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

March 4, 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 were: Drs. Robert Scala (Chairperson), Michael Gallo and Frederica Perera. Members of the Panel of Experts were: Drs. Charles Capen, Vernon Chinchilli, John Crowley, Kim Hooper, Donald Hughes, Franklin Mirer, James Popp, Ian Purchase, and Andrew Sivak. Drs. Crowley, Mirer, Perera, and Purchase 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 July 14, 1987, in Research Triangle Park, North Carolina. For information, contact Dr. Hart, (919) 541-3971; FTS: 629-3971.

CONTENTS

2-Amino-5-Nitrophenol. Dr. R. D. Irwin, NIEHS, NTP Chemical Manager, introduced the toxicology and carcinogenesis studies of 2-amino-5-nitrophenol 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 that received 100 mg/kg 2-amino-5-nitrophenol as shown by the increased incidence of acinar cell adenomas of the pancreas. Reduced survival of male F344/N rats that received 200 mg/kg decreased the sensitivity of this group for detecting carcinogenic activity. There was no evidence of carcinogenic activity for female F344/N rats. Increased incidences of preputial or clitoral gland adenomas or carcinomas (combined) occurred in both male and female F344/N rats administered 200 mg/kg 2-amino-5-nitrophenol. There was no evidence of carcinogenic activity for B6C3F$_1$ mice that received 400 mg/kg 2-amino-5-nitrophenol. Reduced survival of B6C3F$_1$ mice that received 800 mg/kg caused this group to be considered inadequate for detecting carcinogenic activity.

Dr. Gallo, a principal reviewer, agreed with the conclusions as written. He noted that the Maximum Tolerated Dose (MTD) appeared to have been exceeded in both mice and rats, and suggested that the criteria for setting doses based on 13-week studies should be reexamined. Dr. Gallo said the report should note that a structurally related chemical, 2,4-dinitrophenol, is cataractigenic in some animal species and in humans.

As a second principal reviewer, Dr. Hughes agreed with the conclusions for female rats and male and female mice, but thought the conclusions for male rats should be changed to either equivocal evidence of carcinogenic activity or no evidence of carcinogenic activity. The incidence of acinar cell adenomas in low dose male rats was not different from that seen in historical control animals. The lack of dose response and closely associated hyperplastic response was also noted. Dr. Hughes said the lack of chemical stability to water and light made the gavage route appropriate even though the primary route of human exposure was dermal. Dr. Irwin commented that poor survival reduced the sensitivity for detecting an effect in high dose rats. However, 3 of the 13 high dose male rats that survived until week 98 of the study, which is when most of the acinar cell tumors begin to be observed, were found to have pancreatic acinar cell tumors. Dr. J. Huff, NIEHS, emphasized that the primary comparisons should be with concurrent control animals, and Dr. Scala added that historical controls should be used only to supplement the primary analysis.

As a third principal reviewer, Dr. Hooper agreed with the conclusions for male rats and male and female mice but stated that the conclusions in female rats should be equivocal evidence of carcinogenic activity based on the occurrence of clitoral gland adenomas in the high dose group at a rate well above the historical control range along with a positive trend. Since there was an increased incidence of carcinomas of the preputial gland in high dose male rats, he thought some discussion would be helpful on the ontological relationship

between the glands. Dr. S. Eustis, NIEHS, said the clitoral and preputial glands are analogous. Dr. J. Haseman, NIEHS, commented that there were two clitoral gland carcinomas in low dose females but none in the high dose group and when benign and malignant tumors were combined the positive trend was eliminated.

Dr. Gallo moved that the Technical Report on 2-amino-5-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. Hooper seconded the motion and it was approved unanimously with seven votes.

4-Hexylresorcinol - Dr. R. S. Chhabra, NIEHS, NTP Chemical Manager, introduced the toxicology and carcinogenesis studies of hexylresorcinol 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 4-hexylresorcinol for male or female F344/N rats given doses of 62.5 or 125 mg/kg. There was equivocal evidence of carcinogenic activity of 4-hexylresorcinol for male B6C3F$_1$ mice, as shown by marginally increased incidences of pheochromocytomas (and hyperplasia) of the adrenal gland medulla and of harderian gland neoplasms. There was no evidence of carcinogenic activity for female B6C3F$_1$ mice given doses of 62.5 or 125 mg/kg 4-hexylresorcinol. Decreased incidences of several tumors were considered to be related to 4-hexylresorcinol administration: mononuclear cell leukemia in male and female rats, hepatocellular neoplasms in male mice, and circulatory system tumors in male and female mice.

Dr. Perera, a principal reviewer, was unable to attend the meeting; her written comments were read by Dr. L. Hart, NIEHS. Dr. Perera agreed with the conclusions for female rats and male and female mice. She proposed that the conclusions for male rats be changed to equivocal evidence of carcinogenic activity based on the occurrence of rare brain tumors: two astrocytomas and one oligodendroglioma in high dose animals. This incidence exceeded the historical control incidence as well as that seen in any corn oil vehicle control male F344 rat group. Dr. Chhabra opined that the occurrence of a brain tumor in the control group weakened the case for an association of the tumors with chemical administration. Dr. S. Eustis, NIEHS, stated that less import could be given than if the tumors were all of the same cell type. Dr. Hooper observed that the results still support a conclusion of equivocal evidence of carcinogenic activity. Dr. Scala asked that there be a more rigorous treatment in the discussion.

As a second principal reviewer, Dr. Capen agreed with the conclusions as written. Commenting on the conclusion in male mice, he noted that although the mean historical incidence of pheochromocytomas in corn oil gavage control male mice was only 1.3% (19/1443), their range was from zero to 10% (5/49).

As a third principal reviewer, Dr. Sivak also agreed with the conclusions as written. His primary concern related to the rationale for selection of the gavage route given that predominant human exposure is via the skin. Dr. Chhabra responded that 4-hexylresorcinol is still used as an anthelmintic, given orally in tablets, and as an antiseptic used in lozenges and mouthwash. He said more emphasis would be given to the rationale of route selection. Dr. Sivak requested that more information on metabolism and distribution be included if available.

There was some discussion on the decreased incidences of several tumor types, whether this was related to the antiinfective properties of 4-hexylresorcinol, and implications for possible antineoplastic activity.

Dr. Capen moved that the Technical Report on 4-hexylresorcinol be accepted with revisions discussed and with the conclusions as written for male and female rats and female mice, no evidence of carcinogenic activity, and male mice, equivocal evidence of carcinogenic activity. Dr. Popp seconded the motion and it was approved unanimously with seven votes.

Malonaldehyde, Sodium Salt. Dr. J. W. Spalding, NIEHS, NTP Chemical Manager, introduced the toxicology and carcinogenesis studies of malonaldehyde, sodium salt, 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 for male and female F344/N rats administered malonaldehyde, sodium salt, as shown by the increased incidences of follicular cell adenomas or carcinomas (combined) of the thyroid gland in both sexes. Pancreatic islet cell adenomas were also observed at an increased incidence in low dose male rats. There was no evidence of carcinogenic activity for male and female B6C3F$_1$ mice administered 60 or 120 mg/kg malonaldehyde, sodium salt, in distilled water by gavage 5 days per week for 2 years.
>
> Chemically related increased incidences of nonneoplastic lesions in rats included ulcers and inflammation of the glandular stomach and epithelial hyperplasia of the forestomach; corneal inflammation, retinal atrophy, and cataracts of the crystalline lens; and cystic degeneration of the liver, bile duct fibrosis, and bile duct hyperplasia. There was a dose-related increase in the incidences of focal atrophy of the pancreatic acinus in male and female mice. An increased incidence of pigmentation loss in hair shafts was seen in high dose mice.

Dr. Hughes, a principal reviewer, agreed with the conclusions for male and female mice. He proposed that the conclusions for male and female rats be changed to some evidence of carcinogenic activity: because he felt the maximum tolerated dose (MTD) was exceeded in rats, possibly perturbing the endocrine axis and perhaps leading to endocrine tumor response through an indirect mechanism; because the total incidence of adenomas and carcinomas of the thyroid was low; because both short-term tests and initiation/promotion studies yielded mixed results; and because there was not a dose response for pancreatic islet cell tumors in male rats. Dr. Spalding pointed out that the thyroid neoplasms are uncommon, and the incidences in male and female rats at the top dose were well above the historical control range. Commenting on the inconsistencies in the short-term test and initiation/promotion data cited, Dr. Spalding said pre-1980 studies used mixtures of malonaldehyde and intermediates in its synthesis which had mutational activity. This will be expanded on in the report. Dr. J. Huff, NIEHS, commented that the conclusions in rats were based on the thyroid neoplasia, not on the low dose effect for pancreatic tumors.

As a second principal reviewer, Dr. Popp agreed with the conclusions. He agreed with Dr. Hughes that the MTD had been exceeded for high dose male and female rats, but felt the implications were unclear. He opined that the lower incidence of rats with pituitary tumors in high dose groups was probably due to reduced survival and not a primary effect of the chemical, while the non-neoplastic eye lesions probably were chemical related. Dr. Spalding agreed that the eye lesions were chemically related and said the discussion would be expanded.

As a third reviewer, Dr. Gallo agreed with the conclusions but noted that when the MTD is exceeded interpretation of either positive or negative findings is sometimes difficult. However, he added that based on the 13-week studies the doses selected for two-year studies were appropriate. Since the chemical is an intermediate in the biosynthesis of prostaglandins, he suggested that the toxicity may override control mechanisms in the synthesis pathway. Dr. Gallo thought the rationale for deciding to study malonaldehyde was weak.

In other discussions, Dr. Sivak proposed that a statement be included for the rat studies which indicates reduced survival and body weight gain in top dose groups. Dr. Hooper requested that all the genetic toxicology data be organized into a summary table to help the reader draw conclusions about the mutagenic activity of the malonaldehyde salt.

Dr. Hughes moved that the Technical Report on malonaldehyde, sodium salt, be accepted with the conclusions as written for mice, no evidence of carcinogenic activity, but with the conclusions for rats changed to some evidence of carcinogenic activity along with a statement that the MTD had been exceeded. Dr. Sivak asked that the statement be amended to replace the word MTD with a description of the biological alterations themselves, i.e., that there was decreased survival and a >10% decrease in body weight gain in high dose groups. Dr. Hughes agreed. Dr. Gallo seconded the amended motion, and after considerable discussion, it was defeated by 1Y (Dr. Hughes) to 6 No (N) votes. Dr. Gallo moved that the Technical Report be accepted with the conclusions as written for mice, no evidence of carcinogenic activity, and for rats, clear evidence of carcinogenic activity, and with Dr. Sivak's amendment. Dr. Sivak seconded the amended motion and it was approved by 6Y to 1N (Dr. Hughes) votes.

2-Mercaptobenzothiazole. Dr. M. P. Dieter, NIEHS, NTP Chemical Manager, introduced the toxicology and carcinogenesis studies of 2-mercaptobenzothiazole 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 of 2-mercaptobenzothiazole for male F344/N rats, indicated by increased incidences of mononuclear cell leukemia, pancreatic acinar cell adenomas, and adrenal gland pheochromocytomas. There was some evidence of carcinogenic activity for female F344/N rats, indicated by increased incidences of adrenal gland pheochromocytomas and pituitary gland adenomas. There was no evidence of carcinogenic activity of 2-mercaptobenzothiazole for male B6C3F$_1$ mice administered 375 or 750 mg/kg. There was equivocal evidence of carcinogenic activity for female B6C3F$_1$ mice, indicated by increased incidences of hepatocellular adenomas or carcinomas (combined).

Dr. Hooper, a principal reviewer, agreed with the conclusions as written. However, he argued that increased incidences of preputial gland adenomas or carcinomas (combined) should be cited instead of mononuclear cell leukemia (MNCL) in support of the conclusion in male rats. Dr. Dieter suggested that it was valid to include the preputial gland tumors, along with the MNCL, as some evidence of carcinogenic activity, and that the conclusions and other appropriate sections of the technical report could be revised to reflect this change. Dr. S. Eustis, NIEHS, commented that this tumor was not originally included in the list of evidence because although the incidence of preputial gland tumors in this study was twice the historical mean, the incidence also fell within the historical range. Dr. Hooper was still puzzled by the lack of tumors in high dose male rats compared to an elevated tumor incidence in low dose male rats for several neoplasms, including MNCL. Dr. Dieter said there was just one other tumor besides MNCL, pancreatic acinar cell adenomas in male rats, where there was an effect only at the low dose. There were two tumor types where dose-related increases occurred, including adrenal gland tumors in male and female rats, and pituitary gland tumors in female rats.

As a second principal reviewer, Dr. Popp agreed in principle with the conclusions. He said the issue for decision was whether the conclusions in rats should remain as written or be lowered to equivocal evidence of carcinogenic activity.

As a third principal reviewer, Dr. Chinchilli agreed with the conclusions as written. He asked that the incidences table for MNCL in female rats be added to the Results section.

Dr. Harold Grice, Cantox, Inc. Canada, representing the Rubber Additives Program Panel, Chemical Manufacturers Association, mentioned several factors that he felt made interpretation of the increased tumor rates in male rats difficult. These included reduced survival in both dose groups, compound induced kidney

toxicity, gavage stress, and post-gavage lethargy. Dr. Grice thought the conclusion on male rats should be lowered to equivocal evidence of carcinogenic activity.

Since the low dose animals were placed in the cage racks nearest the room fluorescent lights and because cages were not rotated in this study, there was speculation whether photoactivation of the chemical might have been a factor in toxicity/carcinogenicity. While incidence of eye lesions (retinopathy and cataracts) could be correlated with cage position, there was no consensus that increased tumor rates in low dose rats could be associated with exposure to light.

In other discussion, Dr. Hooper thought the small but significant increase in renal neoplasms in male rats (tubular cell adenomas and transitional cell papillomas/carcinomas) was chemically associated. Dr. Eustis said the renal tumors were not considered chemically related because there were two cell types generally not combined and the tumors were split between dose groups.

Dr. Hooper moved that the Technical Report on 2-mercaptobenzothiazole be accepted with the revisions discussed and the conclusions as written for male and female rats, some evidence of carcinogenic activity, for male mice, no evidence of carcinogenic activity, and for female mice, equivocal evidence of carcinogenic activity. He asked that the increased incidences of preputial gland adenomas or carcinomas (combined) in male rats be cited. Dr. Gallo seconded the motion and it was approved unanimously with seven votes. Dr. Scala asked that the flavor of the full and stimulating discussion of a number of points be incorporated into the report.

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

> Under the conditions of these 2-year feed studies of mirex, there is clear evidence of carcinogenic activity for F344/N rats as indicated primarily by marked increased incidences of benign neoplastic nodules of the liver in both males and females, as well as by increased incidences of pheochromocytomas of the adrenal gland and transitional cell papillomas of the kidney in males and by increased incidences of mononuclear cell leukemia in females.

Dr. Popp, a principal reviewer, deferred comment on the conclusions until the Panel discussed some of the major issues with the study. These included: apparent nonreproducibility of liver neoplasms for female rats fed 50 ppm mirex in the original study and a second study started several months later at 50 and 100 ppm; and an unusually high incidence of liver neoplasms in control female animals from the original study. Dr. Huff responded that there was no logical explanation either for the differences between studies or the high control tumor level in females from the first study.

As a second principal reviewer, Dr. Chinchilli agreed with the conclusions as written. He expressed concern over the less than complete record keeping on certain aspects of the study. He asked that more detail be given about the process used for randomization of animals, or it should be stated that detailed records are not available.

As a third principal reviewer, Dr. Hughes thought that the conclusions should be reduced to equivocal evidence of carcinogenic activity because: the primary liver effect was increased nodules; the liver response in females was not the same in both experiments; adrenal gland responses were mainly increases in benign pheochromocytomas; the renal transitional cell papilloma response in males was weak; and mononuclear cell leukemia responses were weak in female and equivocal in males, and there was no evidence of early onset in treated animals. He questioned whether these were valid studies on which to base conclusions since not all records were available.

In response to the reviewers, Dr. Huff stated that staff had confidence that the data were scientifically valid and reportable and that the spectrum of neoplastic responses together supported the category of evidence selected. These lesions are relatively rare occurrences in Fischer rats. Further, these findings in the liver are supported by other long term studies reported in the literature, and ample evidence exists that the target organ for this non-metabolized chemical is the liver. He reminded the Panel of other recent peer reviewed studies with conclusions of clear evidence of carcinogenic activity based on increased incidences of neoplastic nodules, and at rates lower than reported here. Also, the audit revealed that the archived records necessary to support these conclusions are available, as are all the pathology materials and specimens.

In other discussions, Dr. Gallo also emphasized liver as being a primary target organ noting that mirex is known to be a potent inducer of cytochrome P450 enzymes. He speculated that cross-contamination between rooms housing treated and control female animals might have been involved in the high incidence of neoplastic nodules of the liver in female control rats in the first study. Dr. S. Eustis, NIEHS, noted that neoplastic nodules in control animals were primarily composed of basophilic cells while nodules in treated animals were primarily either clear cell or eosinophilic cell types, a clear indication that mirex caused these effects.

Dr. Hooper moved that the Technical Report on mirex be accepted with the revisions discussed and the conclusions as written for male and female rats, clear evidence of carcinogenic activity. Dr. Sivak seconded the motion and it was approved by 6Y to 1N (Dr. Hughes) votes.

N-Phenyl-2-Naphthylamine. Dr. K. M. Abdo, NIEHS, NTP Chemical Manager, introduced the toxicology and carcinogenesis studies of N-phenyl-2-naphthylamine by reviewing the experimental design, results, and proposed conclusions:

> Under the conditions of these 2-year feed studies of N-phenyl-2-naphthylamine, there was no evidence of carcinogenic activity for male or female F344/N rats fed diets containing 2,500 or 5,000 ppm. Decreased incidences of several neoplasms were observed in dosed rats: thyroid gland C-cell neoplasms in male and females, and, in females, mononuclear cell leukemia, pituitary gland adenomas and mammary gland fibroadenomas. There was no evidence of carcinogenic activity for male B6C3F$_1$ mice fed diets containing 2,500 or 5,000 ppm N-phenyl-2-naphthylamine. There was equivocal evidence of carcinogenic activity of N-phenyl-2-naphthylamine for female B6C3F$_1$ mice as indicated by the occurrence of two rare kidney neoplasms. Chemical-related nonneoplastic lesions occurred in the kidney of rats and mice.

Dr. Sivak, a principal reviewer, agreed with the conclusions for male and female rats and male mice. He proposed that the conclusion for female mice be changed to no evidence of carcinogenic activity, arguing that the presence of only one benign and one malignant renal tumor and the absence of any genotoxic response made this designation more appropriate.

As a second principal reviewer, Dr. Capen agreed with the conclusions for male and female rats and male mice while giving support to changing the conclusion for female mice to no evidence of carcinogenic activity.

Dr. Perera, a third principal reviewer, was unable to attend the meeting and her written comments were read by Dr. L. Hart, NIEHS. Dr. Perera agreed with the conclusions for female rats and male and female mice but thought the conclusion for male rats should be changed to equivocal evidence of carcinogenic activity based on the increased incidence of rare tumors of the spleen and two rare tumors of the colon. She said the supporting evidence for the conclusion in female mice should be expanded to include "...as well as karyomegaly of tubular epithelial cells and atypical cell hyperplasia."

In response to Dr. Sivak and Dr. Capen, Dr. Abdo explained that the call of equivocal evidence of carcinogenic activity in female mice was made because the kidney is a target organ for the chemical, the incidence of kidney tumors in the high dose group was four percent while the historical incidence at the performing laboratory is zero, and atypical hyperplasia was present. Dr. Sivak agreed that with mention of the nonneoplastic lesions he could support the original conclusions. He said justification for the conclusion in female mice should cite not only the kidney neoplasms but also the occurrence of hyperplasia and nuclear enlargement as well as enhanced nephropathy in the high dose group. Dr. Abdo also explained that the conclusion chosen for male rats was appropriate because splenic tumors are not as rare as previously thought while the colon

tumors are mesenchymal rather than epithelial in origin and there is no evidence to suggest the colon is a target organ.

Dr. Sivak moved that the Technical Report on N-phenyl-2-naphthylamine be accepted with the revisions discussed and the conclusions as written for male and female rats and male mice, no evidence of carcinogenic activity, and for female mice, equivocal evidence of carcinogenic activity. Dr. Capen seconded the motion and it was approved unanimously with seven votes.

Discussion of Maximum Tolerated Dose (MTD)

by the Panel and NTP Staff, March 4, 1987

Dr. Scala opened the discussion by asking a question of the Panel: Did they view a clear exceeding of the MTD as a watershed event such as to raise questions about all effects, whether in target organ or other organs, at the dose level used, or is it simply an event that occurs with which you have to deal and then are free to speculate on the impact on the study of having exceeded the MTD.

As background, Dr. Scala read the definition of MTD as stated in the Report of the NTP Ad Hoc Panel on Chemical Carcinogenesis Testing and Evalaution ("Doull Report"), and used by the NTP:

> "The maximum tolerated dose is that dose which when given for the duration of the chronic study as the highest dose will not shorten the treated animals' longevity from any toxic effects other than the induction of neoplasms. The MTD should not cause morphological evidence of toxicity of a severity that would interfere with the interpretation of the study. For example, necrosis of a degree to be associated with a significant amount of regeneration may complicate the interpretation of a neoplastic response in that organ. Thus, toxicity and pathology criteria from the subchronic study are the primary criteria for setting the MTD."

Discussion by the Panel: This centered on how data from studies where the MTD was exceeded could be used to examine and understand the mechanisms of toxicity. The point was raised that for each bioassay where the MTD was exceeded there should be an evaluation as to whether tumor responses were logical consequences of exceeding the MTD. It was suggested that there be fewer but more indepth long-term studies with more dose levels aimed at revealing more information on mechanisms. One Panel member noted that the purposes of using the MTD was to achieve the highest sensitivity for tumor response with a limited number of animals.

Response of Staff: The Panel was reminded that setting doses for a long-term study was a prospective exercise that one analyzes retrospectively. Further, it was pointed out that even though the appropriate criteria are used for selecting doses from prechronic studies there frequently still is reduced survival in the chronic studies.

Conclusions: Dr. Scala commented that we needed to consider how to incorporate the facts of exceeding the MTD into the interpretation of the study.

Dr. E.E. McConnell reported that the NIEHS was already conducting some studies around the issues discussed using the large and unique NTP data base. The staff will plan to start presenting some of the findings on a continuing basis at upcoming Panel and Board meetings. Dr. Scala said the Panel would welcome such information. Further he requested that in the future, the Chemical Manager, in his/her introductory remarks for a report review, explicitly discuss toxicity as evidenced by decreased survival, decreased bodyweight gain, and other signs, i.e., "mitigating factors," as well as giving the bases for the doses selected for the chronic studies.

NTP Views on the Use of "Blinded Pathology Evaluations"

Presented by Dr. Scot Eustis, NIEHS, on March 4, 1987

At the last meeting of this panel, we were asked to present our views on the use of "blinded pathology evaluations" in animal toxicological studies. Before I proceed any further, I want to make it clear that we do not believe that a "blinded pathology evaluation" is appropriate for the routine evaluation of histologic sections from animal studies. I want to emphasize routine, because it may be an appropriate technique under specific circumstances that I will describe in a few minutes. First, however, I want to define for you what a "blinded pathology evaluation is and indicate what the advantages and disadvantages of such a technique are.

As most of you know, a "blinded pathology evaluation" is performed by a pathologist without knowledge of the treatment groups to which the animals, microscopic slides, and tissues belong. To accomplish this, all the microscopic slides must be identified with specific code numbers that have no relation to treatment groups, and any ancillary information provided to the pathologist must be similarly identified with code numbers. Ancillary information could include the findings from the gross necropsy, clinical observations or clinical pathology information.

The advantages given for a "blinded evaluation" are the maintenance of the integrity of the control and treatment groups, prevention of bias introduced by the knowledge of which animals belong to the treated groups, and protection of the pathologist from charges of bias. This topic often incites a fairly heated debate, and the reason for that may be that the word bias to some people conjures up the impression that there is an intent to deceive. Much more importantly, however, is the fact that potential bias in a pathology evaluation can be introduced simply because of the time element involved in completing the evaluation. A typical two-year study with two treatment groups and control group may require 8-10 weeks for the pathologist to evaluate one species, rat or mouse. Over this time period, it is certainly possible that a pathologist can inadvertantly make a slight shift or change in criteria that effects his or her diagnosis. This is most apt to occur with very subtle lesions that are difficult to differentiate from spontaneous background variation. Bias may also be introduced because of the "expectation" by the pathologist of lesions in the treated animals whereas they would not be present in the controls. In this situation the pathologist may pay closer attention to the treated animals and mistake spontaneously occurring changes that are also present in controls for unique treatment related lesions.

The disadvantages to the routine performance of "blinded pathology evaluations" are both scientific and economic. The scientific disadvantages relate to the nature in which diagnoses are made. A diagnosis is generally not an evaluation of a single simple event, but an estimate of multiple, variable, individual biological processes which in concert constitute what is termed a lesion. The pathologist must take into consideration a multitude of individual changes such as cell size and shape, size and shape of cell nuclei, changes in the content of the cell cytoplasm, numbers of cells, alteration in growth pattern and architectural features of the organ, and then relate these changes to the expected appearance of these same tissues in a normal animal of the same species, sex, and age. Finally, the pathologist must combine these

multiple changes into a cohesive interpretation that in essence is a prediction of the biological behavior of the lesion. In order to do this efficiently, accurately, and with maximum sensitivity to subtle treatment related lesions, the pathologist must know which animals are the control animals. The pathologist uses the concurrent controls to set baseline values for what is normal or expected in the treated animals under comparable environmental conditions. This is important because of the degree of normal biological variability that is the result of or influenced by species, sex, age, strain, environment, spontaneous disease, and method of death (natural or sacrificed). Even the techniques employed in the fixation and tissue processing can variably affect the histologic appearance of tissues. If the pathologist has no controls to establish baseline values of what is normal, the pathologist will inevitably spend much additional time recording and grading lesions that really are not lesions at all.

Another point to make is that criteria for the grading of nonneoplastic lesions are based on a knowledge of the spectrum of severity of the lesions seen in each particular study. These criteria can be defined much more rapidly and accurately with knowledge of treatment groups. In a blind study, all slides would be evaluated before the criteria would be set, and a second evaluation would likely be necessary to accurately distinguish dose-related increases in severity of lesions.

The second primary disadvantage of routine blinded pathology evaluations is an economic one. Routine application of this technique would add significantly to the direct costs of the pathology evaluation and increase the time required to complete the evaluation. As I mentioned earlier, all slides would have to be coded for the pathologist to evaluate, and then decoded. In a standard chronic study consisting of two species, both sexes, and two dose groups and controls, the number of slides might vary from 9 to 12 thousand. Not only would the slides have to be coded and decoded, but any ancillary information such as gross necropsy records, clinical chemistry results and clinical findings would also have to be coded and decoded. Following the pathology evaluation, the diagnoses would have to be decoded for subsequent analysis. Because of the frequent manipulation of the data, the error rate would most likely be increased. Finely, in addition to the cost incurred to perform these tasks, the efforts in quality assurance would have to be increased, again adding to the cost. Each step in the process of coding and decoding slides and decoding the pathology data would have to be thoroughly reviewed by the laboratory quality assurance unit.

Before I finish, I want to emphasize that there are alternatives to routine blinded pathology evaluations that are commonly followed by pathologists. First, the slides may be evaluated by alternating five control animals, then treated animals animals, then 5 control, and so forth. This process will completely eliminate any effect of a slight shift in diagnostic criteria over an extended time period. Second, individual tissues may be evaluated consecutively rather than evaluating all tissues from each animal consecutively. This often is done after the initial evaluation especially in subchronic studies. Lastly, specific target tissues may be evaluated in a blinded

fashion following the initial evaluation. This is also often done in an informal manner by many pathologists.

We do not require the individual laboratory pathologists evaluating studies for the NTP to follow any of these procedures described above. However, we have encouraged them to review all potential treatment-related changes to ensure the accuracy of their interpretations. In the NTP pathology peer review process, informal blinded pathology evaluations are frequently done by either the quality assessment pathologist or the PWG chairperson when a lesion is particulary subtle or when there is a questionable effect. Also, because of the nature of the pathology peer review process all treatment-related lesions are evaluated within a relatively short time frame, thus precluding any "shift" in diagnostic criteria as a result of time. During the Pathology Working Group reviews, slides are generally evaluated by the participants without knowledge of treatment groups to which they belong, although that information is available upon request. In PWG reviews of subchronic studies, it is sometimes necessary for the participants to know the treatment groups of specific slides so that direct comparisons can be made.

NTP SENTINEL ANIMAL PROGRAM

Presented by Dr. G.N. Rao, NIEHS, on March 4, 1987

The Sentinel Animal Program was started in 1978 with the 13-week studies and the two-year studies. The objectives of the Sentinel Animal Program at that time were to obtain data on the incidence of viral infections in the studies and to aid in the interpretation of the lesions in the toxicity and carcinogenicity studies. This is the reason why we included the viral (serology) profiles in the NTP Technical Reports. A third objective was to evaluate the animal care procedures in the testing facilities.

The Sentinel Animal Program includes collection of serum samples from five males and five females of each species at six, twelve and eighteen months of the two-year study.

The serum samples collected from these animals were tested for antibodies to certain viruses. For the mouse, viruses monitored were Sendai, mouse hepatitis (MHV), pneumonia (PVM), mouse encephalomyelitis, polyoma, mouse adenovirus, REO virus type 3, lymphocytic choriomeningitis (LCM), ectromelia (mouse pox), and the minute virus of mice. In the rat, some of the viruses are the same as in mouse, like the Sendai virus, pneumonia as in mouse, and then rat corona virus (RCV)/sialodacryoadenitis (SDAV), Kilham's rat virus (KRV) and the Toolan's Agent.

These viral serology profiles were then entered into computer files and analyzed for distribution by testing facility by year and other associations between viruses. This information was presented at the Sixth Chemical Industry Institute of Toxicology Conference, and that information was published in the proceedings, "Complications of Viral and Micoplasma Infections in Rodents to Toxicology Research and Testing."

From this historical data base the common viral infections of mice are the Sendai, pneumonia virus of mice, mouse hepatitis virus. In the rats, that is again, the Sendai, PVM, and then the rat corona virus (RCV)/sialodacryoadenitis (SDAV).

In the mouse, Sendai is the most prevalent virus followed by PVM, MHV and occasionally REO 3. In rats, once again, the Sendai is the most prevalent followed by the RCV/SDAV and then PVM. We had occasionally some KRV virus.

Looking at chronic studies, out of 182 studies in the rat, 122 were positive for one or more viruses as determined by viral antibodies. That is 67 percent. Whereas in the mouse 124 out of 170, that's about 73 percent.

With this information on hand in early 1984, we initiated procedures to control and prevent viral infections in the NTP studies. These procedures included supplying animals to the testing facility that are free of viruses and requiring the laboratories to maintain the animals free of viruses throughout the course of the study. And that required quite a bit of change in management, such as changing outer garments of technicians when they move from room to room and also sanitizing all the equipment that is transferred from room to room, and quarantine of any animal room at the first clinical sign of infection.

In addition, if these testing facilities used animals from other sources for other clients, those animals, if indeed they were close to the NTP studies, should have the same microbial quality as the NTP animals. That is, they should be free of viruses. Theses procedures resulted in a dramatic decrease in viral infections in the NTP prechronic and chronic studies during the period 1984, 1985, and 1986.

The Sentinel Animal Program up to now does very well. We established a historical data base and it helped us to monitor the laboratories to control and prevent viral infections. It helped us to understand the lesions that we see in toxicology studies, especially the 90-day studies. At this point we are analyzing our historical data to understand the influence of viral infections on survival and tumor incidences in rats and mice on the two-year studies.