in female rats were only one-fourth those in the other experimental groups. Dr. Bucher said that at the time the two-year study was designed, the convention for setting doses included reduction in body weight gain of 10 per cent or more in 13-week studies, and that was the determinant for the markedly lower doses used.

Dr. McKnight, the second principal reviewer, agreed with the conclusions. She commented on the three lots of PETN and asked how they were used. Dr. Bucher said they were used sequentially with all of the dose preparations being made from the lot in use at a particular time. She asked why there were so many Zymbal glands missing in all three female rat groups. Dr. Bucher said the glands are very small and hard to find unless enlarged with a tumor. The sections are taken through the inner ear and certain other relevant tissues as well; sometimes the Zymbal gland is missed.

Dr. Gold, the third principal reviewer, agreed with the conclusions. She commented on a report in the Discussion that all compounds in the NTP database, except benzene and PETN, that induce tumors of the Zymbal gland are also positive in the Salmonella assay, and requested clarification of the discussion. She indicated that all nine non-NTP chemicals that induced Zymbal gland tumors were also genotoxic. Dr. Bucher responded that this represented one of the first complete assessments of tumor incidence versus genotoxicity that the Program has put together, and was included as a discussion point. He noted that the level of evidence chosen was based on the tumor incidence and not on whether or not PETN was genotoxic.

Dr. Gold moved that the Technical Report on pentaerythritol tetranitrate be accepted with the revisions discussed and with the conclusions as written for male and female rats, equivocal evidence of carcinogenic activity, and for male and female mice, no evidence of carcinogenic activity. Dr. Newberne seconded the motion, which was approved unanimously with seven votes.

Rhodamine 6G. Dr. J.E. French, NIEHS, NTP Chemical Manager, introduced the toxicology and carcinogenesis studies of rhodamine 6G by reviewing the experimental design, results, and proposed conclusions:

> Under the conditions of these 2-year feed studies, there was equivocal evidence of carcinogenic activity for male F344/N rats administered rhodamine 6G, as indicated by a marginally increased incidence of integumentary keratoacanthomas. There was equivocal evidence of carcinogenic activity for female F344/N rats administered rhodamine 6G, as indicated by a marginal increase in pheochromocytomas or malignant pheochromocytomas (combined) of the adrenal gland. There was no evidence of carcinogenic activity for male B6C3F1 mice administered 1,000 or 2,000 ppm rhodamine 6G in the diet. There was no evidence of carcinogenic activity for female B6C3F1 mice administered 500 or 1,000 ppm rhodamine 6G in the diet.
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> There were no significant nonneoplastic lesions attributed to rhodamine 6G administration to male or female rats or male or female mice. Male and female rats might have been able to tolerate a higher concentration of rhodamine 6G in the feed.

Dr. Gold, a principal reviewer, agreed with the conclusions although she would have preferred an evaluation of no evidence of carcinogenic activity for male and female rats. She noted that the historical control rates were quite variable for keratoacanthomas in male rats and for pheochromocytomas in female rats, and the incidences in high dose groups were similar to the highest spontaneous rates at the same laboratory in studies conducted over the same time period. Dr. French acknowleged the variability of the historical controls while noting that concurrent controls are most appropriate for comparisons. With regard to the pheochromocytomas, a contributing factor was the observation of malignant tumors in the high dose group. Dr. Gold said information should be added to the discussion that the International Agency for Research on Cancer evaluated rhodamine 6G as having limited evidence of carcinogenicity from studies reported by others. Dr. J. Huff, NIEHS, indicated that the original research was mentioned in the introduction to the Technical Report, and the IARC evaluation of that research will be included in the Report. Dr. Gold inquired about the statement in the Abstract that the fur of control rats was tinged pink. Dr. French responded that this observation was in error and would be deleted from the final report.

Dr. Gallo, the second principal reviewer, agreed with the conclusions. He speculated that chemical interaction with the epidermal growth factor receptor complex may have played a role in the skin tumors in male rats. The rhodamine compounds are photoactive and many photoactive compounds have been shown to perturb this receptor complex.

Dr. Gold moved that the conclusions for male and female mice be accepted as written, no evidence of carcinogenic activity. Dr. Gallo seconded the motion, which was approved unanimously with seven votes. Dr. Gallo moved that the conclusions for male and female rats be accepted as written, equivocal evidence of carcinogenic activity. Dr. Garman seconded the motion, which was approved by five yes votes (Gallo, Garman, McKnight, Mirer, Popp) to one no vote (Gold) with one abstention (Newberne).

Furosemide: Further Pathology Findings from Kidneys of Male Rats. The NTP toxicology and carcinogenesis studies of furosemide in F344/N rats and B6C3F1 mice (Technical Report No. 356) were peer reviewed and approved by the Panel on April 19, 1988. An important portion of the discussion focused on the marginal increases in renal tubular cell neoplasms in male rats and on the poor survival in male rats. The proposed level of evidence, equivocal evidence of carcinogenesis activity, was approved unanimously by the Panel.

Dr. J. R. Bucher, NIEHS, NTP Chemical Manager, reported that subsequently, furosemide was chosen as one of several studies in which the male rat kidney would be reevaluated by a more extensive sampling procedure to determine if current NTP procedures were giving an accurate assessment of the "true" rates of tubular cell tumors of the kidney. Furosemide was chosen because there was observed an apparent increase in tumors in dosed groups, but a dose response was not evident.

Dr. Bucher described the additional pathology procedures and noted that the numbers of additional kidney sections reviewed were 300 for controls, 301 for low dose, and 299 for high dose. The incidences of tubular cell neoplasms originally reported were: 1/50, control; 4/50, low dose; and 2/50, high dose. All were adenomas except one adenocarcinoma in the low dose group and one in the high dose group. The results of the additional tissue review after eliminating duplicate diagnoses from the original review were: 2, control; 1, low dose; and 4, high dose (all adenomas). When the results were combined, the incidences were still not statistically significant. Dr. Bucher stated that the presence of malignant tumors in dosed animals, and the marginal increase in combined tumors of a target organ for furosemide action still constituted equivocal evidence of carcinogenic activity. Information from the additional studies will be added to the Results section of the Technical Report.

Dr. S. Eustis, NIEHS, noted that the additional results would not be part of the historical control data base. Further, these reevaluations would not be a routine or common event but rather would be considered for studies where there were marginal increases in rare tumors to aid in interpretation of the lesions. Dr. J. Huff, NIEHS, commented that in most cases such additional reevaluations would be performed prior to bringing the studies to the Panel for review.