132-98-9

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

July 14, 1987
Research Triangle Park, North Carolina

The review meeting began at 9:00 a.m. 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. Drs. Perera and Lijinsky were 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 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 November 6, 1987, in Research Triangle Park, North Carolina. For information, contact Dr. Hart, (919) 541-3971; FTS: 629-3971.

CONTENTS

2-Amino-4-Nitrophenol. Dr. R. D. Irwin, NIEHS, NTP Chemical Manager, introduced the toxicology and carcinogenesis studies of 2-amino-4-nitrophenol by reviewing the experimental design, results, and proposed conclusions:

> Under the conclusions of these 2-year gavage studies, there was some evidence of carcinogenic activity of 2-amino-4-nitrophenol for male F344/N rats, as shown by increased incidences of renal cortical (tubular cell) adenomas. The incidences of renal tubular cell hyperplasia were also increased in male rats exposed to 2-amino-4-nitrophenol. The survival of male rats that received 2-amino-4 nitrophenol was reduced compared with survival of vehicle control male rats. There was no evidence of carcinogenic activity of 2-amino-4-nitrophenol for female F344/N rats that received 125 or 250 mg/kg. There was no evidence of carcinogenic activity of 2-amino-4-nitrophenol for male or for female B6C3F$_1$ mice that received 125 or 250 mg/kg.

Dr. Mirer, a principal reviewer, agreed with the conclusions for male and female rats and female mice. He said that the conclusion of some evidence of carcinogenic activity in male rats was strengthened by the fact that there was a statistically significant increase in renal tumors despite a sharply reduced survival in male rats. Dr. Mirer recommended changing the conclusion in male mice from no evidence to equivocal evidence of carcinogenic activity based on a significantly increased incidence of hemangiomas or hemangiosarcomas at multiple sites.

As a second principal reviewer, Dr. Chinchilli agreed with the conclusions, yet asked for more discussion about the circulatory system tumors in male mice. Dr. Irwin responded that the occurrence of these tumors at separate sites and the similarity with historical control incidences at the laboratory where the studies were performed argued against an association with chemical administration.

As a third principal reviewer, Dr. Sivak agreed with the conclusions for female rats and male and female mice but thought the conclusion for male rats should be changed from some evidence to equivocal evidence of carcinogenic activity as the occurrences of renal tumors represented a marginal increase and the diagnosis depends on a continuum of lesions with a high probability of ambiguity. Dr. Irwin stated that the conclusion was based on a small but definite increase in a rare tumor type, a dose-response trend for the tumors, and a corresponding dose related increase in renal tubular cell hyperplasia. Dr. J. Haseman, NIEHS, noted that there was reduced survival in high-dose male rats and that these were late-appearing tumors in this study. Thus, an analysis based on animals surviving until the appearance of the first tumor would further strengthen the significance of the observed increase in kidney neoplasms. At Dr. Scala's request, Dr. Haseman said a supplemental analysis could be added making this comparison. Dr. Sivak wondered about the stability of the dose solutions over time in view of the propensity for ortho amino phenols to condense in solution. Dr. Irwin replied that the dosage formulations were evaluated and shown to be stable in corn oil for at least two weeks.

In other discussion, Dr. Ashby noted that this may be the first mutagenic aromatic nitro compound to not produce liver tumors in either species. He commented on target organ toxicity (in this case, kidney) and possible implications concerning the relationship between tumor formation and mutagenicity. Dr. J. Huff, NIEHS, observed that nonneoplastic lesions were considered as a factor in interpreting the findings for tumorigenicity.

Dr. Mirer moved that the Technical Report on 2-amino-4-nitrophenol be accepted with revisions as discussed and with the conclusions as written for male rats, some evidence of carcinogenic activity, and for female rats and male and female mice, no evidence of carcinogenic activity. Dr. Sivak seconded the motion and the Technical Report was approved unanimously with nine votes.

C.I. Acid Orange 3. Dr. J. H. Mennear, NIEHS, NTP Chemical Manager, introduced the toxicology and carcinogenesis studies of C. I. Acid Orange 3 by reviewing the experimental design, results, and proposed conclusions:

> Under the conclusions of these 2-year gavage studies, there was no evidence of carcinogenic activity of C.I. Acid Orange 3 for male F344/N rats administered 375 mg/kg; because of a marked reduction in survival and no indication of carcinogenicity, the 750 mg/kg group was considered to be inadequate for assessment of carcinogenic activity. There was clear evidence of carcinogenic activity of C.I. Acid Orange 3 for female F344/N rats as shown by the occurrence of transitional cell carcinomas of the kidney in the 750 mg/kg group; this group had reduced survival and chemically related nonneoplastic lesions of the kidney. There was no evidence of carcinogenic activity of C.I. Acid Orange 3 for male B6C3F$_1$ mice administered 125 or 250 mg/kg or for female B6C3F$_1$ mice administered 250 or 500 mg/kg. Nonneoplastic lesions of the kidney were observed in both dosed groups of both sexes of rats and mice.

Dr. Gallo, a principal reviewer, agreed with the conclusions. He thought the increased incidences of rare renal tumors in female rats allowed an opportunity to study mechanisms without having to evoke the caveats for such tumors in male rats. He questioned use of the oral route for studying a chemical used almost exclusively as a hair dye.

As a second principal reviewer, Dr. Popp agreed with the conclusions for male rats and male and female mice. He stated that the high dose for both sexes of rats was clearly excessive. The renal toxicity and poor survival in female rats tended to confound interpretation of the renal tumors in females.

As a third principal reviewer, Dr. Mirer agreed with the conclusions and agreed that the high mortality in male rats reduced the sensitivity of the study to detect a typically late appearing carcinogenic response. He suggested that the presence of kidney toxicity in all four experiments contrasted with occurrence of tumors in only one experiment (female rats) argued against a coupling of toxicity and tumorigenesis. Dr. Mirer asked for clarification of the impurities present in the technical grade chemical used in the studies. Dr. Mennear replied that the commercial product received contained 33% impurities including water, wetting agents, and surfactants. These impurities were not analyzed but were removed by solvent extraction leaving 10% impurities, these being acetone and water used in the extraction procedure.

Dr. Gallo moved that the Technical Report on C.I. Acid Orange 3 be accepted with revisions discussed and with the conclusions as written for male rats and male and female mice, no evidence of carcinogenic activity, and for female rats, clear evidence of carcinogenic activity. Dr. Mirer seconded the motion and it was approved by five Yes to two No votes (Dr. Hooper, Dr. Popp) with one Abstention (Dr. Ashby, for reasons of company affiliation).

Dichlorvos. Dr. P. C. Chan, NIEHS, NTP Chemical Manager, introduced the toxicology and carcinogenesis studies of dichlorvos 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 dichlorvos for male F344/N rats, as shown by increased incidences of adenomas of the exocrine pancreas and mononuclear cell leukemia. There was some evidence of carcinogenic activity of dichlorvos for female F344/N rats, as shown by increased incidences of adenomas of the exocrine pancreas and mammary gland fibroadenomas. There was some evidence of carcinogenic activity of dichlorvos for male and female $B6C3F_1$ mice, as shown by increased incidences of forestomach squamous cell papillomas.

Dr. Hooper, a principal reviewer, agreed with the conclusions for male and female rats and male mice but proposed that the conclusions in female mice be changed to clear evidence of carcinogenic activity based on a dose related increase in a combination of benign and malignant neoplasms (forestomach squamous cell papillomas and carcinomas). No squamous cell carcinomas have been observed in corn oil vehicle control female mice in NTP studies. He opined that male mice likely could have tolerated the same dose level as given to female mice, or twice that given. Dr. Chan agreed and speculated that if the dose levels in males had been the same as in females, the incidence of forestomach papillomas likely would have been increased.

As a second principal reviewer, Dr. Ashby stated that with the possible exception of the female mouse, the conclusions in this report more appropriately might be equivocal evidence of carcinogenic activity. He reasoned that since the chemical is an alkylating agent and direct-acting mutagen, one might expect tumors at the site of exposure, i.e., stomach but not at further sites. The reverse was found in rats, no increases in stomach tumors but increased incidences of pancreatic acinar cell adenomas in males and females, of mononuclear cell leukemias (MNCL) in males, and of mammary gland tumors in females. Confounding the biological significance in rats were the high concurrent control incidences for the male rat tumors (compared with historical control incidence for the laboratory), and conversely the low concurrent control incidence for mammary tumors in females. Dr. S. Eustis, NIEHS, and Dr. J. Haseman, NIEHS, said the incidence of MNCL in rats has been increasing over the last several years so the incidence in concurrent control male rats was probably not unusual. Dr. J. Huff, NIEHS, explained that the level of evidence in male rats was based largely on the high incidence of pancreatic neoplasia, and that the MNCL was contributory. Dr. Ashby said that points supporting equivocal evidence in male mice were no increases in forestomach hyperplasia, equal control and low dose rates for squamous cell papillomas, and no malignant tumors.

As a third reviewer, Dr. Gallo agreed with the conclusions for male rats (but noted the possible effects of corn oil interaction) and with the conclusions for male mice, noting that the increased incidences of forestomach lesions in high dose animals were not statistically significant. He also agreed with the conclusions for female mice. He thought the conclusion for female rats should

be changed to equivocal evidence of carcinogenic activity because the incidence of mammary fibroadenomas was within the historical control rate for both the laboratory and the NTP. Dr. Chan noted that when the most appropriate comparisons are made, with concurrent controls, there are significantly increased incidences for fibroadenomas in both low and high dose groups. Further, there were increased incidences of multiple fibroadenomas in the dose groups which were not seen in the controls. Dr. Huff pointed out that the increase in pancreatic tumors in the high dose females was supported by the same effect in male rats.

Dr. Mirer and other Panel members said there was insufficient information on the methodology used for measuring cholinesterase inhibition as well as lack of adequate interpretation and discussion of the results. Dr. Gallo also questioned the rationale for the dose route; either inhalation or dermal would have been more appropriate.

Professor Paul Grasso, Robens Institute, U. K., representing Shell Internationale Petroleum, suggested that the data did not support association of chemical exposure with increased incidences of mammary gland tumors and MNCL in female rats, while the high incidence of pancreatic tumors in control male rats did not allow a conclusion to be drawn as to causation in dosed animals. He opined that the cluster of forestomach tumors in female control mice obscured any association of chemical with increased incidences of these tumors in treated mice.

Dr. Hooper moved that the conclusions for male rats, clear evidence of carcinogenic activity, be accepted as written with mention made of the high concurrent control incidences for pancreatic tumors and MNCL. Dr. Gallo seconded the motion and it was approved by six Yes to two No (Dr. Ashby, Dr. Popp) votes. Dr. Hooper moved that the conclusions for female rats, some evidence of carcinogenic activity, be accepted as written. The motion failed for lack of a second. Dr. Ashby moved that the conclusion be changed to equivocal evidence of carcinogenic activity. Dr. Sivak seconded the motion. The motion was approved by six Yes to two No votes (Dr. Hooper, Dr. Mirer). Dr. Hooper moved that the conclusions for male mice, some evidence of carcinogenic activity, be accepted as written. Dr. Gallo seconded the motion and it was approved by seven Yes to one No votes (Dr. Sivak). Dr. Hooper moved that the conclusions for female mice be changed to clear evidence of carcinogenic activity. Dr. Ashby seconded the motion and it was approved by seven Yes to one No vote (Dr. Gallo).

Erythromycin Stearate. Dr. J. E. French, NIEHS, NTP Chemical Manager, introduced the toxicology and carcinogenesis studies of erythromycin stearate by reviewing the experimental design, results, and proposed conclusions:

> Under the conditions of these 2-year studies, there was no evidence of carcinogenic activity of erythromycin stearate for male or female F344/N rats administered erythromycin stearate in the diet at 5,000 or 10,000 ppm for 103 weeks. There was no evidence of carcinogenic activity of erythromycin stearate for male or female B6C3F$_1$ mice administered erythromycin stearate in the diet at 2,500 or 5,000 ppm for 103 weeks. Dose-related increases in the incidences of granulomas of the liver were observed in male and female rats. The absence of any biologically important chemical-associated effects in mice suggests that higher doses might have been considered for male and female mice.

Dr. Sivak, a principal reviewer, agreed with the conclusions. He asked why the doses for the two year studies in mice were only half those given to rats, and emphasized the importance of explaining the rationale for the dose selection for the long-term studies. Dr. French responded that the dose selections were based solely on the observed body weight depression, and that mice showed greater body weight differences than did rats during the 13-week studies.

As a second principal reviewer, Dr. Gallo agreed with the conclusions. He commented on the difficulty in evaluating antibiotics by the usual criteria such as changes in body weight as some antibiotics lead to increases in body weights. Dr. Gallo speculated that the granulomas or lymphoid hyperplasias reported in liver, spleen and bladder might be crystalline deposits of the chemical or its metabolites. Dr. French acknowledged this could be an alternative explanation for the lesions, but indicated none were seen on light microscopy.

As a third principal reviewer, Dr. Capen agreed with the conclusions. In other discussion, Dr. Ashby commented on the weak positive genotoxic responses to erythromycin in the mammalian cell assays (mouse lymphoma and Chinese hamster ovary) which may be an artifact resulting from the high doses of the alkaline salt associated with the antibiotic.

Dr. Sivak moved that the Technical Report on erythromycin stearate be accepted with editorial changes discussed and with the conclusions as written for male and female rats and mice, no evidence of carcinogenic activity. Dr. Popp seconded the motion and it was approved unanimously with seven votes.

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

> Under the conditions of these 2-year feed studies, there was some evidence of carcinogenic activity of nitrofurantoin for male F344/N rats as shown by increased incidences of uncommon kidney tubular cell neoplasms. Osteosarcomas of the bone and neoplasms of the subcutaneous tissue were observed in dosed male rats. Incidences of interstitial cell adenomas of the testis and neoplasms of the preputial gland were decreased in the 2,500 ppm group of male rats. There was no evidence of carcinogenic activity of nitrofurantoin for female F344/N rats fed diets containing 600 ppm or 1,300 ppm for 2 years. Female rats may have been able to tolerate higher doses. There was no evidence of carcinogenic activity of nitrofurantoin for male B6C3F$_1$ mice fed diets containing 1,300 ppm or 2,500 ppm for 2 years. There was clear evidence of carcinogenic activity of nitrofurantoin for female B6C3F$_1$ mice as shown by increased incidences of tubular adenomas, benign mixed tumors, and granulosa cell tumors of the ovary.
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> Nonneoplastic lesions considered related to nitrofurantoin exposure were chronic nephropathy and associated lesions (hyperplasia of the parathyroid gland, fibrous osteodystrophy of the bone, and mineralization of the glandular stomach) in male rats and testicular degeneration in male rats and mice. Ovarian atrophy and hyperplasia of the adrenal cortex spindle cells were observed in dosed female mice.

Dr. Popp, a principal reviewer, agreed with the conclusions for female rats and male and female mice; he said the Panel should discuss the concomitant ovarian toxicity in female mice. Dr. Popp opined that the results for male rats, a slight increase in renal tubular neoplasms (controls, 0/50, low dose, 1/50, high dose, 3/50) coupled with a corresponding lack of an increase in renal tubular hyperplasia, more closely supported equivocal evidence of carcinogenic activity. Dr. French acknowledged that the lack of hyperplasia must be considered but felt that the presence of a carcinoma was evidence supporting progression and although the numbers of renal tubular cell tumors were relatively low, there was a 20-fold difference between the high dose and historical control rates.

Dr. Hughes pointed out that there was one study in the historical control data base with two tubular neoplasms. Dr. J. Haseman, NIEHS, reported that for the most recent 73 corn oil gavage and feed studies, 57 had zero incidence in controls, 15 had an incidence of one, and one study had an incidence of two tubular neoplasms.

As a second principal reviewer, Dr. Ashby agreed with the conclusions for male and female rats and male mice, while suggesting the conclusions in female mice be changed to some evidence of carcinogenic activity. He stated that two of the three types of ovarian tumors were observed only in the high dose groups. He questioned whether those tumors could be combined for assessment. Also confounding the interpretation was the presence of ovarian atrophy in almost

all of the treated animals. Dr. French remarked that both the ovarian tubular adenomas and benign mixed tumors were uncommon and histogenetically it was considered appropriate to combine them.

As a third principal reviewer, Dr. Chinchilli agreed with the conclusions for male and female rats and male mice noting that osteosarcomas of the bone and subcutaneous tumors observed in male rats are uncommon. For female mice, he questioned why ovary tissues from only 39 control mice were available for microscopic evaluation while statistical analyses were based on a sample of 50. Dr. S. Eustis, NIEHS, explained that ovaries from all 50 control female mice were examined; however, ovarian abcesses had destroyed much of the tissues from 11 animals. In his opinion, the examination was sufficient to determine whether or not a tumor was present. Dr. Haseman commented that whether one uses a denominator of 39 or 50, the differences in tumor incidences are highly significant and quite striking. Dr. Chinchilli wondered why a statistical comparison test using historical control data could not be used in analysis of uncommon tumors. Dr. Haseman agreed that rare or uncommon tumors might be the one instance in which a formal analysis incorporating historical data should be considered although lack of an agreed upon test was still a problem.

Dr. William H. Butler, of the British Industrial Biological Research Association and representing Norwich Eaton Pharmaceuticals, Inc., presented a review of his observations from examining the slides containing ovary sections from the female mice. He contended that the occurrence of ovarian abcesses in a number of controls obviated a proper analysis, while the possibility that the tubular adenomas may have resulted from hormonal stimulation due to ovarian atrophy, and the existence of other negative studies supported equivocal evidence of carcinogenic activity. Dr. Butler opined further that there was no evidence of carcinogenic activity in male rats because the incidence of renal tubular neoplasms was low and within the expected historical range, there was no evidence of similar lesions in female rats, there was no increase in hyperplasia, and there was high incidence of chronic nephropathy.

Dr. E. McConnell, NIEHS, emphasized that both increases and decreases in hyperplasia are considered in the evaluations. In the case of the renal tumors in male rats, the lack of hyperplasia was noteworthy but did not necessarily offset the increase in very uncommon tumors.

Dr. Popp moved that the conclusions for male rats be changed to equivocal evidence of carcinogenic activity, and that conclusions for female rats, no evidence of carcinogenic activity, be accepted as written. Dr. Ashby seconded the motion, which was defeated by four No (Dr. Ashby, Dr. Chinchilli, Dr. Hooper, Dr. Mirer) to three Yes votes (Dr. Gallo, Dr. Popp, Dr. Sivak) with two Abstentions (Dr. Capen, Dr. Hughes). Dr. Hooper moved that the conclusions be accepted as written for male rats, some evidence of carcinogenic activity, and for female rats, no evidence of carcinogenic activity. Dr. Ashby seconded the motion and it was approved by four Yes (Dr. Ashby, Dr. Chinchilli, Dr. Hooper, Dr. Mirer) to three No votes (Dr. Gallo, Dr. Popp, Dr. Sivak) with two Abstentions (Dr. Capen, Dr. Hughes). Dr. Popp moved that the conclusions for male mice, no evidence of carcinogenic activity, and for female mice, clear

evidence of carcinogenic activity, be accepted as written. Dr. Chinchilli seconded the motion and it was approved by five Yes to two No votes (Dr. Ashby, Dr. Gallo) with two Abstentions (Dr. Capen, Dr. Hughes).

On the various votes, Dr. Capen's abstentions reflected his role as consultant on nitrofurantoin with the manufacturer, and Dr. Hughes abstained for reasons of company affiliation.

Nitrofurazone. Dr. F. Kari, NIEHS, NTP Chemical Manager, introduced the toxicology and carcinogenesis studies of nitrofurazone 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 nitrofurazone for male F344/N rats as shown by the occurrence of sebaceous gland adenomas and trichoepitheliomas of the skin, mesotheliomas of the tunica vaginalis, and preputial gland tumors. There was clear evidence of carcinogenic activity of nitrofurazone for female F344/N rats as shown by a markedly increased incidence of fibroadenomas of the mammary gland. There was no evidence of carcinogenic activity for male B6C3F$_1$ mice fed diets containing nitrofurazone at concentrations of 150 or 310 ppm. There was clear evidence of carcinogenic activity of nitrofurazone for female B6C3F$_1$ mice as shown by increased incidences of benign mixed tumors and granulosa cell tumors of the ovary.
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> Administration of nitrofurazone was associated with decreased incidences of mononuclear cell leukemia in male and female rats, testicular interstitial cell tumors in male rats, and pituitary gland neoplasms in female mice. Convulsive seizures in mice of each sex, ovarian atrophy in female mice, testicular degeneration in rats, and degeneration of articular cartilage in rats were all associated with the administration of nitrofurazone.

Dr. Chinchilli, a principal reviewer, agreed with the conclusions. He suggested a sentence might be added to the conclusions for male rats to emphasize the uncertainty as to whether the increased tumor incidences were chemically related. Dr. Kari indicated that the definition of equivocal evidence of carcinogenic activity already emphasized this uncertainty.

As a second principal reviewer, Dr. Hooper agreed with the conclusions for male and female rats and female mice. He stated that the conclusion for male mice should be equivocal evidence of carcinogenic activity based on marginal increases of subcutaneous tissue fibromas, sarcomas, fibrosarcomas, or neurofibrosarcomas (combined) in high dose animals. Further, reduced survival among the high dose animals limited the sensitivity of the assay to detect carcinogenic effects. Dr. Kari explained that the large variability in the historical control data base for subcutaneous tumors was a prime reason for deciding on no association with chemical exposure. Dr. Hooper asked whether there was any mechanistic explanation for the decreases in incidence of several tumors in dosed rats and mice. Dr. Kari said he could only speculate as to mechanisms in that there is some relationship between body weight differences and incidences of certain endocrine associated tumors.

As a third principal reviewer, Dr. Ashby agreed with the conclusions for male mice. For male rats, he asked whether the incidences of preputial gland adenomas and carcinomas should be analyzed separately, in which case the incidence of carcinomas in treated groups supported a conclusion of some evidence of carcinogenic activity. Dr. Kari said the progression from adenomas

to carcinomas was an established continuum and thus evaluating them combined is more appropriate although the incidence rates for benign and malignant tumors are shown separately. Dr. J. Huff, NIEHS, indicated this was a routine adopted for all tumor sites. Dr. Ashby continued that the conclusion for female rats should be some evidence of carcinogenic activity unless data can be cited that confirm the ability of fibroadenomas to progress to malignancy. Dr. Kari noted that the discussion section of the Technical Report cited other authors who have shown this progression to occur in a small percentage of observations and that the NTP also has compiled evidence for progression, about which a manuscript is being prepared. Dr. S. Eustis, NIEHS, commented that whether a tumor remains benign or becomes malignant depends on both the intrinsic properties of the tumor and on host factors. He further stated that fibroadenomas have been demonstrated to progress to malignant tumors when transplanted into suitable hosts. Dr. Hooper said this made it most important that appropriate documentation be provided supporting the potential for progression to malignancy. For female mice, Dr. Ashby thought the conclusion should be some evidence of carcinogenic activity unless progression to malignancy could be shown for the ovarian granuloma cell tumors. Dr. Kari remarked that in the study of nitrofurantoin, malignant granulosa cell tumors were observed. Dr. Ashby emphasized the importance of good data and exact citations where the distinction between some evidence and clear evidence is supported by evidence of progression from studies of other investigators. Dr. Ashby noted that semicarbazide, a known animal carcinogen which is a substructure of nitrofurazone, was not associated with the carcinogenic effects observed because different site specific neoplasms occur.

Dr. Chinchilli moved that the Technical Report on nitrofurazone be accepted with revisions as discussed to contain more explanation about progression, and with the conclusions as written for male rats, equivocal evidence of carcinogenic activity, for male mice, no evidence of carcinogenic activity, and for female rats and mice, clear evidence of carcinogenic activity. Dr. Hooper seconded the motion and it was approved by seven Yes to zero No votes with two Abstentions (Dr. Capen - served as a consultant with the manufacturer, and Dr. Hughes - company affiliation).

Penicillin VK. Dr. J. K. Dunnick, NIEHS, NTP Chemical Manager, introduced the toxicology and carcinogenesis studies of penicillin VK by reviewing the experimental design, results, and proposed conclusions:

> Under the conditions of these 2-year gavage studies, there was no evidence of carcinogenic activity of penicillin VK for F344/N rats or for B6C3F$_1$ mice administered 500 or 1,000 mg/kg penicillin VK in corn oil by gavage, 5 days per week for 103 weeks. Nonneoplastic lesions were seen in the glandular stomach of dosed mice. Decreased survival of low and high dose male rats and of high dose female rats reduced the sensitivity for determining the presence or absence of a carcinogenic response in this species.

Dr. Capen, a principal reviewer, agreed with the conclusions. He asked for clarification of the significance of the eosinophilic cytoplasmic changes reported in the fundus of the stomach. Dr. S. Eustis, NIEHS, responded that the eosinophilic changes are believed to represent a potential secretory material in the epithelial cells lining the glandular stomach.

As a second principal reviewer, Dr. Sivak agreed with the conclusions. He expressed concern about the selection of corn oil as the vehicle for water soluble substance, and this may influence the pharmacokinetics in relation to human experiences. Dr. Dunnick commented that at the time these studies were designed ampicillin was also selected and because it was less water soluble the vehicle chosen for both studies was corn oil. She said an aqueous gavage solution would have been appropriate, and noted that NTP is monitoring blood levels on some of the newer studies on drugs.

As a third principal reviewer, Dr. Popp agreed with the conclusions. Dr. Capen moved that the Technical Report on penicillin VK be accepted with revisions discussed and with the conclusions as written for male and female rats and mice, no evidence of carcinogenic activity. Dr. Sivak seconded the motion and it was approved unanimously with eight votes.