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

April 18 and 19, 1988
Research Triangle Park, North Carolina

The review meeting began at 9:00 a.m. on April 18 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, Charles Capen, Vernon Chinchilli, Kim Hooper, Donald Hughes, William Lijinsky, Franklin Mirer, James Popp, and Andrew Sivak. Dr. Mirer was unable to attend this meeting. These minutes have been reviewed and approved by all members of the Subcommittee and Panel present. They were written by Dr. Larry G. 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 82-04, 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 October 3 and 4, 1988, in Research Triangle Park, North Carolina. For information, contact Dr. Hart, (919) 541-3971; FTS: 629-3971.

CONTENTS

p-Chloroaniline Hydrochloride. Dr. R.S. Chhabra, NIEHS, NTP Chemical Manager, introduced the toxicology and carcinogenesis studies of p-chloroaniline by reviewing the experimental design, results, and proposed conclusions:

> Under the conditions of these 2-year water gavage studies, there was clear evidence of carcinogenic activity of p-chloroaniline hydrochloride for male F344/N rats, as indicated by increased incidences of uncommon sarcomas of the spleen. Pheochromocytomas of the adrenal gland may also have been associated with chemical administration. There was equivocal evidence of carcinogenic activity of p-chloroaniline hydrochloride for female F344/N rats, as indicated by the presence of uncommon sarcomas of the spleen in one mid and one high dose animal and the increased incidence of pheochromocytomas of the adrenal gland. There was some evidence of carcinogenic activity of p-chloroaniline hydrochloride for male B6C3F1 mice, as indicated by increased incidences of hepatocellular neoplasms and of hemangiosarcomas of the liver or spleen. There was no evidence of carcinogenic activity of p-chloroaniline hydrochloride for female B6C3F1 mice administered 3, 10, or 30 mg/kg by gavage for 2 years.
>
> The incidences of mononuclear cell leukemias in male and female rats and of malignant lymphomas in male and female mice were decreased by administration of p-chloroaniline hydrochloride. Compound-related splenic fibrosis was present in male and female rats.

Dr. Sivak, a principal reviewer, agreed with the conclusions. However, the fact that the hepatic tumor incidence in control male mice was unusually low coupled with a tumor yield in high dose males not much above the historical control range might be mentioned in the Abstract. Dr. Chhabra agreed and pointed out that the effects were mainly due to carcinomas, and, further, there was considerable metastasis of these tumors to the lung (1/50, controls vs. 9/50, high dose). Dr. Sivak stated that inclusion of pharmacokinetic data indicating saturation was not reached, even at the highest dose, was an important addition to the data base available for interpretation of carcinogenic responses. He suggested deleting, as not being supportable, the speculation that increased sensitivity to aniline toxicity was based on differences in a single erythrocyte enzyme. Dr. Chhabra agreed.

Dr. Hughes, the second principal reviewer, agreed with the conclusions. He requested an explanation for poor survival in male and female control rats compared with treated groups. Dr. Chhabra noted that there appeared to be a correlation with a marked negative trend for mononuclear cell leukemias. Dr. Hughes thought the extensive amount of structure-activity data on genetic toxicity, carcinogenicity and other effects for the aniline compounds to be a useful addition.

Dr. Gallo, the third principal reviewer, agreed with the conclusions although he argued that if uncommonness of the tumors is the criterion for the level of evidence then the level in female rats should be the same as in male rats. Dr. J. Haseman, NIEHS, said that to his knowledge the Program had never made a call above equivocal evidence based on a single tumor regardless of uncommonness. Dr. J. Huff, NIEHS, indicated that this is mentioned in the abstract. With regard to dose selection, Dr. Gallo commented that there were marked increases in methemoglobinemia for both rats and mice in 13-week studies even at the lowest doses, and would be for this type of compound an appropriate criterion, i.e., chronic methemoglobinemia as an index for determining a maximum tolerated dose.

In response to inquiries as to why the NCI study (NCI Technical Report No. 189, 1979) was repeated, Dr. Chhabra said p-chloroaniline was considered to be a good candidate for restudy because the findings from the NCI study were unclear as to carcinogenicity, and because of the nature of the chemical and the degree of industrial exposure of humans. Dr. Huff added that three of the four previous experiments were equivocal, the duration of exposure was only 18 months, and the gavage route was used in the current studies.

Dr. Sivak moved that the Technical Report on p-chloroaniline hydrochloride be accepted with the revisions discussed and with the conclusions as written for male rats, clear evidence of carcinogenic activity, for female rats, equivocal evidence of carcinogenic activity, for male mice, some evidence of carcinogenic activity, and for female mice, no evidence of carcinogenic activity. Dr. Hughes seconded the motion, which was approved with eight votes. There was one abstention for reason of company affiliation (Dr. Ashby).

2,4-Dichlorophenol. Dr. R.L. Melnick, NIEHS, NTP Chemical Manager, introduced the toxicology and carcinogenesis studies of 2,4-dichlorophenol by reviewing the experimental design, results, and proposed conclusions:

> Under the conditions of these 2-year feed studies, there was no evidence of carcinogenic activity for male F344/N rats fed diets containing 5,000 or 10,000 ppm 2,4-dichlorophenol or for female F344/N rats fed diets containing 2,500 or 5,000 ppm 2,4-dichlorophenol. There was no evidence of carcinogenic activity for male or female B6C3F1 mice fed diets containing 5,000 or 10,000 ppm 2,4-dichlorophenol.

Dr. Lijinsky, a principal reviewer, agreed with the conclusions. He asked for the rationale for selecting a top dose in male rats that was double that in female rats. Dr. Melnick said the selection of 5,000 ppm as high dose for female rats was based on the observation of bone marrow atrophy in 6/10 given 10,000 ppm in the 13-week study.

Dr. Perera, the second principal reviewer, agreed with the conclusions. She opined that, in retrospect, the chemical might better have been evaluated in an initiation-promotion assay since it had been shown to be positive as a promoter in a mouse skin model.

Dr. Gallo, the third principal reviewer, agreed with the conclusions. He questioned why the dose route was not drinking water since its presence in drinking water was a rationale for doing a study. Dr. Melnick replied that the limited water solubility of 2,4-dichlorophenol would have reduced the top dose to less than half that used in the feed studies.

There was some discussion about the positive trend for forestomach tumors in male mice and why this finding was not given more weight. Dr. J. Huff, NIEHS, explained that the lack of increases in hyperplasias along with the negative trend for these lesions in the female mice suggested to staff that this was not a chemically-related effect.

Dr. Lijinsky moved that the Technical Report on 2,4-dichlorophenol be accepted with minor revisions and with the conclusions as written for male and female rats and mice, no evidence of carcinogenic activity. Dr. Perera seconded the motion, which was approved unanimously with 10 votes.

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

> Under the conditions of these 2-year studies, there was equivocal evidence of carcinogenic activity of furosemide for male F344/N rats, as shown by marginal increases in uncommon tubular cell neoplasms of the kidney and meningiomas of the brain. Survival of all groups of male rats was poor. There was equivocal evidence of carcinogenic activity of furosemide for female F344/N rats, as shown by a marginal increase in C-cell adenomas of the thyroid gland. There was no evidence of carcinogenic activity for male B6C3F1 mice fed diets containing 700 or 1,400 ppm furosemide for 2 years. There was some evidence of carcinogenic activity of furosemide for female mice, as shown by an increase in malignant tumors of the mammary gland.
>
> Nephropathy was more severe in the kidneys of male rats and of male and female mice fed diets containing furosemide than in controls.

Dr. Ashby, a principal reviewer, agreed with the conclusions for male and female mice. Based on the poor survival in all groups, he suggested that the conclusions in male rats be changed to inadequate study of carcinogenic activity. Dr. Bucher commented that survival was adequate in all groups at 93 weeks indicating that the majority of rats were at risk for developing tumors throughout most of the two years. Two of the three meningiomas occurred before one year while the kidney tumors are not known to increase dramatically late in two-year studies. In female rats, the incidences of C-cell tumors of the thyroid gland fell easily within the historical control range making an asssociation with chemical administration uncertain. Dr. Ashby noted that furosemide was genotoxic and that there has been a report that it is a germ cell mutagen. He thought some urgency needed to be given to in vivo bone marrow studies to confirm or refute this observation. Dr. Bucher said furosemide would be considered for bone marrow studies. The link with other furans such as furfural recently reported to be carcinogenic might be explored.

Dr. Chinchilli, the second principal reviewer, agreed with the conclusions for female rats and male and female mice. For male rats, he proposed that a statistical test incorporating historical control data be used to evaluate the two rare tumors, tubular cell neoplasms of the kidney and meningiomas of the brain, on which equivocal evidence of carcinogenic activity was based. Dr. Bucher said such tests would be done and the findings added to the Report. Dr. Haseman added that although the NTP generally does not use historical data in a formal testing mode, he had fewer reservations with rare tumors. In this case with the meningiomas and kidney tumors, the differences relative to the Program-wide historical control rates were significant at the P=0.01 level.

Dr. Capen, the third principal reviewer, agreed with the conclusions for male rats and male and female mice. He thought the conclusion for female rats should be changed to no evidence of carcinogenic activity. Dr. Bucher said the level of evidence chosen was based on the dose-related increases in thyroid gland C-cell adenomas with the high dose incidence double the historical rate, along with a statistically significant trend.

There was some discussion on poor survival in rats and the possible impact on interpretation of carcinogenesis findings that would be borderline between two

categories of evidence. Dr. J. Huff, NIEHS, said there has been a trend over the last few years across all laboratories in decreased survival for the male Fischer rat. Dr. E. McConnell, NIEHS, explained that this could be in part due to increased emphasis on tissue accountability leading to early sacrifice, especially after 91 weeks, to prevent losses due to autolysis or cannabalism. Dr. Huff added that more awareness and attention was being given to humane killing of moribund animals, and pointed out that a table in the Appendix indicates that considerable numbers of control and dosed male rats were removed from the experiment early. Dr. Popp stated that the question of inadequate survival may be given more concern than necessary. Dr. Ashby proposed that this humanely aggressive sacrifice policy be discussed in the text when there is poor survival. Dr. Huff further suggested that the data regarding moribund kills would be moved into the text for all Technical Reports.

Dr. Ashby moved that the conclusion for female rats be changed to no evidence of carcinogenic activity based on the great variability of thyroid C-cell tumors in other studies, no increases in hyperplasias, and lack of effects on the thyroid gland in the other three experimental groups. Dr. Lijinsky seconded the motion, which was approved by six yes votes (Ashby, Capen, Gallo, Hughes, Lijinsky, Sivak) to three no votes (Chinchilli, Hooper, Popp). Dr. Ashby moved that the conclusion for male rats be changed to inadequate study of carcinogenic activity based primarily on the poor survival. The motion failed for lack of a second. Dr. Ashby then moved that the conclusion for male rats be accepted as written, equivocal evidence of carcinogenic activity. Dr. Hooper seconded the motion, which was approved unanimously with nine votes. Dr. Ashby moved that the conclusion for male mice, no evidence of carcinogenic activity, be accepted as written. Dr. Popp seconded the motion, which was approved unanimously with nine votes. Dr. Ashby moved that the conclusion for female mice, some evidence of carcinogenic activity, be accepted as written. Dr. Hooper seconded the motion, which was approved unanimously with nine votes. Dr. Scala said these motions could be considered as approving the report with revisions discussed.

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

nder the conditions of these 2-year feed studies, there was equivocal evidence of carcinogenic activity of hydrochlorothiazide for male F344/N ats, based on a marginal increase in the incidence of neoplasms of the ymbal gland. Survival of all groups of male rats was poor. There was o evidence of carcinogenic activity for female F344/N rats given feed ontaining 250, 500, or 2,000 ppm hydrochlorothiazide. There was some evidence of carcinogenic activity of hydrochlorothiazide for male B6C3F1 nice, based on increased incidences of hepatocellular neoplasms. There was no evidence of carcinogenic activity for female B6C3F1 mice given iets containing 2,500 or 5,000 ppm hydrochlorothiazide.

hronic renal disease was more severe in rats administered ydrochlorothiazide, and increased incidences of secondary lesions parathyroid hyperplasia, fibrous osteodystrophy, and mineralization in ultiple organs) occurred in dosed rats.

: ughes, a primary reviewer, agreed with the conclusions for female rats and nice He stated that the conclusion for male rats should be changed to no evidence of carcinogenic activity in that the incidence of zymbal gland neoplasms in the high dose group was no higher than the highest incidence from historical control group. In addition, recently reported two-year studies by ligansky and Reuber (1987) showed no increases in neoplastic lesions in male or female rats dosed with 1000 ppm hydrochlorothiazide. Dr. J. Huff, NIEHS, mentioned that the dietary concentrations used in those studies were one-half the levels used in the NTP studies. Dr. Hughes thought that in view of the high historical control incidence (30%) of hepatocellular neoplasms in male mice, the conclusion should be changed to no evidence of carcinogenic activity. Dr. Bucher commented that there was a strong dose-related trend for the liver tumors and the incidence in the high dose group was highly significant compared with the controls supporting the interpretation by the staff.

Ivak, the second principal reviewer, agreed with the conclusions.

Chinchilli, the third principal reviewer, agreed with the conclusions in female rats and male and female mice but felt the conclusion in male rats should be changed to no evidence of carcinogenic activity. Had a test been employed directly comparing the data with historical control data, and yielding a lower P value, he could have supported the conclusion. In response to Drs. Chinchilli and Hughes, Dr. Bucher agreed that the evidence in male rats was marginal given the historical rate. All the zymbal gland tumors were observed on gross examination.

other discussion, Dr. Ashby stated that the data from genotoxicology assays are supportive of considering hydrochlorothiazide to be non-genotoxic. Pertaining to the liver tumors in male mice, there was further discussion about the relative weight given to concurrent vs. historical control rates. Dr. Haseman, NIEHS, stated that the NTP position is that the most important comparison is with the concurrent control, while comparisons with historical controls are most useful for rare tumors and also where there is a marginal increase in helping to judge the appropriate level. With regard to the current study he noted that the concurrent control rate of liver tumors in male mice

(7/48) was low relative to historical control values, but the rate in the high dose group (21/50) exceeded all but one of the incidences in the historical control data base. Dr. Hughes commented that he would have more confidence in the association of tumors with chemical administration if there was an adequate historical data base for the laboratory performing the hydrochlorothiazide studies. Dr. Huff indicated that the means for the two contemporary studies were not different from the incidence in the hydrochlorothiazide controls.

Dr. Hughes moved that the conclusions for male rats be changed to no evidence of carcinogenic activity based on the incidence of zymbal gland tumors in dosed rats being no greater than the top rate in historical controls. Dr. Sivak seconded the motion, which was approved by five yes (Capen, Gallo, Hughes, Popp, Sivak) to three no votes (Chinchilli, Hooper, Lijinsky) with one abstention for reasons of company affiliation (Ashby). Dr. Hughes moved that the conclusions for female rats, no evidence of carcinogenic activity, be accepted as written. Dr. Popp seconded the motion, which was approved by eight yes votes with one abstention (Ashby). Dr. Hughes moved that the conclusion for male mice be changed to equivocal evidence of carcinogenic activity based on the variability of liver neoplasms in male mice and lack of adequate historical control data for the performing laboratory. Dr. Lijinsky seconded the motion, which was approved by five yes (Capen, Hughes, Lijinsky, Popp, Sivak) to three no votes (Chinchilli, Gallo, Hooper) with one abstention (Ashby). Dr. Hughes moved that the conclusion for female mice, no evidence of carcinogenic activity, be accepted as written. Dr. Sivak seconded the motion, which was approved by eight yes votes with one abstention (Ashby).

d-Limonene. Dr. C.W. Jameson, NIEHS, NTP Chemical Manager, introduced the toxicology and carcinogenesis studies of d-limonene 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 d-limonene for male F344/N rats, as shown by increased incidences of tubular cell hyperplasia, adenomas, and adenocarcinomas of the kidney. There was no evidence of carcinogenic activity of d-limonene for female F344/N rats that received 300 or 600 mg/kg. There was no evidence of carcinogenic activity of d-limonene for male B6C3F1 mice that received 250 or 500 mg/kg. There was no evidence of carcinogenic activity of d-limonene for female B6C3F1 mice that received 500 or 1000 mg/kg.
>
> An increased severity of nephropathy, increased incidences of mineralization of the renal medulla and papilla, and hyperplasia of the transitional epithelium of the renal papilla were present in dosed male rats.

Dr. Jameson presented results from a short-term in vivo study using a range of doses of d-limonene given by gavage to groups of male and female F344/N rats in 14 daily doses over a three week period. Based on microscopic examination of kidney sections from these rats, d-limonene was shown to cause a dose-related increase in hyaline droplets in tubular epithelial cells in male rats. This effect was not seen in female rat kidneys. Sections of kidney stained by immunohistochemical techniques revealed that the hyaline droplets contained alpha-2u-globulin in male and to a lesser extent in female rats. These data would be included in the Technical Report.

Dr. Popp, a principal reviewer, agreed with the conclusions in female rats and male and female mice but thought the conclusion in male rats should be changed to some evidence of carcinogenic activity based on the hyaline droplet nephropathy and its likely relationship to renal tubular cell neoplasms in the male rat. He asked that the Discussion section mention that humans have not been shown to have alpha-2u-globulin, and that the recent findings with this protein be added to the Abstract.

Dr. Lijinsky, the second principal reviewer, agreed with the conclusions. He considered there to be far too much discussion regarding the presence of hyaline droplets and the associated alpha-2u-globulin proteins and their relationships with renal tubular cell neoplasms in male rats, and noted that the mechanism of carcinogenesis is still not known for this tumor type or for any compound. He indicated this association was a research hypothesis, and much work remains to be done.

Dr. Ashby, the third principal reviewer, agreed with the conclusions, and opined that discussion of the putative mechanism of carcinogenic action was appropriate as part of the hazard-definition process, perhaps in a few less words and could be mentioned in the Abstract. He commented on the well-defined non-genotoxicity of d-limonene.

Dr. Mirer, the fourth principal reviewer, was unable to attend the meeting. Dr. L. Hart, NIEHS, read his review into the record. Dr. Mirer agreed with the proposed conclusions. He argued that the hypothesis that the carcinogenic effect is secondary to renal toxicity has not been proven. He thought inclusion of a review of instances with other chemicals where kidney lesions were present

and neoplasia absent, or where neoplasia was present and toxicity not observed was certainly warranted. Because of the apparent lack of any overt toxicity, body weight decreases or reduced survival, Dr. Mirer noted that male mice might have tolerated a higher dose. Dr. Jameson reported on increases in non-neoplastic effects in high dose male mice that indicated a toxic response in the liver.

Dr. J. Swenberg, Chemical Industry Institute of Toxicology, made a presentation concerning d-limonene, alpha-2u-globulin associated nephropathy and carcinogenesis. He stated that it was likely but not proven that this mechanism of induction of neoplasia was unique to male rats. Dr. Swenberg showed data on the binding of trimethylpentane and components of unleaded gasoline to alpha-2u-globulin in the kidneys of male rats and concomitant cell proliferation. Dr. J. Huff, NIEHS, said the new NTP experimental data would be added to the Technical Report; that is, the design protocols, results, discussion, and a mention in the Abstract.

Dr. Scala pointed out that there were four issues to be resolved by the Panel and asked that discussion focus on these: (1) should a discussion of alpha-2u-globulin be included in the text of the report?; (2) should mention be made in the Abstract?; (3) should mention be made anywhere in the report of the potential uniqueness to male rats?; and (4) should the level of evidence for carcinogenic activity in male rats be reduced?

In the ensuing discussion, Drs. Hooper and Perera said they accepted the association of alpha-2u-globulin with chemically-induced nephropathy but felt the evidence was more circumstantial for association with tumorigenesis. Dr. Perera thought the current report was objectively balanced in the discussion section including the Abstract, but said the potential relevance of alpha-2u-globulin to human risk of cancer should not be noted. She commented that the incidence of kidney tumors in humans is considerably higher in males than in females. Dr. Gallo stated that this fact should be cited in the report. There seemed to be a consensus among Panel members that the statement already in the Abstract was adequate and should be retained: "These lesions have been described as reasonably characteristic of the hyaline droplet nephropathy that is associated with an accumulation of liver-generated alpha-2u-globulin in the cytoplasm of tubular epithelial cells." Also, there was general agreement with staff that a summary of the new data regarding hyaline droplets and alpha-2u-globulin should be included in the results and highlighted in the Abstract.

Dr. Perera moved that the Technical Report on d-limonene be accepted as written with revisions discussed, with no mention of the uniqueness to male rats of the alpha-2u-globulin associated nephropathy, with inclusion of the recent short term *in vivo* results as described, and with a statement about the higher incidence of human kidney tumors in males than in females. Dr. Hooper seconded the motion and it was approved by nine yes to one no (Dr. Popp) votes.

8-Methoxypsoralen. Dr. J.K. Dunnick, NIEHS, NTP Chemical Manager, introduced the toxicology and carcinogenesis studies of 8-methoxypsoralen 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 8-methoxypsoralen for male F344/N rats, as shown by increased incidences of tubular cell hyperplasia, adenomas, and adenocarcinomas of the kidney and carcinomas of the Zymbal gland. Subcutaneous tissue fibromas and alveolar/bronchiolar adenomas of the lung in male F344/N rats may have been related to chemical administration. Dose-related nonneoplastic lesions in male F344/N rats included increased severity of nephropathy and mineralization of the kidney and forestomach lesions. There was no evidence of carcinogenic activity of 8-methoxypsoralen for female F344/N rats given the chemical at 37.5 or 75 mg/kg for 2 years.

Dr. Gallo, a principal reviewer, agreed with the conclusions. His major criticism was that the study should not have been conducted without concurrent administration of ultraviolet light (UV). Use of this drug in humans under FDA approval occurs only with exposure to UV light. The conclusion should state that this is 8-methoxypsoralen in the absence of UV light. Dr. Dunnick reported that the chemical was nominated by the FDA with the request that it be studied without UV light to determine if there were tumorigenic effects of 8-methoxypsoralen alone. She noted that there are appreciable concentrations of 8-methoxypsoralen in some vegetables, up to 1000 ppm in parsnips. Dr. Gallo asked that more detailed discussion be added on the dermal effects especially in treatment of certain conditions and possible mechanisms of action.

Dr. Sivak, the second principal reviewer, agreed with the conclusions. He proposed that a description of the NTP prechronic mouse study with 8-methoxypsoralen and UV light be added to the discussion.

Dr. Chinchilli, the third principal reviewer, agreed with the conclusions. He inquired as to why information on the studies in mice were not included in the report. Dr. Dunnick said the studies in mice were not regular two-year studies but rather a research project. The treatment protocol with UV light was similar to that used in humans, and resulted primarily in skin tumors.

Dr. Lijinsky asked whether there was indication of any involvement of alpha-2u-globulin in the tumorigenic effects. Dr. Dunnick said there was no evidence of kidney toxicity or hyaline droplet formation in the 13-week studies. Dr. Ashby pointed out this was another chemical containing a furan moiety which was clearly genotoxic.

Dr. Gallo moved that the Technical Report on 8-methoxypsoralen be accepted with the revisions discussed and with the conclusions as written for male rats, clear evidence of carcinogenic activity, and for female rats, no evidence of carcinogenic activity. Dr. Hooper seconded the motion, which was approved unanimously with nine votes.

Ochratoxin A. Dr. G.A. Boorman, NIEHS, NTP Chemical Manager, introduced the toxicology and carcinogenesis studies of ochratoxin A 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 ochratoxin A for male F344/N rats as shown by substantially increased incidences of uncommon tubular cell adenomas and of tubular cell carcinomas of the kidney. There was clear evidence of carcinogenic activity for female F344/N rats as shown by increased incidences of uncommon tubular cell adenomas and of tubular cell carcinomas of the kidney and by increased incidences and multiplicity of fibroadenomas of the mammary gland.
>
> Ochratoxin A administration also caused nonneoplastic renal changes including tubular cell hyperplasia, tubular cell proliferation, cytoplasmic alteration, karyomegaly, and degeneration of the renal tubular epithelium.

Dr. Capen, Dr. Popp, and Dr. Hughes, the principal reviewers, agreed with the conclusions. All three reviewers indicated that the inclusion of photomicrographs was most useful. In view of the overwhelming carcinogenic response, Dr. Hughes thought it curious that the genetic toxicity assays did not indicate any strong evidence for interaction of the chemical with DNA or adduct formation. Dr. Boorman said DNA adduct studies had not been done.

Dr. Capen moved that the Technical Report on ochratoxin A be accepted with the conclusions as written for male and female rats, clear evidence of carcinogenic activity. Dr. Popp seconded the motion, which was approved unanimously with 10 votes.

Pentachlorophenol. Dr. E.E. McConnell, NIEHS, NTP Chemical Manager, introduced the toxicology and carcinogenesis studies of pentachlorophenol, technical grade or Dowicide EC-7, by reviewing the experimental design, results, and proposed conclusions:

> Under the conditions of these 2-year feed studies, there was clear evidence of carcinogenic activity for male B6C3F1 mice fed diets containing technical-grade pentachlorophenol, as shown by increased incidences of adrenal medullary and hepatocellular neoplasms. There was some evidence of carcinogenic activity for female B6C3F1 mice exposed to technical-grade pentachlorophenol, as shown by increased incidences of hemangiosarcomas and hepatocellular neoplasms. There was clear evidence of carcinogenic activity for male B6C3F1 mice exposed to pentachlorophenol, EC-7, as shown by increased incidences of adrenal medullary and hepatocellular neoplasms and for female B6C3F1 mice exposed to pentachlorophenol, EC-7, as shown by increased incidences of adrenal medullary and hepatocellular neoplasms and hemangiosarcomas.
>
> Chemically related increased incidences of nonneoplastic lesions included hepatocellular cytomegaly, necrosis, inflammation, pigmentation, and clear cell foci and intrahepatic bile duct hyperplasia.

Dr. Hooper, a principal reviewer, agreed with the conclusions. From the information given in the report about previous studies, he questioned whether pentachlorophenol has been adequately studied for carcinogenicity in rats and asked for clarification. Dr. McConnell said that the chemical was important enough to consider doing an NTP study in rats. Dr. J. Huff, NIEHS, indicated that previous studies in Sprague Dawley rats were less than adequate for negative studies since group sizes were small and since the doses used could likely have been higher. Dr. Hooper commented that results from previous carcinogenesis studies as well as those in the present studies did not support an important role for the impurities of pentachlorophenol, chlorinated dibenzodioxins and dibenzofurans, in the carcinogenic effects observed in mice.

Dr. Ashby, the second principal reviewer, agreed with the conclusions. He cautioned that not too much weight be given to the earlier non-NTP rat study which was negative for carcinogenicity. Since both chemicals were mixtures, Dr. Ashby thought that the pentachlorophenol structure should not be given alone but rather as part of a list of structures including those of the contaminants. He speculated that the tumors were caused by a non-genotoxic mechanism but suggested that in vivo data would be useful.

Dr. Lijinsky, the third principal reviewer, agreed with the conclusions. He concurred that the impurities played little part in causation of liver tumors; however, he thought they may have contributed to development of hemangiosarcomas of the liver and spleen. Dr. McConnell responded that the marked increases in bile duct hyperplasias, as well as the induction of cytochrome P-450 enzymes were characteristic effects of the impurities. He acknowledged the possible role of the impurities in tumor induction was more complicated but thought they did impact on induction of liver tumors. Dr. McConnell presented new comparative dose data, to be added to the report, which compared liver tumor rates in male mice exposed to pentachlorophenol with doses of one impurity, hexachlorodibenzodioxin, compared with liver tumor rates at similar doses in the NCI bioassay of this dioxin.

More discussion followed on the impurities and their contributions to the carcinogenic effects compared with pentachlorophenol. There was consensus that it was appropriate to use the commercial samples in the study as these are what humans are exposed to. Dr. B. Schwetz, NIEHS, warned against overinterpreting the role of the impurities noting that the study was not optimally designed for this purpose. Further, there are contaminants other than hexachlorodibenzo-dioxin for which data are not available.

Dr. Hooper moved that the Technical Report on pentachlorophenol be accepted with revisions discussed and with the conclusions as written for male mice (both technical grade and EC-7), clear evidence of carcinogenic activity, for female mice (technical grade), some evidence of carcinogenic activity, and for female mice (EC-7), clear evidence of carcinogenic activity. Dr. Lijinsky seconded the motion, which was approved unanimously with nine votes.

Tribromomethane. Dr. R.L. Melnick, NIEHS, NTP Chemical Manager, introduced the toxicology and carcinogenesis studies of tribromomethane (bromoform) by reviewing the experimental design, results, and proposed conclusions:

> Under the condidtions of these 2-year gavage studies, there was some evidence of carcinogenic activity of tribromomethane for male F344/N rats and clear evidence of carcinogenic activity for female F344/N rats, based on increased incidences of uncommon neoplasms of the large intestine. Reduced survival for male rats given 200 mg/kg tribromomethane lowered the sensitivity of this group to detect a carcinogenic response. Chemically related nonneoplastic lesions included fatty change and active chronic inflammation of the liver in male and female rats, minimal necrosis of the liver in male rats, and mixed cell foci of the liver in female rats. There was no evidence of carcinogenic activity for male B6C3F1 mice given 50 or 100 mg/kg tribromomethane or for female B6C3F1 mice given 100 or 200 mg/kg; male mice could have been given a higher dose.

Dr. Hooper, a principal reviewer, agreed with the conclusions. He commented on the negative trends for neoplasia at several sites in male rats (mononuclear cell leukemia, preputial gland adenomas/carcinomas) and female rats (mammary fibroadenomas, anterior pituitary adenomas, endometrial stromal polyps).

Dr. Capen, the second principal reviewer, agreed with the conclusions. He noted the fairly striking increased incidence of follicular cell hyperplasia in the thyroid glands of high-dose female mice and wondered about the possible mechanism.

Dr. Perera, the third principal reviewer, agreed with the conclusions for male and female rats and male mice. She said the significantly reduced survival in exposed female mice suggested a change to inadequate study of carcinogenic activity. Dr. Melnick responded that survival in all female mouse groups was greater than 50% at 92 weeks and since with the other trihalomethanes, the only site of significant neoplasia in female mice was the liver, he thought the survival was adequate to have detected an effect. In other discussion, Dr. Sivak commented that since the primary rationale for the study was based on the presence of tribromomethane in drinking water, inclusion of a comparison between drinking water levels and doses used would be helpful to the reader.

Dr. Capen moved that the Technical Report on tribromomethane be accepted with revisions as discussed and with the conclusions as written for male rats, some evidence of carcinogenic activity, for female rats, clear evidence of carcinogenic activity, and for male and female mice, no evidence of carcinogenic activity. Dr. Hooper seconded the motion, which was approved unanimously with 10 votes.

Dichlorvos: Additional Data on Cholinesterase Activities, Pancreatic Acinar Cell Tumors, and Effects on Growth of Transplantable Rat Leukemia. The NTP two year toxicology and carcinogenesis studies of dichlorvos in rats and mice (NTP Technical Report No. 342) were peer reviewed and approved by the Panel on July 14, 1987. At that time, the Panel questioned the data presented on plasma and erythrocyte cholinesterase activities. There was also a request for examination of all remaining pancreata of male and female rats from the studies. Since the level of evidence in male rats was supported by increased incidence of mononuclear cell leukemia, data were presented to the Panel about (i) the effects of dichlorvos administration on the growth of transplantable mononuclear cell leukemias in male F344/N rats, (ii) new data on cholinesterase levels, and (iii) findings from the recut pancreas sections.

Dr. M.P. Dieter, NIEHS, described the biological features of leukemia in the Fischer rat, the development of their leukemia transplant model, and validation of the model with chemicals from the NTP data base. He described the findings with dichlorvos noting that the transplant model showed the same type of positive response as was observed in the two-year studies. He concluded by pointing out the structure-activity relationships among dichlorvos and other phosphoric acid esters as leukemogens. These data would be added to the Technical Report.

Dr. P. Chan, NIEHS, NTP Chemical Manager for dichlorvos, presented data from short-term studies of plasma and erythrocyte cholinesterase activities in male and female rats and mice administered dichlorvos by gavage in corn oil five times weekly for five weeks over a range of doses. The studies showed that dichlorvos suppressed plasma cholinesterase activity in a dose-related manner at all time points during dichlorvos administration to male and female rats and mice. Enzyme activity returned to normal levels within three to four days after cessation of exposure. On the other hand, dichlorvos had no effect on erythrocyte cholinesterase activity in any of the sex/species groups. These results clarified the findings on cholinesterase inhibition contained in the draft Technical Report and have been added to the Technical Report.

Dr. Chan discussed the findings from an additional longitudinal section of the pancreas of male and female rats from the two-year studies. He reviewed the original findings from the Technical Report for pancreatic acinar cell hyperplasias and adenomas in male and female rats (Tables 1 and 4), the findings from the additional sampling (Tables 2 and 5), and the incidences when the original and new data were combined (Tables 3 and 6). As can be seen in Tables 2 and 3, although the incidence of pancreatic adenomas in dosed male rats is still increased, the new data weaken the statistical significance of this response. The conclusion approved by the Panel last July for male rats was clear evidence of carcinogenic activity as shown by increased incidences of adenomas of the exocrine pancreas and mononuclear cell leukemia. The conclusion was based primarily on the strength of the pancreas response. Dr. Chan said the data presented from the leukemia transplant model supported the mononuclear cell leukemia results in the two-year studies. But in light of the new data on pancreatic lesions, the NTP staff requested that the Panel consider a change in the conclusion for male rats to some evidence of carcinogenic activity.

There was some discussion as to why the leukemia findings were supportive only of some evidence. Dr. J. Huff, NIEHS, replied this was because these are quite variable tumors in historical controls, the findings in both dose groups from the two-year studies were only marginally statistically significant, and there was a lack of dose-response.

Dr. Popp moved that the Panel support the staff's recommendation that the conclusion for male rats in the Technical Report on dichlorvos be changed to some evidence of carcinogenic activity. Dr. Hughes seconded the motion, which was approved by nine yes to one no (Perera) votes.

INCIDENCE OF PANCREATIC LESIONS IN MALE RATS

Table 1. Routine sampling - Original

	VH	LH	HH
Pancreas			
Acinar Hyperplasia	9	9	9
Acinar Adenoma (single)	14	18	17
Acinar Adenoma (multiple)	2	7	13
Acinar adenoma (total)	16[1]	25[2]	30[3]

[1] trend, P < 0.001, logistic regression test
[2] vs. VH, P = 0.007, " " "
[3] vs. VH, P = 0.001, " " "

Table 2. Additional Sampling -New

	VH	LH	HH
Pancreas			
Acinar Hyperplasia	33	44	39
Acinar Adenoma (single)	12	13	7
Acinar Adenoma (multiple)	3	10	10
Acinar Adenoma (total)	15	23	17

Table 3. Original and New Data Combined

	VH	LH	HH
Pancreas			
Acinar Hyperplasia	37	45	39
Acinar Adenoma (single)	16	8	13
Acinar Adenoma (multiple)	9[1]	22[2]	20[3]
Acinar Adenoma (total)	25[4]	30[5]	33[6]

[1] trend, P = 0.004, logistic regression test
[2] vs. VH, P < 0.001, " " "
[3] vs. VH, P < 0.006, " " "
[4] trend, P = 0.018, " " "
[5] vs. VH, P = 0.041, " " "
[6] vs. VH, P = 0.026, " " "

INCIDENCE OF PANCREATIC LESIONS IN FEMALE RATS

Table 4. Routine Sampling - Original

Pancreas	VF	LF	HF
Acinar Hyperplasia	2	3	0
Acinar Adenoma (single)	1	1	4
Acinar Adenoma (multiple)	0	0	0

Table 5. Additional Sampling - New

Pancreas	VF	LF	HF
Acinar Hyperplasia	21	22	30
Acinar Adenoma (single)	1	2	1
Acinar Adenoma (multiple)	0	0	1
Acinar Adenoma (total)	1	2	2

Table 6. Original and New Data Combined

Pancreas	VF	LF	HF
Acinar Hyperplasia	21	23	30
Acinar Adenoma (single)	2	3	5
Acinar Adenoma (multiple)	0	0	1
Acinar Adenoma (total)	2	3	6

Nitrofurantoin: Update and Reevaluation of Further Pathology on Kidneys From Male Rats. The NTP toxicology and carcinogenesis studies of nitrofurantoin in F344/N rats and B6C3F1 mice (Technical Report No. 341) were peer reviewed and approved by the Panel on July 14, 1987. An important portion of the discussion focused on the tubular cell neoplasms in the kidneys of dosed male rats and the level of evidence for carcinogenic activity recommended by staff.

Dr. J.E. French, NIEHS, NTP Chemical Manager, summarized the previous discussion. The level of evidence selected for male rats was some evidence of carcinogenic activity based on: (1) a dose-related albeit marginal increased incidence of uncommon neoplasms of the tubular cells of the kidney (0,1,3) (Table 1); (2) possibility of progression to malignancy as evidenced by a tubular cell carcinoma of the kidney in a high dose male rat; and (3) a comparison with historical controls. The Peer Review Panel voted 4 to 3 with 2 abstentions to support the conclusion as written.

Dr. French went on to explain that because of the microscopic size of the majority of these tumors and questions concerning the dose-response relationship additional histological sections of the kidneys were prepared for evaluation. The purpose was to obviate the possibility of bias due to chance and to determine if the number of tumors found in each group would increase proportionally in relation to dose. The data derived only from the additional step sections are shown in Table 2. All of the additional kidney tumors were observed microscopically, and there was an increase in the number of multiple adenomas observed in the high dose male rats. The combined results of both data sets are shown in Table 3. The incidence of tubular cell neoplasms in the kidneys of male rats is: control, 3; low dose, 11; and high dose, 20. The low dose was statistically different from the controls at the 0.05 level and the high dose at the 0.001 level.

Dr. French said the data indicate that male rats administered nitrofurantoin in feed at 0, 1300, or 2500 ppm for 103 weeks developed treatment-related tubular cell neoplasms. These data support the conclusion as written in the Technical Report and approved by the Panel. A summary table and representative photomicrographs of selected tumors will be included in the Results section, and the Discussion section of the Report will be modified to reflect the additional findings.

Discussion among the Panel members and the staff centered around several issues, including: (1) the size and relative numbers of lesions in the recuts versus the original histological sections. It was noted that all of the adenomas were quite small, and there was concern as to why there were not concomitant increases in hyperplasias and carcinomas in the recuts; (2) which sets of numbers could be compared with or added to the historical control data base? (It was agreed that only the original incidences could be compared or added); (3) whether the Panel should move to affirm or change the level of evidence in male rats, or defer this decision; and (4) the generic issue of when and why should the NTP return to a study and do additional sections, etc. There was agreement that this would not be done routinely. The Panel suggested that the NTP develop criteria to use in making such decisions.

Dr. Hooper moved that the conclusion for male rats be changed to clear evidence of carcinogenic activity. Dr. Perera seconded the motion, which was not sustained by five no (Ashby, Capen, Gallo, Popp, Sivak) to four yes (Chinchilli, Hooper, Lijinsky, Perera) votes with one abstention (Hughes) for

TABLE 1

NITROFURANTOIN - TUBULAR CELL TUMORS IN MALE F344 RATS-ORIGINAL EVALUATION

	UM	LM	HM
Tubular Cell Hyperplasia	2	2	1
Tubular Cell Adenoma	0	1	2
Tubular Cell Carcinoma	0	0	1
Tubular Cell Adenoma and Carcinoma, Combined	0	1	3

TABLE 2

NITROFURANTOIN - TUBULAR CELL TUMORS IN MALE F344 RATS

	UM	LM	HM
Tubular Cell Hyperplasia	9	9	7
Tubular Cell Adenoma (Single)	2	9	12
Tubular Cell Adenoma (Multiple)	1	1	5
Tubular Cell Carcinoma	0	0	1
Tubular Cell Adenoma and Carcinoma, Combined	3	10	17

One control male and three low dose males had both hyperplasia and adenoma, while one high dose male had hyperplasia, adenoma, and carcinoma.

TABLE 3

NITROFURANTOIN - TUBULAR CELL TUMORS IN MALE F344 RATS-FINAL COMBINED RESULTS

	UM	LM	HM
Tubular Cell Hyperplasia	10	11	8
Tubular Cell Adenoma	3	11	19
Tubular Cell Carcinoma	0	0	2
Tubular Cell Adenoma and Carcinoma, Combined	3	11	20

Prechronic Technical Reports and Peer Review

Dr. E. McConnell, NIEHS, announced that the NTP soon will begin to report out the results of the prechronic studies on chemicals separately from the two-year study reports ('Blue Books'). The format of the prechronic reports will be similar to the current Technical Reports series. Further, the subsequent two-year reports will also contain prechronic study information but more highlighted and referenced and not in as much detail as in current Blue Books.

Peer review for most of the prechronic studies will be by mail to save Panel meeting time and to expedite moving to the next step if the decision is made that chronic studies should be done. At least three Panel members will be asked to review each report, although they will be sent to all members. If the written responses are uniformly positive, that will be considered the completion of the peer review. However, if the results of a given study appear to be debateable, that report will be submitted to the full Panel for discussion at the next meeting. In addition, members of the Panel can request that any report receive full Panel consideration. Also, the staff may bring a particular report to the Panel meeting if felt that it requires more indepth consideration. Dr. McConnell said the NTP wants to start this procedure on a trial basis realizing that the prechronic reports will evolve as have the two-year study reports. To continue to allow public input, completed prechronic studies will be listed in the Federal Register along with a contact person and an adequate time period for comments.

Proposed Plans for Toxicologic Evaluation of Ozone

Dr. G. Boorman, NIEHS, presented background information, rationale and proposed NTP studies on ozone, as follows:

Ozone has received a lot of attention because of its ubiquitous nature in urban environments. The School of Public Health at the University of North Carolina did a study in 1960 showing that there were low, but detectable, levels of ozone in Chapel Hill at a time when the town had only twelve thousand inhabitants. The author stated that the atmospheric conditions in the area were conducive to ozone problems. The highest levels they found at that time were 0.09 ppm. More recently the EPA has announced that ten North Carolina localities do not meet EPA standards for ozone levels in the air.

Of particular concern is the ozone found in the urban environment as a result of the photo-chemical action on polluted air resulting in increasing ozone levels during the day with peak levels between 2:00 and 4:00 p.m. in most localities. In the Los Angeles air basin the ozone levels exceed the EPA standard of 0.12 ppm on approximately eighty-five out of ninety days in the summer months. And in the United States EPA has estimated that more than a hundred million people are exposed to levels exceeding the EPA standard annually.

Ozone has been the subject of numerous investigations in both man and animal. Most studies were short term and have shown that the centriacinar region of the lung was the most sensitive area. And in that area there was loss of cilia from the respiratory epithelial cells and the dome-like projections on the Clara cells were flattened. Physiological effects have been shown in man and these effects were enhanced by exercise.

It is surprising, given the wide exposure of the human population to ozone levels that are known to cause cellular changes in animals, that adequate carcinogenicity studies have not been completed. There was one study in an A-strain mouse that was considered suggestive, but certainly not conclusive, of a positive pulmonary carcinogenic effect.

Ozone was nominated to the NTP by the State of California and by the Health Effects Institute. The Health Effects Institute is supported in part by EPA funds and deals mainly with research related to air quality.

In the fall of 1987, the NTP Chemical Evaluation Committee evaluated ozone and recommended carcinogenicity studies. It has since been reviewed by the Board of Scientific Counselors and the Executive Committee and been approved for study by the NTP.

A review of the literature has confirmed that while numerous short-term tests are available, adequate carcinogenicity studies have not been done. The NTP convened a group of twelve experts March 25, 1988, to consider issues relative to evaluation of ozone. The plan is to proceed as rapidly as possible on long-term evaluation of ozone.

The NTP Toxicology Design Review Committee was scheduled to review the protocols for ozone on May 18, 1988. The Fischer 344 rat and the B6C3F1 mouse will be used probably with 24- and 30-month exposures. This is because, at least in a study with diesel exhaust, eighty percent of the pulmonary tumors observed occurred after 24 months.

Among the group of experts, there appeared to be a consensus that one part per million was probably a logical top exposure concentration with multiple lower concentrations. There also has been interest in evaluating some markers of lung damage, and many of these parameters have been extensively evaluated at lower concentrations of ozone for shorter time periods. Much of this work has been done very nicely at Duke University.

It is important to be able to correlate the long-term studies with the short-term studies. Ways are being explored for cooperation with the Health Effects Institute so some of the long-term markers can be done in NTP long-term studies and compared with these in the short-term studies.

One of the main interests expressed by the group of experts was in some way of looking at the effect of ozone in combination with a known pulmonary carcinogen and a compound that's been found in the environment, such as benzo(a)pyrene.

At Duke University it has been shown that ozone exposure followed by asbestos exposure results in a greater retention of the asbestos fibers in the lung than with asbestos exposure alone.

Since ozone is so ubiquitous, because it is reactive, and because it occurs in combination with so many other chemicals, there was unanimous agreement that ozone should be tested in combination with other chemicals.

Combination and mixture studies pose extensive health and safety issues, chemistry problems, and the usual difficulty with trying to construct a mixture study. We are continuing to work on the ozone combination study to see if ways can be designed to determine if ozone will enhance the carcinogenicity of certain chemicals.

In the meantime, the NTP will proceed with a more standard ozone study.